THE CONSTITUTION OF OLIGOMERIC BENZOFURANOIDS

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by

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At the end of this road, I wish to mention a few of those who crossed my path during this study as a sign of my appreciation. Many of them left more than a lasting impression and deserve much more than my gratitude.

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A special word of thanks to my family for their continued love, support and interest in my studies.

Finally, I wish to dedicate this work to Madelyn, the love of my life, for her support and love during the completion of this study.

Riaan Bekker

List of publications

The work described in this thesis has already led to the following publications:

1. The absolute configuration of biflavonoids and 2-benzyl-2-hydroxybenzo-furanones. R. Bekker, E.V. Brandt and D. Ferreira, *J. Chem. Soc., Chem. Commun.*, 1996, 957.

2. Absolute configuration of flavanone-benzofuranone-type biflavonoids and 2-benzyl-2-hydroxybenzofuranones. R. Bekker, E.V. Brandt and D. Ferreira, *J. Chem. Soc., Perkin Trans. 1*, 1996, 2535.

3. Benzofuranoids with carbon frameworks reminiscent of products of benzylic acid rearrangement. R. Bekker, R.S. Smit, E.V. Brandt and D. Ferreira, *Phytochemistry*, 1996, **43**, 673.

4. Structure and stereochemistry of the first isoflavanone-benzofuranone biflavonoids. R. Bekker, E.V. Brandt and D. Ferreira, *Tetrahedron Letters*, 1998, **39**, 6407.

5. Biflavonoids. Part 4. Structure and stereochemistry of novel flavanone - and the first isoflavanone-benzofuranone biflavonoids. R. Bekker, E.V. Brandt and D. Ferreira, *Tetrahedron*, 1999, submitted.

TABLE OF CONTENTS

DISCUSSION

EXPERIMENTAL

PHYSICAL DATA

CHAPTER 1

INTRODUCTION

Investigations[1,2] into the contents of the heartwood of red ivory, *Berchemia zeyheri* (Sond.) Grubov [= *Phyllogeiton zeyheri* (Sond.) Suesseng = *Rhamnus zeyheri* (Sond.)][3] revealed a rich source of unique biflavonoids containing one or more benzofuranoid constituent units. The unusual nature of these compounds prevented their full structural elucidation at the time and sparked renewed interest in the flavonoid content of this tree. Thus, the isolation and identification of the phenolic content of *B. zeyheri* are the focus of this study with special attention on the absolute configuration of the isolated compounds. As part of the latter goal, an asymmetric synthesis of maesopsin, the main metabolite in the heartwood of *B. zeyheri*, was attempted.

Red ivory belongs to the *Rhamnaceae*[4] family with approximately 600 species that can be divided into 51 genera, nine of which are located in Southern Africa. The name of the species was derived from the famous German botanist and collector, K.J.P. Zeyher, while the genus was named after the French botanist, M. Berchem.[3]

Berchemia zeyheri can be classified as follows[4]:

Class	:	*Magnoliatae*
Subclass	:	*Rosidae*
Order	:	*Rhamnales*
Family	:	*Rhanbaceae*
Genus	:	*Berchemia*
Species	:	*B. zeyheri*

Red ivory is a species of Southern Africa only and it grows in Zimbabwe, Mozambique, Kwazulu-Natal and Transvaal, mainly in a subtropical climate. It is usually smallish and dense with delicate and luxuriant foliage and leaves that are simple and opposite. The leaves are neat oval or oblong with tips that are round or bluntly pointed. They are bright green when young and grey-green when mature. The tree is sometimes evergreen but

usually deciduous and in winter, the leaves often turn a clear golden colour.

The small pale green flowers are borne singly or a few grouped together and give rise to small, edible fruit that are long, oval and green, turning yellow or red when mature.

The fruit is a great delicacy for the Africans who collect and store the surplus fruit in grain-storing baskets where they form a solid brown, sugary mass. They also smoke the powdered root as a headache cure and the powdered bark as an enema. The bark is said to be poisonous.[3]

Red ivory timber is famous for its quality and colour. The sapwood is yellow and the heartwood pink to bright red becoming duller on exposure. It has a particular fine texture and is extremely hard, strong, durable and resistant to insect attack and decay.[3]

Literature survey

CHAPTER 2

BENZOFURANOIDS AND BIFLAVONOIDS

2.1 INTRODUCTION

Flavonoids are highly conspicuous compounds in nature and especially the anthocyanins (1) are intensely coloured plant pigments found throughout vascular plants as an attraction to pollinating plant insects[5]. Most often, however, the important roles are played by the invisible flavonoids that have effects in plant biochemistry and physiology, acting as antioxidants, enzyme inhibitors and growth regulators[6]. Flavonoids also affect some aspects of mammalian metabolism and possess antiallergic, anti-inflammatory, antiviral and anticarcinogenic activities while certain members of this group of compounds may significantly affect the function of multiple mammalian cellular systems[6]. Flavonoids have wide industrial use, especially in the food industry as natural colourants to replace synthetic red dyes[7] while they are also used extensively in the leather industry[8]. Inhibition of larval growth in insects has been ascribed to anthocyanins, for example in the tobacco budworm.[7]

1
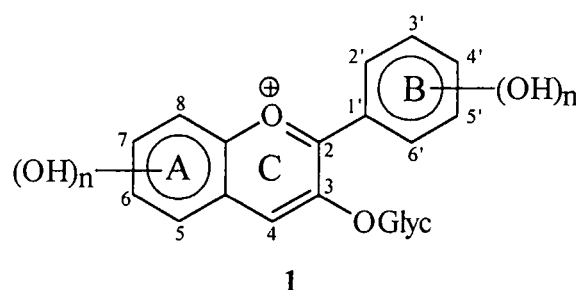
2.1.1 Variations in flavonoid structure

A variety of flavonoid type compounds (more than 4000 chemically unique examples[6]) has been identified in plant sources. All of these have the characteristic C_6-C_3-C_6 carbon skeleton in common while the observed variety originates from several structural and oxidative features. These include:

(a)   cyclization of chalcones (2) and α-hydroxychalcones (3) give rise to benzopyranoids (4) and benzofuranoids (5) and (6);

2

3

4
flavanone

5
aurone

6
auranol
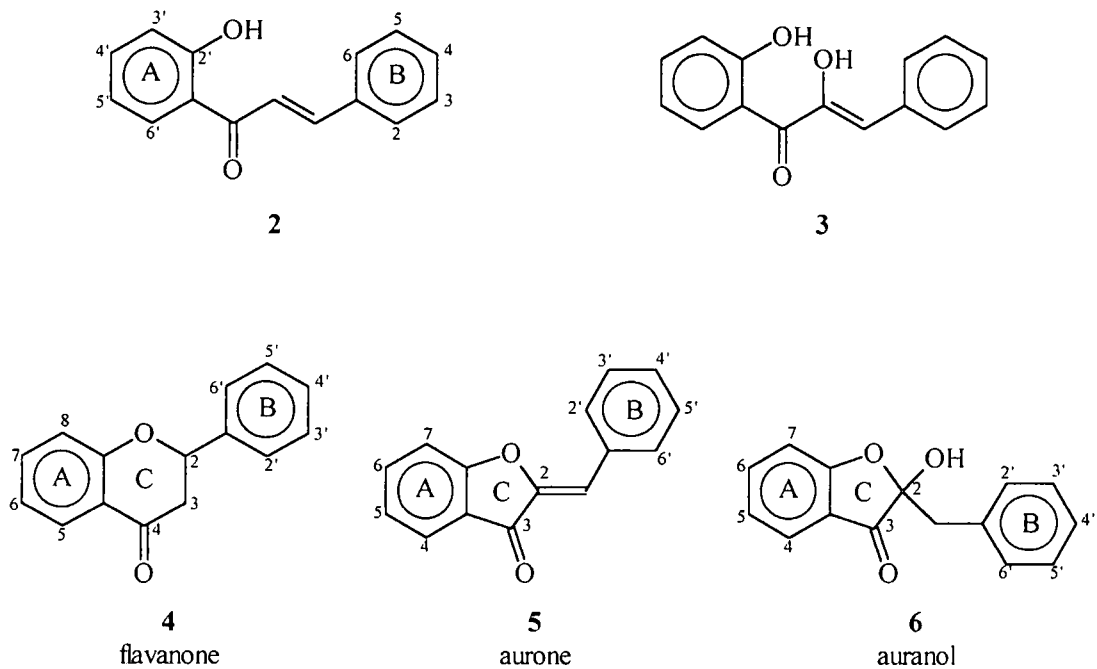
(b)   isomerisation of the B-ring of benzopyranoids to the 3-position to form isoflavonoids (**7**) and then to the 4-position to form neoflavonoids (**8**);

7

8
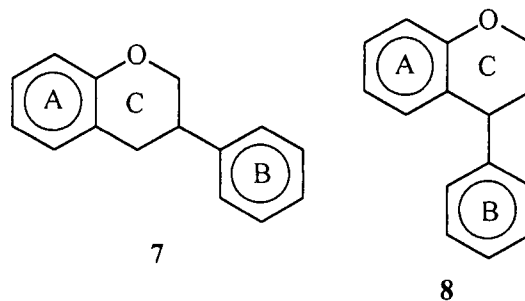
(c)   a change in the oxidation state of the C-ring or, in the case of chalcones, of the C_3-bridge [for example α-hydroxychalcone (**3**)] leading to flavans (**9**), flavan-3-ols (**10**), flavan-3,4-diols (**11**), flavanones (**4**), etc. [see also structures (**12**) - (**14**)] while stereoisomers arise from the presence of stereocentres at 2-C, 3-C and/or 4-C;

9

10

11
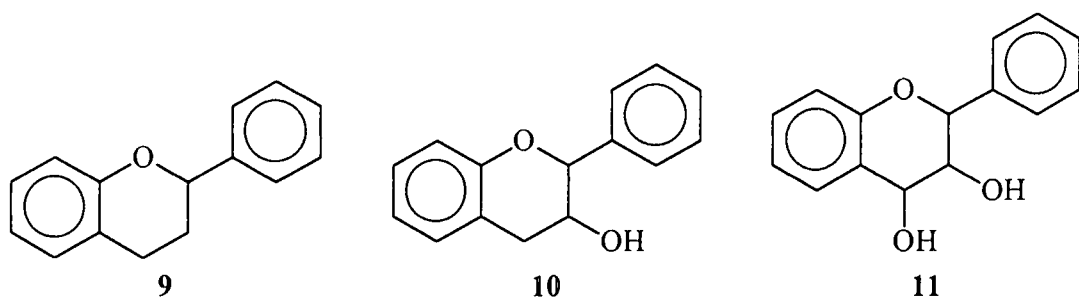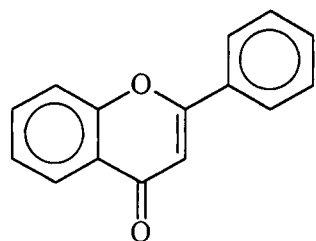
12
flavone
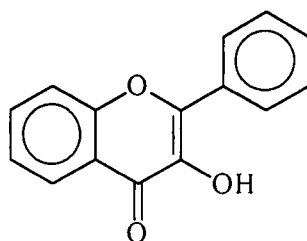
13
flavonol
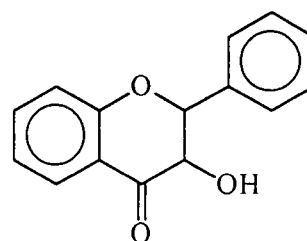
14
dihydroflavonol

(d)   various oxygenation patterns on the aromatic A- and B-rings are encountered in nature, phloroglucinol- (**15**) and resorcinol type (**16**) A-rings being common variations. B-ring patterns frequently occur as 4'-, 3',4'-di- or 3',4',5'-trihydroxy substitutions.
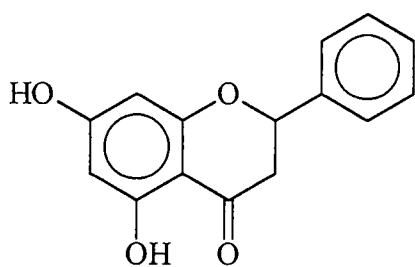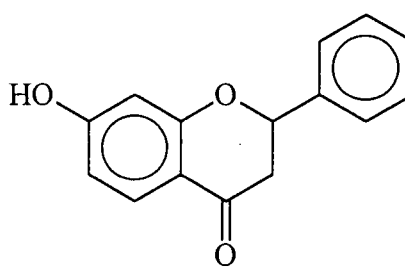
15   **16**

Examples of further variations are the occurrence of flavonoids with C- and O-glycoside substituents, as well as the occurrence of dimeric, trimeric and polymeric flavonoids (see § 2.3).

2.1.2 Biosynthesis of flavonoids

Although there are still some areas of uncertainty, the various pathways to the different flavonoid classes are fairly well documented[9,10]. The past few years have seen a rapid development in the knowledge of the enzymology responsible for individual steps in the biosynthetic process. One example is the identification of chalcone synthase, the enzyme responsible for the formation of chalcones from malonyl coenzyme A and *p*-coumaryl coenzyme A.[11]

All other flavonoid classes are derived from the chalcone intermediate in a sequential manner (see **Scheme 2.1**). For example, a stereospecific transformation of the chalcone by chalcone isomerase provides a (2*S*)-flavanone[12] that undergoes further transformations by known enzymatic processes as summarized in **Scheme 2.1**.

Chalcone   Flavonol

(cyclization) | Chalcone isomerase   (dehydrogenation) | Flavonol synthase

Flavone ← Flavone synthase / (dehydrogenation) — Flavanone — Flavanone 3-hydroxylase / (hydroxylation) → Dihydroflavonol

(bioreduction) | Flavanone 4-reductase   (bioreduction) | Dihydroflavonol 4-reductase

Flavan-4-ol   Flavan-3,4-diol

(dehydroxylation) | Leucoanthocyanidin 4-reductase

Flavan-3-ol

Scheme 2.1

One of the areas of uncertainty is the exact process involved in the biosynthesis of aurones (**5**) and auranols (**6**). The identification of the first α-hydroxychalcone, $\alpha,2',3,4,4'$-pentahydroxychalcone (**17**) by Ferreira *et al.*[13] from the gum copal tree (*Trachylobium verrucosum*) opened possible routes to the formation of auranols by α-cyclization as well as alternative routes for the biosynthesis of dihydroflavonols *via* cyclization of the enolic form of the α-hydroxy-chalcone (see **Scheme 2.2**).[14]

17   Keto-enol tautomerism   18

19   20
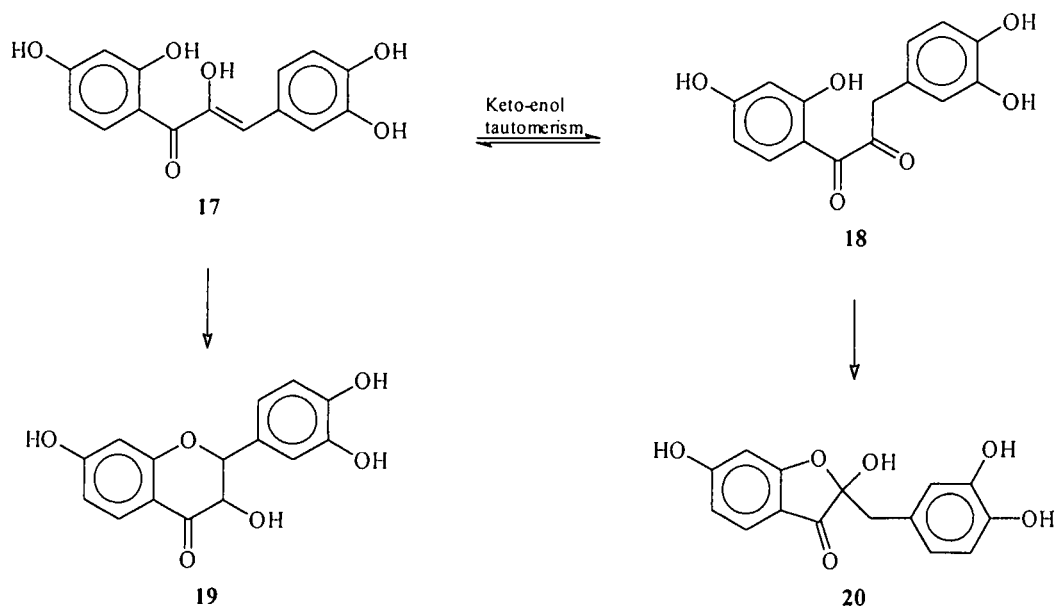
Scheme 2.2

2.2 FLAVONOIDS BASED ON 2-BENZYLCOUMARANONES

This small group of flavonoids consists of only two types, namely aurones and auranols. Aurones are yellow coloured, relatively rare aromatic compounds, based on the 2-benzylidenecoumaranone structure (5), while auranols are colourless compounds based on 2-hydroxy-2-benzylcoumaranones (6). Auranols are more rare than aurones and can be seen as hydrated aurones [dehydration of auranols with boiling sulphuric acid (5%) in acetic acid yields the corresponding aurone[15]].

2.2.1 Natural auranols

Although the number of known auranols is limited, most of the common variations in the aromatic oxygenation patterns of the flavonoids can be found in this small group of natural products [see structures (20) - (25)].
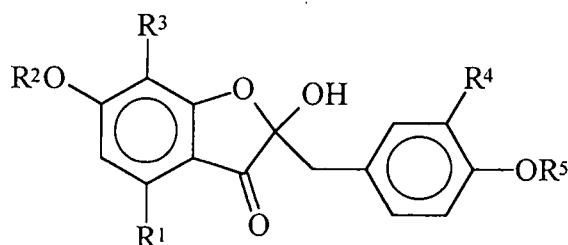
20 R^4 = OH; R^1 = R^2 = R^3 = R^5 = H
21 R^1 = R^4 = OH; R^2 = R^3 = R^5 = H
22 R^4 = OH; R^1 = R^2 = R^3 = H; R^5 = Me
23 R^1 = OH; R^2 = R^3 = R^4 = R^5 = H
24 R^3 = R^4 = OH; R^1 = R^2 = R^5 = H
25 R^2 = R^3 = R^4 = R^5 = H; R^1 = OMe

Alphitonin (21), isolated by Read and Smith[16] from the heartwood of the Australian "red ash" (*Alphitonia excelsa*), represents the first example of this class of compound from nature. Although its isolation dates back to 1922, the structure was only determined in 1960 when Birch et al.[17] reduced the pentamethyl ether derivative (26) to the benzylcoumaranone (27) and subsequently to the 2'-hydroxy-3,4,4',6'-tetramethoxychalcone (28) (see **Scheme 2.3**), the structure of which was determined by comparison with an authentic sample. Final conformation was obtained by synthesis of the 2'-hydroxy-α,3,4,4',6'-pentamethoxychalcone (29) from 3,4-dimethoxybenz-aldehyde (30) and 2-hydroxy-ω,4,6-trimethoxyacetophenone (31) (**Scheme 2.3**). This chalcone was cyclized to alphitonin pentamethyl ether that was identical to the one

isolated from nature.
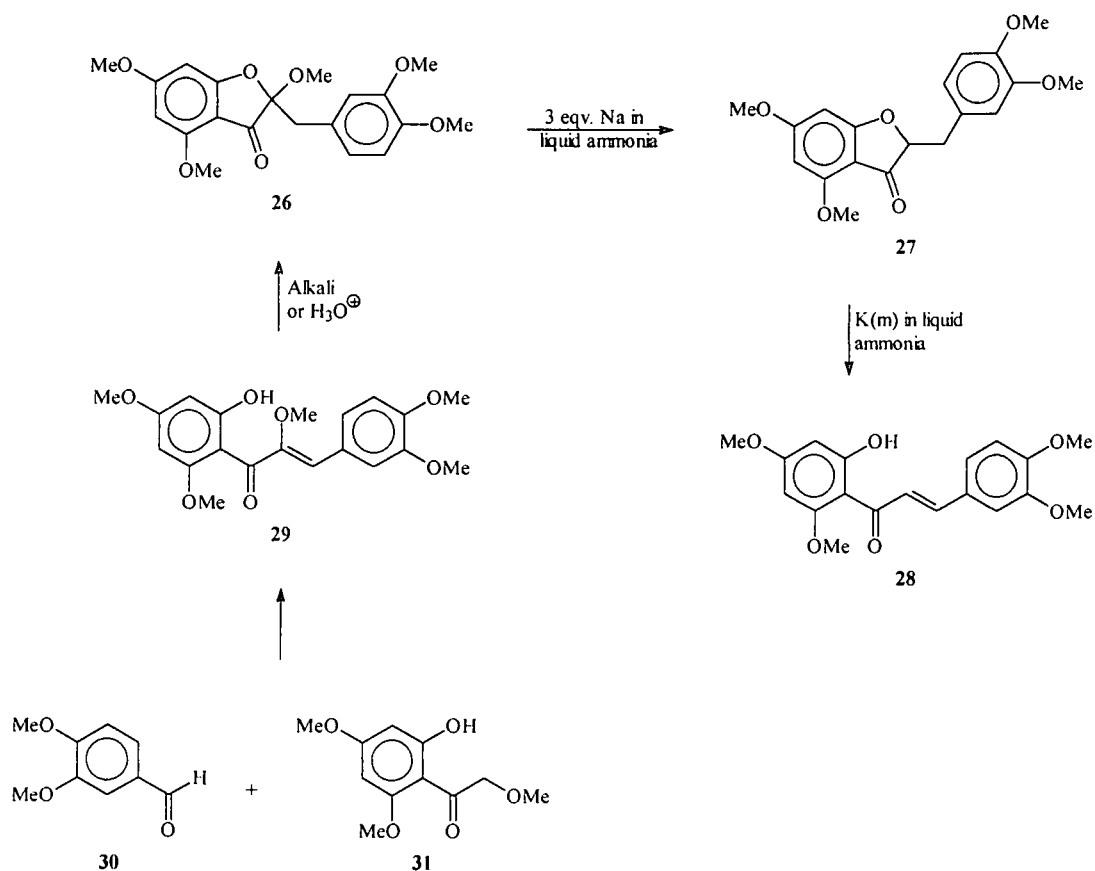
Scheme 2.3

In 1957 King and White[18] announced the isolation of two auranols, 2-benzyl-2,3',4',6-tetrahydroxybenzo[b]furan-3(2H)-one **(20)** and the 2,3',6-trihydroxy-4'-methoxy analogue **(22)**, from the Quebracho tannin extract (the aqueous heartwood extract of *Schinopsis balansae* and *S. lorentzii*). The characterization and synthesis of these compounds were complicated by the absence of the 4-hydroxyl group, a factor that was claimed to affect the ease of synthesis and reactivity of these substances[19]. Full characterization was only achieved when the auranols were treated with concentrated

32 R = Me
33 R = H
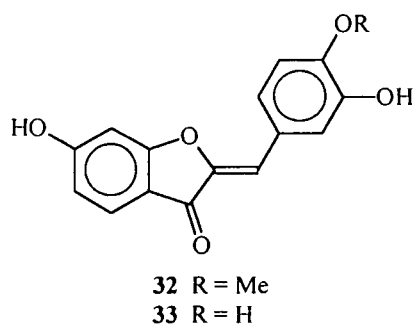
sulphuric acid to yield the corresponding aurones, 6,3'-dihydroxy-4'-methoxyaurone

(32) and sulphuretin (33).[19] Confirmation of the structures was obtained by the synthesis of the chalcone and subsequent cyclization as discussed above. 2-Benzyl-2,3',4',6-tetrahydroxybenzo[b]furan-3(2H)-one (20) was also isolated by Ferreira *et al.*[13] from the gum copal tree (*Trachylobium verrucosum*).

Maesopsin (23) is the most widespread of the auranols and was isolated from *Maesopsis eminii*[15], *Alphitonia whitei*[20], *Berchemia zeyheri*[21] and *Colubrina granulosa*[22]. Janes *et al.*[15] identified the yellow compound obtained from the reaction of maesopsin with boiling sulphuric acid as the 4,4',6-trihydroxyaurone, thus disclosing the structure of the hydrated analogue, maesopsin. The reaction of dihydrokaempferol (34) with hot alkaline solution in the absence of oxygen to yield maesopsin (23) (**Scheme 2.4**) supported the structure. Guise *et al.*[20] provided further proof for the structure by using the same reductive conditions as Birch *et al.*[17] (**Scheme 2.3**) to form the 2'-hydroxy-4,4',6'-trimethoxychalcone.
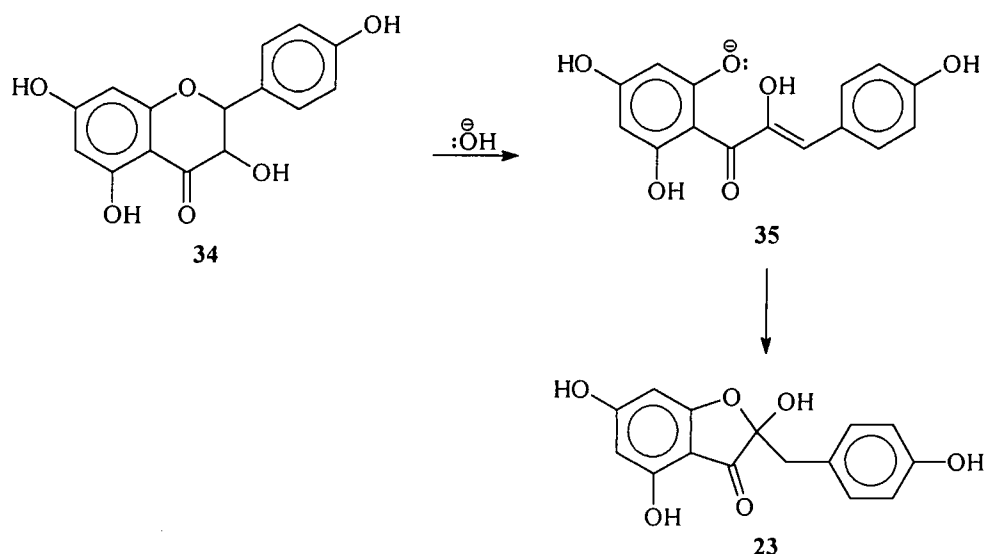
Scheme 2.4

The first auranol exhibiting optical activity, nigrescin (24), was isolated from *Acacia nigrescens* by Fourie *et al.*[23] Structural proof was provided by conversion of 3',4',7,8-tetramethoxy-2,3-*trans*-dihydroflavonol (37) into nigrescin by brief treatment with alkali in a process similar to that described in **Scheme 2.4**.

Although the CD-curve of this auranol exhibited a positive Cotton effect in the 280 - 320 nm area, no interpretation of this effect in terms of the absolute configuration at C-2 was possible at the time since no analogies existed. Since the dihydroflavonol (36)

and flavanone (**38**) accompanying nigrescin in the heartwood of *A. nigrescens* were completely racemized, it was speculated that the five-membered ring of the auranol was more stable than the six-membered ring system of the flavanone.[23]

36 R = H
37 R = Me

38
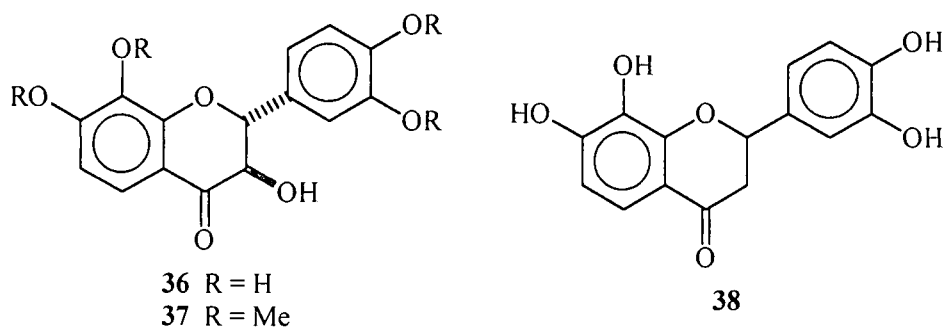
The 4-*O*-methyl analogue of maesopsin, carpusin (**25**), isolated from the heartwood of *Pterocarpus marsupium*[24], was identified by ^{1}H NMR while it was also treated with sulphuric acid (5%) in acetic acid to give the aurone. The tri-*O*-methyl ether of carpusin would naturally be the same as the tetra-*O*-methyl ether of maesopsin while the α,2',4,4',6'-pentamethoxychalcone (**39**), formed as by-product during the methylation of maesopsin[15], was also detected when carpusin was methylated with dimethyl sulphate and potassium carbonate.[24] Carpusin was also reported as a constituent of *Berchemia racemosa*.[25] More recently, it was discovered that the acetone extract of the heartwood of this tree protected the liver against injuries induced by CCl₄ and α-naphthylisothiocyanate in rats. Carpusin was identified as one of the active substances.[26]

39
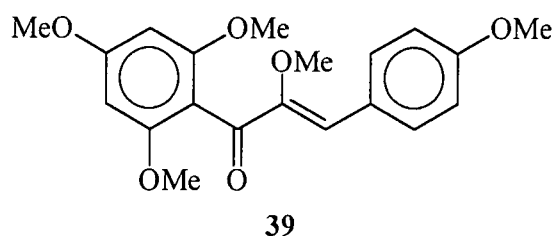
The latest example of auranol type compounds from nature is the four castillenes [castillene A (**40**), B (**41**), C (**42**) and D (**43**)] isolated from *Lonchocarpus castilloi*[27] along with the biosynthetically related α-hydroxydihydrochalcone, castillene E (**44**). The compounds were identified by ^{1}H NMR and mass spectral methods.

The [α]D values recorded for the four auranols all reflected a marked degree of optical activity. Despite this fact, no attempt was made to determine the stereochemistry at
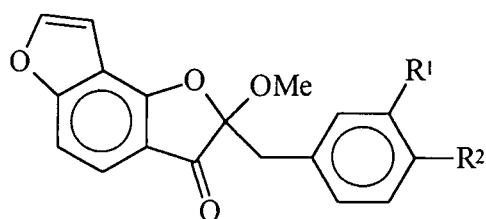
40 R¹ = R² = H
42 R¹ + R² = -OCH₂O-
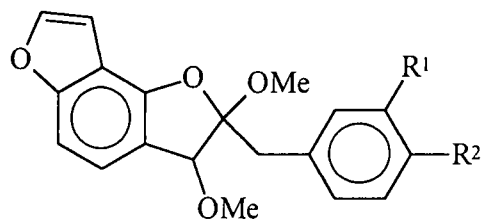
41 R¹ = R² = H
43 R¹ + R² = -OCH₂O-

any of the stereocentra. Since the heartwood of *L. castilloi* is known for its resistance against several wood-rotting fungi, the five phenols were tested for antifungal activity against *Lenzites trabea*. Each of the castillenes partially inhibited the growth of the fungus, while castillene E (**44**) exhibited the highest degree of activity.[27]
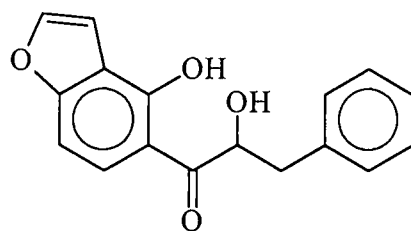
44

2.3 BIFLAVONOIDS WITH STEREOCHEMISTRY OTHER THAN ATROPISOMERISM

Biflavonoids exhibit several interesting chemical[28] and pharmacological[29,30] properties, including antihistamine[31] and antifungal[32] activities. Thus, in the recent past, the emphasis of biflavonoid chemistry has shifted from a purely phytochemical interest to a search for biological active compounds. The wide structural variety observed in natural biflavonoids include variations in the position of the interflavonoid bond and the oxidation state of the constituent monomers.

The biflavones[33] [e.g. sahranflavone (**45**) and 3,8"-biapigenin (**46**)], comprising about half of the known biflavonoids, are often found as atropisomers due to restrictions of rotation about the interflavonoid bond. The remainder of this class of natural products, consisting of biflavanones and dimers with mixed flavone, flavanone and dihydroflavonol, and to a lesser extend aurone, chalcone and dihydrochalcone constituent units, may contain up to four stereocenters.[33] This introduces an

interesting dimension to the study of these biflavonoids, the structural elucidation and natural occurrence of which will be discussed in sections 2.3.1, 2.3.2 and 2.3.3.
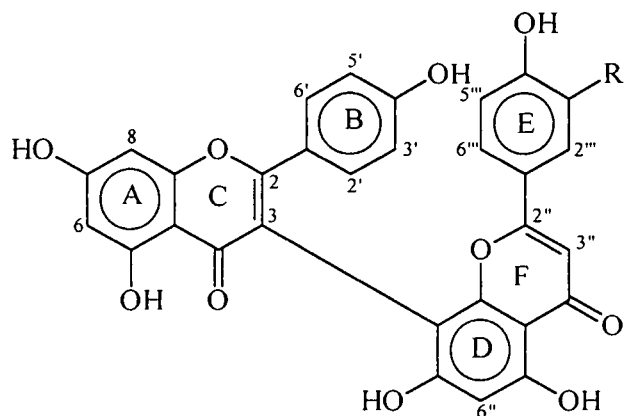
45 R = H
46 R = OH

2.3.1 <u>3,3"-Coupled biflavonoids</u>

The natural occurrence of 3,3"-coupled biflavonoids is limited to the various derivatives and stereoisomers of 3,3"-binaringenin isolated primarily from *Stellera chamaejasme*. The first, named chamaejasmin (**47a**), was isolated in 1979 by Hwang and Chang[34] (and later by Castro and Valverde[35] from *Diphysa robinioides*) who determined the structure from the ^{1}H NMR and mass spectra. The ^{1}H NMR spectrum of this dimer is similar to that of naringenin and the dimeric character of the substance was only apparent from the mass spectrum. The *trans* relative stereochemistry at 2-C(C)/3-C(C) and 2-C(F)/3-C(F) follows from the coupling constants [J 12.0 Hz for both 2-H(C)/3-H(C) and 2-H(F)/3-H(F)]. This dimer was isolated as a racemic mixture.

In 1984, Hirata and co-workers[36] isolated three derivatives of chamaejasmin from *S. chamaejasme*. Chamaejasmenin A (**48a**) and B (**49d**) contain two methoxy groups each and the symmetrical character of both was again evident from the ^{1}H NMR spectra which display a single set of signals corresponding to the monomeric unit. The dimeric character followed from the mass spectra and ^{13}C NMR was used to confirm the coupling position. Comparison of the ^{13}C NMR spectra of 7-*O*-methyl- and 4'-*O*-methylnaringenin with those of the two dimers identified 4'-*O*-methylnaringenin as the constituent unit. Despite the element of symmetry present in the molecule, both have

large $[\alpha]_D$ values, indicating that both chamaejasmenins A (**48a**) and B (**49d**) have no plane of symmetry. The relative stereochemistry at 2-C(C)/3-C(C) and 2-C(F)/3-C(F) was evident from the coupling constants of the corresponding protons - chamaejasmenin A (**48a**) possessing a *trans-trans* geometry (J 12.0 Hz) while chamaejasmenin B (**49d**) is the first example with a *cis-cis* relative configuration (J 2.0 Hz).

a

b

c

d
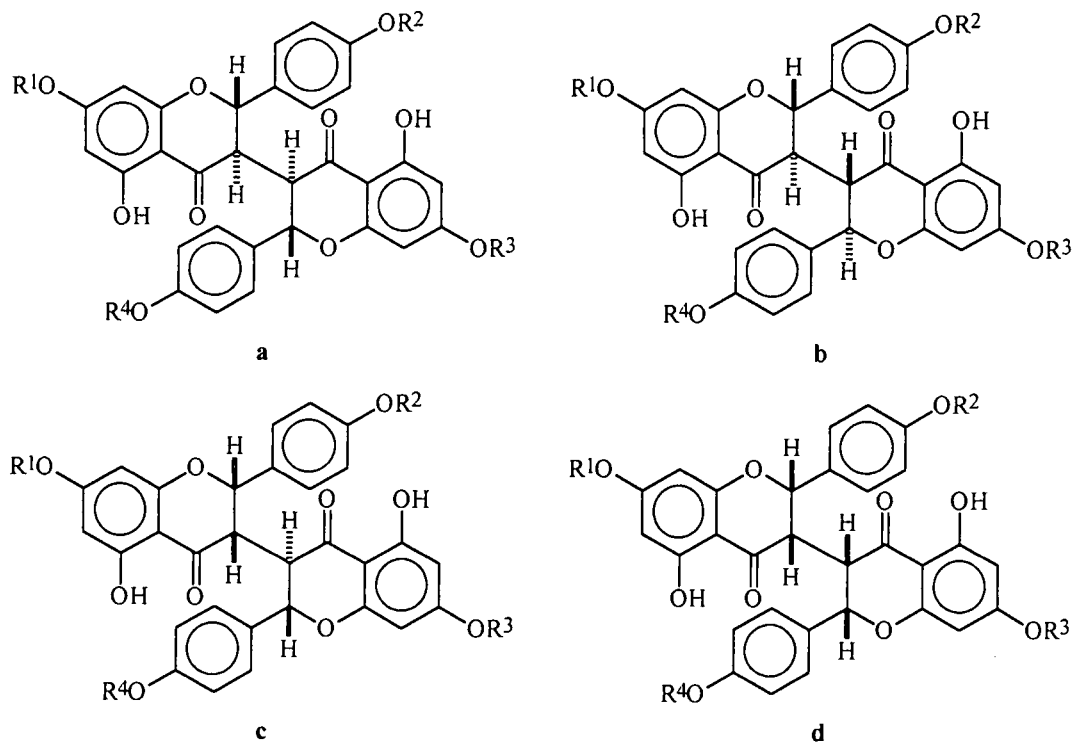
	R^1	R^2	R^3	R^4		R^1	R^2	R^3	R^4
47a	H	H	H	H	**55b**	H	H	H	H
48a	H	Me	H	Me	**56b**	Me	Me	Me	Me
49d	H	Me	H	Me	**57d**	H	Me	H	H
50d	Me	Me	H	Me	or	H	H	H	Me
51d	H	H	H	H	**58c**	H	Me	H	H
52c	H	H	H	H	**59a**	Glc	H	Glc	H
53d	Me	Me	Me	Me	**60b**	H	Me	H	H
54a	H	Me	H	H					

Table 2.1

Chamaejasmenin C[36] (**50d**) was identified as the 7-*O*-methylether derivative of chamaejasmenin B based on evidence from the ^{1}H NMR and mass spectra. Again the

comparison of the ^{13}C NMR spectrum of chamaejasmenin C with those of 4',7-di-*O*-methyl- and 4',5-di-*O*-methylnaringenin identified the position of the methoxy group. This derivative has the same *cis-cis* relative stereochemistry (J 1.0 Hz) as chamaejasmenin B (**49d**).

Continued investigation of *S. chamaejasme*[37] revealed two additional examples of 3,3"-biflavanones named neochamaejasmin A (**51d**) and B (**52c**), both stereoisomers of chamaejasmin. The structure of neochamaejasmin A (**51d**) was determined from the ^{1}H and ^{13}C NMR and mass spectra as described above while the relative stereochemistry was resolved as *cis-cis* (J 4.0 Hz). In the case of neochamaejasmin B (**52c**), similar proton and carbon resonances were observed in the ^{1}H and ^{13}C NMR spectra but the stereochemistry was determined as *cis-trans* (J 4.0 and 12.0 Hz). The 7,4',7",4'''-tetramethyl ether of neochamaejasmin A (**53d**) was isomerized with 10% NaOH to yield a substance identical to 7,7"-di-*O*-methylchamaejasmenin A (**54a**).

The same source also yields isochamaejasmin[38] (**55b**), an optically inactive isomer of chamaejasmin with *trans-trans* relative stereochemistry (J 12.0 Hz). When the 7,4',7",4'''-tetra-*O*-methyl ether (**56b**) of this biflavonoid was isomerized with 10% NaOH, a mixture consisting of starting material (**56b**) and 7,7"-di-*O*-methylchamaejasmenin A (**54a**) was obtained. This corresponds to the results of the isomerization of 7,4',7",4'''-tetra-*O*-methylneochamaejasmin A (**53d**) described above.

Since Hwang and Chang used NaOH during the initial isolation of chamaejasmin[34], the natural product was probably racemized during the isolation process. It was not until 1986 that the reinvestigation of *S. chamaejasme* yielded optically active chamaejasmin (**47a**).[39] Both chamaejasmin and isochamaejasmin (**55b**) were easily isomerized into a mixture of the two isomers. Considering that isochamaejasmin was isolated in an optically inactive form, it now became clear that chamaejasmin is the (+)- or (-)-form while isochamaejasmin is the *meso*-form of 3,3"-binaringenin with *trans-trans* stereochemistry. Resolution of chamaejasmin by means of HPLC on a chiral substrate showed that the optically inactive form was a 50:50 mixture of two enantiomers while the optically active form consisted of a 68:32 mixture of the (-)- and (+)-forms.[39]

Sikokianin A[40] (**57d**) is an optically active form of chamaejasmin with an *O*-methyl

group on 4'-C(B) or 4'''-C(E) and a *cis-cis* geometry (J 2.0 Hz). It was isolated along with sikokianin B (**58c**), an optically active isomer of sikokianin A with a *cis-trans* relative configuration (J 4.0 and 9.0 Hz), from the roots of *Wikstroemia sikokiana.*[40] The structures of these derivatives where determined in the same way as those of chamaejasmenins A, B and C.

Up to 1990, no attempt to determine the absolute stereochemistry of the chamaejasmins had been reported in the literature. This changed when Nyandat *et al.*[41] isolated ormocarpin (**59a**), a 7,7''-di-*O*-β-diglucoside of chamaejasmin, form *Ormocarpum kirkii*. Hydrolysis with β-glucosidase yielded D-glucose and optically active chamaejasmin (**47a**). In order to establish the positions of the two glucose units, tetra-*O*-methylormocarpin was hydrolysed and the resulting tetra-*O*-methylchamaejasmin acetylated to give the corresponding di-*O*-acetyl derivative. The position of the acetoxy groups was then determined by comparison of the ^{1}H NMR and ^{13}C NMR spectra of this compound with the spectra of tetra-*O*-methyl- (obtained from the hydrolysis) and hexa-*O*-methylchamaejasmin.

In an attempt to determine the absolute stereochemistry of ormocarpin (**59a**), the authors recorded the CD curve of methylated ormocarpin[41] which exhibited a strong positive Cotton effect at 323 nm (the n→π* transition for carbonyls) and a negative Cotton effect at 275 nm (the π→π* transition). Application of the octant rule modified for aryl ketones[42] indicated an apparent 2S,3R-configuration for the diequatorial substituents. However, while this rule is useful for monomeric flavonoids, its application to biflavonoids is untested and considering that CD effects are cumulative, the influence of a second heterocyclic ring that introduces two additional asymmetric carbons is unknown. Furthermore, no consideration was given to the possible restriction of rotation about the interflavonoid bond and the resulting conformations that could influence the CD curve.

Detailed investigation of *Wikstroemia sikokiana* by Baba *et al.*[43] identified this deciduous Japanese shrub to be another source of 3,3''-binaringenin dimers. It's flavonoid content included chamaejasmenins A (**48a**) and B(**49d**), sikokianins A (**57d**) and B(**58c**), neochamaejasmins A (**51d**) and B (**52c**) and isochamaejasmin (**55b**) as well as a new biflavonoid named sikokianin C (**60b**). This was identified as a 4'-*O*-

methyl ether of isochamaejasmin by means of n.O.e. The similarity between the ^{1}H NMR spectra of sikokianin C and isochamaejasmin was quoted as the basis for the structural assignment of sikokianin C.

2.3.2 3,8''-Coupled biflavonoids

Although the identification of flavonoids dates back to the turn of the century, it was not until ^{1}H NMR techniques became available that the structural elucidation of biflavonoids became viable. Thus, the structure of fukugetin (61), isolated by Perkin and Phipps[44] in 1904 from *Garcinia spicata*, was only determined in 1969 by Konoshima *et al.*[45] The approach the authors followed is typical of that used by other workers and included the degradation of the dimer and spectroscopic studies.

Alkali fusion of fukugetin (61) yielded phloroglucinol (63), protocatechuic acid (64) and *p*-hydroxybenzoic acid (65). A heptamethyl ether (62), obtained by methylation of (61), gave veratric acid (66), anisaldehyde (67) and a β-diketone (68) upon hydrolysis with boiling ethanolic potassium hydroxide (see **Scheme 2.5**). The β-diketone was identified by means of ^{1}H NMR and mass spectrometry.[45]

The ^{1}H NMR spectrum of the heptamethyl ether (62) showed signals for seven methoxy groups and a pair of doublets (J 12.0 Hz) for 2-H(C) and 3-H(C). A doublet of doublets (J 2.5 and 8.5 Hz) along with two doublets in the aromatic region (J 2.5 Hz and 8.5 Hz respectively) correspond to the three protons of the E-ring while two doublets (J 9.0 Hz) in the same region coincide with the four protons of the B-ring. Two *meta*-coupled doublets (J 2.5 Hz) at higher field and a residual singlet fit the criteria required for two phloroglucinol type rings, one of which is substituted in the 6''- or 8''-position. A second singlet in the same region was assigned to 3''-H(F).

The position of the interflavonoid bond was not established in any rigorous way. It was suggested that since the hydroxyl group at 5''-C(D) was not exceptionally difficult to methylate, the bulky substituent (ring C) is positioned at 8''-C(D) rather than at 6''-C(D). In the latter case, it is expected that the large C-ring will prevent methylation of this hydroxyl group to a certain degree.

The large coupling constants of 2-H(C) and 3-H(C) indicated a *trans*-diaxial

arrangement for the heterocyclic protons. Fukugetin (**61**) was isolated in racemic form as well as in an optically pure form ($[\alpha]_D = +170°$). Although ORD data was available for this compound, the authors did not attempt to determine the absolute stereochemistry of (+)-fukugetin.
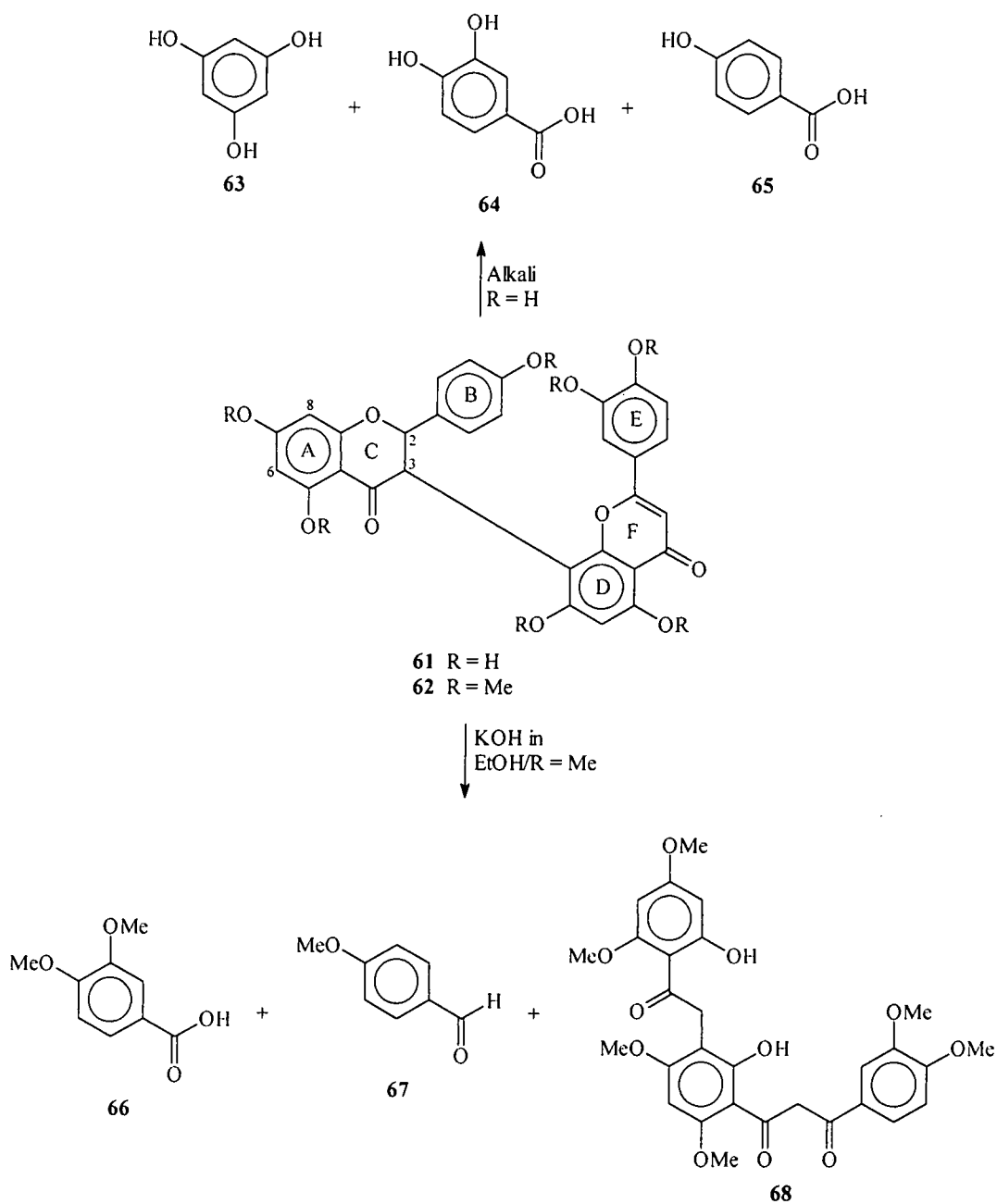
Scheme 2.5

Fukugetin was found to be identical with morelloflavone[45] isolated earlier form *G. morella* by Karanjgaokar et al.[46]

The uncertainty concerning the point of linkage of some biflavonoids was partially

addressed by Joshi *et al.*[47] and Pelter *et al.*[48] When CDCl₃ is replaced with C_6D_6 in a ¹H NMR experiment, it is expected that all methoxy groups with an *ortho*-proton will exhibit pronounced shift to higher field. Application of this method to talbotaflavone[47] (**70**) and fukugetin[48] (**61**) indicated the 8"-C(D) position as the point of linkage. Attempts by Jackson *et al.*[49] to apply the nuclear Overhauser effect (n.O.e.) on fukugetin (**61**) failed. Thus, the position of the interflavonoid bond remained ambiguous until Ikeshiro and Konoshima[50] synthesized hepta-*O*-methyl-fukugetin (**62**) from the appropriate flavone. Several other examples of this class of biflavonoids are summarized in **Table 2.2**. The procedures for the identification of these compounds are very similar to those used for fukugetin (**61**) described above.

Name	Substituents			Source
	R1	R2	R3	
Fukugetin (**61**) (BHG-II or morelloflavone)	H	H	OH	*G. spicata*[45], *G. morella*[46], *G. talboti*[47], *G. livingstonii*[48], *G. volkensii*[51], *G. xanthochymus*[52], *G. linii*[52], *G. multiflora*[52,53], *G. densivenia*[54], *Allanblackia floribunda*[55], *G. echinocarpa*[56]
Spicatiside (**69**)	H	Glc	H	*G. spicata*[52], *G. multiflora*[57]
Volkensiflavone (**70**) (BGH-III or talbotaflavone)	H	H	H	*G. talboti*[47], *G. livingstonii*[48], *G. volkensii*[51], *G. spicata*[52], *G. xanthochymus*[52], *G. multiflora*[53], *G. echinocarpa*[56]
3-*O*-methylfukugetin (**71**)	H	H	OMe	*G. spicata*[45], *G. densivenia*[54]
Fukugiside (**72**)	H	Glc	OH	*G. xanthochymus*[52], *G. multiflora*[57], *G. spicata*[58]
I-5,II-5,I-7,II-7,I-3',I-4',II-4'-heptahydroxy-[I-3,II-8]-flavanonyl-flavone (**73**)	OH	H	H	*G. Nervosa*[59]

Table 2.2

It is noteworthy that the relative stereochemistry of the heterocyclic C-ring is *trans*-diaxial in all the cases studied. Although the optical activity (or lack thereof) was noted for most of the above substances, no attempts were made to determine the absolute configuration of any of these compounds.

The group of 3,8"-biflavonoids includes several biflavanones, isolated primarily from the *Garcinia* genus (see **Table 2.3**). The methods used for identification of these biflavanones are similar to those described above, utilizing ^{1}H NMR, mass spectrometry and the degradation of the biflavanone.

Name	Substituents						Source
	R^1	R^2	R^3	R^4	R^5	R^6	
GB1a(**74**)	H	H	H	H	H	H	*G. eugeniifolia*[49], *G. vol-kensii*[51], *G. spicata*[52], *G. xanthochymus*[52], *G. linii*[52], *G. multi-flora*[52,53], *G. terpnophylla*[56], *G. buchananii*[60], *G. kola*[61,62]
GB1a-7"-*O*-glucoside (**75**)	H	H	H	Glc	H	H	*G. multiflora*[57]
GB1 (**76**)	H	H	OH	H	H	H	*G. eu-geniifolia*[49], *G. multiflora*[52], *G. linii*[52], *G. terpnophylla*[56], *G. buchananii*[60], *G. kola*[61,63]
GB2a (**77**)	H	H	H	H	OH	H	*G. eugeniifolia*[49], *G. volkensii*[51], *G. xanthochymus*[52], *G. linii*[52], *G. spicata*[52], *G. multiflora*[52,53], *G. buchananii*[60], *G. kola*[61,62,63]
Manniflavanone (**81**)	OH	H	OH	H	OH	H	*G. kola*[62], *G. mannii*[64]

Table 2.3

Name	Substituents						Source
	R^1	R^2	R^3	R^4	R^5	R^6	
GB2 (78)	H	H	OH	H	OH	H	*G. eugeniifolia*[49], *G. xanthochymus*[52], *G. multiflora*[52], *G. linii*[52], *G. terpnophylla*[56], *G. buchananii*[60],
Xanthochymusside (80)	H	H	OH	Glc	OH	H	*G. xanthochymus*[52], *G. multiflora*[57],
Kolaflavanone (79)	H	H	OH	H	OH	Me	*G. kola*[61,62]
Garciniflavanone (82)	OH	H	OH	H	OH	H	*G. kola*[62]
GB3 (83)	OH	Me	OH	H	OH	H	*G. kola*[62,65]

Table 2.3 (continued)

GB1 (**76**), GB1a (**74**), GB2 (**78**) and GB2a (**77**) were first isolated by Jackson *et al.*[49] in the late sixties. However, soon after the first publication of these results, Pelter[66] pointed out that the structure (**76**) proposed by Jackson *et al.* was not the only possibility. He suggested a structure (**84**) where the second biflavanone is coupled to the 2-position of an isoflavanone, rather than to the 3-position of a flavanone as proposed by Jackson *et al.* He based his proposal primarily on biosynthetic arguments.[66] In order to distinguish between these structures, the degradation of the heptamethyl ether of GB-1 was studied[66] and the reaction mixture was scrutinized for traces of deoxybenzoins (**85**) and (**86**). Isolation of the former from the reaction mixture indicated that the structure proposed by Jackson *et al.* was indeed correct.

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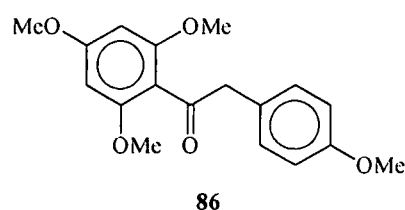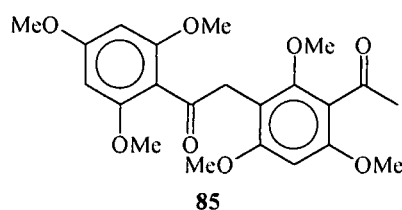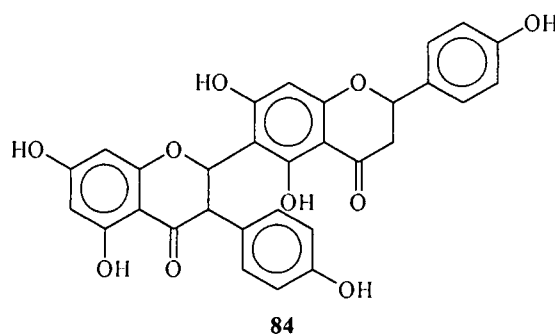
The study of these biflavonoids was extended by utilization of ^{13}C NMR spectroscopy[61,67] in order to address some areas of uncertainty, e.g. the determination

of the position of the interflavonoid bond. Thus, Crichton and Waterman[64], using the observation that the resonances of sterically hindered methoxy substituents occur above 59.5 ppm as compared with 55 - 57 ppm for normal substituents, determined the point of linkage for manniflavanone (**81**). Since 2D NMR methods were unavailable at the time, the assignments of the ^{13}C NMR signals were primarily made by comparison with the spectra of the appropriate monomers[67] and by use of empirical methods[61].

Owing to the complex nature of the CD phenomenon in flavonoids, their interpretation necessitates an empirical rather than a theoretical approach. The CD spectra of monomeric flavanones have been studied thoroughly and at least four Cotton effects are generally observed.[68] However, only two of these are usually unambiguous, namely the $\pi \rightarrow \pi^*$ (270 - 290 nm) and $n \rightarrow \pi^*$ (320 - 330 nm) transitions and therefore it is generally these Cotton effects which are considered. Empirically it is frequently observed that 3-hydroxyflavanones with a (2R,3R)-configuration and flavanones with a 2S-configuration exhibit a positive Cotton effect for the $n \rightarrow \pi^*$ transition and a negative Cotton effect for the $\pi \rightarrow \pi^*$ transition.[68]

In the case of biflavonoids, the influence of the second flavonoid unit must be considered. In dimers with a chalcone substituent (e.g. **87**) that are often produced during the methylation of biflavanone dimers, only the flavanone moiety is chiral. Although the achiral chalcone unit is chirally perturbed by the flavanone unit, relative small $\Delta\varepsilon$-values can be expected for this chromophore with achiral first and second spheres. This is confirmed by experimental data and the CD spectra of these dimers can be compared to those of the relevant monomers with known stereochemistry.[67]

87
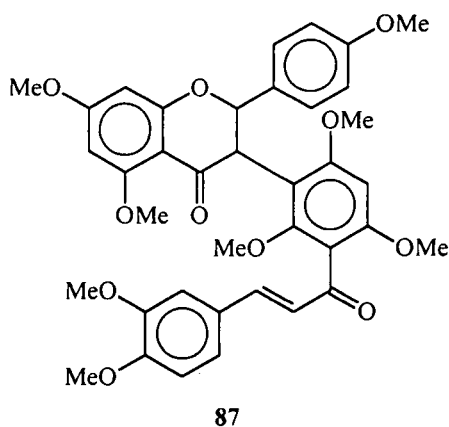
In fukugetin (**61**) the flavone moiety is also achiral but due to ring closure not flexible

and therefore much more susceptible to chiral perturbation from the flavanone substituent.[67] This is equivalent to the case of flavone C- and O-glycosides that may give CD effects of considerable magnitude comparable to those of the flavanones.[69,70,71] Comparison of the CD data of fukugetin (**61**) with that of the chalcone-flavanone dimer (**87**) shows that the Cotton effects for the π→π* transition is very similar for the two dimers. However, the n→π* transition has a well defined negative Cotton effect in the case of (**87**) while fukugetin has a positive Cotton effect in the same region. It is thus obvious that a flavone unit coupled to 3-C(C) of a flavanone has a definite influence on the flavanone n→π* transition.[67] It is now clear that the influence of a second flavanone unit on the CD transitions of the first is unpredictable and until more detailed studies become available only tentative conclusions can be made from the CD data of biflavonoids.

Only two cases have been recorded where authors attempted to determine the stereochemistry of biflavanones. The CD curves of BG1 (**76**) and GB2 (**78**) isolated by Sonnenbichler and Madubunyi[63] from *G. kola* showed a negative Cotton effect at 290 nm (π→π*) and positive Cotton effect at 330 nm (n→π*). After comparison with CD data of monomeric flavanones, the stereochemistry was determined as 2S,3R for the flavanone unit and 2R,3R for the dihydroflavonol unit. Four additional well defined Cotton effects at 210 (-), 230 (+), 245 (-) and 275 (+) nm were ignored and need to be clarified. In the second case, Iwu *et al.*[62] came to the same conclusion regarding GB1 and GB2 isolated by them from *G. kola*. These authors also isolated manniflavanone (**81**) and garciniflavanone (**82**), two isomeric biflavanones from the same source. Unfortunately, no chiroptical data is available for these two isomers and therefore the relationship between them are still unknown. The assignments made by these authors may indeed be correct, but in view of the above discussion must be regarded as tentative.

2.3.3 Other biflavonoids

The biflavonoids (excluding biflavones) with different coupling positions than those discussed above are limited in number. Since little can be added to the discussion of structural elucidation and determination of stereochemistry, each mode of coupling is demonstrated by suitable representative examples.

In some rare cases, the interflavonoid bond is located on the A-rings of both constituent units (A- and D-rings in the biflavonoid). Succedaneaflavanone (**88**), a 6,6"-linked binaringenin, was isolated from *Rhus succedanea* by Chen and Lin[72]. The same source yielded rhusflavone[73] (**89**) and rhusflavanone[74] (**90**), two 6,8"-coupled biflavonoids. Included in this category are mesuaferrone B[75] (**91**) and neorhusflavanone[76] (**92**), two 8,8"-linked biflavonoids.
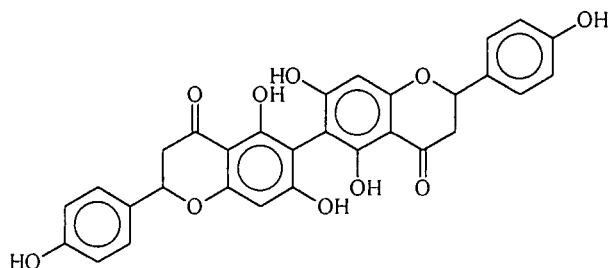
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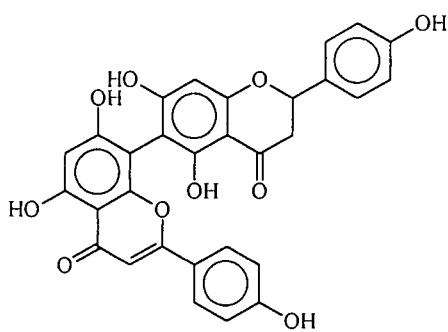
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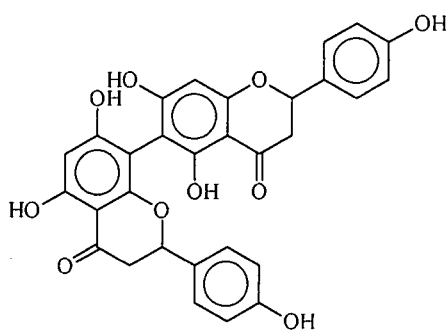
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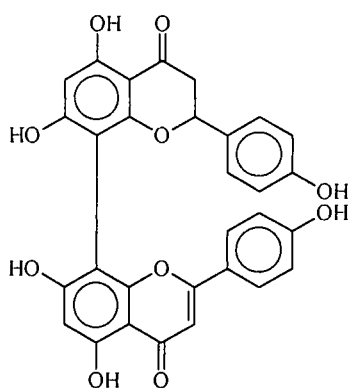
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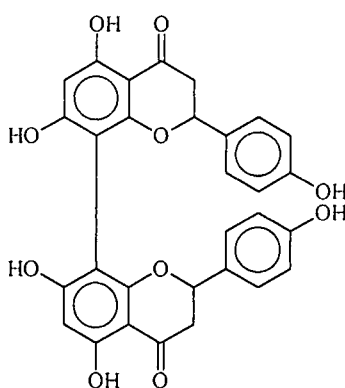
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Several biflavonoids with the coupling position located on the B-ring of the one unit and the A-ring of the other have been isolated. The linkage position on the B-ring is either the 2'- or 3'-position and the 6"- or 8"-position on the D-ring. Similar to the other groups, only a few dimers linked to the 6"-position are known. Examples of these are 2,3-dihydrodicranolomin[77] [2',6"-coupled (**93**)] and 2",3"-dihydro-5',6"-

biluteolin[77] [3',6"-coupled (**94**)]. There is only one example of a 2',8"-linked biflavonoid, namely 2,3-dihydrophilonotisflavone[78] (**95**). The 3',8"-coupled dimers are more numerous and include tetrahydroamentoflavone[79,80] (**96**) and 2,3-dihydroamentoflavone[81] (**97**).

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96   **97**
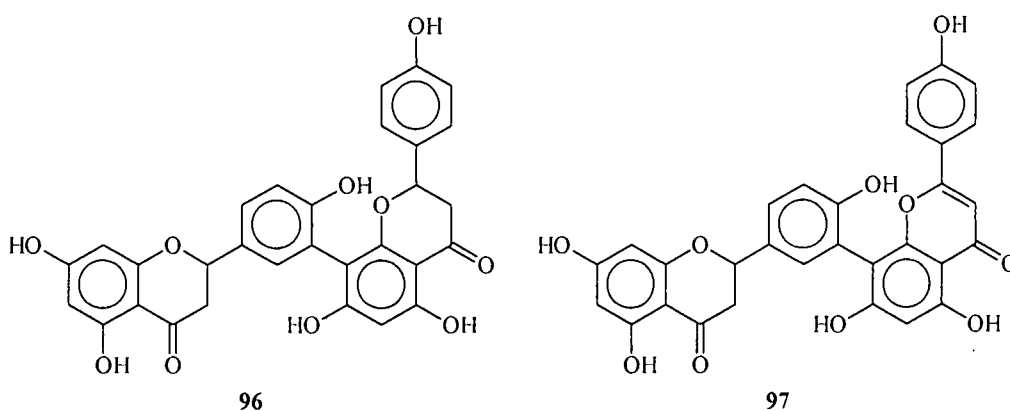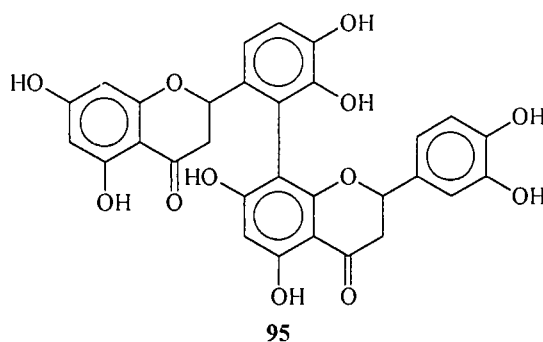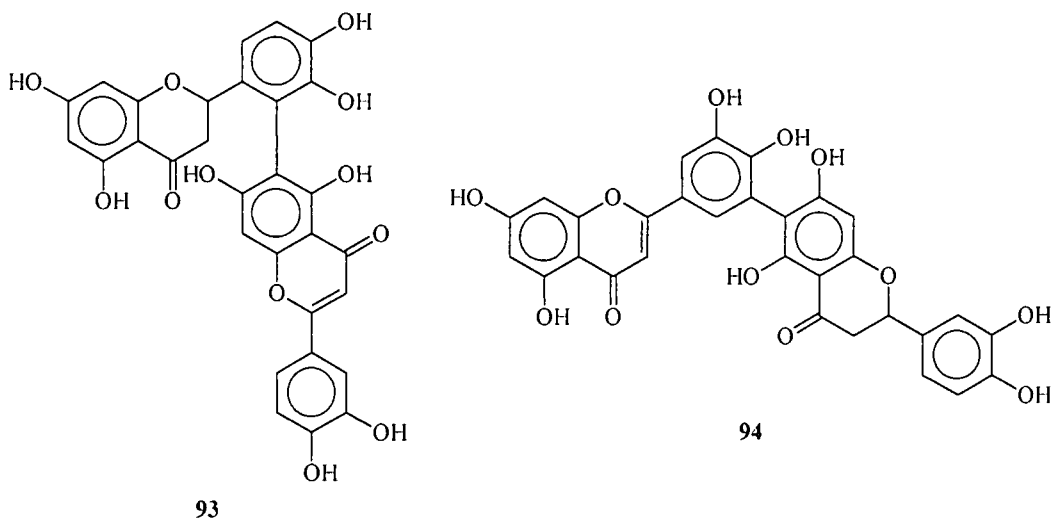
The group of biflavonoids with B-ring to E-ring biphenyl interflavonoid linkages from *Hypnum cupressiforme* was named hypnogenols by Sievers *et al.*[82,83] The three published hypnogenols are denoted as A (**98**) , B (**99**) and B1 (**100**) (3',3'''-binaringenin). The other example in this group is [5',5']-bisdihydroquercetin (**101**)

isolated by Foo *et al.*[84] from *Pseudotsuga menziesii.*
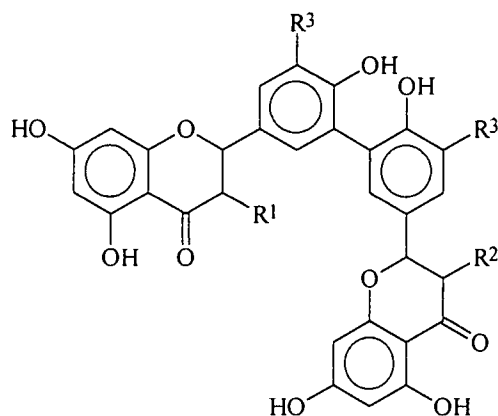
98 $R^1 = R^2 = OH, R^3 = H$
99 $R^1 = OH, R^2 = R^3 = H$
100 $R^1 = R^2 = R^3 = H$
101 $R^1 = R^2 = R^3 = OH$

The last example in this group of flavonoids is the unusual biflavonoid derivatives with an interflavonoid ether linkage. The first of these, 2,3-dihydrohinokiflavone (**102**), was isolated by Beckmann *et al.*[85] from *Metasequoia glyptostroboides*. Other examples are 2'',3''-dihydroisocryptomerin (**103**) from *Selaginella willdenowii*[86], tetrahydro-hinokiflavone (**104**) from *Cycas beddomei*[87] and 3,5,7,4',3'',5'',7''-heptahydroxy-3'-*O*-4'''-biflavanone (**105**) from *Hypnum cupressiforme*[82]. As usual, the structures were identified primarily with the aid of mass spectrometry and ^{1}H NMR studies. The ^{13}C NMR data of these compounds are especially useful for structural clarification.

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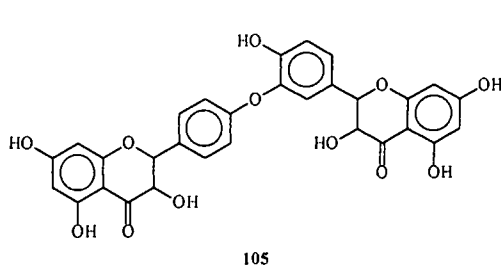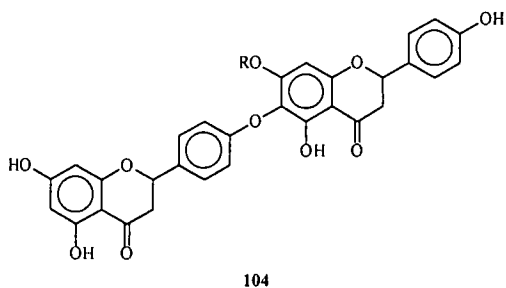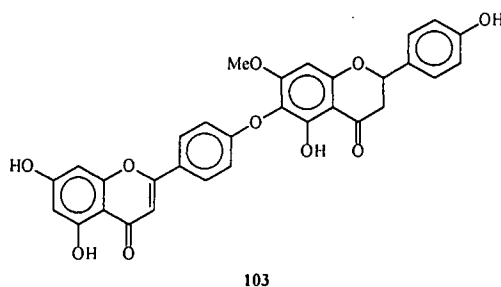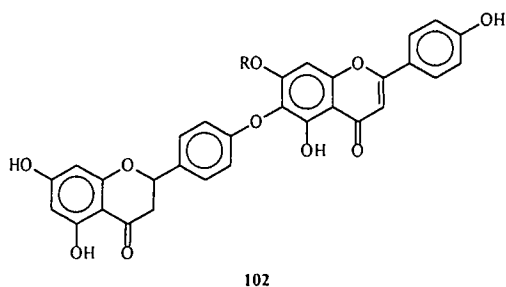
Modern NMR techniques, for instance n.O.e and COSY experiments, have the

potential to greatly facilitate the structural elucidation of biflavonoids. Since the majority of the biflavonoids were isolated before the advent of these techniques, the reliance on the simpler methods described above was inevitable. The use of n.O.e., 1D HETCOR and selective INEPT NMR techniques were demonstrated more recently by Sievers *et al.*[82] and Silva *et al.*[86] Furthermore, the lack of chiroptical data for the majority of biflavonoids leaves a noticeable gap in the study of these compounds, a situation that will continue until a better understanding of the CD behaviour of these compounds is established.

CHAPTER 3

THE SYNTHESIS OF BENZOFURANOIDS

The synthesis of benzo[b]furan-3(2H)-ones (hereafter referred to as benzofuranones) and aurones has received only limited attention since the beginning of the century. Although several routes for the synthesis of each exist, serious deficiencies in these routes justify continued research in this field. Thus, attempts to simplify the synthesis of these relatively simple molecules continue.

3.1   THE SYNTHESIS OF BENZOFURANONES

The synthesis of benzofuranones commonly involves the formation of the five-membered ring *via* an aromatic oxygen nucleophile *ortho* to a suitable electrophile. A variety of methods can be used to establish the electrophile and this is often the most challenging phase of the synthesis.

Considering that the methods used at the beginning of the century were frequently rather crude and that authors often did not indicate yields, it is difficult to assess the usefulness and relevance of some of the routes proposed by these authors. Therefore, these are only included for their historic importance.

3.1.1   Cyclization of ω-haloacetophenones

One of the more common methods to achieve ring closure is by nucleophilic attack of the 2-hydroxy group on the ω-carbon of 2-hydroxy-ω-haloacetophenones, which are, however, not always easily accessible.

Friedländer[88] used this method in 1897 to convert 2-hydroxyacetopenone (106) in three steps to the desired benzofuranone (109) (see **Scheme 3.1**). Since bromination of hydroxyaryl ketones with free bromine predominantly leads to aromatic substitution,[89] it was necessary to acetylate the 2-hydroxy group before bromination. A four-step process to synthesize 2-hydroxyacetopenone from *o*-nitrophenylpropionic acid, which was not

freely available at the time, complicated this route. While the cyclization of ω-haloacetophenones is perhaps the most frequently used route to benzofuranones, this is the only instance in the literature where direct bromination was used to form the ω-bromoacetophenone.
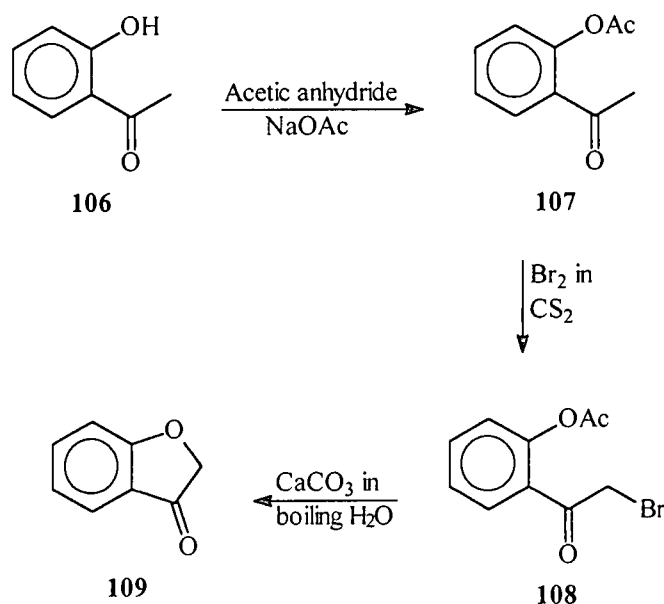
Scheme 3.1

In 1905, Blom and Tambor[90] claimed to form 2,4-dimethoxy-ω-bromoacetophenone (**111**) when the dimethylether of resorcinol (**110**) was treated with bromoacetyl bromide in the presence of AlCl₃ (Friedel-Crafts acylation). Ring closure was then effected under basic conditions to yield the benzofuranone (**113**). However, when Auwers and Pohl[91] repeated these experiments with chloroacetyl chloride, they found that 2-hydroxy-4-methoxy-ω-chloroacetophenone (**112**) is formed in an 86% yield under such conditions. The subsequent ring closure was again effected under basic conditions in an 80% yield (**Scheme 3.2**). They also prepared the hydroquinone equivalent (5-methoxybenzo[*b*]furan-3(2H)-one) in 45% yield, using the same methods.

In the case of phloroglucinol, it is necessary to use chloroacetonitrile as the two-carbon source in the presence of dry HCl and ZnCl₂ (the Houben-Hoesch reaction[92]) in order to form the ω-chloroacetophenone.
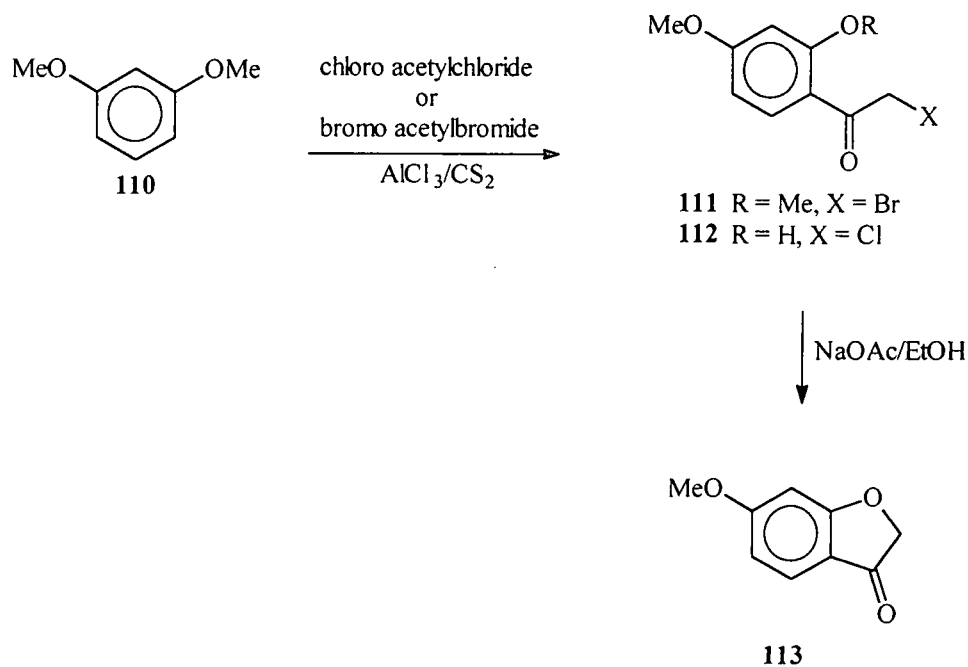
Scheme 3.2

Another method for the formation of ω-chloroacetophenone makes use of the Fries rearrangement[93] of phenolic esters (**Scheme 3.3**). Fries and Pfaffendorf[94] used this method to synthesize unsubstituted benzofuranones from phenol (**115**) in approximately 45% yield while Fries *et al.*[95] prepared 6-chlorobenzofuran-3(2H)-one from *p*-chlorophenol (**114**).
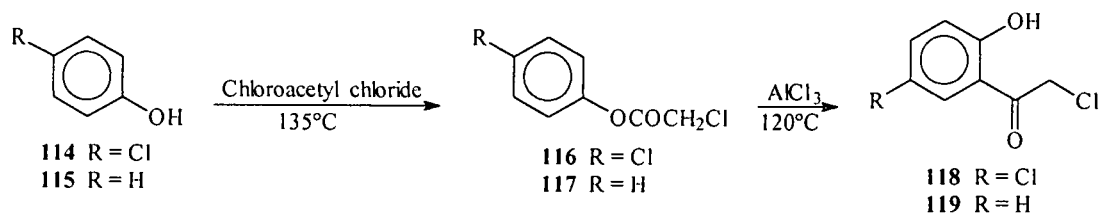
Scheme 3.3

3.1.2 Cyclization of ω-diazoacetophenones

More recently, a synthetic method utilizing the cyclization of ω-diazoacetophenones (**122**) was developed. The origins of this reaction can be traced back to studies by Clibbens and Nierenstein[96], Marshall *et al.*[97] and by Richtzenhain and Alfredsson[98]. During this sequence *o*-methoxybenzoic acid (**120**) is converted into the acid chloride and then treated

with diazomethane. Presumably, the resulting diazo ketone (**122**) is then protonated to form an acyl diazonium acetate (**123**). Intramolecular displacement of nitrogen by the lone pair of the *o*-methoxy group would then give the oxonium acetate (**124**) which would lead to the desired product by loss of methyl acetate[99] (**Scheme 3.4**). Dallacker and Korb[100] used this method to synthesize a large number of benzofuranones in moderate yield (~50-60%), thus demonstrating the value of this method. However, the method failed for 2,4,6-trimethoxy-ω-diazoacetophenone since the reaction conditions only led to decomposition.
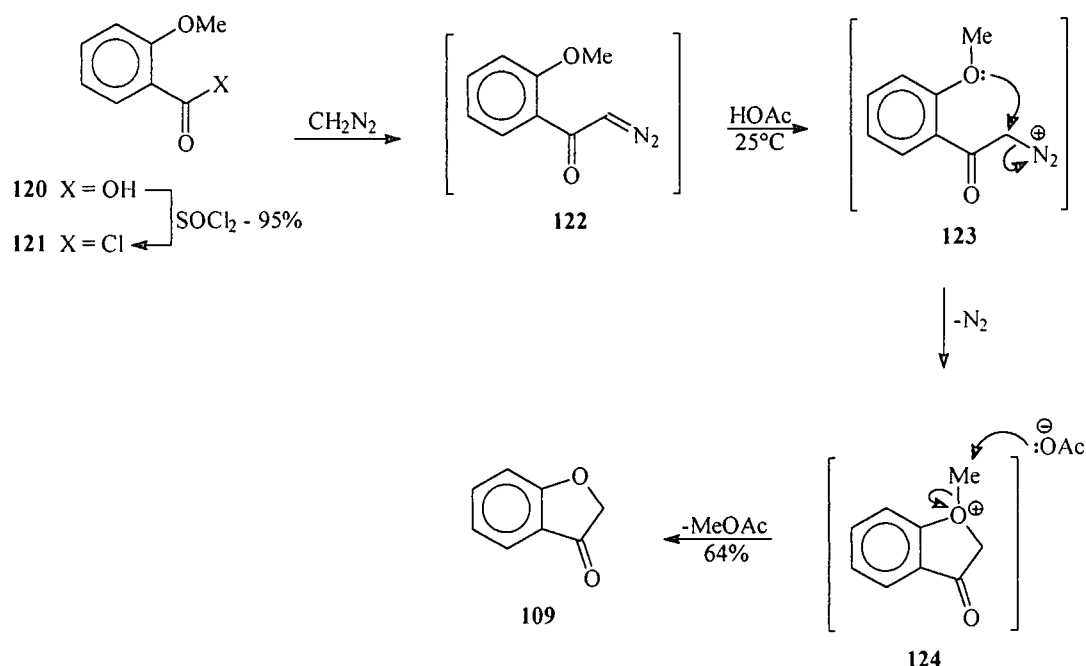
Scheme 3.4

3.1.3 Cyclization of phenoxyacetic acid derivatives

In 1899, Friedländer[101] reported that an attempt to form benzofuranones utilizing the cyclodehydration of phenoxyacetic acid (**125**) was less successful due to the low yield. In spite of this observation, Stoermer and Bartsh[102] conducted a study of this method, stating that the 8 – 10% yield obtained from the reaction was sufficient for the synthesis of small amounts of benzofuranone. At least the starting material was relatively cheap. Phosphorus pentoxide in dry benzene proved to be the most effective dehydration agent and several analogues are also accessible under these conditions (**Scheme 3.5**).
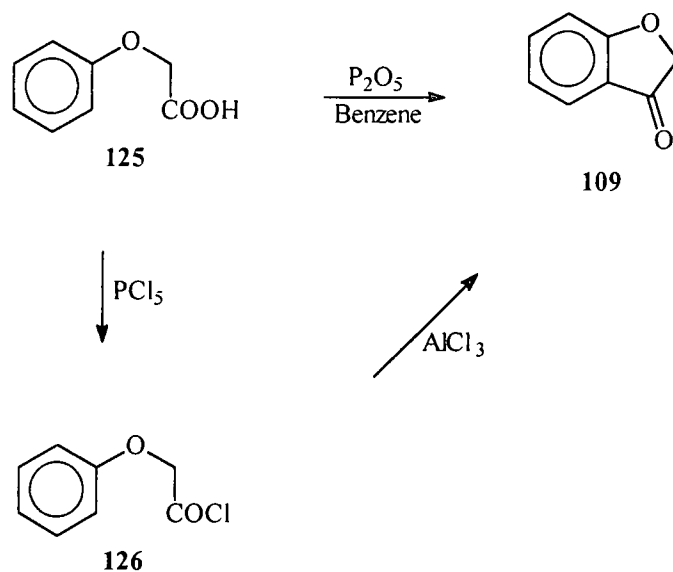
Scheme 3.5

In an attempt to improve the yield, the phenoxyacetic acid was first converted into the acid chloride (**126**) by the action of phosphorus pentachloride[103]. Ring closure was then effected by means of AlCl₃ in 15% yield. These methods were later evaluated by Kalinowski and Kalinowski[104] who found that they compared unfavourably with the cyclization of ω-chloroacetophenones especially with chloro substituted phenoxyacetic acids.

An alternative procedure suggested by Friedländer[101] consists of a Dieckmann reaction performed on methyl *o*-carbmethoxymethylsalicylate (**127**) (**Scheme 3.6**). The resulting benzofuranone carboxylic ester (**128**) is hydrolyzed to the benzofuranone carboxylic acid (**129**), which is easily converted to the benzofuranone (**109**) by decarboxylation. Schroeder *et al.*[105] evaluated most of the preceding methods and found the last process to be most satisfactory. They obtained several benzofuranone analogues in 27 − 90% yield using this method. They claimed that the yield for the other methods was low and that the products obtained were so badly contaminated that purification became tedious.

Bryant and Huhn[106] adapted the above method in order to address the need for an easy large-scale synthesis of benzofuranones by converting the ester (**127**) into the diacid (**130**), which was cyclized to the 3-acetoxybenzofuran (**131**). Finally, the benzofuranone (**109**)

was obtained by hydrolysis in a 65% yield (**Scheme 3.6**). The advantage of this method lies in the fact that each intermediate product can be isolated by crystallization.
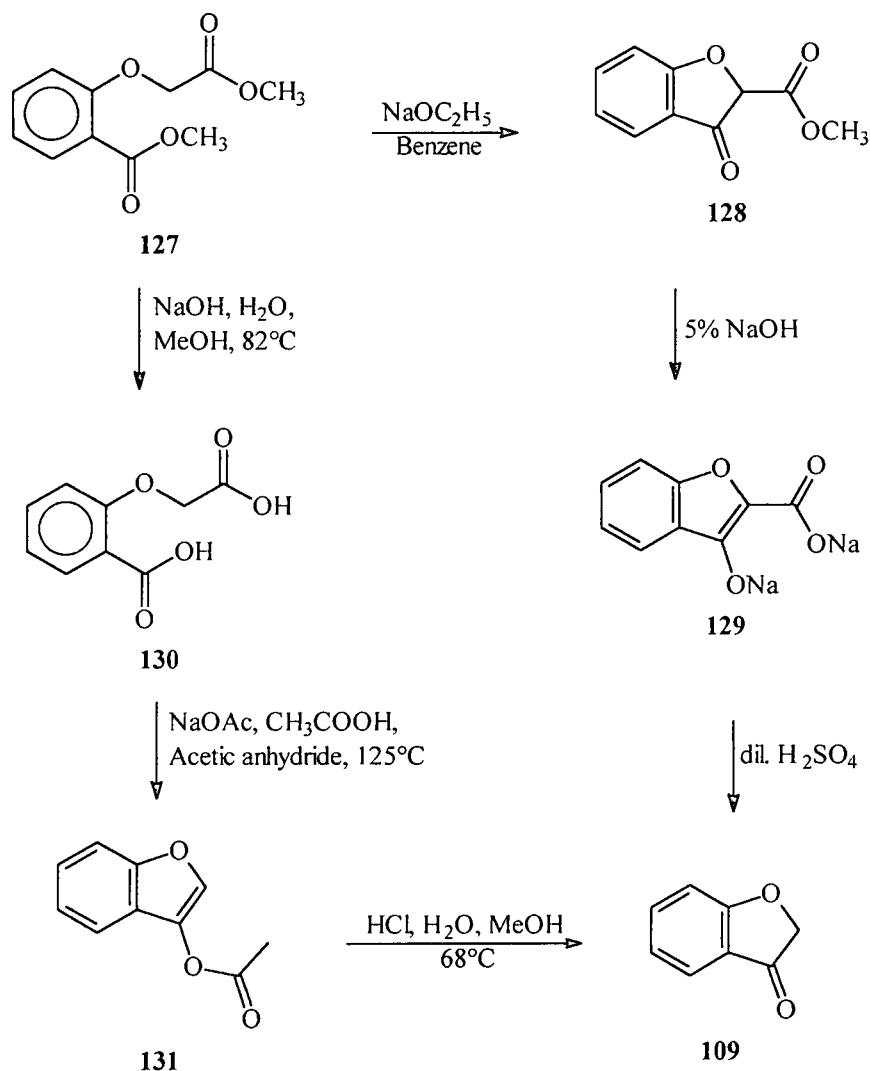
Scheme 3.6

3.2 THE SYNTHESIS OF AURONES

Aurones have a bright golden yellow colour and are important contributors to the pigmentation of flowers. Because of their limited occurrence in nature, aurone synthesis has received limited attention when compared to the related flavones and isoflavones. The majority of useful aurone syntheses, for example the Wheeler synthesis, involves chalcones as intermediates.

3.2.1   <u>Chalcones as aurone precursors</u>

The cyclization of chalcones is a focal point in the synthesis of many flavonoids, serving as precursors for among others, dihydroflavonols and flavonols. Reactions in this category are the Algar-Flynn-Oyamada (A.F.O.) reaction[107,108], the Emilewicz-von Kostanecki reaction[109,110,111] and the Rasoda reaction[112,113]. Depending on the substitution pattern of the chalcones, aurones can also be the main product of these reactions. Accordingly, chalcones and their dihalides have been divided into two classes based on the product obtained from the von Kostanecki reaction[114]. Class 1 constitutes those that yield only six-membered flavonoids on cyclization and Class 2 those that yield aurones under similar reaction conditions. The most important group of Class 2 chalcones is those with a 6'-OMe substituent on the A-ring.

The A.F.O. reaction is the oxidation of 2'-hydroxychalcones to flavonols by means of alkaline hydrogen peroxide. The reaction is general and can be applied to chalcones with a variety of substitution patterns, failing only when a 6'-OMe group is present, in which case the main product is an aurone.[115] The steric effect caused by the 6'-substituent forces the carbonyl group out of the plane of the nucleus, producing steric inhibition of resonance from the 2'-O$^{\ominus}$ group. This disruption of resonance in the system causes the 2'-O$^{\ominus}$ phenolate to become more nucleophilic while the α-carbon becomes more electrophilic.[115,116] A secondary factor may be that β-cyclization is stereoelectronically disfavored in comparison with α-cyclization. However, this reaction did not lead to a general synthesis for aurones.

The cyclization of 2'-hydroxychalcone dihalides in aqueous alcoholic alkali, the von Kostanecki reaction, is another general synthesis of flavones that yield significant amounts of aurone under certain conditions. Studies of 6'-substituted dihalides showed that aurone formation is enhanced when the base concentration is increased[117] and thus two separate mechanisms for the formation of the two possible products were proposed. For Class 1 chalcone dihalides, an intermediate 3-haloflavanone (**137**) is formed by cyclization of the dihalide followed by elimination of the halogen to form a flavone (**12**). In the case of 6'-

substituted dihalides the acidity of the α-hydrogen is increased by the disruption of conjugation described above and hydrogen halide will be readily eliminated from the side chain. It is suggested that a halohydrin (134) is then formed at the expense of the flavone precursor and that this cyclizes to an epoxide (135) before forming the aurone (136) (Scheme 3.7).[117]
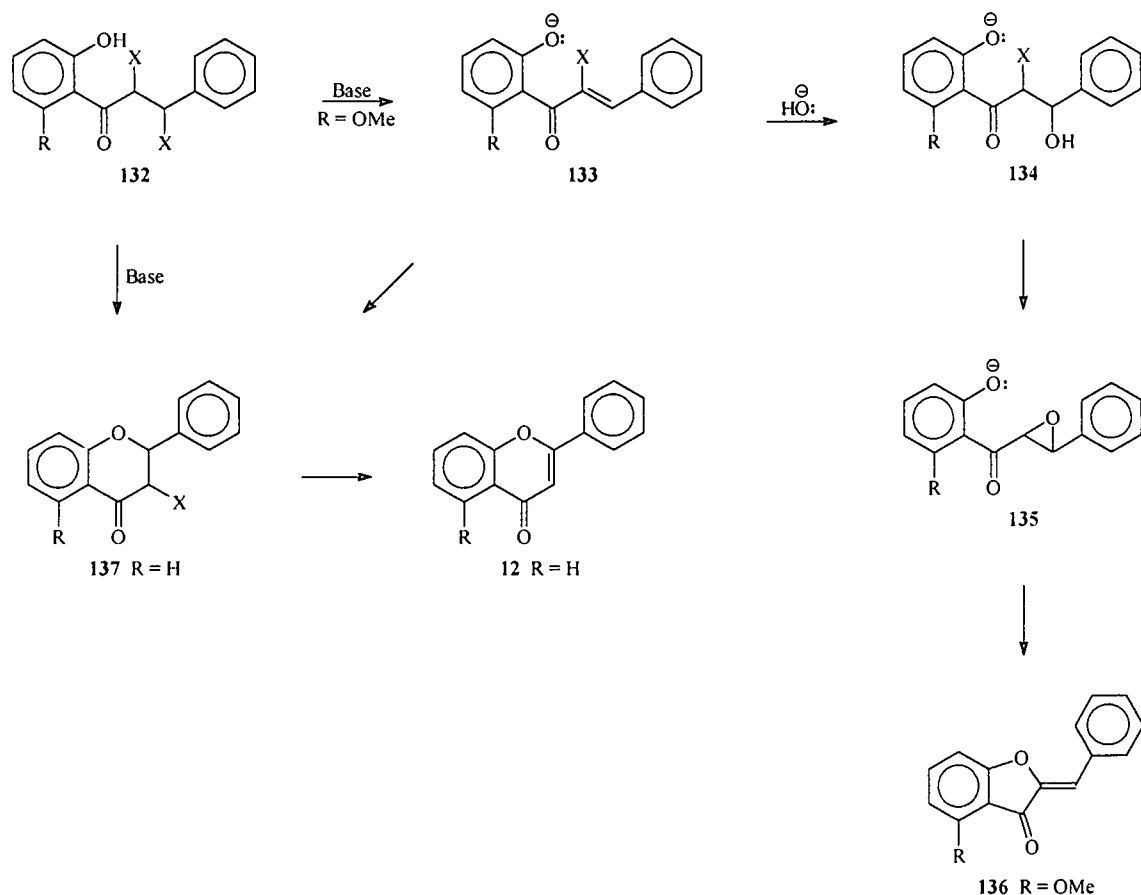
Scheme 3.7

Closely related to the above method, is the reaction of dihalides with aqueous acetone and sodium carbonate to form dihydroflavonols. This, the Rasoda reaction, proceeds *via* a halohydrin (134) and was originally limited to dihalides with 2- or 4-oxygenation on the B-ring, as only these dihalides were hydrolyzable to halohydrins. The method was extended to other dihalides by Donnelly *et al.*[118] who found that the reaction is generally applicable, except for 6'-substituted dihalides, which again yield aurones. Since the postulated mechanism for the reaction again involves an epoxide, the reasons for this phenomenon are probably the same as for the previous two cases.

Chalcone dihalides with 2- or 4-oxygenation on the B-ring react with hot alcohol to form α-halo-β-alkoxydihydrochalcones, which are transformed to aurones with the addition of base. This, the Wheeler synthesis[119], is a particularly useful method for the synthesis of aurones, utilizing the greater effectiveness of the halide, compared with the alkoxide, as a leaving group. The reaction was generalized by Donnelly et al.[119] who found that methanolic N-bromosuccinimide (NBS) is an effective bromomethoxylation agent, converting chalcones smoothly into the desired α-halo-β-methoxydihydrochalcones (139). It is, however, necessary to protect the 2-hydroxy group, usually by acetylation, in order to prevent aromatic bromination of the A-ring. The 2'-acetoxy-α-bromo-β-methoxydihydrochalcone was cyclized to the aurone with aqueous sodium hydroxide in 88% yield, the overall yield from the chalcone being 64% (**Scheme 3.8**).
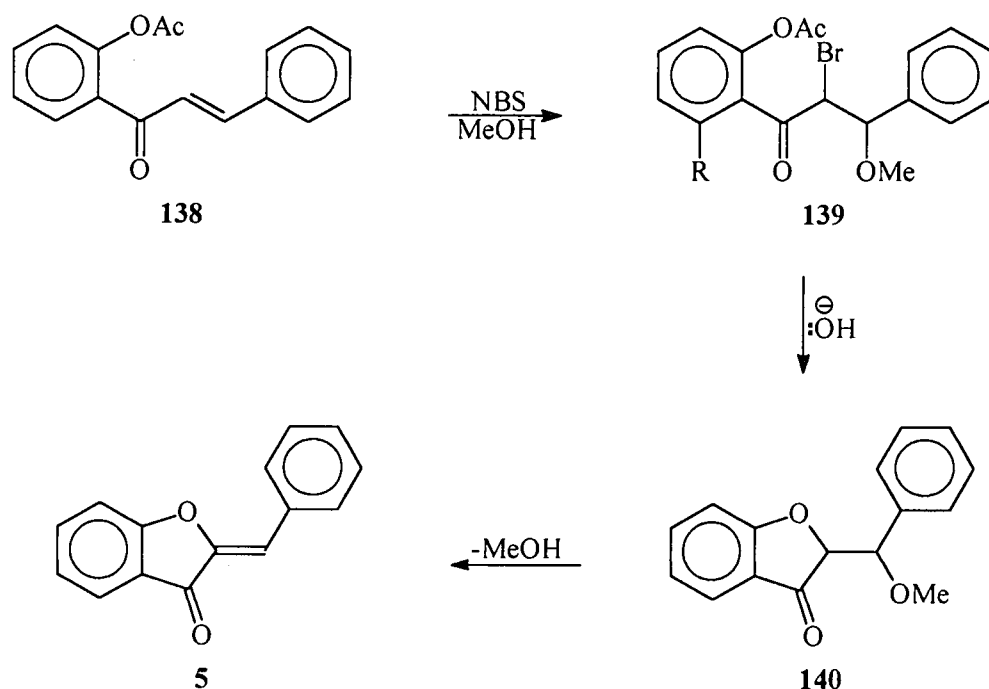
Scheme 3.8

Studies[120] showed that flavones can also be formed during the course of the Wheeler reaction and that flavone production increases with increasing base concentration. These studies showed that the optimum base concentration for aurone formation was less than 1%.

The oxidative cyclization of 2'-hydroxychalcones with thallium (III) nitrate[121,122,123] is a recent addition to chalcone cyclization that yields aurones. This method is also restricted to chalcones with specific substitution patterns.

3.2.2 Condensation reactions of acetophenones

If the appropriately substituted benzofuranone (109) is available, the acid or base condensation of benzofuranones with benzaldehydes (141)[91,124] is the simplest aurone synthesis available (Scheme 3.9). The relevance of this reaction was extended by Varma and Varma[125] who found that this condensation proceeds fast (10 - 15 min.) and in high yield (86 - 93%) under mild conditions if absorption grade neutral alumina is used as solid support. A wide range of functional groups, including ester groups, are tolerated.

109	141	5
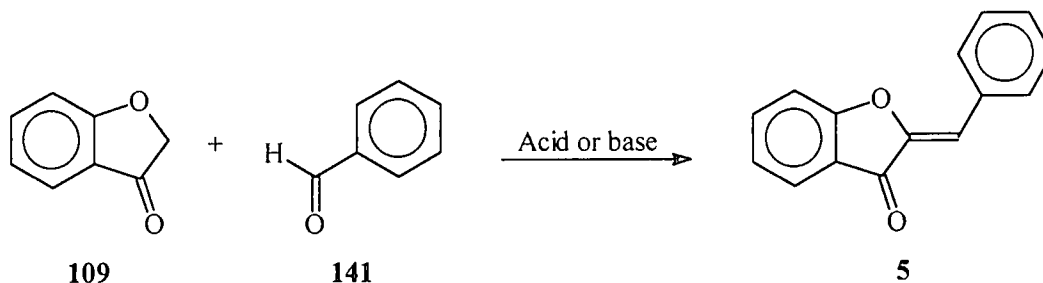
Scheme 3.9

3.2.3 The configuration of aurones

Despite the fact that only one aurone isomer is obtained from the reactions described above, the determination of this configuration has received almost no attention from the authors involved in these studies. Attempts by O'Sullivan and co-workers[126,127] to differentiate between the (*E*)- and (*Z*)-isomers by means of NMR studies have met with little success. Since, unlike other α,β-unsaturated carbonyl compounds, there is little difference between the chemical shifts of the β-hydrogen of the (*E*)- and (*Z*)-isomers of aurones, these workers attempted to use the chemical shifts of the 2',6'-protons to determine the configuration of unsubstituted aurones.

Independent studies by Hastings and Heller[128] showed that these results are dubious at best while the u.v. spectra of the aurone isomers are also similar. They managed to

establish the stereochemistry of several 4'-chloroaurones by dipole measurements, using the principle that the *p*-chlorophenyl substituent will oppose the group moment of the benzofuranone system in the (*Z*)-isomer, while reinforcing it in the (*E*)-isomer. I.r. studies were also used to distinguish between the isomers, the (*Z*)-isomer exhibiting a characteristic absorption (1650 - 1670 cm^{-1}) which is absent in the spectra of the (E)-isomers.

I.r. studies on aurones obtained as the sole product from condensation reactions indicated that the (*Z*)-isomer was obtained in al the cases. This was later confirmed by an X-ray study of the (*Z*)-isomer[129].

ASYMMETRIC HYDROXYLATION OF ENOLATES

4.1 INTRODUCTION

The synthesis of natural products often involves α-hydroxy carbonyl compounds as intermediates or starting materials and therefore, many methods for their synthesis have been devised.[130-132] One of the most practical routes is the direct oxidation of enolates with a suitable source of oxygen. One such source is molecular oxygen[133-135] that can also be used in conjunction with chiral phase transfer catalysts[136] to give α-hydroxy ketones in 90% yield and up to 79% enantiomeric excess. Another method is the use of Vedejs' reagent,[137,138] molybdenum peroxide-pyridine-hexamethylphosphoramide (MoOPH). However, this reagent fails to oxidize 1,3-dicarbonyl enolates and overoxidation to α-dicarbonyl compounds is common.[137,138]

The two methods convenient for the asymmetric hydroxylation of enolates involve the use of chiral oxaziridines and the osmium catalyzed dihydroxylation of alkenes. In this short discussion, the application of these two methods will be considered.

4.2 CHIRAL OXAZIRIDINES

The synthetic utility of 2-sulfonyloxaziridine (145), an aprotic and neutral oxidizing reagent, is demonstrated by its ability to oxidize several substrates. In addition to epoxidation of alkenes[139,140] and heteroatom (S, Se, N)[141-143] oxidation, it is capable of oxidizing lithium and Grignard reagents to alcohols and phenols.[144] Extension of these capabilities to enolates was logical since the proposed mechanism for the oxidation of an anion involves a nucleophilic S_N2-type attack of the enolate on the electrophilic oxaziridine oxygen atom.[145] The aprotic nature of the oxaziridine implies that it will not be destroyed by the enolate prior to oxidation. Furthermore, the oxaziridines are easy to prepare and stable, requiring no special conditions for storage.[145]
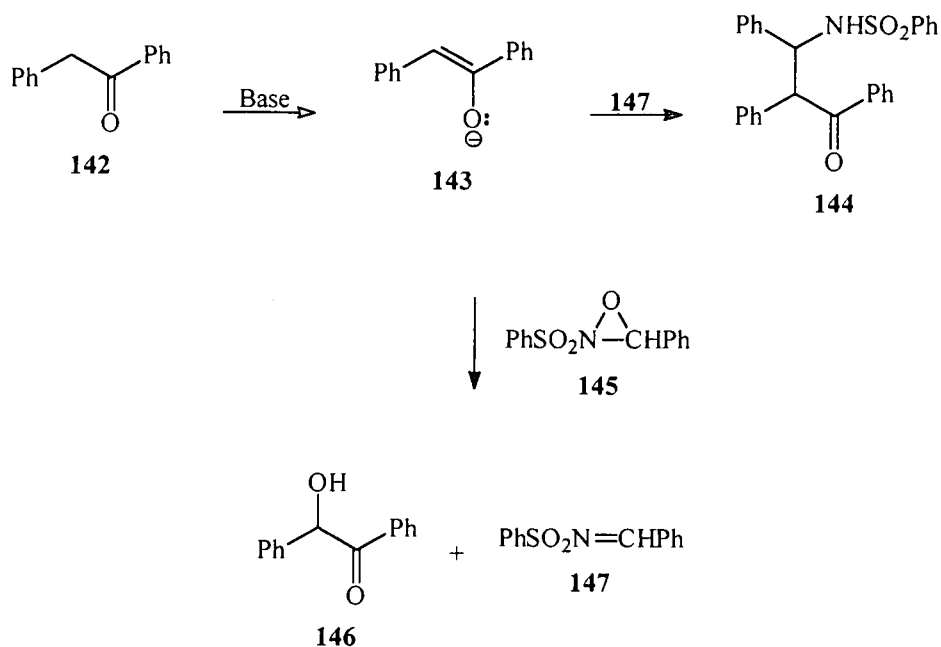
Scheme 4.1

The only observed side reaction is the formation of compound (**144**), the adduct of the sulfonimine (**147**) and the enolate (**Scheme 4.1**). However, this reaction is only detected when t-BuOK/HMPA is used to generate the enolate and is only significant at room temperature. Lithium diisopropylamide (LDA), the base most often used to generate enolates, gives low yields with most substrates since the isopropylamine competes with the enolate for the oxaziridine. The best results are obtained when potassium bis(trimethylsilyl)amide (KHMDS) is used as base.[145]

In order to demonstrate the superiority of 2-sulfonyloxaziridine (**145**) compared to MoOPH or O_2, the reaction in **Scheme 4.2** can be considered. The enolate of lactone (**148**), formed with LDA, is converted to the hydroxy lactones (**149a**) and (**149b**) (a 3:1 mixture) in 15% yield with MoOPH. Treatment of the lithio enolate with 2-sulfonyloxaziridine (**145**) at -78°C afforded only hydroxy lactone (**149a**) in 62% yield. When KHMDS is used to generate the enolate the isolated yield of (**149a**) increased to 91%. Attack of the enolate by the oxaziridine and MoOPH from the sterically less hindered direction, results in the *cis* stereochemistry of (**149a**).[145]
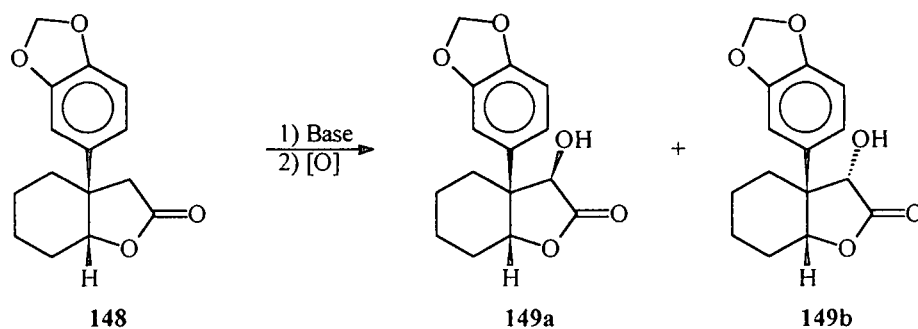
Scheme 4.2

This reaction was extended to include reagent controlled asymmetric oxidation by application of enantiopure N-sulfonyloxaziridines (**150**) - (**154**). Enantiomerically enriched α-hydroxy carbonyl compounds, sulfoxides, selenoxides and epoxides are accessible with these reagents.[146,147] The (camphorylsulfonyl)oxaziridines (**152**) - (**154**) are superior to the other two types for the asymmetric hydroxylation of enolates and the dichloro (**153**) and dimethoxy (**154**) analogues have been employed in the enantioselective syntheses (> 95% ee) of biologically significant α-hydroxy carbonyl compounds.[148-150]
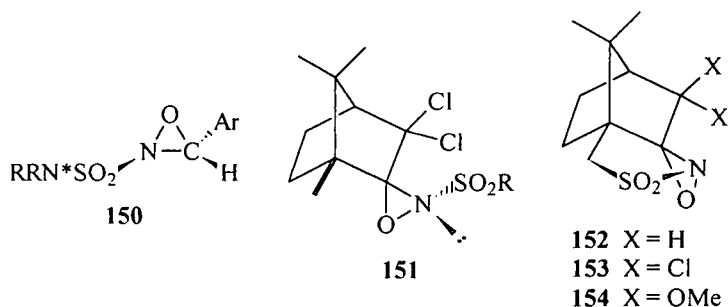
The enantioselective oxidation of several substrates with (camphorylsulfonyl)oxaziridines (**152**) and (**153**) were studied by Davis *et al.* (**Scheme 4.3**)[151]. The results of these studies suggested that [(8,8-dichlorocamphoryl)sulfonyl]oxaziridine (**153**) is more reactive than the dihydro reagent (**152**). Most of the reactions progress smoothly at -78°C and are complete within 20 minutes. In the case of propiophenone (**155a**) and butyrophenone (**155b**) oxaziridine (**153**) gave the best results, yielding the corresponding (*S*)-hydroxy ketones (**156a**) and (**156b**) in >95% ee. In the case of 2,2-dimethyl-3-pentanone (**155c**) and deoxybenzoin (**155d**) the oxaziridine (**153**) gave much poorer results (2 - 36% ee) while the best results were obtained with analogue (**152**) (90 - 95% ee). In contrast to the

other substrates, oxidation of 2,2-dimethyl-3-pentanone (**155c**) with (**152**) yielded the (*R*)-enantiomer of (**156c**) as the product. Oxaziridine (**153**) also afforded (*R*)-2-hydroxy-2-methyl-1-tetralone (**158**) in >95% ee (**Scheme 4.3**).

a) R^1 = Ph, R^2 = Me, b) R^1 = Ph, R^2 = C_2H_2, c) R^1 = Me_3C, R^2 = Me, d) R^1 = Ph, R^2 = Ph
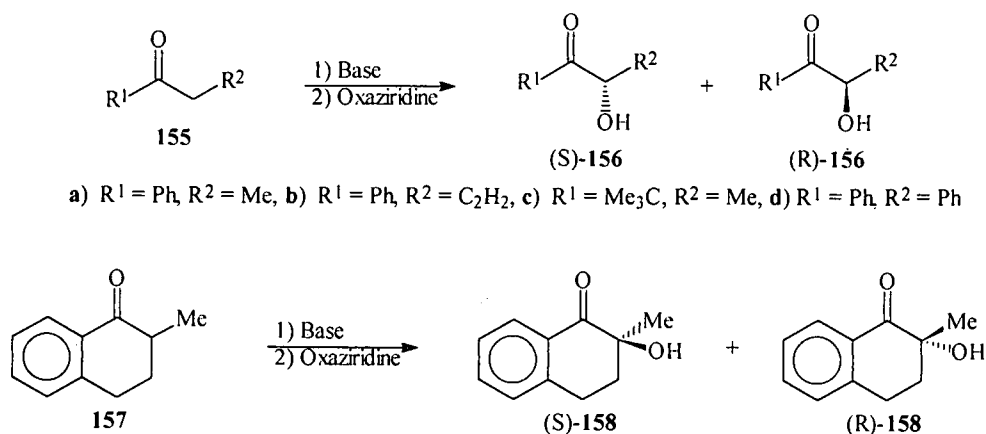
Scheme 4.3

Molecular recognition in the above reactions has been interpreted in terms of "open" or "nonchelated" transition state structures (**Scheme 4.4**).[151] It is argued that the primary transition-state control element is steric in origin, as was observed for the enantioselective oxidation of sulfides and alkenes.[146,147] Furthermore, it was assumed that, regardless of the actual structure of the enolate in solution, the oxygen-metal aggregate is sterically the most demanding group in the vicinity of the enolate C-C bond.[151] While these models (**Scheme 4.4**) are useful in a number of cases, the influence of reaction parameters (counter ion, solvent, etc.) is often unpredictable.

In an alternative and more realistic "closed" transition state, the lithium cation is coordinated to the enolate oxygen as well as the oxaziridine oxygen and nitrogen atoms. The geometric constraints of this transition state can be approximated by transition state structures **TS-1** to **TS-4** (**Scheme 4.4**).[151] From a purely steric perspective, **TS-1** and **TS-4** are favored to give the observed stereoisomers. This must mean that adverse nonbonded interactions between the phenyl group (R^1) and the sulfonyl group oxygen, or in the case of (**157**) between the aromatic tetralone ring and this oxygen, are insignificant. When R^1 is a *tert*-butyl group, this interaction becomes unfavourable leading to (R)-(**156c**) *via* the less favourable **TS-2**. The chlorine substituents in (**153**) increase the size of the

quadrant where they are located, and therefore the energy of **TS-2** and **TS-4** relative to **TS-1** and **TS-3,** resulting in the observed higher ee's. When R^2 (**Scheme 4.3**) is a Ph group, the ee's are lower because of unfavourable interactions between the Cl substituent and R^2 in the enolate. Likewise, the lower ee's on oxidation of the enolate of (**155c**) ($R^1 =$ *tert*-butyl) with (**153**) can be explained by the adverse interaction of the bulky *tert*-butyl group and the Cl substituent.[151]

The marked increase in ee observed for the oxidation of (**155a**) and (**155b**) with (**153**) cannot only be ascribed to favourable nonbonded interactions in the transition state, but also to the fact that (**153**) is more reactive when compared to the analogue without the chlorine groups (**152**).[151]
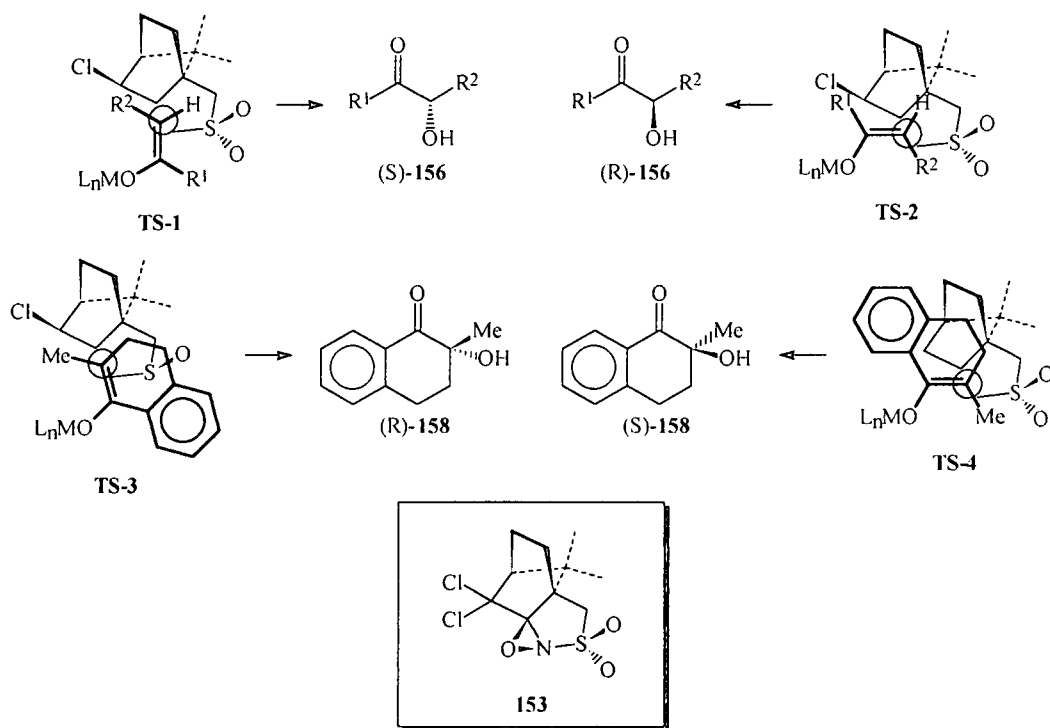
Scheme 4.4

4.3 ASYMMETRIC DIHYDROXYLATION

The asymmetric reaction of osmium tetroxide with alkenes (**159**) to afford *syn*-diols (**161**) has emerged as one of the most selective and general transformations in organic chemistry. The fundamental principle governing the selectivity of this reaction is the ligand
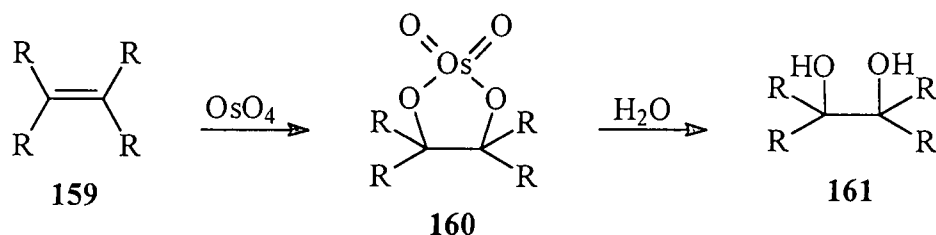
Scheme 4.5

acceleration effect (LAE)[152-154] which means that the rate of the reaction is greatly enhanced in the presence of an appropriate ligand (**Scheme 4.6**), ensuring that the reaction is funneled through a pathway involving the chiral catalyst. This phenomenon was first observed by Criegee[155-157] who showed that pyridine accelerates the reaction considerably.
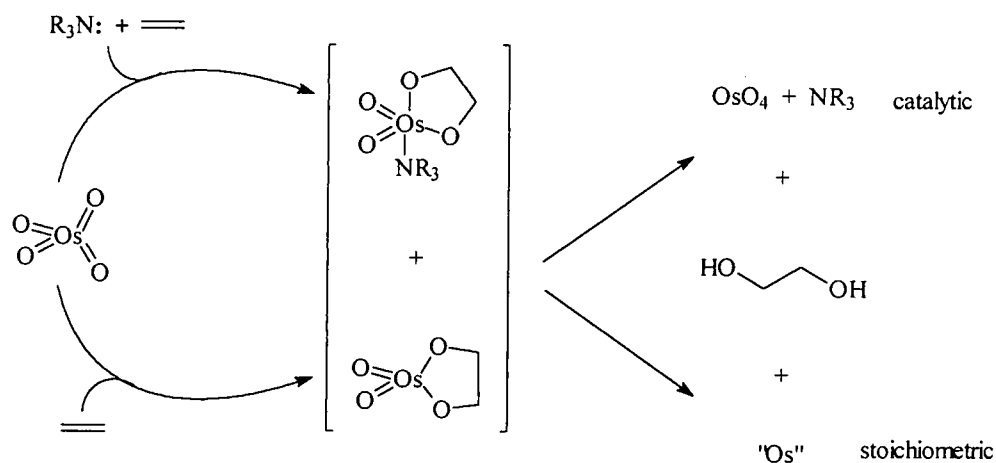
$$LAE = \frac{\text{Saturation rate with Ligand}}{\text{Rate without Ligand}}$$

Scheme 4.6

Initially, osmium tetroxide was used in stoichiometric amounts and although the resultant osmate ester (**160**) could be hydrolyzed oxidatively to regenerate the osmium tetroxide, financial considerations made the development of catalytic variations of this reaction inevitable. These variations employ inexpensive co-oxidants to reoxidize the osmium(VI) glycolate products. Initial use of potassium chlorate[158] and hydrogen peroxide[159,160] as co-oxidants lead to diminished yields due to over-oxidation and while alkaline *tert*-butyl hydroperoxide[161] and N-methylmorpholine N-oxide (Upjohn process)[162,163] give better

results, it is $K_3Fe(CN)_6$ in the presence of K_2CO_3[164] that provides a powerful system for reoxidation of the osmium.

l-2-(2-Menthyl)pyridine (**162**)[165] was the first chiral ligand used in attempts to introduce stereoselectivity to this reaction. However, the low affinity of this ligand for OsO_4 resulted in unsatisfactory enantioselectivities (3 - 18% ee). The cinchona alkaloid acetates dihydroquinidine (DHQD) (**165**) and dihydroquinine (DHQ) (**166**) were the first chiral ligands used with success, yielding diols in moderate to good enantiomeric excesses (34 - 99% ee).[165]
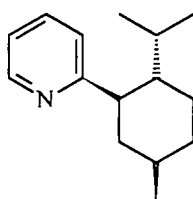
162
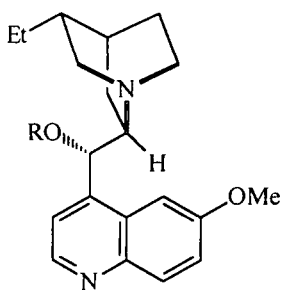
163 R = H
165 R = Ac
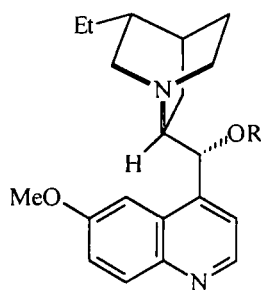
164 R = H
166 R = Ac

Good results have also been achieved with several other ligands[166], notably with certain chiral diamine ligands, e.g. (**167**)[167] and (**168**)[168]. Despite the good enantioselectivities that can be obtained with these ligands, a serious drawback, however, results from their bidentate nature - they form stable chelate complexes with the osmium (VI) glycolate products which prevent *in situ* recycling of the osmium and the ligand. Consequently, all reactions involving these ligands are stoichiometric for both OsO_4 and the chiral ligand.

Attempts to find catalytic conditions for the asymmetric dihydroxylation (AD) reaction was only realized in 1988 when Sharpless and co-workers[169] found that the process became catalytic when N-methylmorpholine N-oxide was employed as the co-oxidant.

167

168
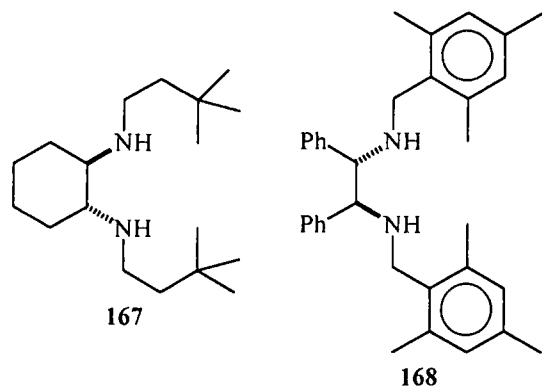
The enantiomeric excesses of the diols obtained under these conditions were lower than those produced by the stoichiometric reactions. This was explained by the identification of a second catalytic cycle that exhibited only low or no enantioselectivity (**Scheme 4.7**).[170]
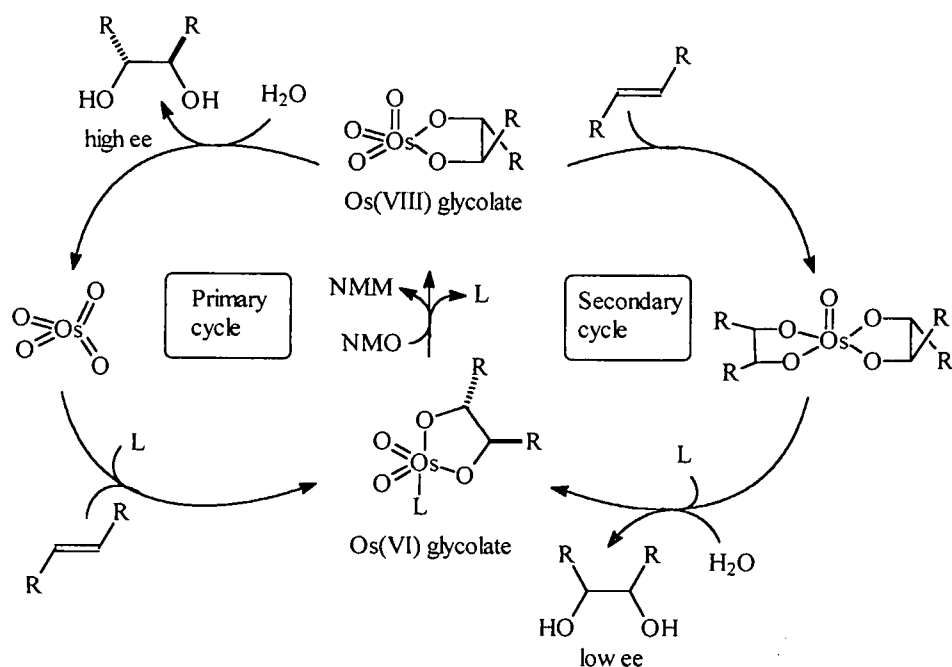
Scheme 4.7

Three important developments by Sharpless and co-workers dramatically increased the value of this synthetic protocol in terms of general applicability, reduced financial considerations and increased enantioselectivity. The first of these was the elimination of the secondary catalytic cycle by performing the reaction in a two-phase reaction system with $K_3Fe(CN)_6$ as the re-oxidant (**Scheme 4.8**).[171] Under these conditions, OsO_4 remains the only oxidant in the organic layer, resulting in a considerable increase in the

enantioselectivity of the reaction. Since the actual osmylation and subsequent hydrolysis take place in the organic layer, the diol and ligand are released into the organic layer and the Os(VI) into the aqueous layer before re-oxidation can occur. Consequently, entry of the osmium glycolate into the second cycle is prevented.[166]
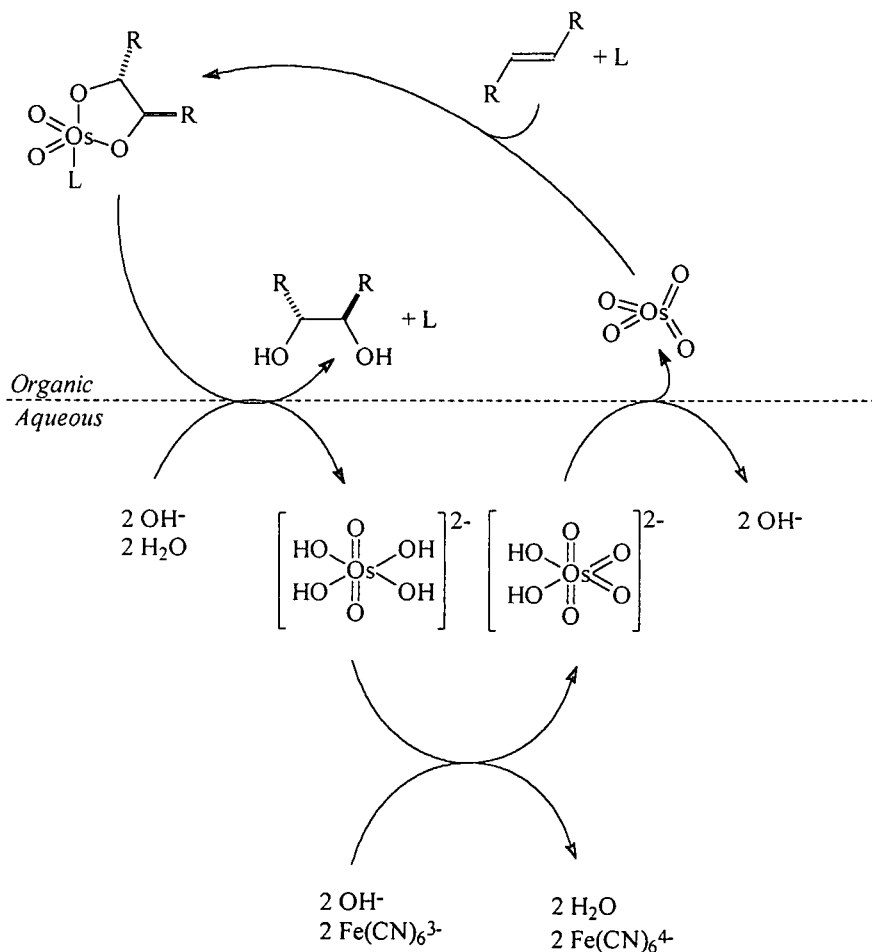
Scheme 4.8

The hydrolysis of the osmium (VI) glycolate can be accelerated substantially (up to 50 times) by addition of methanesulfonamide $(MeSO_2NH_2)$.[172] Subsequently, most AD reactions can be carried out at 0°C rather than room temperature. This has a beneficial influence on the selectivity of the reaction.[173]

Finally, the development of phthalazine (PHAL) alkaloid derivatives, $(DHQD)_2$-PHAL (169) and $(DHQ)_2$-PHAL (170) as chiral ligands led to a substantial increase in both the

enantioselectivity and scope of the reaction.[173,174] These developments allowed the formulation of commercially available premix, AD-mix-β with (DHQD)$_2$-PHAL (**169**) and AD-mix-α with (DHQ)$_2$-PHAL (**170**) as chiral ligands. The mixture contains K$_2$OsO$_2$(OH)$_4$ as the non-volatile Os source and K$_3$Fe(CN)$_6$/K$_2$CO$_3$ as co-oxidant.[166]
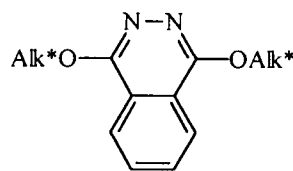
169 Alk* = DHQD
170 Alk* = DHQ

Extensive mechanistic investigations[175], ligand structure-activity studies[176] and high level *ab initio* calculations[177] led to a rationale for predicting the enantiofacial selectivity in these AD reactions (**Scheme 4.9**).
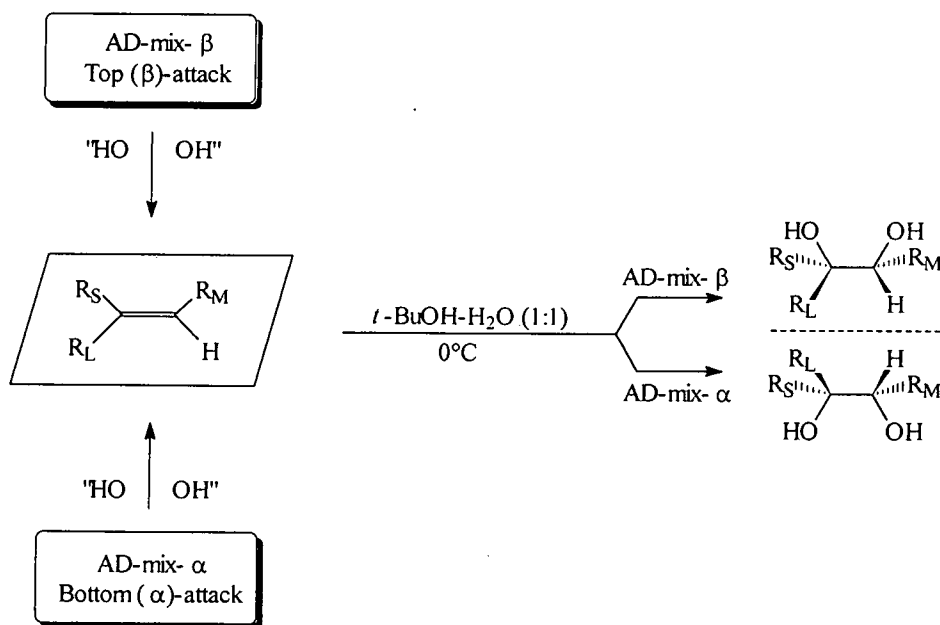
Scheme 4.9

With the improvements described above, it became possible to use the AD protocol for tetrasubstituted olefins. A few modifications were necessary to compensate for the slow hydrolysis of the osmate esters. Thus, the presence of MeSO$_2$NH$_2$ is crucial for sufficient catalytic turnover while slightly increased amounts of OsO$_4$ and of the chiral ligand appeared to be necessary for acceptable results.[178]

A consequence of this development is the stereoselective accessibility of α-hydroxy carbonyl compounds from the appropriate enol ethers in moderate to good enantiomeric excesses and yields (see **Scheme 4.10** and **Table 4.1**).[178]
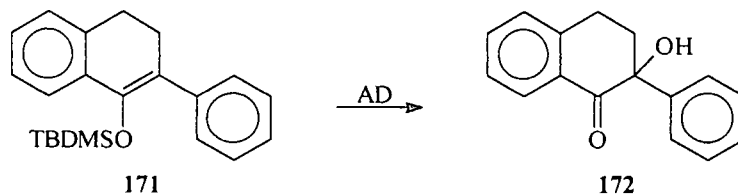
Scheme 4.10
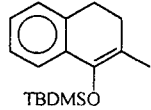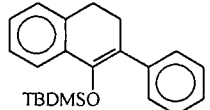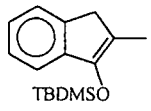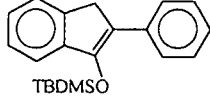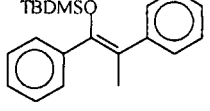
Enol ether	Ligand (ee)		Isolated
	DHQD	DHQ	yield (%)
	67(*S*)	65(*R*)	89-92
	93(*R*)	95(*S*)	94-98
	85(*S*)	81(*R*)	64-85
	89(*R*)		23-32
	75(*R*)	63(*S*)	15-22

Table 4.1[178]

Discussion

CHAPTER 5

INTRODUCTION

Since the first identification of dimeric flavonoids in the late sixties, much progress has been made in the isolation and identification process associated with these compounds. However, attempts to assess the absolute stereochemistry of biflavonoids possessing chirality other than that originating from atropisomerism of the interflavonoid bond have hitherto met with little success (see § 2.3.2). This can be ascribed to the limitations imposed by the existing strategies available for determination of the absolute stereochemistry of oligomeric flavonoids. The three strategies most likely to be of use and the limitations associated with these methods include the following:

(a)   The interpretation of physical data, e.g. the CD data of a compound, is often the starting point for the determination of the absolute stereochemistry of monomeric flavonoids. Due to the mutual interference of the additional stereocentres and chromophores it is, however, seldom possible to interpret the same physical data for dimeric and oligomeric analogues with any degree of accuracy.

(b)   For some classes of flavonoids, for example A-type proanthocyanidins[179], it is possible to obtain the monomeric building blocks of the oligomers by means of chemical degradation of the appropriate oligomer. This method, though still limited in its scope, is useful if the stereocentres of at least one of the constituent units are unchanged by the chemical procedure.

(c)   The utilization of synthetic methods to synthesize dimeric and oligomeric flavonoids in an enantiomerically pure form has the potential to be very powerful. However, this is rarely feasible due to the inaccessibility of enantiomerically pure monomeric starting materials. This limits synthetic endeavors to those natural products that are available in sufficient quantities and enantiomeric purity and which are obtainable through repetitive isolation from the natural source. The recent successful asymmetric syntheses of dihydroflavonols[180] and flavan-3-ols[181] by Van Rensburg *et al.* open the way for the lifting of such restrictions.

Owing to the complexity involved in this subject, it is conceivable that it may be necessary to use these methods collectively in order to achieve acceptable results. The results reported here demonstrate these concepts.

Several novel biflavonoids coexist in the red heartwood of *Berchemia zeyheri* with the predominant benzofuranone, maesopsin (**23**), along with several other monomeric flavonoids. A characteristic feature of these biflavonoids is the presence of at least one benzofuranoid moiety, making this a unique group of natural products. Furthermore, all the examples of these compounds encountered thus far, were isolated as diastereomeric mixtures. These compounds fall into two major groups, comprising either a flavanone and a benzofuranone unit or two benzofuranone constituent units.

The shortcomings in the available methods for the determination of absolute stereochemistry also precluded the stereochemical assignment of these compounds. The collective utilization of chemical degradation, ^{1}H NMR and CD spectroscopy and computational data now permits estimation of the absolute stereochemistry of several of these dimeric compounds and for the first time also of the two enantiomers of maesopsin (**23**).

In an attempt to augment the results obtained from the physical data of these compounds and to facilitate the possible synthesis of the dimers in an enantiomerically pure form, we attempted to develop an asymmetric synthesis towards maesopsin (**23**). Although unexpected problems prevented the full realization of this goal, two new routes for the synthesis of this compound were developed, one of which has notable potential for further development.

CHAPTER 6

FLAVANONE-BENZOFURANONE-TYPE BIFLAVONOIDS

6.1 INTRODUCTION

One group of biflavonoids from *Berchemia zeyheri* is those consisting of a flavanone constituent unit coupled through the heterocyclic C-ring to the A-ring of a maesopsin moiety. The identification of the first example in this class dates back more than 25 years to the isolation of a naringenin-maesopsin dimer, called zeyherin[21], from the heartwood of *B. zeyheri*. Renewed interest was sparked by the identification, also from *B. zeyheri*, of an epimer of zeyherin. The collective study of the epimers by means of chemical degradation, ^{1}H NMR, CD spectroscopy and computational studies finally permitted estimation of the absolute stereochemistry of these biflavonoids.

Supplementing this duo are the epimers of a regio-isomer of zeyherin, the stereochemistry of which was assigned by extension of the results obtained for zeyherin. Finally, a unique pair of biflavonoids, dihydrogenistein-$(2\alpha \rightarrow 5)$-maesopsin and its epimer, representing the first isoflavanone-benzofuranone-type biflavonoids, was also isolated from this natural source. Again, application of the techniques developed during the study of the zeyherin epimers allowed "direct" assessment of the stereochemistry of these compounds.

6.2 ZEYHERIN AND ITS EPIMER

Owing to the complexity of the mixture of polyphenols in the heartwood of *B. zeyheri*, several fractionation steps were required to effect the isolation of the relevant biflavonoids. Having established the absence of natural methoxy groups in the refined fractions by ^{1}H NMR, the polyphenols were isolated and identified as their permethylaryl ethers. The derivatization with dimethylsulphate under anhydrous conditions proved to be a prerequisite for sample purity.

Initially, the two diastereomers of zeyherin were isolated as a yellow amorphous solid, the ^{1}H NMR spectrum of which displayed well-defined signal doubling. The possibility of rotational isomerism was ruled out only after extended ^{1}H NMR studies at a variety of temperatures (-20 to 80°C). Subsequently, the mixture was separated by means of PLC to yield hepta-O-methylzeyherin (175) and its epimer (176).

173 R = H
175 R = Me

174 R = H
176 R = Me
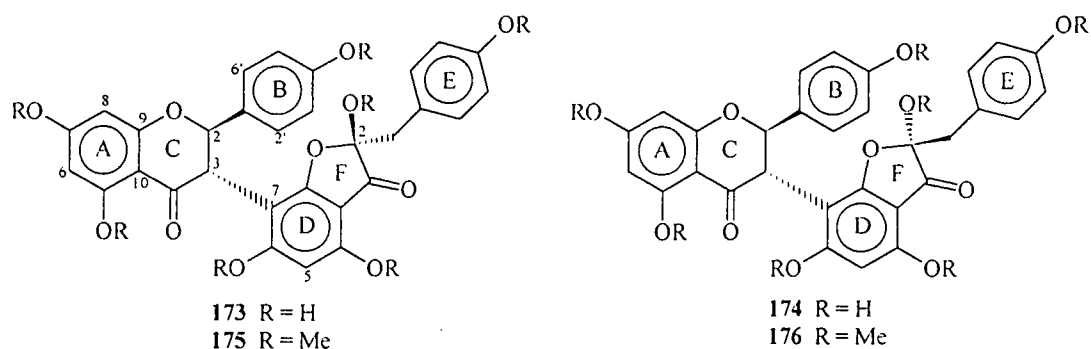
A conspicuous feature of the ^{1}H NMR spectra [NMR-plates 1 and 3, Table 6.1] of these two diastereomers, notably free of the effects of dynamic rotational isomerism about the interflavonoid bond, is the presence of a tetra-O-methylmaesopsin unit coupled to a tri-O-methylnaringenin moiety. The naringenin unit, substituted at 3-C(C-ring), is characterized by an AA'BB' spin system (J 8.5 Hz) typical of a $para$-substituted aromatic B-ring along with an aromatic AB-system (J 2.0 Hz) representing the phloroglucinol-type A-ring. An AM spin system represents 2-H(C) [δ 5.86 and 5.64 for (175) and (176) respectively] and 3-H(C) [δ 4.69 and 4.47 for (175) and (176) respectively] while the coupling constant (J$_{2,3}$ 12.0 Hz) for this system indicates a 2,3-$trans$ relative configuration for the heterocyclic C-ring of both (175) and (176). The presence of the maesopsin unit is disclosed by a second AA'BB'-system and the characteristic methylene doublets [δ 3.03 and 2.80 for (175) and δ 3.03 and 3.09 for (176) respectively; J 13.5 Hz] along with a methoxy singlet [δ 3.15 and 2.93 for (175) and (176) respectively] with pronounced acetal character, indicating the unique features of this moiety. The interflavonoid bond is located on the A-ring of the maesopsin unit (D-ring of the dimer) as indicated by the residual singlet [δ 5.91 and 5.93 for (175) and (176) respectively] in the aromatic region. The bonding position was identified as 7-C(D) in both derivatives (175) and (176) by the n.O.e. association of 5-H(D) with the two D-ring O-methyl resonances [δ 3.84, 3.91 and δ 3.73, 3.86 for (175)

Ring	H	(175), CDCl₃, 298K	(176), CDCl₃, 298K
A	6-H	6.16 (d, J 2.0 Hz)	6.17 (d, J 2.0 Hz)
	8-H	6.18 (d, J 2.0 Hz)	6.21 (d, J 2.0 Hz)
	5-OMe	3.84 (s)	3.88 (s)
	7-OMe	3.93 (s)	3.91 (s)
B	2-H/6-H	7.31 (d, J 8.5 Hz)	7.11 (d, J 8.0 Hz)
	3-H/5-H	6.80 (d, J 8.5 Hz)	6.73 (d, J 8.0 Hz)
	4-OMe	3.76 (s)	3.73 (s)
C	2-H	5.86 (d, J 12.0 Hz)	5.64 (d, J 12.0 Hz)
	3-H	4.69 (d, J 12.0 Hz)	4.47 (d, J 12.0 Hz)
D	5-H	5.91 (s)	5.83 (s)
	4-OMe/6-OMe	3.84/3.91 (each s)	3.73/3.86 (each s)
E	2-H/6-H	7.16 (d, J 8.5 Hz)	7.08 (d, J 8.0 Hz)
	3-H/5-H	6.69 (d, J 8.5 Hz)	6.71 (d, J 8.0 Hz)
	4-OMe	3.75 (s)	3.73 (s)
F	2-OMe	3.15 (s)	2.93 (s)
	α-C$\underline{H}_2$	3.03 (d, J 13.5 Hz)	3.03 (d, J 13.5 Hz)
	α-C$\underline{H}_2$	2.80 (d, J 13.5 Hz)	3.09 (d, J 13.5 Hz)

Table 6.1: ^{1}H NMR (δ_H) data of (2R,3S)-4',5,7-tri-*O*-methylnaringenin-(3α→7)-(2R)-2,4,4',6-tetra-*O*-methylmaesopsin (175) and (2R,3S)-4',5,7-tri-*O*-methylnaringenin-(3α→7)-(2S)-2,4,4',6-tetra-*O*-methylmaesopsin (176). Splitting patterns and J values (Hz) are given in parenthesis.

and (176) respectively]. N.O.e. correlations also assisted in identifying 6-H(A) [δ 6.16 and 6.17 for (175) and (176) respectively] and 8-H(A) [δ 6.18 and 6.21 for (175) and (176) respectively], following from the correlations of 6-H(A) with 5-OMe(A) [δ 3.84 and 3.88 for (175) and (176) respectively] and 7-OMe(A) [δ 3.93 and 3.91 for (175) and (176) respectively] and a single correlation of 8-H(A) with 7-OMe(A).

Differentiation between the very similar spin systems of the B- and E-rings was effected by way of COSY spectra using the benzylic methylene and 2-H(C) resonances, respectively, as reference signals. Thus, long-distance coupling ($^4J_{HH}$) in the 2D COSY spectra correlates the methylene group with 2/6-H(E) [δ 7.16 and 7.08 for (175) and (176) respectively] while the rest of the E-ring protons are defined by coupling ($^3J_{HH}$) between 2/6-H(E) and 3/5-H(E) [δ 6.69 and 7.16 for (175) and (176) respectively] along with a n.O.e. association of the latter with 4-OMe(E) (δ3.75 and 3.73). Long-distance coupling ($^4J_{HH}$) between 2-H(C) and 2/6-H(B) [δ 7.31 and 7.11 for (175) and (176) respectively] served to distinguish between the heterocyclic protons while the rest of the B-ring system was identified by correlations similar to those described above. The position of the residual proton was confirmed by long-distance coupling ($^5J_{HH}$) between 5-H(D) and two methoxy groups.

The exploitation of modern NMR techniques permitted the assignment of the signals in the ^{13}C NMR spectra of (175) and (176) [NMR-plates 2 and 4, Table 6.2] without having to resort to empirical methods, the carbon-hydrogen correlations in the HMQC spectra and long-range carbon-hydrogen correlations in the HMBC spectra providing ample information for the assignment of the majority of the signals. The close relationship of derivatives (175) and (176) is again demonstrated by the similarity in their ^{13}C NMR spectra.

Carbons bonded directly to a hydrogen atom were identified from the HMQC spectra of (175) and (176). Classification of 1-C(B) [δ 130.6 and 130.9 for (175) and (176) respectively] followed from correlations with 2-H(C) ($^2J_{CH}$) and 3/5-H(B) ($^2J_{CH}$) while 1-C(E) showed correlations with the α-methylene protons and with 3/5-H(E) (both $^2J_{CH}$).

Ring	C	(175)	(176)	Ring	C	(175)	(176)
A/C	C-2	81.6	81.7	D/F	C-2	109.8	110.0
	C-3	49.7	50.6		C-3	194.4	194.5
	C-4	188.9	188.8		C-4	159.1	158.9
	C-5	166.2	166.0		C-5	88.8	88.7
	C-6	93.8	94.0		C-6	168.2	167.9
	C-7	163.0	163.0		C-7	101.2	101.1
	C-8	93.6	94.0		C-8	170.5	170.6
	C-9	165.5	165.3		C-9	104.2	104.6
	C-10	106.1	106.5		C-α	40.7	40.9
B	C-1	130.6	130.9	E	C-1	126.2	125.8
	C-2/C-6	129.1	128.7		C-2/C-6	132.4	132.0
	C-3/C-5	114.1	114.0		C-3/C-5	113.6	113.9
	C-4	160.2	160.0		C-4	158.7	158.8
	OMe	55.4	55.5		OMe	56.4	56.4
		55.6	55.6			56.5	56.6
		56.0	56.0			56.7	

Table 6.2: ^{13}C NMR (δ_C) data of (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→7)-(2R)-2,4,4',6-tetra-O-methylmaesopsin **(175)** and (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→7)-(2S)-2,4,4',6-tetra-O-methylmaesopsin **(176)** at 300 MHz (25°C) in CDCl$_3$.

Together with the associations of 2/6-H(B) ($^3J_{CH}$) and 3/5-H(B) ($^2J_{CH}$) with 4-C(B) [δ 160.2 and 160.0 for **(175)** and **(176)** respectively] and of 2/6-H(E) ($^3J_{CH}$) and 3/5-H(E) ($^2J_{CH}$) with 4-C(E) [δ 158.7 and 158.8 for **(175)** and **(176)** respectively], the above mentioned correlations serve to identify the signals of the B- and E-aromatic rings. The

resonance at δ 109.8 (175) and δ 110.0 (176) was identified in each case as 2-C(F) by means of correlations with the methylene protons as well as 2-OMe(F) (both $^2J_{CH}$). Correlation of 4-C(C) [δ 188.9 and 188.8 for (175) and (176) respectively] with 2-H(C) ($^3J_{CH}$) and 6/8-H(A) ($^4J_{CH}$) and of 3-C(F) [δ 194.4 and 194.5 for (175) and (176) respectively] with 5-H(D) ($^4J_{CH}$) was used to distinguish between the carbonyl signals. The 5/7-C(A) and 4/6-C(D) oxygenated carbons was identified by association with the appropriate methoxyl resonances ($^2J_{CH}$) and with 6/8-H(A) or 5-H(D). A correlation between 6-C(D) and 3-H(C) ($^3J_{CH}$) in the HMBC spectrum of (176) served to distinguish between 4-C(D) [δ 159.1 and 158.9 for (175) and (176) respectively] and 6-C(D) [δ 168.2 and 167.9 for (175) and (176) respectively]. By elimination the two remaining signals [δ 165.5, 170.5 for (175) and 165.3, 170.6 for (176)] then represent the oxygenated 9-C(A) and 8-C(D). However, there is no evidence of any correlation involving these peaks in the HMBC spectrum of derivative (175), while only a single correlation in the spectrum of derivative (176) between 8-C(D) (δ 170.6) and 3-H(C) ($^3J_{CH}$) allowed differentiation of these peaks. The three remaining peaks are 7-C(D) [δ 101.2 and 101.1 for (175) and (176) respectively], identified by correlation with 5-H(D) ($^3J_{CH}$) and 3-H(C) ($^2J_{HC}$), 9-C(D) [δ 104.2 and 104.6 for (175) and (176) respectively], identified by correlation with 5-H(D) ($^3J_{CH}$) and 10-C(A) [δ 106.1 and 106.5 for (175) and (176) respectively], identified by correlation with 6/8-H(A).

The above structural assignments are consistent with the EI mass spectral fragmentation data [M/S Scheme 2, Table B2] of derivatives (175) and (176). Apart from the molecular ion M⁺, m/z 656 [15.1 and 6.2% for (175) and (176) respectively], prominent peaks occurred at m/z 535 (42.2, 29.5%), 476 (100, 100%), 355 (75.2, 88.4%), 313 (10.6, 10.1%), 181 (21.0, 11.7%) and 121 (75.6, 63.0%). The latter fragments reflect the loss of a 4-methoxybenzyl radical involving the E-ring, hence affording the m/z 535 ion, while the base peak (m/z 476) results from the equivalent of a retro-Diels-Alder (RDA) fragmentation of the naringenin C-ring.

Owing to the complexity imposed by the three stereocentres, the well-defined CD spectra (Figure 6.1) did not permit stereochemical assignment to either of the zeyherin derivatives
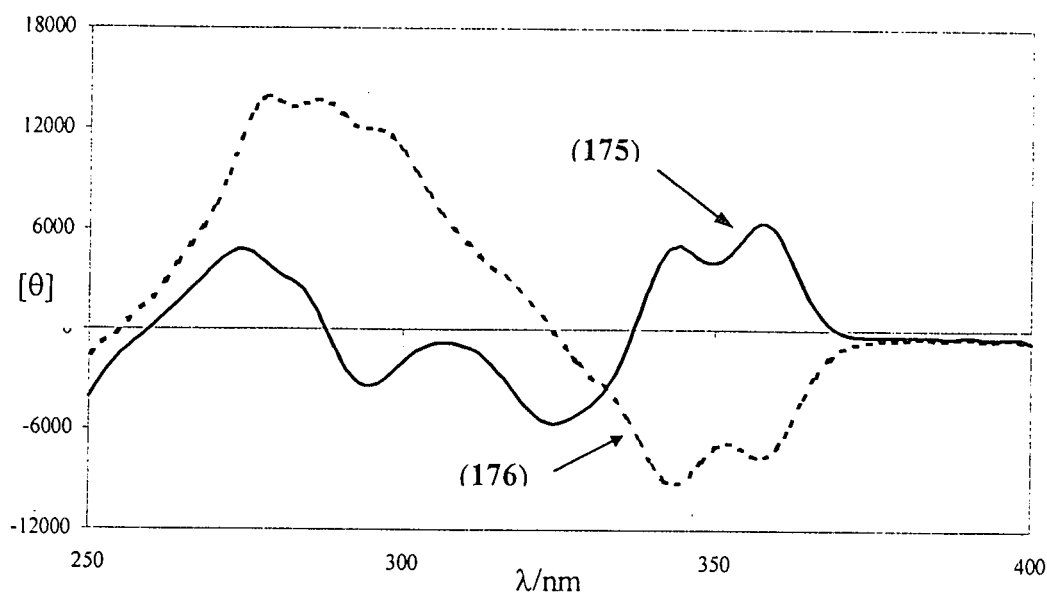
Figure 6.1 : CD curves of the biflavonoid derivatives (175) and (176).

(175) and (176). In the absence of direct stereochemical levers, an approach of chemical degradation was adopted in an attempt to reduce the number of stereocentres. Whereas the interflavonoid bond in epimers (175) and (176) proved to be stable towards acid-catalyzed thiolytic cleavage[182,183], treatment of these compounds with sodium cyanoborohydride [Na(CN)BH₃][184] (12 molar excess) in trifluoroacetic acid (TFA) for 2 hours at 0°C under nitrogen[179] led to the formation of the 7-(4-methoxyphenethyl)-2,4,4',6-tetra-O-methylmaesopsin enantiomers (188) and (190) (Scheme 6.1).

The structures of these degradation products were confirmed by the presence of the distinct elements of a tetra-O-methylmaesopsin unit in the identical ¹H NMR spectra of (188) and (190) [NMR-Plate 5, Table 6.3]. The maesopsin unit remains substituted on the A-ring (residual singlet at δ 5.91) and the coupling position is confirmed by n.O.e. associations between 5-H(D) and two methoxyl resonances (δ 3.83 and 3.92). A four proton multiplet (δ 2.76) along with an AA'BB' spin system (δ 7.11 and 6.83, J 8.5 Hz) represents those protons that originated from the naringenin unit of derivatives (175) and (176). Differentiation between the B- and E-ring protons was achieved by means of n.O.e. and COSY experiments as described above.

Ring	H	(188)/(190), CDCl₃, 298K	(189), CDCl₃, 298K
D	5-H	5.91 (s)	5.91 (s)
	4-OMe/6-OMe	3.83/3.92 (each s)	3.83/3.92 (each s)
E	2-H/6-H	7.17 (d, J 8.5 Hz)	7.17 (d, J 8.5 Hz)
	3-H/5-H	6.73 (d, J 8.5 Hz)	6.73 (d, J 8.5 Hz)
	4-OMe	3.73 (s)	3.73 (s)
F	2-OMe	3.21 (s)	3.21 (s)
	α-C$\underline{H}_2$	3.18 (d, J 13.0 Hz)	3.18 (d, J 13.0 Hz)
	α-C$\underline{H}_2$	3.11 (d, J 13.0 Hz)	3.11 (d, J 13.0 Hz)
B	2-H/6-H	7.11 (d, J 8.5 Hz)	7.11 (d, J 8.5 Hz)
	3-H/5-H	6.83 (d, J 8.5 Hz)	6.83 (d, J 8.5 Hz)
	4-OMe	3.80 (s)	3.80 (s)
	4 x α-C$\underline{H}_2$	2.76 (m)	2.79 (d, 8.0, 3-H)
			2.73 (d, 8.0, 2-H)

Table 6.3: ^{1}H NMR (δ_H) data of the 7-phenethylmaesopsin enantiomers **(188)**/**(190)** and the dideuteriomaesopsin derivative **(189)**. Splitting patterns and J values (Hz) are given in parenthesis.

Again, these structural assignments were confirmed by the EI mass spectral fragmentation data **[M/S Schemes 1, Table B1]** of derivatives **(188)** and **(190)**. Besides the low intensity molecular ion M$^+$, m/z 478 (2.7%), the fragments m/z 121 (48.0%) and m/z 357 (100.0%) again reflects the loss of a 4-methoxybenzyl radical involving the "E-ring".

The genesis of the degradation products requires the breaking of both the O-1–C-2 and C-3–C-4 bonds of the C-rings of the zeyherin epimers **(175)** and **(176)**. The initial step
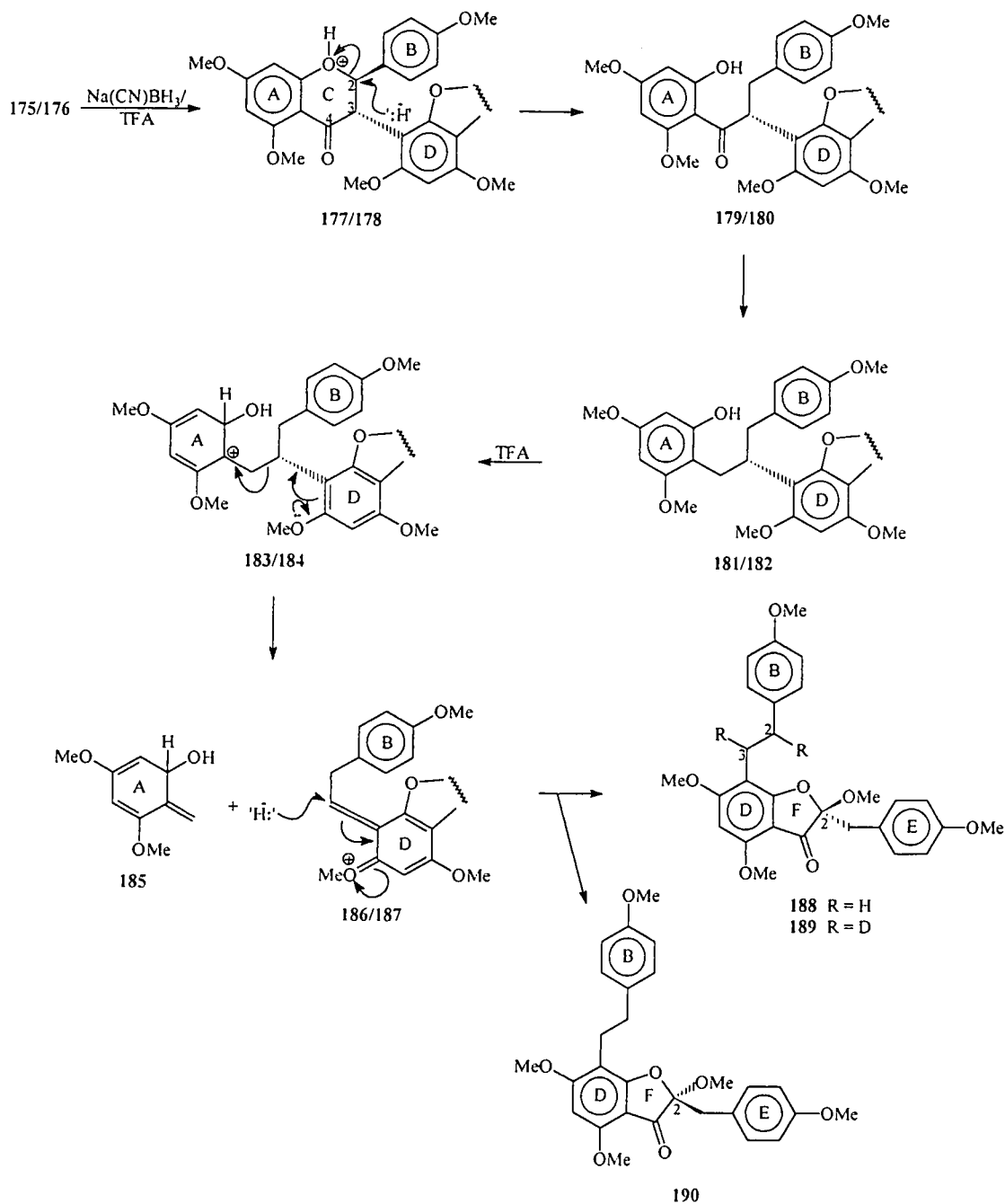
Scheme 6.1

presumably involves reductive cleavage of the O-1–C-2 bond *via* the protonated species (177)/(178) hence leading to the formation of the 2,3-diarylpropiophenones (179)/(180) which are subsequently reduced to the 1,2,3-triarylpropanes (181)/(182).[185,186] Protonation of the electron-rich phloroglucinol-type A-ring could impart lability to the equivalent of the C-3–C-4 bond which then breaks under the influence of the electron-

releasing D-ring intermediate (183)/(184). Aromatization of the A-ring fragment (185) *via* [1,3]-sigmatropic rearrangement results in the formation of 2-hydroxy-4,6-dimethoxytoluene while reduction of the *o*-quinomethane type intermediates (186)/(187) affords the 7-(4-methoxyphenethyl)-tetra-*O*-methylmaesopsin enantiomers (188) and (190). This mechanism was confirmed by utilizing sodium cyanotrideuterioborohydride [Na(CN)BD₃] under the same conditions used for the original reduction of the zeyherin epimer (175). This resulted in the formation of the dideuteriomaesopsin analogue (189), the ¹H NMR data of which are collated in **Table 6.3**.
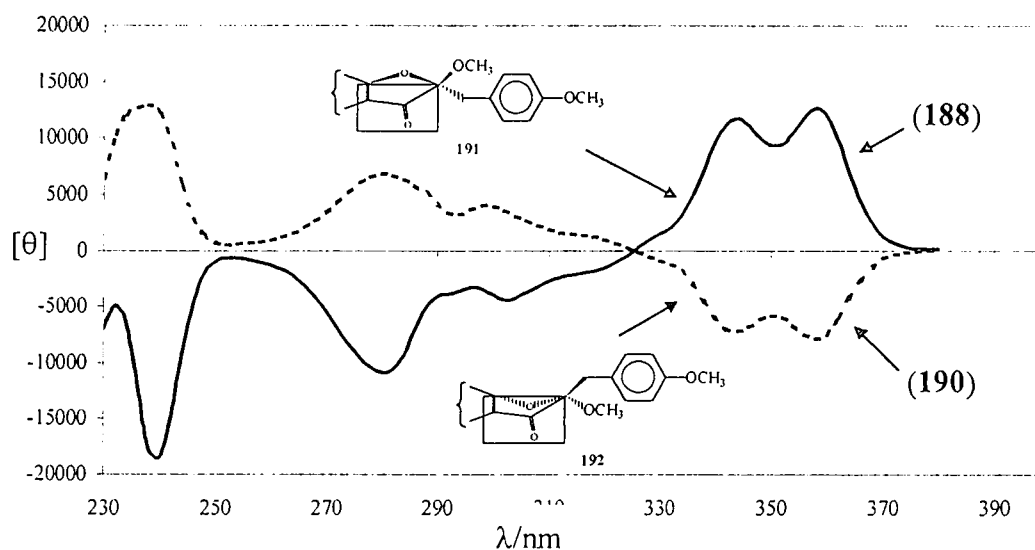
Figure 6.2 : CD spectra of maesopsin enantiomers (188) and (190).

The CD spectra (**Figure 6.2**) of the maesopsin enantiomers (188) and (190) may in principle be used to define the absolute configuration at C-2 providing that the preferred conformations of their benzofuranone moieties are known. Estimation of the latter by semi-empirical methods[187] (AM1) indicates that the oxacyclopentenone ring preferentially adopts a β-*O*-1-envelope conformation (191) with the heteroatom projecting above the plane (dihedral angle C*O*–C-3–C-2–O-1 = -178.8°) of the enone ring system in the 2*R* enantiomer (188). For the 2*S* enantiomer (190) an α-*O*-1-envelope conformation (192) with the heteroatom projecting below the plane (dihedral angle C*O*–C-3–C-2–O-1 = 179.5°) of the enone ring system is adopted. To account, however, for the dependence of

the CD curves on the total ensemble of conformers significantly populated at ambient temperature rather than a single preferred conformer, a global search routine[187] (GMMX 1.0) was employed to explore the potential energy surface (PES) of both enantiomers (188) and (190). The results (Table 6.4) conformed to those of the initial calculations, indicating conformers (Boltzmann population, 99.72%) with a β-O-1-envelope conformation (191) for the 2R enantiomer (188) and conformers (Boltzmann population, 99.77%) with an α-O-1-envelope conformation (192) for the 2S enantiomer (190) within a 3 kcal mol^{-1} energy window of the minimum.

191   192
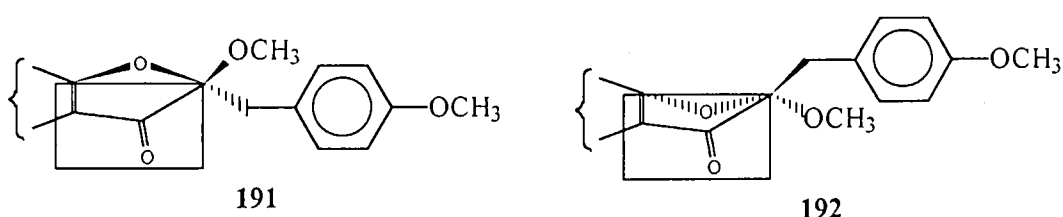
The observed positive and negative Cotton effects for the n→π* transition in the 330 - 365 nm region of the CD spectra of the maesopsin analogues (188) and (190) are thus in accord with the β-O-1- and α-O-1-envelope conformations for (188) and (190) respectively. By application of Snatzke's chirality rule for cyclopentenones[42] that correlates the sign of the Cotton effect with the absolutes stereochemistry of the cyclopentenone, a 2R absolute configuration can be assigned to (188) and a 2S configuration to (190). The CD curves (Figure 6.2) for derivatives (188) and (190) should additionally also permit the unambiguous assessment of the absolute stereochemistry of the 2-benzyl-2-hydroxybenzofuranone group of naturally occurring flavonoids.

Comparison of this CD data with the CD data for derivatives (175) and (176) subsequently also permitted tentative assignment of a 2R (C-ring) configuration to both the zeyherin derivatives via the high-amplitude positive Cotton effects ($[\theta]_{274.3}$ + 4.8 x 10^3 and ($[\theta]_{278.3}$ + 1.4 x 10^4 respectively) for the π→π* transitions in their CD spectra.[68] A global conformational search routine (GMMX 1.0)[187] was used to estimate the dihedral angle [2-C(C)–3-C(C)–7-C(D)–6-C(D)] about the 3-C(C)–7-C(D) bond for conformers of zeyherins (193) and (194) significantly populated within 3 kcal mol^{-1} of the minimum

Structure	Total conformers[a]	Unique conformers[b]	Final ensemble[c]	Dihedral angle[d]/°	E_{min}[e]/kcal mol^{-1}
(188)	7464	2993	1961	-168 to -179	52.09
(190)	6547	2616	1582	168 to 179	52.04
(193)	8989	2804	613[f]	100 to 110	77.70
(194)	3183	1098	331[f]	50 to 60	76.02

[a] Total number of conformers considered during search. [b] Number of unique conformers (carbon skeleton only) within 3 kcal mol^{-1} of the most stable conformer. [c] Final ensemble of conformers (hydrogens attached) within 3 kcal mol^{-1} of the most stable conformer. [d] Dihedral CO–C-3–C-2–O-1 for (188) and (190); dihedral 2-C(C)–3-C(C)–7-C(D)–6-C(D) for (193) and (194). [e] E_{min} of the most stable conformer in the final ensemble. [f] Resulting conformers further refined by four additional cycles.

Table 6.4

energy (**Table 6.4**). The results indicated preferred E-conformers (C-ring) for both these conformers with interflavonoid dihedral angles of 100-110° (Boltzmann population, 99.98%) and 50-60° (Boltzmann population, 99.90%), respectively.

193   194
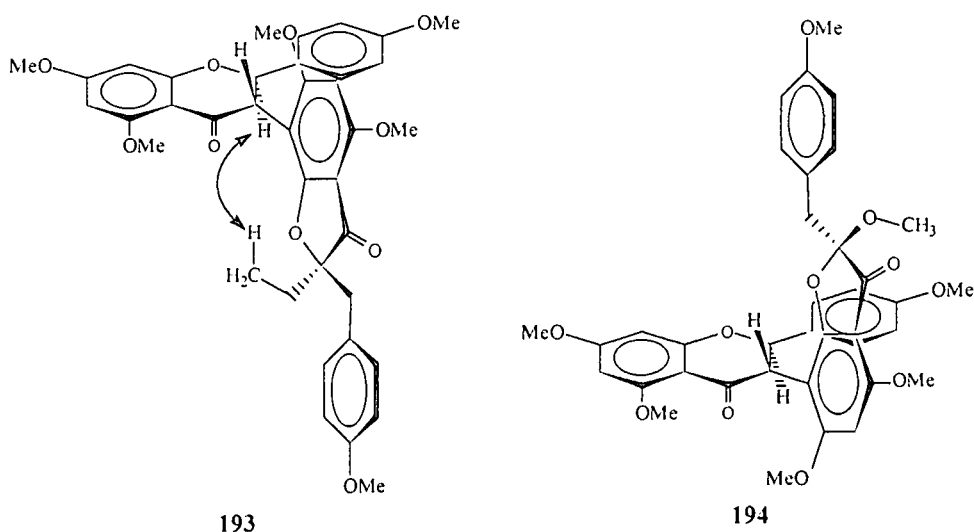
When taken in conjunction with the observed n.O.e. association (0.7%) of 2-OMe(F) (δ 3.15) with 2-H(C) (δ 5.86) in conformer (193) of derivative (175), with the (2R)-benzofuranoid constituent unit, and its conspicuous absence in the diastereomer (176) with the 2S(F) configuration, these data unequivocally confirms the 2R(C) absolute configuration of the zeyherins (173) and (174). The 3S(C) configuration of both these natural products is then evident from the ^{1}H NMR coupling constants ($^3J_{2,3}$ 12.0 Hz) establishing a 2,3-*trans* (C) relative configuration for both the zeyherin epimers.

6.3 <u>REGIO-ISOMERS OF ZEYHERIN</u>

A second pair of epimers from the heartwood of *B. zeyheri*, isolated as a yellow amorphous substance following methylation, proved to be regio-isomers of zeyherin. The ¹H NMR spectra [**NMR-plates 6 and 8, Table 6.5**] of the hepta-*O*-methyl derivatives **(197)** and **(198)** exhibits the typical effects of dynamic rotational isomerism about the interflavonoid bond at ambient temperature. At elevated temperature (70°C) the close structural relationship with the zeyherin epimers becomes unmistakable and again, the presence of a tetra-*O*-methylmaesopsin unit coupled with a tri-*O*-methylnaringenin moiety can be clearly discerned from the ¹H NMR data.

195 R = H
197 R = Me

196 R = H
198 R = Me
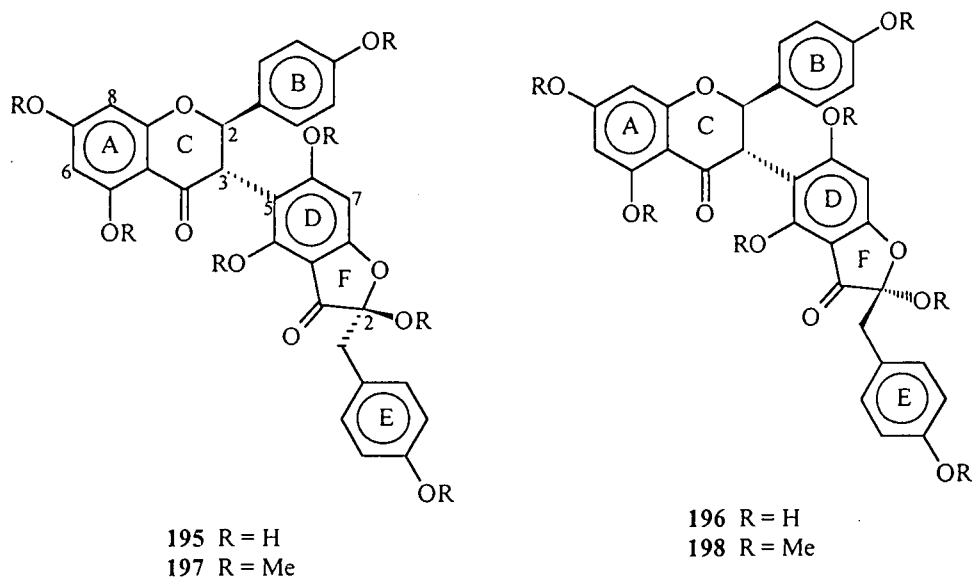
The spectra of both epimers **(197)** and **(198)** exhibit the characteristic signals of a naringenin unit substituted at 3-C(C) with an aromatic AB-system (J 2.0 Hz) representing the A-ring, the AM spin system associated with 2-H(C) [δ 5.51, 5.65 for **(197)** and **(198)** respectively] and 3-H(C) [δ 4.47, 4.57 for **(197)** and **(198)** respectively; $J_{2,3\text{-}trans}$ 12.0 Hz for both epimers] and an AA'BB' spin (J 9.0 Hz) system originating from the *para*-substituted B-ring. N.O.e.-associations between 2-H(C) and 2/6-H(B) [δ 7.02, 7.15 for **(197)** and **(198)** respectively] identify 2/6-H(B). While it is possible to observe coupling ($^3J_{HH}$) between 2/6-H(B) and 3/5-H(B) in the 2D COSY spectra of **(197)** and **(198)**, the close proximity of 3/5-H(B) and 3/5-H(E) [δ 6.75, 6.76 and 6.74, 6.74 for **(197)** and

Ring	H	(197), CDCl₃, 343K	(198), CDCl₃, 343K
A	6-H/8-H	6.16/6.18 (each d, J 2.0 Hz)	6.15/6.18 (each d, J 2.0 Hz)
	5-OMe/7-OMe	3.84/3.89 (each s)	3.84/3.89 (each s)
B	2-H/6-H	7.02 (d, J 9.0 Hz)	7.15 (d, J 9.0 Hz)
	3-H/5-H	6.75 (d, J 9.0 Hz)[a]	6.76 (d, J 9.0 Hz)[a]
	4-OMe	3.79 (s)[b]	3.80 (s)[b]
C	2-H	5.51 (d, J 12.0 Hz)	5.65 (d, J 12.0 Hz)
	3-H	4.47 (d, J 12.0 Hz)	4.57 (d, J 12.0 Hz)
D	7-H	5.99 (s)	6.08 (s)
	4-OMe	3.93 (s)	3.75 (s)
	6-OMe	3.55 (s)	3.69 (s)
E	2-H/6-H	7.12 (d, J 9.0 Hz)	7.11 (d, J 9.0 Hz)
	3-H/5-H	6.74 (d, J 9.0 Hz)[a]	6.74 (d, J 9.0 Hz)[a]
	4-OMe	3.77 (s)[b]	3.78 (s)[b]
F	2-OMe	3.24 (s)	3.22 (s)
	α-CH₂	3.18 (d, J 14.0 Hz)	3.15 (d, J 14.0 Hz)
	α-CH₂	3.11 (d, J 14.0 Hz)	3.04 (d, J 14.0 Hz)

[a,b] May be interchanged

Table 6.5: ^{1}H NMR (δ_H) data of (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→5)-(2R)-2,4,4',6-tetra-O-methylmaesopsin (**197**) and (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→5)-(2S)-2,4,4',6-tetra-O-methylmaesopsin (**198**). Splitting patterns and J values (Hz) are given in parenthesis.

(198) respectively] precludes the differentiation of these peaks and subsequently also the differentiation of 4-OMe(B) and 4-OMe(E). Although n.O.e. data associates 6/8-H(A) [δ 6.16, 6.18 and 6.15, 6.18 for (197) and (198) respectively] with 5-OMe/7-OMe [δ 3.84, 3.89 for both (197) and (198)], the nature of the ^{1}H NMR data again did not allow full differentiation between these peaks.

The maesopsin unit, again substituted on the A-ring, is characterized by the conspicuously shielded 2-OMe(F) [δ 3.24, 3.22 for (197) and (198) respectively] and the α-methylene [δ 3.18, 3.11 for (197) and δ 3.15, 3.04 for (198); J_{AB} 14.0 Hz] resonances reminiscent of the benzofuranoid moiety along with an AA'BB' spin system and a residual proton [δ 5.99, 6.08 for (197) and (198) respectively]. A n.O.e. association between this proton and only one methoxy group, 6-OMe(D) [δ 3.55, 3.69 for (197) and (198) respectively], define these as (3→5)-linked dimers. The n.O.e. association between 2/6-H(E) [δ 7.12, 7.11 for (197) and (198) respectively] and the methylene resonances identifies the E-ring, but again, it is not possible to distinguish between the remaining signals of the epimers as discussed above.

The ^{13}C NMR spectra of (197) and (198) [NMR-plates 7 and 9, Table 6.6] are very similar to those of the zeyherin epimers (175) and (176) (see Table 6.2), exhibiting, with a few exceptions, the same general pattern. The data obtained from the HMQC and HMBC spectra proved, however, less diagnostic due to the broadening of some peaks at room temperature. It was therefore necessary to repeat the HMQC and HMBC experiments at 70°C in order to identify all the carbon signals.

All the carbons bonded directly to a hydrogen atom were identified from the HMQC spectra of (197) and (198). The signal assigned to 7-C(D) [δ ±89 for both (197) and (198)] is not discernible in the ^{13}C NMR spectrum but was identified from a correlation in the high temperature HMQC spectrum of (197). The remaining resonances of the naringenin unit were identified from the HMBC spectra by means of correlation ($^4J_{CH}$) between 4-C(C) [δ 190.4 and 190.2 for (197) and (198) respectively] and 6-H(A) and correlations ($^3J_{CH}$) of 5-C(A) [δ 165.9 for both (197) and (198)] and 7-C(A) [δ 163.0 for

Ring	C	(197)	(198)	Ring	C	(197)	(198)
A/C	C-2	82.3	82.2	D/F	C-2	109.3	109.3
	C-3	51.3	51.1		C-3	193.3	193.6
	C-4	190.4	190.2		C-4	$\pm 156^c$	$\pm 156^c$
	C-5	165.9	165.9		C-5	111.2	111.1
	C-6	94.0^a	94.0^a		C-6	168.2	168.0
	C-7	163.0	163.0		C-7	$\pm 89^c$	$\pm 89^c$
	C-8	93.8^a	93.8^a		C-8	173.3	173.3
	C-9	165.4	165.3		C-9	106.7	106.4^b
	C-10	106.7	106.5^b		C-α	41.0	40.9
B	C-1	130.8	130.9	E	C-1	125.6	125.6
	C-2/C-6	128.5	128.7		C-2/C-6	132.0	132.0
	C-3/C-5	113.7	113.8		C-3/C-5	113.7	113.8
	C-4	160.0	160.0		C-4	158.9	159.0
	OMe	52.5	52.4		OMe	56.0	56.0
		55.5	55.5			56.5	56.5
		55.7	55.7				

[a,b] May be interchanged. [c] No peaks observed for this carbon - identified from correlations in the HMQC and HMBC spectra.

Table 6.6: ^{13}C NMR (δ_C) of (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3$\alpha\rightarrow$5)-(2*R*)-2,4,4',6-tetra-*O*-methylmaesopsin (**197**) and (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3$\alpha\rightarrow$5)-(2*S*)-2,4,4',6-tetra-*O*-methylmaesopsin (**198**) at 300 MHz (25°C) in CDCl₃.

both (**197**) and (**198**)] with 6/8-H(A) and 5-OMe/7-OMe(A). Differentiation between 5-C(A) and 7-C(A) is based on similarities with the ^{13}C NMR spectra of (**175**) and (**176**).

Analogous to the spectra of (175) and (176), no correlations identifying 9-C(A) can be observed in HMBC spectra of either (197) nor (198) and therefore the peak at δ 165.4 (197) and δ 165.3 (198) was reserved for this carbon in accordance with the assignments made for (175) and (176) [δ 165.5 and 165.3 respectively]. The peak at δ 106.7 in the ^{13}C NMR spectrum of (197) [resonating as two peaks at δ 106.4 and 106.5 in the spectrum of (198)] show correlation with both 6-H(A) and 7-H(D), indicating that both 10-C(A) and 9-C(D) is represented by this peak. Although the same correlations are observed in the HMBC spectrum of (198), the close proximity of the peaks prevents differentiation between 10-C(A) and 9-C(D).

Identification of the B- and E-ring carbon resonances followed from identical correlations to those observed in the HMBC spectra of the zeyherin dimers.

The maesopsin carbons are identified by correlations of 2-C(F) [δ 109.3 for both (197) and (198)] with 2-OMe(F) and the methylene protons and between the carbonyl carbon 3-C(F) [δ 193.3 and 193.6 for (197) and (198) respectively] and the methylene protons. Correlations of 4-C(D) [δ ±156 for both (197) and (198)] with 4-OMe(D), of 6-C(D) [δ 168.2 and 168.0 for (197) and (198) respectively] with 6-OMe(D) and 7-H(D) and of 8-C(D) [δ 173.3 for both (197) and (198)] with 7-H(D) along with a correlation between 5-C(D) [δ 111.2 and 111.1 for (197) and (198) respectively] and 7-H(D) identify the remainder of the maesopsin carbons.

These structural assignments correlated with the EI mass spectral fragmentation data [**M/S Scheme 3, Table B3**] of derivatives (197) and (198). The molecular ion M$^+$, m/z 656 [20.2 and 12.1% for (197) and (198) respectively] and several other prominent peaks correspond to the peaks observed for (175) and (176). These fragments once again reflect the loss of a 4-methoxybenzyl radical to afford the m/z 535 ion while the base peak (m/z 476) results from a retro-Diels-Alder (RDA) fragmentation of the naringenin C-ring.

The CD-curves (**Figure 6.3**) of the 5-linked zeyherin derivatives (197) and (198) are very similar to those of the zeyherin derivatives (see **Figure 6.1**) and the epimeric relationship

72

is again clear form this data. From the results obtained for the latter derivatives it is clear that the positive Cotton effect observed for the n→π* transition in the 340 - 380 nm region in the case of (197) corresponds with a 2R(F) absolute configuration for the maesopsin unit of this epimer. Similarly, the negative Cotton effect in the same region observed for (198) indicates a 2S(F) absolute configuration for the maesopsin moiety.
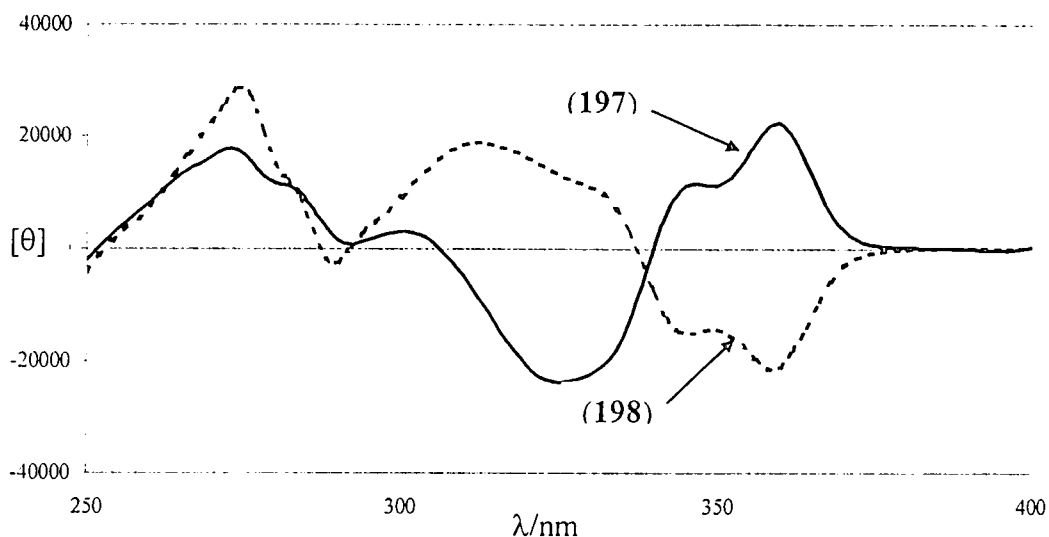
Figure 6.3 : CD curves of the biflavonoid derivatives (197) and (198).

Similar to the case of the zeyherins (175) and (176) (**Figure 6.1**), it can be concluded that the positive Cotton effect observed in the π→π* region (260 - 290 nm) indicates a 2R(C) absolute configuration for this moiety in both (197) and (198). Together with the 2,3-*trans* configuration of the C-ring, designated by the coupling constants of the protons of the heterocyclic ring ($^3J_{2,3}$ 12.0 Hz), this indicates that the absolute stereochemistry of the flavanone unit of the 5-linked zeyherin derivatives can be assigned as 2R,3S(C).

Several possible proposals can be forwarded to rationalize the biosynthetic origin of the four zeyherin dimers. These involve attack of a suitable electrophile on the 7- or 5-position of the phloroglucinol-type ring of either maesopsin (23) or the α-hydroxychalcone (35). Possible electrophiles include the highly unstable 3-carbocation[21] originating from the dihydroflavonol, aromadendrin, or a flavanone radical (199)[188]. It also appears reasonable to propose that a quinomethane radical[19] (201), initiated by

oxidation of 2',4,4',6'-tetrahydroxychalcone (**200**), may act as electrophile. Since the HOMO is located predominantly on the 7-position of maesopsin (**23**), the 7-carbon is more susceptible to electrophilic attack, thus explaining the greater abundance of the 7-coupled zeyherin dimers (**175**) and (**176**) in the heartwood of *B. zeyheri*.
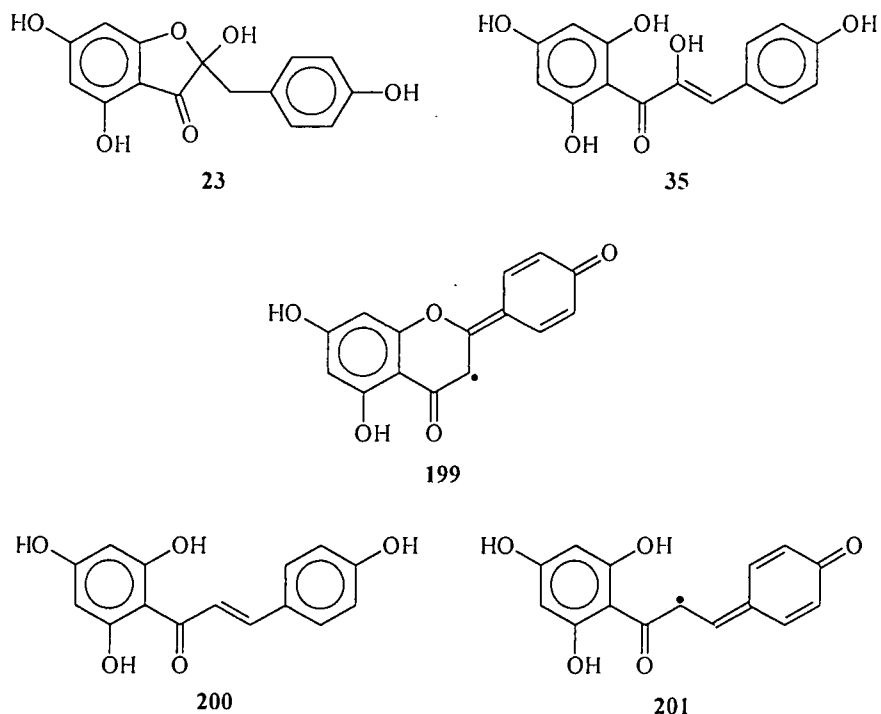
Evidence from the synthetic studies (see § 8.3) indicated that the hemiacetal bond of maesopsin is very labile, especially under basic conditions. Consequently, the interconversion[189] between the 2-benzyl-2-hydroxybenzofuranone and the corresponding α-hydroxychalcone may feature prominently in the heartwood of *B. zeyheri* thereby explaining the array of enantiomerism depicted above.

6.4 THE FIRST ISOFLAVANONE-BENZOFURANONE BIFLAVONOIDS

An additional pair of dimers in the group of flavanone-benzofuranone-type biflavonoids, isolated as yellow amorphous compounds from the heartwood of *B. zeyheri*, following methylation, was identified as (2*S*,3*R*)-dihydrogenistein-(2β→7)-(2*R*)-maesopsin (**202**) and its 2*S*(F)-epimer (**203**). These dimers represent the first natural isoflavanone-

benzofuranoid biflavonoids.

202 R = H
204 R = Me

203 R = H
205 R = Me
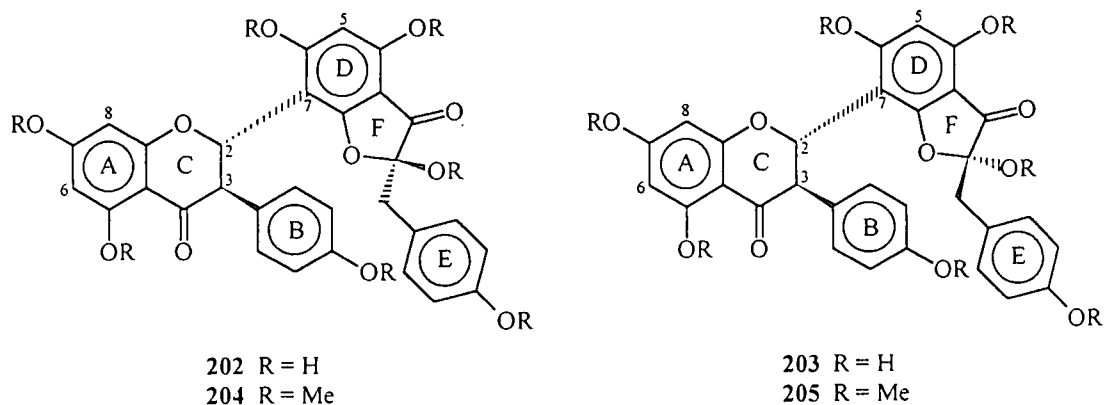
The close structural relationships between the derivatives (204) and (205) and the permethylaryl ethers of the zeyherin epimers (175) and (176) are evident from the resemblance of their ^{1}H NMR spectra [NMR-plates 10 and 11, Table 6.7]. The spectra of both epimers (204) and (205) exhibit an AM spin system associated with 2-H(C) [δ 5.62, 5.63 for (204) and (205) respectively] and 3-H(C) [δ 4.67, 4.63 for (204) and (205) respectively; $J_{2,3\text{-}trans}$ 10.5 Hz for both (204) and (205)] of the heterocyclic C-rings and the shielded 2-OMe(F) [δ 3.25, 3.18 for (204) and (205) respectively] together with the α-methylene (δ 3.15, 3.06 and 3.18, 3.06 for (204) and (205) respectively, J_{AB} 14.0 Hz] resonances typical of the benzofuranoid moieties. The B- and E-ring protons, appearing as two AA'BB' spin systems, are common to both derivatives while the A- and D-ring protons resonate respectively as an AB spin system [J_{AB} 2.0 Hz for both (204) and (205)] and a residual singlet [δ 5.94, 5.95 for (204) and (205) respectively]. N.O.e.-association of the latter proton with two methoxy groups [4-OMe(D) and 6-OMe(D)] define these compounds as 7(D)-linked dimers.

The B- and E-ring systems were differentiated on the grounds of n.O.e. associations and long-range COSY experiments in the same way as in the previous two cases. Thus, 2/6-H is identified by means of a n.O.e. association with either the α-methylene or the 3-H(C) resonances. Subsequently, the rest of the system can be identified with the long-range coupling ($^3J_{HH}$) between 2/6-H and 3/5-H and a n.O.e. association between 3/5-H and the 4-OMe resonance. The correlation of 2/6-H(B) [δ 7.33 and 7.38 for (204) and (205)

Ring	H	(204), CDCl₃, 298K	(205), CDCl₃, 298K
A	6-H	5.99 (d, J 2.0 Hz)	6.00 (d, J 2.0 Hz)
	8-H	6.00 (d, J 2.0 Hz)	6.05 (d, J 2.0 Hz)
	5-OMe	3.80 (s)	3.88 (s)
	7-OMe	3.87 (s)	3.82 (s)
B	2-H/6-H	7.33 (d, J 8.5 Hz)	7.38 (d, J 8.5 Hz)
	3-H/5-H	6.85 (d, J 8.5 Hz)	6.83 (d, J 8.5 Hz)
	4-OMe	3.79 (s)	3.79 (s)
C	2-H	5.62 (d, J 10.5 Hz)	5.63 (d, J 10.5 Hz)
	3-H	4.67 (d, J 10.5 Hz)	4.63 (d, J 10.5 Hz)
D	5-H	5.94 (s)	5.95 (s)
	4-OMe/6-OMe	3.91/3.88 (each s)	3.88/3.92 (each s)
E	2-H/6-H	7.02 (d, J 8.5 Hz)	7.16 (d, J 8.5 Hz)
	3-H/5-H	6.66 (d, J 8.5 Hz)	6.62 (d, J 8.5 Hz)
	4-OMe	3.74 (s)	3.71 (s)
F	2-OMe	3.25 (s)	3.18 (s)
	α-CH₂	3.15 (d, J 10.5 Hz)	3.18 (d, J 14.0 Hz)
	α-CH₂	3.06 (d, J 10.5 Hz)	3.06 (d, J 14.0 Hz)

Table 6.7: ¹H NMR (δ_H) data of (2S,3R)-4',5,7-tri-*O*-methyldihydrogenistein-(2α→7-(2R)-2,4,4',6-tetra-*O*-methylmaesopsin (**204**) and (2S,3R)-4',5,7-tri-*O*-methylnaringenin-(2α→7)-(2S)-2,4,4',6-tetra-*O*-methylmaesopsin (**205**). Splitting patterns and J values (Hz) are given in parenthesis.

respectively] with 3-H(C) [4.67, 4.63 for **(204)** and **(205)** respectively] rather than with 2-H(C) as was the case with the zeyherin dimers, is indicative of 2(C)→7(D)-linked oligomers based on a 2-substituted isoflavanone unit. N.O.e associations between 6-H(A) [δ 5.99 and 6.00 for **(204)** and **(205)** respectively] and two methoxyl resonances [5-OMe and 7-OMe(A)] and association of 8-H(A) with only one methoxyl resonance [7-OMe(A)] were used to differentiate between the A-ring protons.

Owing to the small amounts of material available for NMR studies, it was impossible to record the ^{13}C NMR data of these compounds.
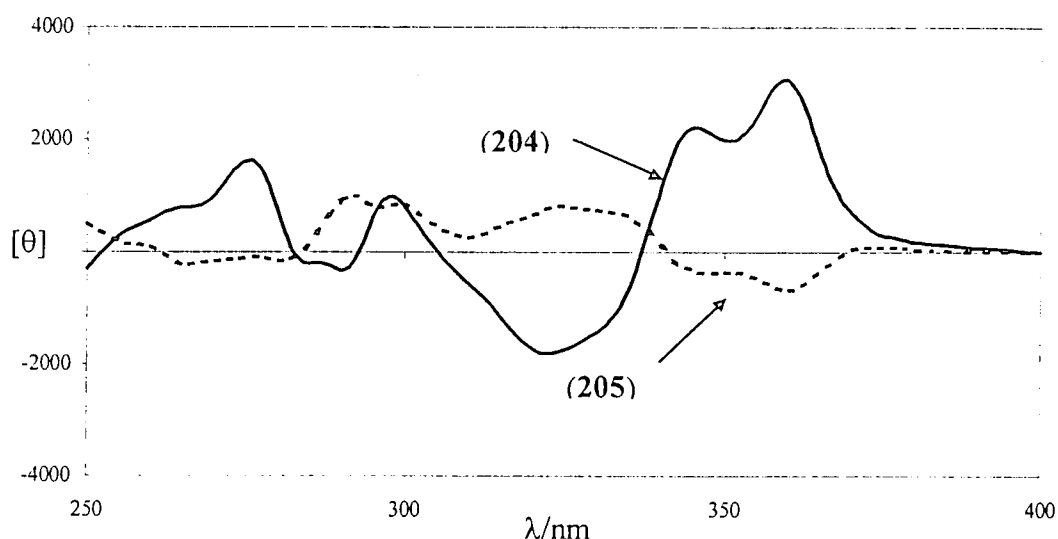
Figure 6.3 : CD curves of the biflavonoid derivatives **(197)** and **(198)**.

High-amplitude positive and negative Cotton effects for the n→π* transition in the 350 - 370 nm region of the CD spectra (**Figure 6.4**) of derivatives **(204)** and **(205)**, respectively, are in accord with 2R(F) and 2S(F) absolute configuration for the maesopsin moieties, thus illustrating their epimeric relationship. Isoflavanones, however, display Cotton effects for their n→π* transitions in the same region of their CD curves, a positive Cotton effect reflecting a 3R-configuration and a negative Cotton effect a 3S-configuration.[190,191] Thus, the positive Cotton effect at these wavelengths in the spectrum of **(204)** is reminiscent of a 3R(C) configuration, its high amplitude being the result of the cumulative effects of the

chirality at this centre and that at 2-C(F). The much reduced negative Cotton effect for derivative (205) similarly reflects a $3R$(C) configuration, the effects due to the chirality at 3-C(C) and 2-C(F) now opposing each other with that of the latter slightly more dominant. When taken in conjunction with the coupling constants of the protons of the heterocyclic C-ring ($^3J_{2,3}$ 10.5 Hz) indicating a 2,3-*trans* configuration for both (204) and (205), the absolute configuration of these novel biflavonoid derivatives may be assigned as $2S,3R$(C):$2R$(F) for (204) and $2S,3R$(C):$2S$(F) for (205).

Biogenetically, the novel biflavonoids (202) and (203) presumably originate *via* 2,3-phenyl migration of the $(2R)$-4',5,7-trihydroxyflavanone (206) (naringenin) which coexists in *B. zeyheri*.[192] Irrespective of the particular mechanism involved, *i.e.* ionic or radical,[193-195] such a migration proceeds in a *suprafacial* mode thus leading to an isoflavanone intermediate with a 3β aryl group and an electron deficiency at C-2. The less hindered α-face at C-2 of such an intermediate is then prone to nucleophilic attack by the phloroglucinol-type A-ring of maesopsin (23), thus leading to the $2S,3R$(C)-configuration. Since maesopsin coexists as a racemate in *B. zeyheri*[192] the two epimers are formed.

CHAPTER 7

BIFLAVONOIDS WITH TWO BENZOFURANONE UNITS

7.1 INTRODUCTION

After fractionation and methylation, six biflavonoids, consisting of two benzofuranoid moieties, were isolated from the heartwood of *B. zeyheri* representing three pairs of epimers.

The first pair consists of a maesopsin unit coupled *via* the A-ring to the C-ring of a second maesopsin unit. After establishment of the racemic nature of the epimers, they were resolved by means of HPLC using a chiral column. The foundation laid in the previous chapter along with information obtained from a crystal structure of one of the epimers permitted the rationalization of the CD spectra in terms of the absolute configuration of each of the four stereoisomers.

A second dimer and its epimer, also consist of two benzofuranoid moieties but evidence strongly suggests the presence of a γ-lactone functionality in the upper benzofuranoid unit. This is probably the result of a benzylic acid rearrangement of the upper unit, a conclusion substantiated by the previous isolation of similar monomeric compounds from *B. zeyheri*.[196]

Analogous to the above novel compounds, the final pair of epimeric biflavonoids also contains a γ-lactone functionality and proved to be regio-isomers of the above compounds.

7.2 MAESOPSIN

Maesopsin[15], [2-(4-hydroxybenzyl)-2,4,6-trihydroxybenzo[*b*]furan-3(2H)-one] (23) is the predominant metabolite (*ca.* 7% of the dry extract) in the heartwood of *B. zeyheri*.

The ^{1}H NMR spectrum [NMR-plate 12, Table 7.1] of maesopsin (23) is identical to that

Ring	H	(23), (CD$_3$)$_2$CO, 298K
A	5-H	5.83 (d, J 2.0 Hz)
	7-H	5.87 (d, J 2.0 Hz)
B	2-H/6-H	7.05 (d, J 9.0 Hz)
	3-H/5-H	6.65 (d, J 9.0 Hz)
	α-C$\underline{H}_2$	3.10 (s)

Table 7.1: ^{1}H NMR (δ_H) data of maesopsin (**23**)

of an authentic sample. Two *meta*-coupled doublets (J 2.0 Hz) depict the AB spin system of the phloroglucinol-type A-ring while an AA'BB' spin system (J 9.0 Hz) represents the four protons of the *para*-substituted B-ring. The two methylene protons resonate as a singlet despite their diastereotopic character. As was expected from previous reports[21] on the isolation of maesopsin, it was isolated as a racemate.

Methylation of maesopsin with dimethyl sulfate under anhydrous conditions yields the tetra-*O*-methyl[21] derivative (**206**). The ^{1}H NMR spectrum of this compound [**NMR-plate 13**] confirms the tetra-oxygenation while the diastereotopic character of the methylene protons, which now resonate as a non-equivalent geminal pair (J 14.0 Hz), is clarified. Another conspicuous feature of this spectrum is the presence of one methoxy resonance with pronounced acetal character hence resonating at higher field (δ 3.28) than the phenolic methoxyl resonances (δ 3.76, 3.86 and 3.88).

MeO

O

OMe

OMe

OMe

OMe

O
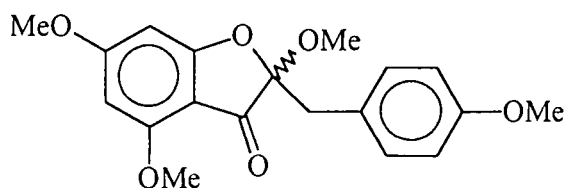
206 Racemate
207 ⁀ = ▬
208 ⁀ = ⫶⫶⫶

Resolution of tetra-*O*-methylmaesopsin (**206**) by means of HPLC using a chiral column gave access to the two enantiomers of maesopsin (**207**) and (**208**) in high enantiomeric purity (>99%). The CD spectra (**Figure 7.1**) of these enantiomers exhibit similar Cotton effects for the n→π* transition in the 330→360 nm region as that observed in the CD spectra (**Figure 6.2**) of the maesopsin derivatives (**188**) and (**190**) (see § 6.2). It has already been established that a positive Cotton in this region depicts 2*R* absolute configuration. Enantiomer (**207**) is thus identified as 2*R*-maesopsin and (**208**) as 2*S*-maesopsin.
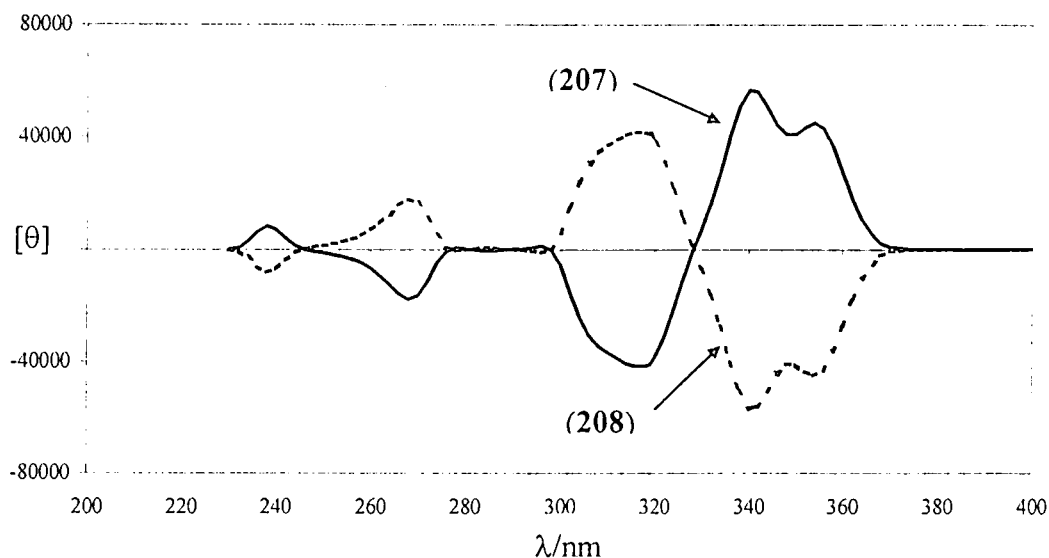
Figure 7.1 : CD spectra of the maesopsin enantiomers (**207**) and (**208**).

7.3 BIFLAVONOIDS WITH TWO MAESOPSIN UNITS

The two biflavonoids consisting of two maesopsin units were identified as 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**211/212**) and its epimer (**215/216**). [Henceforth the numbers (**211**) and (**215**) will be used to indicate the racemic mixtures of the two dimers until the separations of these into the enantiomeric pairs are discussed.] The ^{1}H NMR spectra [**NMR-plates 14 and 16, Table 7.2**] of these diastereomers, which again show no signs of dynamic rotational isomerism about the interflavonoid bond, exhibit the distinct characteristics associated with the presence of two

maesopsin moieties. Two AA'BB' spin systems (J 9.0 Hz) along with an AB spin system (J 2.0 Hz) and a residual proton in the aromatic region define the four benzenoid ring systems. The residual proton also designates one of the coupling positions on the D-ring. Two methylene groups (J 14.0 Hz), typical of two maesopsin units, one shielded methoxy resonance and six aromatic methoxy resonances represent the remainder of the protons. The loss of the second 2-OMe group indicates its replacement by the interflavonoid bond on 2-C(C).
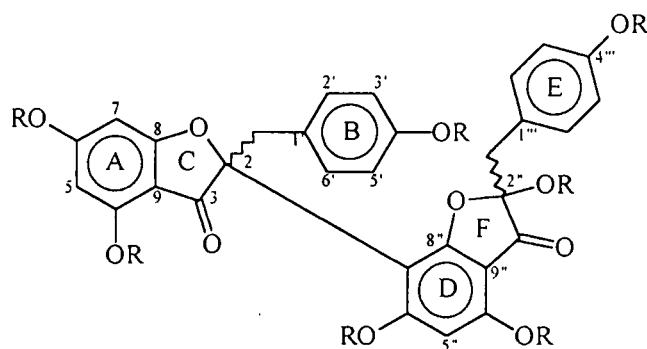
209 R = H;
211 R = Me;
213 R = H
215 R = Me;

210 R = H;
212 R = Me;
214 R = H
216 R = Me;

A n.O.e association between 5-H(D) [δ 5.88 and 5.87 for (211) and (215) respectively] and two OMe(D) resonances [δ 3.58 and 3.90; δ 3.57 and 3.90 for (211) and (215) respectively] identified the bonding position as 7-C(D). Additional n.O.e. associations between 6-OMe(A) [δ 3.79 and 3.80 for (211) and (215) respectively] and two protons, 7-H(A) [δ 5.93 and 5.95 for (211) and (215) respectively] and 5-H(A) [δ 5.79 for both (211) and (215)], and between 4-OMe(A) and only one proton, 5-H(A), identify the A-ring resonances.

In order to distinguish between the B/C- and E/F-ring systems, long-range coupling in a HMBC spectrum was utilized to differentiate between the carbonyl carbons and between the two methylene groups. Coupling of 3-C(F) [δ 194.4 and 194.3 for (211) and (215) respectively] with 5-H(D) ($^4J_{CH}$) as well as with α-CH$_2$(F) [δ 3.04 and 3.16; δ 3.09 and 3.17 for (211) and (215) respectively] ($^3J_{CH}$) partially identifies the resonances of the lower

Ring	H	(211), CDCl₃, 298K	(215), CDCl₃, 298K
A	7-H	5.93 (d, J 2.0 Hz)	5.95 (d, J 2.0 Hz)
	5-H	5.79 (d, J 2.0 Hz)	5.79 (d, J 2.0 Hz)
	4-OMe	3.80 (s)	3.81 (s)
	6-OMe	3.79 (s)	3.80 (s)
B	2-H/6-H	7.13 (d, J 9.0 Hz)	7.13 (d, J 9.0 Hz)
	3-H/5-H	6.63 (d, J 9.0 Hz)	6.63 (d, J 9.0 Hz)
	4-OMe	3.70 (s)	3.71 (s)
C	α-C$\underline{H}_2$	3.60 (d, J 14.0 Hz)	4.18 (d, J 14.0 Hz)
	α-C$\underline{H}_2$	4.05 (d, J 14.0 Hz)	3.54 (d, J 14.0 Hz)
D	5-H	5.88 (s)	5.87 (s)
	4-OMe/6-OMe	3.90/3.58 (each s)	3.90/3.57 (each s)
E	2-H/6-H	7.20 (d, J 9.0 Hz)	7.21 (d, J 9.0 Hz)
	3-H/5-H	6.77 (d, J 9.0 Hz)	6.78 (d, J 9.0 Hz)
	4-OMe	3.72 (s)	3.75 (s)
F	α-C$\underline{H}_2$	3.16 (d, J 14.0 Hz)	3.17 (d, J 14.0 Hz)
	α-C$\underline{H}_2$	3.04 (d, J 14.0 Hz)	3.09 (d, J 14.0 Hz)
	2-OMe	3.24 (s)	3.32 (s)

Table 7.2: ^{1}H NMR (δ_H) data of the 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**211**)/(**212**) and 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**215**)/(**216**). Splitting patterns and J values (Hz) are given in parenthesis.

unit. Likewise, 3-C(C) [δ 197.5 and 197.7 for (211) and (215) respectively] shows coupling with 7/5-H(A) and with α-C$\underline{H}_2$(C) [δ 3.60 and 4.05; δ 3.54 and 4.18 for (211) and (215) respectively]. Association of α-C$\underline{H}_2$(C) with 2/6-H(B) [δ 7.13 for both (211) and (215)] and of the latter with 3/5-H(B) [δ 6.63 for both (211) and (215)] in a 2D COSY experiment along with a n.O.e. association of 4-OMe(B) [δ 3.70 and 3.71 for (211) and (215) respectively] with 3/5-H(B) facilitate identification of the B-ring system. Similar correlations identify the E-ring system.

Carbon atoms in the ^{13}C NMR spectra of (211) and (215) [NMR-plates 15 and 17, Table 7.3] bonded directly to a hydrogen atom were identified from proton-carbon correlations in the HMQC spectra of derivatives (211) and (215). 2-C(C) [δ 91.3 and 91.1 for (211) and (215) respectively] was recognized by its association with α-C$\underline{H}_2$(C) ($^2J_{CH}$), 2/6-H(B) and 5-H(D) (each $^4J_{CH}$) while 4-C(A) [δ 158.5 for both (211) and (215)] was identified by correlation with 5-H(A) and 4-OMe(A). Correlation of 6-C(A) [δ 169.2 and 169.1 for (211) and (215) respectively] with both 5-H(A) and 7-H(A) as well as 6-OMe(A) distinguishes this carbon from 4-C(A) while coupling ($^2J_{CH}$) between 7-H(A) and 8-C(A) [δ 174.5 and 174.4 for (211) and (215) respectively] identifies the latter. Finally, 9-C(A) is distinguished by coupling ($^3J_{CH}$) with both 7-H(A) and 5-H(A). Concerning the B-ring, 1-C(B) [δ 127.2 and 126.9 for (211) and (215) respectively] is identified by means of coupling with 3/5-H(B) ($^4J_{CH}$) and α-C$\underline{H}_2$(C) while 4-C(B) [δ 158.4 for both epimers] shows coupling with 4-OMe(B) ($^2J_{CH}$) and 2/6-H(B) ($^3J_{CH}$). On account of the proximity of the carbon resonances assigned to 4-C(B) and 4-C(A), these assignments may be interchanged.

With a few exceptions, the carbon resonances of the lower maesopsin unit can be identified with similar correlations as those described above. 2-C(F) [δ 109.7 and 110.0 for (211) and (215) respectively] couples with 2/6-H(E) ($^4J_{CH}$), α-C$\underline{H}_2$(F) ($^2J_{CH}$) and 2-OMe(F) ($^3J_{CH}$) while 4/6-C(D) [δ 160.1 and 169.4; δ 160.2 and 169.3 for (211) and (215) respectively] relate to 5-H(D) and to either 4-OMe(D) or 6-OMe(D). Distinction between 4-C(D) and 6-C(D) is, however not possible. The resonances reserved for 8-C(D) show

no correlations in the HMBC spectra of either (211) and (215) and are assigned by a process of elimination. The only remaining carbon signals are those of 7-C(D) [δ 103.4

Ring	C	(211)	(215)	Ring	C	(211)	(215)
A/C	C-2	91.3	91.1	D/F	C-2	109.7	110.0
	C-3	197.5	197.7		C-3	194.4	194.3
	C-4	158.5[a]	158.5[a]		C-4	160.1[c]	160.2[c]
	C-5	92.2	92.2		C-5	89.4	89.5
	C-6	169.2	169.1		C-6	169.4[c]	169.3[c]
	C-7	88.3	88.3		C-7	103.4	103.7
	C-8	174.5	174.4		C-8	171.6	172.0
	C-9	106.2	106.4		C-9	105.3	105.4
	C-α	41.6	41.4		C-α	40.8	40.9
					OMe	52.6	52.8
B	C-1	127.2	126.9	E	C-1	125.6	125.7
	C-2/C-6	132.2[b]	132.2[b]		C-2/C-6	132.1[b]	132.1[b]
	C-3/C-5	113.4	113.4		C-3/C-5	113.9	113.9
	C-4	158.4[a]	158.4[a]		C-4	158.9	158.9
	OMe	55.2	55.5		OMe	56.3	56.6
		55.6	56.1			56.6	56.7
		56.2	56.4			56.7	

[a,b,c] May be interchanged

Table 7.3: ^{13}C NMR (δ_C) data of the 4,4',6-tri-O-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-O-methylmaesopsins (211)/(212) and (215)/(216) at 300 MHz (25°C) in CDCl_3.

and 103.7 for (211) and (215) respectively] and 9-C(D) [δ 105.3 and 105.4 for (211) and (215) respectively]. Both show correlation with 5-H(D) ($^3J_{CH}$ in both cases) while the correlation between 7-C(D) and α-C$\underline{H}_2$(C) ($^3J_{CH}$) distinguishes between the two carbons.

The EI mass spectral data of compounds (211) and (215) [M/S Scheme 5, Table B5] are essentially identical and exhibit the molecular ion M$^+$, m/z 656 [1.9 and 2.9% for (211) and (215) respectively]. The base peak, m/z 535 (100, 100%) originates from the loss of a p-methoxybenzyl radical [m/z 121 (54.3, 19.6%)] and subsequent cleavage of the interflavonoid bond results in the formation of a deoxymaesopsin radical [m/z 313 (3.3, 5.8%)]. This fragmentation pattern is consistent with the structural assignments made above.
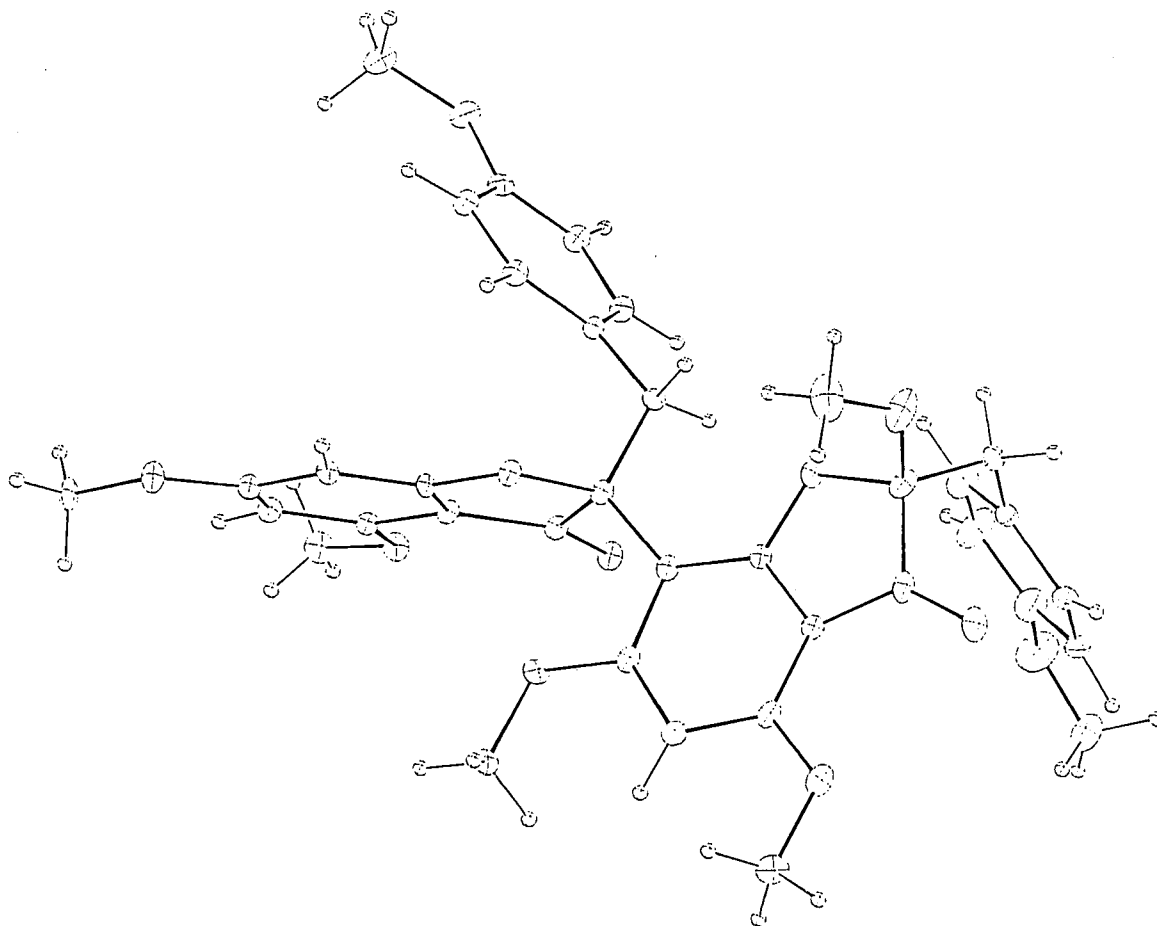

Figure 7.2: The crystal structure of 4,4',6-tri-O-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-O-methylmaesopsin (211)/(212).

The proposed structure of (211) was further confirmed by means of an X-ray diffraction study (Figure 7.2) of a crystal obtained from ethanol-water (9:1 v/v). The biflavonoid crystallized in an orthorhombic crystal system exhibiting a P_{bca} point group. The two constituent units are arranged almost perpendicular to each other [dihedral angle O-1(C)–C-2(C)–C-7(D)–C-6(D) = 45.86] and the B- and E-ring systems are folded over the A- and D-rings respectively, the angle produced by 2-C(C)–α-C–1-C(B) being 118.91° for the upper constituent unit and 108.06° for the lower unit.

While the symmetrical nature of the point group and orientation of the crystal prevent the direct assignment of the stereochemistry from the X-ray data, it is possible to limit the possible absolute configurations to that of the 2R(C):2S(F) or 2S(C):2R(F) diastereomers. The CD curve (Figure 7.3) of (211) contributes little towards differentiation between these possibilities, especially when it is compared with the CD curve of its diastereomer (215). It is furthermore unclear whether the observed Cotton effects are indicative of the combined effects of the constituent units of the dimer or of a partial racemate. Since maesopsin is isolated as a racemate from the heartwood of *B zeyheri* and all previous studies of dimers from this source (see Chapter 6) have identified maesopsin as the source of diastereoisomerism, the latter possibility seems very likely.
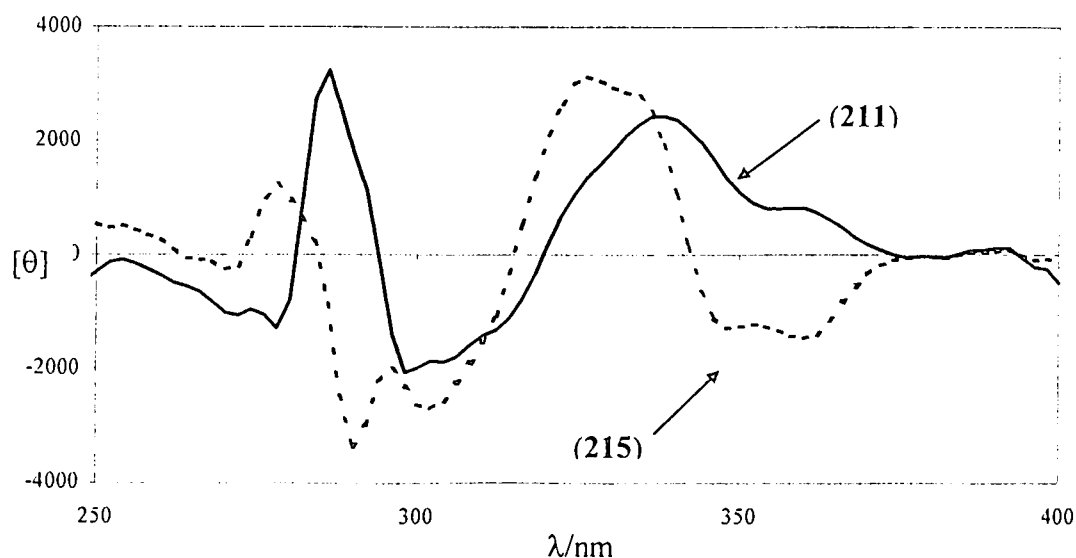

Figure 7.3: CD spectra of the biflavonoid diastereomers (211) and (215).

Attempts at degradation of these dimers were unsuccessful - the methyl ether derivatives (211) and (215) were stable under both acidic and basic reaction conditions, including those used for the degradation of compound (175) (see Chapter 6).

^{1}H NMR analysis of (211) and (215) using tris[3-heptafluoropropylhydroxymethylene-d-camphorato] europium (III) [Eu(hfc)$_3$] as chiral shift reagent revealed that both compounds are indeed racemic. Addition of about 1 mg of the shift reagent to compound (211) causes the D-ring methoxy group at δ 3.90 to resolve into two peaks at δ 3.95 and 3.97 indicating the presence of the two enantiomers in equal amounts. Upon addition of a further portion of the shift reagent, the majority of the signals in the ^{1}H NMR spectrum exhibit a resolution of about 0.5 ppm. Similarly, the methoxy group at δ 3.90 in the spectrum of (215) yields two peaks at δ 3.93 and 3.97 upon addition of about 1 mg of the shift reagent.

For the first time, the enantiomers of both dimers became accessible *via* resolution of the racemates by means of HPLC using a chiral column. Thus, the (211/212) racemate was resolved to give the enantiomerically pure derivatives (211) and (212) while racemate (215/216) was resolved into derivatives (215) and (216), each in high enantiomeric purity (> 99%).
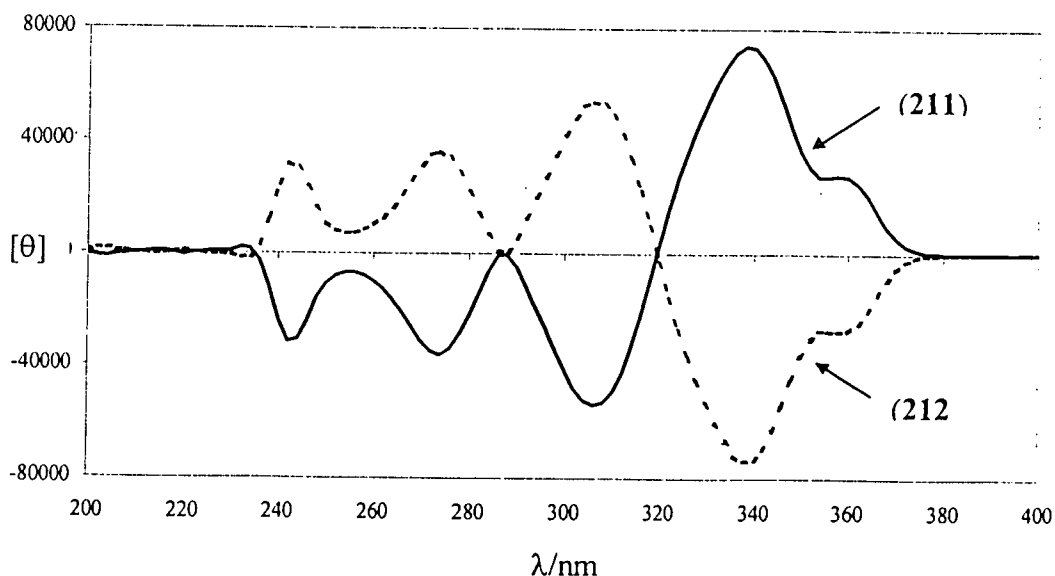

Figure 7.4: CD spectra of the biflavonoid enantiomers (211) and (212).

The CD curves of enantiomers (211) and (212) (Figure 7.4) exhibit five Cotton effects in the 230 to 380 nm regions. While it is impossible to link the respective effects to specific chromophores, the Cotton effects in the n→π* transition in the 330-365 nm region have been linked to the absolute configuration of maesopsin, positive and negative Cotton effects indicating 2R and 2S configurations, respectively. In the present case the influence of the upper constituent units of dimers (211) and (212) must be considered in order to differentiate between the 2R(C):2S(F) and 2S(C):2R(F) absolute configurations that was assigned to this pair of enantiomers from the X-ray crystal structure.

The potential energy surface (PES) of both the benzofuranoid moieties of the enantiomer with 2S(C):2R(F) absolute configuration was explored by means of a global search routine[187] (GMMX 1.0) in order to determine the total aggregate of conformers significantly populated at ambient temperature The results indicate conformers (Boltzmann population, 98.21%) with a β-O-1-envelope conformation for the lower maesopsin unit of this enantiomer. While this result corresponds with that obtained for the enantiomeric maesopsin derivative (188) (see § 6.2), the result for the upper constituent unit indicates a mixture of conformers with the conformer (Boltzmann population, 64.38%) exhibiting an α-O-1 envelope conformation dominating. Effectively this means a 28.76% excess of the α-O-1 conformer and its contribution to the observed Cotton effect, compared to that of the lower maesopsin unit, would therefore be insignificant.

Therefore, the positive Cotton effect for the n→π* transition in the CD curve of (211) indicates 2R absolute configuration for the lower maesopsin unit of this enantiomer and thus a 2S(C):2R(F) absolute stereochemistry. The stereochemistry of its enantiomer (212) is then 2R(C):2S(F).

Similar exploration of the PES of the 2S(C):2S(F) enantiomer indicates conformers (Boltzmann population, 99.11%) with an α-O-1 envelope conformation for the lower unit and again a mixture of conformers (Boltzmann population, 56.30% for the β-O-1 conformer and 43.70% for the α-O-1 conformer) for the upper unit. Again the contribution by the upper unit is negligible and therefore the positive Cotton effect for the

n→π* transition in the CD curve of (215) (**Figure 7.5**) indicates 2R absolute configuration for the lower unit and thus 2R(C):2R(F) configuration for compound (215). Its enantiomer (216) then exhibits 2S(C):2S(F) stereochemistry as reflected by the negative Cotton effect in the 350 - 370 nm region.
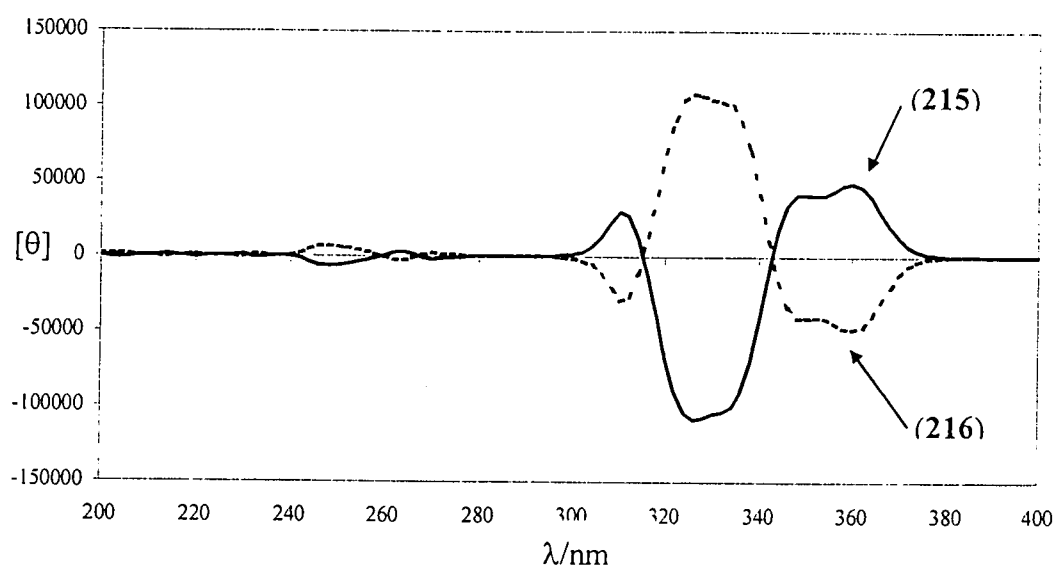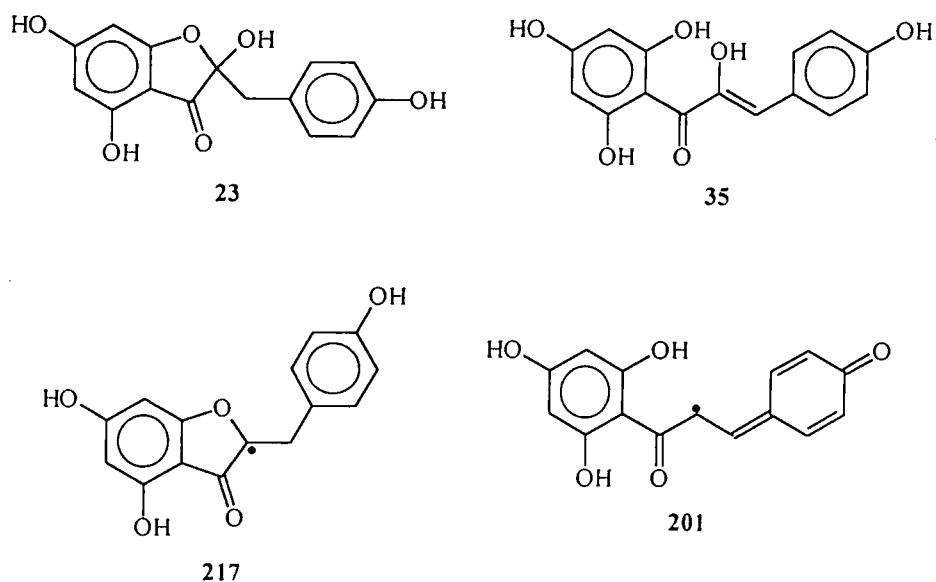

**Figure 7.5**: CD spectra of the biflavonoid enantiomers (215) and (216).

While the biosynthetic origin of these compounds is still unclear, it is possible to envisage an attack of a quinomethane radical[49] (201) (see § 6.3) on the 7-position of the phloroglucinol-type ring of either maesopsin (23) or the α-hydroxychalcone (35).

Subsequent α-cyclization, as opposed to β-cyclization in the case of the zeyherin dimers, will then lead to biflavonoids (211/212) and (215/216). Although a benzofuranonyl radical (217) or carbocation may also be considered as possible electrophiles, no evidence exists to support this route.

7.4 BIFLAVONOIDS WITH CARBON FRAMEWORKS REMINIS-CENT OF PRODUCTS OF BENZYLIC ACID REARRANGEMENT

The spin systems in the ^{1}H NMR spectra [NMR-plates 18 and 20, Table 7.4] of compounds (219) and (221) closely resemble those of the epimeric dimers (211/215) described above (§ 7.5) with the notable exception of a doublet belonging to one of the AA'BB' spin systems resonating at much higher field than expected. The presence of two benzofuranoid moieties follows from an AB spin system and a residual proton in the aromatic region along with two pairs of the characteristic methylene doublets associated with maesopsin. This evidence in conjunction with the two AA'BB' doublets [J 9.0 Hz for both (219) and (221)] depicting the B- and E-ring systems and the seven methoxy resonances, again indicate a dimer consisting of two maesopsin-type units coupled through the C- and D-rings, respectively.
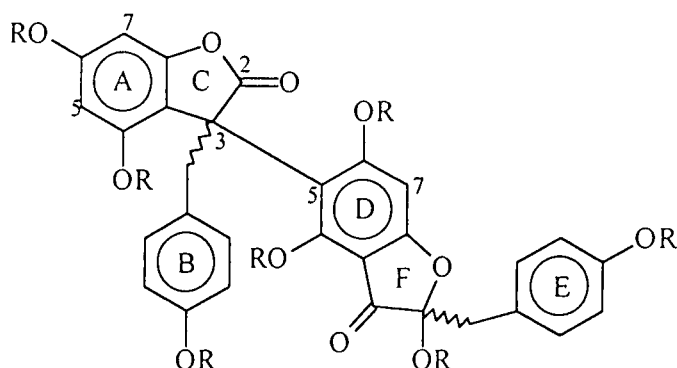

218 R = H;
219 R = Me;
220 R = H
221 R = Me;

*May be interchanged

A second pair of dimers accompanies the above pair of biflavonoids in the heartwood of

Ring	H	(219), CDCl₃, 298K	(221), CDCl₃, 298K
A	7-H	5.92 (d, J 2.0 Hz)	6.01 (d, J 2.0 Hz)
	5-H	6.25 (d, J 2.0 Hz)	6.27 (d, J 2.0 Hz)
	4-OMe	3.85 (s)	3.85 (s)
	6-OMe	3.76 (s)	3.79 (s)
B	2-H/6-H	5.76 (d, J 9.0 Hz)	6.12 (d, J 9.0 Hz)
	3-H/5-H	6.40 (d, J 9.0 Hz)	6.39 (d, J 9.0 Hz)
	4-OMe	3.70 (s)	3.67 (s)
C	α-C$\underline{H}_2$	3.33 (d, J 13.0 Hz)	3.33 (s)
	α-C$\underline{H}_2$	3.02 (d, J 13.0 Hz)	3.33 (s)
D	7-H	6.02 (s)	6.05 (s)
	4-OMe	3.78 (s)	3.77 (s)
	6-OMe	4.13 (s)	4.03 (s)
E	2-H/6-H	7.19 (d, J 9.0 Hz)	7.14 (d, J 9.0 Hz)
	3-H/5-H	6.82 (d, J 9.0 Hz)	6.82 (d, J 9.0 Hz)
	4-OMe	3.74 (s)	3.73 (s)
F	α-C$\underline{H}_2$	3.23 (d, J 14.0 Hz)	3.11 (d, J 13.0 Hz)
	α-C$\underline{H}_2$	3.28 (d, J 14.0 Hz)	3.21 (d, J 13.0 Hz)
	2-OMe	3.29 (s)	3.33 (s)

Table 7.4: ^{1}H NMR (δ_H) data of the 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsins (**219**) and (**221**). Splitting patterns and J values (Hz) are given in parenthesis.

B. zeyheri. The ^{1}H NMR spectra [**NMR-plates 22 and 24, Table 7.5**] of both compounds (**223**) and (**225**) are very similar to the spectra of (**219**) and (**221**) including the unexpected up-field shift of one of the AA'BB' doublets. Thus, the spectra of these compounds exhibit the now familiar AB spin system accompanied by the residual proton in the high field aromatic region along with two AA'BB' doublets. Taken in conjunction with the seven methoxy resonances and the characteristic methylene doublets [J 14.0 Hz and 13.0 Hz for (**223**) and (**225**) respectively], the analogy with the above compounds is clear.

222 R = H;
223 R = Me;

224 R = H
225 R = Me;
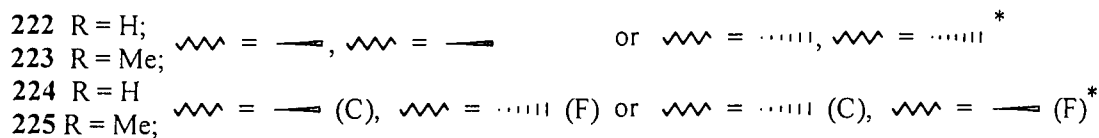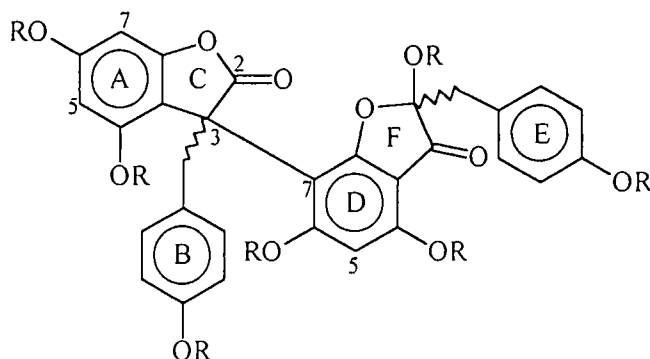
*May be interchanged

A n.O.e. association between the residual singlet [δ 5.84 and 5.80 for (**223**) and (**225**) respectively] of the latter pair of compounds and only one methoxy resonance apparently indicates that the bonding position of these dimers is located at 5-C(D). However, the residual singlet [δ 6.02 and 6.05 for (**219**) and (**221**) respectively] of the first pair of dimers shows no correlation with any methoxy group in either the 2D COSY or the 2D NOESY spectra. A weak correlation between this singlet and one methoxy group can, however, be observed in a long-range COSY experiment hence suggesting (2→5)-linkages for compounds (**219**) and (**221**).

The close resemblance of the ^{13}C NMR spectra of derivatives (**219**) and (**221**) [**NMR-plates 19 and 21, Table 7.6**] is consistent with an epimeric relationship of the two dimers. A similar resemblance may be observed in the ^{13}C NMR spectra of derivatives (**223**) and

Ring	H	(223), CDCl₃, 298K	(225), CDCl₃, 298K
A	7-H	5.98 (d, J 2.0 Hz)	5.94 (d, J 2.0 Hz)
	5-H	6.31 (d, J 2.0 Hz)	6.29 (d, J 2.0 Hz)
	4-OMe	3.91 (s)	3.88 (s)
	6-OMe	3.79 (s)	3.76 (s)
B	2-H/6-H	6.15 (d, J 9.0 Hz)	5.68 (d, J 8.0 Hz)
	3-H/5-H	6.45 (d, J 9.0 Hz)	6.36 (d, J 8.0 Hz)
	4-OMe	3.68 (s)	3.68 (s)
C	α-C$\underline{H}_2$	3.52 (d, J 14.0 Hz)	3.25 (d, J 13.0 Hz)
	α-C$\underline{H}_2$	3.46 (d, J 14.0 Hz)	3.34 (d, J 13.0 Hz)
D	5-H	5.84 (s)	5.80 (s)
	4-OMe	3.84 (s)	3.86 (s)
	6-OMe	3.82 (s)	3.78 (s)
E	2-H/6-H	7.20 (d, J 9.0 Hz)	7.28 (d, J 8.0 Hz)
	3-H/5-H	6.76 (d, J 9.0 Hz)	6.73 (d, J 8.0 Hz)
	4-OMe	3.73 (s)	3.57 (s)
F	α-C$\underline{H}_2$	3.24 (d, J 14.0 Hz)	3.25 (d, J 13.0 Hz)
	α-C$\underline{H}_2$	3.17 (d, J 14.0 Hz)	3.35 (d, J 13.0 Hz)
	2-OMe	3.50 (s)	3.23 (s)

Table 7.5: ^{1}H NMR (δ_H) data of the 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsins (**223**) and (**225**). Splitting patterns and J values (Hz) are given in parenthesis.

(225) [NMR-plates 23 and 25, Table 7.7], while the similarities between these spectra and those of compounds **(219)** and **(221)** are also unmistakable. The only differences between the ^{13}C NMR spectra of derivatives **(219/221)** and **(223/225)** are the up-field shift of one of the D-ring methoxy carbon resonances [δ 62 in the case of **(219/221)** versus δ 56 for **(223/225)**] and of the carbon resonance associated with the D-ring coupling position [δ 109 for **(219/221)** versus δ 100 for **(223/225)**]. This is comparable to the differences observed when the ^{13}C NMR spectra of **(175/176)** are compared to those of **(197/198)** (see Chapter 6). Here, the (2→5)-coupled dimers **(197)** and **(198)** each exhibit a methoxy carbon resonance at δ 62.2 and 62.1 respectively while the D-ring carbon at the coupling position resonates at δ 111.2 for **(197)** and at δ 111.1 for **(198)**. In the case of the (2→7)-coupled dimers **(175)** and **(176)**, all the methoxy carbons resonate below δ 57 and 7-C(D) resonates at δ 101.2 in the case of **(175)** and δ 101.1 in the case of **(176)**. From the above comparisons it can be inferred that dimers **(219)** and **(221)** are (2→5)-coupled while dimers **(223)** and **(225)** are the (2→7)-linked analogues despite the absence of compelling evidence usually provided by n.O.e. data. From Dreiding models it is clear that 6-OMe(D) will be influenced considerably by the upper constituent unit in both the (2→5)- and (2→7)-linked dimers and, presumably, this influence prevents the observation of any correlations with this methoxy group. Thus, in the (2→7)-coupled dimers, the residual proton shows only one correlation with 4-OMe(D) [δ 3.84 and 3.86 for **(223)** and **(225)** respectively] while in the (2→5)-coupled dimers this proton shows no correlations with 6-OMe(D). The remainder of this discussion will assume the proposed structures in order to facilitate the structural elucidation of compounds **(219/221)** and **(223/225)**, while alternative structures will be eliminated at the end of this chapter.

The correlations for the (2→5)-coupled dimers **(219)** and **(221)** are very similar to those observed for the dimers described in § 7.3. Association of 5-H(A) [δ 6.25 and 6.27 for **(219)** and **(221)** respectively] with two methoxy signals, 4-OMe(A) [δ 3.85 for both **(219)** and **(221)**] and 6-OMe(A) [δ 3.76 and 3.79 for **(219)** and **(221)** respectively] along with association of 7-H(A) [δ 5.92 and 6.01 for **(219)** and **(221)** respectively] with only 6-OMe(A) in a 2D NOESY experiment distinguish between the A-ring resonances. The

correlation of 7-H(D) with a methoxy resonance observed in a long-range COSY experiment identifies 6-OMe(D) [δ 4.13 and 4.03 for (219) and (221) respectively].

Ring	C	(219)	(221)	Ring	C	(219)	(221)
A/C	C-2	170.9	171.0	D/F	C-2	109.7[b]	109.8[b]
	C-3	48.4	48.4		C-3	193.9	193.9
	C-4	158.1	158.2		C-4	156.6	156.9
	C-5	95.3	95.3		C-5	109.4	109.3
	C-6	160.7[a]	160.7[a]		C-6	175.3	175.2
	C-7	93.2	93.2		C-7	93.9	93.9
	C-8	151.6	151.5		C-8	160.6[a]	160.4[a]
	C-9	104.3	104.4		C-9	108.4[b]	108.1[b]
	C-α	42.1	41.6		C-α	41.2	40.9
B	C-1	129.0	129.6	E	C-1	125.3	125.2
	C-2/C-6	131.2	131.3		C-2/C-6	132.2	132.0
	C-3/C-5	113.0	113.0		C-3/C-5	113.9	113.9
	C-4	158.4	158.4		C-4	159.0	159.0
	OMe	52.5	52.2		OMe	55.8	55.8
		52.6	52.5			56.2	56.2
		55.4	55.4			62.4	62.2
		55.5	55.5				

[a,b] May be interchanged

Table 7.6: ^{13}C NMR (δ_C) data of the 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsins (**219**) and (**221**) at 300 MHz (25°C) in CDCl$_3$.

In the absence of any direct correlations between 2-OMe(F) and α-C$\underline{H}_2$(F) in the 2D COSY or 2D NOESY spectra of (219) and (221), the ^{13}C NMR data [NMR-plates 19 and 21, Table 7.6] of these compounds permit differentiation between the B/C- and E/F-ring systems. The carbonyl resonances at δ 193.9 for both (219) and (221) show coupling ($^4J_{CH}$) with the residual singlet [7-H(D)] and a three-bond correlation with α-C$\underline{H}_2$(F) [δ 3.24 and 3.28; δ 3.11 and 3.21 for (219) and (221) respectively] in the HMBC spectra of (219) and (221), thus identifying 3-C(F) and α-C$\underline{H}_2$(F). A n.O.e. association between α-C$\underline{H}_2$(F) and 2/6-H(E) [δ 7.19 and 7.14 for (219) and (221) respectively] along with the correlation between the latter and 3/5-H(E) [δ 6.82 for both (219) and (221)] and a n.O.e association between 3/5-H(E) and 4-OMe(E) [δ 3.74 and 3.73 for (219) and (221) respectively] permit identification of the E-ring spin systems of the two epimers. Similar correlations identify the B-ring spin systems.

Correlations in the HMQC spectra of (219) and (221) were used to identify the carbons bonded directly to a hydrogen atom. The carbons of the lower maesopsin unit exhibit similar correlations to those observed for the lower maesopsin unit of (211) and (215). Thus, the correlations of 2-C(F) [δ 109.7 and 109.8 for (219) and (221) respectively] with α-C$\underline{H}_2$(F) ($^2J_{CH}$) and 2-OMe(F) ($^3J_{CH}$) along with the correlations of the carbonyl carbon described above identify the five-membered F-ring. Correlations between 4-C(D) [δ 156.6 and 156.9 for (219) and (221) respectively] and 4-OMe(D) ($^3J_{CH}$) and α-C$\underline{H}_2$(C) ($^4J_{CH}$) together with the association of 6-C(D) [δ 175.3 and 175.2 for (219) and (221) respectively] with 6-OMe(D) ($^3J_{CH}$) and 7-H(D) ($^2J_{CH}$) as well as a two-bond coupling between 8-C(D) [δ 160.6 and 160.4 for (219) and (221) respectively] and 7-H(D) were used to identify the oxygenated carbons of the D-ring. Both 9-C(D) [δ 108.4 and 108.1 for (219) and (221) respectively] and 5-C(D) [δ 109.4 and 109.3 for (219) and (221) respectively] show three-bond coupling with 7-H(D) while the association of 5-C(D) with α-C$\underline{H}_2$(C) distinguishes between the two carbons.

The carbons of the E-ring are identified by the association of 1-C(E) [δ 125.3 and 125.2 for (219) and (221) respectively] with 3/5-H(E) ($^3J_{CH}$) and α-C$\underline{H}_2$(F) ($^2J_{CH}$) and of 4-C(E)

Ring	C	(223)	(225)	Ring	C	(223)	(225)
A/C	C-2	170.5	170.5	D/F	C-2	110.8	110.2
	C-3	47.4	47.1		C-3	194.0	194.4
	C-4	158.2[a]	158.1[a]		C-4	158.0[a]	157.7[a]
	C-5	95.4	95.4		C-5	93.2	93.1
	C-6	160.8[b]	160.7[b]		C-6	174.2	174.8
	C-7	93.5	93.5		C-7	100.5	100.9
	C-8	151.6	152.0		C-8	160.7[b]	161.0[b]
	C-9	104.1	104.1		C-9	106.5	106.5
	C-α	41.0	41.2[b]		C-α	40.6	40.8[b]
B	C-1	129.4	128.9	E	C-1	125.3	125.5
	C-2/C-6	131.2	131.5		C-2/C-6	132.0	132.2
	C-3/C-5	113.2	112.8		C-3/C-5	113.9	114.2
	C-4	158.4[a]	158.3[a]		C-4	158.9[a]	159.1[a]
	OMe	52.8	52.3		OMe	55.8	55.8
		52.9	52.7			56.3	56.3
		55.4	55.3			56.6	56.6
		55.5	55.4				

[a,b] May be interchanged

Table 7.7: ^{13}C NMR (δ_C) data of the 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[b]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-O-methylmaesopsins (**223**) and (**225**) at 300 MHz (25°C) in CDCl₃.

[δ 159.0 for both (**219**) and (**221**)] with 3/5-H(E) ($^2J_{CH}$), 2/6-H(E) ($^2J_{CH}$) and 4-OMe(E) ($^2J_{CH}$).

The diamagnetic shift of the second carbonyl resonance from approximately δ 194 to δ 170.9 and 171.0 in the ^{13}C NMR spectra of (219) and (221) respectively, strongly indicates the presence of a γ-lactone functionality and, hence, a 3-benzylbenzo[b]furan-2(3H)-one molecular framework for the upper unit of this novel biflavonoid. The carbonyl peak shows a five-bond correlation with the residual singlet, 7-H(D). This unique structural feature is confirmed by the correlations of 3-C(C) [δ 48.4 for both (219) and (221)] with 7-H(D), 5- and 7-H(A), 2/6-H(B) (each $^4J_{CH}$) and α-C$\underline{H}_2$(C) ($^2J_{CH}$). Correlation of 4-C(A) [δ 158.1 and 158.2 for (219) and (221) respectively] with 4-OMe(A) ($^3J_{CH}$) and with 5-H(A) ($^2J_{CH}$) and the similar correlations of 6-C(A) [δ 160.7 for both (219) and (221)] with 6-OMe(A), 5-H(A) and 7-H(A) along with coupling between 8-C(A) [δ 151.6 and 151.5 for (219) and (221) respectively] and 7-H(A) identify the A-ring oxygenated carbon resonances. Finally, 9-C(A) [δ 104.3 and 104.4 for (219) and (221) respectively] is identified by correlations with 5- and 7-H(A) and with α-C$\underline{H}_2$(C) (each $^3J_{CH}$). The B-ring carbons are identified by the same correlations observed for the E-ring spin systems.

The pair of (2→7)-coupled dimers exhibits very similar correlations as those observed for the (2→5)-coupled analogues. The A-ring protons were identified by using correlations of 5-H(A) [δ 6.31 and 6.29 for (223) and (225) respectively] with two methoxyl signals, 4-OMe(A) [δ 3.91 and 3.88 for (223) and (225) respectively] and 6-OMe(A) [δ 3.79 and 3.76 for (223) and (225) respectively] and of 7-H(A) [δ 5.98 and 5.99 for (223) and (225) respectively] with only 6-OMe(A) in a 2D NOESY spectrum. The single n.O.e. correlation observed from the residual singlet [δ 5.84 and 5.80 for (223) and (225) respectively] in the NOESY spectra of these compounds identifies 4-OMe(D) [δ 3.84 and 3.86 for (223) and (225) respectively].

The correlation in the HMBC spectra between the carbonyl resonance at δ 194.0 and 194.4 for (223) and (225) respectively and the residual singlet [5- H(D)] and a three-bond correlation with α-C$\underline{H}_2$(F) [δ 3.24 and 3.17; δ 3.25 and 3.35 for (223) and (225) respectively] again identify 3-C(F) and also permit differentiation between the B/C- and

E/F-ring systems. These systems may now be fully identified using the correlations in the 2D NOESY and 2D COSY spectra of compounds (223) and (225) in the same way as described for the (2→5)-coupled dimers.

Again, all carbons bonded directly to a hydrogen atom were identified from the correlations in the HMQC spectra of compounds (223) and (225). The correlations in the HMBC spectra of these compounds are essentially the same as those observed in the HMBC spectra of compounds (219) and (221) and 2-C(F) [δ 110.8 and 110.2 for (223) and (225) respectively] is again identified by the correlations with α-C$\underline{H}_2$(F) and 2-OMe(F). The remaining D-ring carbons were identified as described above and 7-C(D) [δ 100.5 and 100.9 for (223) and (225) respectively] by coupling with 5-H(D) and α-C$\underline{H}_2$(C). The carbons belonging to the E-ring, not identified by correlations in the HMQC spectra, are identified by the association of 1-C(E) [δ 125.3 and 125.5 for (223) and (225) respectively] with 3/5-H(E) and α-C$\underline{H}_2$(F), and of 4-C(E) [δ 158.9 and 159.1 for (219) and (221) respectively] with 3/5-H(E) ($^2J_{CH}$), 2/6-H(E) and 4-OMe(E).

Similar to the (2→5)-coupled analogues, the carbonyl resonance [δ 170.5 for both (223) and (225)] associated with the upper constituent unit is reminiscent of a γ-lactone functionality in this unit. The rest of the correlations exhibited by these dimers are identical to those observed in the case of the (2→5)-coupled analogues and the B-ring carbons are identified by similar correlations as those observed for the E-ring spin systems.

The single correlation of the residual singlet in (223) and (225) or the absence of correlations between the residual proton and any other entity in (219) and (221) cast some doubt regarding the proposed structures and therefore several alternative structures were considered. In order to account for the observed correlations, or the absence thereof, of the residual singlet, structures (226) and (227) were considered. While the correlations in the 2D COSY, NOESY, HMQC and HMBC spectra of these compounds can be reconciled with the proposed structures, the latter may also be correlated with products expected from the Wessely-Moser rearrangement[197]. This acid or base catalyzed rearrangement involves the cleavage of the heterocyclic ring of appropriately substituted

5-hydroxy flavonoids. Subsequent ring closure then gives access to the two possible regio-isomers, depending on the hydroxy group involved in the cyclization process.

A second possibility is to accept the pair of dimers exhibiting only one n.O.e. association for the residual proton as the (2→5)-coupled analogues (219) and (221) and to propose structure (228) for the pair of dimers that shows no correlations for the residual proton. Again, the correlations observed in the 2D NMR experiments of these compounds would support the structures.

226

227

228
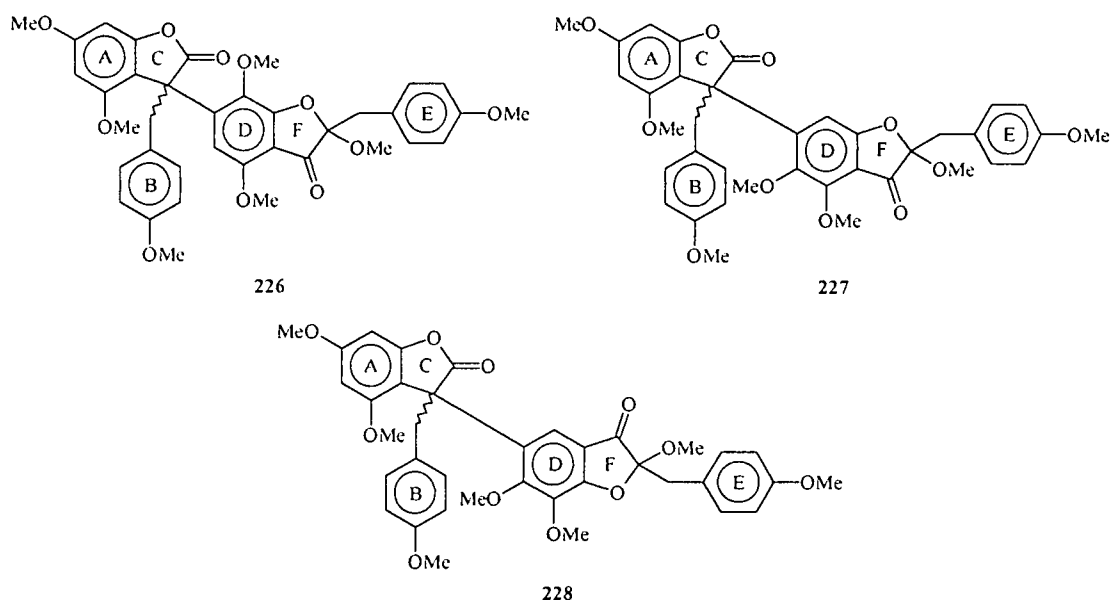
However, since the alternative structures (226), (227) and (228) all incorporate a D-ring oxygenation pattern that differs substantially from the phloroglucinol-type ring system, several objections to these structures may be lodged. Firstly, the pattern exhibited by the carbon signals of the D-ring in the ^{13}C NMR spectra of these compounds would be expected to differ substantially from those exhibited by a phloroglucinol-type ring. Considering the close resemblance in the ^{13}C NMR spectra of all the dimers studied here, with the oxygenated carbons of the phloroglucinol-type rings resonating between δ 150 and 180 and the remaining ring carbons resonating between δ 80 and 100, the alternative structures are not reflected in this data. Furthermore, the fact that the residual proton resonates consistently between δ 5.80 and 6.10 in the ^{1}H NMR spectra of all the dimers

discussed in this and the previous chapter also disqualifies these structures. Finally, the alternative structures have no biochemical precedent. The oxygenation pattern of structures (226) and (227) are unknown in flavonoid chemistry and while the oxygenation pattern of compound (228) is known[23], no compound with this pattern has been isolated from the heartwood of *B. zeyheri*. In view of this evidence, it is therefore feasible to discard these alternative structures.

Another alternative that must be considered is that, instead of (2→7)-coupled, compounds (223) and (225), showing the single n.O.e. association, are (2→5)-coupled. If the regio-isomeric relationship indicated by the ^{13}C NMR spectra of compounds (219) and (221) is accepted, the latter two dimers must be (2→7)-coupled and both n.O.e. correlations normally observed for (2→7)-coupled flavonoids are not detected. The steric influence or "buttressing effect"[198] of the upper unit can be stated as reason for this phenomenon although it is unlikely that 4-OMe(D) will be influenced to such an extend by the upper constituent unit if it is located on 7-C(D). In the light of the evidence offered by the ^{13}C NMR data, the original hypothesis can be accepted as more likely.

A last alternative is the possibility of atropisomerism. From Dreiding models it is clear that there will be a large steric interaction between the constituent units, especially in the case of the (2→5)-coupled analogues. The absence of the effects of dynamic rotational isomerism about the interflavonoid bond in the ^{1}H NMR spectra of the above compounds is significant, especially when compared to the ^{1}H NMR spectra of the (2→5)-coupled zeyherins (197) and (198) that do exhibit this phenomenon. Although it is unlikely that atropisomerism can account for the differences in the ^{13}C NMR spectra of the epimeric pairs, this possibility was evaluated by heating a small sample of compound (219) to above 100°C in DMSO. The solution was analyzed by means of TLC, but no trace of either (223) or (225) was observed. This evidence precludes the possibility of atropisomerism.

The EI mass spectral data of the four dimers [M/S Schemes 6 and 7, Table B6 and B7] offer no additional evidence to differentiate between the proposed structures. The fragmentation pattern exhibited by this data is inherently analogous to that observed for

compounds (211) and (215) and is thus consistent with the structural assignments made above. Each of the four dimers exhibits the molecular ion M^+, m/z 656 and, in the case of compounds (219), (221) and (225) the base peak, m/z 535 originates from the loss of a p-methoxybenzyl radical [m/z 121]. For compounds (223) the base peak is m/z 121. The other major fragment, m/z 625, exhibited by all four dimers, originates from the loss of a methoxy radical.

Considering that the four dimers were isolated as diastereomeric pairs and that each contains two benzofuranoid moieties, it is feasible that each diastereomer is racemic, as was the case for compounds (211) and (215). ^{1}H NMR analysis of the four dimers using Eu(hfc)$_3$ as chiral shift reagent revealed that this is indeed the case. Preliminarily attempts to separate these compounds into their respective enantiomers were unsuccessful and therefore their stereochemistry remains unknown.

Some of the unusual phenomena observed in the ^{1}H NMR spectra of dimers (219), (221), (223) and (225) suggest that these compounds exhibit some exceptional conformational characteristics. The most conspicuous of these is the shielding of 2/6-H(B) from δ 7.13 in the ^{1}H NMR spectrum of (211) to δ 5.76 in the spectrum of (219). Similar shielding effects can be observed in the spectra of compounds (221), (223) and (225). Inspection of Dreiding models reveal that, due to steric constraints, the B-ring will most likely be folded over the A-ring. This unique position of the B-ring substituent subjects the 2/6-B protons to anisotropic shielding by the A-ring. Furthermore, the absence of associations with 6-OMe(D) in the NOESY and COSY spectra of these dimers seems to suggest that this group is forced from its usual position by the "buttressing effect"[198] of the upper constituent unit. This is confirmed by n.O.e. associations in the NOESY spectrum of (223) of 6-OMe(D) with 2/6-H(E) and 3/5-H(E), an association that is only possible if the E-ring is again folded over the D-ring and if 6-OMe(D) is displaced from the plane formed by the aromatic D-ring in the direction of the E-ring.

Despite the simple *in vitro* benzylic acid rearrangement of benzofuranones [e.g. maesopsin (23)] to 3-benzyl-3-hydroxybenzo[*b*]furan-2(3H)-ones (232), presumably *via* an α-

diketone intermediate (229)[15,20,199,200], flavonoids exhibiting the carbon framework of compound (232) have, until recently[196], been unknown in natural sources. This changed with the isolation of 4,6-dihydroxy-3-(4-hydroxybenzyl)-3-methylbenzo[b]furan-2(3H)-one (233), along with two other compounds exhibiting the same structural features, from the heartwood of *B. zeyheri*.[196] The formation of this compound (**Scheme 7.1**) provides a direct biochemical precedent for the formation of dimers (219), (221), (223) and (225) although it is unclear whether benzylic acid rearrangement precedes formation of the interflavonoid bond.
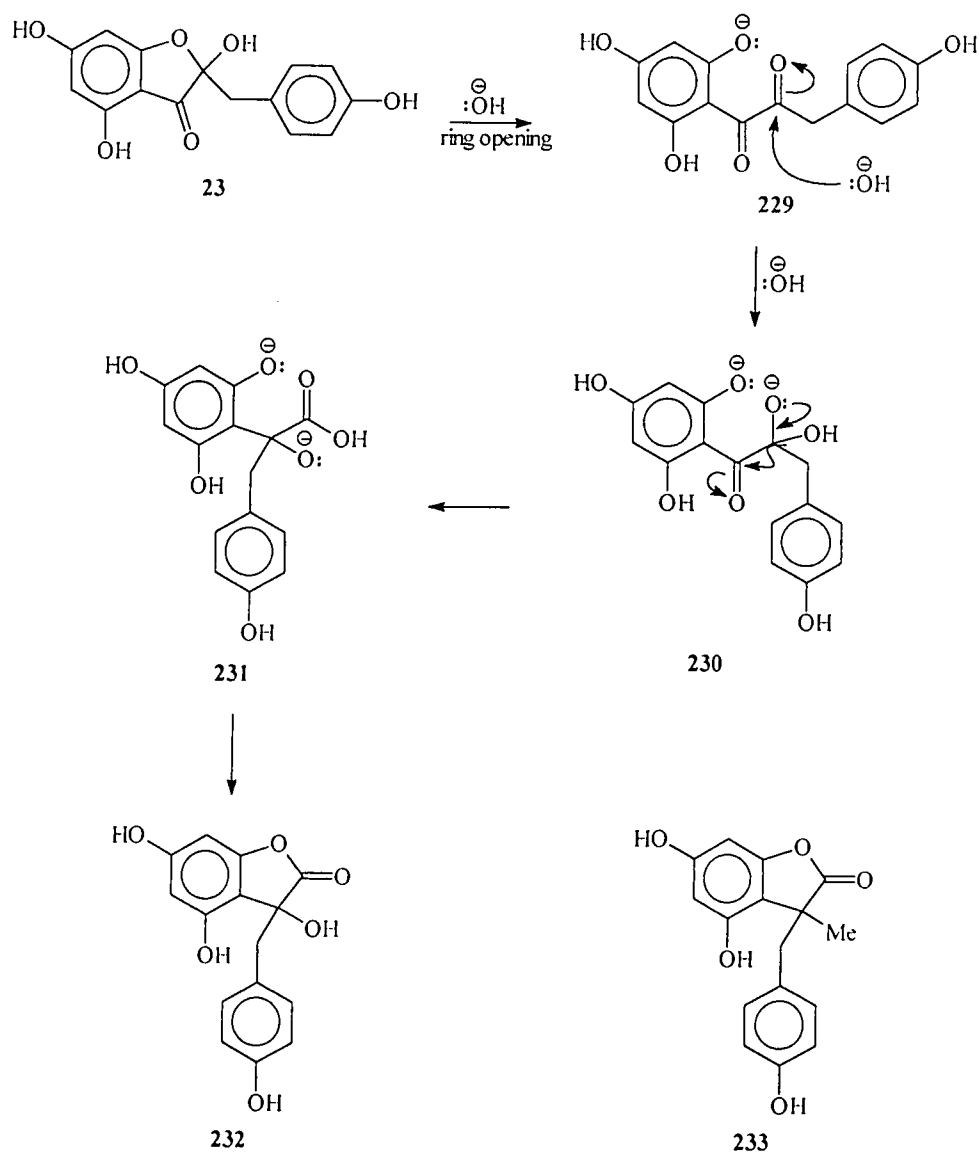
Scheme 7.1

CHAPTER 8

AN ASYMMETRIC SYNTHESIS OF MAESOPSIN

8.1 INTRODUCTION

CD data have become the single most important tool in the determination of the absolute stereochemistry of monomeric flavonoids.[68,201,202] However, since benzofuranoids are usually isolated as racemates (see § 2.2.1), nigrescin[23] (24) from *Acacia nigrescens* being the sole exception, no correlations between configuration and Cotton effect which permit the interpretation of the CD data of these compounds exist at present. The availability of an asymmetric synthesis of benzofuranoids would therefore facilitate a study of the Cotton effects exhibited by these compounds. Furthermore, such a synthesis could be utilized to gain access to the enantiomeric pure dimers discussed in Chapters 6 and 7. These considerations prompted attempts at the development of an asymmetric synthesis of tetra-*O*-methylmaesopsin (206).

Although the relevant benzofuranoids are readily available by means of α-cyclization of α-methoxy chalcones [e.g. (234)] (**Scheme 8.1**) under acidic conditions[189], this approach leaves little scope for the development of an asymmetric synthesis.

$$3N \text{ HCl or } 3N \text{ H}_2\text{SO}_4$$

234   206
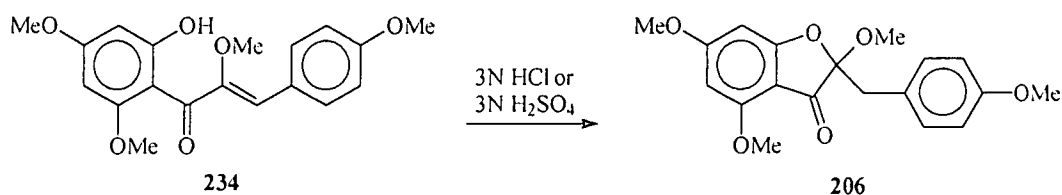
Scheme 8.1

In the light of recent developments in the preparation of optically active α-hydroxy carbonyl compounds[151,166], a study to evaluate the feasibility of the direct oxidation of 2-benzylbenzofuranones was proposed (**Scheme 8.2**). The latter compound (236) is readily obtainable from the corresponding aurone (235) by means of catalytic hydrogenation in moderate yield (50%). Oxidation of the silyl enol ether (237) of this compound with the

stereoselective catalyst AD-mix-α[166] afforded the desired compound (238) in low yield (20%) but with no optical activity. Attempts to optimize the reaction conditions were unsuccessful. Introduction of the oxygen by means of chiral oxaziridines[151] gave much-improved yields (up to 85%), but still without any stereoselectivity.
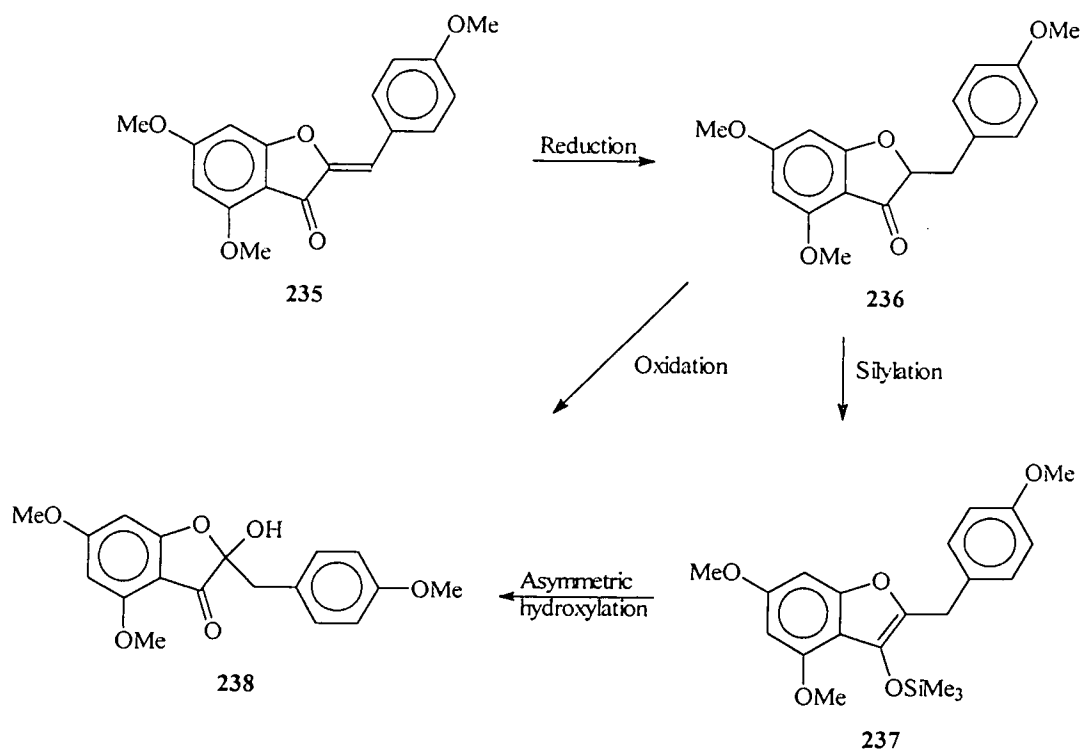
Scheme 8.2

This unexpected failure to introduce chirality prompted a revision of protocol and an attempt was made to introduce the oxygen before the benzyl fragment (**Scheme 8.3**). Thus, oxidation of the benzofuranone (239) with TTN in methanol[203,204] yielded the 2-methoxybenzofuranone (240) (65%) and subsequent benzylation (85%) of the enolate afforded maesopsin (206). Among the few useful methods towards asymmetric carbon addition to enolates are the procedures involving chiral bases such as chiral lithium amides[205,206]. However, since these bases are not commercially available, (-)-sparteine[207] was evaluated as chiral auxiliary, but several experiments utilizing various conditions showed that the addition of (-)-sparteine had no influence on the course of the benzylic

coupling reaction. At this time, the resolution of maesopsin (**206**) in its enantiomers (see §
7.2) was accomplished and consequently further attempts to develop an asymmetric route
towards maesopsin (**206**) were temporarily abandoned.
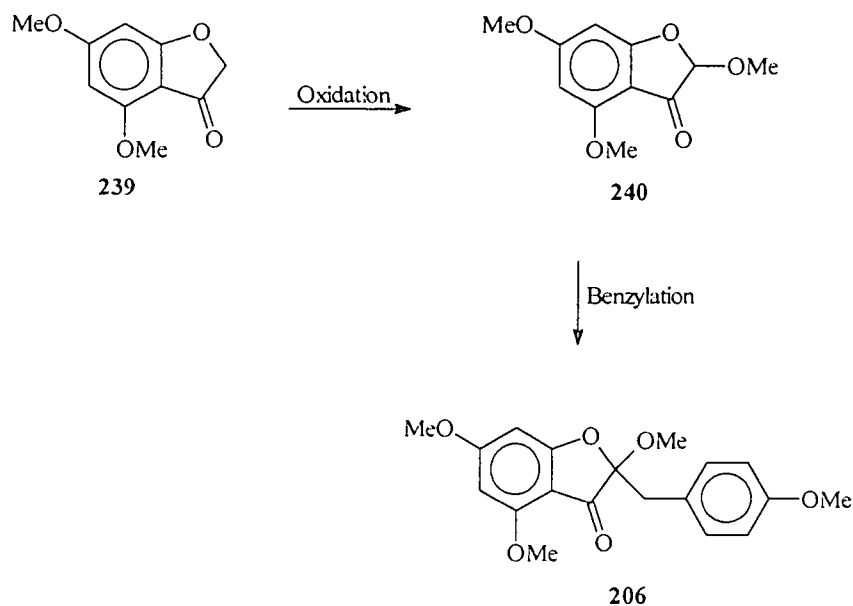
Scheme 8.3

8.2 THE SYNTHESIS OF BENZOFURANONE PRECURSORS

8.2.1 The synthesis of aurones

The most obvious route to 2-benzylbenzofuranones (*e.g.* **236**) involves benzylation of the
enolate of a benzofuranone (*e.g.* **239**). However, previous studies[208,209] in this group have
indicated that the predominant formation of the dialkylated product (**241**) (**Scheme 8.4**)
negates the usefulness of this route.
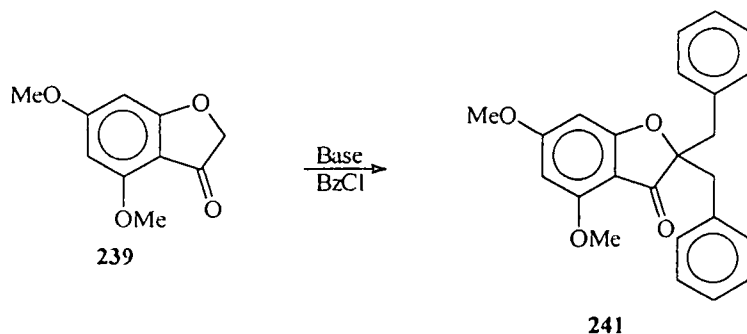
Scheme 8.4

Alternatively, the 2-benzylbenzofuranones may be accessed *via* catalytic hydrogenation of the appropriate aurone (**Scheme 8.5**). The Wheeler synthesis of aurones as modified by Donnelly *et al.*[119] (see § 3.2.1) seems to be the most effective method for the synthesis of aurones. Condensation of 2-hydroxy-4,6-dimethoxy-acetophenone (**242**) [**NMR-plate 26**] with 4-methoxybenzaldehyde (**243**) and subsequent acetylation produced the 2'-acetoxy-4',4,6'-trimethoxychalcone (**245**) [**NMR-plate 27**] in 72.9% yield. Acetylation of the crude mixture obtained from the condensation followed by recrystallisation facilitated purification of the chalcone (**245**). The acetate derivative was converted to the α-bromo-β-methoxy derivative[119] (**246**) (71.9%), a white amorphous solid, using NBS in methanol. The structure of this compound is confirmed by its ^{1}H NMR spectrum [**NMR-plate 28**]
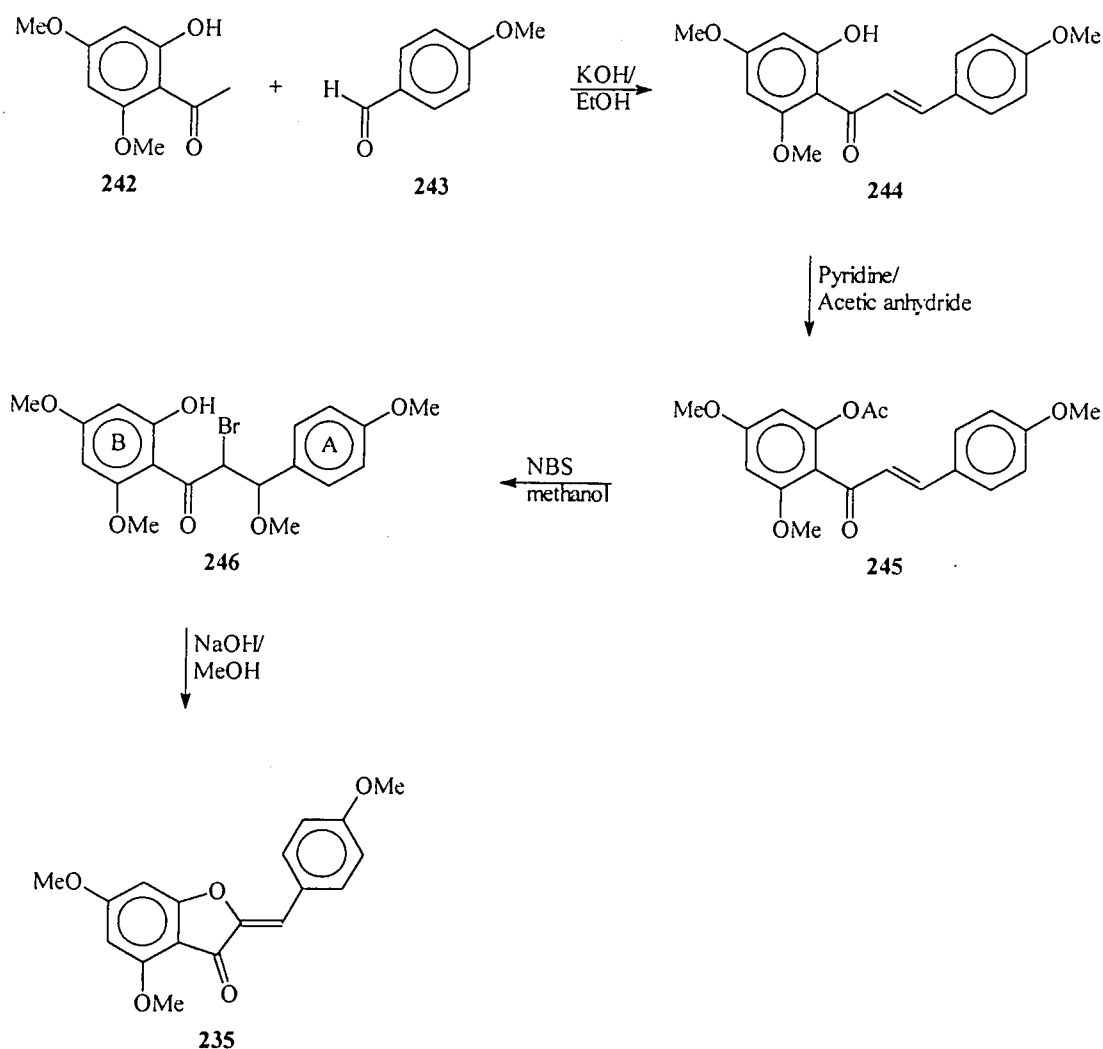
Scheme 8.5

which exhibits two AA'BB' doublets for the A-ring protons and two AB doublets for the two B-ring protons along with three aromatic methoxy signals and an acetoxy signal. Another methoxy signal at δ 3.19 and two additional doublets (J 10.0 Hz) confirm the presence of the β-bromo ether group. Correlations in a 2D COSY spectrum show coupling between β-C$\underline{H}$ (δ 4.67) and 2/6-H(A) (δ 7.34) as well as with the aliphatic methoxy group. Treatment of this derivative with base yielded the aurone (235) [NMR-plate 29] in good yield (75.1%) with an overall yield of 39.4%. The ^{1}H NMR spectrum of aurone (235) is identical to that of an authentic sample.[192]

Subsequent reduction of the 4,4',6-trimethoxyaurone (235) using Pd/C (5%) as catalyst yielded the desired product (236) [NMR-plate 30] along with the unexpected formation of 4,4',6-tri-O-methylmaesopsin (238) [NMR-plate 31] as by-product (Scheme 8.6).
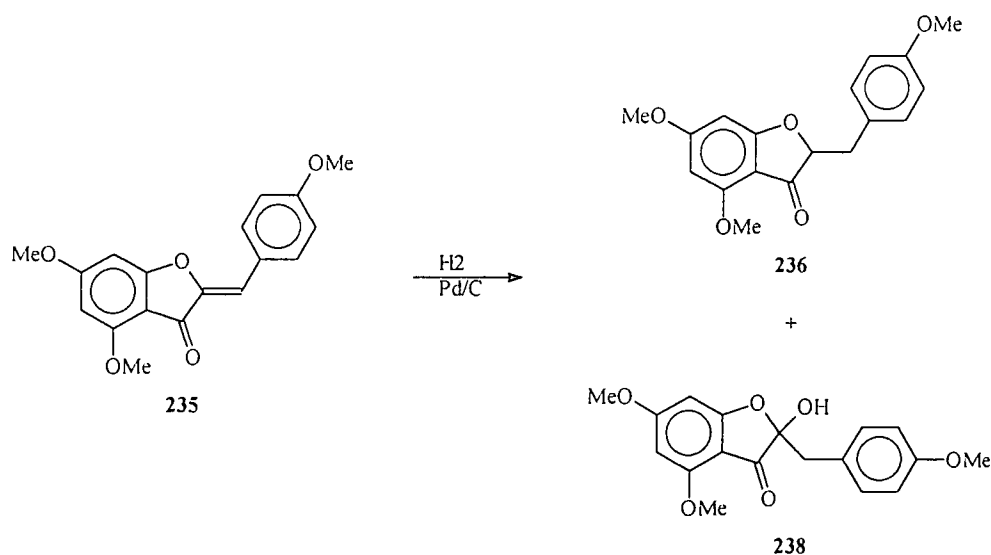
Scheme 8.6

The initial reaction in ethanol afforded product (236) in only 47% yield and (238) in 25% yield. Elimination of excess water from the solvents made no difference to the product distribution while the utilization of dry acetone as solvent similarly made little difference. However, it was found that the desired product is favored with an increase in the catalyst concentration with an optimum concentration of 20% (Table 8.1). The hydration of alkenes during catalytic hydrogenation is unknown and in the present case the possible

presence of trace amounts of $PdCl_2{}^{210}$ in the catalyst may explain the formation of 4,4',6-tri-*O*-methylmaesopsin (238). However, when the aurone (235) was stirred for 24 hours with the Pd/C catalyst in ethanol, no trace of compound (238) was observed.

Solvent	Amount of catalyst	% Products	
		(236)	4,4',6-tri-*O*-methylmaesopsin (238)
EtOH	10%	47.2	25.8
EtOH	15%	56.3	21.6
EtOH	20%	70.2	12.8
EtOH	25%	69.2	13.5
Acetone	10%	44.6	27.9
Acetone	20%	61.2	15.4

Table 8.1: Conditions used during the catalytic hydrogenation of compound (235).

The ^{1}H NMR spectrum [NMR-plate 30] of compound (236) exhibits the characteristic AA'BB'- and AB-aromatic spin systems and three methoxy signals while the two α-C$\underline{H}_2$-protons (δ 3.30 and δ 2.91) as well as 2-H(C) (δ 4.73) each resonate as doublet of doublets.

8.2.2   The synthesis of benzofuranones

A literature survey showed that the direct ω-halogenation of acetophenones to afford ω-haloacetophenones, which can subsequently be cyclized to the desired benzofuranone, are often troublesome (see § 3.1.1). This was confirmed by preliminarily attempts to brominate 2-hydroxy-4,6-dimethoxyacetophenone with cupric bromide[211,212] and bromine using dioxane as catalyst[213], which afforded only products exhibiting aromatic substitution. The tendency of cupric bromide to effect nuclear bromination rather than ω-bromination in the case of 2-hydroxy-4,6-dimethoxyacetophenone was also noted by Jemison[214].

In contrast to this method, the Houben-Hoesch reaction[92], utilizing phloroglucinol (**247**) and chloroacetonitrile as the two-carbon source, afforded 2,4,6-trihydroxy-ω-chloro-acetophenone (**248**) [NMR-plate 32] in high yield (84.2%) (**Scheme 8.7**). A one-pot reaction employing K_2CO_3 in dry acetone to effect cyclization and subsequent addition of dimethyl sulfate yielded 4,6-dimethoxybenzofuranone (**239**) [NMR-plate 33] (62.5%) in 52.6% overall yield.
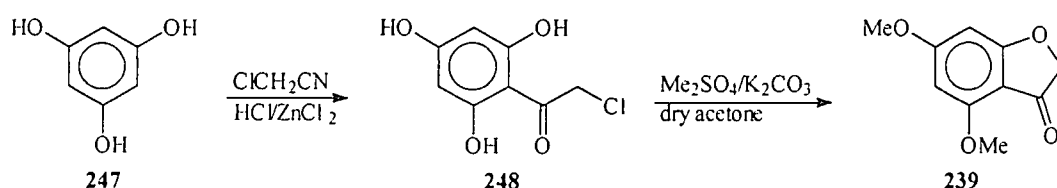
Scheme 8.7

8.2.3   The oxidation of benzofuranones

Since the synthesis of 2-methoxybenzofuranone (**240**) ideally called for a simple, effective procedure, the methods reported in Chapter 4 were not considered for this reaction. Instead, the first attempt involved the oxidation of the silyl enol ether of benzofuranone (**239**) with m-chloroperbenzoic acid (**Scheme 8.8**).[215]
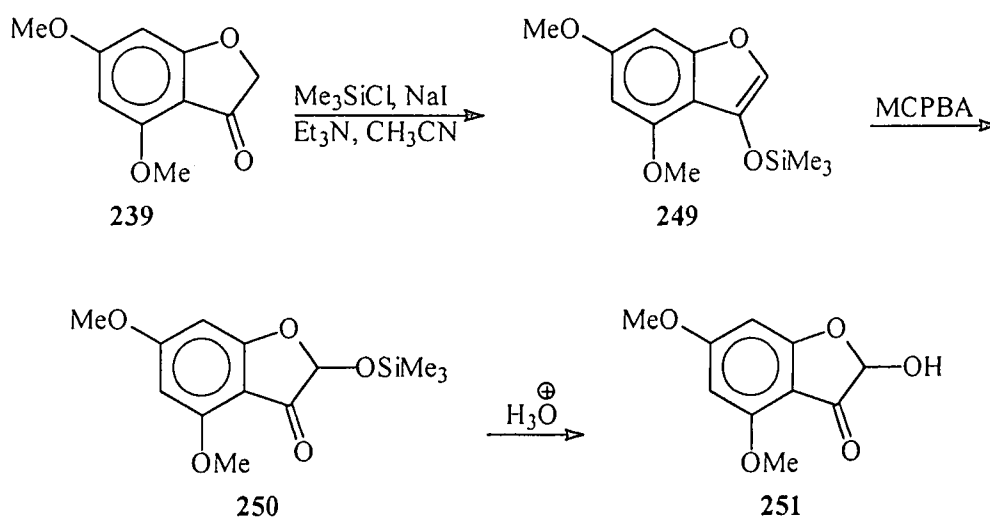
Scheme 8.8

Silylation of compound (**239**) with the method of Cazeau et al.[216] was unsuccessful with the t-butyldimethylsilyl group, probably due to the steric interaction between this bulky

group and the 4-methoxy group of the benzofuranone. However, the reaction with TMSCl proceeded in high yield (99%) to provide the silyl enol ether (**249**) [**NMR-plate 34**]. The ^{1}H NMR spectrum of this compound exhibits the expected aromatic doublets (J 2.0 Hz) and two methoxy signals along with the vinylic proton at δ 7.21 as well as a nine-proton singlet at δ 0.44. The silyl enol ether (**249**) was subsequently treated with *m*-chloroperbenzoic acid. Studies of this reaction indicated the formation of the α-trimethylsiloxy ketone (**250**) as intermediate, a compound that is converted to the α-hydroxy analogue under acidic conditions.[18] Despite reports of good yields, the reaction with compound (**249**) afforded a complex mixture of products with only traces of the desired compound (**251**) [**NMR-plate 35**].

McKillop and Hunt[217] showed that cyclohexanones could be converted to *o*-hydroxy-cyclohexanones (adipoins) in good yield (84%) using thallium (III) nitrate (TTN) in acetic acid. Application of this reaction to substrate (**239**) again gave a complex mixture of
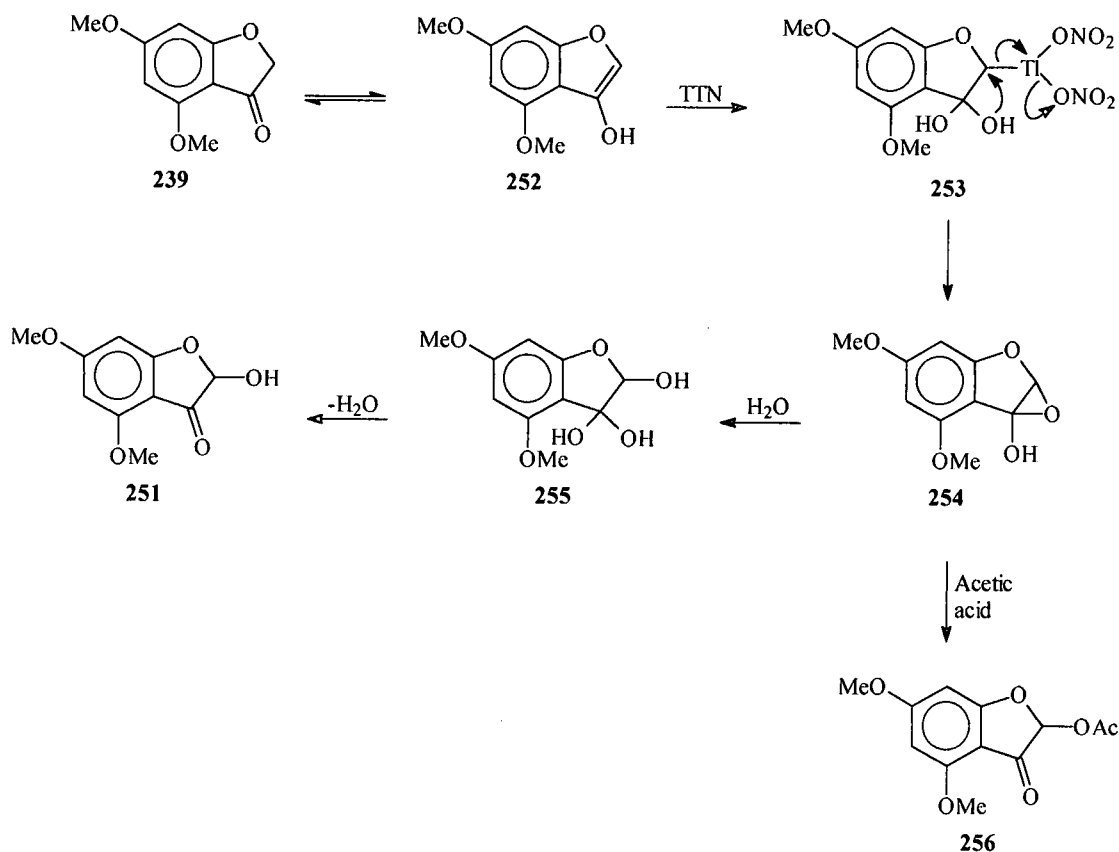
Scheme 8.9

products, two of which were identified as compound (251) and the 2-acetoxy-benzofuranone (256) [NMR-plate 36] (Scheme 8.9). Commonly, reactions with TTN involve participation of the solvent and/or migration of a suitable group in the substrate.[218] The proposed mechanism for the current transformation, therefore, must likely involves the formation of epoxide (254) followed by nucleophilic attack by water to afford the α-hydroxy carbonyl compound.[217] α-Acetylation is likewise a known reaction, but only if thallium (III) acetate is used.[219] In the present case, nucleophilic attack by acetic acid will furnish compound (256).

The above introduction of the solvent in one of the products prompted the idea to use acidic methanol as solvent. Thus, the benzofuranone (239) was treated with TTN in
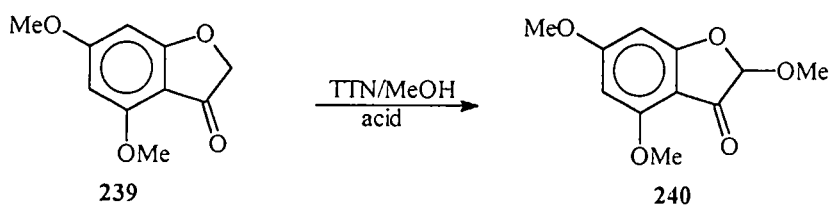
Scheme 8.10

Proton	(251)	(256)	(240)
2-H	5.56 (s)	6.24 (s)	5.26 (s)
5-H	6.02 (d, J 2.0 Hz)	6.06 (d, J 2.0 Hz)	6.01 (d, J 2.0 Hz)
7-H	6.15 (d, J 2.0 Hz)	6.16 (d, J 2.0 Hz)	6.13 (d, J 2.0 Hz)
2 x OMe	3.89/3.92 (both s)	3.89/3.94 (both s)	3.88/3.91 (both s)
2-OH	2.19 (s)	-	-
2-OAc	-	2.21 (s)	-
2-OMe	-	-	3.59 (s)

Table 8.2: ^{1}H NMR (δ_H) data of compounds (251), (256) and (240). Splitting patterns and J values (Hz) are given in parenthesis.

methanol containing *p*-toluenesulphonic acid to afford 2-methoxybenzofuranone (**240**) [**NMR-plate 37**] in 64.9% yield (**Scheme 8.10**). The mechanism of this reaction is presumably similar to that suggested for the oxidation of cyclohexanones described above. Considering that this reaction eliminates the need for a second derivatisation reaction, the moderate yield (64.9%) was acceptable. α-Methoxylation was also observed when 1-tetralone was treated with TTN absorbed on K-10, a montmorillonite clay, to afford 2-methoxy-1-tetralone in 50% yield.[204]

The ^{1}H NMR data for 2-hydroxy-4,6-dimethoxybenzofuranone (**251**), 2-acetoxy-4,6-dimethoxybenzofuranone (**256**) and 2,4,6-trimethoxybenzofuranone (**240**) is in each instance in accordance with the proposed structure and is summarized in **Table 8.2**.

8.3 THE α-HYDROXYLATION OF BENZOFURANONES

The extension of the asymmetric dihydroxylation (AD) process to tetrasubstituted olefins (see § 4.3) opened the way for the stereoselective oxidation of 2-benzylbenzofuranones. Therefore, 2-(4-methoxybenzyl)-4,6-dimethoxybenzofuranone (**236**) was converted into the silyl enol ether (**237**) [**NMR-plate 38**] in good yield (99%), again with the method of
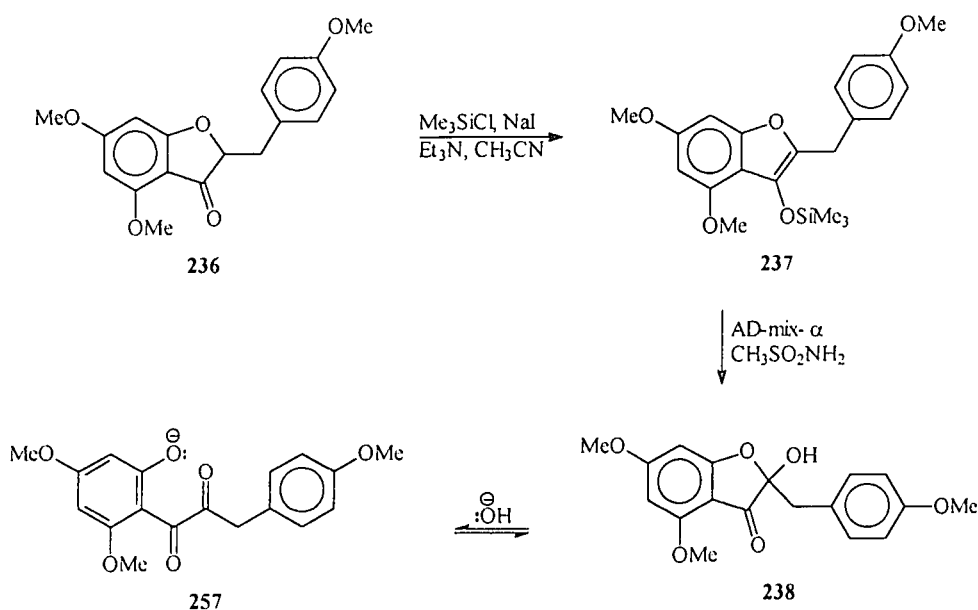
Scheme 8.11

Cazeau et al.[216] using TMSCl (**Scheme 8.11**). The conditions described by Morikawa et al.[178] for tetrasubstituted olefins were applied to the silyl enol ether with 1 molar equivalent of methanesulfonamide ($CH_3SO_2NH_2$) at 0°C. 4,4',6-Tri-O-methylmaesopsin (**238**) was isolated from this reaction in only 20.1% yield and analysis of the product with Eu(hfc)₃ as chiral shift reagent showed no enantiomeric enrichment.

Although this lack of selectivity may be contributed to the incompatibility of the substrate with the chiral catalyst, the high K_2CO_3 content[166] of the AD-mix may cause the formation of a substantial amount of the α-diketone (**257**) during the course of the reaction. This equilibrium will effectively destroy any chirality imparted by the catalyst. Attempts to minimize the reaction time and to complete the work-up procedure at 0°C or to neutralize the reaction mixture before work-up did not improve the enantiomeric constitution of the product.

While this unexpected complication seemed to indicate that the present route is inappropriate for the purpose of this synthesis, the oxidation of the 2-benzylbenzofuranone (**236**) with chiral oxaziridines was also evaluated. Thus, the enolate of compound (**236**), generated with lithium diisopropylamide (LDA) at -78°C, was treated with 2-(phenyl-sulfonyl)-3-(4-nitrophenyl)oxaziridine (**259**)[145] to afford 4,4',6-tri-O-methylmaesopsin (**238**) in 22.5% yield along with an unknown byproduct (**Scheme 8.12**).

The ¹H NMR spectrum of the latter contains all the signals associated with 2,4,4',6-tetra-O-methylmaesopsin (**206**) with exception of the 2-methoxy resonance. Two additional AA'BB' doublets along with five protons resonating as two two-proton multiplets and a one-proton multiplet in the aromatic region suggest the formation of compound (**261**) [**NMR-plate 39**], the adduct of sulfonimine (**260**) and the enolate of 2-benzylbenzofuranone (**236**).[145] Since studies showed that this side reaction is limited to certain conditions (see § 4.2), it seems that the enolate of 2-benzylbenzofuranone (**236**) is more susceptible to this reaction than other substrates. The use of sodium bis(trimethylsilyl)amide (NaHMDS) to generate the enolate made no difference to the product distribution.
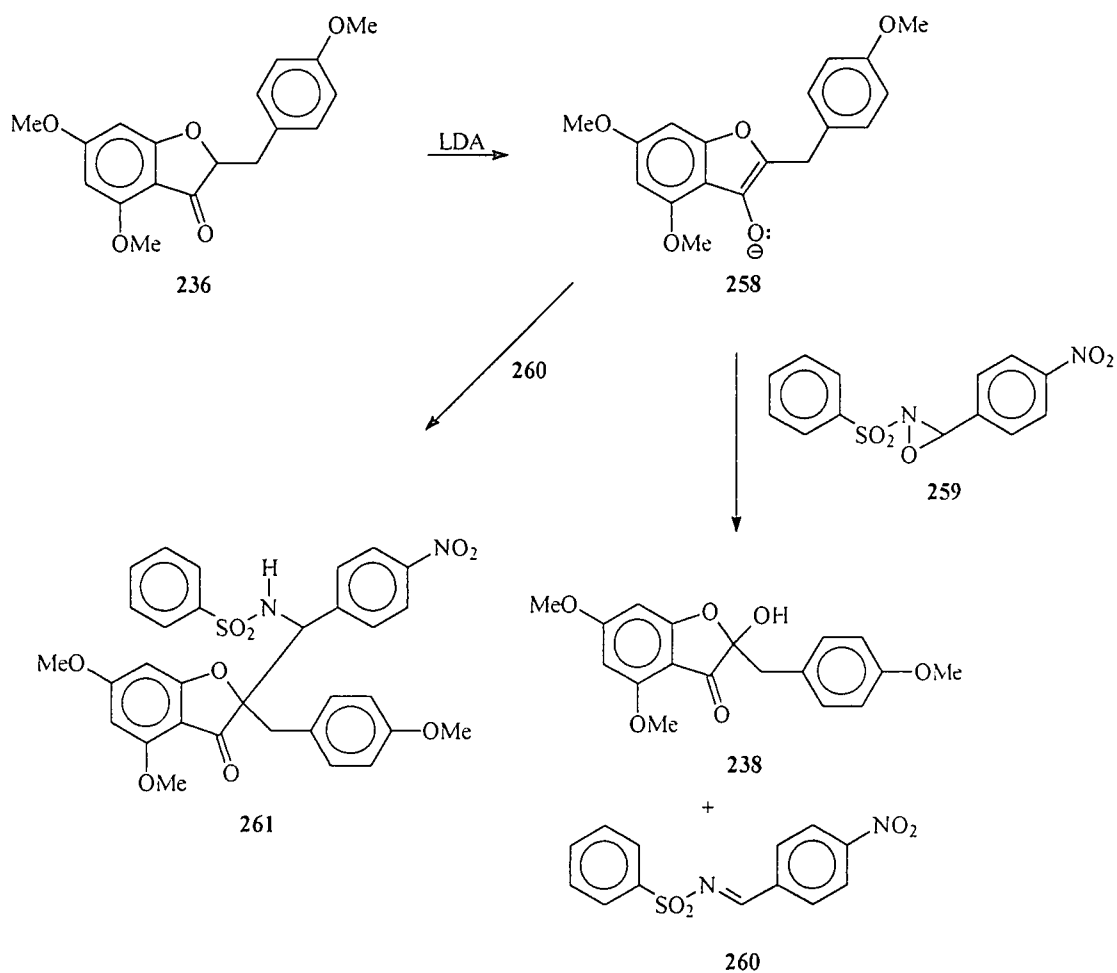
Scheme 8.12

Repetition of this reaction with NaHMDS as base and the chiral oxaziridine (152)[151] afforded 4,4',6-tri-*O*-methylmaesopsin (238) in good yield as the only product, but still without any stereoselectivity. Although the exact selectivity that can be expected from this reaction is unknown, the lack of enantiomeric excess in the product may also be attributed to the formation of the α-diketone (257) (Scheme 8.11).

152
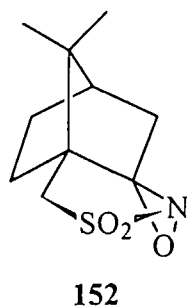
The initial reaction with 1.5 molar equivalents of oxaziridine (152), 1.2 equivalents base and a reaction time of 30 minutes afforded the product in only 65.3% yield. Attempts to prevent the formation of the α-diketone (257) included limiting the amount of NaHMDS used to generate the enolate to 0.95 equivalents while using only 1.2 equivalents of the oxaziridine and limiting the reaction time to as little as 10 minutes. Under these conditions, the yield increased to 80.4% with no influence on the selectivity of the reaction. Neutralization of the reaction mixture at -78°C after ten minutes gave the same result. In an attempt to trap the product in a stable form by methylation, methyl iodide was added at -78°C after completion of the reaction and the reaction mixture was slowly warmed to 0°C. However, no methylation occurred under these conditions. The addition of acetic anhydride under similar conditions did yield some of the acetylated product (262) [NMR-plate 40] (28.5%) that was identical to an authentic sample obtained by acetylation of 4,4',6-tri-O-methylmaesopsin. ¹H NMR experiments with a chiral shift reagent revealed that this derivative was still racemic and this route was subsequently abandoned.

262
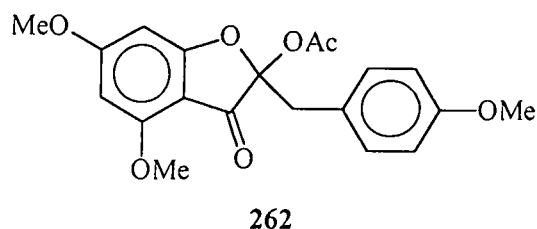
8.4   THE α-ALKYLATION OF BENZOFURANONES

The successful synthesis of 2,4,6-trimethoxybenzofuranone (240) (see § 8.2.3) opened an alternative route to maesopsin. Since the 2-hydroxy group of the product will be protected, any chirality introduced during the course of the reaction should stay intact. In order to explore the conditions required for carbon-carbon bond formation, the enolate of benzofuranone (240), generated with sodium hydride, was trapped with methyl iodide (Scheme 8.13). Initial experiments showed that compound (240) is highly susceptible to self-condensation and compound (264) [NMR-plate 42] (30.2%) was isolated along with the methylated benzofuranone (263) [NMR-plate 41] (20.6%). Prevention of self-

condensation was achieved by slowly adding a solution of the benzofuranone to a stirred suspension of sodium hydride (1.5 equiv.) in THF. Under these conditions, compound (263) (65.2%) was isolated as sole product together with unreacted starting material.

240   263   264 R = R^1 = R^2 = OMe
                          265 R = OMe, R^1 = H, R^2 = OH
                          266 R = R^1 = H. R^2 = OH
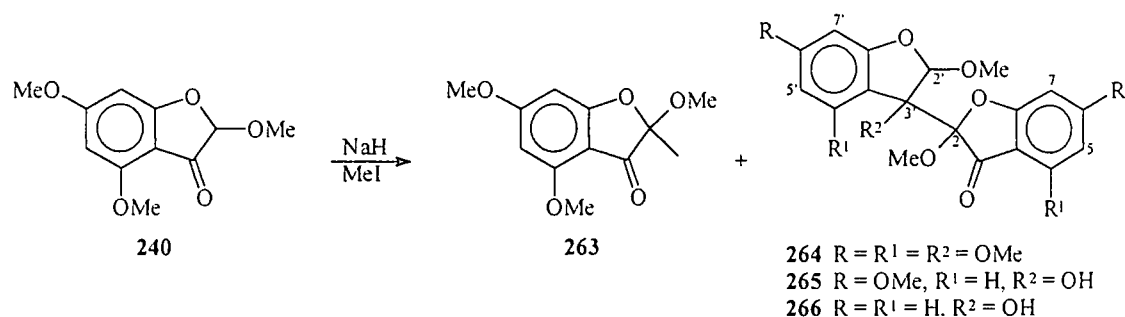
Scheme 8.13

The ^{1}H NMR spectrum of compound (264) exhibits seven methoxy resonances, two pairs of AB doublets and a one-proton singlet in the aromatic region. Since only six methoxy groups are introduced by the substrate, the seventh methoxy group presumably originates from methylation of the 3'-hydroxy group. Correlations in a 2D NOESY experiment indicated the relationships between the respective aromatic resonances but were unable to distinguish between the two ring systems. However, correlation between the carbonyl resonance and 5-H (δ 5.97) ($^4J_{CH}$) in an HMBC experiment identified the lower unit of the

Proton	(264)	Proton	(264)
5-H	5.97 (d, J 2.0 Hz)	5'-H	5.98 (d, J 2.0 Hz)
7-H	6.03 (d, J 2.0 Hz)	7'-H	6.10 (d, J 2.0 Hz)
4-OMe	3.91 (s)	4'-OMe	3.79 (s)
6-OMe	3.86 (s)	6'-OMe	3.76 (s)
		2'-H	6.05 (s)
2-Ome	3.28 (s)	2'-OMe	3.66 (s)
		3'-OMe	3.27 (s)

Table 8.3: ^{1}H NMR (δ_H) data of compound (264). Splitting patterns and J values (Hz) are given in parenthesis.

dimer. Association of 5-H with two methoxy resonances, 4-OMe (δ 3.91) and 6-OMe (δ 3.86) and of 7-H (δ 6.03) with 6-OMe in the NOESY spectrum defines the remainder of this system. The upper unit is likewise characterized by the correlation ($^4J_{CH}$) of 3'-C with 5'-H (δ 5.98) in the HMBC spectrum and similar correlations to those described above in the NOESY spectrum. 2'-OMe (δ 3.66) was identified by its correlation with 2'-C in the HMBC spectrum and its correlation with 2'-H in the NOESY spectrum. Differentiation between 2-OMe (δ 3.28) and 3'-OMe (δ 3.27) depends on the correlation between the former methoxy resonance and 2'-H and the absence of any correlations with the latter in the NOESY spectrum.

Notably, only one diastereomer out of a possible four was obtained for the dimeric compound (264). The self-condensation of 2-methoxybenzofuranones was also observed by Burke and O'Sullivan[220] during an aldol condensation of 2,6-dimethoxybenzofuranone using bromomagnesium diisopropylamide (BMDA) as base. As in the present case, only one diastereomer of compound (265) was formed during the reaction. Earlier, Antus *et al.*[221] had synthesized the analogue (266) by treating 2-methoxy-2-(4-methoxybenzoyl)-3(2H)-one with 0.1M sodium methoxide to afford two diastereomers of (266) *via* a *retro*-Claisen condensation/aldol reaction sequence. Although tentative stereochemical assignments were made for both the above cases, the stereochemical relationships with dimer (264) are unclear and no attempt to elucidate the stereochemistry was made.
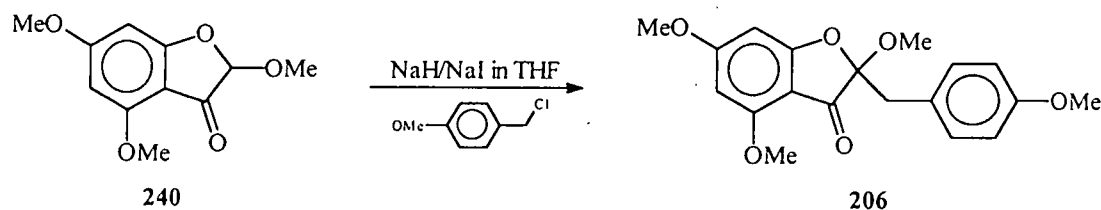
Scheme 8.14

Following the successful introduction of the methyl group, the reaction was repeated with 4-methoxybenzyl chloride but without any success. This was thought to be a consequence of the low reactivity of the benzyl chloride and attempts to activate the electrophile by the addition of sodium iodide confirmed this. Under these conditions, 2,4,4',6-tetra-*O*-metylmaesopsin (206) was formed in good yield (77.5%) (**Scheme 8.14**). Similar results

were obtained when LDA was used as base, again only after activation of the electrophile.

Asymmetric substitution in which a racemic organolithium intermediate, complexed to a chiral ligand, reacts with high enantioselectivity in carbon-carbon bond formation, is a very appealing approach for efficient asymmetric synthesis. Thus, chiral lithium amides, e.g. compound (267), have found application in several enantioselective reactions such as enantioselective deprotonation of prochiral cyclohexanones[222] and *meso*-type cycloalkanones[223] as well as kinetic reaction of racemic 2-substituted cyclohexanones[224]. Furthermore, enantioselective aldol reactions[225], enantioselective alkylation at the α-position of cyclic ketones[226] and enantioselective protonations[227] are also possible with these reagents.

267   268
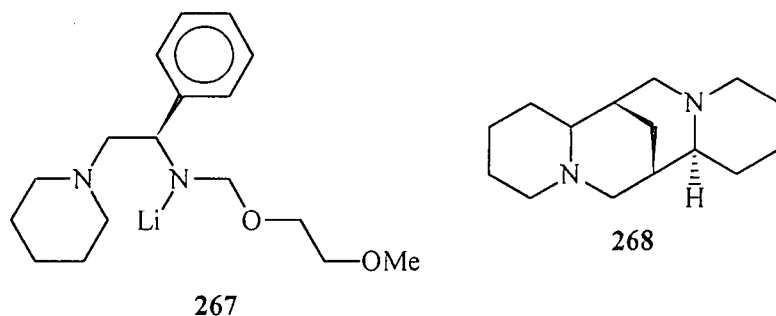
It has also been shown that an efficient methodology for such asymmetric synthesis is provided by enantioselective lithiation-substitution sequences mediated by (-)-sparteine (268)[207]. Asymmetry in these sequences can be introduced by energy differences in either the formation or reaction of the diastereomeric lithiated intermediates,[228] *i.e.*, two different pathways can be observed for these reactions. The first, described as asymmetric deprotonation, involves the formation of an enantio-enriched organolithium intermediate that would react faster than it would racemize. During the second pathway, asymmetric substitution, deprotonation of the substrate yields a racemic organolithium intermediate which would then react enantioselectively under the influence of (-)-sparteine (268).

These pathways are illustrated in **Scheme 8.15** for the cyclization of (arylmethyl)(3-chloropropyl)-Boc-amine (269) to Boc-(S)-2-phenylpyrrolidine (272). Studies showed that the former pathway is dominant in this case.[228] Enantioselectivity in the this sequence is a function of the nucleofuge, ligand, solvents as well as the method and the temperature

used for formation of the organolithium intermediate.[229] It has been found that the ee of some reactions are greatly increased if a mixture of the organolithium intermediate and (-)-sparteine is stirred at -25°C prior to cooling (-78°C) and addition of the electrophile. This requirement is justified by the postulate that the diastereomeric organolithium/(-)-sparteine complexes are equilibrating at -25°C but non- equilibrating at -78°C.[229]
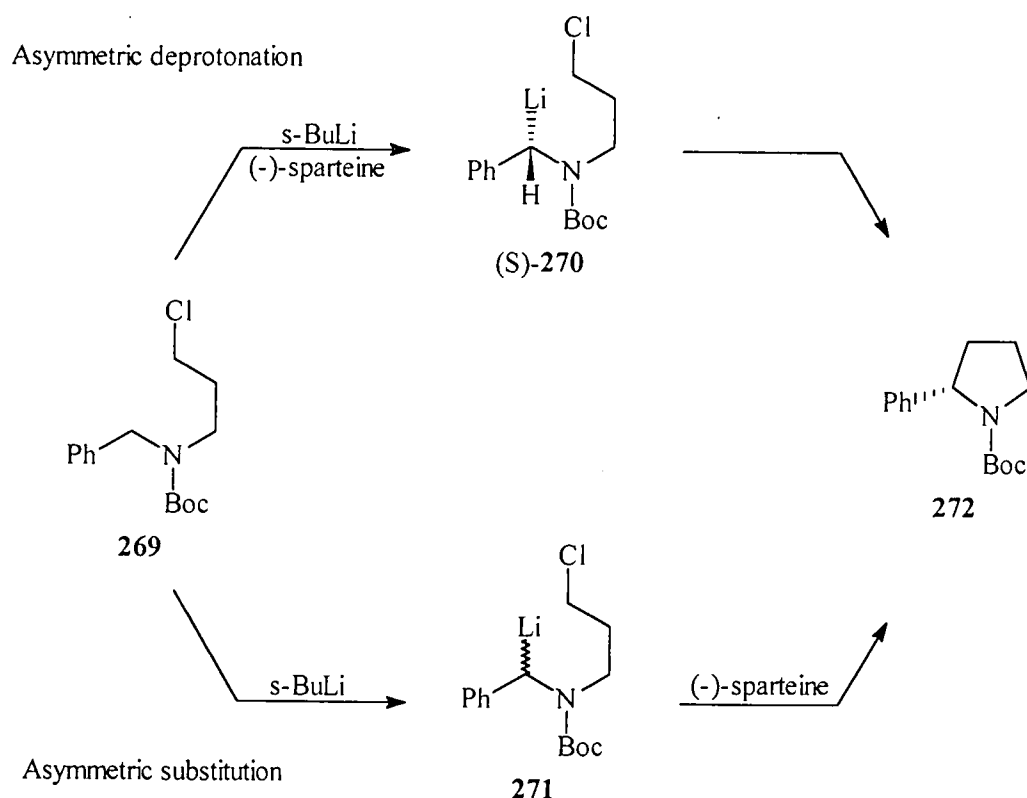
Scheme 8.15

The utility of this method is further illustrated by the alkylations of racemic *N,N*-diisopropyl-*o*-(1'-lithioethyl)-benzamide **(274)**, prepared from *N,N*-diisopropyl-*o*-ethylbenzamide **(273)** in the presence of (-)-sparteine, which occur with high and opposite enantioselectivities with different leaving groups (see **Scheme 8.16**). Studies showed that the pathway of this reaction can be designated as asymmetric substitution, but no explanation for the opposite selectivities obtained for the alkyl tosylates were offered.[230]

In light of these results and the commercial availability of (-)-sparteine, the above method was considered for the benzylic coupling of benzofuranones. In order to investigate the
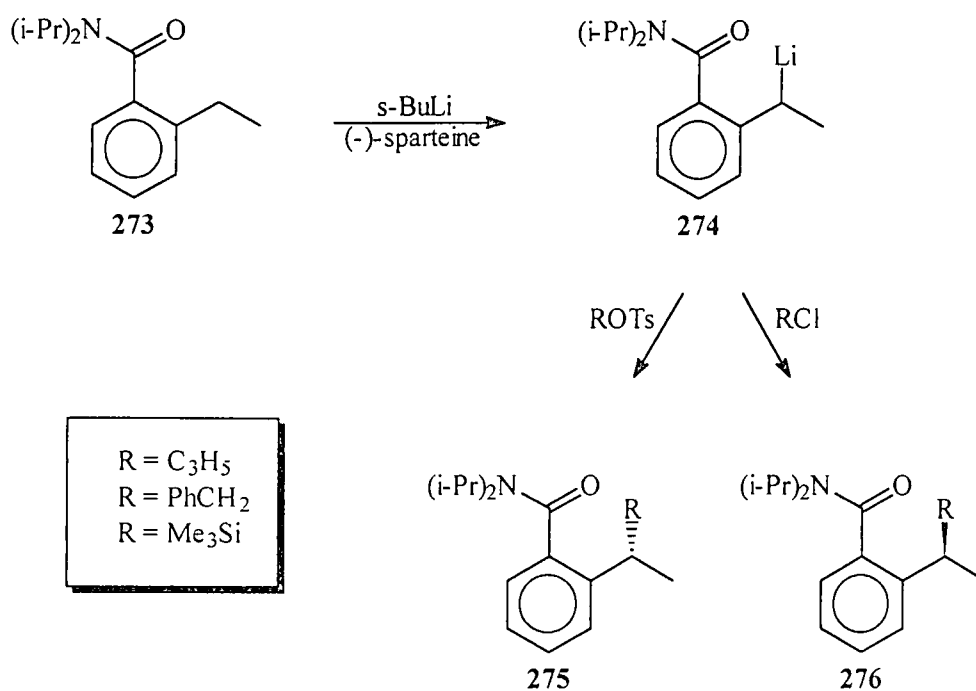
Scheme 8.16

possibility of carbon-carbon bond formation when *s*-BuLi is used as base, benzofuranone (**240**) was treated with this reagent at -78°C for one hour after which methyl iodide was
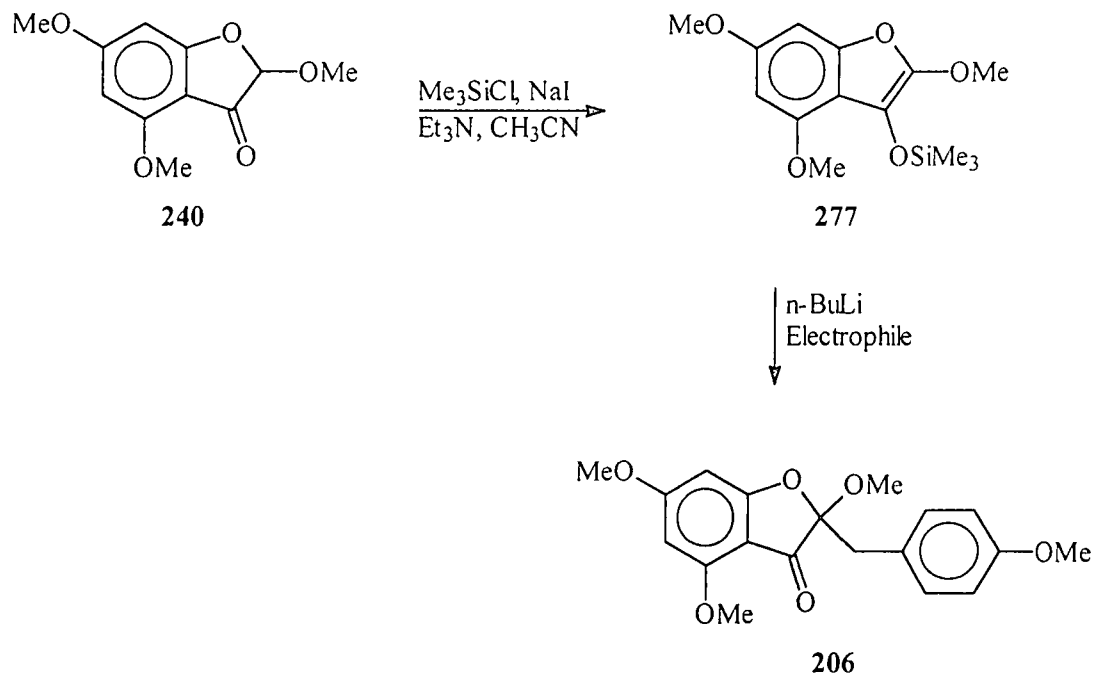
Scheme 8.17

Run	Electrophile	Conditions prior to addn of electrophile	Conditions after addn of electrophile	Solvent	Yield (%)
1	4-OMePhCH₂Cl	-78°C; 30 min	-78°C; 24h	Ether	4.1
2	4-OMePhCH₂Cl	-78°C; 30 min	-78°C; 24h	THF	4.8
3	4-OMePhCH₂Cl	-78°C; 30 min	-78°C; 24h	DCM	-
4	4-OMePhCH₂I	-78°C; 30 min	-78°C; 8h	THF	59.2
5	4-OMePhCH₂I	-25°C; 30 min then -78°C; 45 min	-78°C; 8h	THF	58.1

Table 8.4: Reaction conditions for the alkylation of silyl enol ether (**277**).

added. Only starting material was isolated from this reaction even after the reaction temperature was allowed to rise to 0°C over a period of 3 hours during a subsequent experiment. Although this indicated that the enolate of (**240**) probably did not form, the substrate was nevertheless treated with *s*-BuLi in the presence of (-)-sparteine for 30 minutes before addition of 4-methoxybenzyl chloride. Again, no reaction was observed.

The possibility of competition for the lithium group between (-)-sparteine and the diisopropylamide group of LDA excluded the use of LDA as base. In several communications, Koga *et al.*[226] reported the utility of silyl enol ethers in reactions with alkyllithiums in the presence of chiral amines, for example compound (**267**). Although the reaction conditions were unproven for (-)-sparteine, the silyl enol ether (**277**) [**NMR-plate 43**] of 2,4,6-trimethoxybenzofuranone (**240**) was treated with *n*-BuLi and (-)-sparteine in several solvents (see **Scheme 8.17** and **Table 8.4**). Again, 4-methoxybenzyl chloride proved to be inadequate as electrophile and the yield of maesopsin (**206**) again increased greatly when the electrophile was first treated with sodium iodide.

The 2,4,4',6-tetra-*O*-methylmaesopsin (**206**) isolated from these reactions showed no trace of enantiomeric enrichment. The lack of selectivity can possibly be attributed to the lack of a suitable coordination site for the lithium group that prevents the formation of a diastereomeric transition state that is capable of reacting with the electrophile.

Experimental

CHAPTER 9

STANDARD EXPERIMENTAL TECHNIQUES

Unless specified otherwise the following techniques were applied throughout the course of this study.

9.1 CHROMATOGRAPHY

9.1.1 Paper chromatography

Two dimensional paper chromatographs on Whatman no. 1 paper (28.5 x 46 cm) was developed in two directions, namely with water saturated butan-2-ol in the first and a 2% (v/v) acetic acid solution in the second direction. After the chromatograms were dried in a fast air current, they were investigated by UV-light and spraying reagents.

9.1.2 Thin layer chromatography

Qualitative thin layer chromatography (TLC) was conducted on Merck TLC-plastic sheets: Silica Gel F_{254} (0.2 mm layer) divided into strips of *ca.* 3 x 5 cm. R_f values reported are those observed in these qualitative TLC assessments.

Preparative scale thin layer chromatography (PLC) was conducted on glass plates (20 x 20 cm) coated with a layer (1.0 mm) of unactivated Merck Kieselgel 60 PF_{254} and dried overnight at room temperature. After development in the appropriate eluent the plates were dried in a fast stream of air and the bands identified by either UV-light (254 nm) or by the appropriate spraying reagent. The bands were eluted with acetone and the acetone removed under reduced pressure on a water bath at *ca.* 30°C. The plates were charged with 10 - 15 mg of crude product. Small-scale separations were conducted on Merck Pre-coated (0.25 mm) TLC Plates Silica Gel 60 F_{254} with each plate charged with 3-5 mg of the crude product.

9.1.3   Column chromatography

Separations on **Sephadex** LH-20 were performed on various column sizes and differing flow rates as specified. The Sephadex LH-20 was prepared by soaking it in the eluent (ethanol or ethanol/water mixtures) for 24 hours. The glass column was charged with this suspension of Sephadex LH-20 and the crude extract, dissolved in a minimum of the eluent, was applied to the column.   Columns were developed at a flow rate of approximately 0.5 ml/min. and fractions of 15 ml were collected with an ISCO (model 273) automatic fraction collector.

Flash column chromatography (FCC) was performed on a glass column (5 cm diameter) charged with 100 g of Merck Kieselgel 60 (230-400 mesh) for column chromatography for every 1 g of crude product. Air was disposed by elution with the appropriate solvent under N_2-pressure (*ca.* 50 kPa). The crude product was dissolved in a minimum amount of the appropriate solvent and carefully applied to the column. The purified product was recovered by elution under N_2-pressure with the appropriate solvent system and collected in 15 ml fractions.

High performance liquid chromatography (HPLC) was performed on a Waters Liquid Chromatograph equipped with a Waters 600 Controller and a Waters 486 Tunable Absorbance Detector. A Chiralcel OD (4.6 x 250 mm, 10 μ) stainless steel column (Daicel [Europa] GmbH, Düsseldorf, Germany) was used at ambient temperature and fractions were collected by hand.

9.2   SPRAY REAGENTS

9.2.1   Formaldehyde-sulfuric acid

Thin layer chromatograms were gently sprayed with a 2% v/v solution of formaldehyde (37 wt. % solution in water) and concentrated sulfuric acid and gently heated to *ca.* 120°C to effect maximum development of colour.

9.2.2   Bis-diazotated benzidine

Benzidine (5g) was dissolved in 14 ml of concentrated hydrochloric acid and added to distilled water (980 ml). Paper chromatograms were gently sprayed with a freshly prepared mixture of this solution (30 ml) and a 10% (m/v) sodium nitrite solution (20 ml). The papers were subsequently washed for 1 hour under flowing tap water. (The lifetime of the solutions in the dark is approximately 3 weeks.)

9.3   SPECTROSCOPICAL METHODS

9.3.1   Nuclear magnetic resonance spectroscopy (NMR)

NMR spectra were recorded on an AVANCE DPX$_{300}$ Bruker spectrometer with tetramethylsilane as internal standard. Deuteriochloroform (CDCl$_3$) was used as solvent at 298 K unless specified otherwise. Chemical shifts are reported in parts per million (ppm) on the δ-scale and coupling constants were measured in Hz. Abbreviations used are as follows:

Abbreviation	Signal multiplicity	Abbreviation	Signal multiplicity
s	singlet	d	doublet
t	triplet	dd	doublet of doublets
m	multiplet	br.	broadened

Table 9.1: Abbreviations used in describing ^{1}H NMR signal multiplicities.

9.3.2   Circular dichroism (CD)

CD spectra were recorded on a Jasco J-710 spectropolarimeter in spectrophotometric grade 1,2-dichloroethane (~ 1 mg/10 ml DCE). The formula used to calculate the molecular ellipticity [θ] was:

$$[\theta] = \frac{(L)(scale)(molecular\ weight)(100)}{[length\ of\ tube\ (cm)][concentration\ (g/l)]}$$

where L is the difference (at any given wavelength) between the reading (in cm) of the compound in solution and the reading (in cm) of pure solvent.

9.3.3 Fast atom bombardment (FAB) mass spectrometry

All FAB mass spectra were recorded on a VG70-70E double-focusing mass spectrometer using a VG-250J data system and iontech saddlefield FAB gun. M^+ denotes the molecular ion.

9.3.4 Enantiomeric excess (ee)

The ee determinations were done in deuteriochloroform ($CDCl_3$) utilizing tris[3-heptafluoropropylhydroxy-methylene-d-camphorato] europium (III) [$Eu(hfc)_3$] as chiral shift reagent.

9.4 ANHYDROUS SOLVENTS AND REAGENTS

Acetone was left over dry K_2CO_3 (oven-dried, 24 hours, 200°C) for 24 hours. The K_2CO_3 was filtered off and the solvent distilled over 3Å molecular sieves and stored under N_2.

Benzene, diethyl ether, THF, triethylamine and diisopropylamine were refluxed over sodium/benzophenone under N_2 until a dark blue colour persisted with subsequent fresh distillation under N_2 prior to use.

Acetonitrile, dichloromethane and DMF were refluxed over CaH_2 under N_2 for 12 hours with subsequent fresh distillation under N_2 before use.

9.5 CHEMICAL METHODS

9.5.1 Methylation with dimethylsulfate[231]

Phenolic material was dissolved in dry acetone, dry K_2CO_3 (8 equiv.) was added and dimethylsulfate (3 - 10 equiv.) was subsequently added drop-wise over a period of 30 minutes under N_2. The reaction mixture was refluxed for 8 hours after which the mixture

was filtrated and the acetone removed under reduced pressure. The excess dimethylsulfate was destroyed by addition of dilute ammonia and extraction with ethyl acetate afforded the methylated product(s).

9.5.2 Reduction with sodium cyanoborohydride[179]

The substrate was dissolved in trifluoracetic acid (1.5 ml) under N_2 at 0°C. $Na(CN)BH_3$ (12 equiv.) was added in portions over a period of 30 minutes. The reaction was followed at 0°C by means of TLC until completion after about 2 hours. The reaction mixture was added to water and neutralized with 2% $NaHCO_3$. Subsequent extraction with ethyl acetate and purification *via* PLC afforded the product.

9.6 FREEZE-DRYING

Phenolic material in aqueous solution was freeze-dried using a Virtis Freezemobile 12 SL (40 millitorr).

9.7 MOLECULAR MODELLING

A SUN SPARCstation 10 utilizing GMMX 1.0, a global search routine based on the MMX-force field of PC-MODEL 3.0 (both from Serena Software, Bloomington, USA) was employed to explore conformational space. Input was prepared with PC-MODEL's graphical user interface treating aromatic carbons as type 40 and the search run *via* the statistical option, alternating between internal (bonds) and external (cartesian) coordinates. The hydrogen bond function was activated and standard dielectric constants ($\varepsilon = 1.5$) were used. Searches were allowed to run until standard criteria were reached and the Boltzmann populations were calculated at 25°C for the final ensemble of conformers with an energy distribution within 3.0 kcal/mol. of the minimum energy.

Semi-empirical calculations were performed by means of MOPAC 93 2.0 (JJP Stewart, Fujitsu Limited, Tokyo, Japan, 1993). AM1- and PM3-procedures were used with gradient minimization to a gnorm = 0.001.

9.8   MELTING POINTS

Melting points were determined with a Reichert Thermopan microscope with a Koffler hot-stage and are uncorrected.

9.9   ABBREVIATIONS

The following abbreviations for solvents are used throughout the experimental section:

A	=	acetone
B	=	benzene
C	=	chloroform
DCM	=	dichloromethane
DMF	=	N,N-dimethylformamide
E	=	diethyl ether
EA	=	ethyl acetate
EtOH	=	ethanol
H	=	hexane
M	=	methanol
MEK	=	methylethylketone
T	=	toluene
THF	=	tetrahydrofuran

CHAPTER 10

ISOLATION OF COMPOUND FROM THE HEARTWOOD OF *BERCHEMIA ZEYHERI*

10.1 ENRICHMENT OF THE HEARTWOOD EXTRACT OF *B. ZEYHERI*

Drillings (3.49 kg) from the heartwood if *B. zeyheri* sond. were extracted with hexane (2 x 2l; 2 x 24 h) at room temperature in order to remove fats and waxes. Drillings were then air-dried and extracted with Me_2CO-H_2O (4:1,5, 5 x 15.0 *l*, 48 hours) at 22°C. Extracts were combined, the Me_2CO evaporated at 40°C and the aqueous solution freeze-dried to give a red amorphous powder (377 g, 10.8% of mass drillings).

This (6 x 40 g) was partitioned in *sec.*-BuOH–H_2O–hexane (5:3,5:1.5, v/v/v) in a twenty tube, 100 ml under-phase, Craig countercurrent assembly. Following qualitative TLC (B:A:M, 7:2:1, v/v/v) and 2D paper chromatography analysis, the fractions were combined to afford eight fractions (see **Table 10.1**).

Fraction	Tubes	Mass (g)
A1	1 - 2	13.4
A2	3 - 4	4.5
A3	5 - 7	5.0
A4	8 - 10	7.1
A5	11 - 12	10.9
A6	13 - 16	42.5
A7	17 - 19	38.6
A8	20	12.9

Table 10.1: The eight fractions from the twenty tube Craig countercurrent distribution.

Fractions A7 (38.6 g) and A8 (12.9 g) from the 20-tube Craig assembly were combined and subjected to countercurrent distribution on a Quickfit Model 20 machine [25 cm^3 top and bottom phases; H_2O–*sec.*-BuOH–hexane (5:4:1, v/v/v)]. Following 103 transfers of top phase and qualitative TLC (B:A:M, 7:2:1, v/v/v) and 2D paper chromatography, the fractions were combined to afford nine fractions (see **Table 10.2**).

Fraction	Tube	Mass (g)
B1	1 - 11	0.05
B2	12 - 24	0.08
B3	25 - 37	0.12
B4	38 - 47	0.16
B5	48 - 57	1.32
B6	58 - 70	14.5
B7	71 - 83	6.0
B8	84 - 93	5.4
B9	94 - 103	14.5

Table 10.2: The nine fractions from the 103 tube Craig countercurrent distribution on the Quickfit Model 20 machine.

10.2 ISOLATION OF COMPOUNDS

Fraction A1 (13.4 g) was subjected to column chromatography on Sephadex LH-20 (4 x 150 cm column, flow rate 30 ml/hr) in ethanol to give 10 fractions after analysis with TLC (B:A:M, 7:2:1, v/v/v) (see **Table 10.3**).

Fraction	Test tubes	Mass (g)
C1	1 - 9	0.1601
C2	10 - 22	0.1737

Table 10.3: Fractions obtained from the column separation of fraction A1.

Fraction	Test tubes	Mass (g)
C3	23 - 44	0.7004
C4	45 - 53	0.3185
C5	54 - 79	0.3510
C6	80 - 104	0.5011
C7	105 - 123	0.2901
C8	124 - 132	0.4979
C9	133 - 149	0.2712
C10	150 - 179	0.1923

Table 10.3 (continued)

TLC analysis of fractions C1, C2 and C7 - C10 showed that these fractions were still comprised of complex mixtures and were not investigated further. The results of the investigation of fraction C3 have previously been described in reference 196.

Methylation and FCC [C:A (95:5, v/v)] of fraction C4 (300 mg) yielded four fractions (see **Table 10.4**).

Fraction	Test tubes	Mass (g)
C4.1	1 - 20	61.8
C4.2	21 - 38	64.7
C4.3	39 - 54	25.8
C4.4	55 - 64	129.2

Table 10.4: Fractions obtained from the column separation of fraction C4.

Fraction C4.1 was resolved by PLC (H:EA, 3:1, v/v, x 8) into three fractions, C4.1.1. (R_f 0,63, 11.1 mg), C4.1.2 (R_f 0.57, 6.2 mg) and C4.1.3 (R_f 0.35, 8.1 mg).

10.2.1 4,6-Dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin (219)

Fraction C4.1.1 afforded the title compound as an *orange amorphous solid.*

Found	:	M$^+$, 656.2257, C$_{37}$H$_{36}$O$_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 18, Table 7.4
^{13}C NMR data	:	NMR-plate 19, Table 7.6
MS	:	Scheme 6, Table B6

10.2.2 4,6-Dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin (221)

Fraction C4.1.2 afforded the title compound as an *orange amorphous solid.*

Found	:	M$^+$, 656.2257, C$_{37}$H$_{36}$O$_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 20, Table 7.4
^{13}C NMR data	:	NMR-plate 21, Table 7.6
MS	:	Scheme 6, Table B6

10.2.3 4,6-Dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (223)

Fraction C4.1.3 afforded the title compound as a *yellow amorphous solid.*

Found	:	M$^+$, 656.2257, C$_{37}$H$_{36}$O$_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 22, Table 7.5
^{13}C NMR data	:	NMR-plate 23, Table 7.7
MS	:	Scheme 7, Table B7

Fractions C4.2 - C4.4 were still comprised of complex mixtures and were not investigated further.

Methylation of fraction C5 (250 mg) and subsequent PLC (H:B:A:M, 40:40:15:5, v/v/v/v) afforded two fractions, fraction C5.1 (R_f 0.83, 18.5 mg) and C5.2 (R_f 0.63, 44.3 mg). Fraction C5.1 was a combination of compounds (219) (see § 10.2.1) and (221) (see § 10.2.2) and was not investigated further. Fraction C5.2 was resolved by means of PLC (C:H:A, 90:6:4, v/v/v, x 2) into two fractions, C5.2.1 (R_f 0.85, 14.0 mg) and C5.2.2 (R_f 0.78, 27.7 mg). Fraction C5.2.1 was compound (223) (see § 10.2.3).

10.2.4 4,6-Dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (225)

Fraction C5.2.2 afforded the title compound as a *yellow amorphous solid.*

Found	:	M^+, 656.2257, $C_{37}H_{36}O_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 24, Table 7.5
^{13}C NMR data	:	NMR-plate 25, Table 7.7
MS	:	Scheme 7, Table B7

Column chromatography of fraction B6 (14.5 g) on Sephadex LH-20 (4 x 150 cm column, flow rate 30 ml/hr) in ethanol gave 7 fractions after analysis with TLC (B:A:M, 7:2:1, v/v/v) (see **Table 10.5**).

Fraction	Test tubes	Mass (g)
D1	1 - 38	7.0665
D2	39 - 49	0.5598
D3	50 - 92	0.8560
D4	93 - 112	0.1334
D5	113 - 133	0.0399
D6	134 - 161	0.6740
D7	162 - 347	0.1483

Table 10.5: Fractions obtained from the column separation of fraction B6.

10.2.5 2-(4-Hydroxybenzyl)-2,4,6-trihidroxybenzo[*b*]furan-3(2H)-one (maesopsin) (23)

Fraction D1 (R$_f$ 0.45 in B:A:M, 7:2:1, v/v/v) afforded compound (**23**) as a white amorphous solid.

^{1}H NMR data   :   NMR-plate 12, Table 7.1

TLC and ^{1}H NMR analysis of fraction D2 indicated that this fraction is mainly comprised of maesopsin and the same metabolites as contained in fraction D3. This fraction was therefore not investigated.

Methylation of fraction D1 (100 mg) followed by PLC (B:A, 9:1, v/v) yielded two bands, D1.1 (R$_f$ 0.48, 56.2 mg) and D1.2 (R$_f$ 0.26, 21.3 mg).

10.2.6 2,4,4',6-Tetra-*O*-methylmaesopsin (206)

Fraction D1.1 afforded the title compound as a yellow amorphous compound.

^{1}H NMR [CDCl$_3$, NMR plate 13]:   δ 7.18 (d, J 8.0 Hz, 2/6-H(B)), 6.75 (d, J 8.0 Hz, 3/5-H(B)), 6.09 (d, J 2.0 Hz, 7-H(A)), 5.92 (d, J 2.0 Hz, 5-H(A)), 3.88 (s, OMe), 3.86 (s, OMe), 3.76 (s, OMe), 3.28 (s, OMe), 3.19 (d, J 15.0 Hz, α-C$\underline{H}_2$), 3.07 (d, J 15.0 Hz, α-C$\underline{H}_2$).

10.2.7 2-Hydroxy-4,4',6-tri-*O*-methylmaesopsin (238)

Fraction D1.2 afforded the title compound as a yellow amorphous compound.

^{1}H NMR [CDCl$_3$, NMR plate 31]:   δ 7.25 (d, J 9.0 Hz, 2/6-H(B)), 6.82 (d, J 9.0 Hz, 3/5-H(B)), 6.07 (d, J 2.0 Hz, 7-H(A)), 5.93 (d, J 2.0 Hz, 5-H(A)), 3.87 (s, OMe), 3.85 (s, OMe), 3.78 (s, OMe), 3.19 (d, J 14.0 Hz, α-C$\underline{H}_2$), 3.10 (d, J 14.0 Hz, α-C$\underline{H}_2$).

Fraction D1.1 (2 mg) was dissolved in chloroform (1 mg/ml) and resolved by means of HPLC (H:EtOH:EtOAc, 250:250:0.6, v/v/v, flow rate 0.1 ml/s) using a chiral column to

yield two fractions, D1.1.1 (retention time 249 s, 0.78 mg) and D1.1.2 (retention time 304 s, 0.66 mg).

10.2.8 *(R)*-2,4,4',6-Tetra-*O*-methylmaesopsin (207)

Fraction D1.1.1 yielded the title compound in *high enantiomeric purity* (> 99%).

^{1}H NMR :   See § 10.2.6

CD   :   Figure 7.1; $[\theta]_{354.1}$ 4.511 x 10^4, $[\theta]_{340.7}$ 5.725 x 10^4, $[\theta]_{316.8}$ -4.210 x 10^4, $[\theta]_{268.3}$ -1.805 x 10^4, $[\theta]_{238.2}$ 8.448 x 10^3.

10.2.9 *(S)*-2,4,4',6-Tetra-*O*-methylmaesopsin (208)

Fraction D1.1.2 yielded the title compound in *high enantiomeric purity* (> 99%).

^{1}H NMR :   See § 10.2.6

CD   :   Figure 7.1; $[\theta]_{353.9}$ -3.100 x 10^4, $[\theta]_{340.7}$ -3.837 x 10^4, $[\theta]_{316.8}$ 2.897 x 10^4, $[\theta]_{268.2}$ 1.219 x 10^4, $[\theta]_{238.6}$ -4.994 x 10^3.

Methylation of fraction D3 (500 mg) and subsequent FCC (H:A:EA 55:30:15, v/v/v/v) afforded four fractions (see **Table 10.6**).

Fraction	Test tubes	Mass (g)
D3.1	1 - 9	93.9
D3.2	10 - 23	130.7
D3.3	24 - 30	131.6
D3.4	31 - 40	212.5

Table 10.6: Fractions obtained from the column separation of fraction D3.

10.2.10 4,4',6-Tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (211/212)

PLC of fraction D3.2 (H:A:EA 55:30:15, v/v/v) afforded compound (**211/212**) (R$_f$ 0.16,

28 mg) as a *yellow crystalline solid*, melting point 215.6°C (lit[1] 216.8°C). Fraction D3.3 (R$_f$ 0.17 in B:A, 9;1, v/v) similarly yielded the title compound.

Found	:	M$^+$, 656.2258, C$_{37}$H$_{36}$O$_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 14, Table 7.2
^{13}C NMR data	:	NMR-plate 15, Table 7.3
MS	:	Scheme 5, Table B5
CD	:	Figure 7.3; [θ]$_{366.6}$ 8.083 x 10^2, [θ]$_{337.5}$ 2.446 x 10^3, [θ]$_{298.5}$ -2.087 x 10^3, [θ]$_{285.6}$ 3.268 x 10^3.

X-ray crystallographic data: Suitable crystals were obtained from ethanol/water 9:1 (v/v).

Empirical formula	:	C$_{37}$H$_{36}$O$_{11}$	
Crystal system	:	Orthorhombic	
Point group	:	P$_{bca}$	
Unit cell dimensions	:	a = 6.193(2) Å	α = 90°
		b = 25.8080(5)	β = 90°
		c = 27.3020(5)	γ = 90°
Final R index	:	R$_1$ = 0.0570	

See **Appendix C, Tables C1 and C2** for further details regarding the crystal data of compound (**211/212**).

Fraction D3.2 (2 mg) was dissolved in chloroform (1 mg/ml) and resolved by means of HPLC (H:EtOH:EtOAc, 350:150:0.6, v/v/v, flow rate 0.1 ml/s) using a chiral column to yield two fractions, D3.2.1 (retention time 285 s, 0.83 mg) and D3.2.2 (retention time 312 s, 0.92 mg).

10.2.11 (2*S*)-4,4',6-Tri-*O*-methyl-2-deoxymaesopsin-(2→7)-(2*R*)-2,4,4',6-tetra-*O*-methylmaesopsin (211)

Fraction D3.2.1 yielded the title compound in *high enantiomeric purity* (> 99%).

CD : Figure 7.4; $[\theta]_{358.5}$ 2.801 x 10^4, $[\theta]_{338.6}$ 7.383 x 10^4, $[\theta]_{306.3}$ -5.415 x 10^4, $[\theta]_{273.7}$ -3.627 x 10^4, $[\theta]_{242.8}$ -3.232 x 10^4.

10.2.12 (2R)-4,4',6-Tri-O-methyl-2-deoxymaesopsin-(2→7)-(2S)-2,4,4',6-tetra-O-methylmaesopsin (212)

Fraction D3.2.2 yielded the title compound in *high enantiomeric purity* (> 99%).

CD : Figure 7.4; $[\theta]_{358.5}$ -1.774 x 10^4, $[\theta]_{338.4}$ -5.038 x 10^4, $[\theta]_{305.8}$ 3.650 x 10^4, $[\theta]_{274.2}$ 2.663 x 10^4, $[\theta]_{241.5}$ 2.378 x 10^4.

10.2.13 4,4',6-Tri-O-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-O-methylmaesopsin (215/216)

PLC of fraction D3.4 (H:A:EA 55:30:15, v/v/v) afforded two fractions, D3.4.1 (R_f 0.16, 18.0 mg) and D3.4.2 (R_f 0.10, 33.0 mg). Fraction D3.4.1 is compound (211/212) (see § 10.2.10). Fraction D3.4.2 afforded the title compound as a *yellow amorphous solid.*

Found	:	M^+, 656.2261, $C_{37}H_{36}O_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 16, Table 7.2
^{13}C NMR data	:	NMR-plate 17, Table 7.3
MS	:	Scheme 5, Table B5
CD	:	Figure 7.3; $[\theta]_{360.1}$ -1.470 x 10^3, $[\theta]_{348.7}$ -1.287 x 10^3, $[\theta]_{326.2}$ 3.120 x 10^3, $[\theta]_{301.8}$ -2.711 x 10^3, $[\theta]_{290.3}$ -3.380 x 10^3, $[\theta]_{277.7}$ 1.241 x 10^3.

Fraction D3.4.2 (2 mg) was dissolved in chloroform (1 mg/ml) and resolved by means of HPLC (H:EtOH:EtOAc, 350:150:0.6, v/v/v, flow rate 0.1 ml/s) using a chiral column to yield two fractions, D3.4.2.1 (retention time 252 s, 0.87 mg) and D3.4.2.2 (retention time 306 s, 0.71 mg).

10.2.14 (2R)-4,4',6-Tri-O-methyl-2-deoxymaesopsin-(2→7)-(2R)-2,4,4',6-tetra-O-methylmaesopsin (215)

Fraction D3.4.2.1 yielded the title compound *in high enantiomeric purity* (> 99%).

CD : Figure 7.4; $[\theta]_{359.8}$ 2.475 x 10^4, $[\theta]_{349.0}$ 2.100 x 10^4, $[\theta]_{326.4}$ -5.460 x 10^4, $[\theta]_{309.2}$ 2.288 x 10^4.

10.2.15 (2S)-4,4',6-Tri-O-methyl-2-deoxymaesopsin-(2→7)-(2S)-2,4,4',6-tetra-O-methylmaesopsin (216)

Fraction D3.4.2.2 yielded the title compound in *high enantiomeric purity* (> 99%).

CD : Figure 7.4; $[\theta]_{359.8}$ -4.918 x 10^4, $[\theta]_{349.2}$ -4.179 x 10^4, $[\theta]_{326.1}$ 1.082 x 10^5, $[\theta]_{310.6}$ -3.008 x 10^4.

10.2.16 (2S,3R)-4',5,7-tri-O-methyldihydrogenistein-(2α→7)-(2R)-2,4,4',6-tetra-O-methylmaesopsin (204)

Methylation and subsequent purification with PLC (B:A, 9:1, v/v) of fraction D4 (see **Table 10.5**) yielded fraction D4.1 (R_f 0.24, 12 mg) which was resolved into two fractions by a further PLC separation (B:EA, 3:1, v/v, x 7). Fraction D4.4.1 (R_f 0.63, 5.1 mg) afforded the title compound as yellow amorphous solid.

Found	:	M^+, 656.2257, $C_{37}H_{36}O_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 10, Table 6.7
MS	:	Scheme 4, Table B4
CD	:	Figure 7.3; $[\theta]_{359.0}$ 3.068 x 10^3, $[\theta]_{345.3}$ 2.218 x 10^3, $[\theta]_{322.1}$ -1.819 x 10^3, $[\theta]_{297.7}$ 9.785 x 10^2, $[\theta]_{275.2}$ 1.627 x 10^3.

10.2.17 (2*S*,3*R*)-4',5,7-tri-*O*-methyldihydrogenistein-(2α→7)-(2*S*)-2,4,4',6-tetra-*O*-methylmaesopsin (205)

Fraction D4.4.2 (R_f 0.53, 5.6 mg) afforded the title compound as yellow amorphous solid.

Found	:	M^+, 656.2257, $C_{37}H_{36}O_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 11, Table 6.7
MS	:	Scheme 4, Table 4
CD	:	Figure 7.3; $[\theta]_{359.7}$ -6.894 x 10^2, $[\theta]_{347.6}$ -3.900 x 10^2,
		$[\theta]_{324.5}$ 8.048 x 10^2, $[\theta]_{291.5}$ 1.002 x 10^3.

Fraction B9 (14.5 g) was subjected to column chromatography on Sephadex LH-20 (4 x 150 cm column, flow rate 30 ml/hr) in ethanol to give 25 fractions after analysis with TLC (B:A:M, 7:2:1, v/v/v) (see **Table 10.7**).

Fraction	Test tubes	Mass (g)	Fraction	Test tubes	Mass (g)
E1	1 - 51	0.0037	E14	206 - 213	0.4713
E2	52 - 80	0.0294	E15	214 - 230	2.5658
E3	81 - 84	0.0161	E16	231 - 236	0.7974
E4	85 - 93	0.0532	E17	237 - 249	1.4502
E5	94 - 104	0.0786	E18	250 - 280	1.7131
E6	105 - 122	0.0391	E19	281 - 302	0.4019
E7	123 - 144	0.0167	E20	303 - 317	0.1459
E8	145 - 159	0.0237	E21	318 - 329	0.1287
E9	160 - 172	0.0377	E22	330 - 343	0.2093
E10	173 - 181	0.0273	E23	344 - 390	1.1079
E11	182 - 191	0.0355	E24	391 - 408	0.4643

Table 10.7: Fractions obtained from the column separation of fraction B9.

Fraction	Test tubes	Mass (g)	Fraction	Test tubes	Mass (g)
E12	192 - 196	0.0426	E25	409 - 485	0.9555
E13	197 - 205	0.03102			

Table 10.7 (continued)

Fractions E1 to E11 still comprised complex mixtures and the small available amounts prevented further investigation of these fractions. The investigation of the other fractions have previously been described in references 192 and 196.

Methylation of fraction E15 (600 mg) followed by FCC (H:B:A:M 40:40:15:5, v/v/v) yielded three fractions (see **Table 10.8**).

Fraction	Test tubes	Mass (g)
E15.1	1 - 18	219.5
E15.2	19 - 28	111.6
E15.3	36 - 51	183.4

Table 10.6: Fractions obtained from the column separation of fraction D3.

Successive PLC of fraction E15.1 with B:A (9:1, v/v) [yielding fraction E15.1.1 (R_f 0.27, 89.2 mg)], B:MEK (8:2, v/v, x 2) [yielding fraction E15.1.1.1 (R_f 0.64, 40.6 mg)] and C:E (98:2, v/v, x 4) yielded fractions E15.1.1.1.1 (R_f 0.66, 15.7 mg) and E15.1.1.1.2 (R_f 0.62, 12.9 mg).

10.2.18 (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→5)-(2R)-2,4,4',6-tetra-O-methylmaesopsin (197)

Fraction E15.1.1.1.1 afforded the title compound as yellow amorphous solid.

Found	:	M^+, 656.2257, $C_{37}H_{36}O_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 6, Table 6.5
^{13}C NMR data	:	NMR-plate 7, Table 6.6
MS	:	Scheme 3, Table B3

CD   :   Figure 6.3; $[\theta]_{359.3}$ 2.244 x 10^4, $[\theta]_{346.2}$ 1.156 x 10^4, $[\theta]_{325.1}$

                             -2.380 x 10^4, $[\theta]_{272.9}$ 1.783 x 10^4.

10.2.19 (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3α→5)-(2*S*)-2,4,4',6-tetra-*O*-methylmaesopsin (198)

Fraction E15.1.1.1.2 afforded the title compound as yellow amorphous solid.

Found   : M^+, 656.2257, $C_{37}H_{36}O_{11}$ requires M, 656.2258

^{1}H NMR data   :   NMR-plate 8, Table 6.5

^{13}C NMR data   :   NMR-plate 9, Table 6.6

MS   :   Scheme 3, Table B3

CD   :   Figure 6.3; $[\theta]_{358.6}$ -2.157 x 10^4, $[\theta]_{345.4}$ -1.520 x 10^4,

                             $[\theta]_{312.3}$ 1.880 x 10^4, $[\theta]_{274.4}$ 2.863 x 10^4.

10.2.20 (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3α→7)-(2*R*)-2,4,4',6-tetra-*O*-methylmaesopsin (175)

Fraction E15.3 was resolved with PLC (C:A, 95:5, v/v, x 4) to yield two fractions, E15.3.1 (R_f 0.80, 26.7 mg) and E15.3.2 (R_f 0.74, 19.7 mg). Fraction E15.3.1 gave the title compound as a yellow amorphous solid.

Found   : M^+, 656.2258, $C_{37}H_{36}O_{11}$ requires M, 656.2258

^{1}H NMR data   :   NMR-plate 1, Table 6.1

^{13}C NMR data   :   NMR-plate 2, Table 6.2

MS   :   Scheme 2, Table B2

CD   :   Figure 6.1; $[\theta]_{357.5}$ 6.441 x 10^3, $[\theta]_{344.0}$ 4.994 x 10^3, $[\theta]_{324.1}$

                             -5.661 x 10^3, $[\theta]_{294.7}$ -3.430 x 10^3, $[\theta]_{274.3}$ 4.778 x 10^3.

10.2.21 (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→7)-(2S)-2,4,4',6-tetra-O-methylmaesopsin (176)

Fraction E15.3.2 yielded the title compound as a yellow amorphous solid.

Found	:	M^r, 656.2258, $C_{37}H_{36}O_{11}$ requires M, 656.2258
^{1}H NMR data	:	NMR-plate 3, Table 6.1
^{13}C NMR data	:	NMR-plate 4, Table 6.2
MS	:	Scheme 2, Table B2
CD	:	Figure 6.1; $[\theta]_{357.4}$ -7.663 x 10^3, $[\theta]_{343.5}$ -9.271 x 10^3, $[\theta]_{286.3}$ 1.375 x 10^4, $[\theta]_{278.3}$ 1.391 x 10^4.

10.2.22 (2R)-7-[2-(4-methoxyphenyl)ethyl]-2,4,4',6-tetra-O-methylmaesopsin (188)

Fraction E15.3.1 (15 mg) was reduced by means of Na(CN)BH₃ (see § 9.5.3) and the product resolved by means of PLC (B:A, 9:1, v/v) to afford the title compound as a white amorphous solid (R_f 0.56, 1.64 mg, 15%).

Found	:	M^+, 478.1984, $C_{28}H_{30}O_7$ requires M, 478.1992
^{1}H NMR data	:	NMR-plate 5, Table 6.3
MS	:	Scheme 1, Table B1
CD	:	Figure 6.1; $[\theta]_{358.0}$ 1.263 x 10^4, $[\theta]_{343.5}$ 1.171 x 10^4, $[\theta]_{302.3}$ -4.360 x 10^3, $[\theta]_{280.5}$ -1.087 x 10^4.

10.2.23 (2S)-7-[2-(4-methoxyphenyl)ethyl]-2,4,4',6-tetra-O-methylmaesopsin (190)

Fraction E15.3.2 (15 mg) was similarly reduced to afford, after PLC separation (B:A, 9:1, v/v) of the product, the title compound as a white amorphous solid (R_f 0.56, 2.2 mg, 20%).

Found	:	M^+, 478.1983, $C_{28}H_{30}O_7$ requires M, 478.1992
^{1}H NMR data	:	NMR-plate 5, Table 6.3
MS	:	Scheme 1, Table B1
CD	:	Figure 6.1; $[\theta]_{358.1}$ -7.891 x 10^3, $[\theta]_{343.6}$ -7.256 x 10^3.
		$[\theta]_{298.7}$ 4.032 x 10^3, $[\theta]_{280.1}$ 6.839 x 10^3.

CHAPTER 11

THE SYNTHESIS OF MAESOPSIN

11.1 THE SYNTHESIS OF 2-BENZYLBENZOFURANONES

11.1.1 2-Hydroxy-4,6-dimethoxyacetophenone (242)

2,4,6-Trihydroxyacetophenone (10 g, 0.06 mol) was methylated as described in § 9.5.2 to yield the title product (8.5 g, 72.8%) after recrystallisation from ethanol as a white crystalline solid, m. p. 86 °C (lit.[232] 82 - 83°C). R_f 0.74 (B:A, 9:1, v/v)

^{1}H NMR [CDCl$_3$, Plate 26]:   δ 14.06 (s, 2-O$\underline{H}$), 6.07 (d, J 2.0 Hz, 7-H), 5.94 (d, J 2.0 Hz, 5-H), 3.87 (s, OC$\underline{H}_3$), 3.83 (s, OC$\underline{H}_3$), 2.62 (s, COC$\underline{H}_3$)

11.1.2 2'-Acetoxy-4',4,6'-trimethoxycalcone (245)

2-Hydroxy-4,6-dimethoxyacetophenone (242) (8 g, 0.04 mol) was dissolved in ethanol (100 ml) and 50% (m/v) *aq.* KOH (16.4 ml, 0.4 ml/mmol acetophenone) was added. The reaction mixture was stirred for 10 minutes before the addition of 4-methoxybenzaldehyde (243) (6 ml, 0.049 mol, 1.2 equiv.). The resulting mixture was stirred for 12 hours and water (50 ml) was added to the solution, which was then extracted with ethyl acetate (4 x 20 ml). The combined extracts were dried (Na$_2$SO$_4$) and evaporated. The resulting crude product was dissolved in pyridine (20 ml), acetic anhydride (20 ml, excess) was added and the reaction mixture was kept at 30°C for 8 hours. Ice was added and the resulting precipitate was collected and crystallized from ethanol to yield compound (245) as light yellow crystals (10.4 g, 72.9%), m.p. 109°C (lit.[233] 108 - 109°C). R_f 0.50 (B:A, 9:1, v/v)

^{1}H NMR [CDCl$_3$, Plate 27]:   δ 7.50 (d, J 9.0 Hz, 2/6-H(A)), 7.40 (d, J 16.0 Hz, β-C$\underline{H}$), 6.92 (d, J 9.0 Hz, 3/5-H(A)), 6.89 (d, J 16.0 Hz, α-C$\underline{H}$), 6.43 (d, J 2.0 Hz, 7-H(B)), 6.32 (d, J 2.0 Hz, 5-H(B)), 3.85 (s, 2 x OC$\underline{H}_3$), 3.81 (s, OC$\underline{H}_3$), 2.18 (s, OCOC$\underline{H}_3$)

11.1.3 2'-Acetoxy-α-bromo-β,4',4,6'-tetramethoxydihydrocalcone[119] (246)

NBS (6 g, 0.034 mol, 1.2 equiv.) was added to a solution of 2'-acetoxy-4',4,6'-trimethoxycalcone (245) (10 g, 0.028 mol) in methanol (200 ml). After 12 hours, the precipitate was collected and a single fraction from FCC (H:EA, 3:1, v/v) afforded the title compound (7.8 g, 71.9%) as a white amorphous solid. R_f 0.21 (H:EA, 3:1, v/v)

^{1}H NMR [CDCl3, Plate 28]: δ 7.34 (d, J 9.0 Hz, 2/6-H(A)), 6.92 (d, J 9.0 Hz, 3/5-H(A)), 6.40 (d, J 2.0 Hz, 7-H(B)), 6.31 (d, J 2.0 Hz, 5-H(B)), 5.04 (d, J 10.0 Hz, α-CH), 4.67 (d, J 10.0 Hz, β-CH), 3.88 (s, OCH3), 3.85 (s, OCH3), 3.83 (s, OCH3), 3.19 (s, OCH3), 2.32 (s, OCOCH3)

11.1.4 4,4',6-Trimethoxyaurone[192] (235)

Aqueous NaOH (2.0 M, 30 ml) was added to a suspension of the dihydrochalcone (246) (3.7 g, 9.5 mmol) in methanol (100 ml), stirred for 1 hour and diluted with water (200 ml), after which the precipitate was collected. A single fraction from FCC (B:A, 9:1, v/v) gave the title compound (2.23g, 75.1%) as a yellow amorphous solid. R_f 0.33 (B:A, 9:1, v/v)

^{1}H NMR [CDCl3, Plate 29]: δ 7.83 (d, J 9.0 Hz, 2/6-H(B)), 6.96 (d, J 9.0 Hz, 3/5-H(B)), 6.76 (s, α-CH), 6.38 (d, J 2.0 Hz, 7-H(A)), 6.13 (d, J 2.0 Hz, 5-H(A)), 3.96 (s, OCH3), 3.91 (s, OCH3), 3.86 (s, OCH3)

11.1.5 2-(4-Methoxybenzyl)-4,6-dimethoxybenzo[b]furan-3(2H)-one (236)

The aurone (235) (100 mg, 0.32 mmol) was dissolved in ethanol (10 ml) or acetone (10 ml) (see **Table 8.1**) and 5% Pd/C catalyst (10 - 25 mg, 10 - 25%) was added. The reaction mixture was stirred (1 - 3 hours) (TLC) under a H_2-atmosphere (1 atm) after which it was filtered through Celite to remove the catalyst. Subsequent PLC (B:A, 9:1, v/v) gave two fractions, the first of which afforded the title compound (44.6 - 70.2%, R_f

0.43, **Table 8.1**).

¹H NMR [CDCl₃, Plate 30]:   δ 7.23 (d, J 9.0 Hz, 2/6-H(B)), 6.83 (d, J 9.0 Hz, 3/5-H(B)), 6.13 (d, J 2.0 Hz, 7-H(A)), 5.98 (d, J 2.0 Hz, 5-H(A)), 4.73 (dd, J 3.0 and 9.0 Hz, 2-H(C)), 3.90 (s, OC$\underline{H}_3$), 3.85 (s, OC$\underline{H}_3$), 3.79 (s, OC$\underline{H}_3$), 3.30 (dd, J 3.0 and 15.0 Hz, α-C$\underline{H}_2$), 2.91 (dd, J 9 and 15.0 Hz, α-C$\underline{H}_2$)

The second fraction afforded 2-hydroxy-4,4',6-tri-*O*-methylmaesopsin (**238**) (R$_f$ 0.26, 12.8 - 27.9%, **Table 8.1**) as a yellow amorphous solid.

¹H NMR [CDCL₃, Plate 31]:   See § 10.2.7.

11.2 THE SYNTHESIS OF 2-METHOXYBENZOFURANONES

11.2.1 2,4,6-Trihydroxy-ω-chloroacetophenone[92] (248)

HCl gas [dried over H_2SO_4(c)] was bubbled through a mixture of freshly distilled chloroacetonitrile (4.5 ml, 0.07 mol, 1.1 equiv.) and freshly fused zinc chloride (16 g, 0.12 mol, 2.0 equiv.) in dry ether (50 ml) at 0°C under an inert N_2 atmosphere for 1 hour. Phloroglucinol (8 g, 0.06 mol) in dry ether (30 ml) was subsequently added over 1 hour under N_2 and HCl-bubbling was continued for 6 hours to yield an orange paste. The mixture was left at 4°C for 12 hours after which HCl was again bubbled through for an additional 3 hours. The mixture was again left at 4°C for 12 hours, the solvent decanted and H_2O (excess) added to give an orange solution. This solution was stirred at 80°C for 1 hour and left at 4°C for 5 hours to yield a yellow precipitate (10.2 g, 84.2%) which was used directly in the cyclization step (*vide infra*). R$_f$ 0.48 (B:A:M, 7:2:1, v/v/v)

¹H NMR [(CD₃)₂CO, Plate 32]:   δ 11.68 (s, OH), 11.65 (s, OH), 9.54 (s, OH), 5.99 (s, 5/7-H), 4.98 (s, COC$\underline{H}_2$Cl)

11.2.2 4,6-Dimethoxybenzo[*b*]furan-3(2*H*)-one (239)

2,4,6-Trihydroxy-ω-chloroacetophenone (**248**) (8 g, 0.04 mol) was dissolved in dry

acetone, dry K$_2$CO$_3$ (22 g, 0.16 mol, 4 equiv.) was added and the resulting slurry was stirred at 60°C for 30 minutes. Dimethylsulfate (8.5 ml, 0.088 mol, 2.2 equiv.) was subsequently added drop-wise over a period of 30 minutes under N$_2$. The reaction mixture was refluxed for 8 hours after which it was filtered and the acetone removed under reduced pressure. The excess dimethylsulfate was destroyed by addition of dilute ammonia and extracted with ethyl acetate. The crude product was purified with FCC (B:A, 9:1, v/v) to afford a single fraction, compound (239) (4.8 g, 62.5%). R$_f$ 0.31 (B:A, 9:1, v/v)

^{1}H NMR [CDCl$_3$, Plate 33]: δ 6.17 (d, J 2.0 Hz, 7-H), 6.03 (d, J 2.0 Hz, 5-H), 4.61 (s, 2-CH$_2$), 3.93 (s, OCH$_3$), 3.88 (s, OCH$_3$)

11.2.3 4,6-Dimethoxy-3-trimethylsilyloxybenzo[*b*]furan[216] (249)

4,6-Dimethoxybenzo[*b*]furan-3(2H)-one (239) (200 mg, 1.03 mmol), triethylamine (220 µl, 1.55 mmol, 1.5 equiv.) and trimethylsilylchloride (200 µl, 1.55 mmol, 1.5 equiv.) was successively introduced into a reaction flask containing dry acetonitrile (5 ml) under N$_2$. Sodium iodide (230 mg, 1.55 mmol, 1.5 equiv.) in dry acetonitrile (5 ml) was added drop-wise (15 minutes) at room temperature and the reaction mixture was stirred until the reaction was complete (^{1}H NMR, 1 hour). Cold pentane (20 ml) and ice water was added successively and after removal of the organic layer, the aqueous layer was extracted twice more with pentane (20 ml). The combined organic layers were washed with ice water (2 x 20 ml) and dried (Na$_2$SO$_4$). The solvent was removed under a fast N$_2$ stream to yield the title compound (274 mg, 99%) as a colorless oil.

^{1}H NMR [C$_6$D$_6$, Plate 34]: δ 7.21 (s, 2-H), 6.63 (d, J 2.0 Hz, 7-H), 6.42 (d, J 2.0 Hz, 5-H), 3.43 (s, 2-CH$_2$), 3.39 (s, OCH$_3$), 0.31 (s, OSi(CH$_3$)$_3$)

11.2.4 2-Hydroxy-4,6-dimethoxybenzo[*b*]furan-3(2H)-one[215] (251)

A solution of MCPBA (176 mg, 1.02 mmol, 1 equiv.) in dry hexane (5 ml) was cooled in an ice methanol bath and a solution of the silyl enol ether (249) (274 mg, 1.02 mmol) in

dry hexane (5 ml) was added drop-wise. After the addition was complete, the resulting slurry was stirred for 45 minutes and filtered to remove the *m*-chlorobenzoic acid. The filtrate was concentrated and the residue was partitioned between ether and 10% (m/v) aqueous NaOH for 3 hours to effect hydrolysis. The title compound (21.2 mg, 9.9%, R_f 0.14) was obtained after PLC (B:A, 9:1, v/v) as a yellow amorphous solid along with 4,6-dimethoxybenzo[*b*]furan-3(2H)-one (**239**) (40 mg) (see § 11.2.2) and a complex mixture of compounds that were not investigated further.

^{1}H NMR [CDCl$_3$, Plate 35]:   δ 6.15 (d, J 2.0 Hz, 7-H), 6.02 (d, J 2.0 Hz, 5-H), 5.56 (s, 2-H), 3.92 (s, OC$\underline{H}_3$), 3.89 (s, OC$\underline{H}_3$), 2.19 (s, 2-OH).

11.2.5 2-Acetoxy-4,6-dimethoxybenzo[*b*]furan-3(2H)-one[217] (256)

TTN (253 mg, 0.57 mmol, 1.1 equiv.) was added to a solution of 4,6-dimethoxy-benzo[*b*]furan-3(2H)-one (**239**) (100 mg, 0.51 mmol) in acetic acid (20 ml). The reaction mixture was stirred for 2 hours and the thallium (I) nitrate formed during the reaction was removed by filtration. The filtrate was neutralized with sodium bicarbonate, extracted with chloroform (3 x 30 ml) and resolved by means of PLC to yield three main bands. The first band (R_f 0.43) yielded the title compound (18.7 mg, 14.5%) as a white amorphous solid.

^{1}H NMR [CDCl$_3$, Plate 36]:   δ 6.24 (s, 2-H), 6.16 (d, J 2.0 Hz, 7-H), 6.06 (d, J 2.0 Hz, 5-H), 3.94 (s, OC$\underline{H}_3$), 3.89 (s, OC$\underline{H}_3$), 2.20 (s, 2-OCOC$\underline{H}_3$).

The second band (R_f 0.31) yielded starting material (**239**) (20 mg).

^{1}H NMR [CDCl$_3$, Plate 33]:   See § 11.2.2

The third band (R_f 0.14) afforded 2-hydroxy-4,6-dimethoxybenzo[*b*]furan-3(2H)-one (**251**) (22.3 mg, 20.8%).

^{1}H NMR [CDCl$_3$, Plate 35]:   See § 11.2.4

11.2.6 2,4,6-Trimethoxybenzo[*b*]furan-3(2H)-one (240)

TTN (253 mg, 0.57 mmol, 1.1 equiv.) was added to a solution of 4,6-dimethoxy-benzo[*b*]furan-3(2H)-one (239) (100 mg, 0.51 mmol) and *p*-toluenesulphonic acid (108 mg, 0.57 mmol, 1.1 equiv.) in methanol (20 ml). The reaction mixture was stirred for 12 hours and the thallium (I) nitrate formed during the reaction was removed by filtration. The filtrate was neutralized with sodium bicarbonate, extracted with chloroform (3 x 30 ml) and resolved by means of PLC to yield two bands. The first band (R_f 0.34) yielded the title compound (240) (74.2 mg, 64.9%) as a white amorphous solid.

^{1}H NMR [CDCl$_3$, Plate 37]: δ 6.13 (d, J 2.0 Hz, 7-H), 6.01 (d, J 2.0 Hz, 5-H), 5.26 (s, 2-H), 3.91 (s, OC$\underline{H}_3$), 3.88 (s, OC$\underline{H}_3$), 3.59 (s, 2-OC$\underline{H}_3$).

The second band (R_f 0.31) is starting material (239) (24 mg).

^{1}H NMR [CDCl$_3$, Plate 33]: See § 11.2.2

11.3 THE α-HYDROXYLATION OF BENZOFURANONES

11.3.1 2-(4-Methoxybenzyl)-4,6-dimethoxy-3-trimethylsilyloxy-benzo[*b*]furan[216] (237)

2-(4-Methoxybenzyl)-4,6-dimethoxybenzo[*b*]furan-3(2H)-one (236) (100 mg, 0.32 mmol), triethylamine (0.07 ml, 0.48 mmol, 1.5 equiv.) and trimethylsilylchloride (0.06 ml, 0.48 mmol, 1.5 equiv.) was successively introduced into a reaction flask containing dry acetonitrile (3 ml) under N$_2$. Sodium iodide (72 mg, 0.48 mmol, 1.5 equiv.) in dry acetonitrile (5 ml) was added drop-wise (15 minutes) at room temperature and the reaction mixture was stirred until the reaction was complete (^{1}H NMR, 2 hours). The work-up procedure described in § 11.2.3 afforded the title compound (122 mg, 99%) as a colorless oil.

^{1}H NMR [C$_6$D$_6$, Plate 38]: δ 7.34 (d, J 9.0 Hz, 2/6-H(B)), 6.88 (d, J 9.0 Hz, 3/5-H(B)), 6.55 (d, J 2.0 Hz, 7-H(A)), 5.43 (d, J 2.0 Hz, 5-H(A)), 4.15 (s, α-C$\underline{H}_2$), 3.43 (s,

OCH$_3$), 3.39 (s, OCH$_3$), 3.38 (s, OCH$_3$), 0.38 (s, OSi(CH$_3$)$_3$)

11.3.2 α-Hydroxylation using AD-mix-α[178]

A 10-ml round-bottomed flask was charged with *tert*-butyl alcohol (2 ml), water (2 ml) and AD-mix-α (518 mg, 1.4 g/mmol of the enol ether). The mixture was stirred at room temperature to produce two clear phases. The lower aqueous phase appeared bright yellow. Methanesulfonamide (29 mg, 0.31 mmol, 1 equiv.) was added to the mixture which was then cooled to 0°C and treated with silyl enol ether (**237**) (122 mg, 0.31 mmol). The resulting heterogeneous slurry was stirred vigorously at 0°C for 18 hours (TLC).

Three different work-up procedures were employed. The first involved the addition of sodium sulfite (200 mg) to the reaction mixture, which was then allowed to warm to room temperature and stirred for 30 minutes. Ethyl acetate (10 ml) was added to the mixture and the organic layer was separated. The aqueous phase was extracted with ethyl acetate (3 x 15 ml) and the combined organic layers were dried (Na$_2$SO$_4$).

Alternatively, the ethyl acetate was added to the cold reaction mixture and after separation of the organic layer, the mixture was exhaustively extracted with ethyl acetate at 0°C.

A third method involved the neutralization of the reaction mixture with 1M HCl at 0°C and subsequent extraction with ethyl acetate (3 x 20 ml). In each instance PLC (EA:H 6:4, v/v) afforded 4,4',6-tri-*O*-methylmaesopsin (**238**) (R$_f$ 0.45, 20.6 mg, 20.1%).

^{1}H NMR [CDCL$_3$, Plate 31]:   See § 10.2.7.

A second fraction from PLC is 2-(4-methoxybenzyl)-4,6-dimethoxybenzo[*b*]furan-3(2H)-one (**236**) (R$_f$ 0.32, 33.6 mg, 34.5%)

^{1}H NMR [CDCl$_3$, Plate 30]:   See § 11.1.5

11.3.3 α-Hydroxylation using 2-(phenylsulfonyl)-3-(4-nitrophenyl) oxaziridine[145] (259)

n-Butyllithium (138 μl of a 1.52 mol/dm³ solution, 0.21 mmol, 1.2 equiv. relative to the amine base) was slowly added to a solution of diisopropylamine (25 μl, 0.18 mmol, 1.1 equiv. relative to the starting material) in THF (2 ml) at 0°C and the mixture was stirred for 15 minutes. Alternatively, sodium bis(trimethylsilyl)amide (NaHMDS) (175 μl, 0.18 mmol, 1.1 equiv.) was dissolved in THF (2 ml). The resulting solution was cooled to -78°C, a solution of 2-(4-methoxybenzyl)-4,6-dimethoxybenzo[*b*]furan-3(2H)-one (236) (50 mg, 0.16 mmol) in THF (2 ml) was added and the mixture was stirred at this temperature or 15 minutes. Subsequently, 2-(phenylsulfonyl)-3-(4-nitrophenyl) oxaziridine (259) (70 mg, 0.18 mmol, 1.1 equiv.) was added and the mixture was stirred until the reaction was completed (TLC, 1 hour). The reaction was quenched at -78°C by addition of 2 ml of a saturated NH₄Cl solution and the aqueous phase was extracted with ethyl acetate (3 x 20 ml). PLC (B:A, 9:1, v/v) of the product afforded three main fractions. The first (R_f 0.43) fraction is starting material (3.5 mg).

¹H NMR [CDCl₃, Plate 30]:   See § 11.1.5

The second fraction (R_f 0.33) afforded 2-[phenylsulfonylamino(4-nitrophenyl)methyl]-2-deoxymaesopsin (261) (11.4 mg, 18.6%) as a *white amorphous solid*.

¹H NMR [CDCl₃, Plate 39]:   δ 7.79 (d, J 9.0 Hz, 2/6-H(D)), 7.66 (m, 2/6-H(E)), 7.39 (m, 4-H(E)), 7.28 (m, 3/5-H(E)), 7.19 (d, J 9.0 Hz, 2/6-H(B)), 7.01 (d, J 9.0 Hz, 3/5-H(D)), 6.61 (d, J 9.0 Hz, 3/5-H(B)), 6.06 (d, J 10.0 Hz, N-H̲), 5.85 (d, 2.0 Hz, 7-H(A)), 5.61 (d, 2.0 Hz, 5-H(A)), 4.99 (d, J 10.0 Hz, α-CH̲), 3.75 (s, OMe), 3.69 (s, OMe), 3.62 (s, OMe), 3.50 (d, J 14.0 Hz, α-CH̲₂), 3.21 (d, J 14.0 Hz, α-CH̲₂)

The third fraction (R_f 0.26) yielded 4,4',6-tri-*O*-methylmaesopsin (238) (11.8 mg, 22.5%).

¹H NMR [CDCL₃, Plate 31]:   See § 10.2.7.

11.3.4 α-Hydroxylation using (+)-(camphorylsulfonyl)oxaziridine[151] (152)

Sodium bis(trimethylsilyl)amide (NaHMDS) (190 µl, 0.19 mmol, 1.2 equiv.) in THF (2 ml) was cooled to -78°C and a solution of 2-(4-methoxybenzyl)-4,6-dimethoxybenzo[b]furan-3(2H)-one (236) (50 mg, 0.16 mmol) in THF (2 ml) was added. The mixture was stirred at this temperature for 15 minutes and (+)-(camphorylsulfonyl)oxaziridine (152) (55 mg, 0.24 mmol, 1.5 equiv.) was added. The mixture was stirred for 30 minutes and quenched at -78°C by addition of 2 ml of a saturated NH_4Cl solution. The aqueous phase was extracted with ethyl acetate (3 x 20 ml), the combined extracts were dried (Na_2SO_4) and the solvent was removed under reduced pressure. PLC (B:A, 9:1, v/v) of the product afforded two fractions, the first (R_f 0.43) of which is starting material (236) (12.3 mg).

^{1}H NMR [CDCl$_3$, Plate 30]:   See § 11.1.5

The second fraction (R_f 0.26) afforded 4,4',6-tri-O-methylmaesopsin (238) (34.5 mg, 65.3%).

^{1}H NMR [CDCL$_3$, Plate 31]:   See § 10.2.7.

Repetition of this procedure with 0.95 equiv. (NaHMDS) (150 µl, 0.15 mmol) and 1.2 equiv. (+)-(camphorylsulfonyl)oxaziridine (152) (44 mg, 0.19 mmol) on 50 mg of 2-(4-methoxybenzyl)-4,6-dimethoxybenzo[b]furan-3(2H)-one (236) (0.16 mmol) with a reaction time of 10 minutes followed by work-up as described above and PLC (B:A, 9:1, v/v) yielded 2-hydroxy-4,4',6-tri-O-methylmaesopsin (238) (42.5 mg, 80.4%).

^{1}H NMR [CDCL$_3$, Plate 31]:   See § 10.2.7.

The above procedure was repeated and MeI (30 µl, 0.48 mmol, 3 equiv.) was added after 10 minutes. The temperature of the reaction mixture was allowed to rise to 0°C over the period of 1 hour. Work-up as described above and PLC (B:A, 9:1, v/v) afforded 2-hydroxy-4,4',6-tri-O-methylmaesopsin (238) (41.9 mg, 79.3%).

The above procedure was repeated and acetic anhydride (60 µl, 0.64 mmol, 4.0 equiv.) was added after 10 minutes. The temperature of the reaction mixture was allowed to rise to 0°C over the period of 1 hour. Work-up as described above and PLC (B:A, 9:1, v/v) afforded two fractions, the first (R_f 0.45) is 2-*O*-acetyl-4,4',6-tri-*O*-methylmaesopsin (**262**) (17.4 mg, 29.2%).

¹H NMR [CDCl₃, NMR plate 40]:   δ 7.20 (d, J 9.0 Hz, 2/6-H(B)), 6.78 (d, J 9.0 Hz, 3/5-H(B)), 6.06 (d, J 2.0 Hz, 7-H(A)), 5.97 (d, J 2.0 Hz, 5-H(A)), 3.88 (s, OMe), 3.84 (s, OMe), 3.78 (s, OMe), 3.26 (d, J 14.0 Hz, α-C$\underline{H}_2$), 3.06 (d, J 14.0 Hz, α-C$\underline{H}_2$), 2.09 (s, 2-COC$\underline{H}_3$)

The second fraction (R_f 0.26) afforded 2-hydroxy-4,4',6-tri-*O*-methylmaesopsin (**238**) (25.4 mg, 48.0%).

¹H NMR [CDCL₃, Plate 31]:   See § 10.2.7.

11.4   THE α-ALKYLATION OF BENZOFURANONES

11.4.1   2,4,6-Trimethoxy-2-methylbenzo[*b*]furan-3(2H)-one (263)

Sodium hydride (8 mg of a 80% suspension, 0.27 mmol, 1.2 equiv.) was added to a solution of 2,4,6-trimethoxybenzo[*b*]furan-3(2H)-one (**240**) (50 mg, 0.22 mmol) in THF (3 ml) at room temperature under N₂. The reaction mixture was stirred for 30 minutes and MeI (17 µl, 0.27 mmol, 1.2 equiv.) was added. After 2 hours ice was added to the mixture and the resulting aqueous phase was extracted with ethyl acetate (3 x 20 ml). The combined extracts were dried (Na₂SO₄) and the solvent was removed under reduced pressure. PLC (B:A, 9:1, v/v) afforded three fractions, the first of which (R_f 0.45) afforded the title compound (10.8 mg, 20.6%) as a white amorphous solid.

¹H NMR [CDCl₃, Plate 41]:   δ 6.15 (d, J 2.0 Hz, 7-H), 6.02 (d, J 2.0 Hz, 5-H), 3.93 (s, OC$\underline{H}_3$), 3.90 (s, OC$\underline{H}_3$), 3.29 (s, 2-OC$\underline{H}_3$), 1.57 (s, 2-CH₃).

The second fraction (R_f 0.34) is starting material (**240**) (10.1 mg).

¹H NMR [CDCl₃, Plate 37]:   See § 11.2.6

The third fraction (R$_f$ 0.21) afforded 2-(2,3,4,6-tetramethoxy-2,3-dihydrobenzofuran-3-yl)-2,4,6-trimethoxybenzo[*b*] furan-3(2H)-one (**264**) (30.7 mg, 30.2%) as a *white amorphous solid.*

¹H NMR data   :   NMR-plate 42, Table 8.3

A second method involved the addition of a solution of 2,4,6-trimethoxybenzo[*b*]furan-3(2H)-one (**240**) (50 mg, 0.22 mmol) in THF (2 ml) to a suspension of sodium hydride (10 mg of a 80% suspension, 0.33 mmol, 1.5 equiv.) in THF (2 ml) under N₂. The mixture was stirred for 5 minutes and MeI (17 μl, 0.27 mmol, 1.2 equiv.) was added drop-wise at room temperature. The resulting reaction mixture was stirred for 30 minutes and worked up as described above. PLC (B:A, 9:1,v/v) afforded the title compound (34.1 mg, 65.2%) and starting material (10.4 mg).

11.4.2 2,4,4',6-Tetra-*O*-methylmaesopsin (206)

A solution of 2,4,6-trimethoxybenzo[*b*]furan-3(2H)-one (**240**) (50 mg, 0.22 mmol) in THF (2 ml) was added to a suspension of sodium hydride (10 mg of a 80% suspension, 0.33 mmol, 1.5 equiv.) in THF (2 ml) under N₂. The mixture was stirred for 5 minutes and 4-methoxybenzylchloride (37 μl, 0.27 mmol, 1.2 equiv.) was added drop-wise at room temperature. The resulting reaction mixture was stirred for 12 hours, ice was added and the resulting aqueous phase was extracted with ethyl acetate (3 x 20 ml). The combined extracts were dried (Na₂SO₄) and the solvent was removed under reduced pressure. PLC (B:A, 9:1, v/v) afforded only starting material (**240**) (34.9 mg).

¹H NMR [CDCl₃, Plate 37]:   See § 11.2.6

A second attempt involved preparation of the enolate as described above and 4-methoxybenzylchloride (37 μl, 0.27 mmol, 1.2 equiv.) was added to a solution of NaI (50 mg, 0.33 mmol, 1.5 equiv.) in dry acetonitrile (2 ml). This solution was stirred for 5 minutes and added to the enolate in THF (2 ml). The resulting mixture was stirred for 30

minutes and worked up as described above. PLC (B:A, 9:1, v/v) afforded the title compound (58.7 mg, 77.5%, R_f 0.48) as a yellow amorphous compound.

¹H NMR [CDCl₃, Plate 12]:   See § 10.2.6

A third method involved preparation of the enolate with LDA [0.27 mmol, prepared from *n*-Butyllithium (178 µl of a 1.52 mol/dm³ solution in hexane, 0.27 mmol, 1.2 equiv.) and diisopropylamine (37 µl, 0.27 mmol, 1.2 equiv.) in THF (2 ml) at 0°C] at -78°C. 4-Methoxybenzylchloride (37 µl, 0.27 mmol, 1.2 equiv.) was added to a solution of NaI (50 mg, 0.33 mmol, 1.5 equiv.) in dry acetonitrile (2 ml) and stirred for 5 minutes. This solution was cooled to -78°C and added to the enolate. The temperature of the reaction mixture was allowed to rise to 0°C over the period of 1 hour and quenched by addition of 2 ml of a saturated NH₄Cl solution. The aqueous phase was extracted with ethyl acetate (3 x 20 ml), the combined extracts were dried (Na₂SO₄) and the solvent was removed under reduced pressure. PLC (B:A, 9:1, v/v) of the product afforded the title compound (55.1 mg, 72.2%, R_f 0.48).

11.4.3 Reaction of 2,4,6-Trimethoxybenzo[*b*]furan-3(2H)-one (240) with *s*-BuLi and (-)-sparteine.

A solution of 2,4,6-trimethoxybenzo[*b*]furan-3(2H)-one (**240**) (50 mg, 0.22 mmol) in THF (2 ml) was cooled to -78°C and *s*-butyllithium (270 µl of a 0.9 mol/dm³ solution in hexane, 0.24mmol, 1.1 equiv.) was added. This mixture was stirred for 15 minutes at -78°C after which (-)-sparteine (55 µl, 0.24 mmol, 1.1 equiv.) was added and the reaction mixture was stirred at -78°C for 30 minutes. MeI (21 µl, 0.33 mmol, 1.5 equiv.) was added drop-wise and the mixture was stirred for 1 hour at -78°C and quenched by addition of 2 ml of a saturated NH₄Cl solution. The aqueous phase was extracted with ethyl acetate (3 x 20 ml), the combined extracts were dried (Na₂SO₄) and the solvent was removed under reduced pressure. PLC (B:A, 9:1, v/v) of the product afforded only starting material (**240**) (13.4 mg).

¹H NMR [CDCl₃, Plate 37]:   See § 11.2.6

The experiment was repeated as above, but after addition of the MeI the temperature of the reaction mixture was allowed to rise to 0°C over a period of 3 hours. Again only starting material (240) (5.2 mg) was isolated from the reaction mixture.

During a final repetition of the experiment, 4-methoxybenzylchloride (44 μl, 0.33 mmol, 1.5 equiv.) was added to the reaction mixture after the addition of the (-)-sparteine (see above). The mixture was stirred for 24 hours at -78°C and worked-up as described above. PLC (B:A, 9:1, v/v) again afforded only starting material (240) (17.5 mg).

11.4.4 2,4,6-Trimethoxy-3-trimethylsilyloxybenzo[b]furan[216] (277)

2,4,6-Trimethoxybenzo[b]furan-3(2H)-one (240) (100 mg, 0.45 mmol), triethylamine (93 μl, 0.67 mmol, 1.5 equiv.) and trimethylsilylchloride (85 μl, 0.67 mmol, 1.5 equiv.) were successively introduced into a reaction flask containing dry acetonitrile (5 ml) under N_2. Sodium iodide (100 mg, 0.67 mmol, 1.5 equiv.) in dry acetonitrile (5 ml) was added drop-wise (15 minutes) at room temperature and the reaction mixture was stirred until the reaction was complete (^{1}H NMR, 1 hour). Cold pentane (20 ml) and ice water was added successively and after removal of the organic layer, the aqueous layer was extracted twice more with pentane (20 ml). The combined organic layers were washed with ice water (2 x 20 ml) and dried (Na_2SO_4). The solvent was removed under a fast N_2 stream to yield the title compound (116 mg, 99%) as a colorless oil.

^{1}H NMR [C_6D_6, Plate 43]:   δ 6.58 (d, J 2.0 Hz, 7-H), 6.46 (d, J 2.0 Hz, 5-H), 3.72 (s, OCH₃), 3.44 (s, OCH₃), 3.41 (s, OCH₃), 0.43 (s, OSi(CH₃)₃).

11.4.5 Reaction of 2,4,6-trimethoxy-3-trimethylsilyloxybenzo[b]furan (277) with s-BuLi and (-)-sparteine.

A solution of the silyl enol ether (277) (0.22 mmol - 0.44 mmol) was dissolved in the appropriate solvent (see **Table 8.4**) and cooled to -78°C (-25°C in the case of entry 5, **Table 8.4**). n- butyllithium (0.22 - 0.44 mol, 1 equiv.) and (-)-sparteine ((0.22 - 0.44 mol, 1 equiv.) was added and the mixture was stirred for 30 minutes. The electrophile, 4-

methoxybenzyl chloride treated with NaI before addition, (2.2 mmol - 4.4 mmol, 10 equiv.) was added at -78°C and the mixture was stirred at this temperature for 8 - 24 hours. The reaction was quenched at -78°C by addition of 2 ml of a saturated NH₄Cl solution and the aqueous phase was extracted with ethyl acetate (3 x 20 ml). PLC (B:A, 9:1, v/v) of the product afforded 2,4,4',6-tetra-*O*-methylmaesopsin (**206**) (see § 10.2.6) in 4.1 - 59.2% yield (see **Table 8.4**).

¹H NMR [CDCl₃, Plate 12]:   See § 10.2.6

Physical Data

Appendix A
NMR-Plates 1 – 43

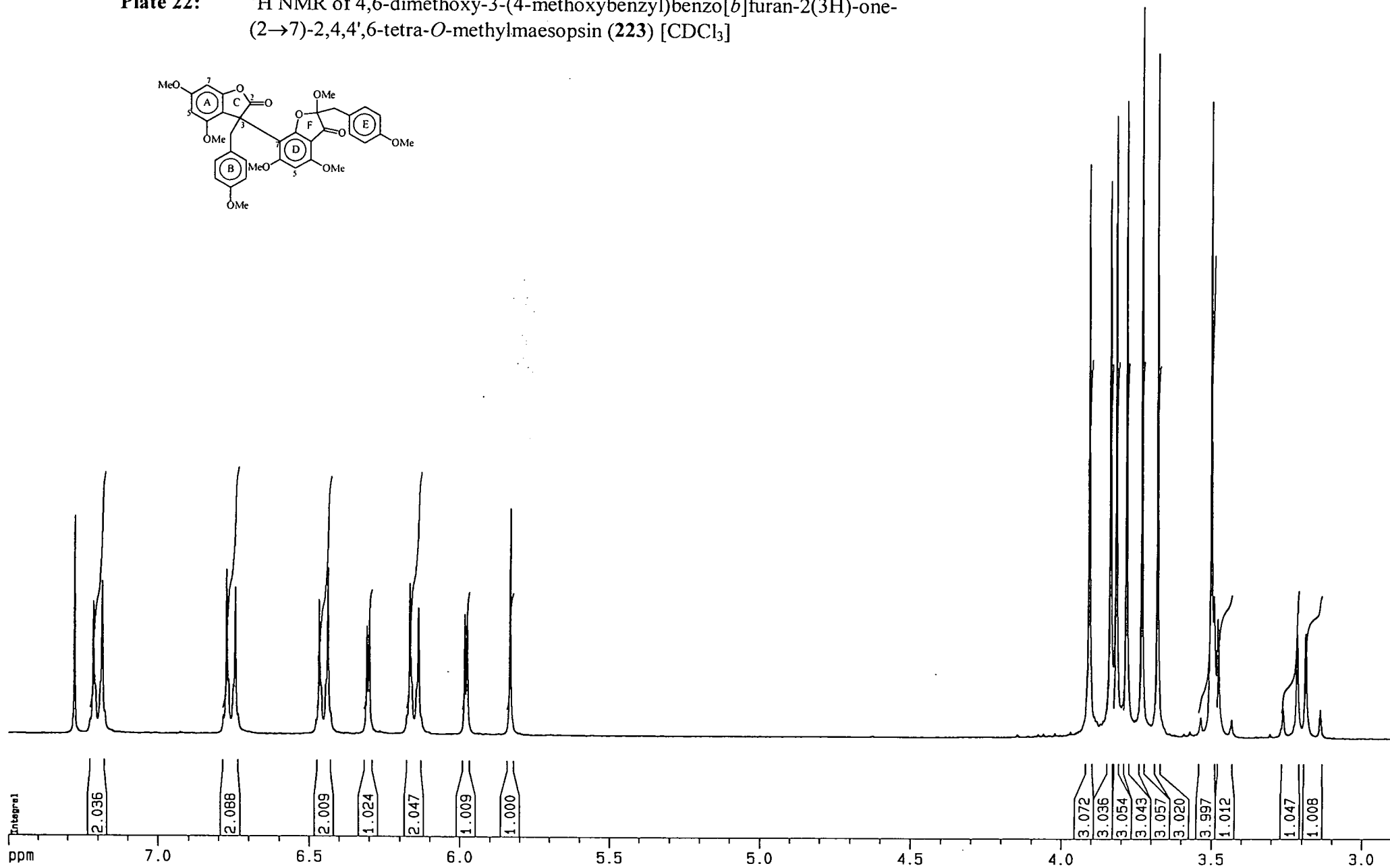**Plate 1:** ^{1}H NMR of (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3α→7)-(2*R*)-2,4,4',6-tetra-*O*-methylmaesopsin (**175**) [CDCl$_3$]

3–H(C)

OMe OMe 2 x OMe OMe OMe 2–OMe(F) α–CH₂ α–CH₂

4.70 ppm 3.95 3.90 3.85 3.80 3.75 ppm 3.20 3.15 ppm 3.05 3.00 ppm 2.85 2.80 ppm

2/6–H(B) 2/6–H(E) 3/5–H(B) 3/5–H(E) 8–H(A) 6–H(A) 5–H(D) 2–H(C)

7.35 7.30 7.25 7.20 7.15 ppm 6.85 6.80 6.75 6.70 ppm 6.20 ppm 5.90 5.85 ppm

Plate 2: ^{13}C NMR of (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→7)-(2R)-2,4,4',6-tetra-O-methylmaesopsin (175) [CDCl$_3$]

Top spectrum labels:
6–C(A), 8–C(A), 5–C(D), 2–C(C), CDCl$_3$, 7 x OMe, α–CH$_2$, 3–C(C)

Bottom spectrum labels:
3–C(F), 4–C(C), 5–C(A), 9–C(A), 4–C(B), 6–C(D), 7–C(A), 4–C(D), 4–C(E), 8–C(D), 2/6–C(E), 1–C(B), 2/6–C(B), 1–C(E), 3/5–C(B), 3/5–C(E), 2–C(F), 10–C(A), 9–C(D), 7–C(D)

Plate 3: ^{1}H NMR of (2R,3S)-4',5,7-tri-*O*-methylnaringenin-(3α→7)-(2S)-2,4,4',6-tetra-*O*-methylmaesopsin (**176**) [CDCl$_3$]
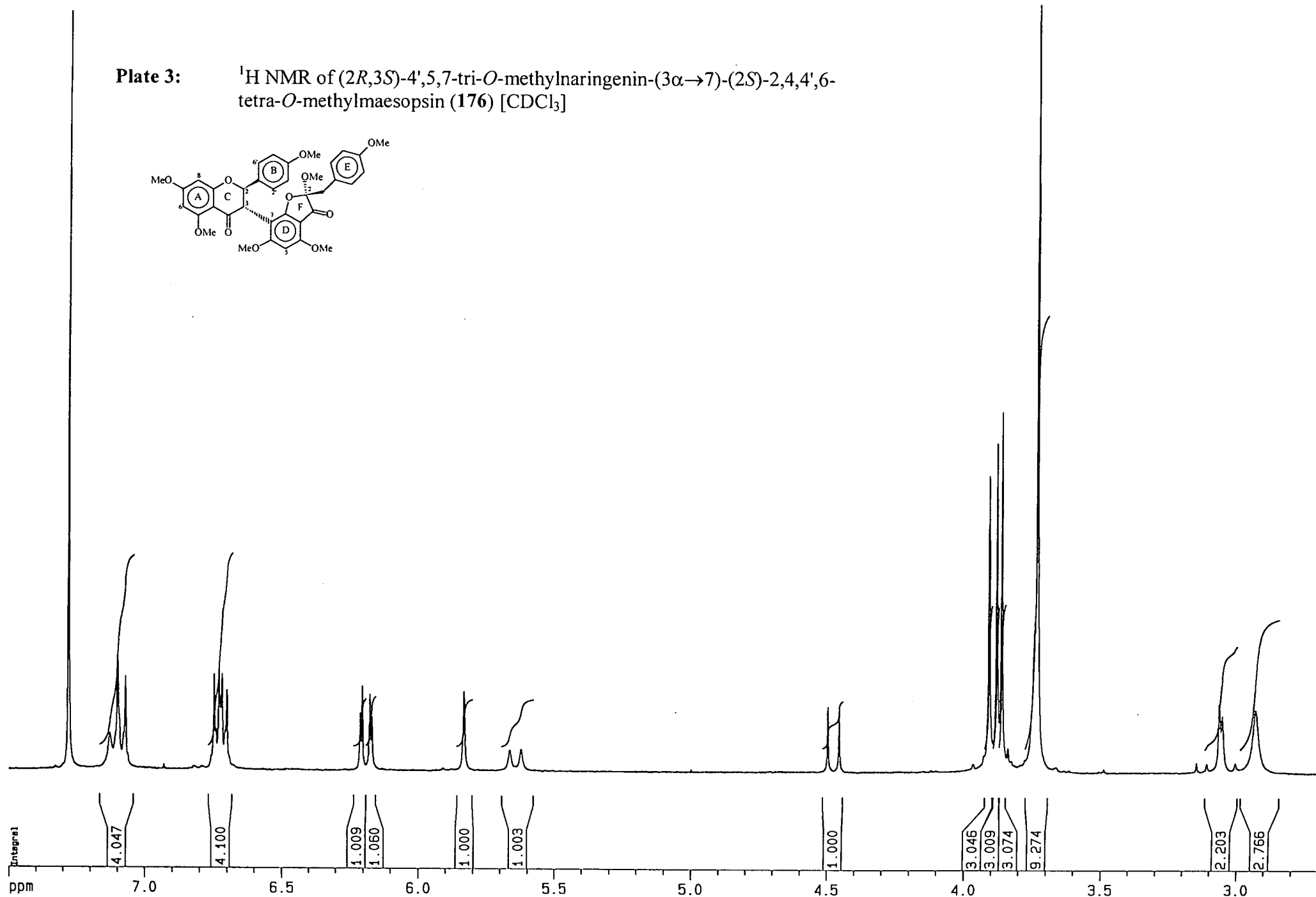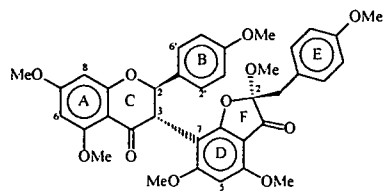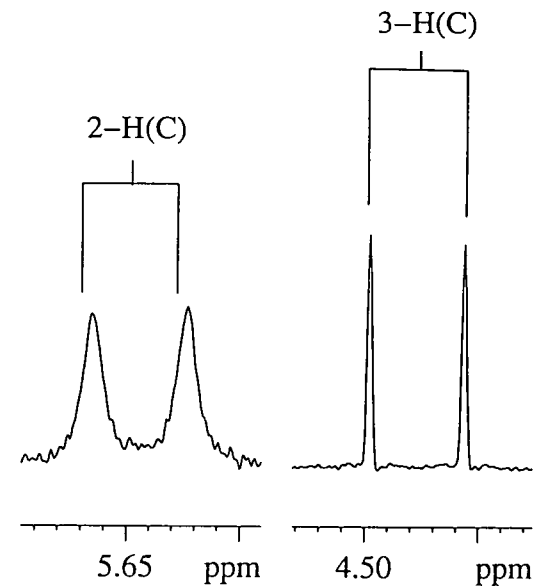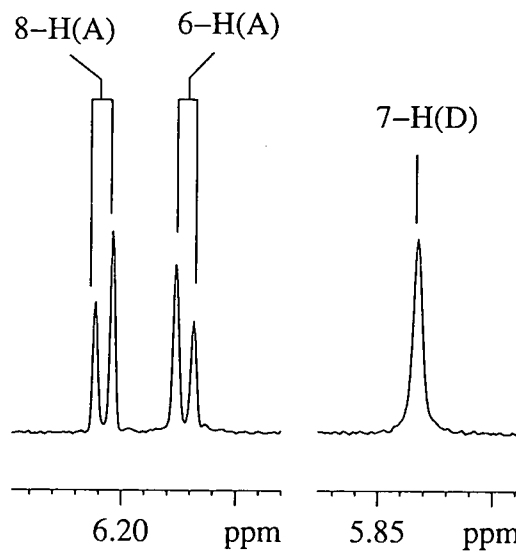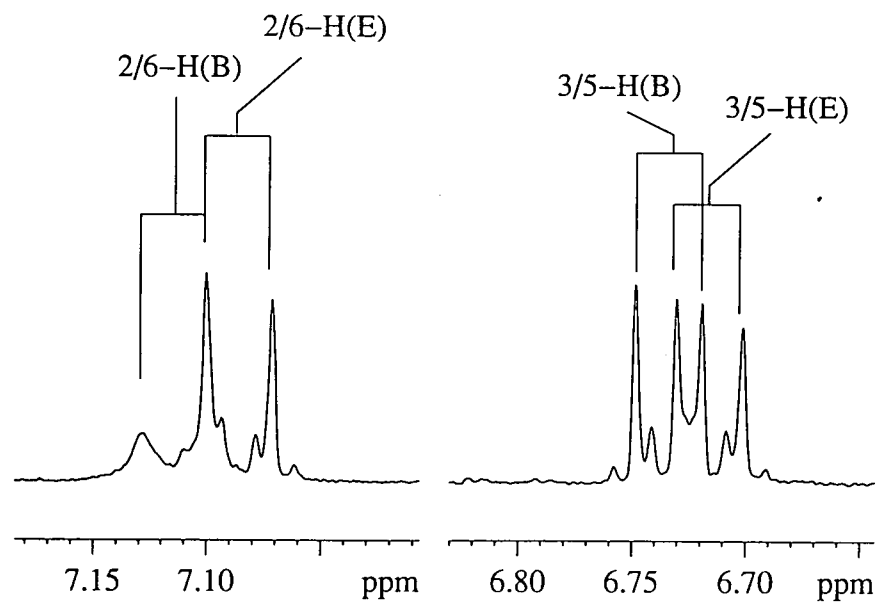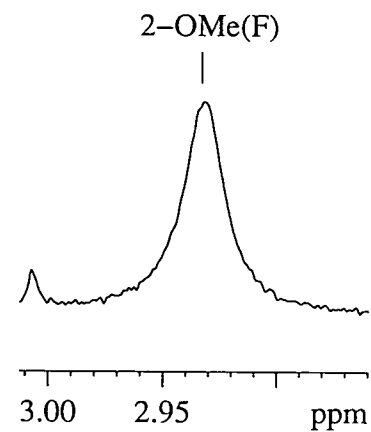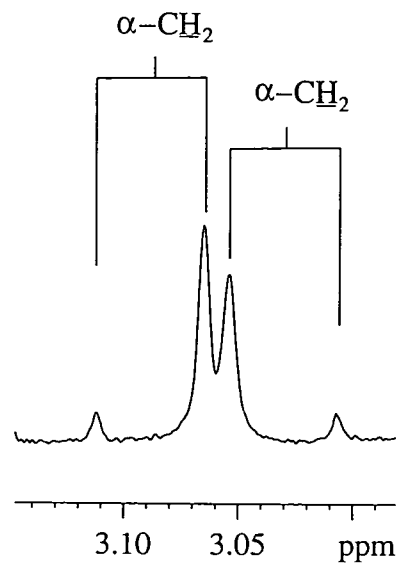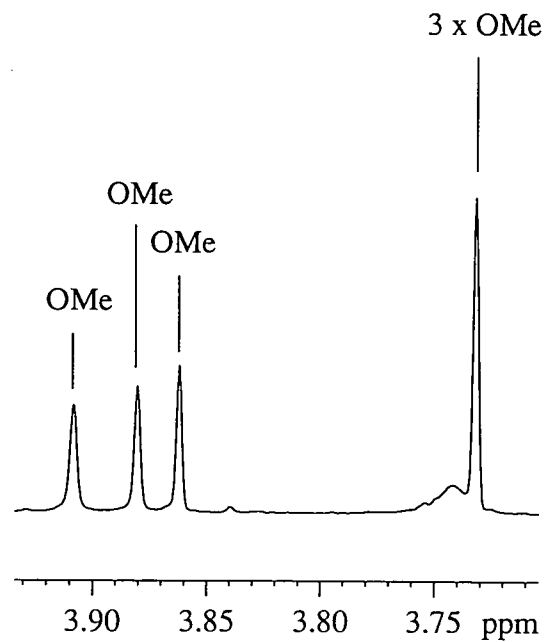
Plate 4: ^{13}C NMR of (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3α→7)-(2*S*)-2,4,4',6-tetra-*O*-methylmaesopsin (**176**) [CDCl₃]

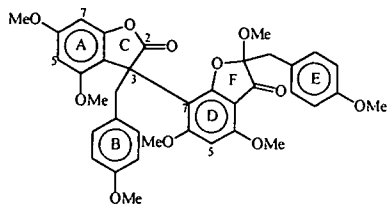Top spectrum labels: 6–C(A), 8–C(A), 5–C(D), 2–C(C), CDCl₃, 7 x OMe, 3–C(C), α–$\underline{C}$H₂

Bottom spectrum labels: 3–C(F), 4–C(C), 8–C(D), 6–C(D), 5–C(A), 9–C(A), 7–C(A), 4–C(B), 4–C(D), 4–C(E), 2/6–C(E), 1–C(B), 2/6–C(B), 1–C(E), 3/5–C(B), 3/5–C(E), 2–C(F), 10–C(A), 9–C(D), 7–C(D)
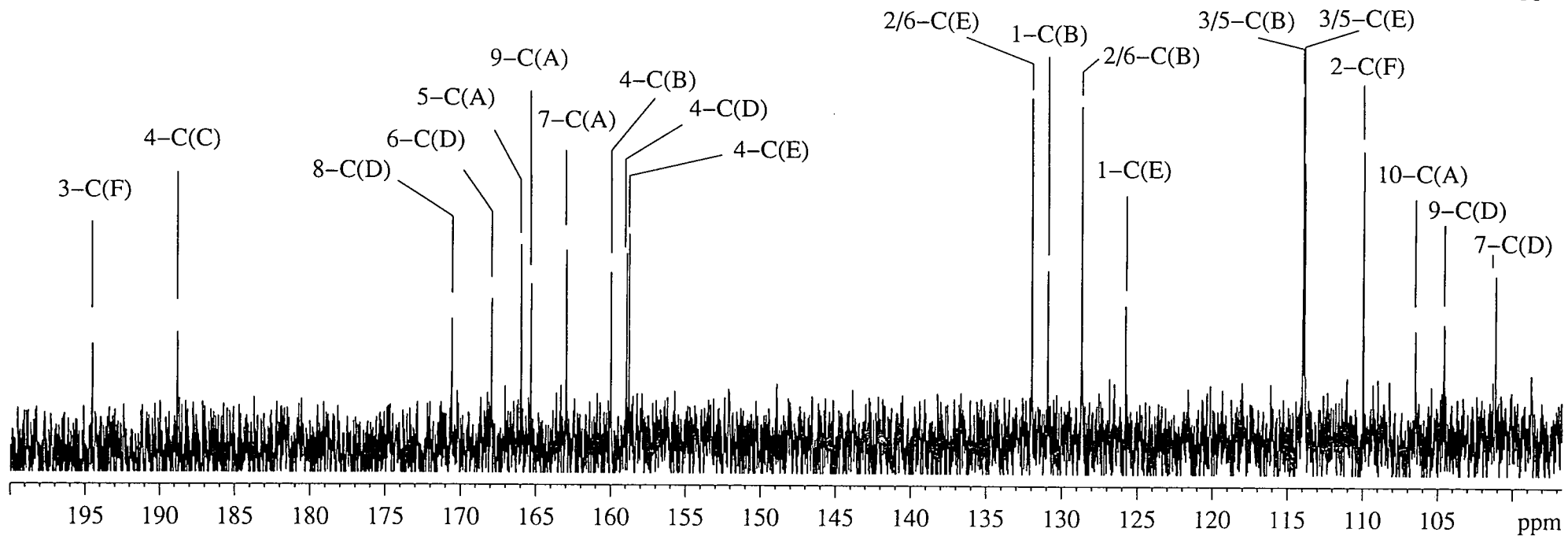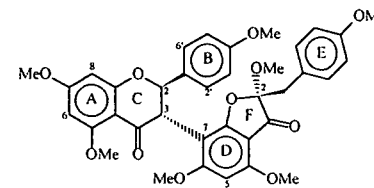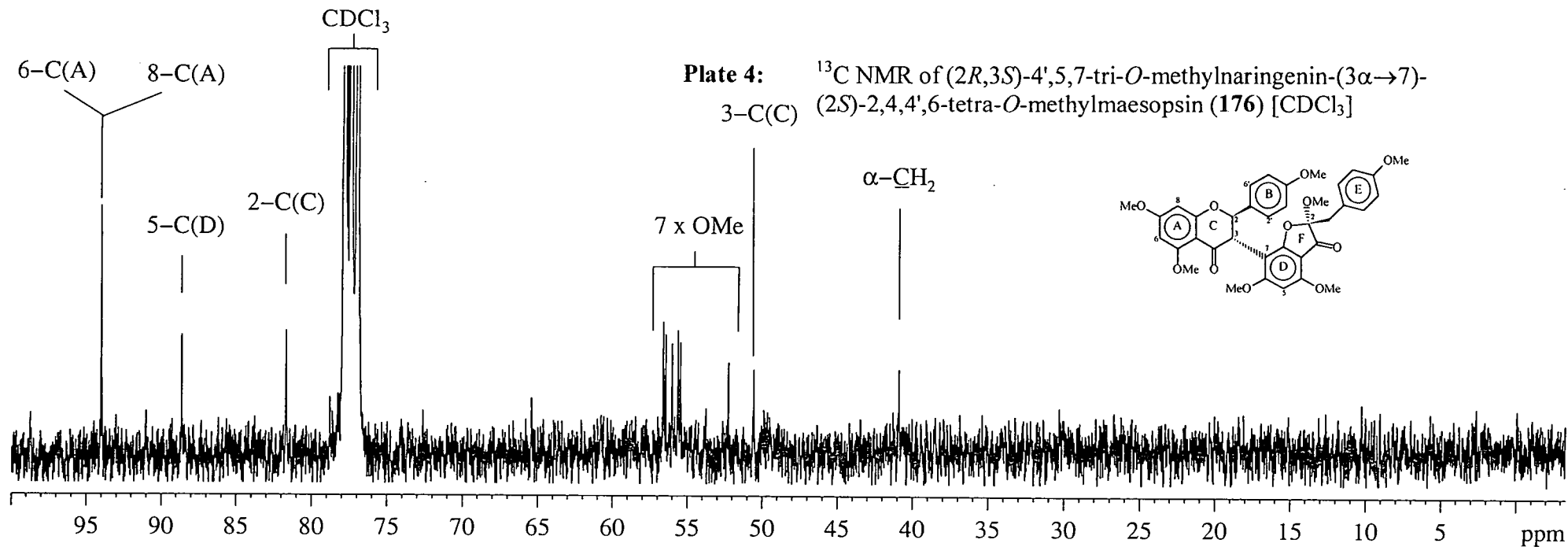
Plate 5: ¹H NMR of 7-[2-(4-methoxyphenyl)ethyl]-2,4,4',6-tetra-O-methylmaesopsin (**188**)/(**190**) [CDCl₃]

OMe   OMe OMe   OMe

3.95   3.90   3.85   3.80   3.75   ppm

2-OMe(F)

α-CH$_2$(F)   α-CH$_2$(F)

3.20   3.15   ppm

4 x α-CH$_2$

2.85   2.80   2.75   2.70   ppm

2/6-H(E) 2/6-H(B)

7.20   7.15   7.10 ppm

3/5-H(B)   3/5-H(E)

6.85   6.80   6.75   ppm

5-H(D)

5.95   5.90   ppm
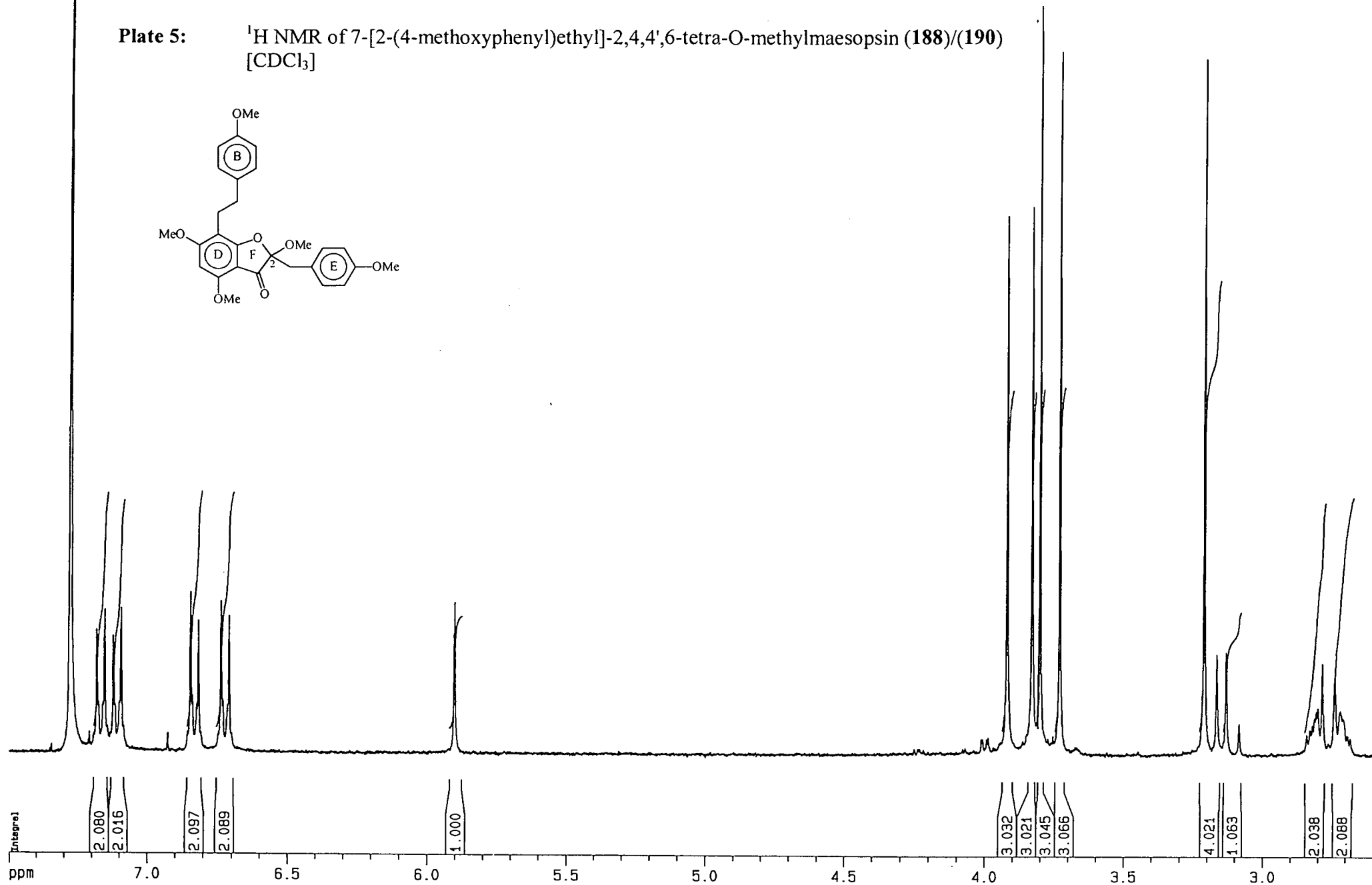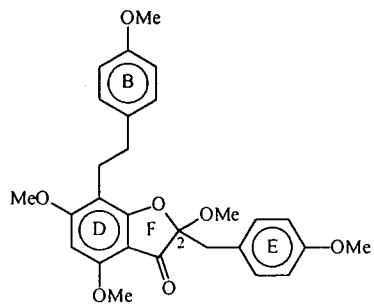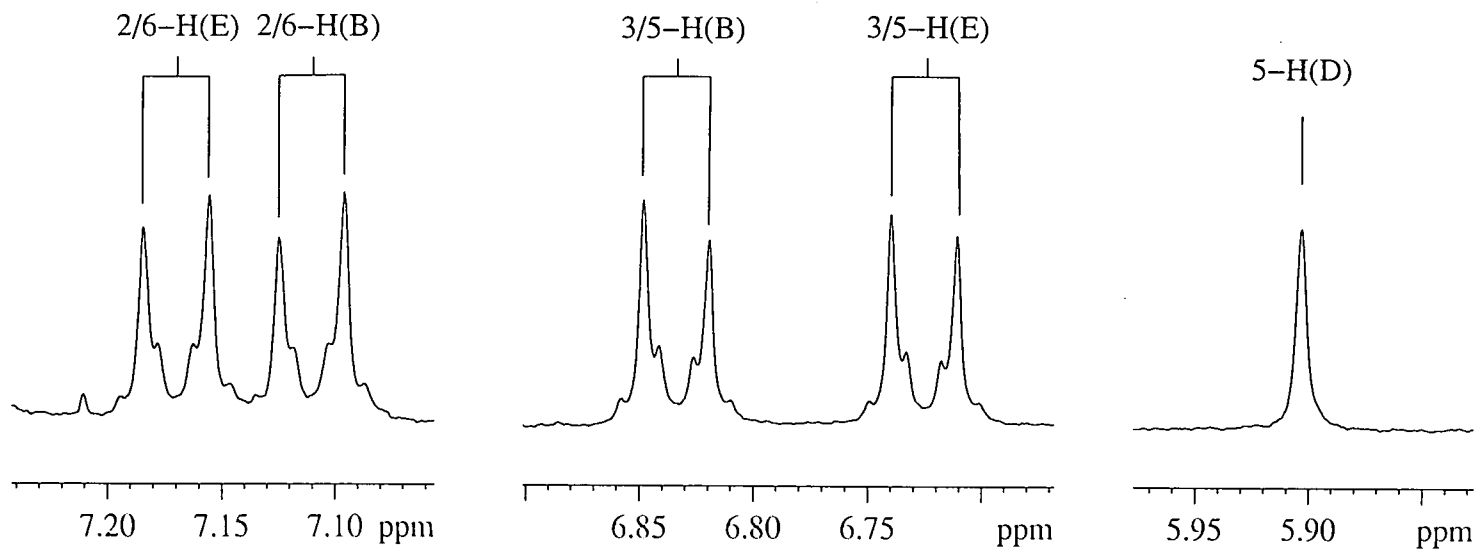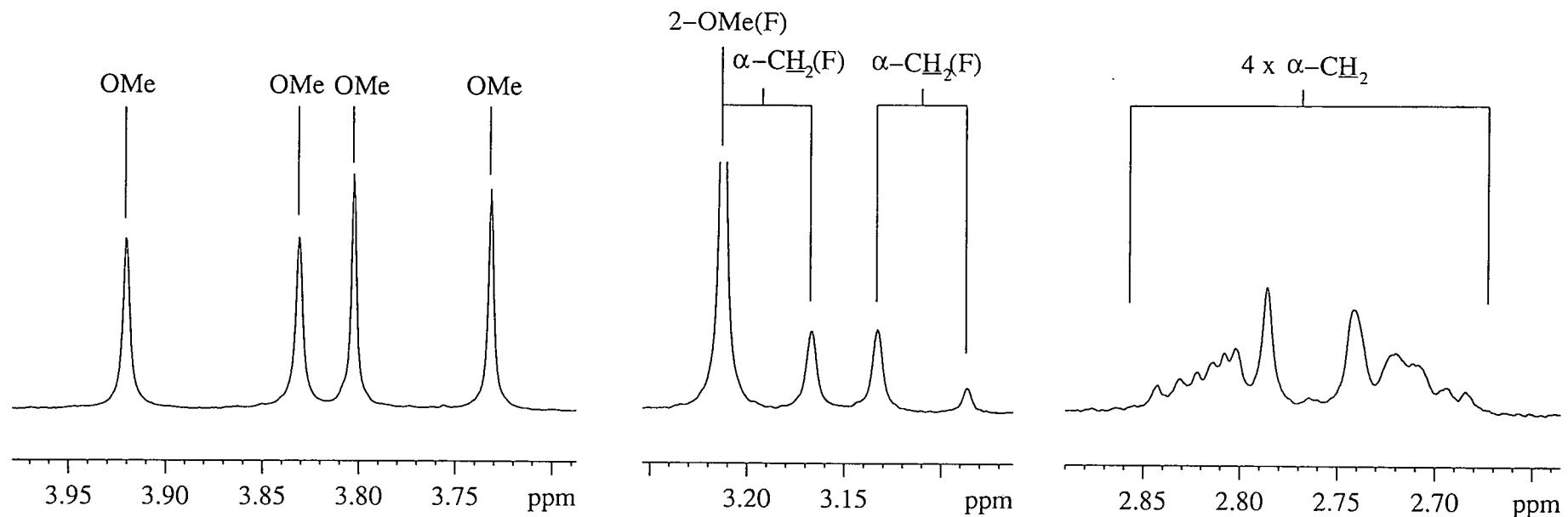
Plate 6: ^{1}H NMR of (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3α→5)-(2*R*)-2,4,4',6-tetra-*O*-methylmaesopsin (**197**) [CDCl$_3$, 343K]
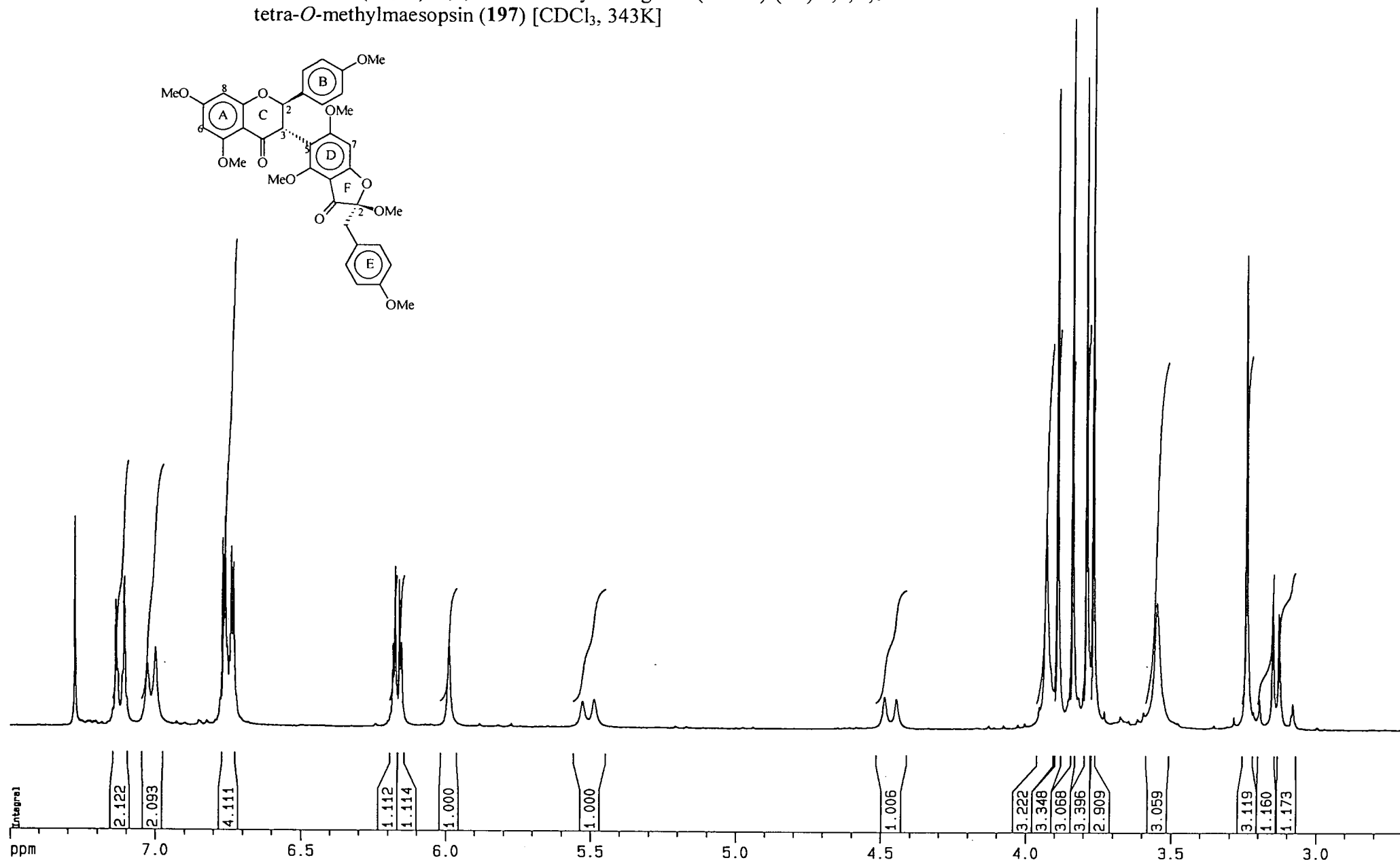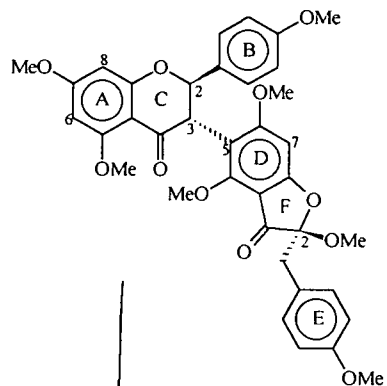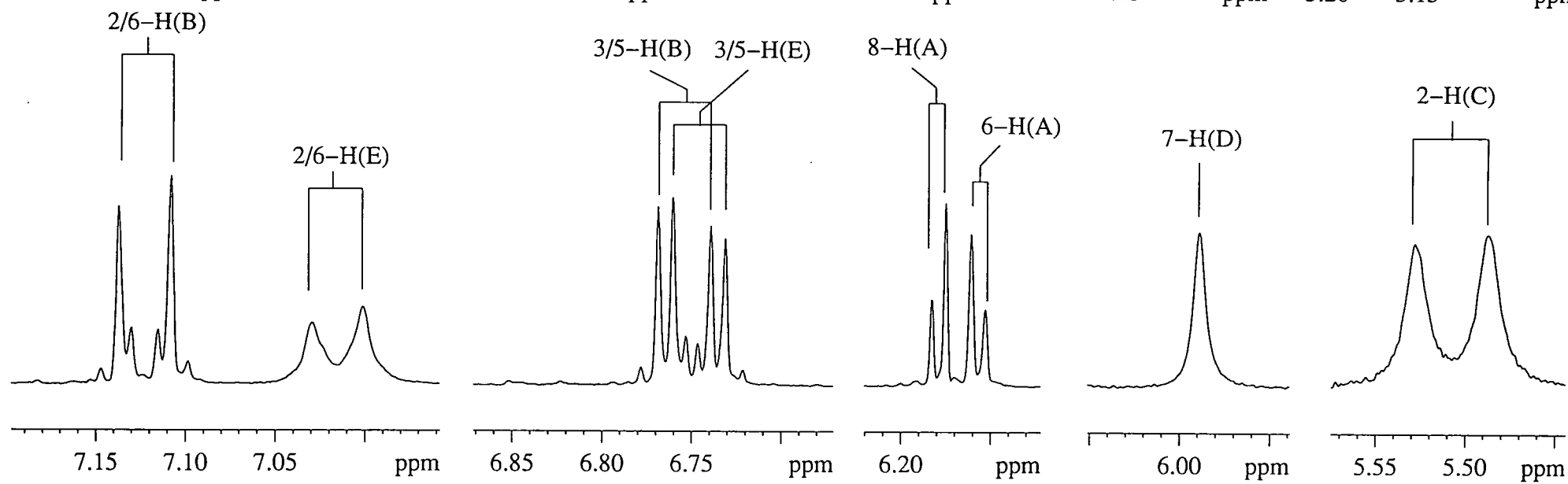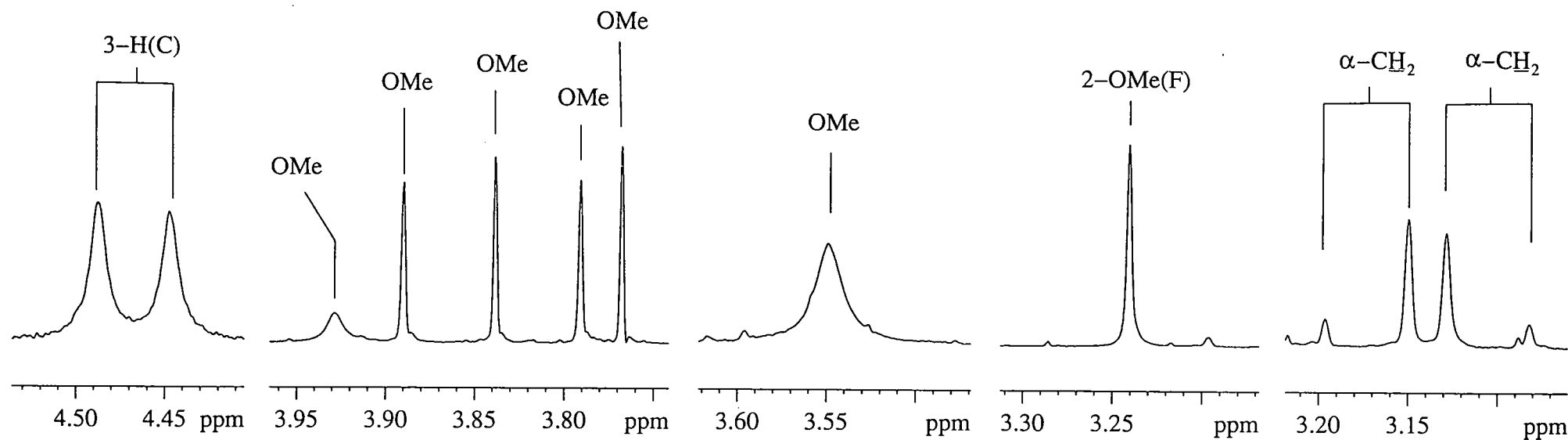
Plate 7: ^{13}C NMR of (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→5)-(2R)-2,4,4',6-tetra-O-methylmaesopsin (**197**) [CDCl$_3$]
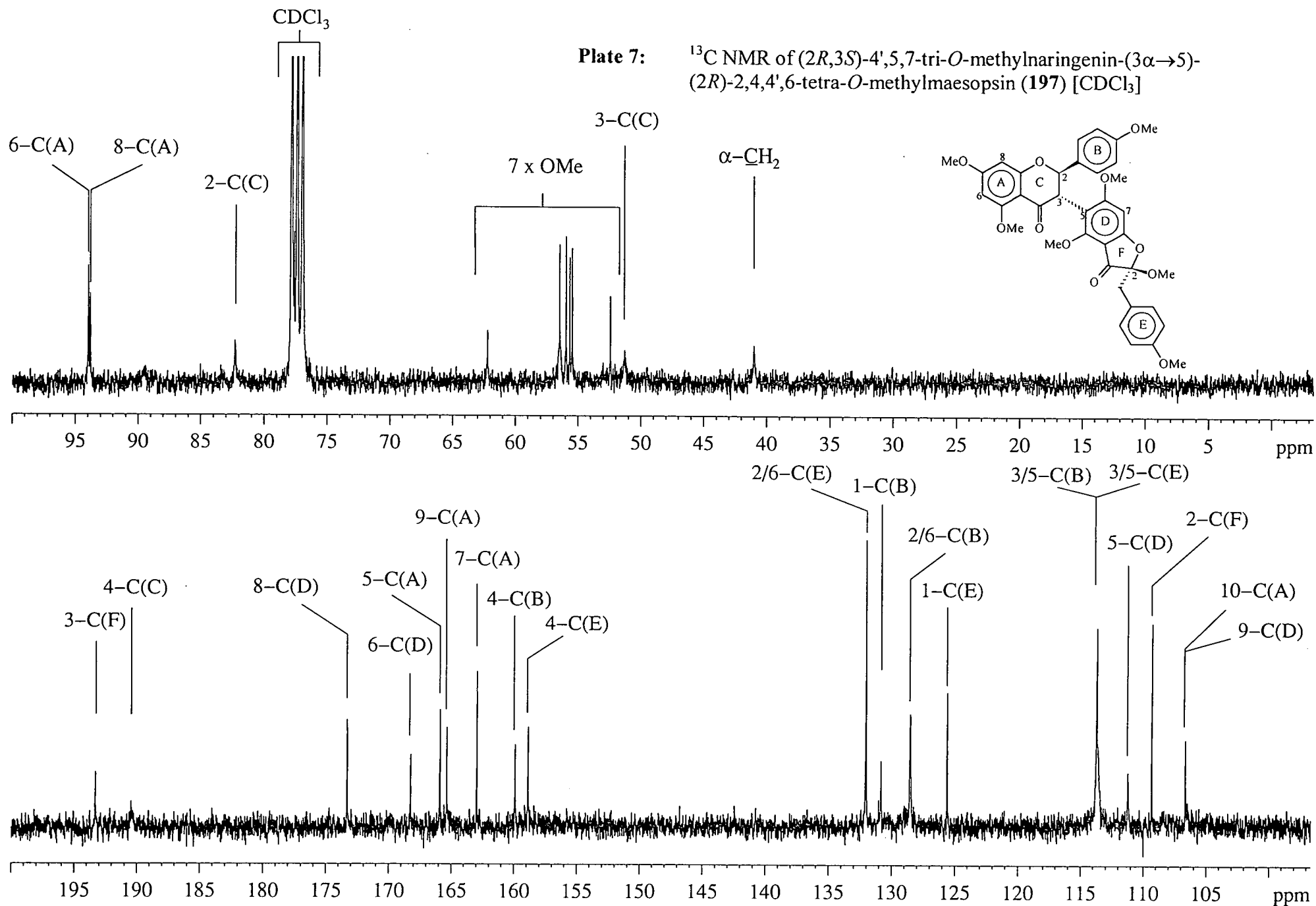
^{1}H NMR of (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→5)-(2S)-2,4,4',6-tetra-O-methylmaesopsin (**198**) [CDCl$_3$, 343K]
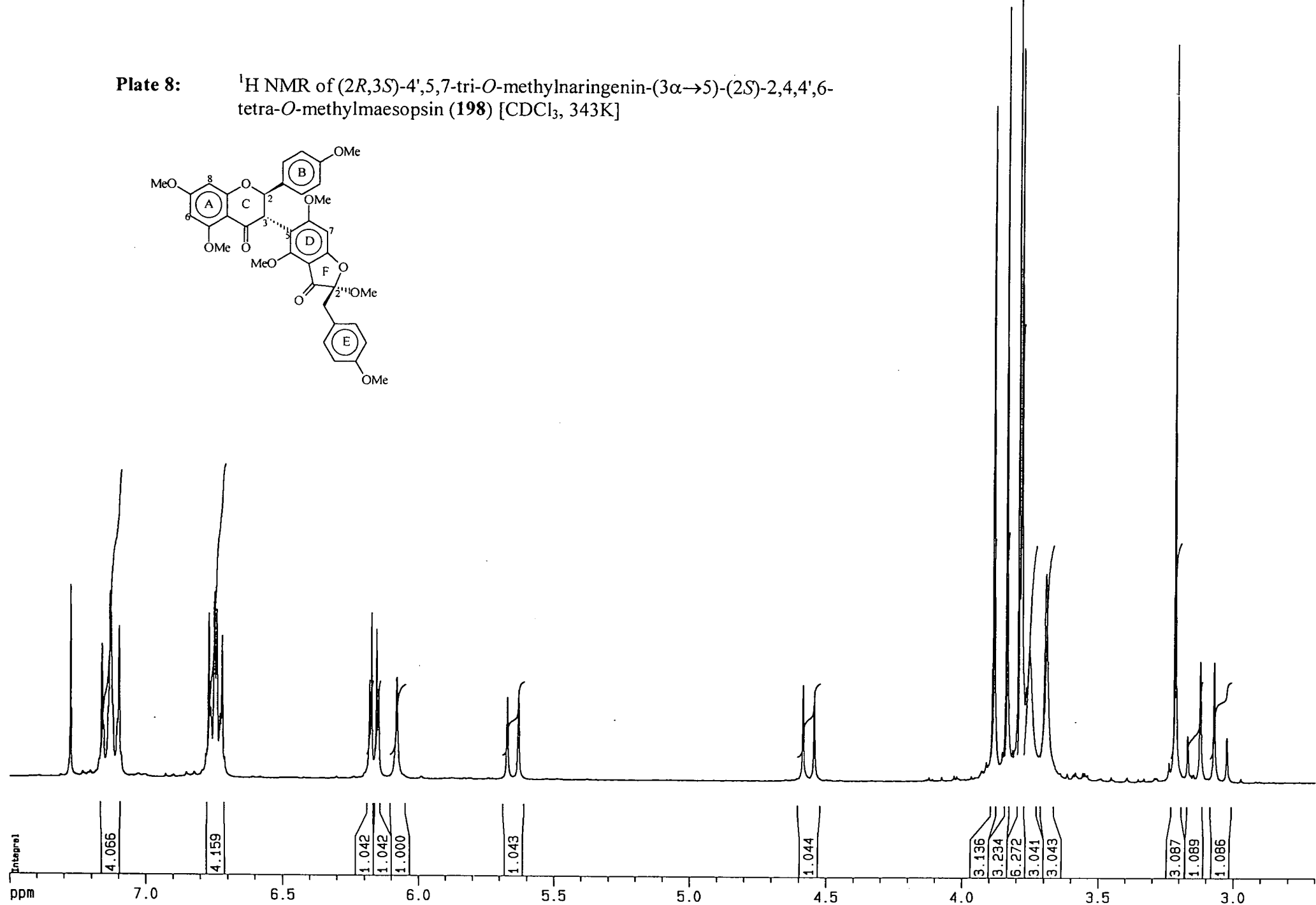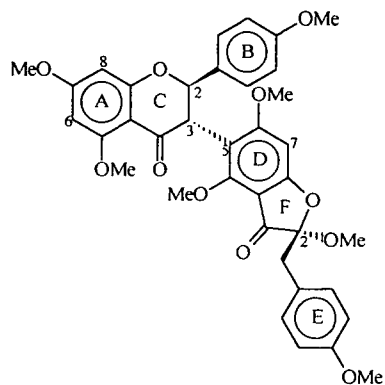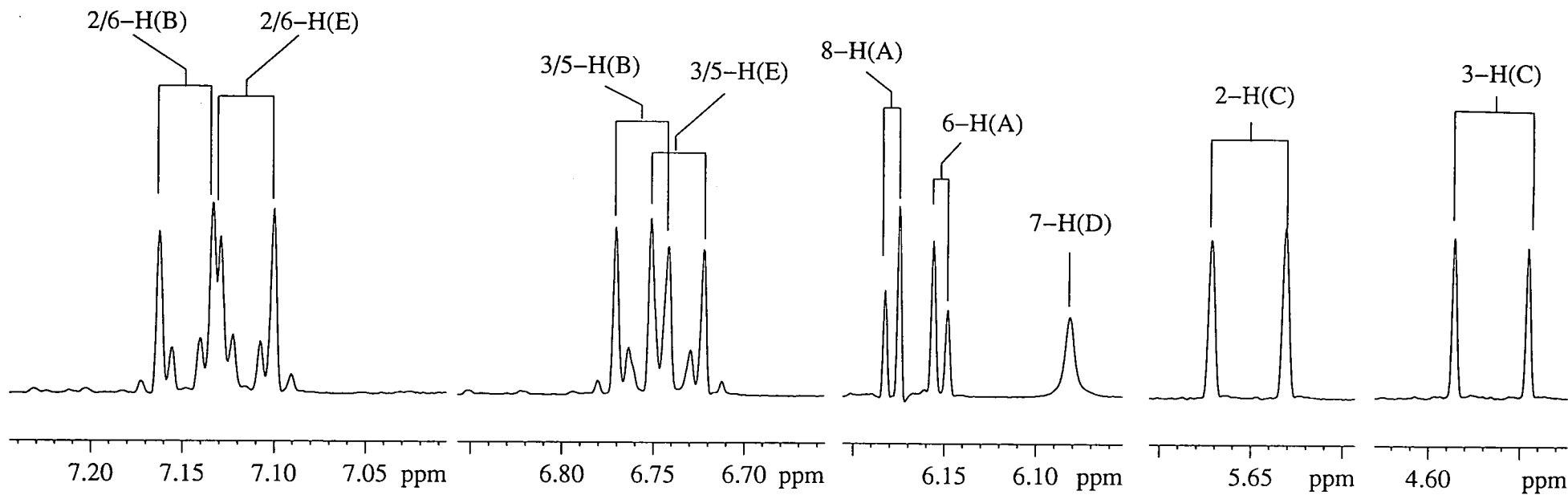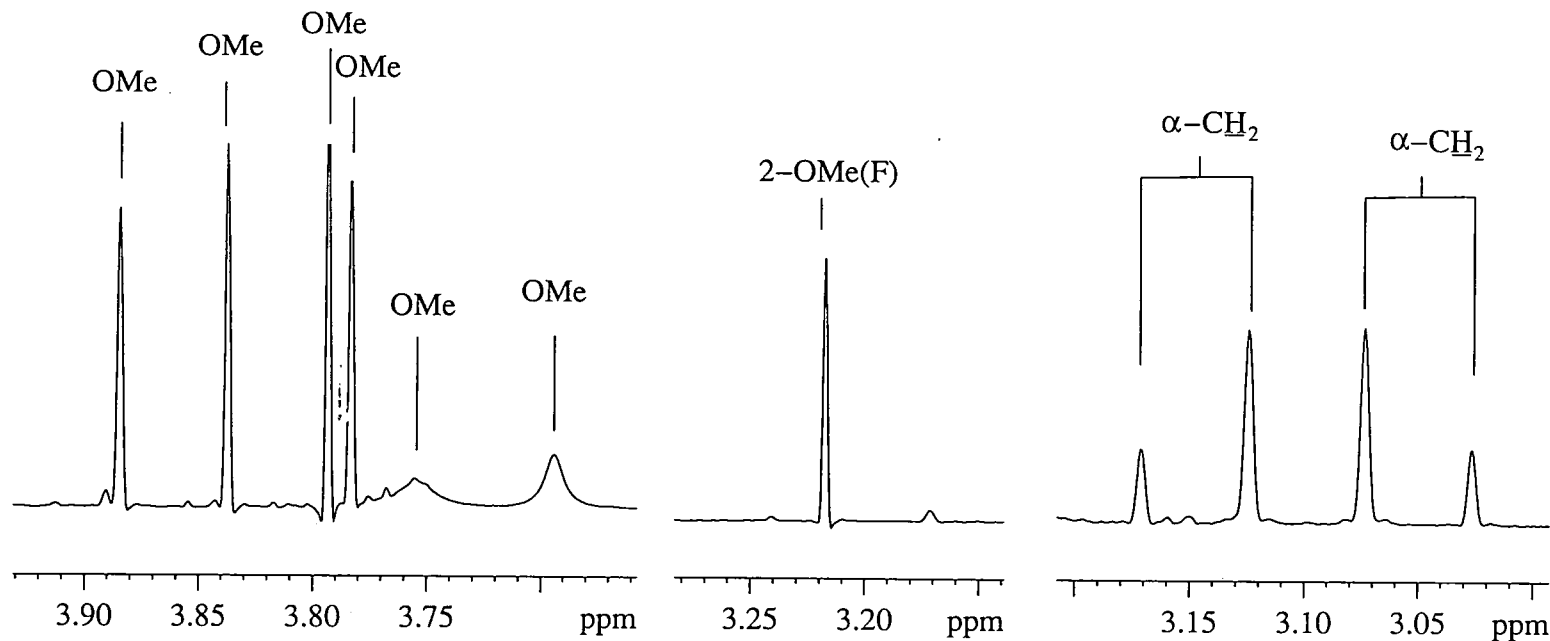
Plate 9: ^{13}C NMR of (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→5)-(2S)-2,4,4',6-tetra-O-methylmaesopsin (**198**) [CDCl₃]

Top spectrum labels: 6–C(A), 8–C(A), 2–C(C), CDCl₃, 7 x OMe, 3–C(C), α–CH₂

Bottom spectrum labels: 3–C(F), 4–C(C), 8–C(D), 6–C(D), 5–C(A), 9–C(A), 7–C(A), 4–C(B), 4–C(E), 2/6–C(E), 1–C(B), 2/6–C(B), 1–C(E), 3/5–C(B), 3/5–C(E), 5–C(D), 2–C(F), 10–C(A), 9–C(D)
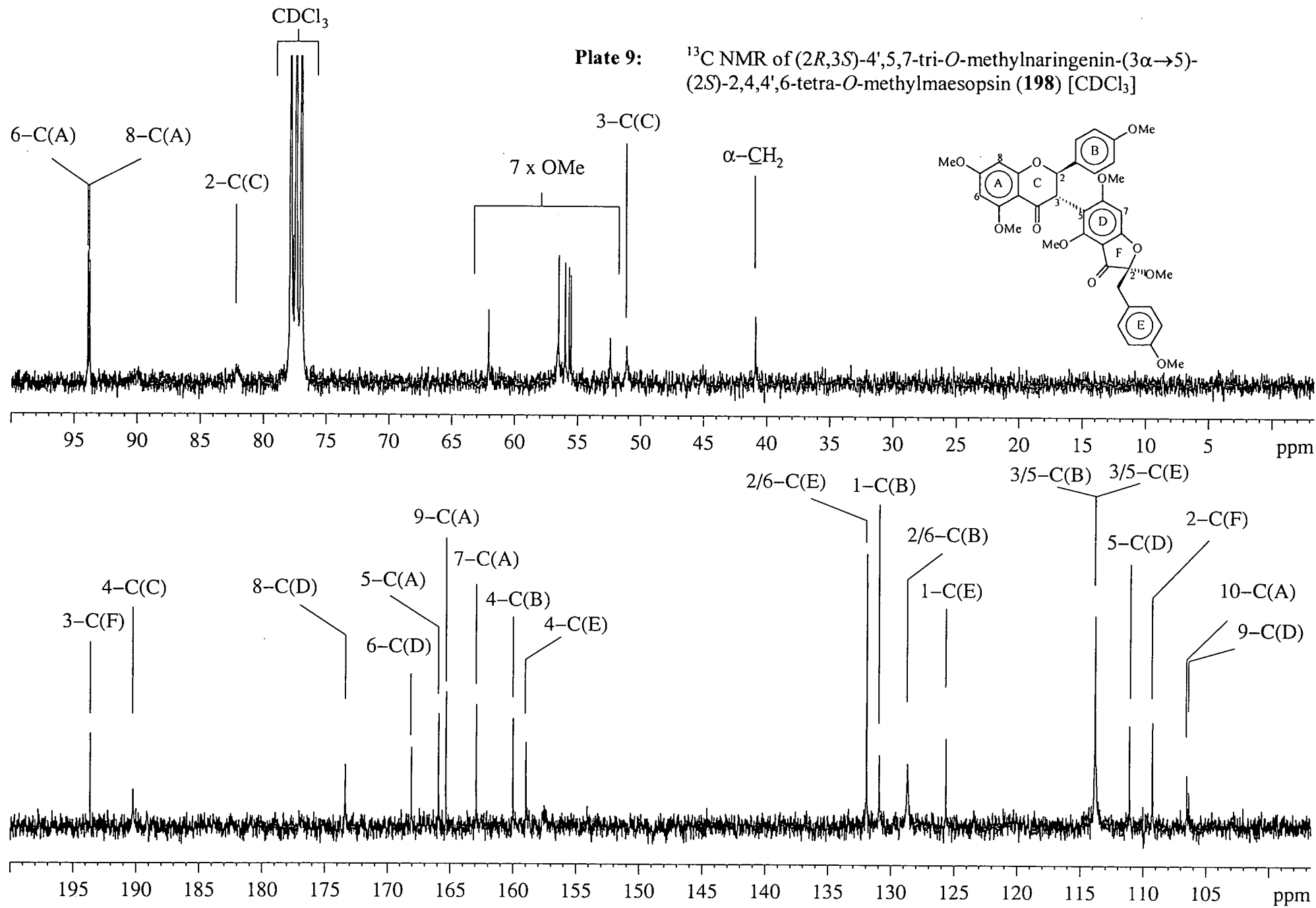
Plate 10: ^{1}H NMR of (2*S*,3*R*)-4',5,7-tri-*O*-methyldihydrogenistein-(2α→7)-(2*R*)-2,4,4',6-tetra-*O*-methylmaesopsin (**204**) [CDCl3]

3-H(C)

OMe OMe OMe OMe OMe OMe

2-OMe(F)

α-CH₂ α-CH₂

4.70 ppm   3.90 3.85 3.80 3.75 ppm   3.25 ppm   3.15 3.10 ppm

2/6-H(B)   2/6-H(E)   3/5-H(B)   3/5-H(E)   8-H(A) 6-H(A) 5-H(D)   2-H(C)

7.35 ppm   7.05 ppm   6.85 ppm   ppm   6.05 6.00 5.95 ppm   5.65 ppm
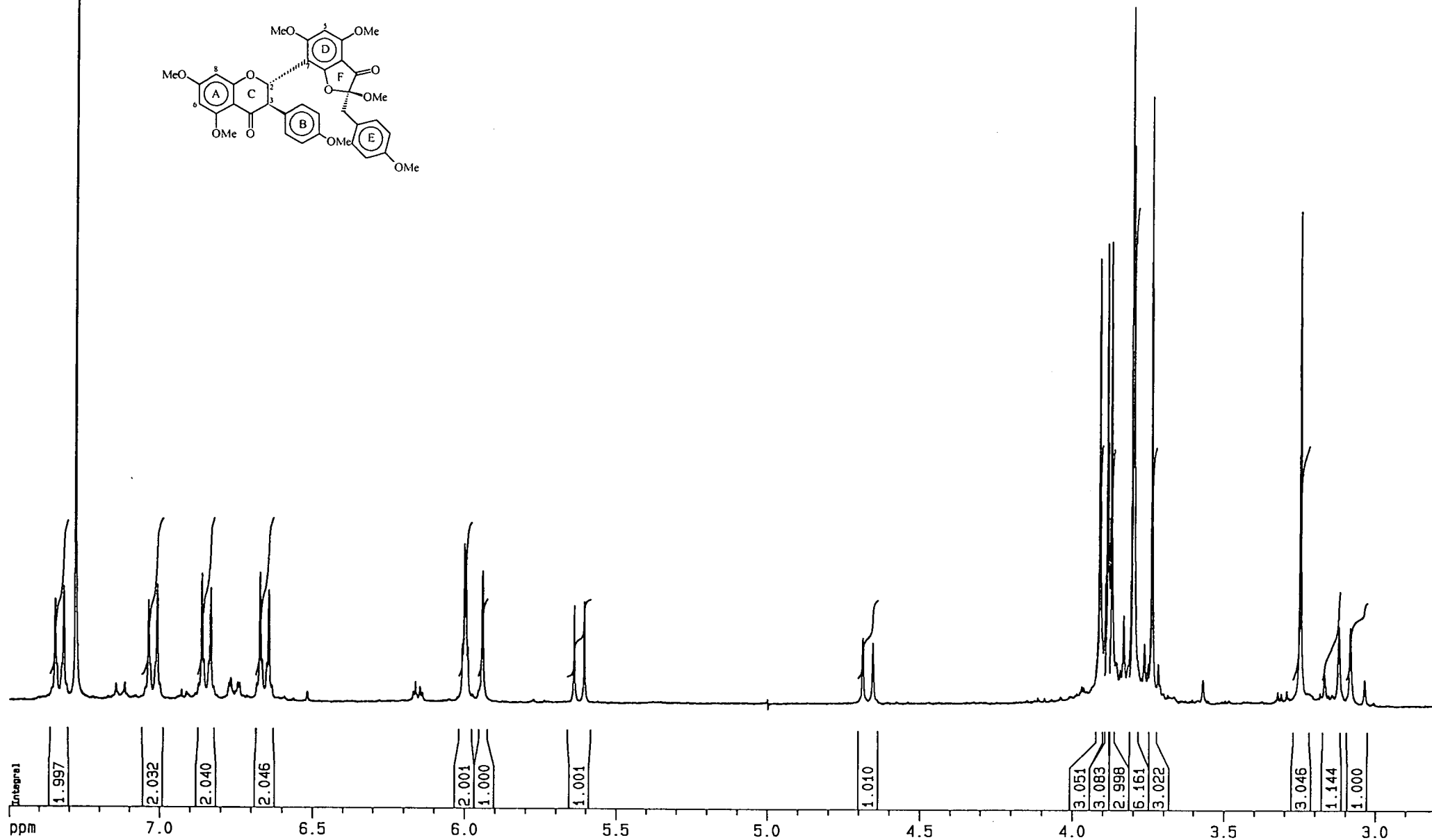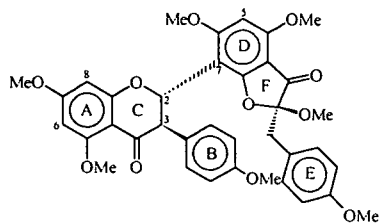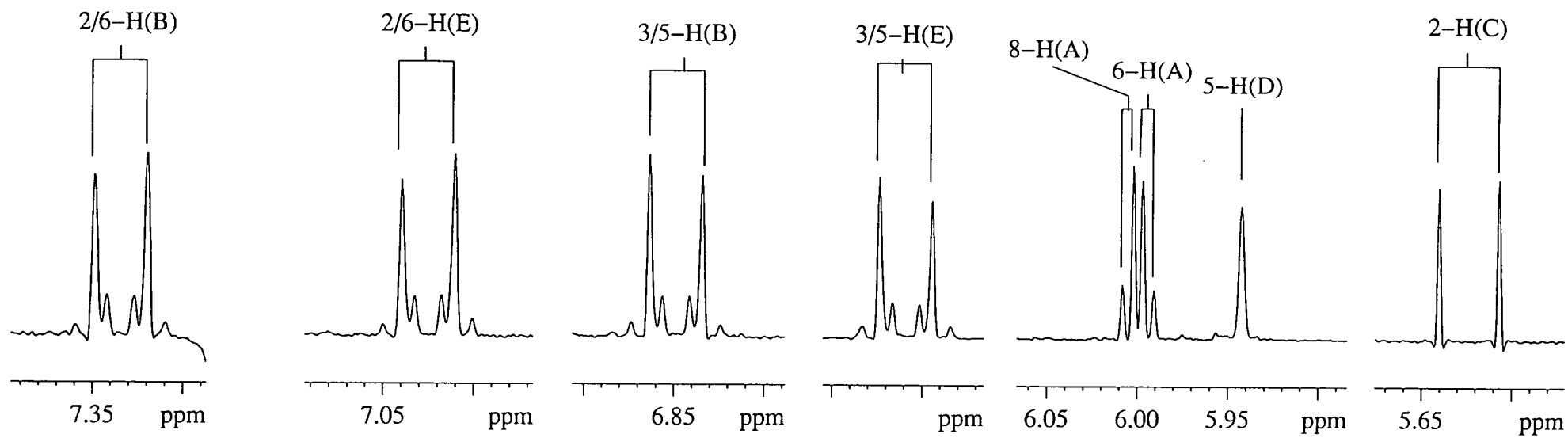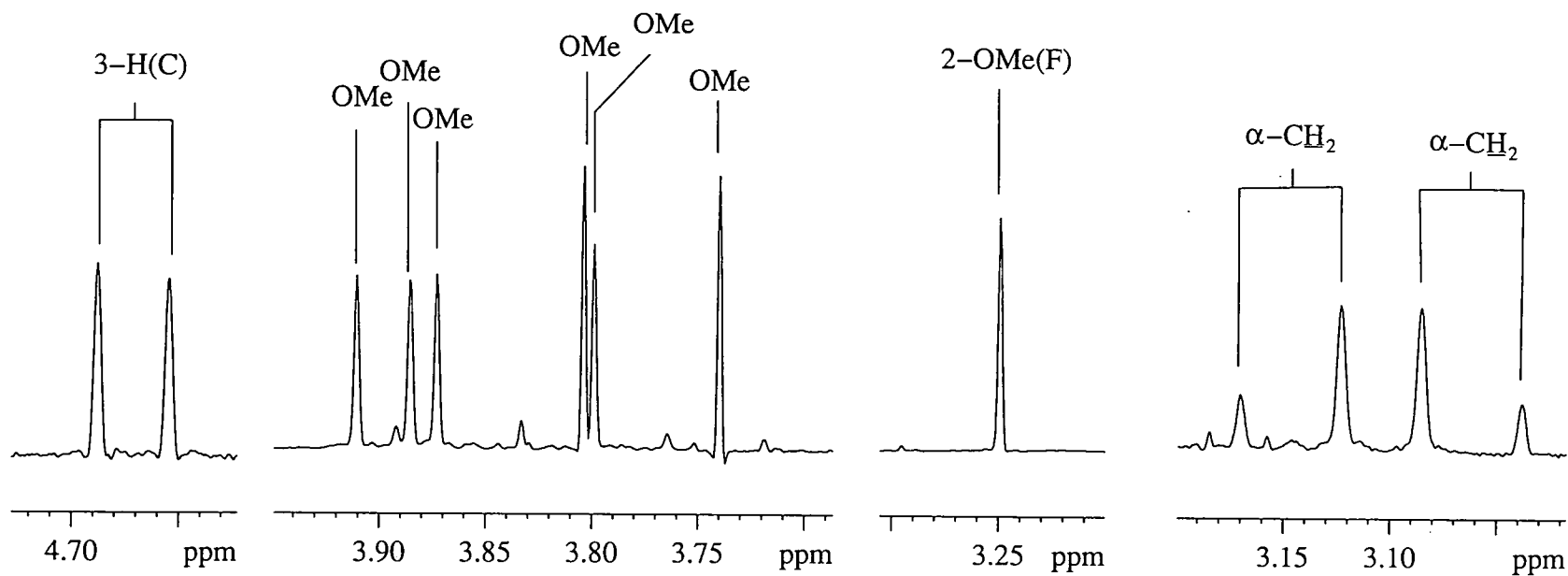
Plate 11: ^{1}H NMR of (2*S*,3*R*)-4',5,7-tri-*O*-methyldihydrogenistein-(2α→7)-(2*S*)-2,4,4',6-tetra-*O*-methylmaesopsin (**205**) [CDCl3]

3-H(C)

4.65 ppm

OMe 2 x OMe OMe OMe OMe

3.90 3.85 3.80 3.75 ppm

2-OMe(F) α-CH₂ α-CH₂

3.25 3.20 3.15 3.10 3.05 ppm

2/6-H(B)

7.40 ppm

2/6-H(E)

7.20 7.15 ppm

3/5-H(B)

6.85 ppm

3/5-H(E)

6.65 ppm

8-H(A) 6-H(A) 5-H(D)

6.05 6.00 ppm

2-H(C)
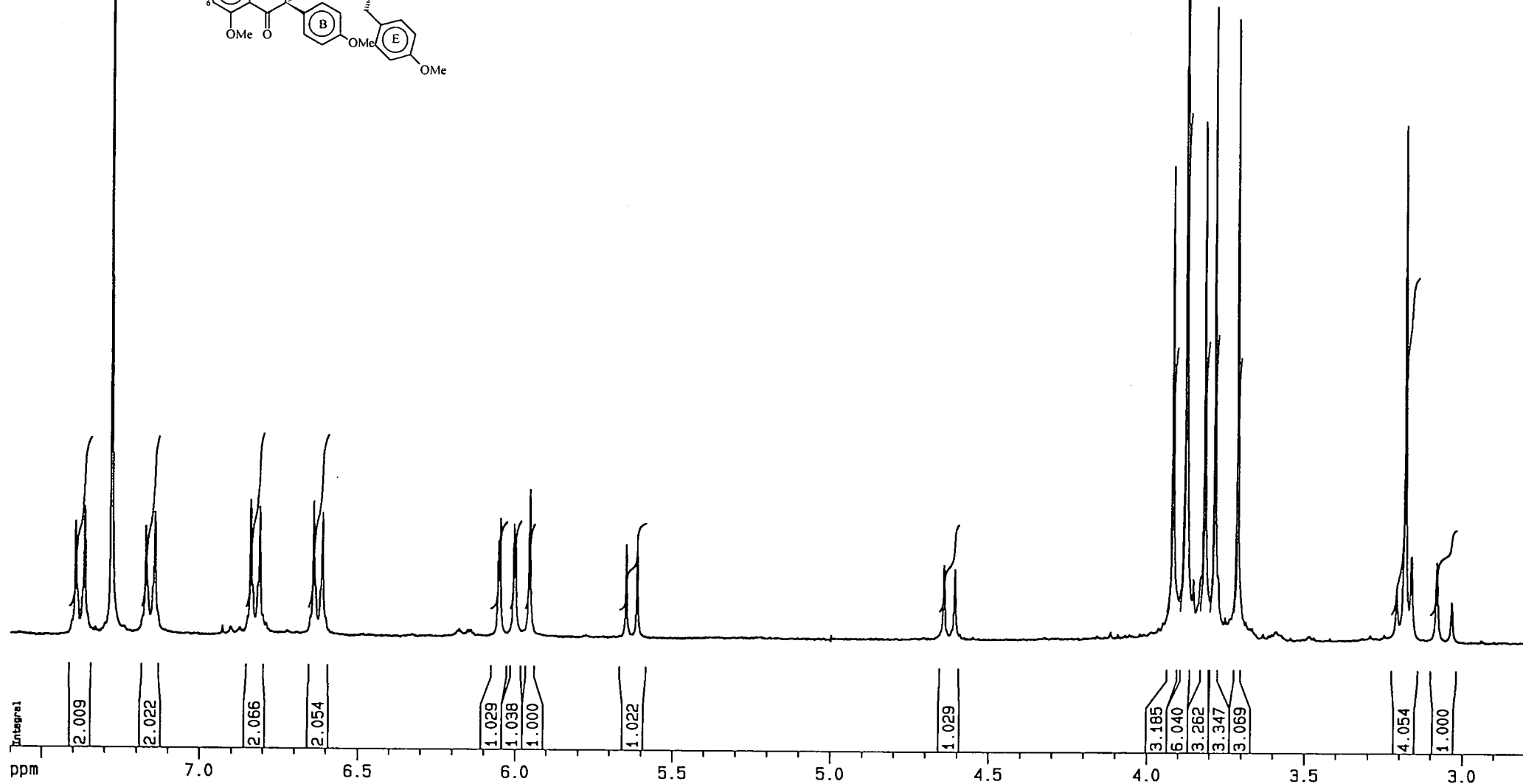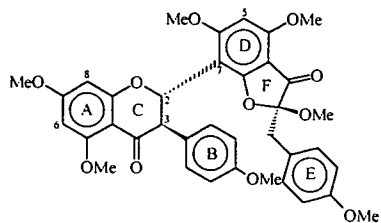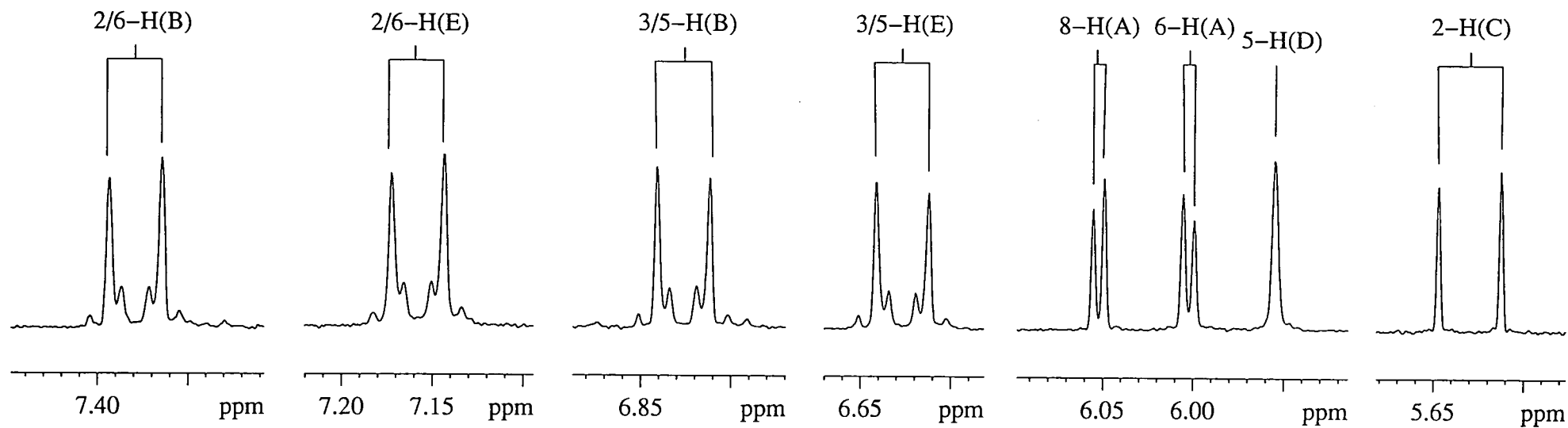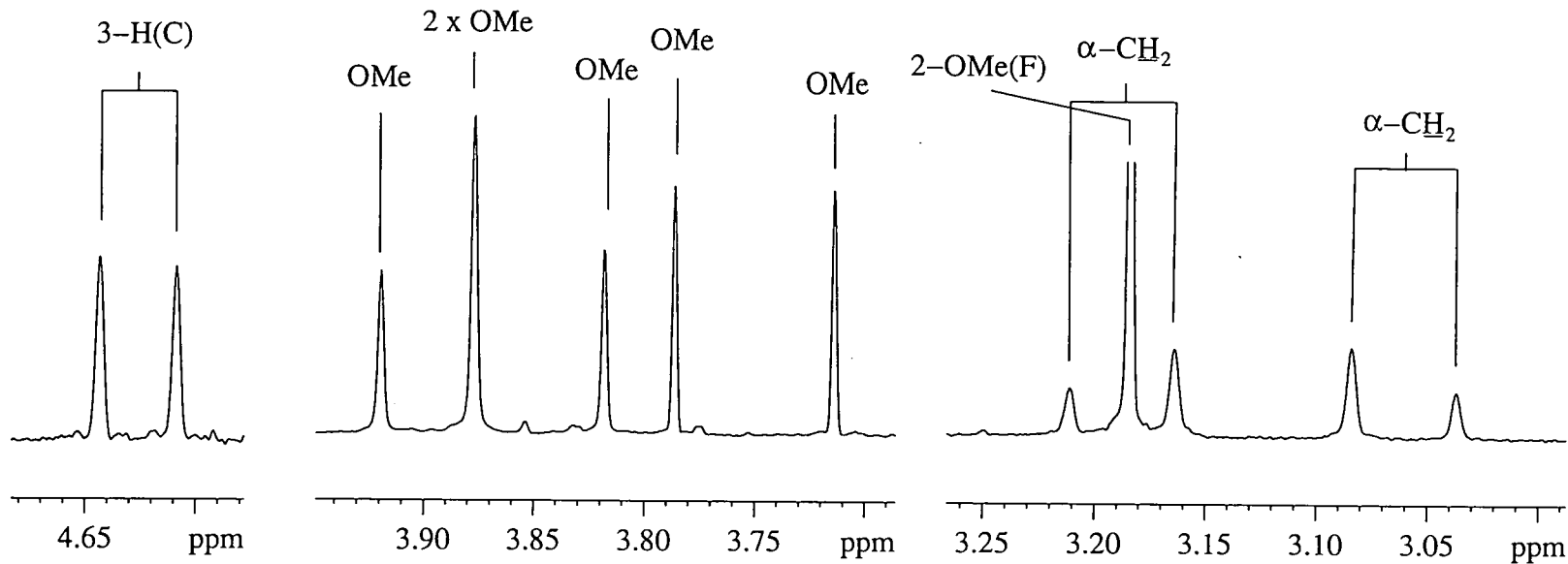
5.65 ppm

Plate 12: ^{1}H NMR of maesopsin (**23**) [(CD$_3$)$_2$CO]
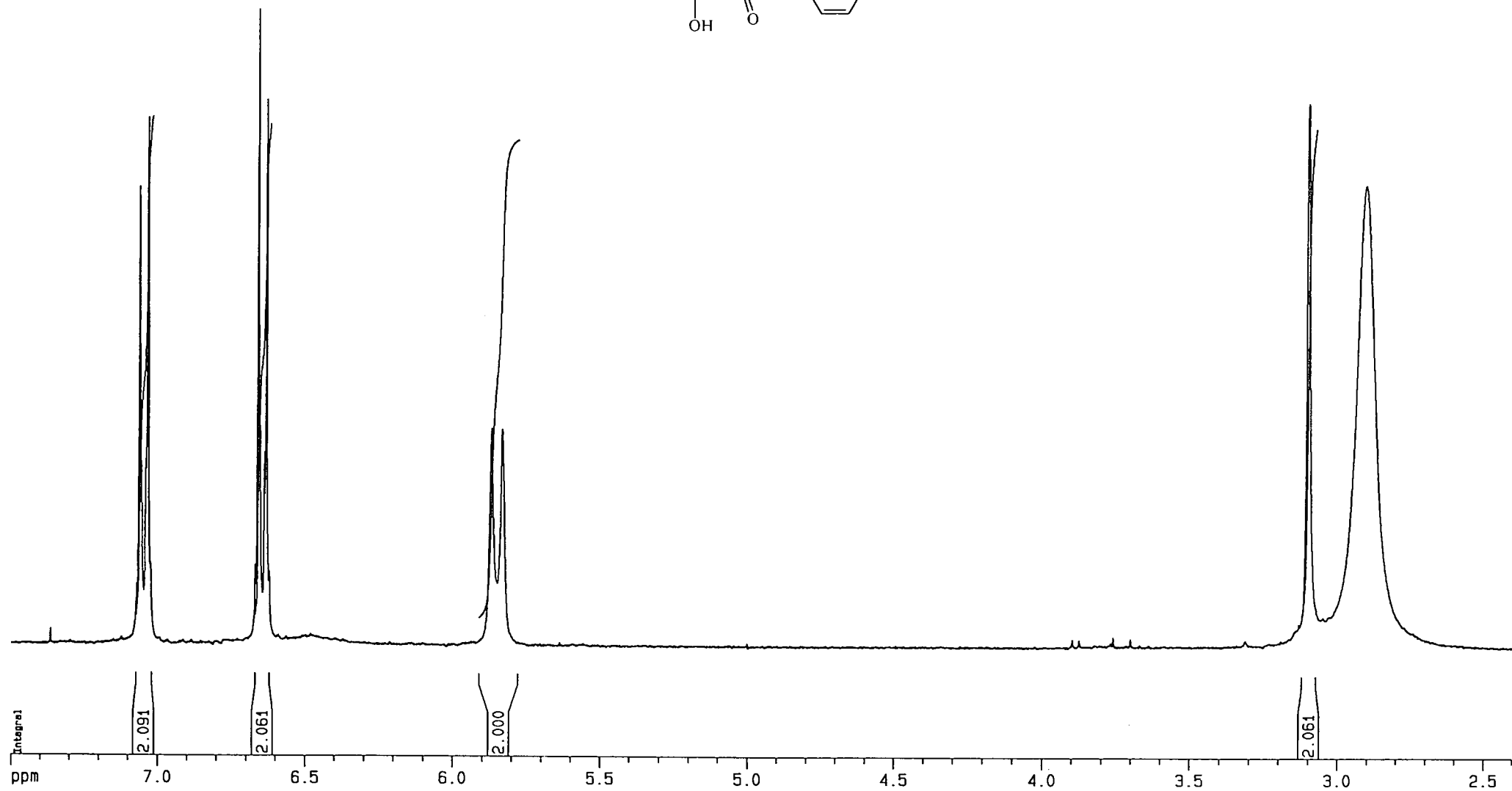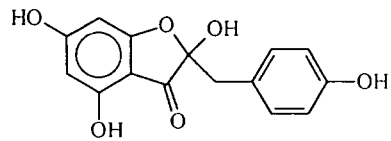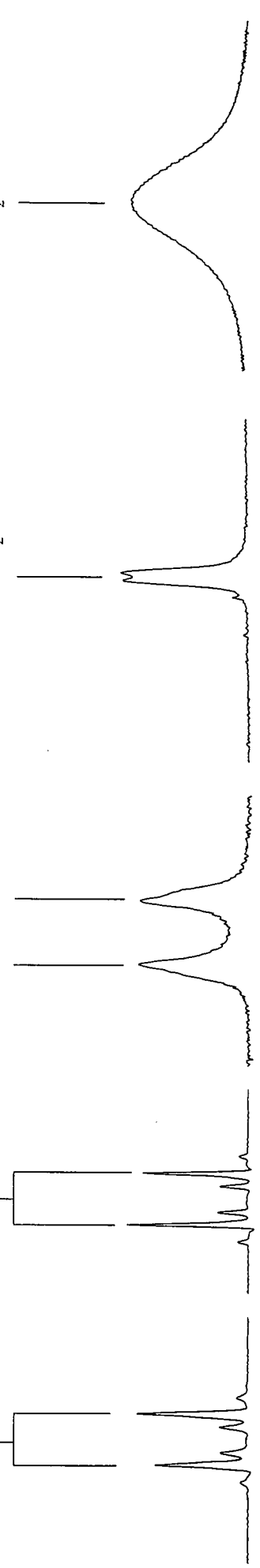
Plate 13: ^{1}H NMR of 2,4,4',6-tetra-O-maesopsin (**206**) [CDCl$_3$]
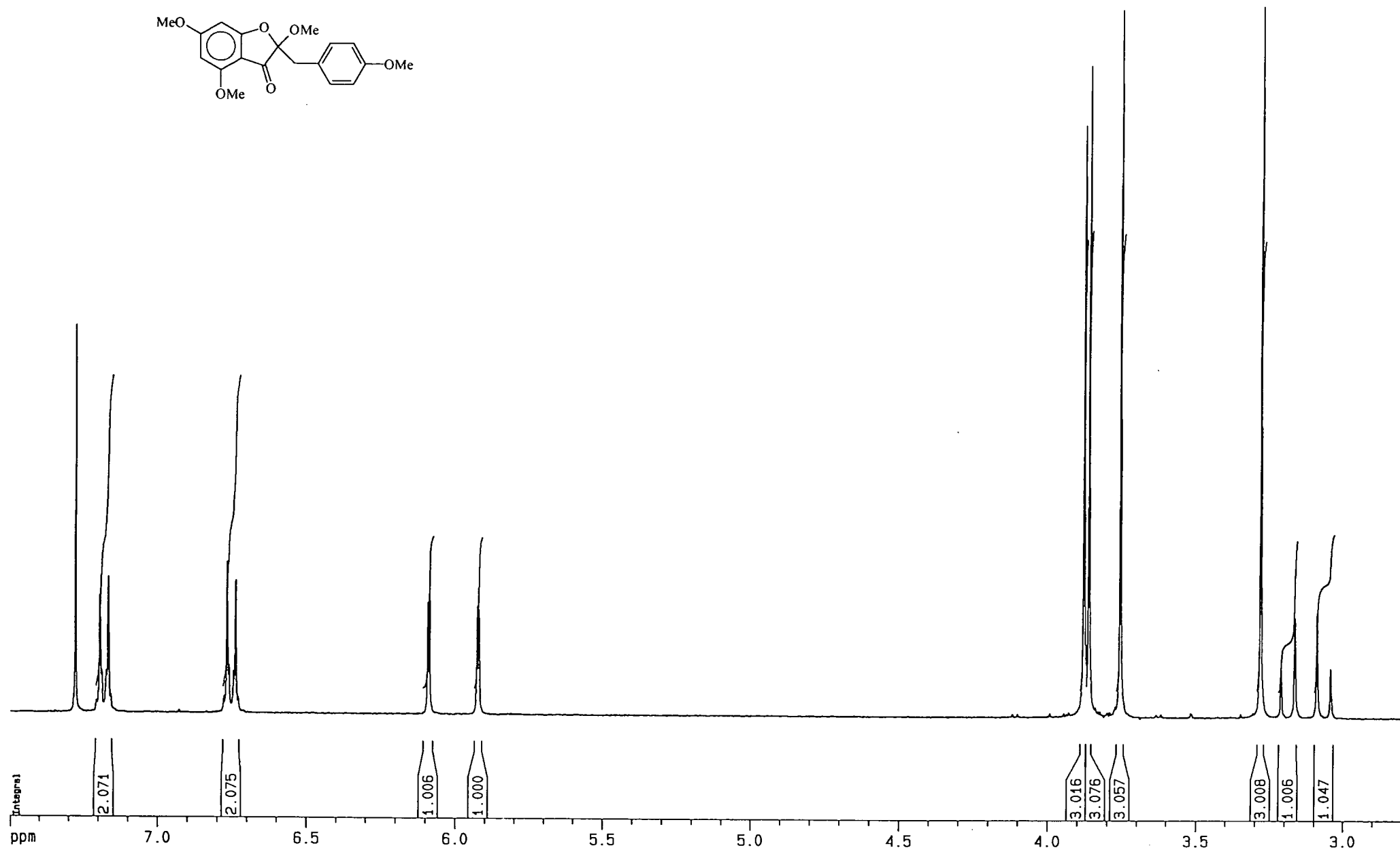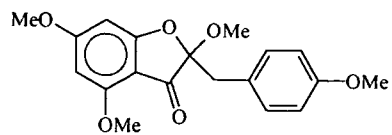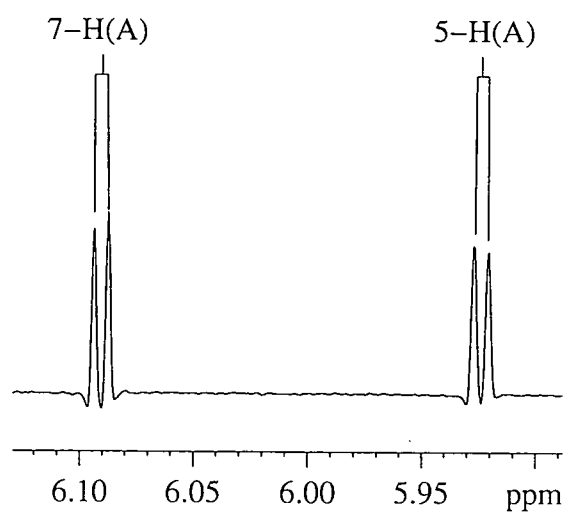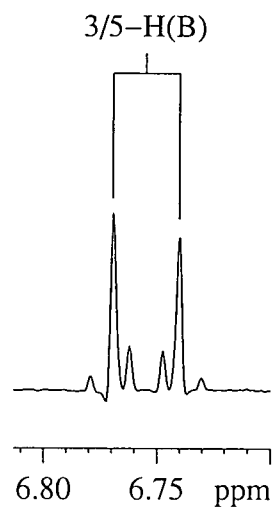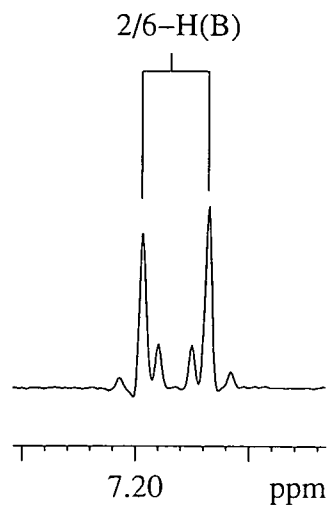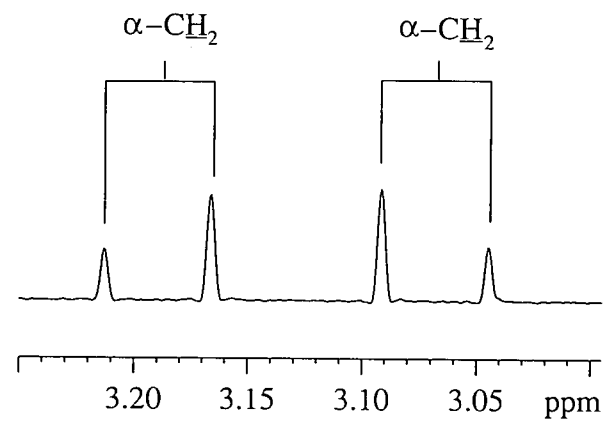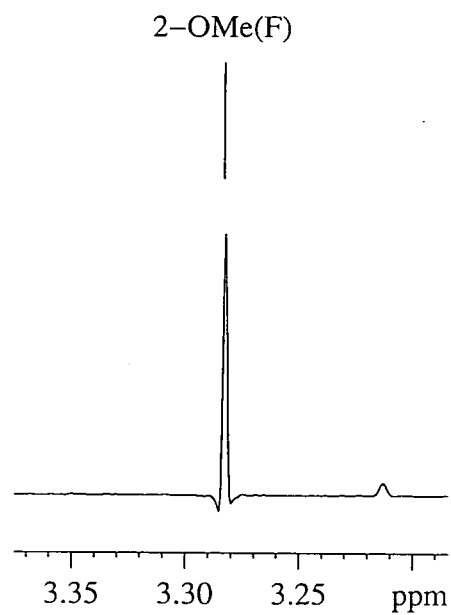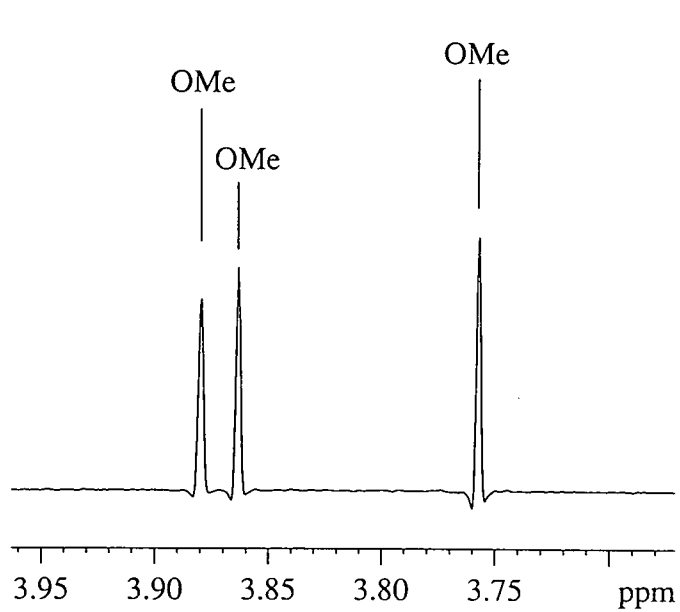
Plate 14: ¹H NMR of 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**211/212**) [CDCl₃]

Plate 15: ^{13}C NMR of 4,4',6-tri-O-methyl-2-deoxy-maesopsin-(2→7)-2,4,4',6-tetra-O-methyl-maesopsin (211/212) [CDCl$_3$]

Top spectrum labels:
5–C(A), 2–C(C), 5–C(D), 7–C(A), CDCl$_3$, 7 x OMe, α–CH$_2$(C), α–CH$_2$(F)

Bottom spectrum labels:
3–C(C), 3–C(F), 8–C(A), 8–C(D), 6–C(D), 6–C(A), 4–C(D), 4–C(E), 4–C(A), 4–C(B), 2/6–C(B), 2/6–C(E), 1–C(B), 1–C(E), 3/5–C(E), 3/5–C(B), 2–C(F), 9–C(A), 9–C(D), 7–C(D)
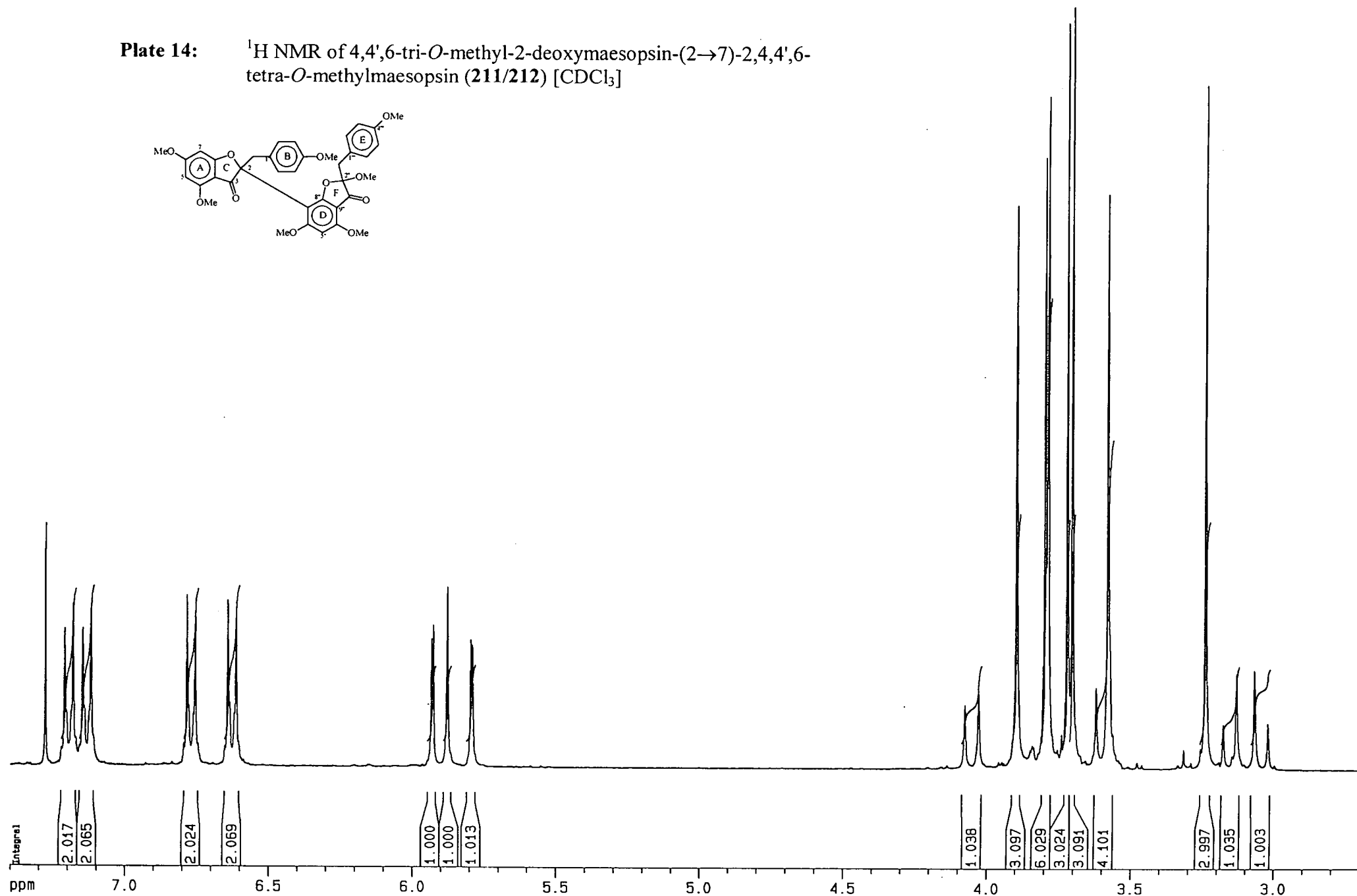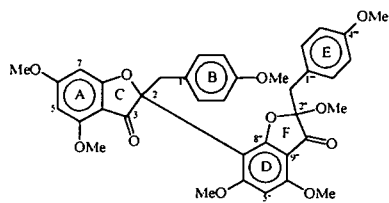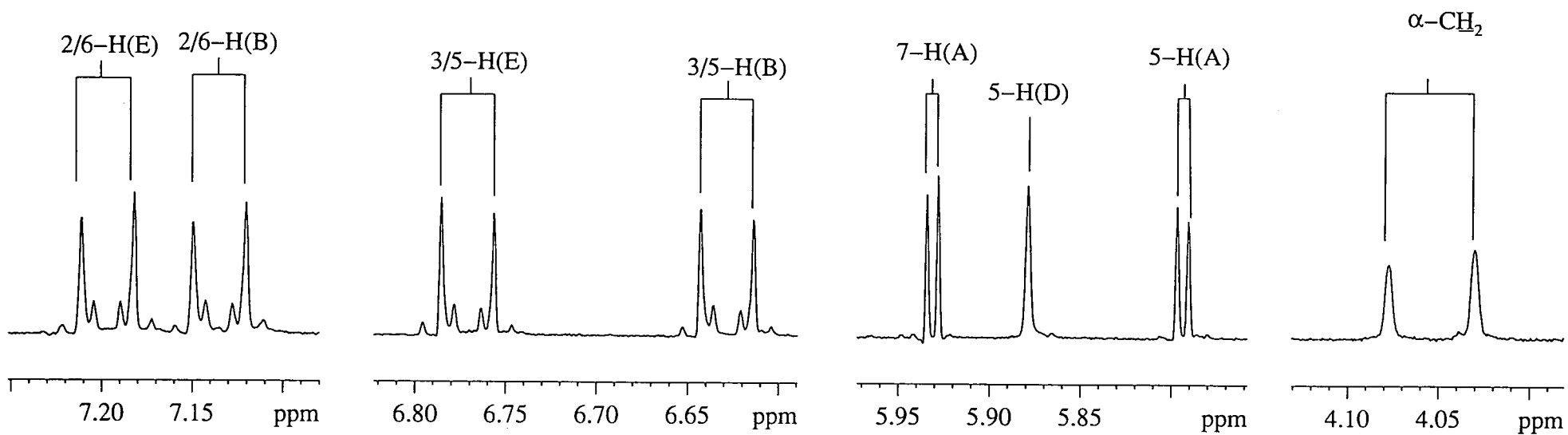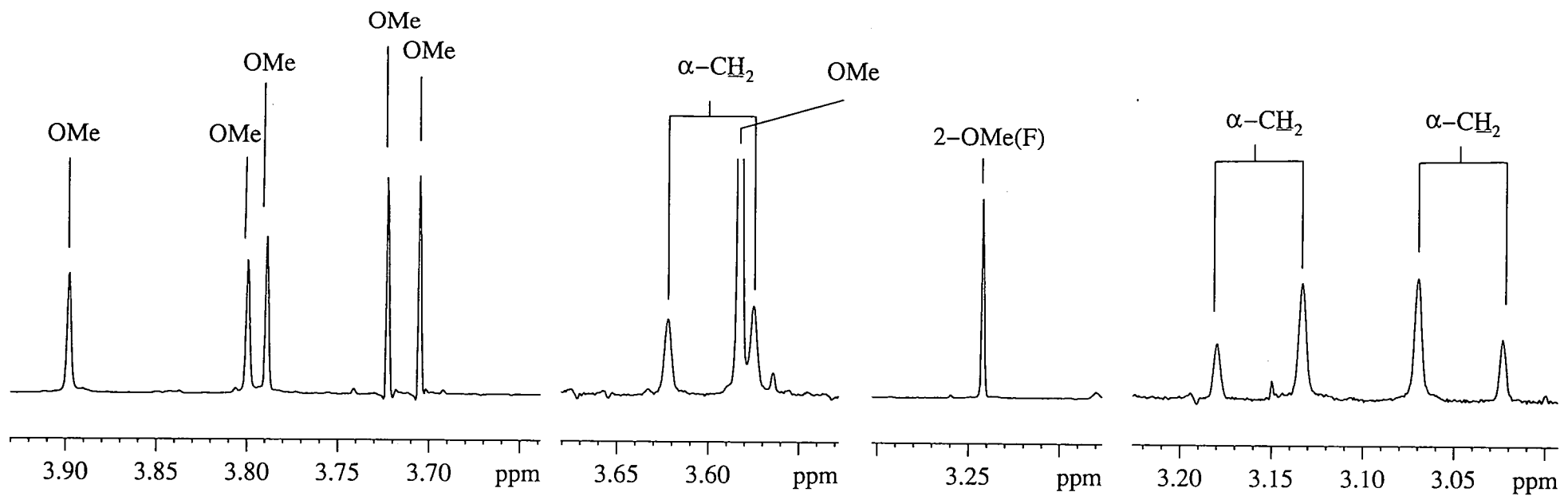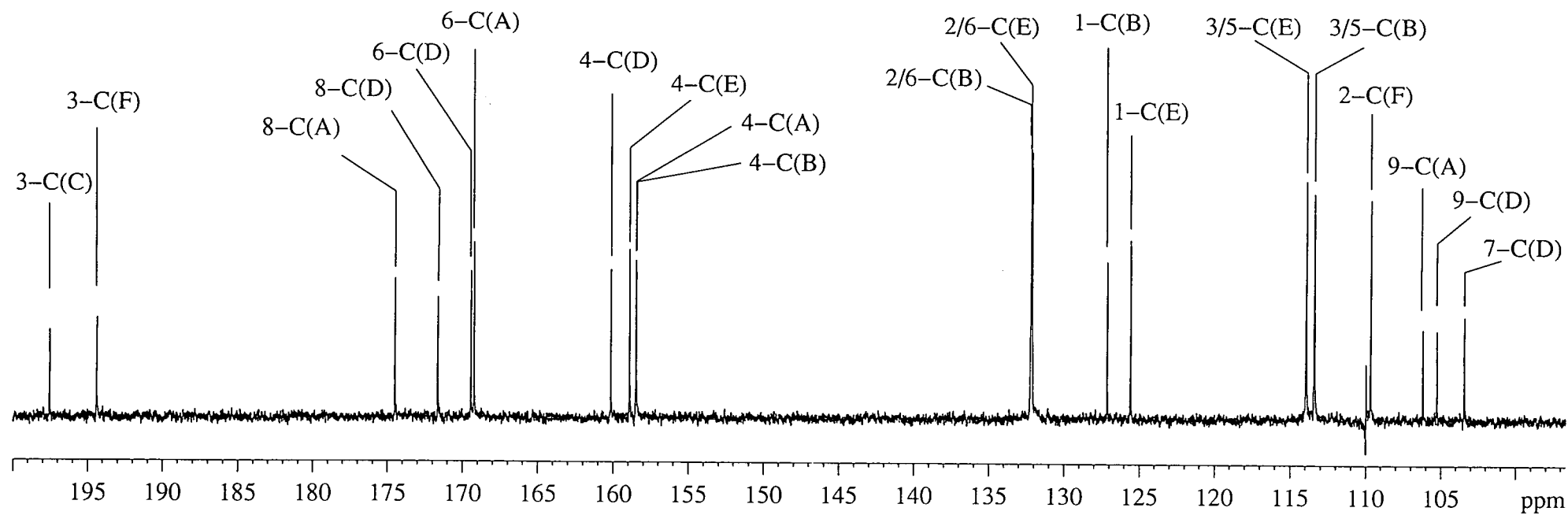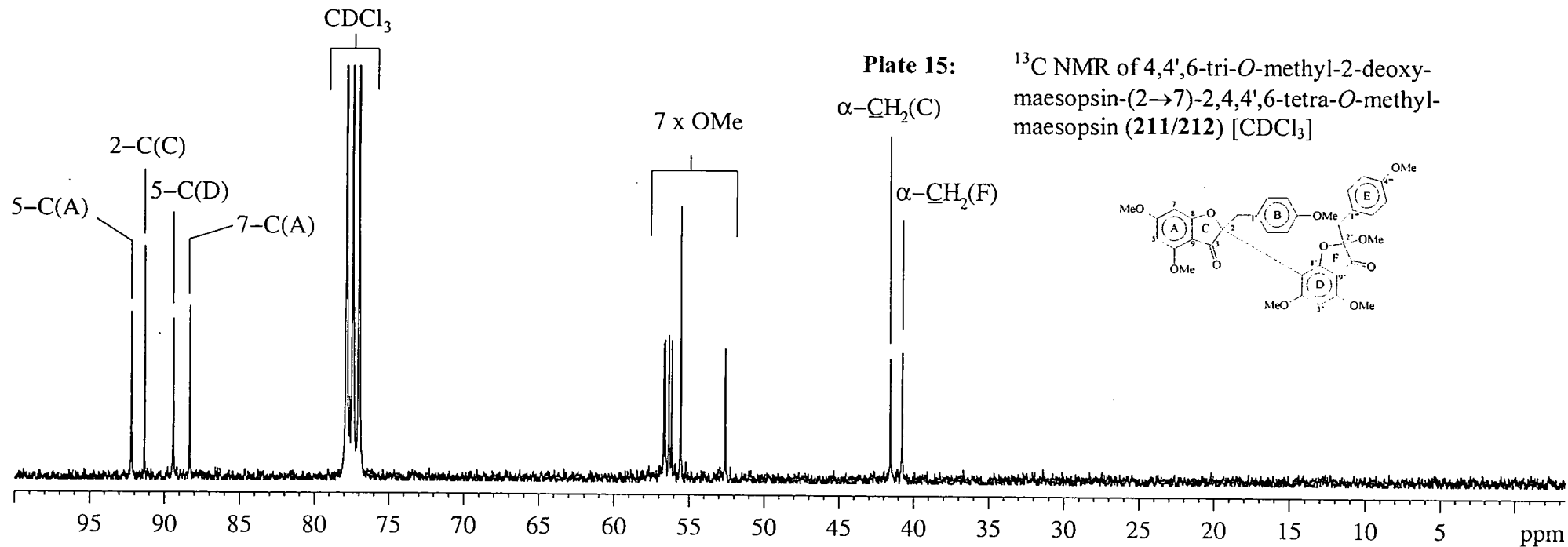
Plate 16: ^{1}H NMR of 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**215/216**) [CDCl$_3$]
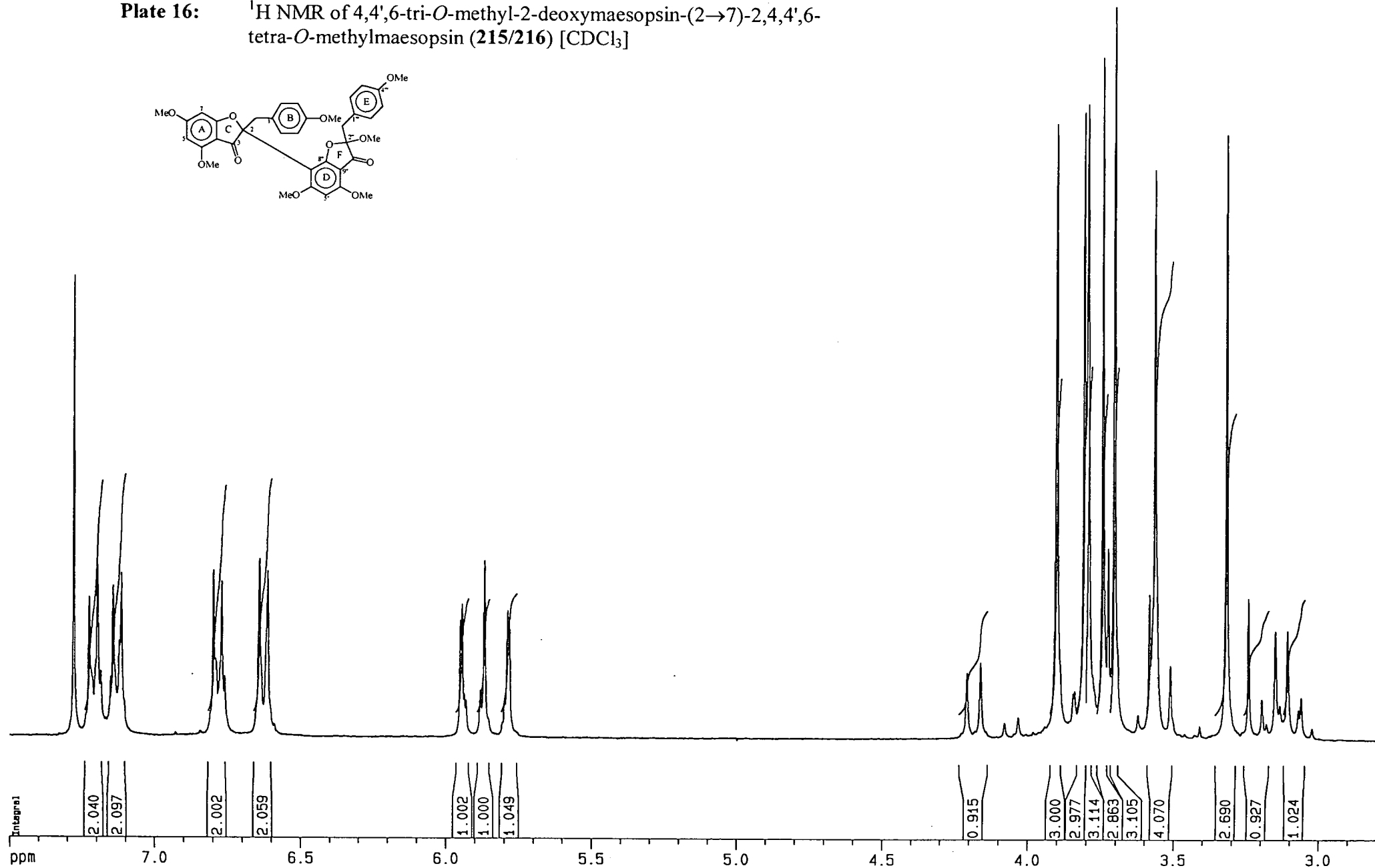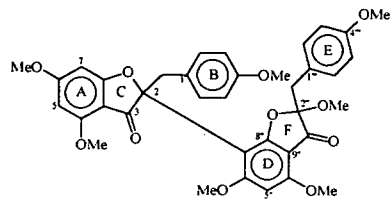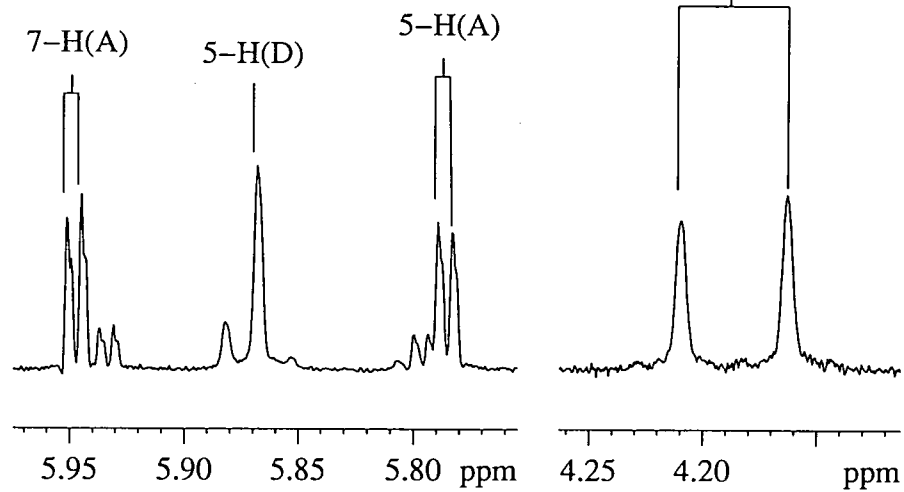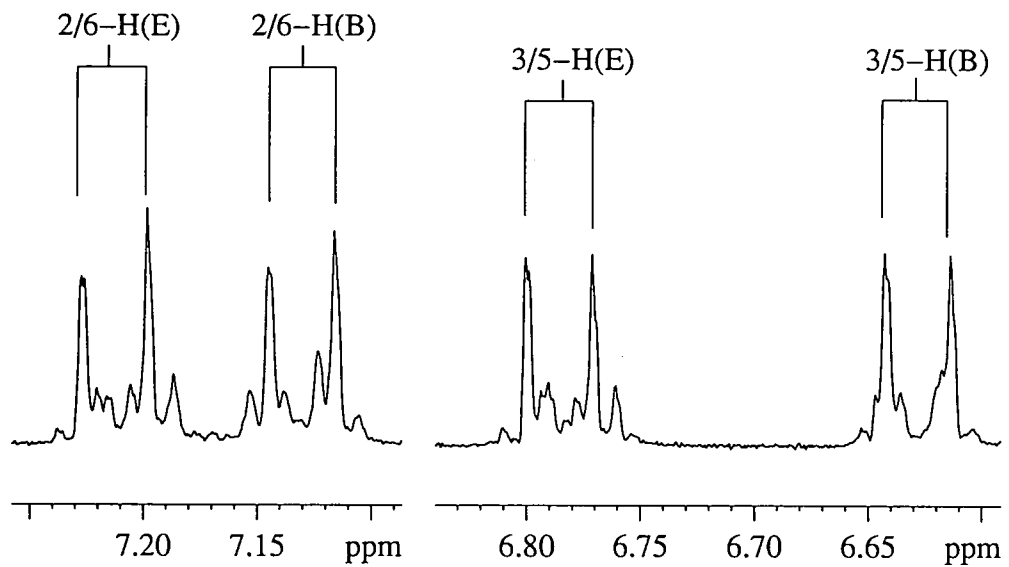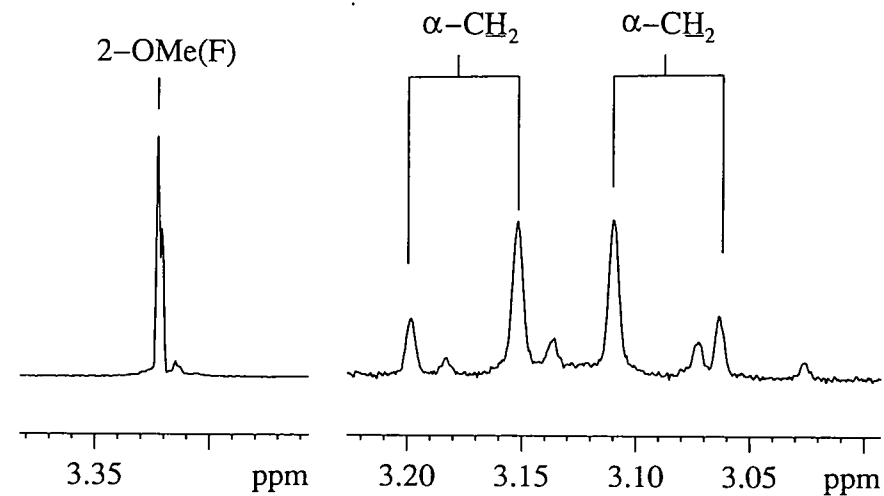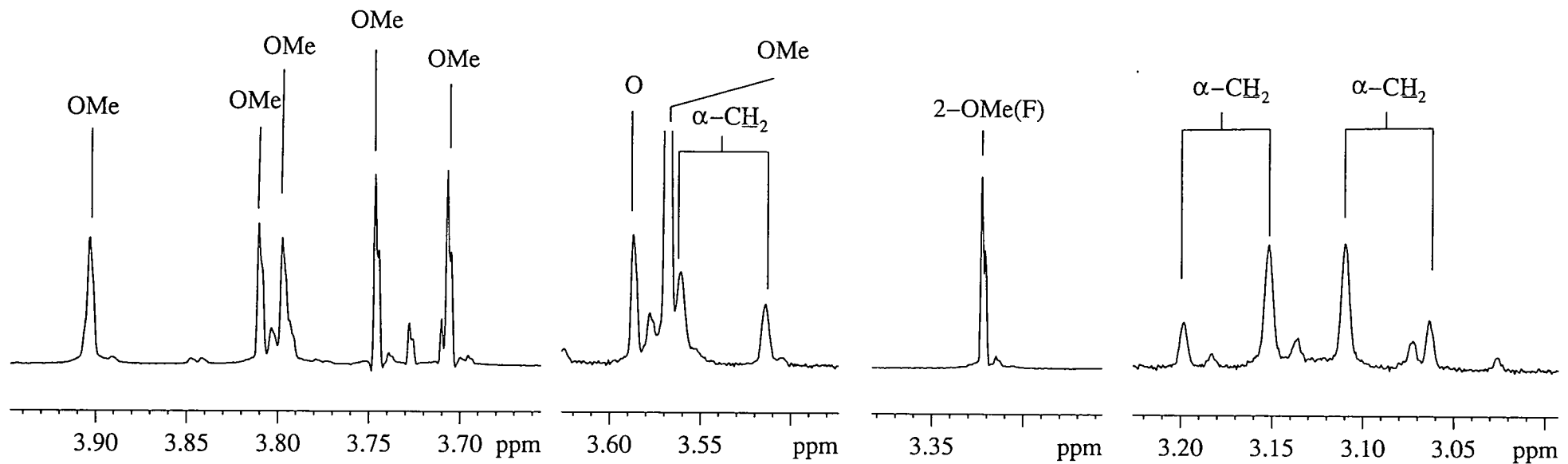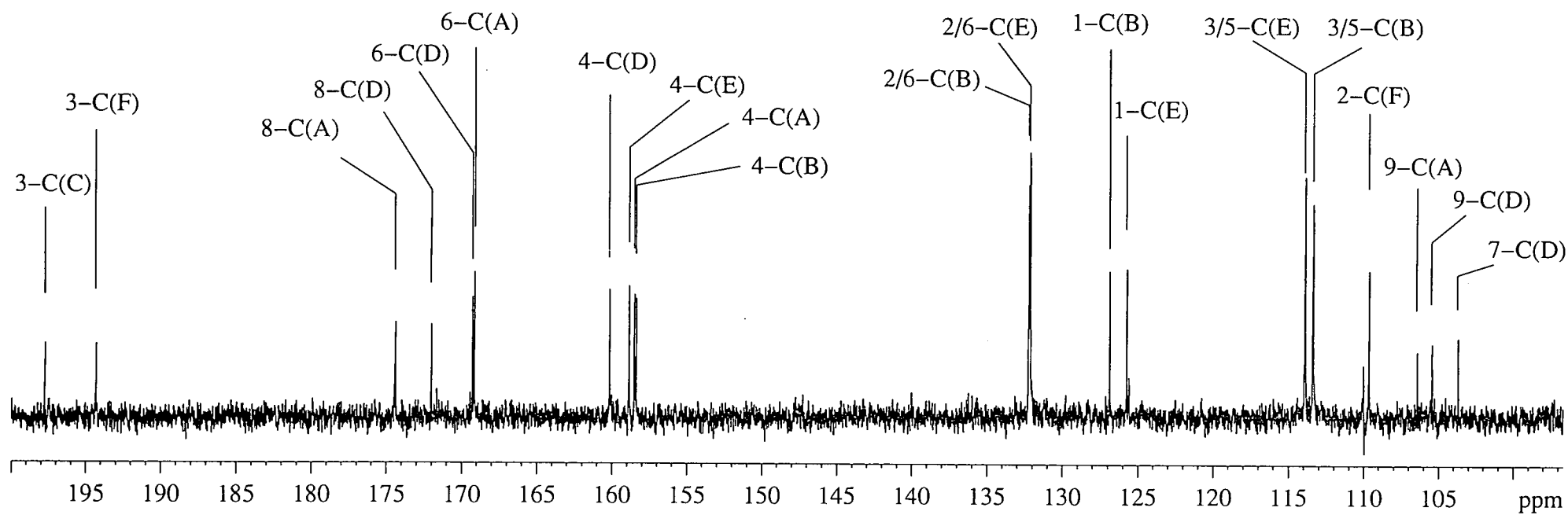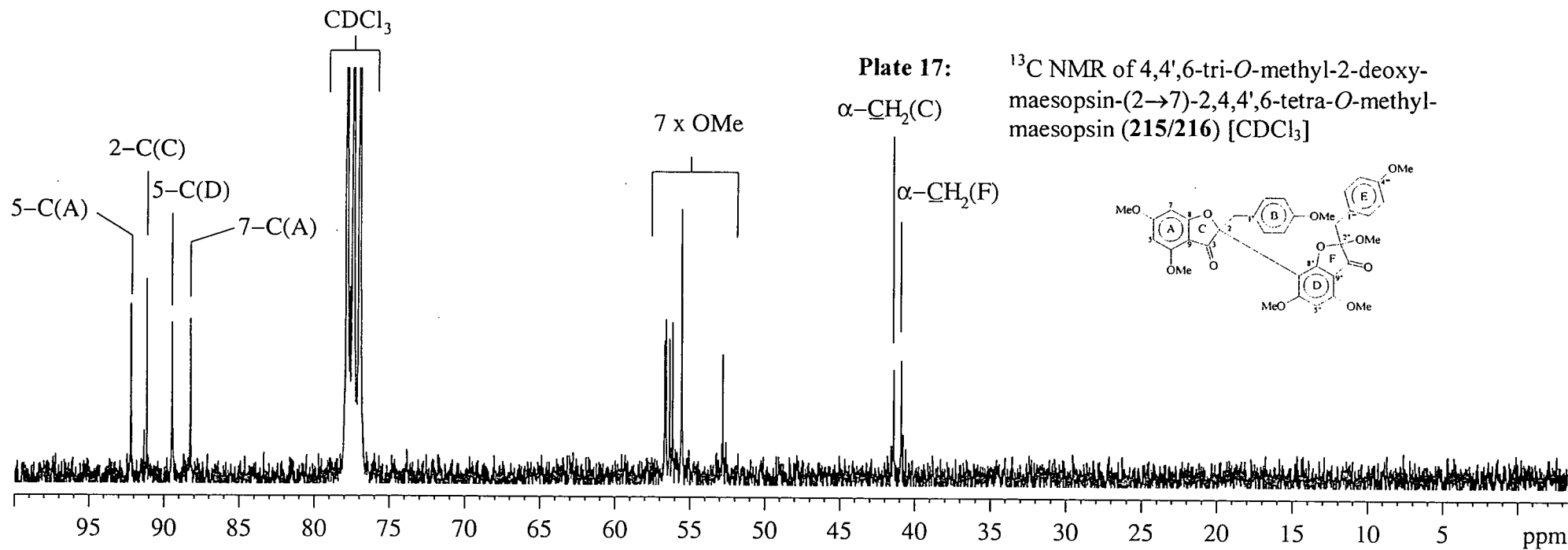
Plate 17: ^{13}C NMR of 4,4',6-tri-*O*-methyl-2-deoxy-maesopsin-(2→7)-2,4,4',6-tetra-*O*-methyl-maesopsin (**215/216**) [CDCl$_3$]

Top spectrum labels: CDCl$_3$, 5–C(A), 2–C(C), 5–C(D), 7–C(A), 7 x OMe, α–CH$_2$(C), α–CH$_2$(F)

Bottom spectrum labels: 3–C(C), 3–C(F), 8–C(A), 8–C(D), 6–C(D), 6–C(A), 4–C(D), 4–C(E), 4–C(A), 4–C(B), 2/6–C(B), 2/6–C(E), 1–C(B), 1–C(E), 3/5–C(E), 3/5–C(B), 2–C(F), 9–C(A), 9–C(D), 7–C(D)

¹H NMR of 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin (**219**) [CDCl₃]

Plate 19: ^{13}C NMR of 4,6-dimethoxy-3-(4-methoxy-benzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin (**219**) [CDCl₃]
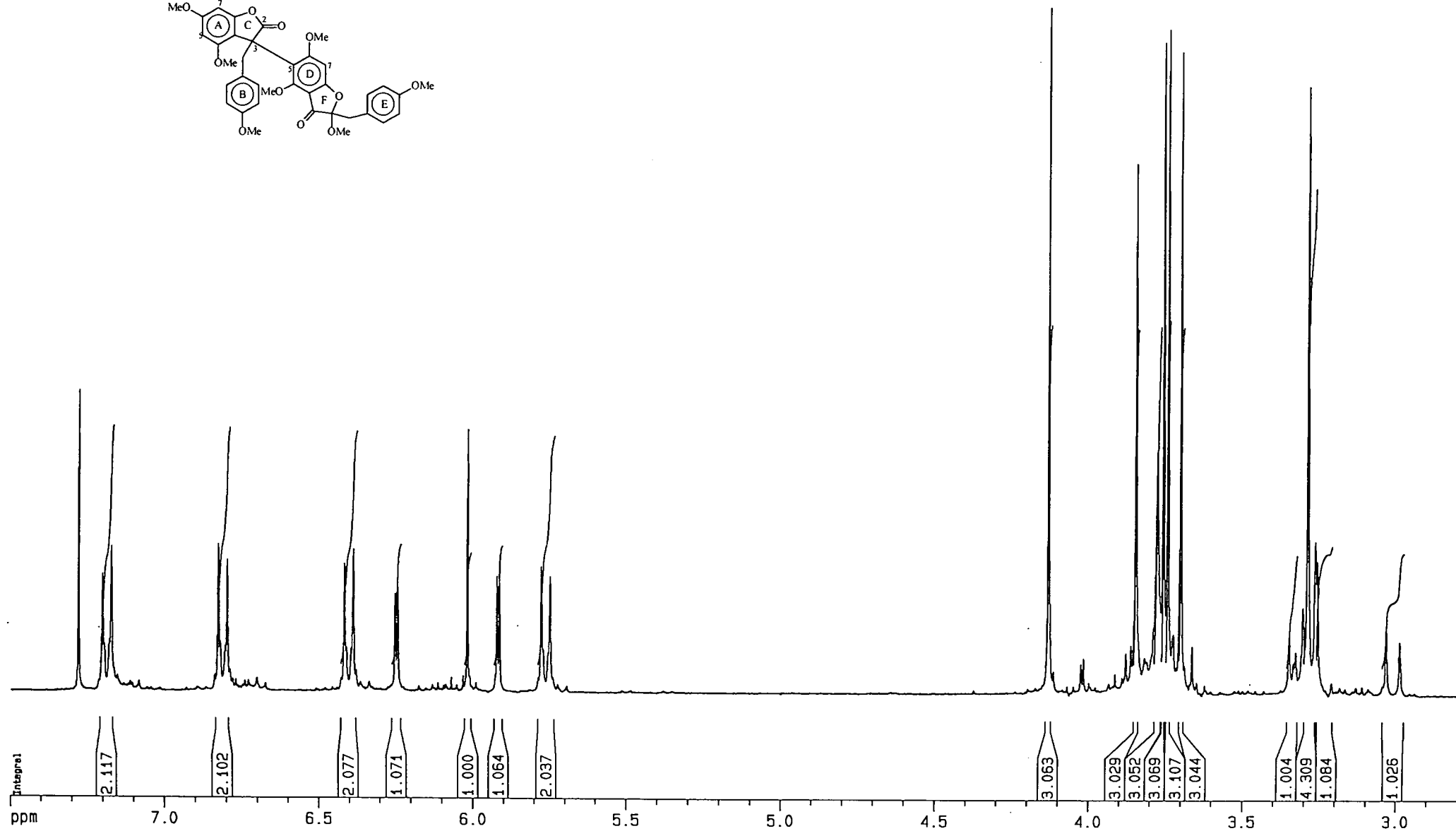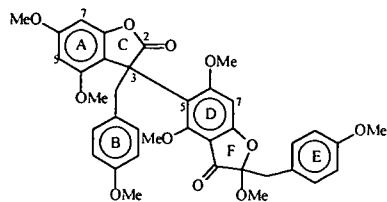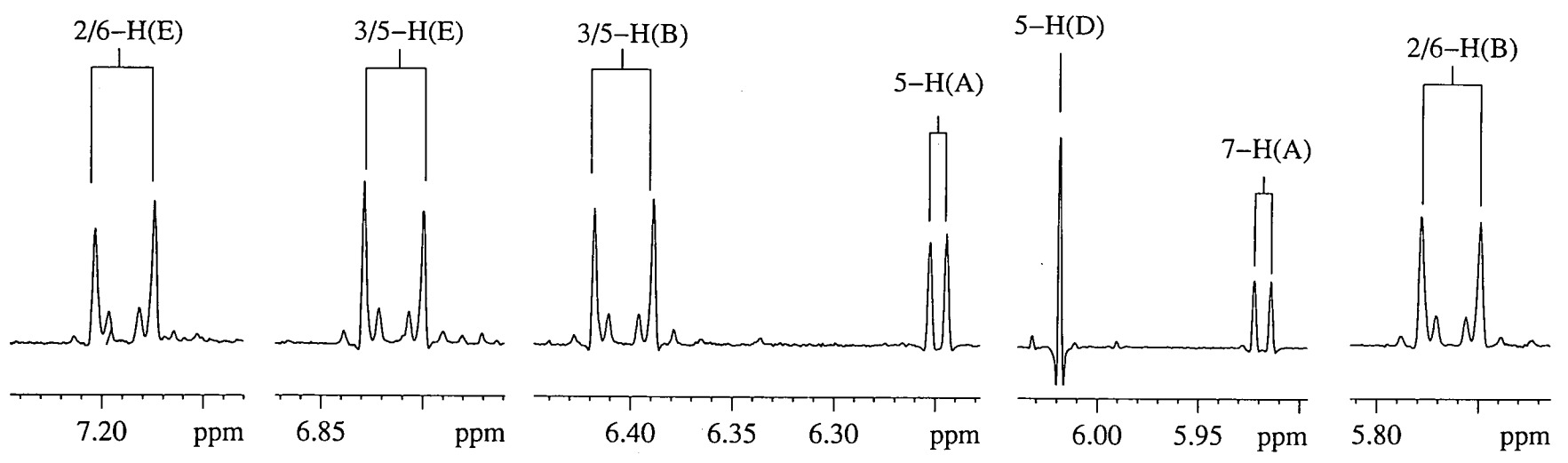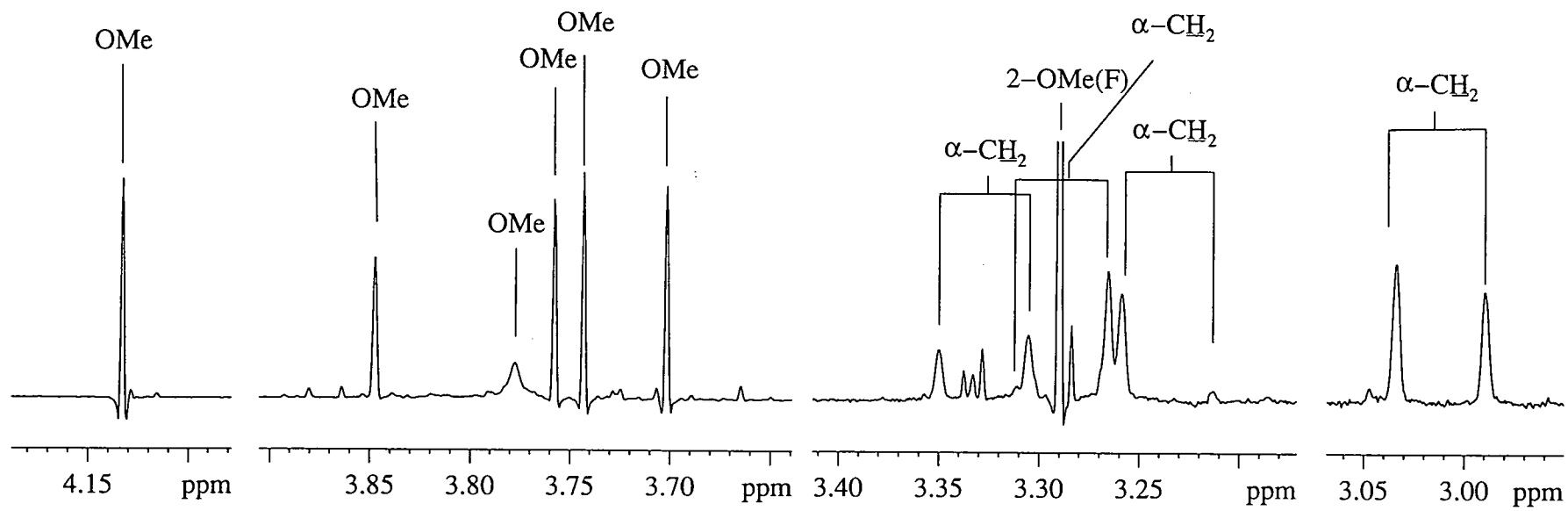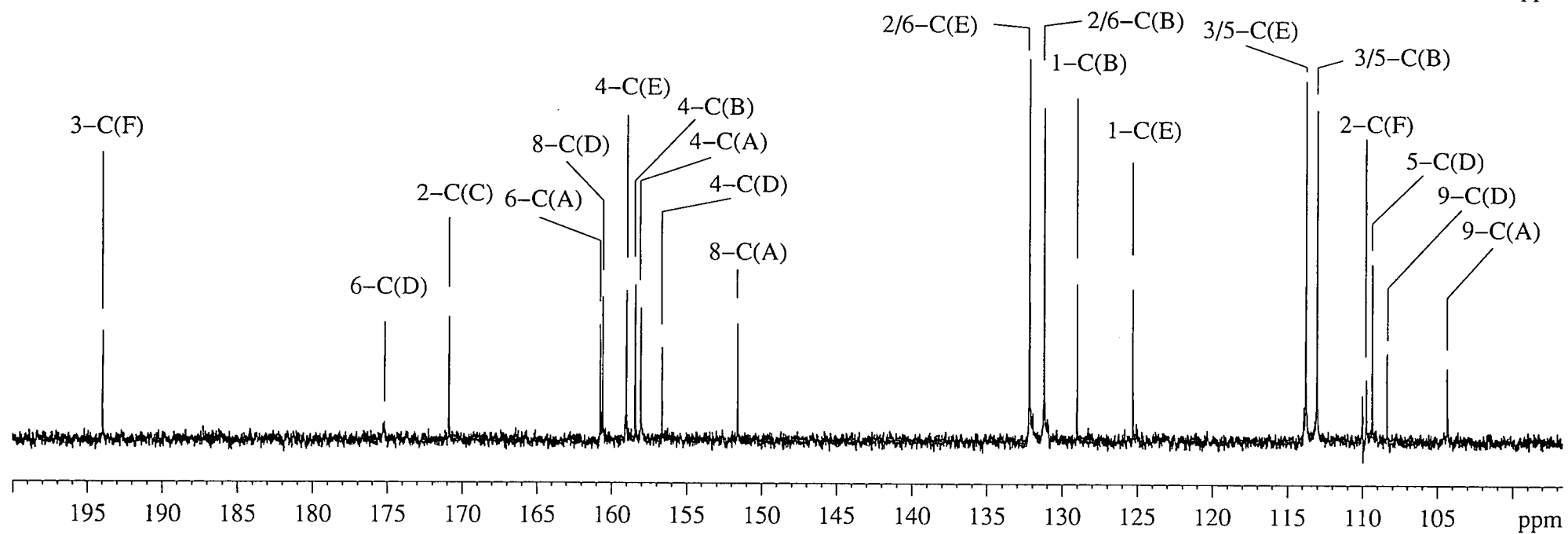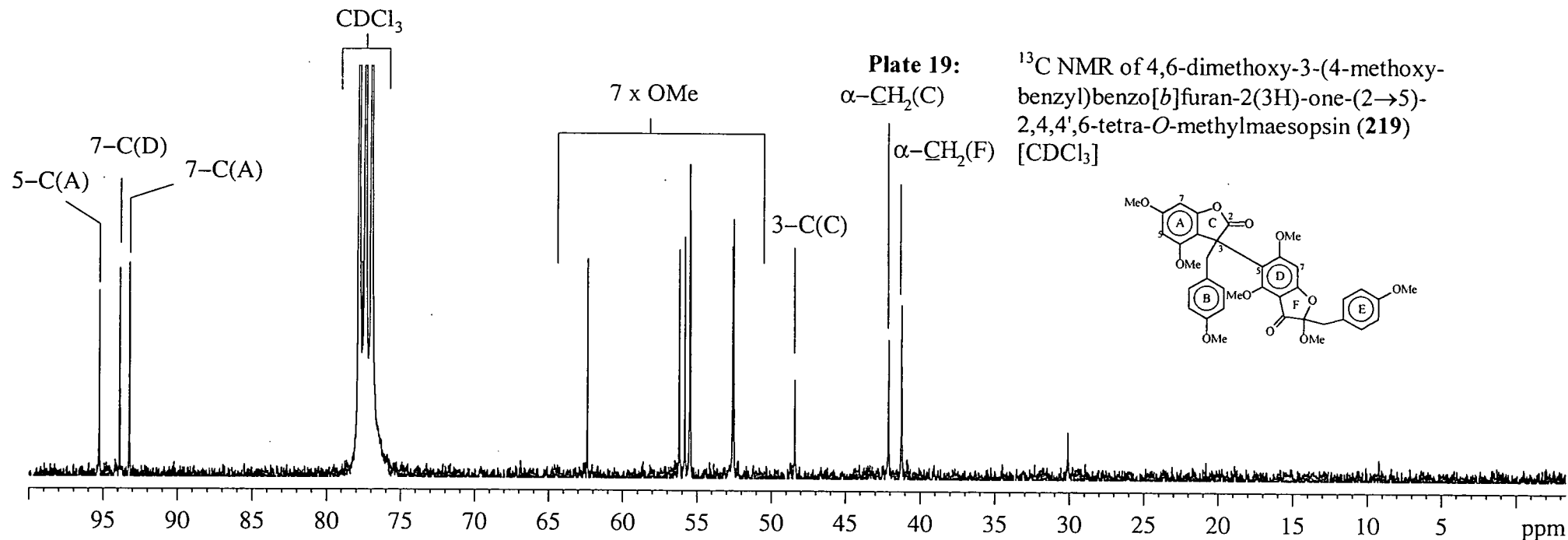
Plate 20: ^{1}H NMR of 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin (**221**) [CDCl₃]

Plate 21: ^{13}C NMR of 4,6-dimethoxy-3-(4-methoxy-benzyl)benzo[b]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-O-methylmaesopsin (221) [CDCl₃]
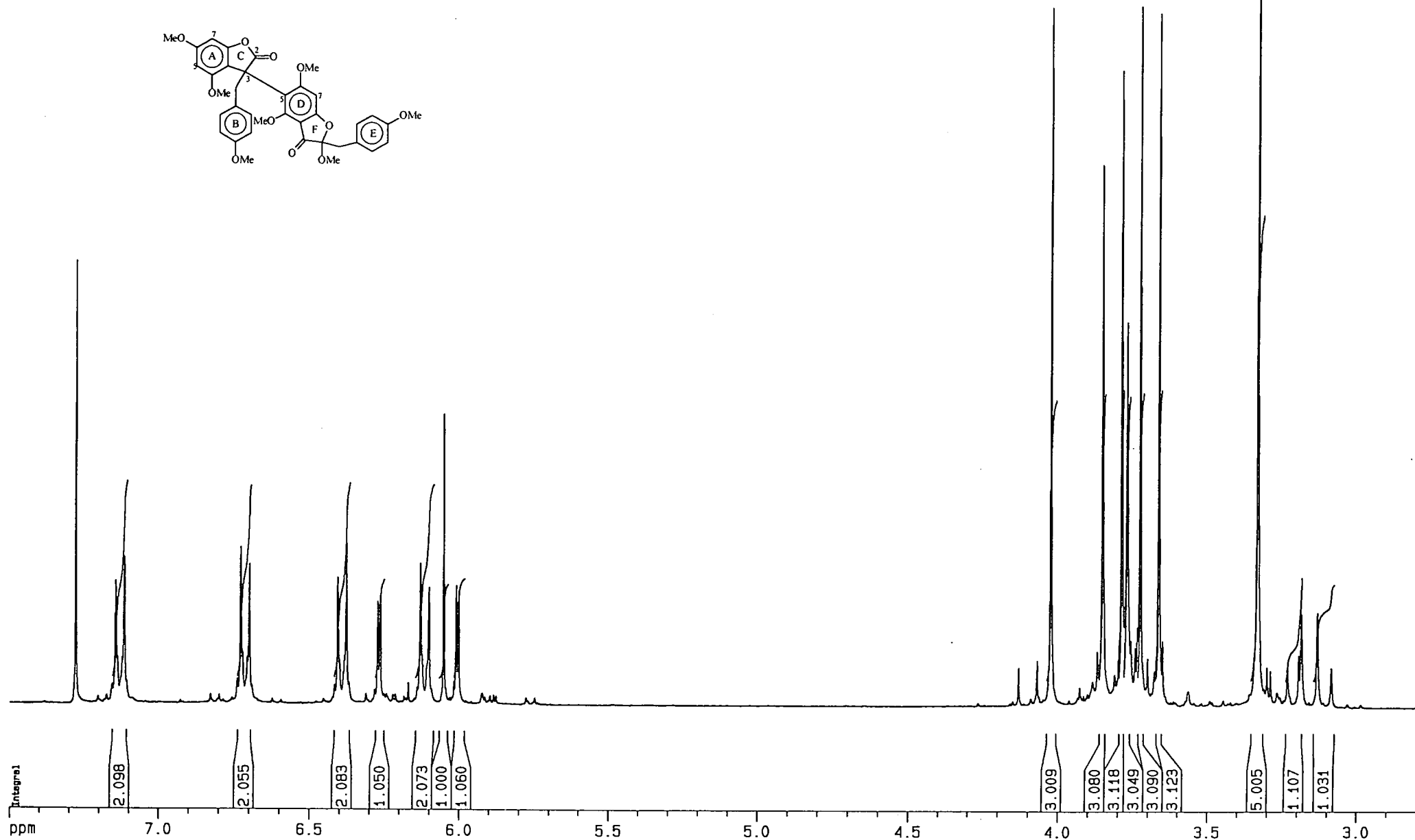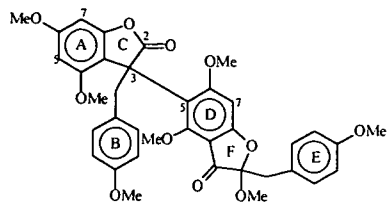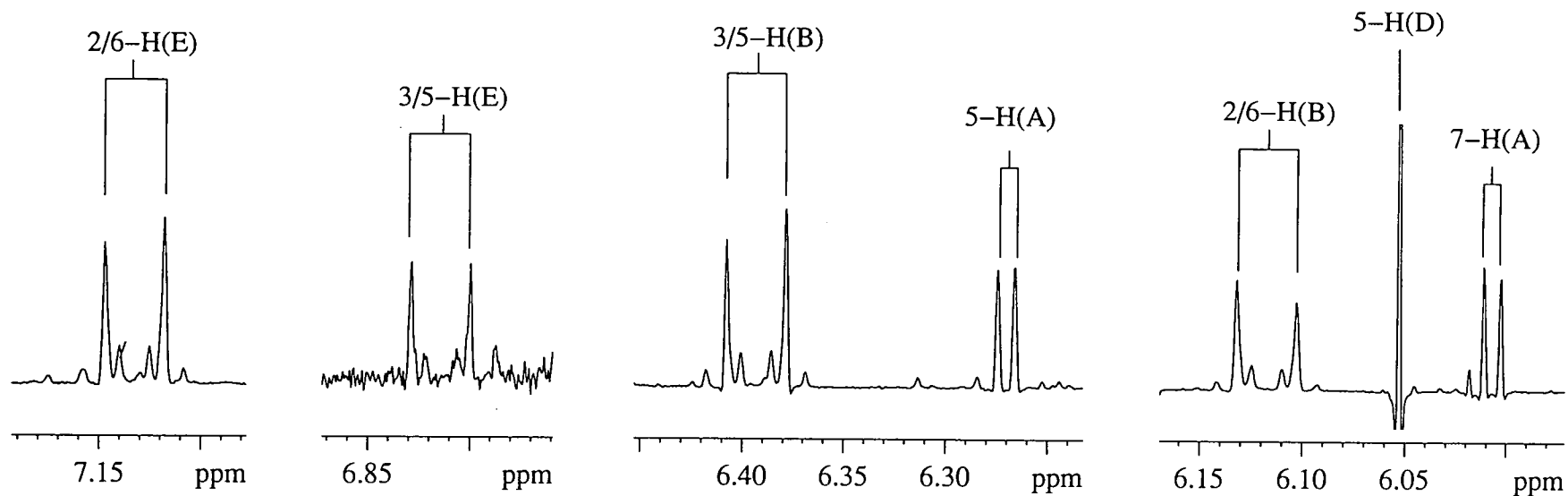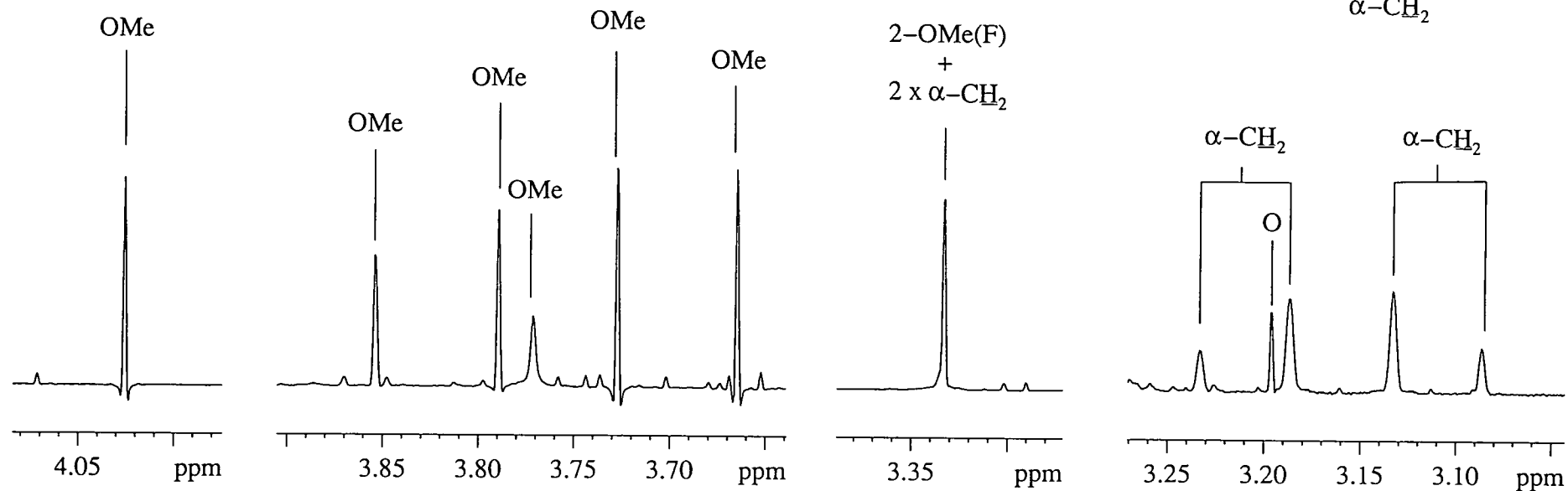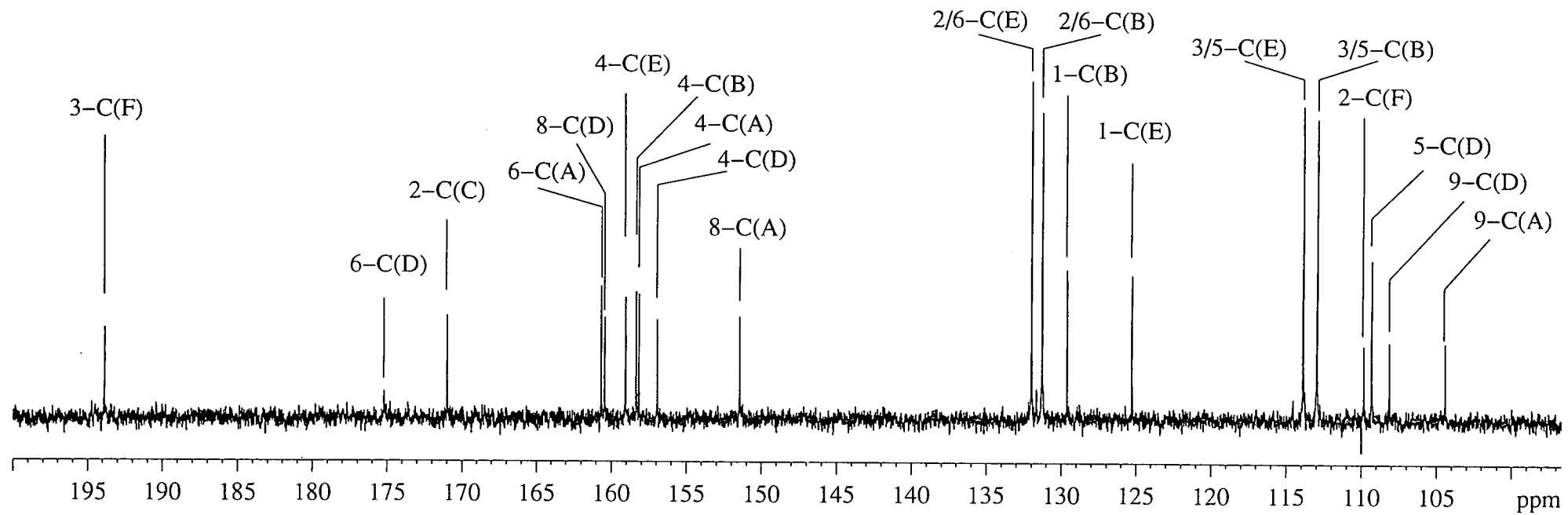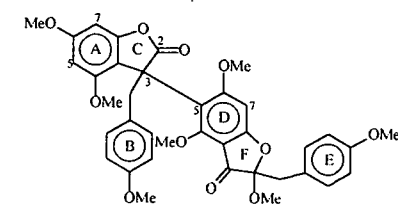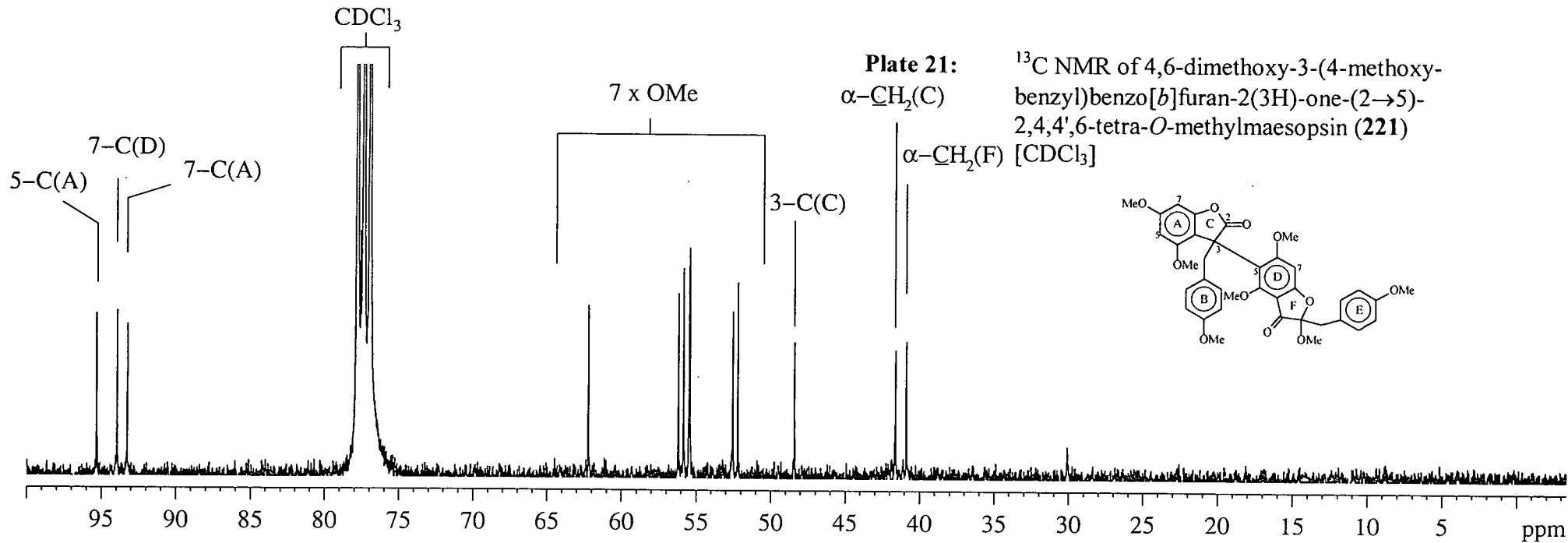
Plate 22: ^{1}H NMR of 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**223**) [CDCl$_3$]

Plate 23: ¹³C NMR of 4,6-dimethoxy-3-(4-methoxy-benzyl)benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**223**) [CDCl₃]

Top spectrum labels: 5–C(A), 7–C(A), 5–C(D), CDCl₃, 7 x OMe, 3–C(C), α–C̲H₂(C), α–C̲H₂(F)

Bottom spectrum labels: 3–C(F), 6–C(D), 2–C(C), 6–C(A), 8–C(D), 4–C(E), 4–C(B), 4–C(A), 4–C(D), 8–C(A), 2/6–C(E), 2/6–C(B), 1–C(B), 1–C(E), 3/5–C(E), 3/5–C(B), 2–C(F), 7–C(D), 9–C(D), 9–C(A)
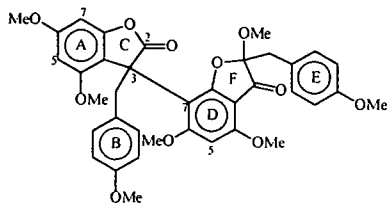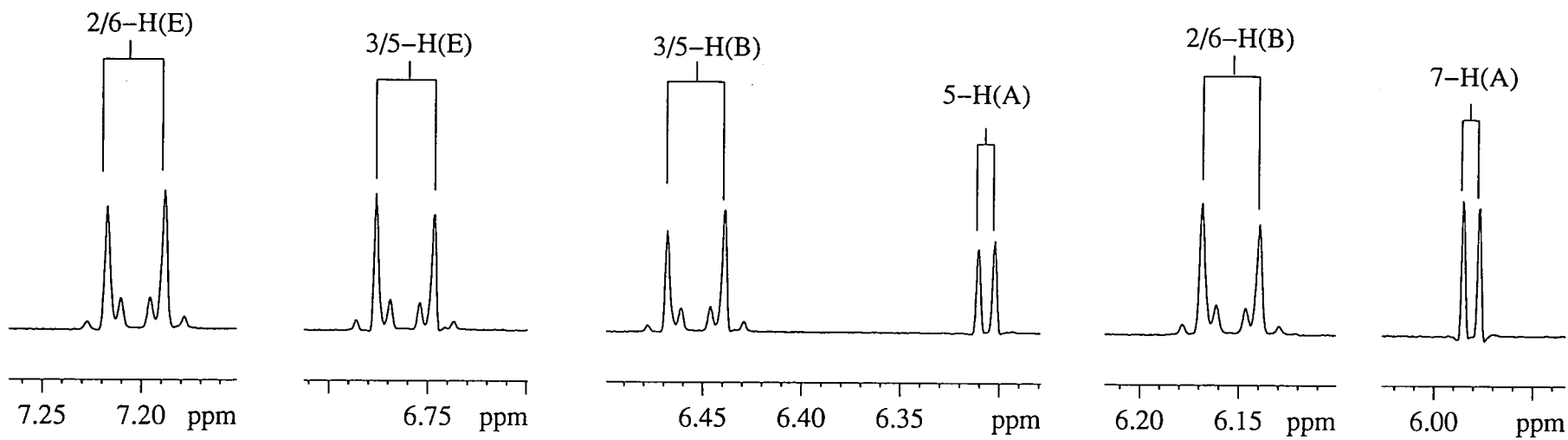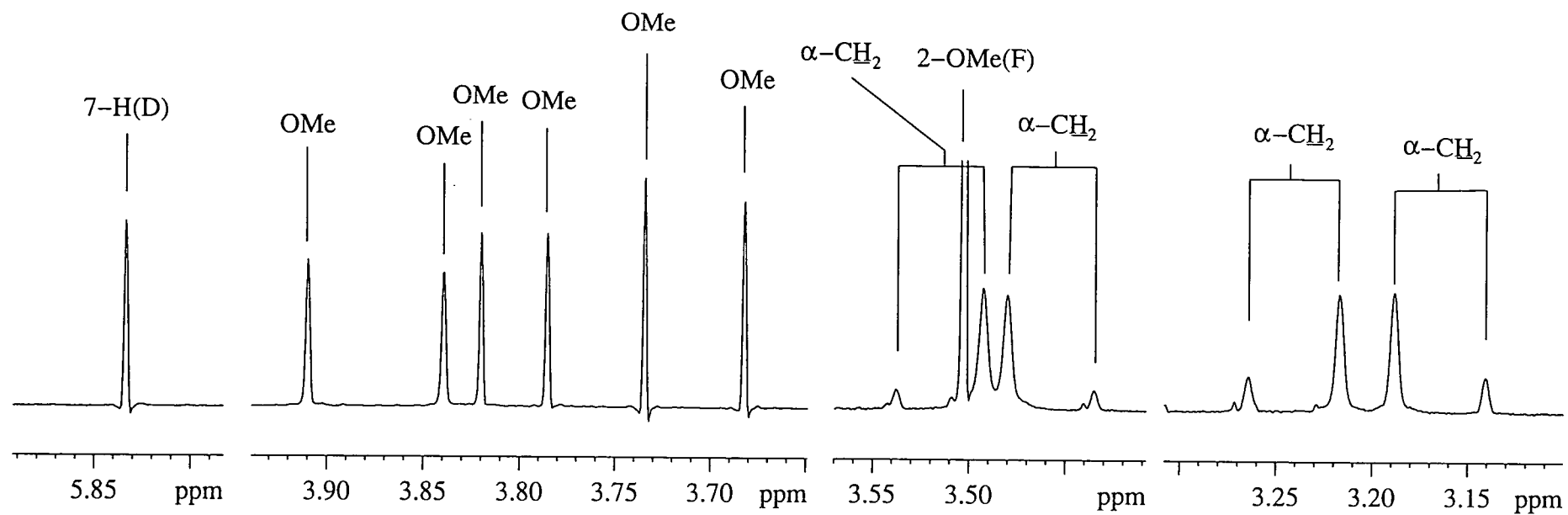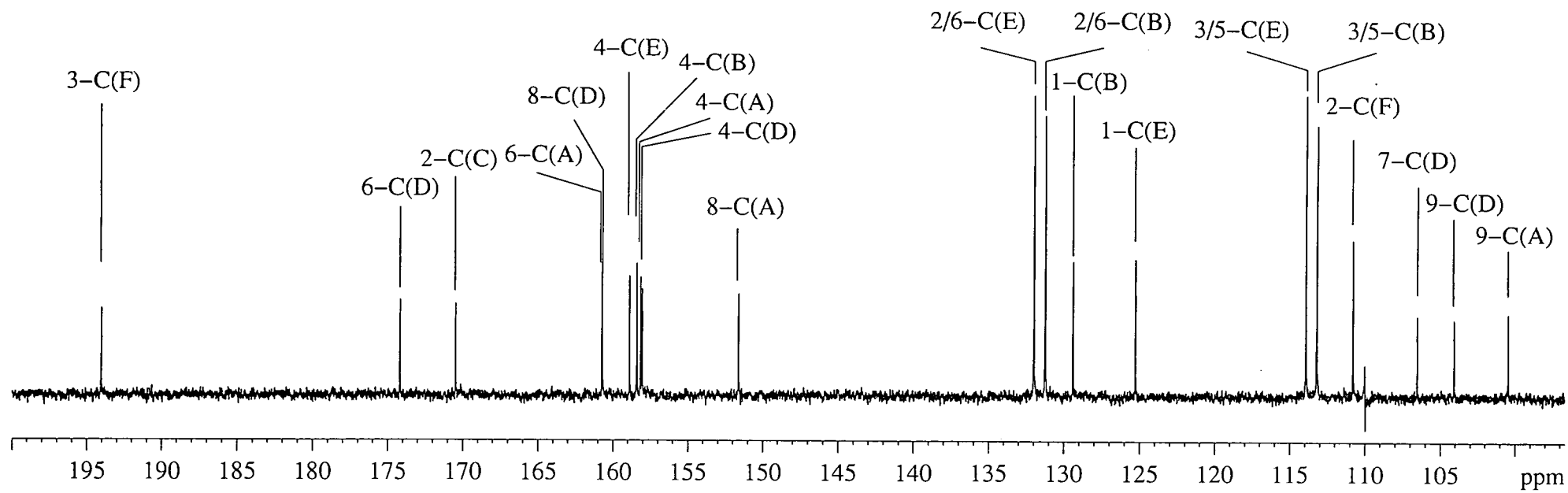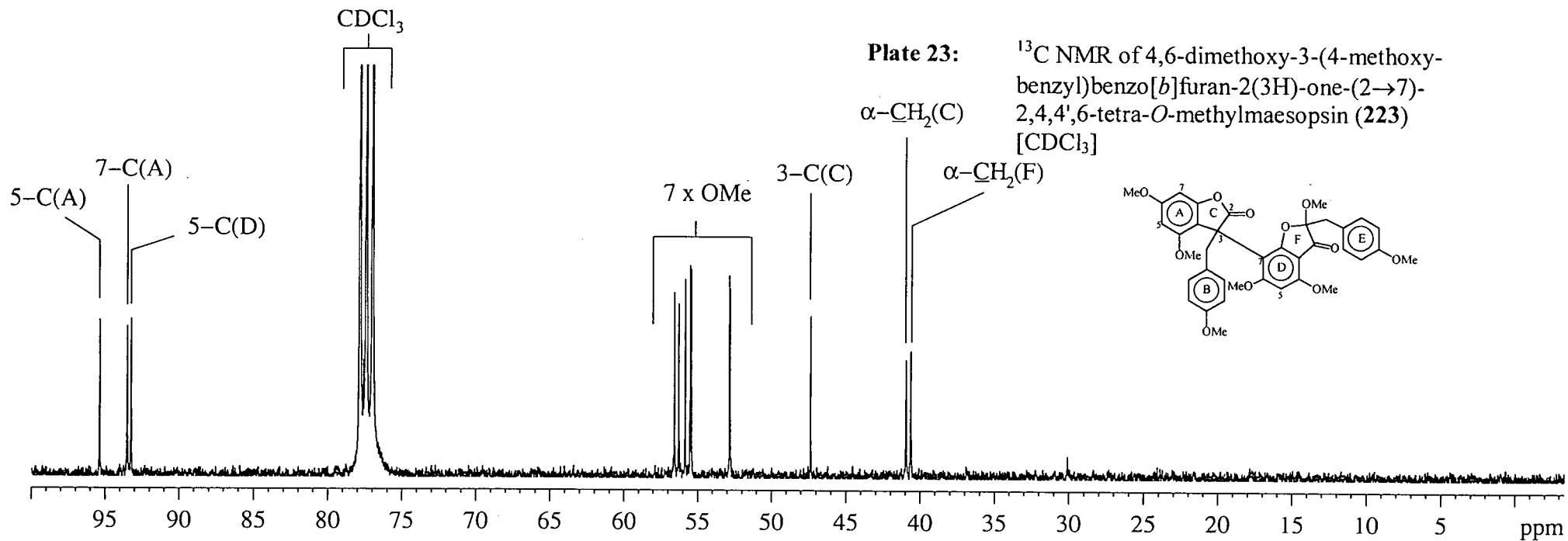
Plate 24: ^{1}H NMR of 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**225**) [CDCl$_3$]

OMe OMe OMe OMe OMe OMe

4 x α–CH₂

2–OMe(F)

3.90 3.85 3.80 3.75 3.70 3.65 3.60 ppm

3.35 3.30 ppm

3.25 ppm

2/6–H(E) CHCl₃ 3/5–H(E)

3/5–H(B) 5–H(A)

7–H(A)

7–H(D) 2/6–H(B)

7.30 ppm

6.75 ppm

6.40 6.35 6.30 ppm

5.95 ppm

5.80 5.75 5.70 ppm

Plate 25: ^{13}C NMR of 4,6-dimethoxy-3-(4-methoxy-benzyl)benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (225) [CDCl$_3$]
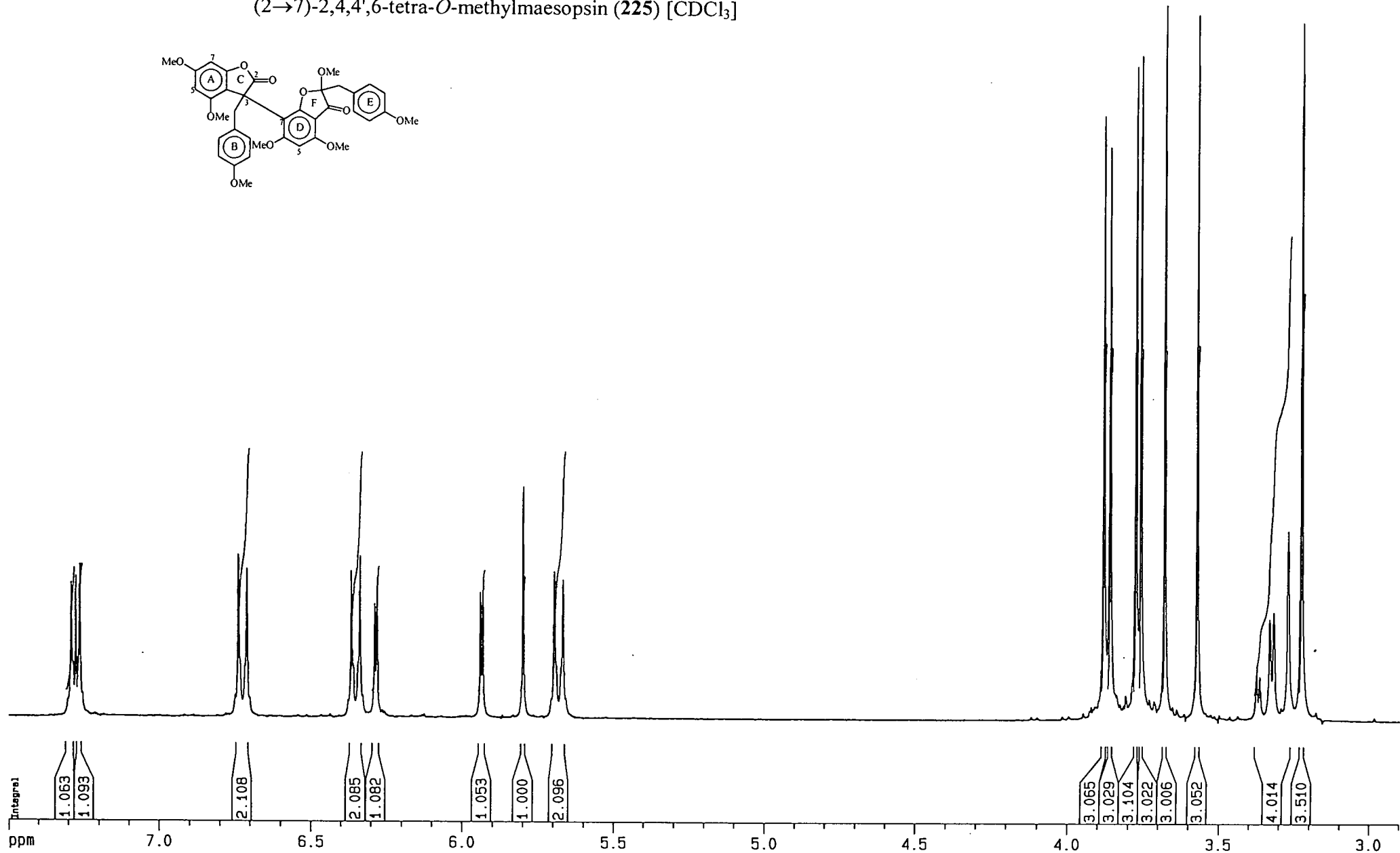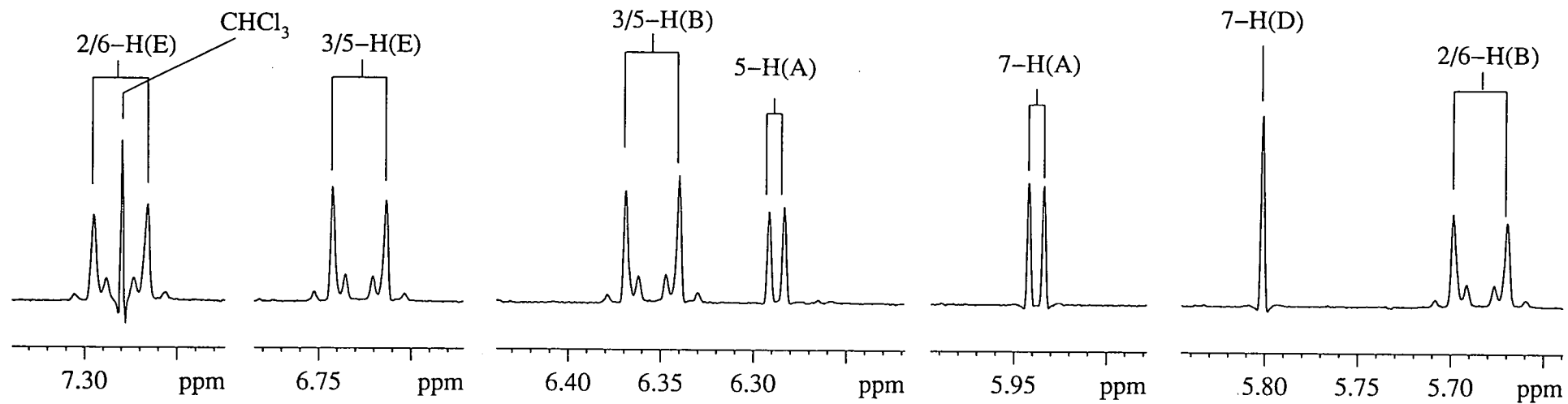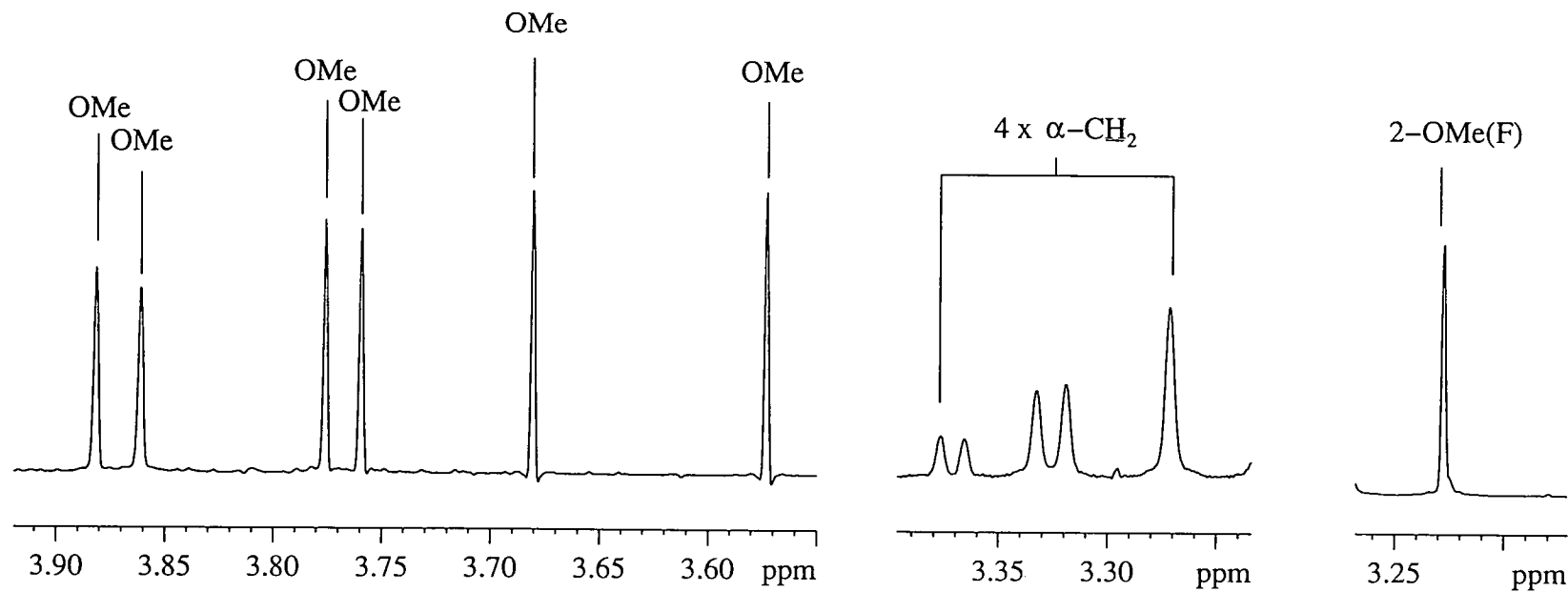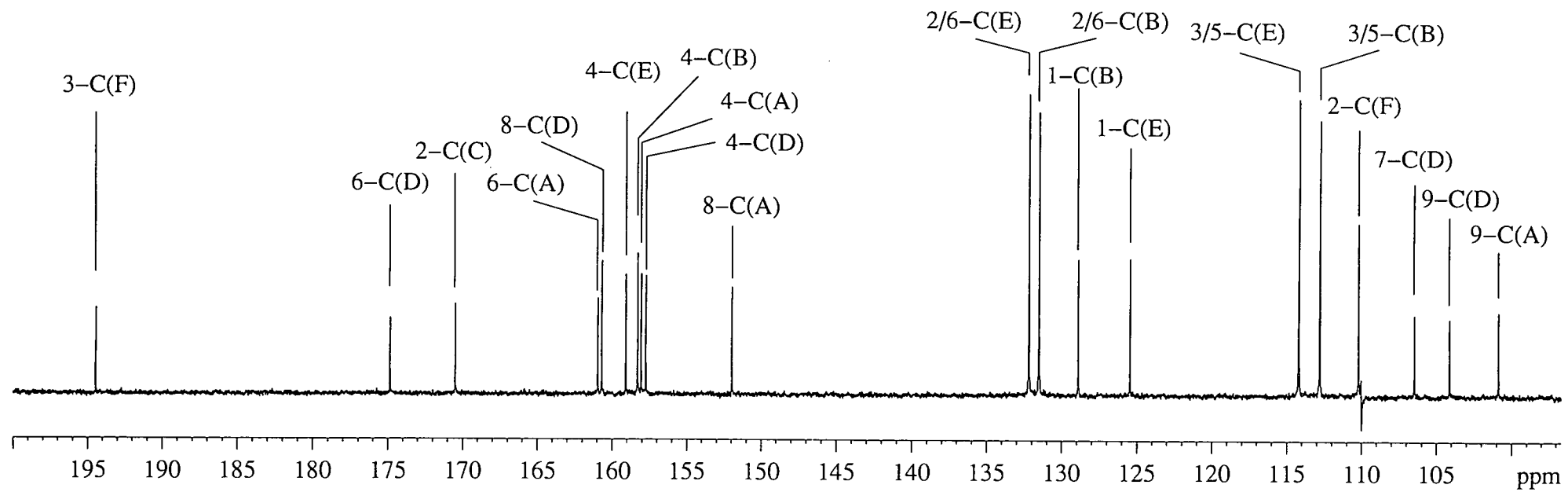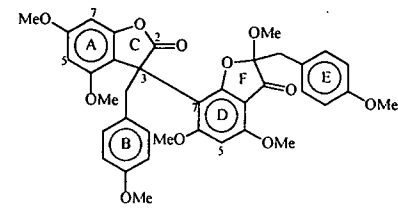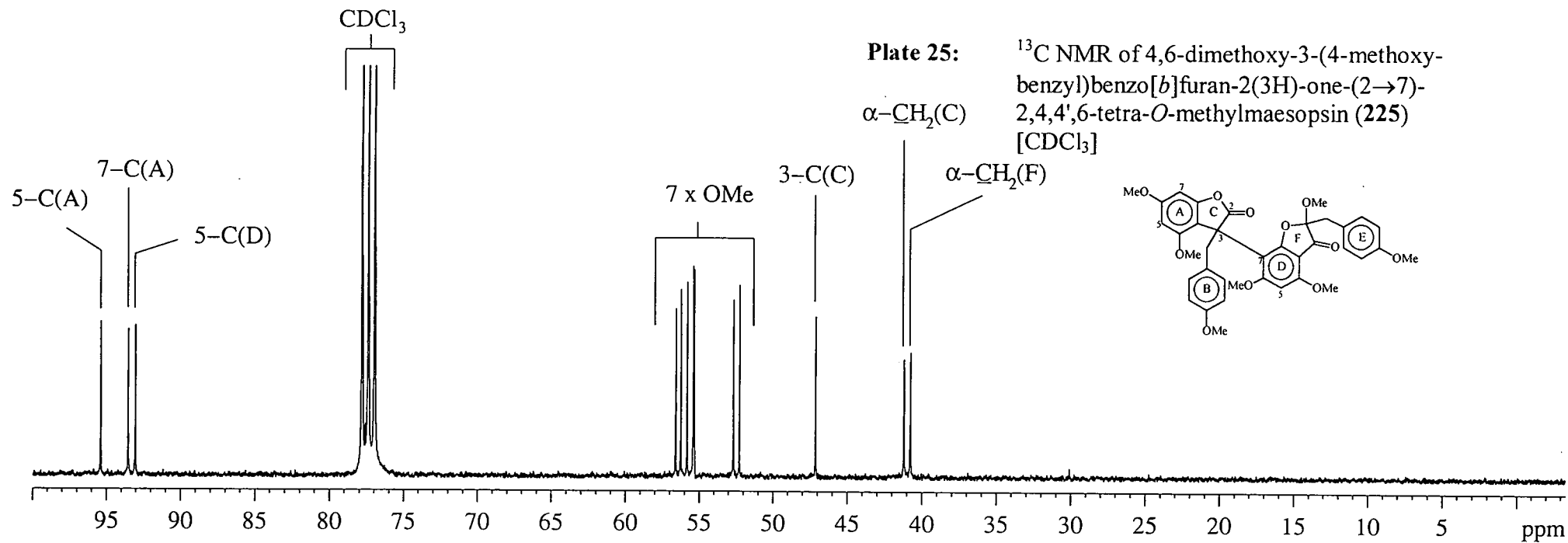
Plate 26: ^{1}H NMR of 2-hydroxy-4,6-dimethoxyacetophenone (**242**) [CDCl$_3$]
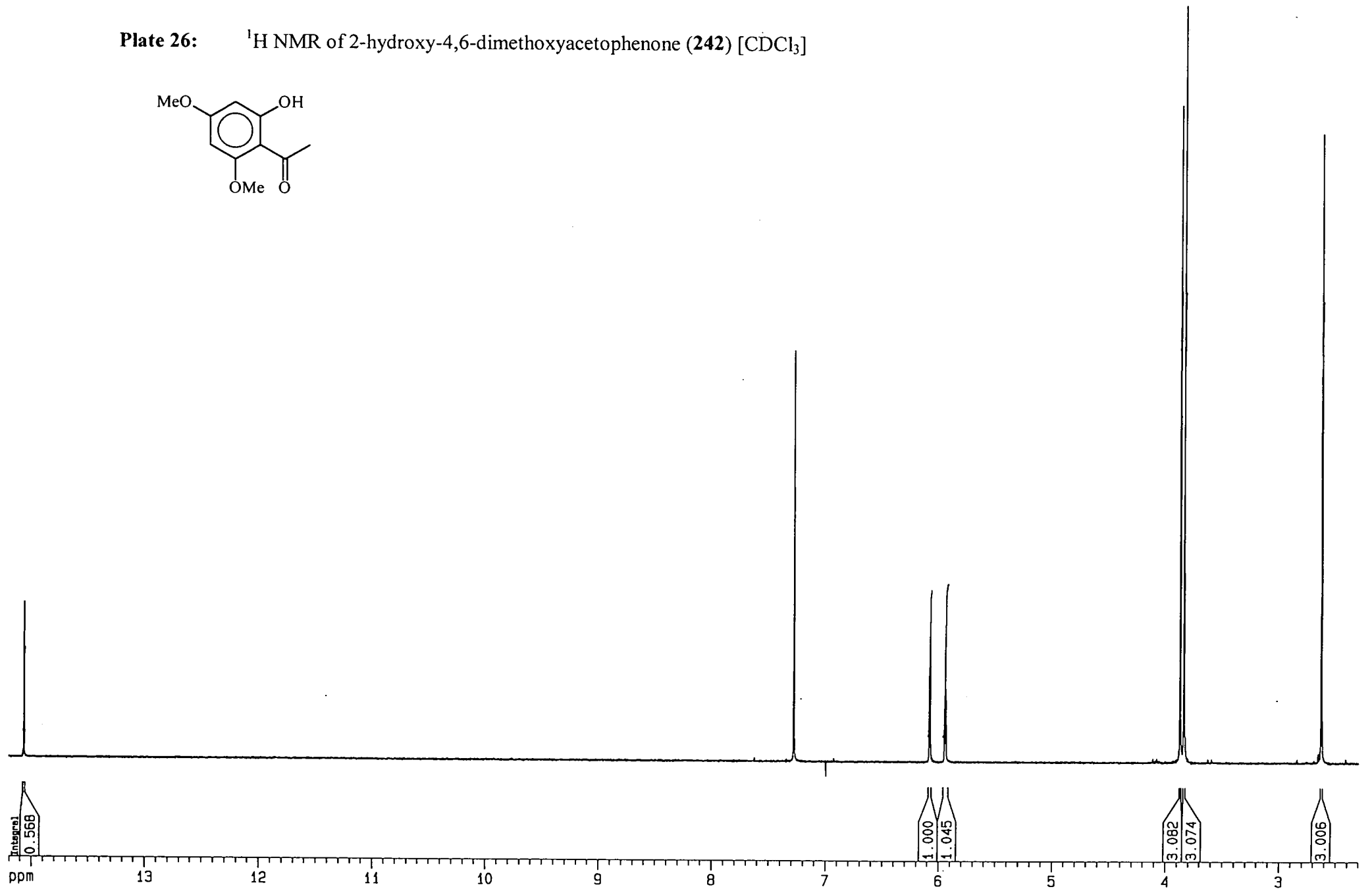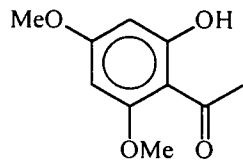
2-OH

7-H

5-H

OMe

OMe

COCH₃

14.10 14.05 ppm

6.05 6.00 5.95 ppm

3.80 3.85 3.90 ppm

2.60 2.65 2.70 ppm
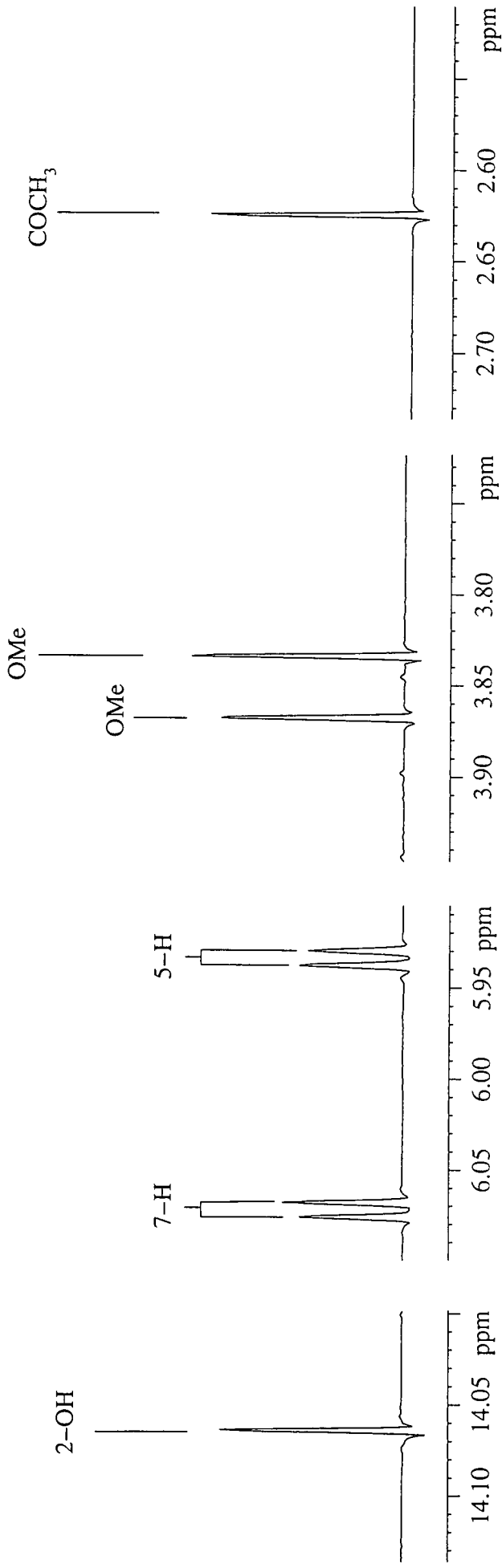
Plate 27: ¹H NMR of 2'-acetoxy-4,4',6'-trimethoxychalcone (**245**) [CDCl₃]

2/6–H(B) α–CH 3/5–H(B) α–CH 7–H(A) 5–H(A) 2 x OMe OMe OCOCH₃

7.50 7.45 7.40 ppm   6.95 6.90 ppm   6.45 6.40 6.35 ppm   3.85 ppm   2.20 ppm
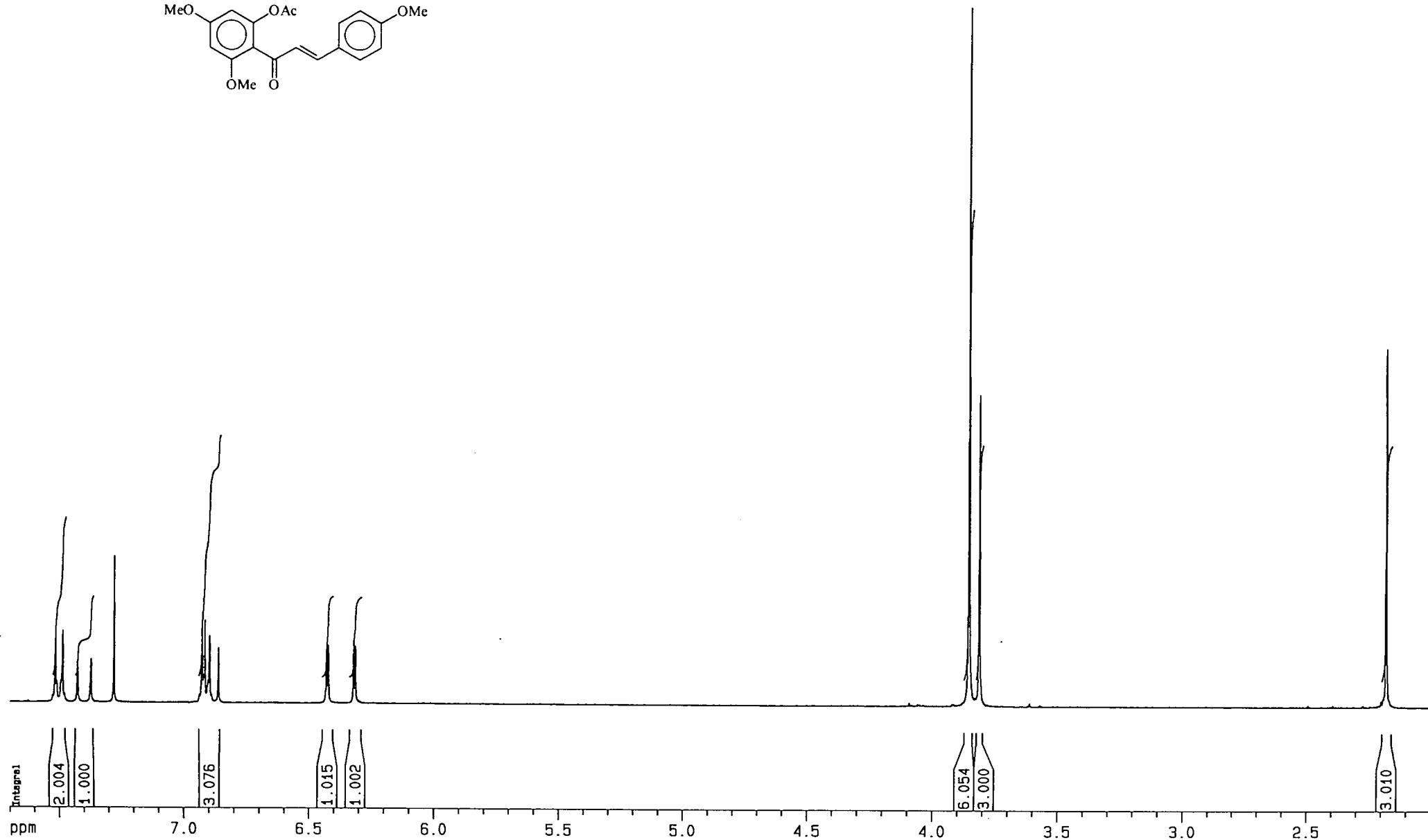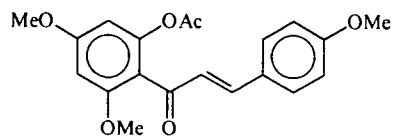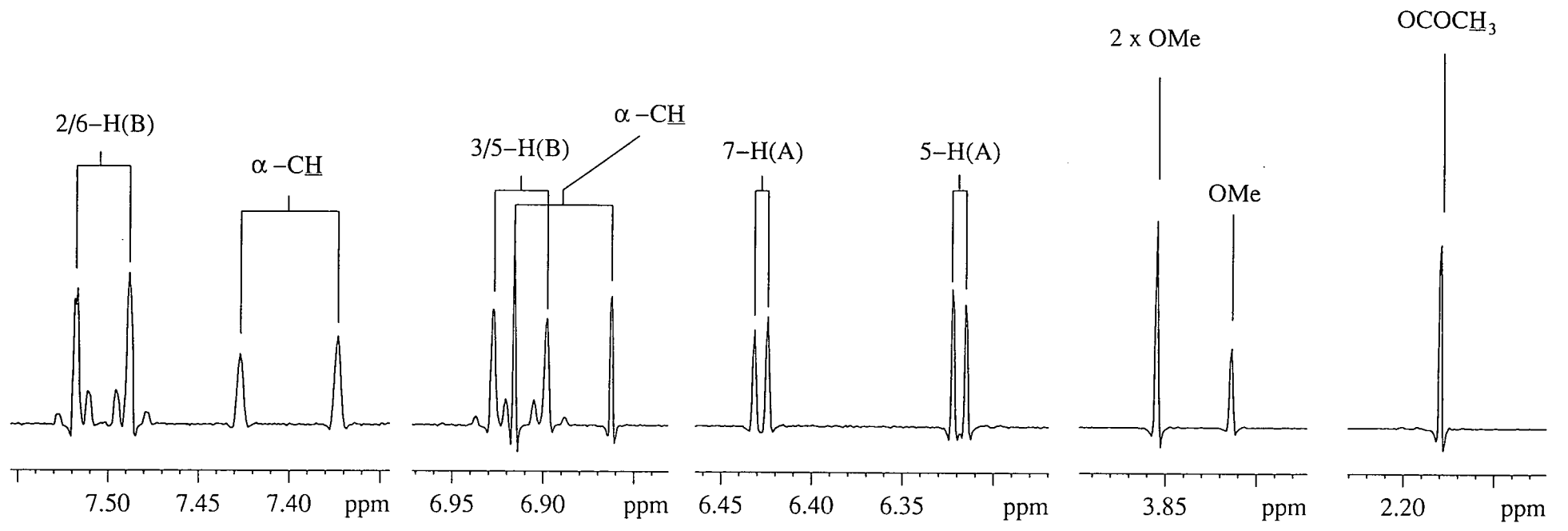
Plate 28: ^{1}H NMR of 2'-acetoxy-α-bromo-β,4,4',6'-tetramethoxydihydrochalcone (**246**) [CDCl$_3$]

OMe

OMe

OMe

OMe

OCOC$\underline{H}_3$

3.90   3.85   ppm

3.20   ppm

2.35   ppm

2/6–H(B)

3/5–H(B)

7–H(A)

5–H(A)

α–C$\underline{H}$

β–C$\underline{H}$

7.40   7.35   ppm

6.95   ppm

6.40   6.35   6.30 ppm

5.05   ppm

4.70   4.65 ppm
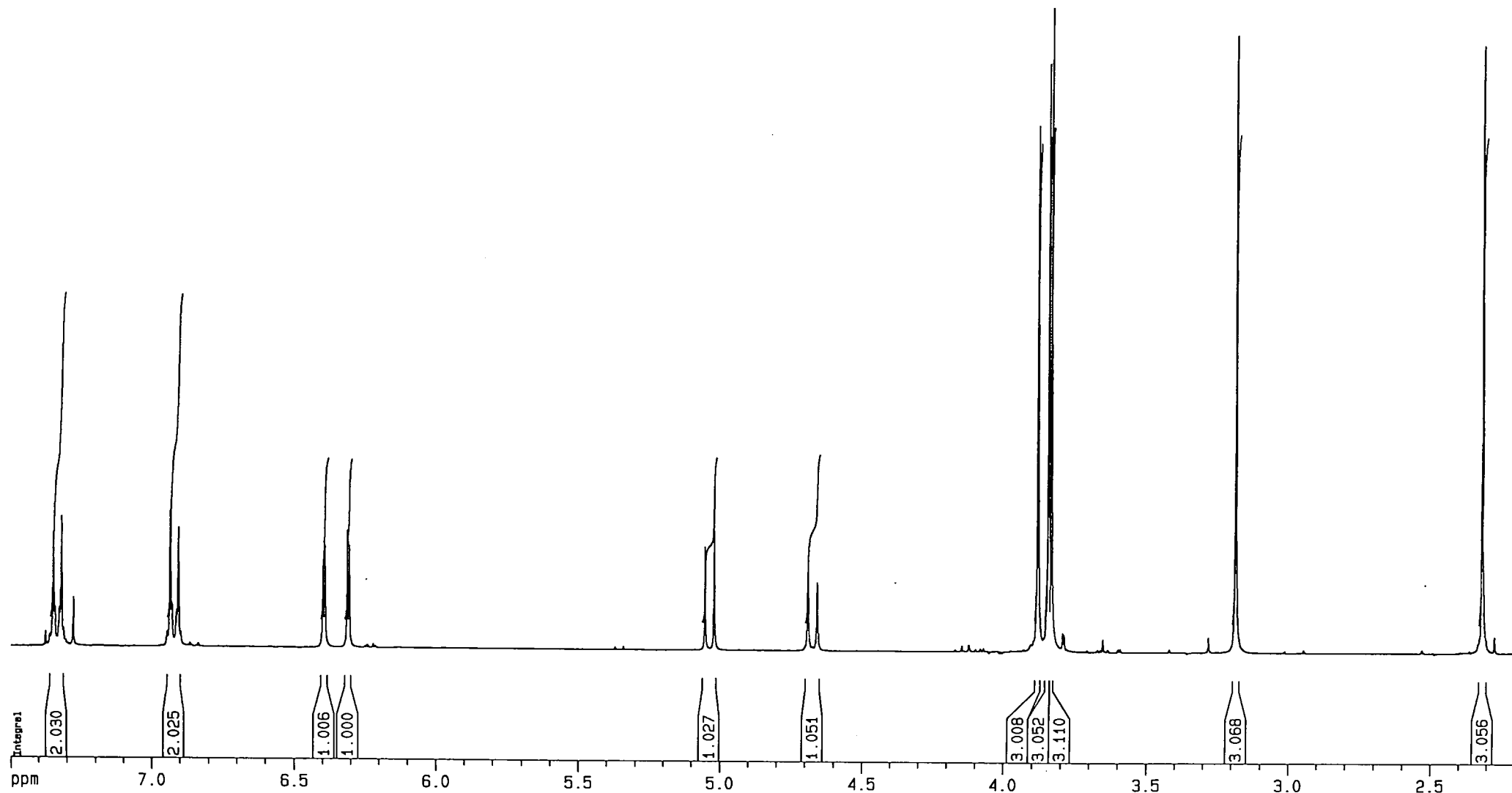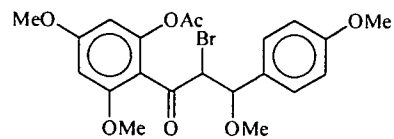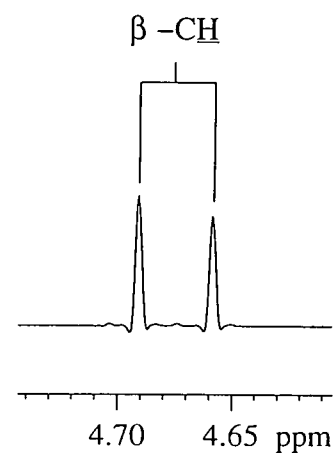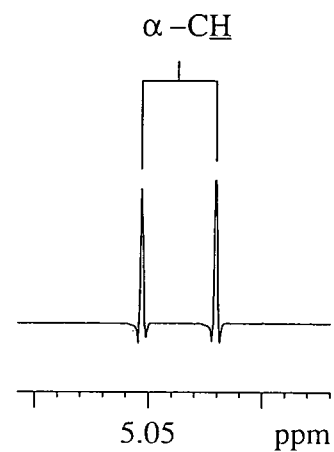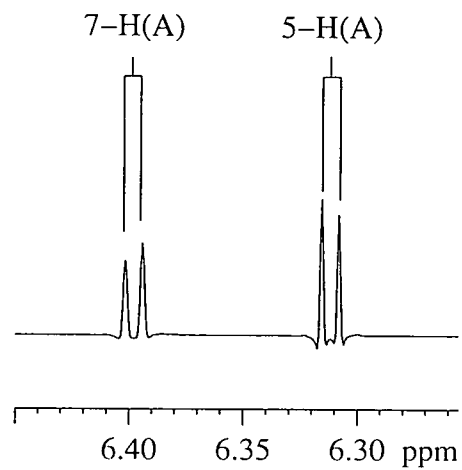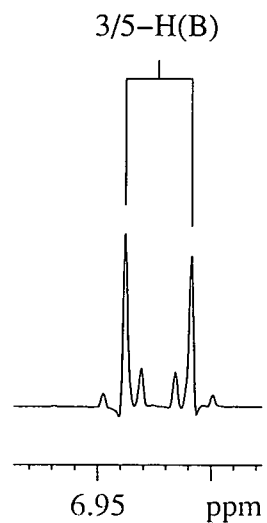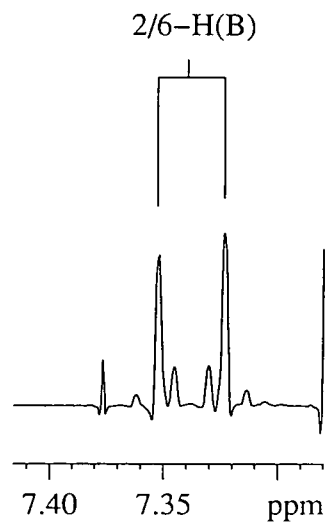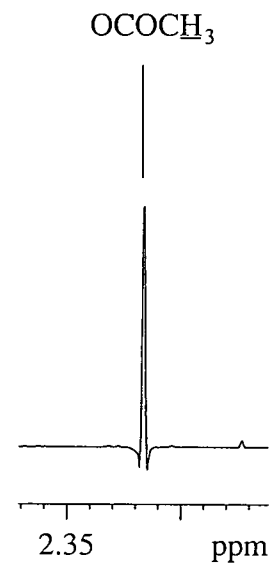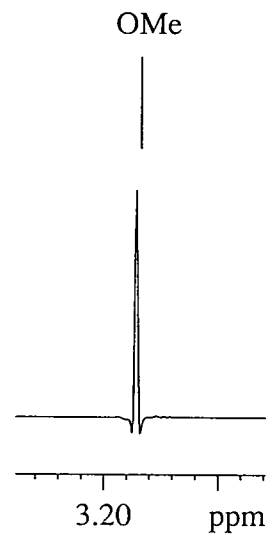
Plate 29: ^{1}H NMR of 4,4',6-trimethoxyaurone (**235**) [CDCl$_3$]
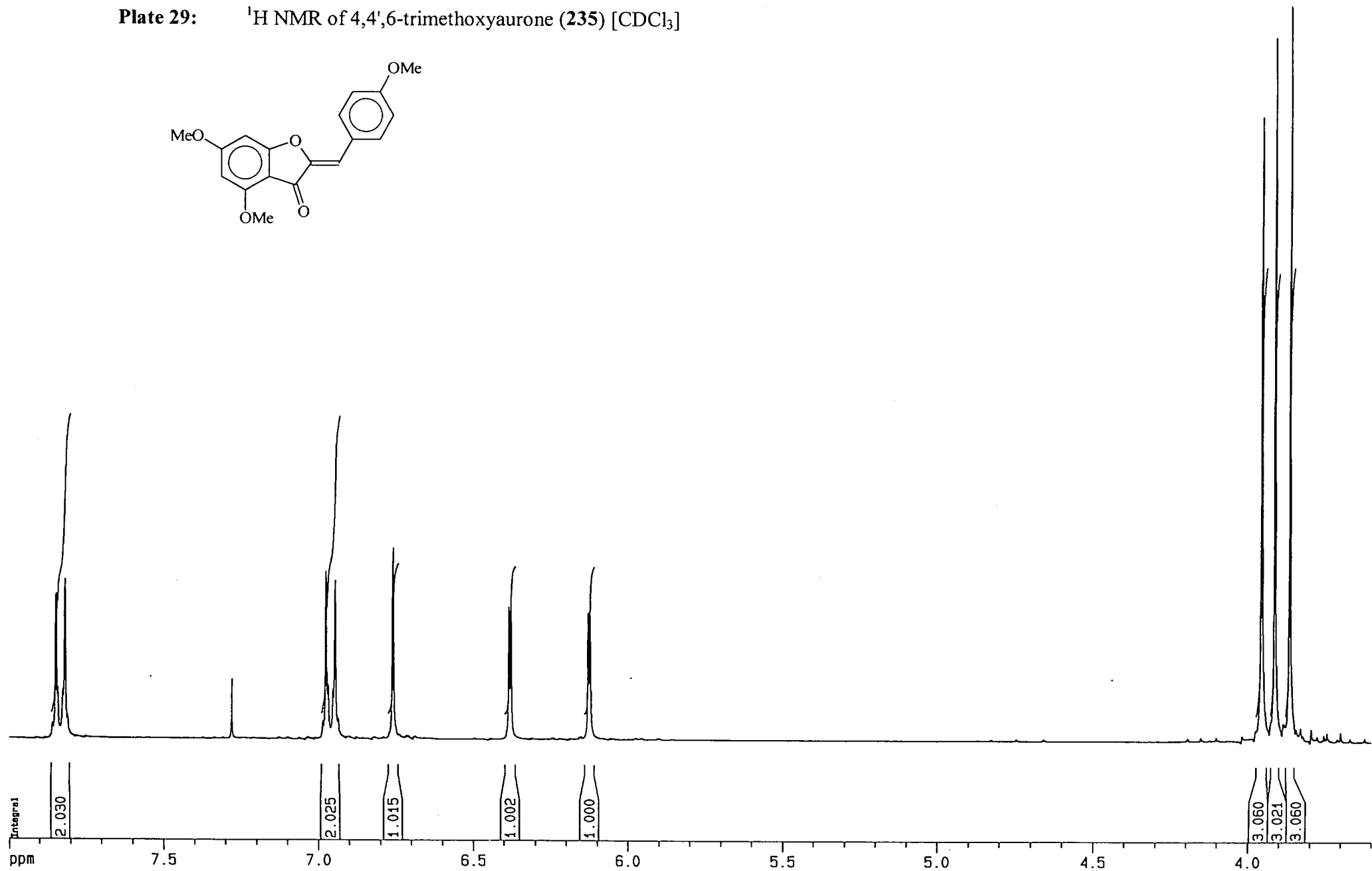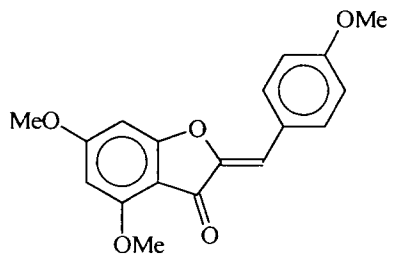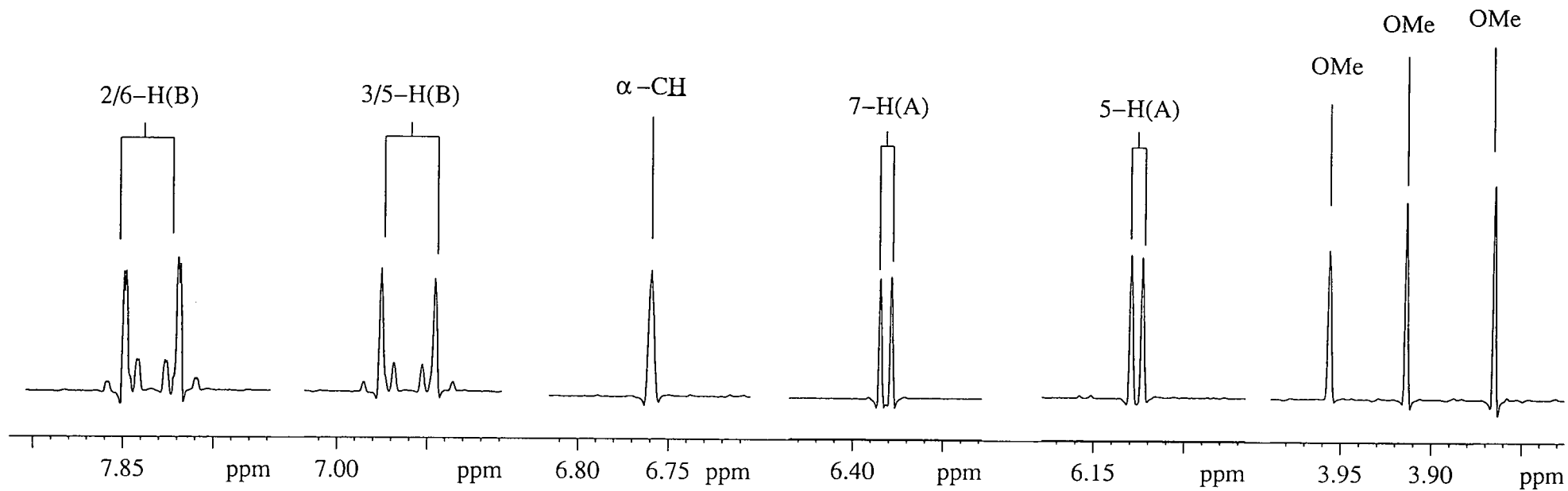
Plate 30: ¹H NMR of 2-(4-metoxybenzyl)-4,6-dimethoxybenzo[*b*]furan-3(2H)-one (**236**) [CDCl₃]

OMe OMe OMe

α–CH₂

α–CH₂

3.90 3.85 3.80 ppm

3.35 3.30 ppm

2.95 2.90 ppm

2/6–H(B) 3/5–H(B) 7–H(A) 5–H(A) 2–H(C)

CHCl₃

7.30 7.25 ppm

6.85 ppm

6.15 6.10 6.05 6.00 ppm

4.75 ppm
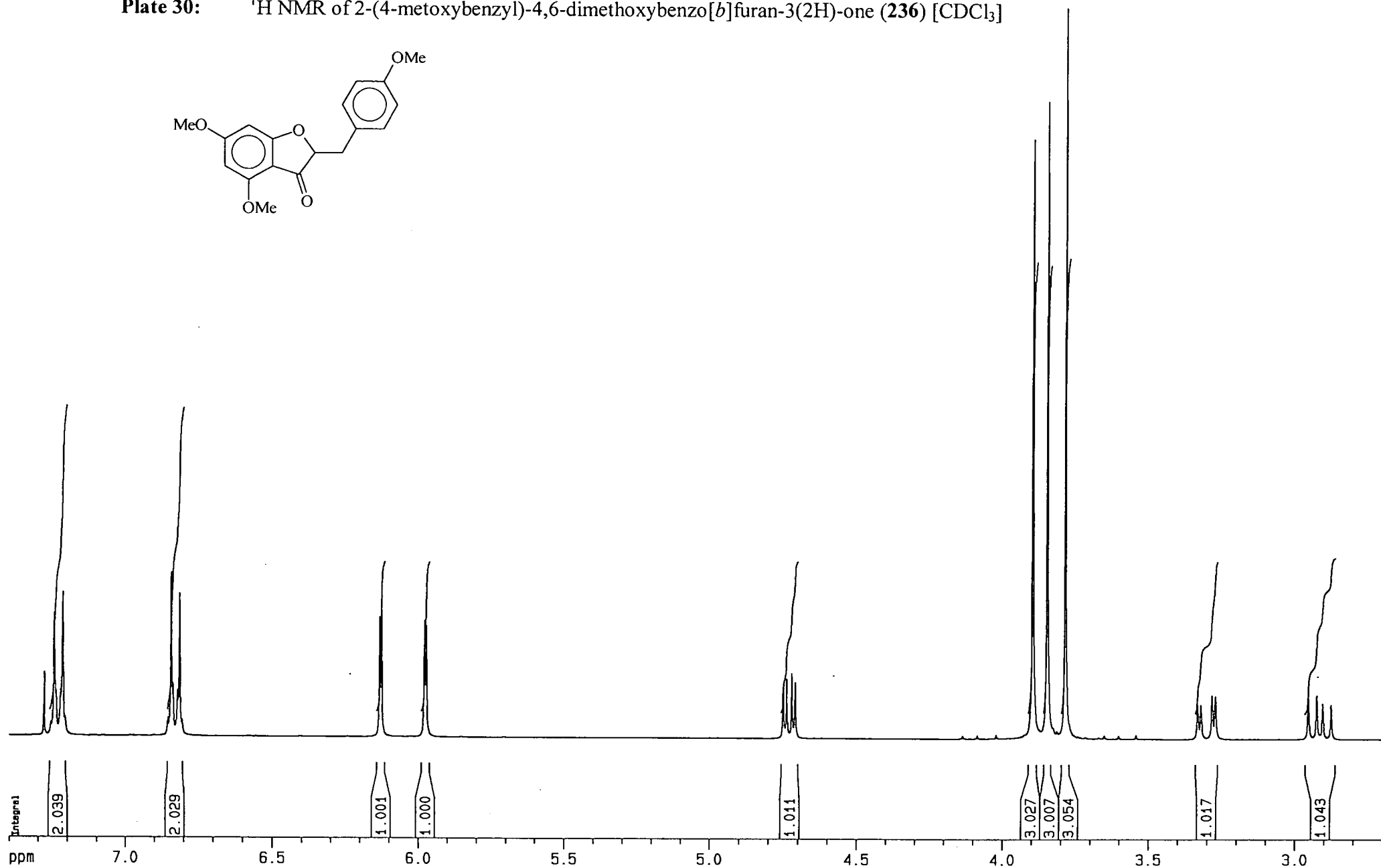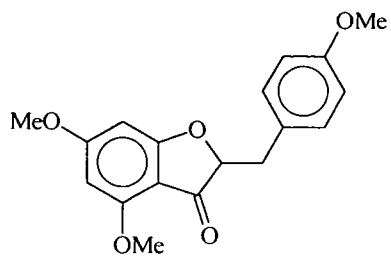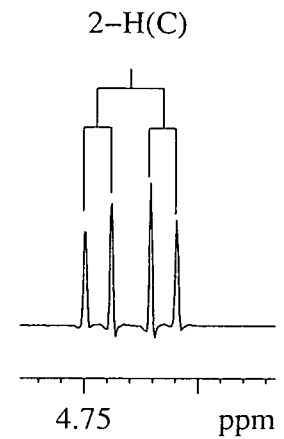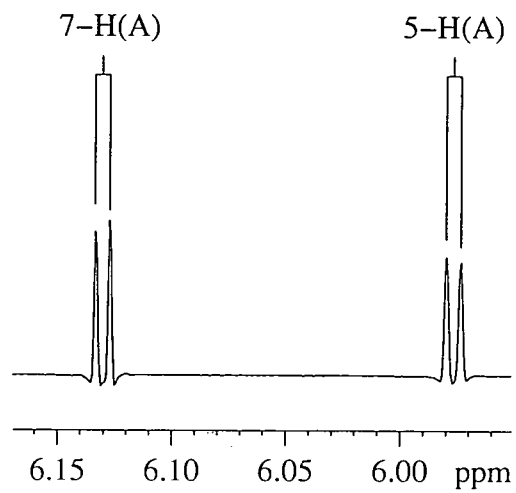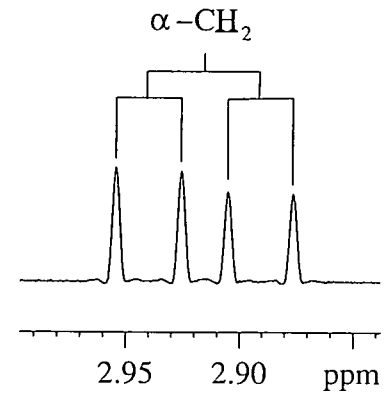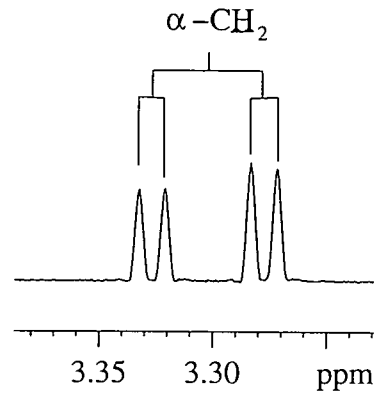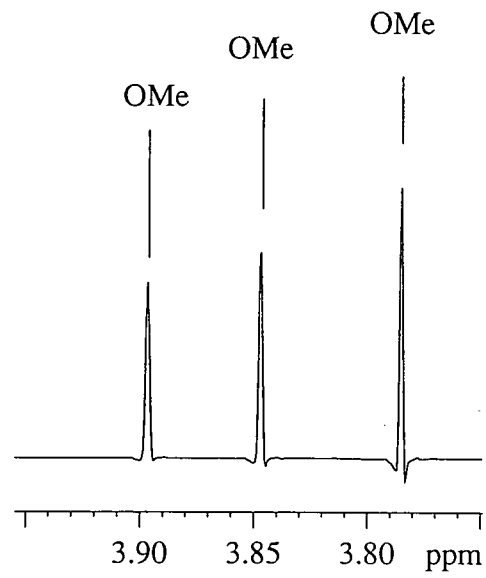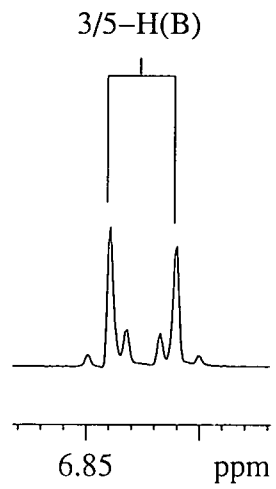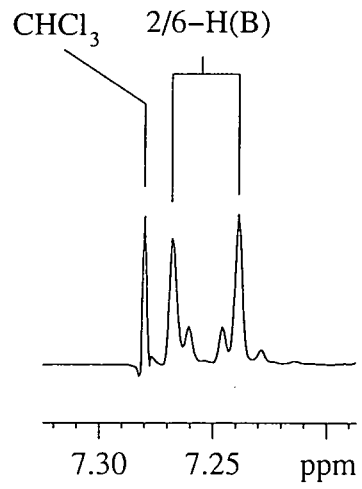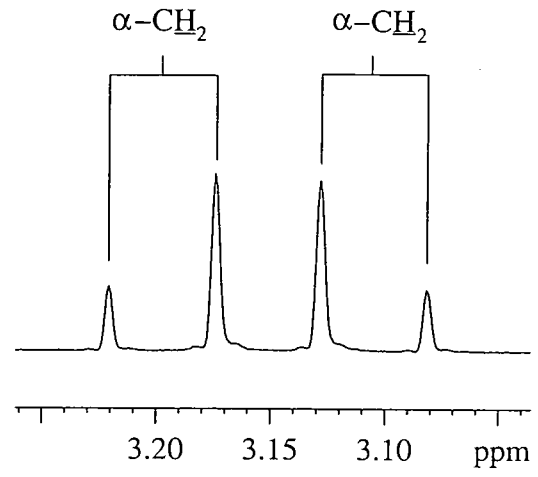
Plate 31: ^{1}H NMR of 4,4',6-tri-O-maesopsin (**238**) [CDCl$_3$]

OMe
OMe
OMe

3.90 3.85 3.80 ppm

α–CH₂
α–CH₂

3.20 3.15 3.10 ppm

CHCl₃ 2/6–H(B)

3/5–H(B)
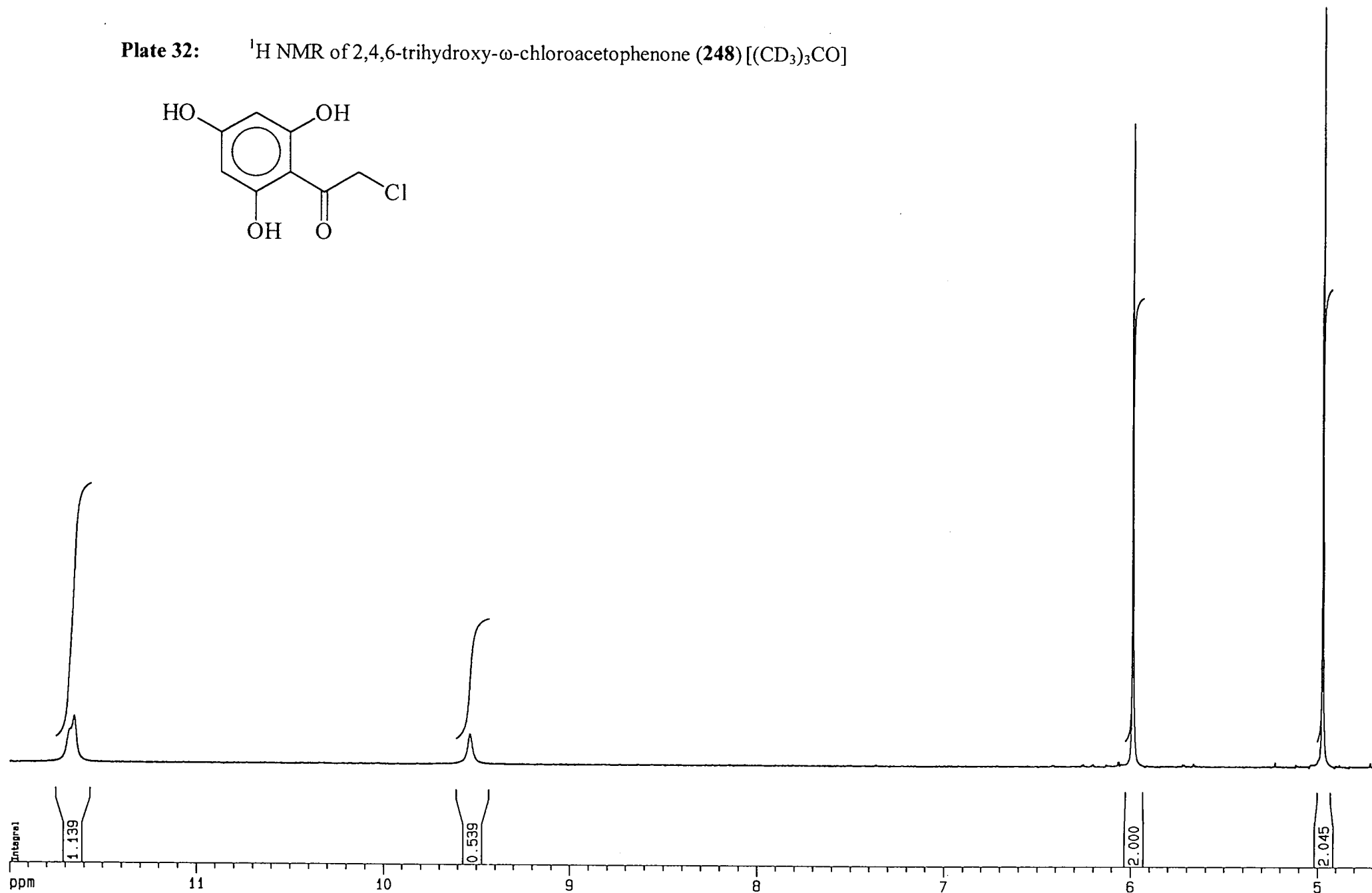
7–H(A) 5–H(A)

7.30 7.25 ppm

6.85 ppm

6.05 6.00 5.95 ppm
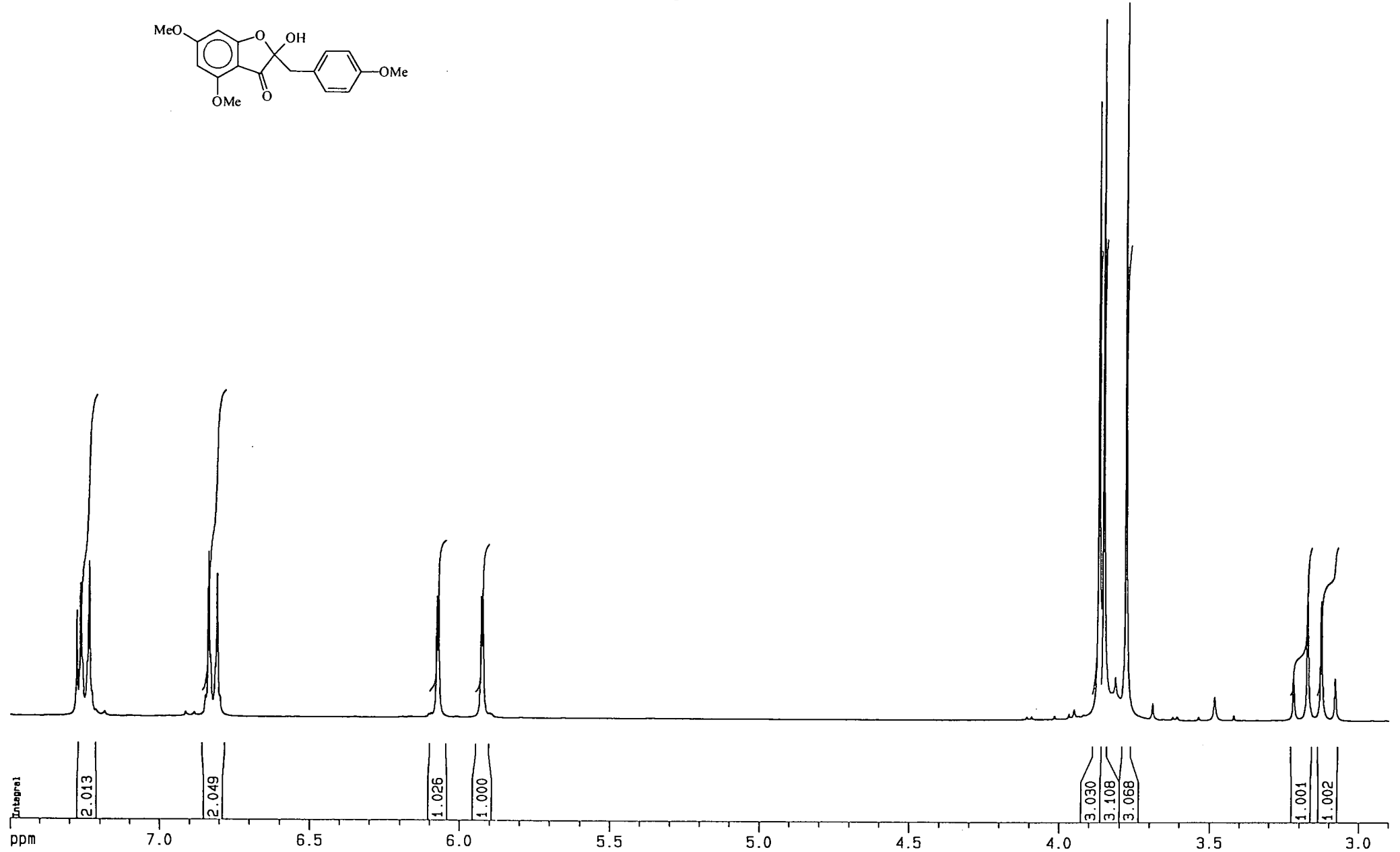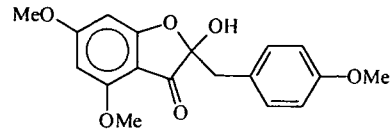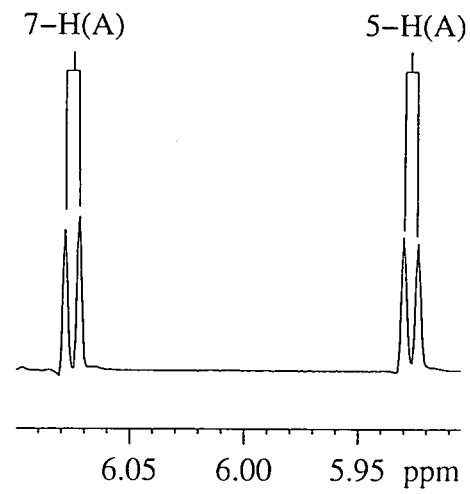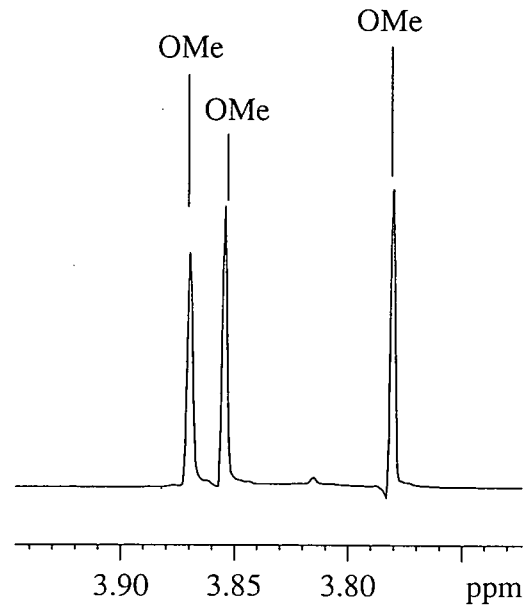
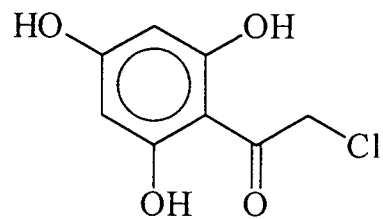**Plate 32:** ^{1}H NMR of 2,4,6-trihydroxy-ω-chloroacetophenone (**248**) [(CD$_3$)$_3$CO]

1.139	0.539		2.000	2.045
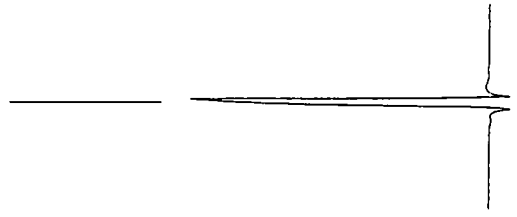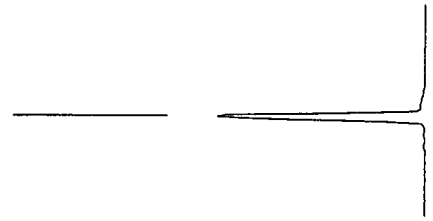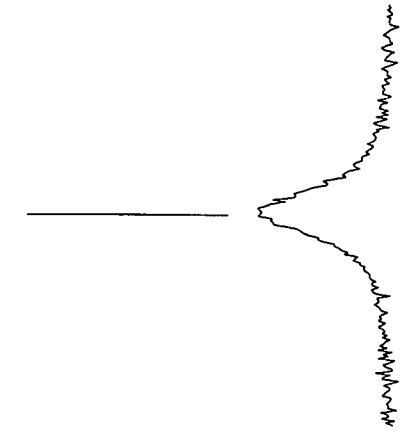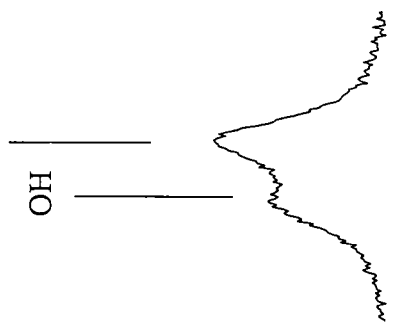
Plate 33: ^{1}H NMR of 4,6-dimethoxybenzo[*b*]furan-3(2H)-one (**239**) [CDCl$_3$]
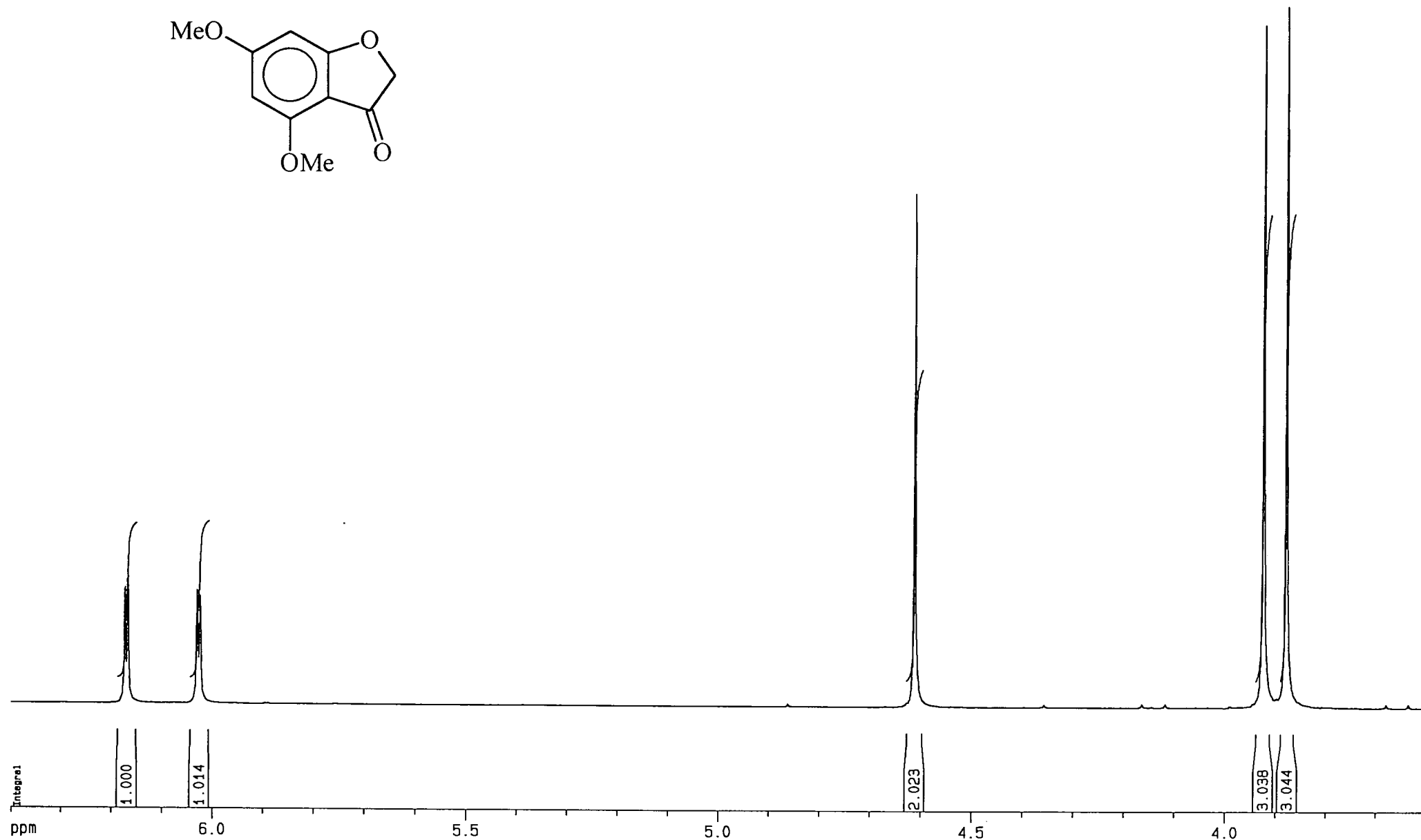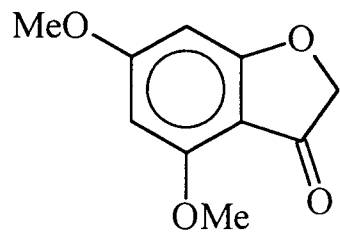
7-H   5-H   2-H₂   OMe   OMe

ppm   6.15 6.10 6.05 ppm   4.65 4.60 ppm   3.95 3.90 ppm
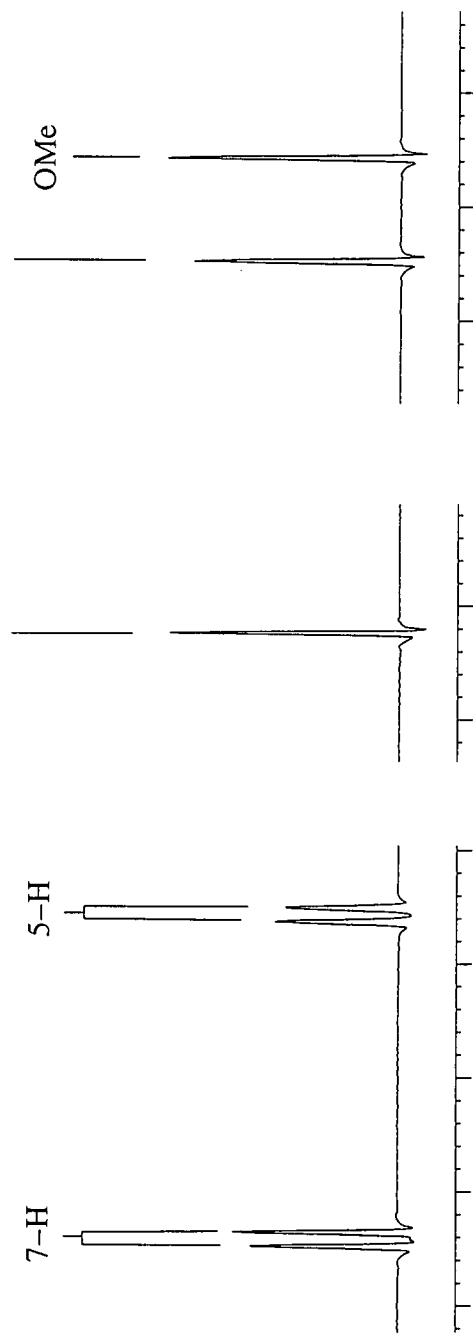
Plate 34: ^{1}H NMR of 4,6-dimethoxy-3-trimethylsilyloxybenzo[*b*]furan (**249**)[C$_6$D$_6$]

| 2–H | 7–H | 5–H | OMe | OMe | SiO(CH₃)₃ |

7.25   ppm   6.65   6.60   6.55   6.50   6.45   ppm   3.45   3.40   ppm   0.35   ppm
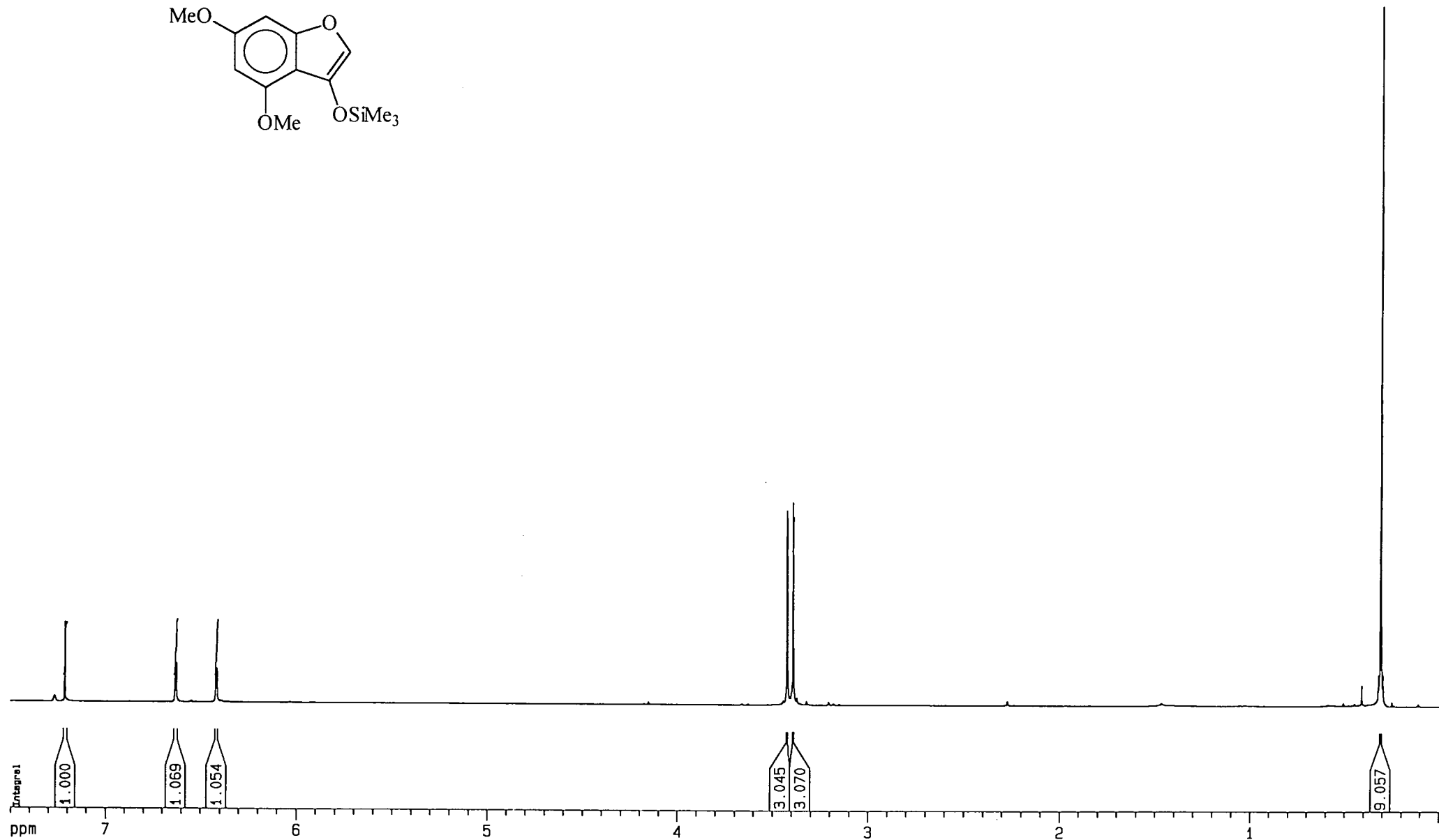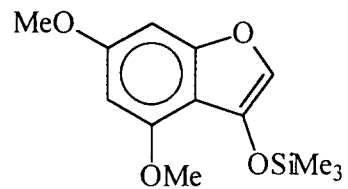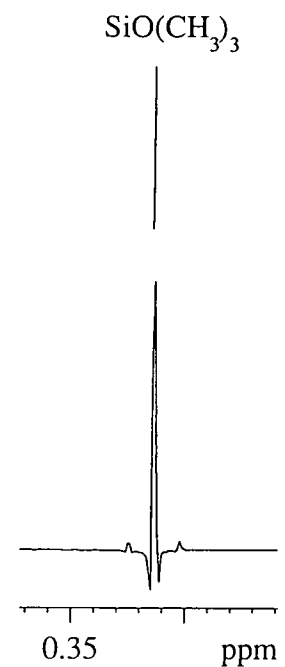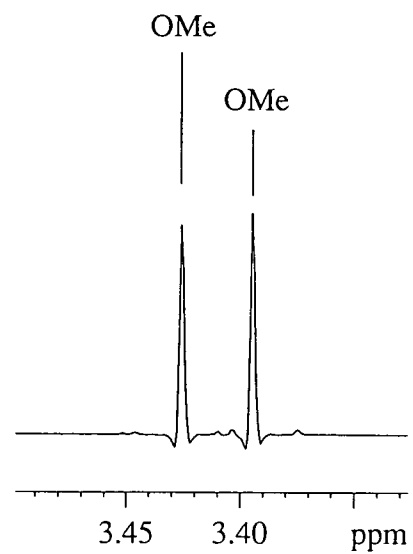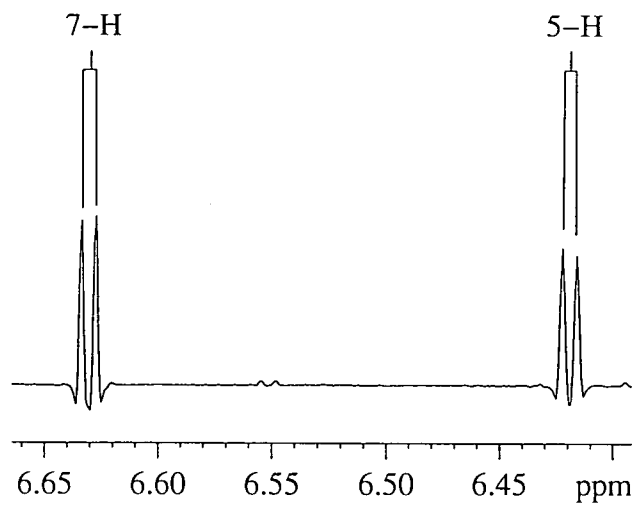
Plate 35: ^{1}H NMR of 2-hydroxy-4,6-dimethoxy-benzo[*b*]furan-3(2H)-one (**251**) [CDCl$_3$]

2–OCOCH₃

OMe

OMe

2–H

7–H

5–H

2.20 ppm

3.95 3.90 ppm

6.25 6.20 6.15 6.10 ppm
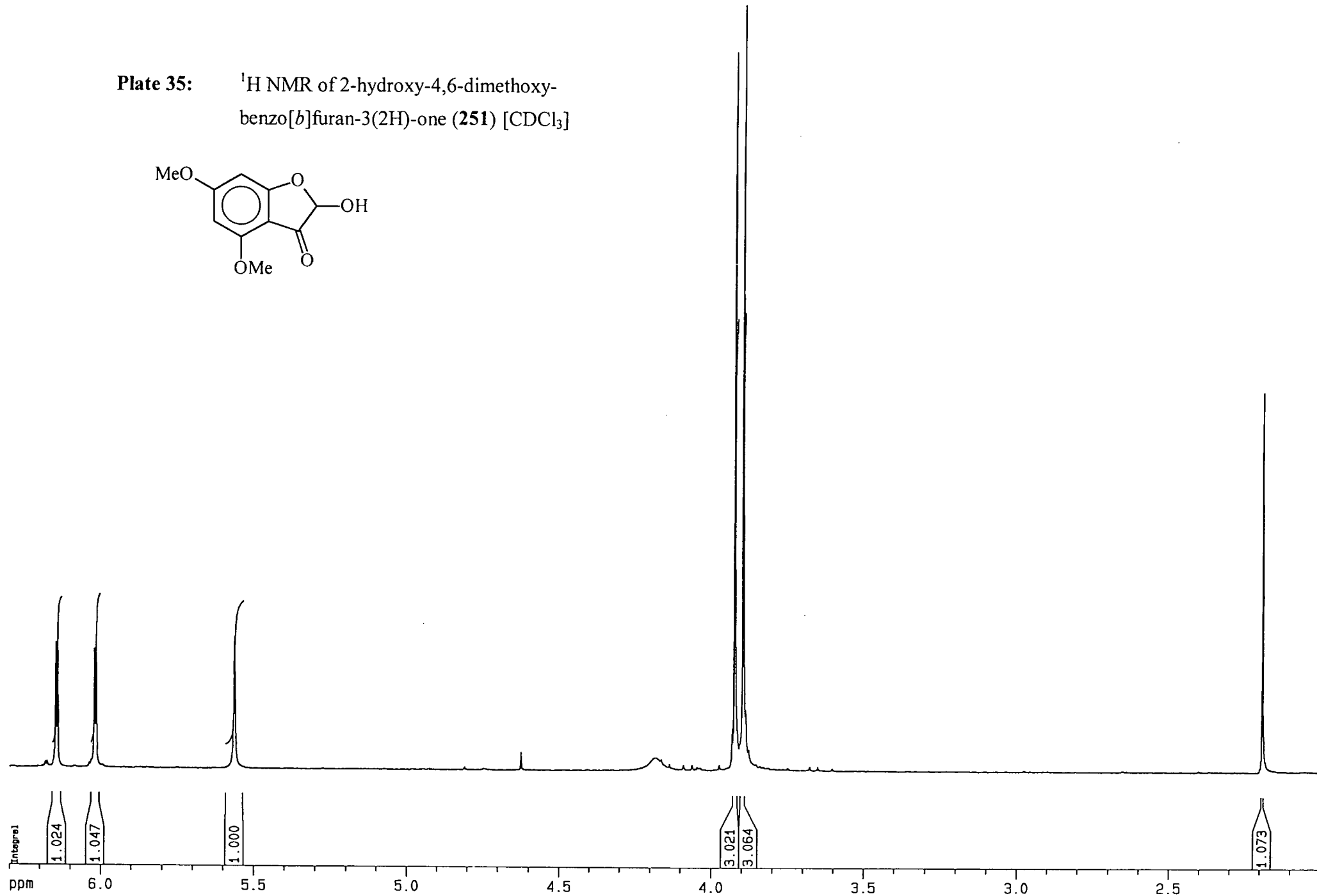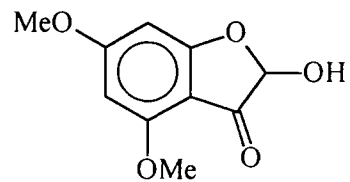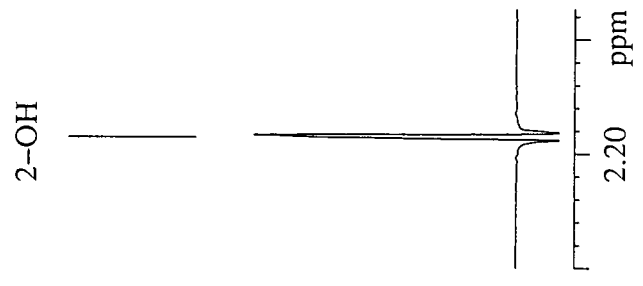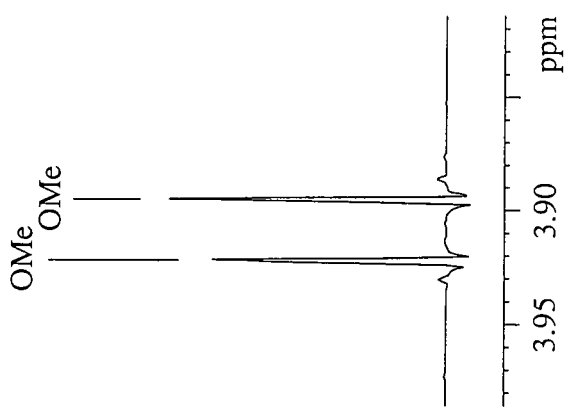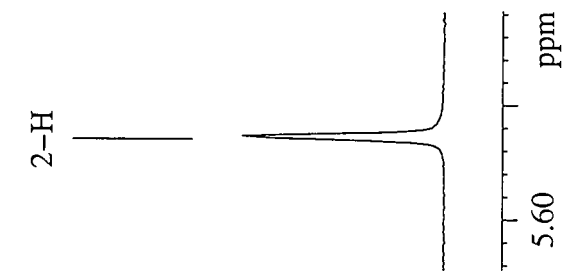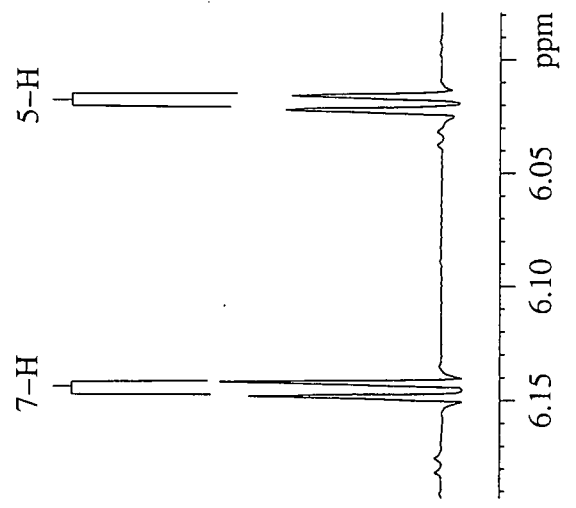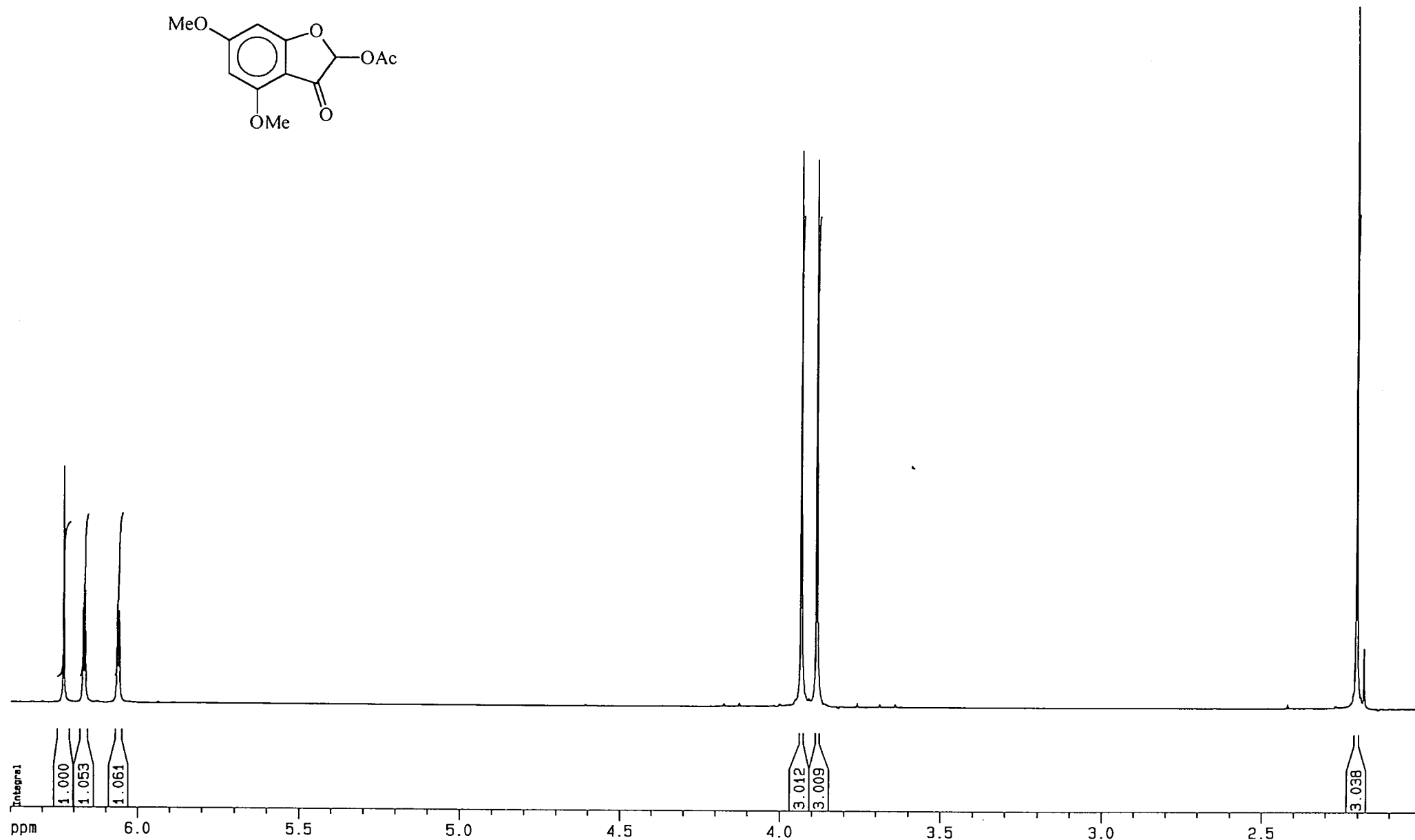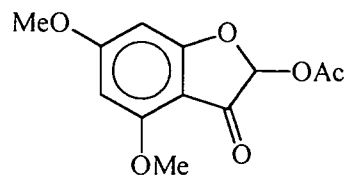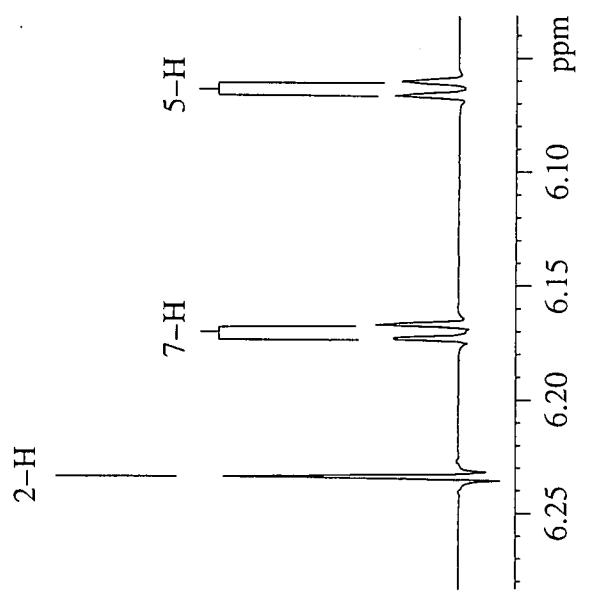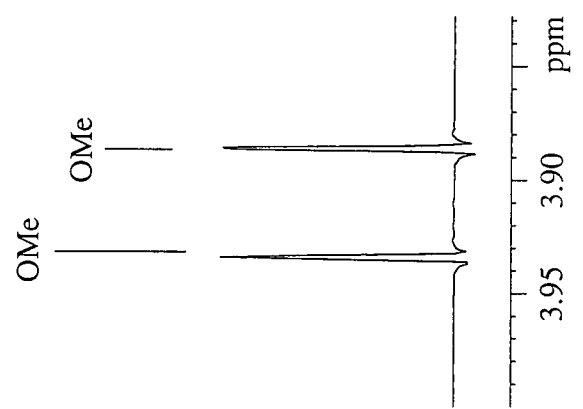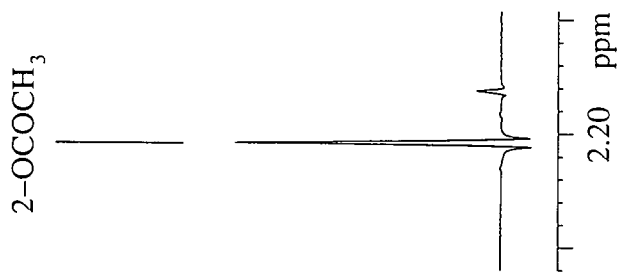
Plate 37: ^{1}H NMR of 2,4,6-trimethoxybenzo[*b*]furan-3(2H)-one (**240**) [CDCl$_3$]
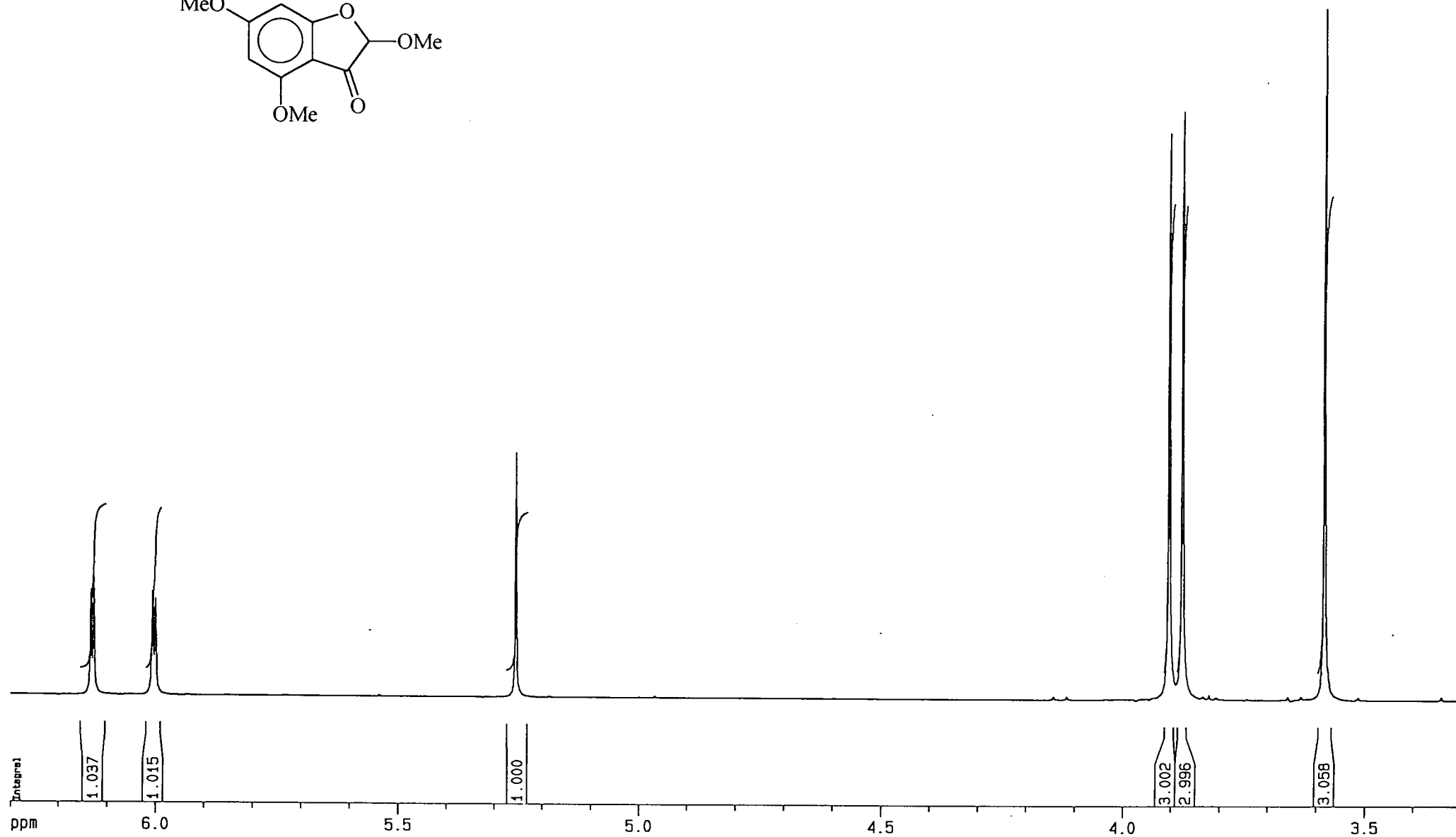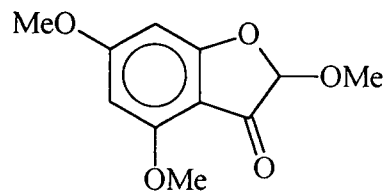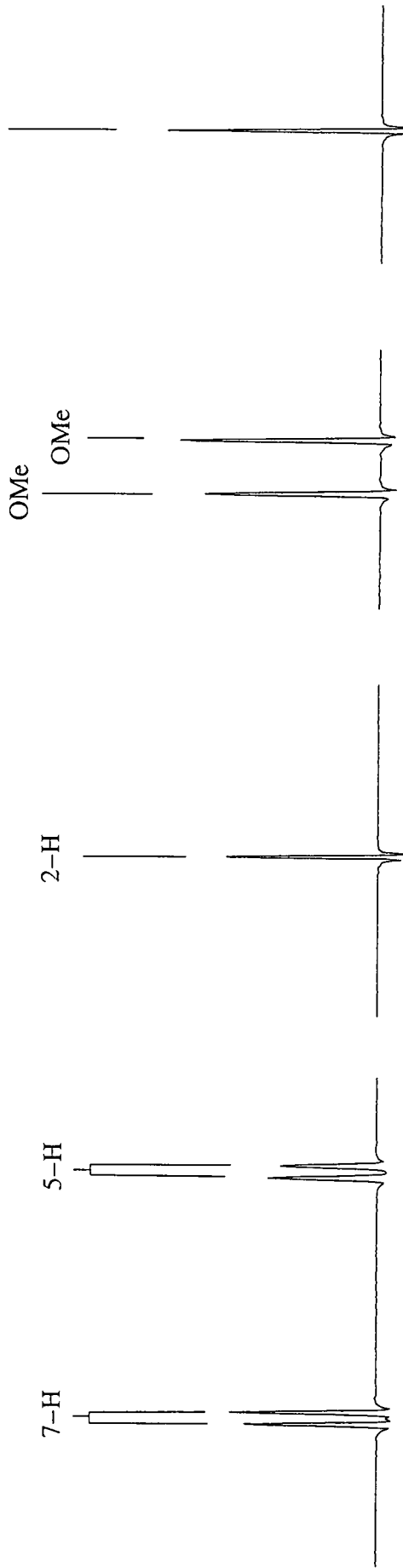
Plate 38: ^{1}H NMR of 2-(4-methoxybenzyl)-4,6-dimethoxy-3-trimethylsilyloxy-benzo[*b*]furan (**237**) [C$_6$D$_6$]

2/6–H(B) 3/5–H(B) 7–H(A) 5–H(A) α–CH₂ OMe OMe OMe OSI(CH₃)₃

7.35 ppm   6.90 ppm   6.55 6.50 6.45 ppm   4.15 ppm   3.45 3.40 ppm   0.40 ppm
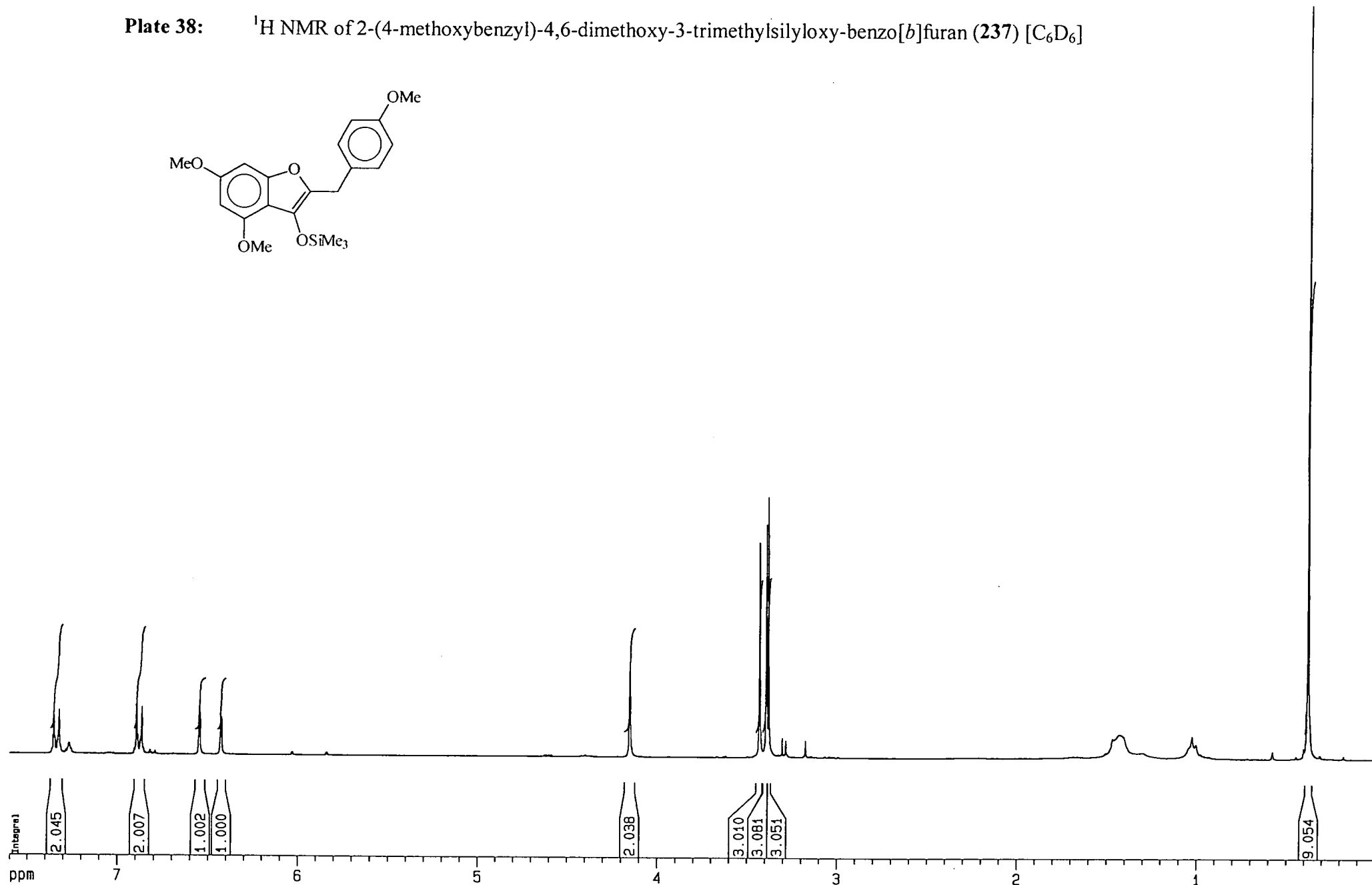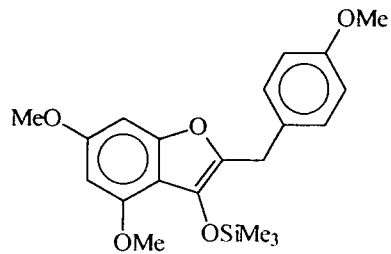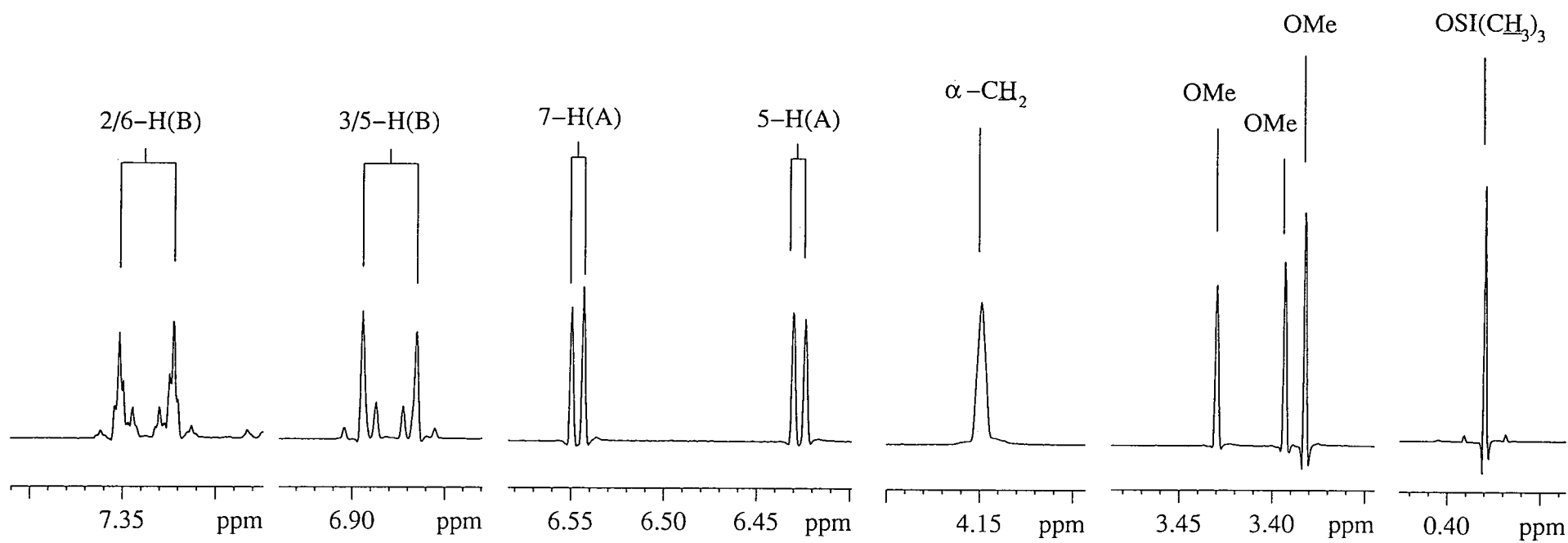
Plate 39: ^{1}H NMR of 2-[phenylsulfonylamino(4-nitrophenyl)methyl]-2-deoxymaesopsin (**261**) [CDCl$_3$]

Top row (left to right):
7-H(A) 5-H(A) α-CH O OMe OMe OMe α-CH₂ α-CH₂

4.90 ppm 3.75 3.70 3.65 ppm 3.55 3.50 ppm 3.25 3.20 ppm

Bottom row (left to right):
2/6-H(D) 2/6-H(E) 4-H(E) CHCl₃ 3/5-H(E) 2/6-H(B) 3/5-H(D) 3/5-H(B) H-N

7.80 7.75 7.70 ppm 7.40 ppm 7.25 7.20 ppm 7.05 ppm 6.65 ppm 6.10 ppm
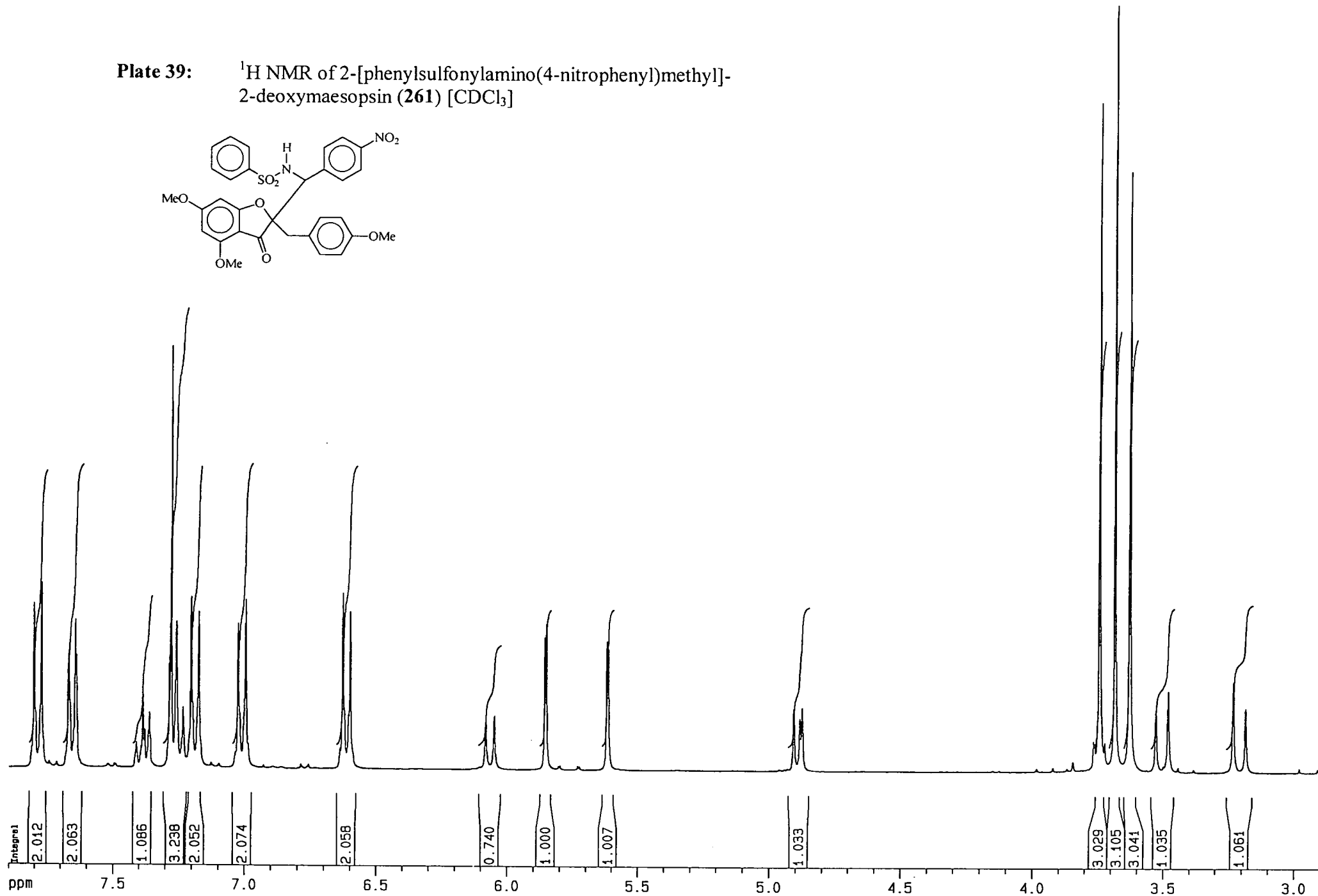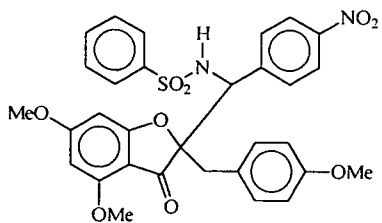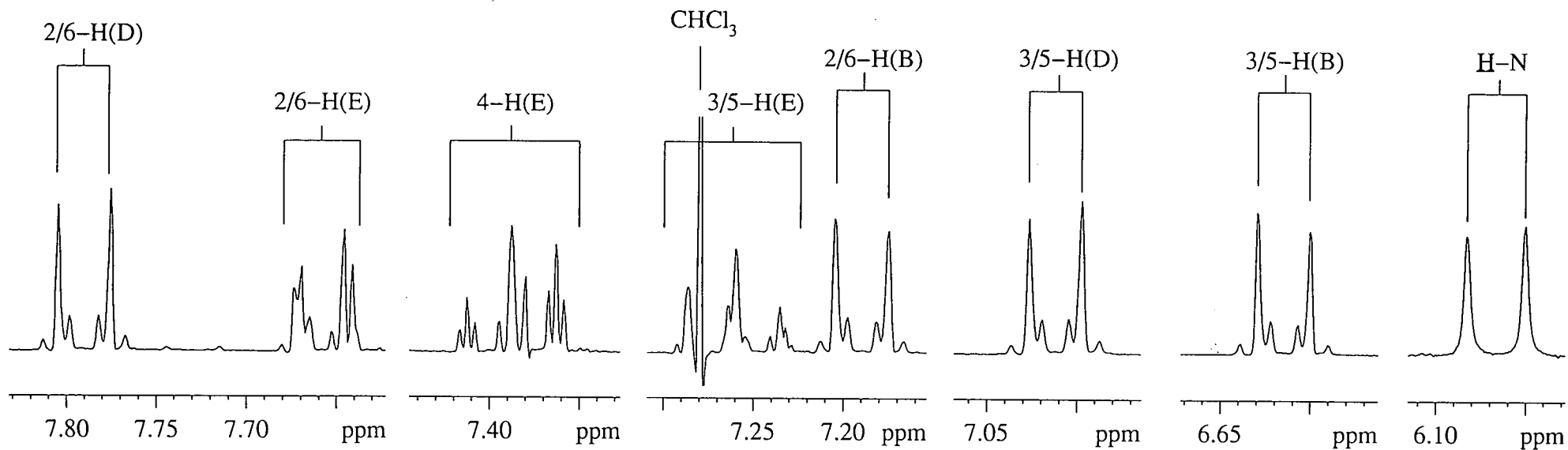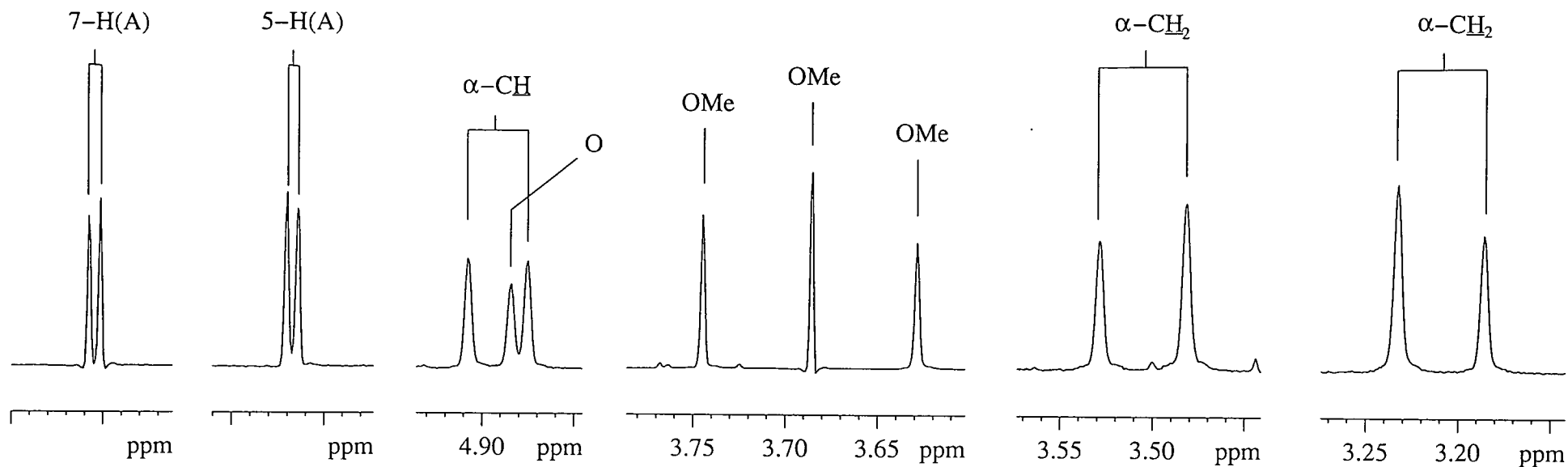
Plate 40: ¹H NMR of 2-*O*-acetyl-4,4',6-tri-*O*-methylmaesopsin (**262**) [CDCl₃]
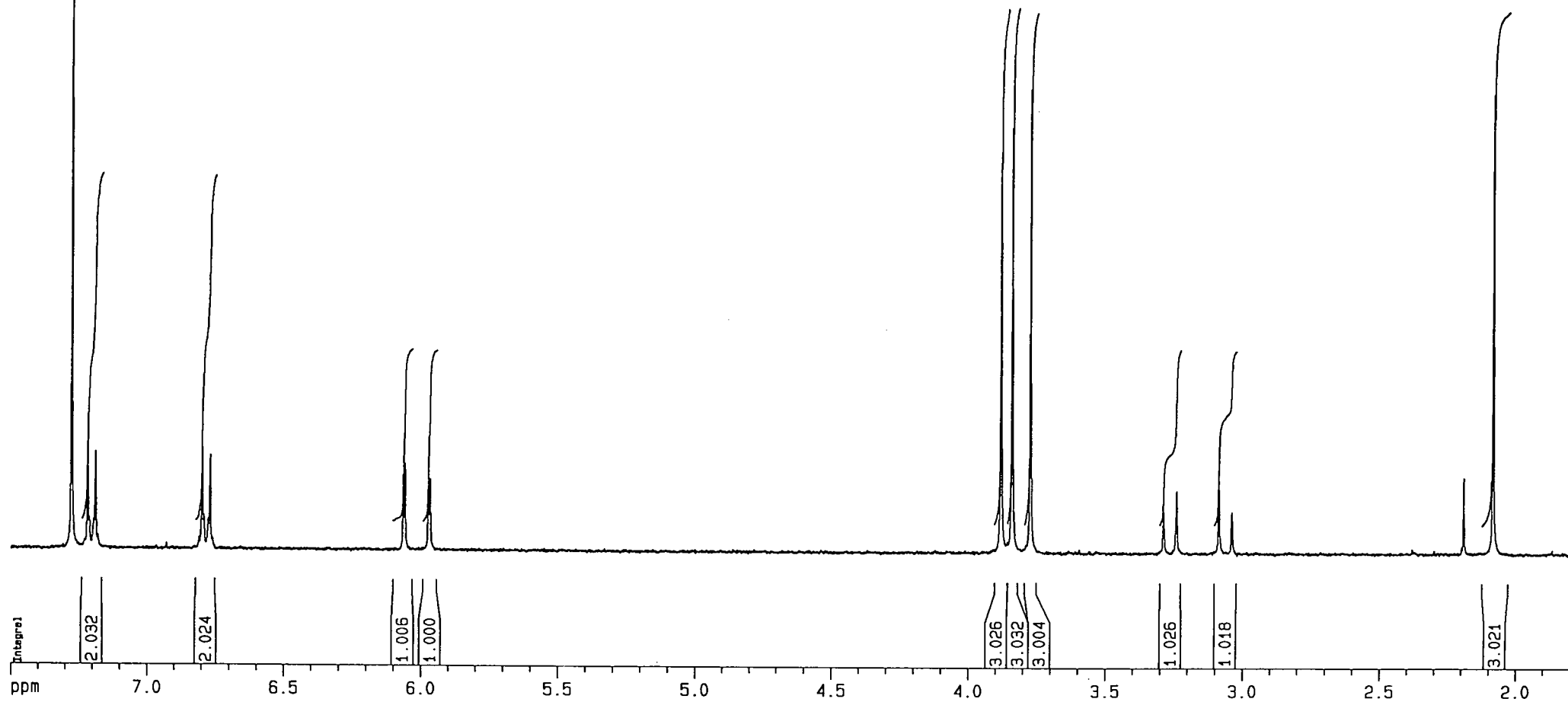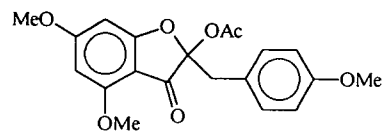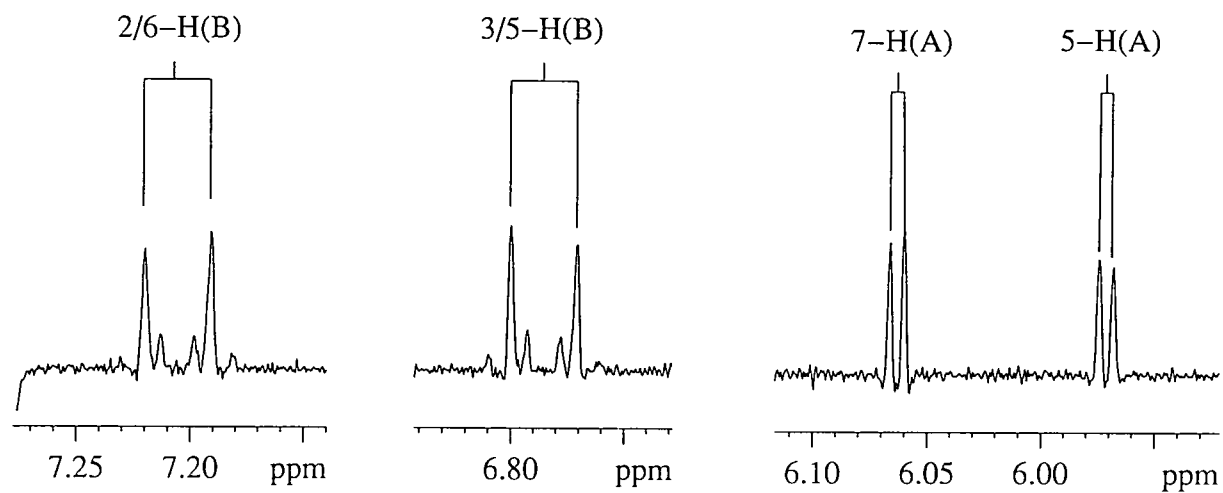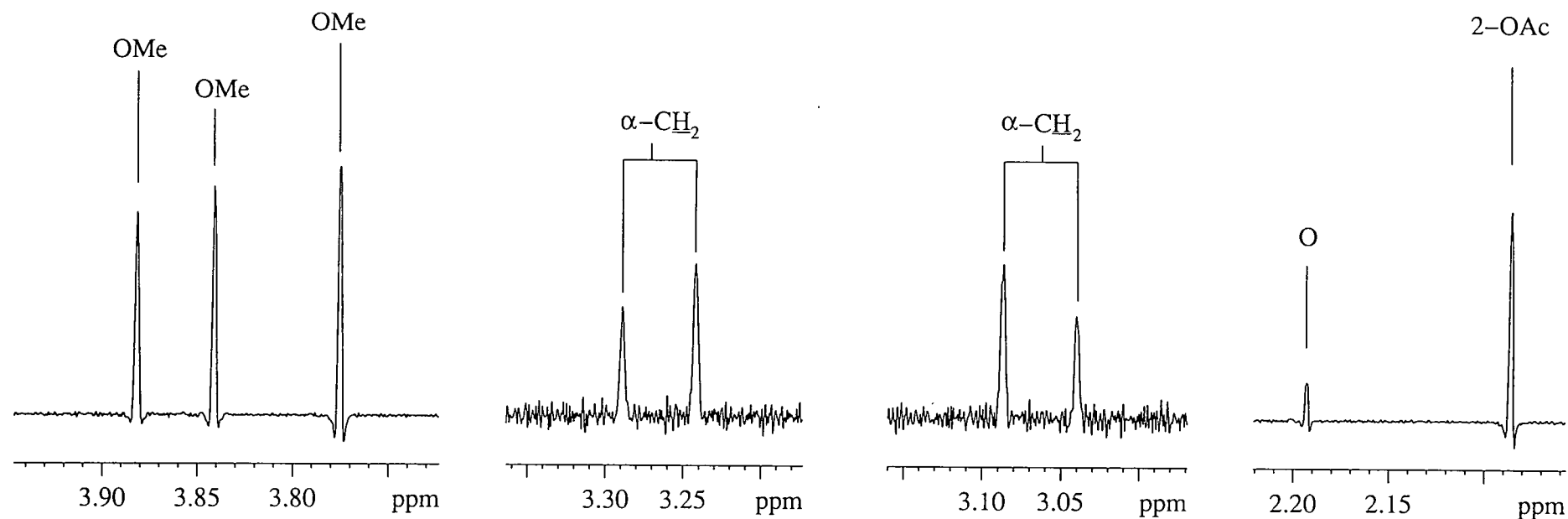
Plate 41: ¹H NMR of 2,4,6-trimethoxy-2-methylbenzo[*b*]furan-3(2H)-one (**263**) [CDCl₃]

Integral
1.000 1.028 3.039 3.036 2.998 3.015

ppm   6.0   5.5   5.0   4.5   4.0   3.5   3.0   2.5   2.0
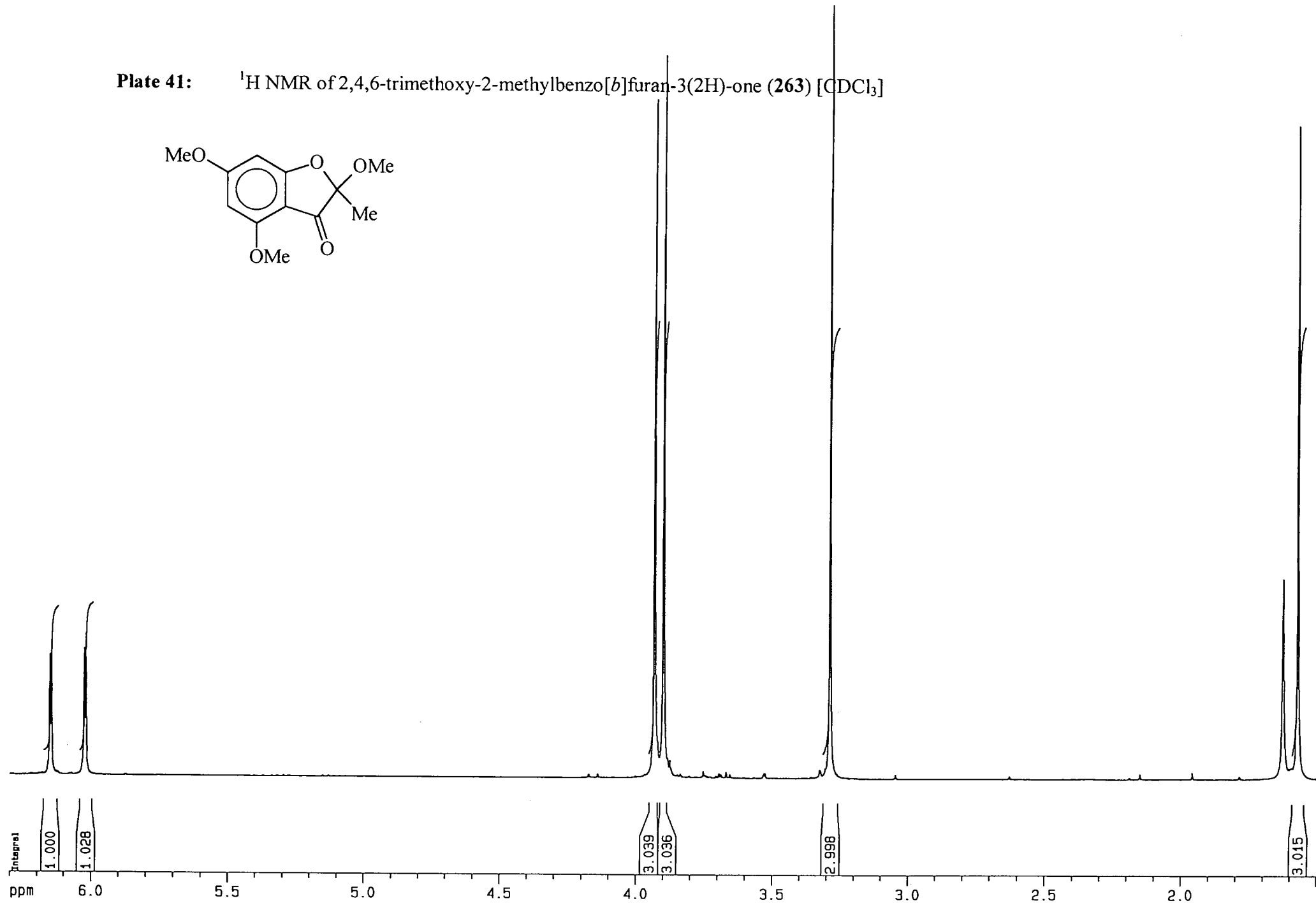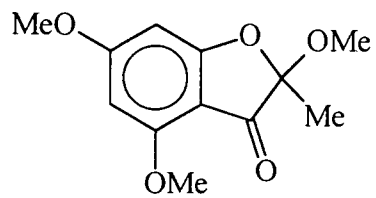
7–H   5–H

OMe   OMe   OMe

2–Me

6.20 6.15 6.10 6.05 ppm

3.95 3.90 ppm

3.30 ppm

1.60 ppm
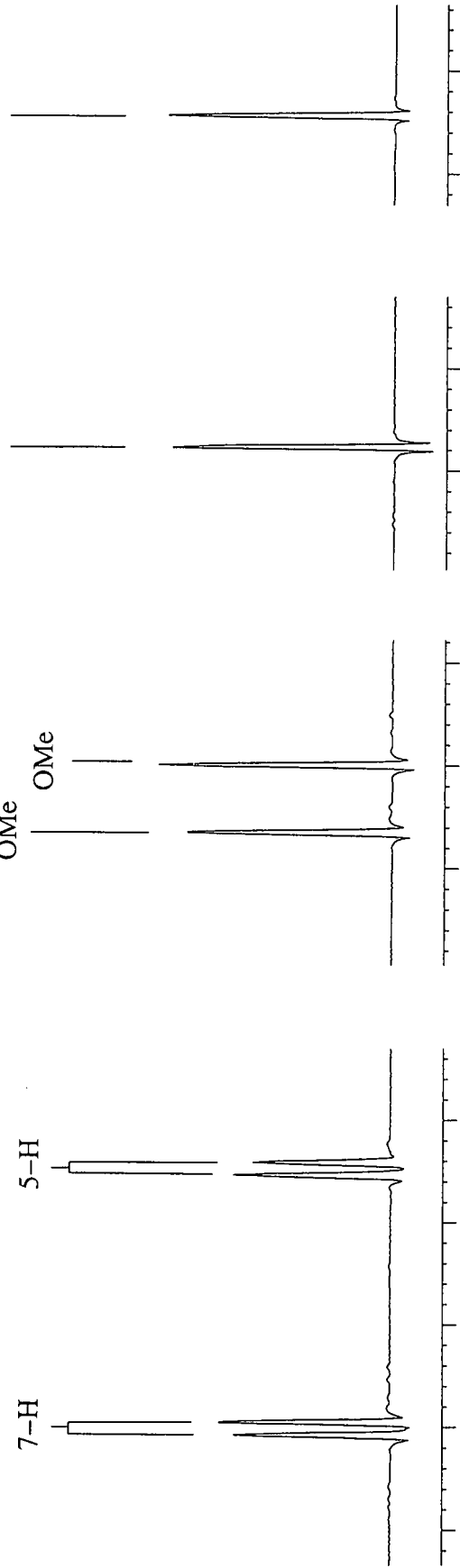
Plate 42: ^{1}H NMR of 2-(2,3,4,6-tetramethoxy-2,3-dihydrobenzofuran-3-yl)-2,4,6-trimethoxybenzo[*b*] furan-3(2H)-one (**264**) [CDCl$_3$]

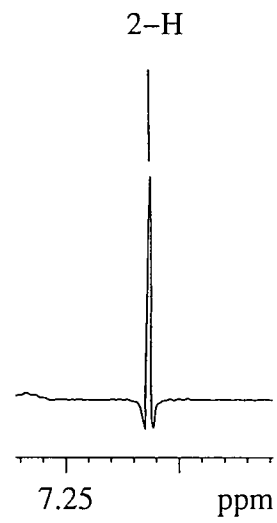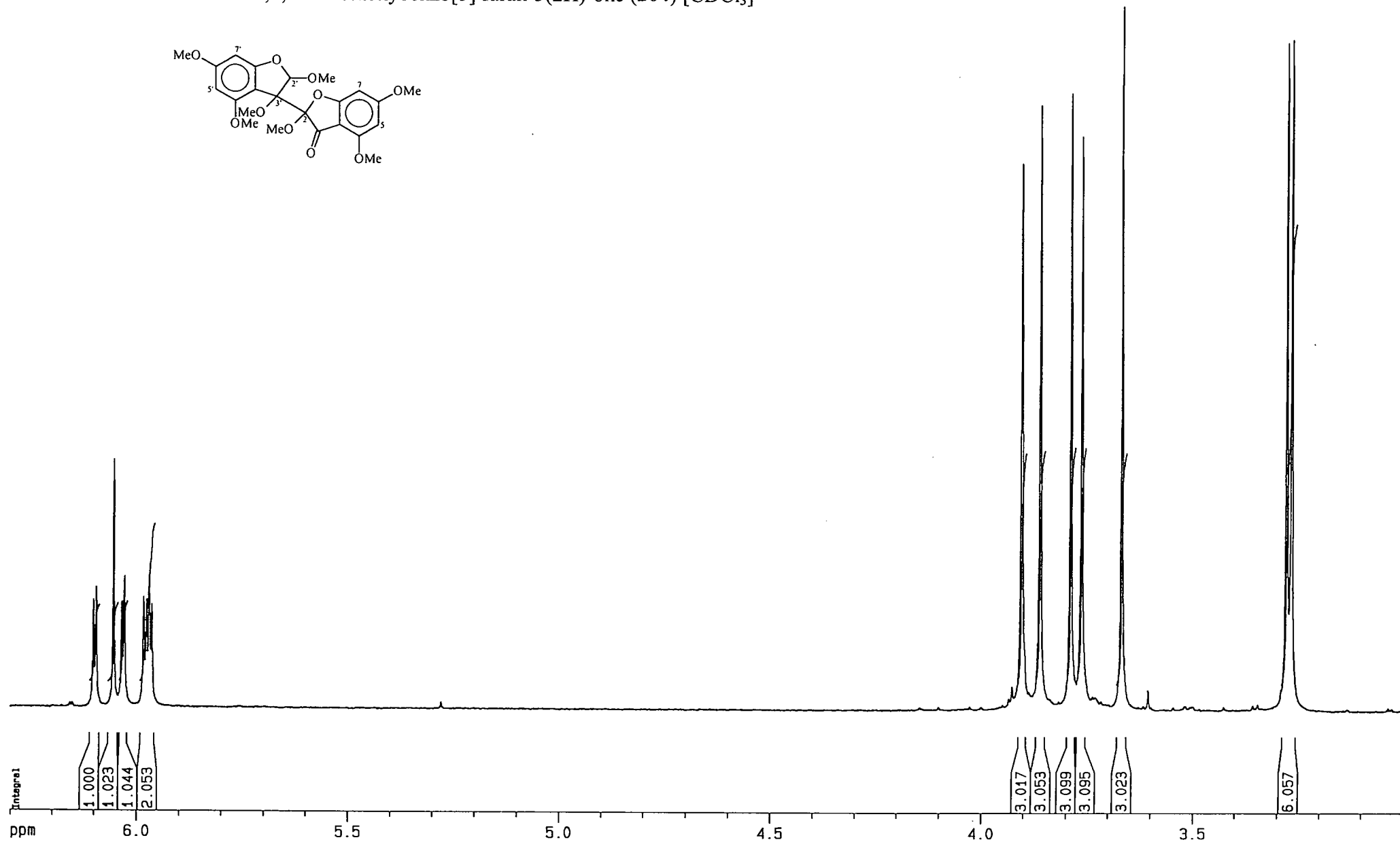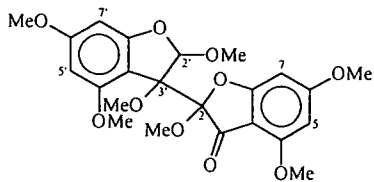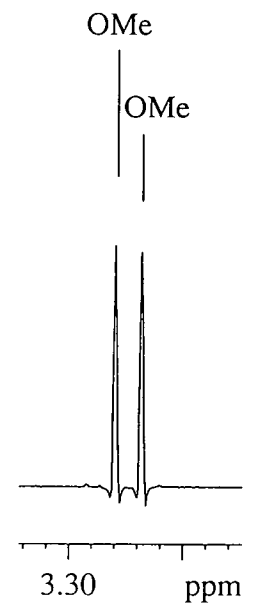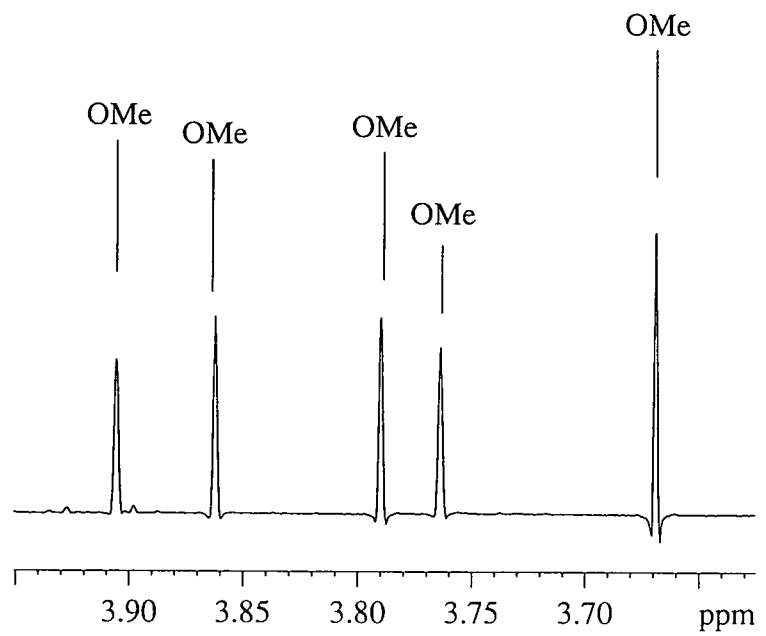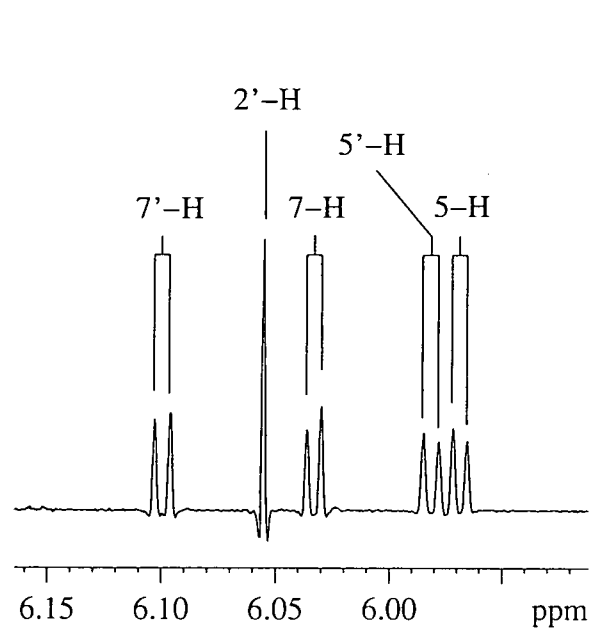**Plate 43:** ^{1}H NMR of 2,4,6-trimethoxy-3-trimethylsilyloxybenzo[*b*]furan (**277**) [C$_6$D$_6$]

7–H   5–H   OMe   OMe   OMe   OSi(CH₃)₃

6.60 6.55 6.50 6.45 ppm   3.75 3.70 ppm   3.45 ppm   0.45 ppm
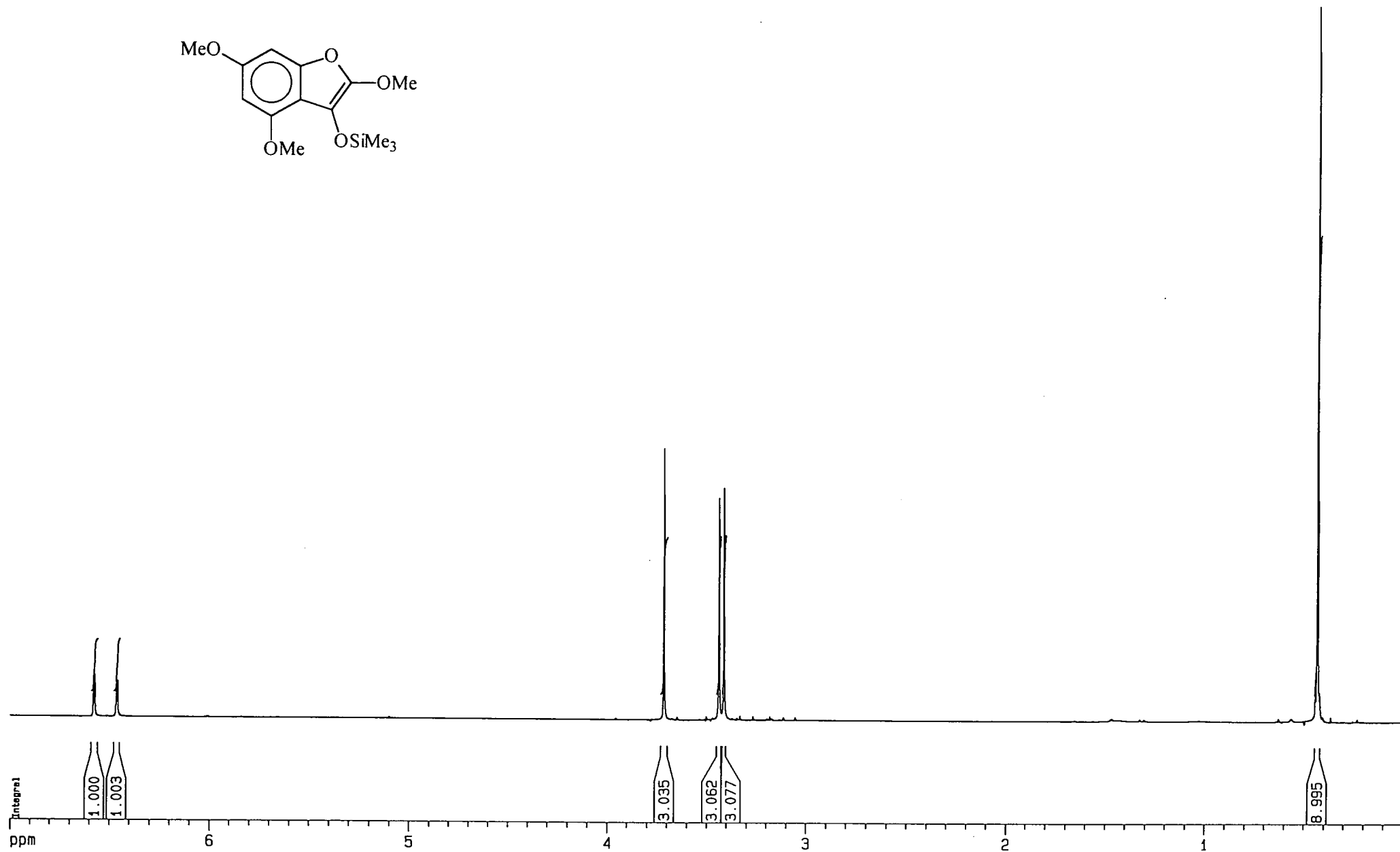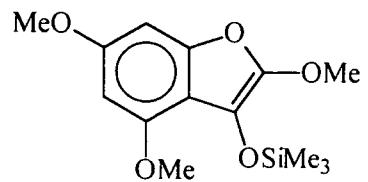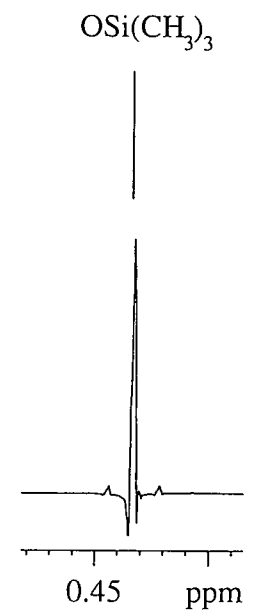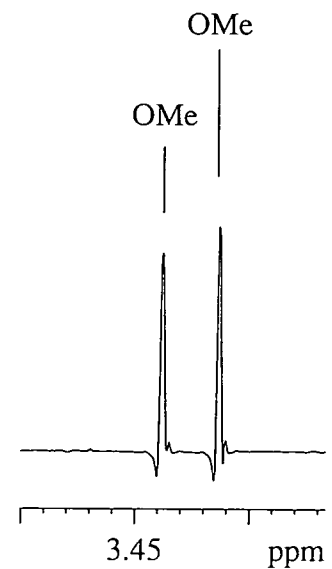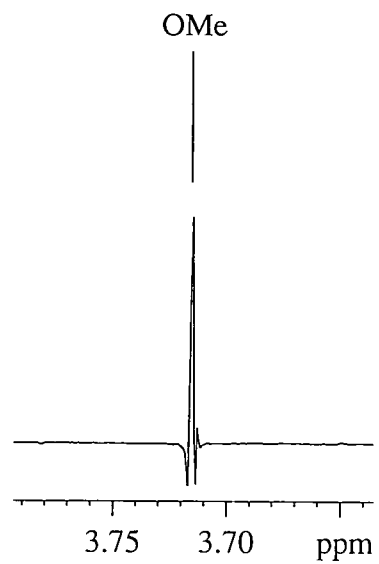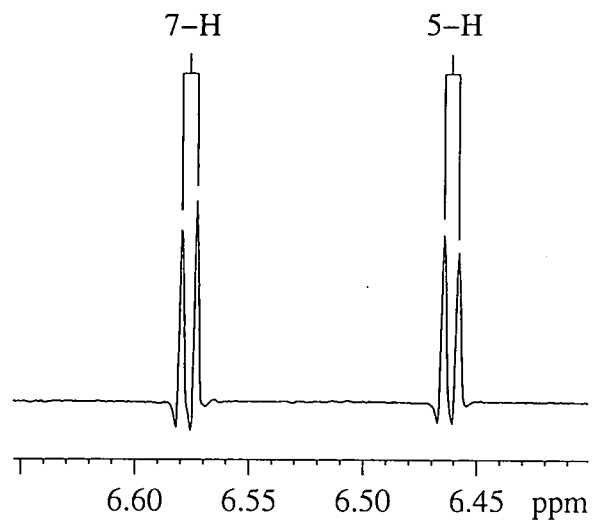
Appendix B

Scheme 1 - 7

Tables B1 - B7

MASS SPECTROMETRY

Fragment (m/z)	Relative intensity (%)
478 (M^+)	2.7
447	3.0
357	100.0
193	89.3
121	48.0

Table B1: Fragments for 7-[2-(4-methoxyphenyl)ethyl]-2,4,4',6-tetra-*O*-methylmaesopsin (**190**).
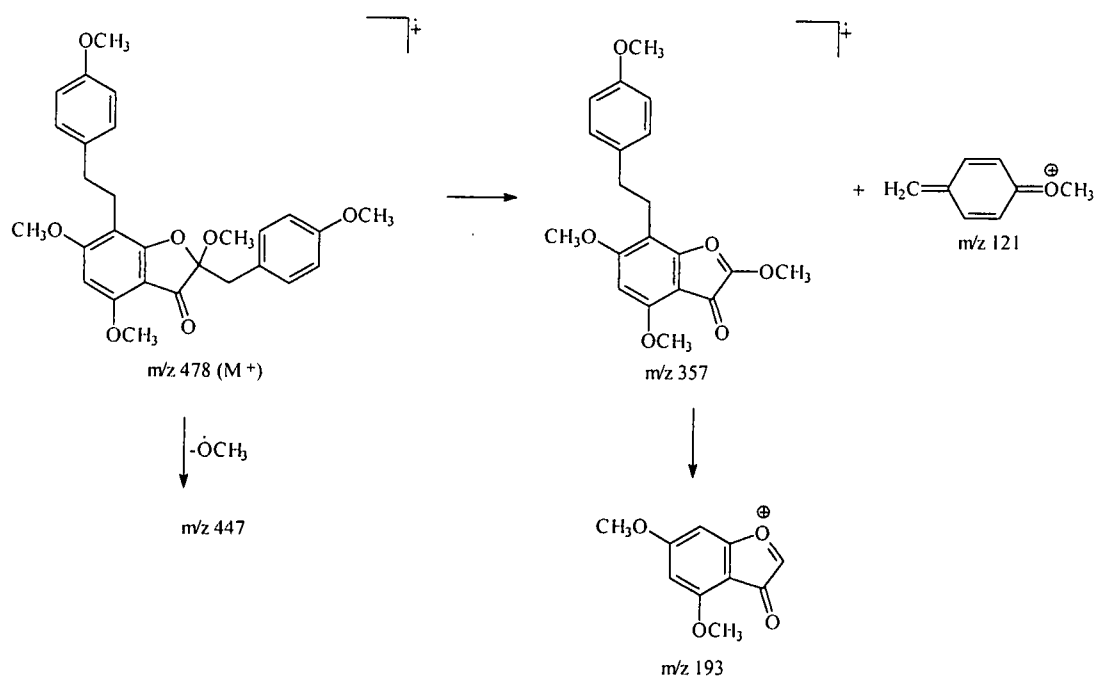
Scheme 1: Fragmentation pattern for 7-[2-(4-methoxyphenyl)ethyl]-2,4,4',6-tetra-*O*-methylmaesopsin (**190**)

Fragment (m/z)	(175)	(176)
	Relative intensity (%)	Relative intensity (%)
656 (M$^+$	15.1	6.2
535	42.2	29.5
476	100.0	100.0
355	75.2	88.4
327	5.0	5.3
313	10.6	10.1
195	28.8	26.9
181	21.0	11.7
121	75.6	63.0

Table B2: Fragments for (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→7)-(2R)-2,4,4',6-tetra-O-methylmaesopsin **(175)** and (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→7)-(2S)-2,4,4',6-tetra-O-methylmaesopsin **(176)**
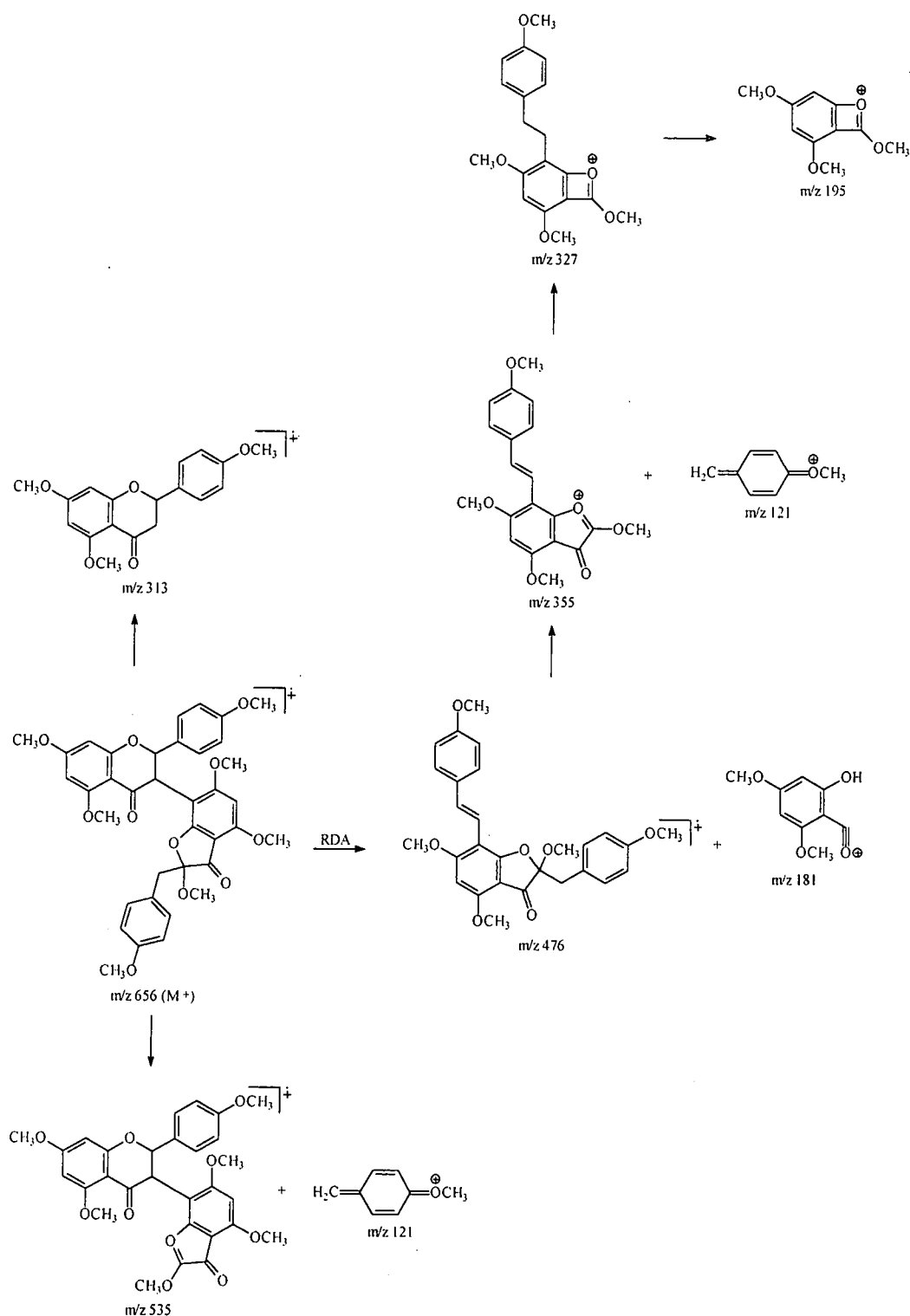
Scheme 2: Fragmentation pattern for (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3α→7)-(2*R*)-2,4,4',6-tetra-*O*-methylmaesopsin (**175**) and (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3α→7)-(2*S*)-2,4,4',6-tetra-*O*-methylmaesopsin (**176**).

Fragment (m/z)	(197 Relative intensity (%)	(198 Relative intensity (%)
656 (M$^+$	20.2	12.1
535	38.1	41.5
476	100.0	100.0
355	74.7	77.5
327	5.2	5.9
313	11.3	12.5
195	29.7	27.2
181	25.0	19.8
121	71.6	61.0

Table B3: Fragments for (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→5)-(2R)-2,4,4',6-tetra-O-methylmaesopsin (**197**) and (2R,3S)-4',5,7-tri-O-methylnaringenin-(3α→5)-(2S)-2,4,4',6-tetra-O-methylmaesopsin (**198**)
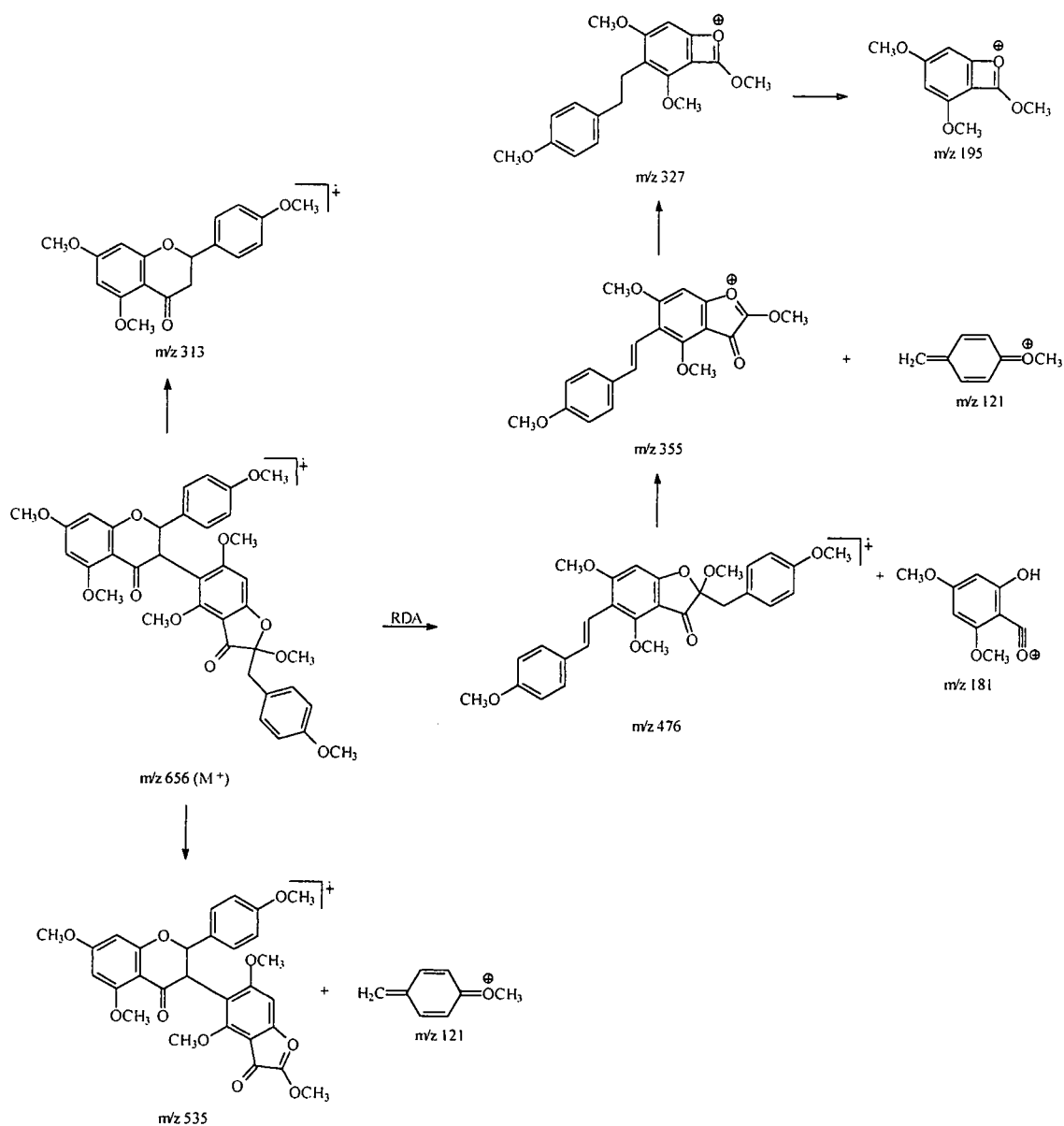
Scheme 3: Fragmentation pattern for (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3α→5)-(2*R*)-2,4,4',6-tetra-*O*-methylmaesopsin (**197**) and (2*R*,3*S*)-4',5,7-tri-*O*-methylnaringenin-(3α→5)-(2*S*)-2,4,4',6-tetra-*O*-methylmaesopsin (**198**)

Fragments (m/z)	(204) Relative intensity (%)	(205) Relative intensity (%)
656	18.2	21.2
289	12.5	11.9
273	8.5	9.6
154	100.0	100.0
121	46.8	25.7

Table B4: Fragments for (2S,3R)-4',5,7-tri-O-methyldihydrogenistein-(2α→7)-(2R)-2,4,4',6-tetra-O-methylmaesopsin (**204**) and (2S,3R)-4',5,7-tri-O-methyldihydrogenistein-(2α→7)-(2S)-2,4,4',6-tetra-O-methylmaesopsin (**205**).
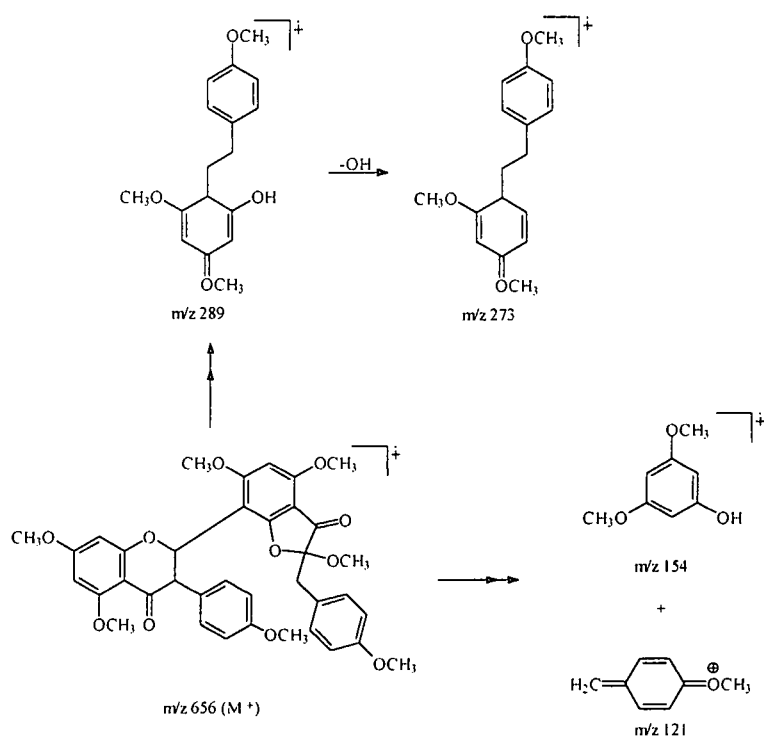
Scheme 4: Fragmentation pattern for (2S,3R)-4',5,7-tri-O-methyldihydrogenistein-(2α→7)-(2R)-2,4,4',6-tetra-O-methylmaesopsin (**204**) and (2S,3R)-4',5,7-tri-O-methyl-dihydrogenistein-(2α→7)-(2S)-2,4,4',6-tetra-O-methylmaesopsin (**205**).

Fragments (m/z)	(211/212) Relative intensity (%)	(215/216) Relative intensity (%)
656 (M⁺)	1.9	2.9
535	100..0	100.0
371	23.3	22.4
313	3.3	5.8
121	54.3	19.6

Table B5: Fragments of 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**211/212**) and 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**215/216**)
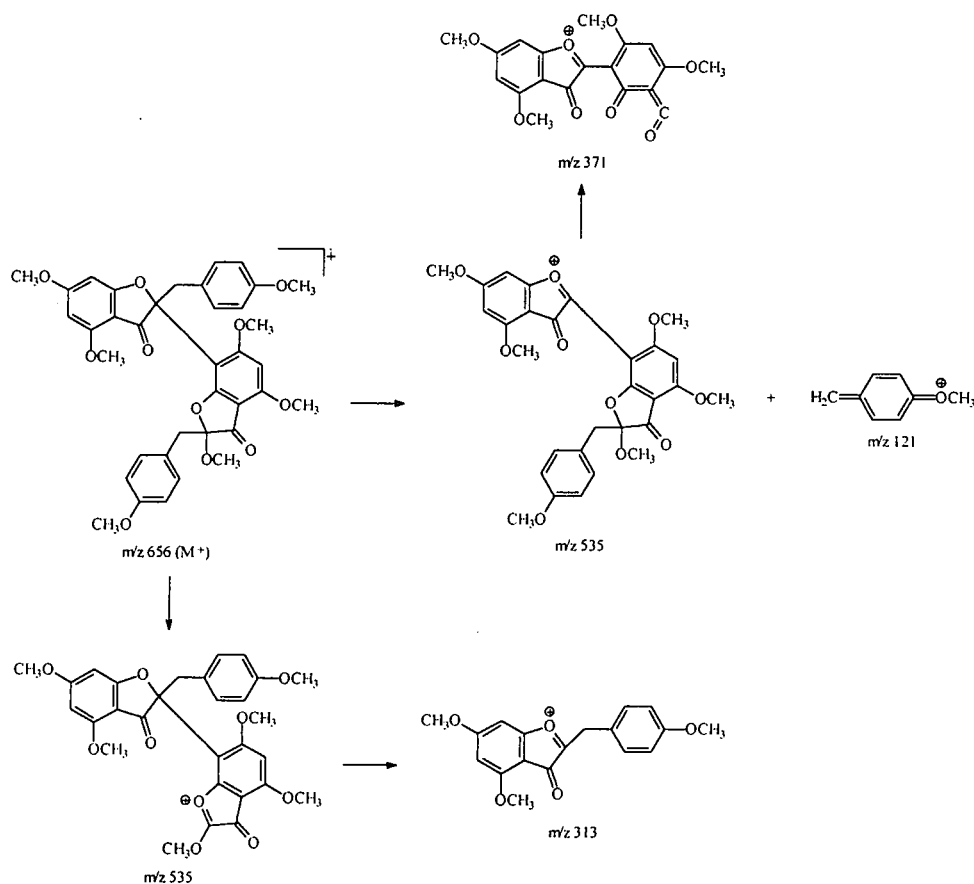
Scheme 5: Fragmentation pettern of 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**211/212**) and 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**215/216**)

Fragments (m/z)	(219) Relative intensity (%)	(221) Relative intensity (%)
656	42.5	62.4
625	23.0	25.8
535	100.0	100.0
371	22.7	4.2
154	24.1	23.7
121	53.9	77.4

Table B6: Fragments of 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin **(219)** and 4,6-dimethoxy-3-(4-methoxybenzyl)-benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin **(221)**.
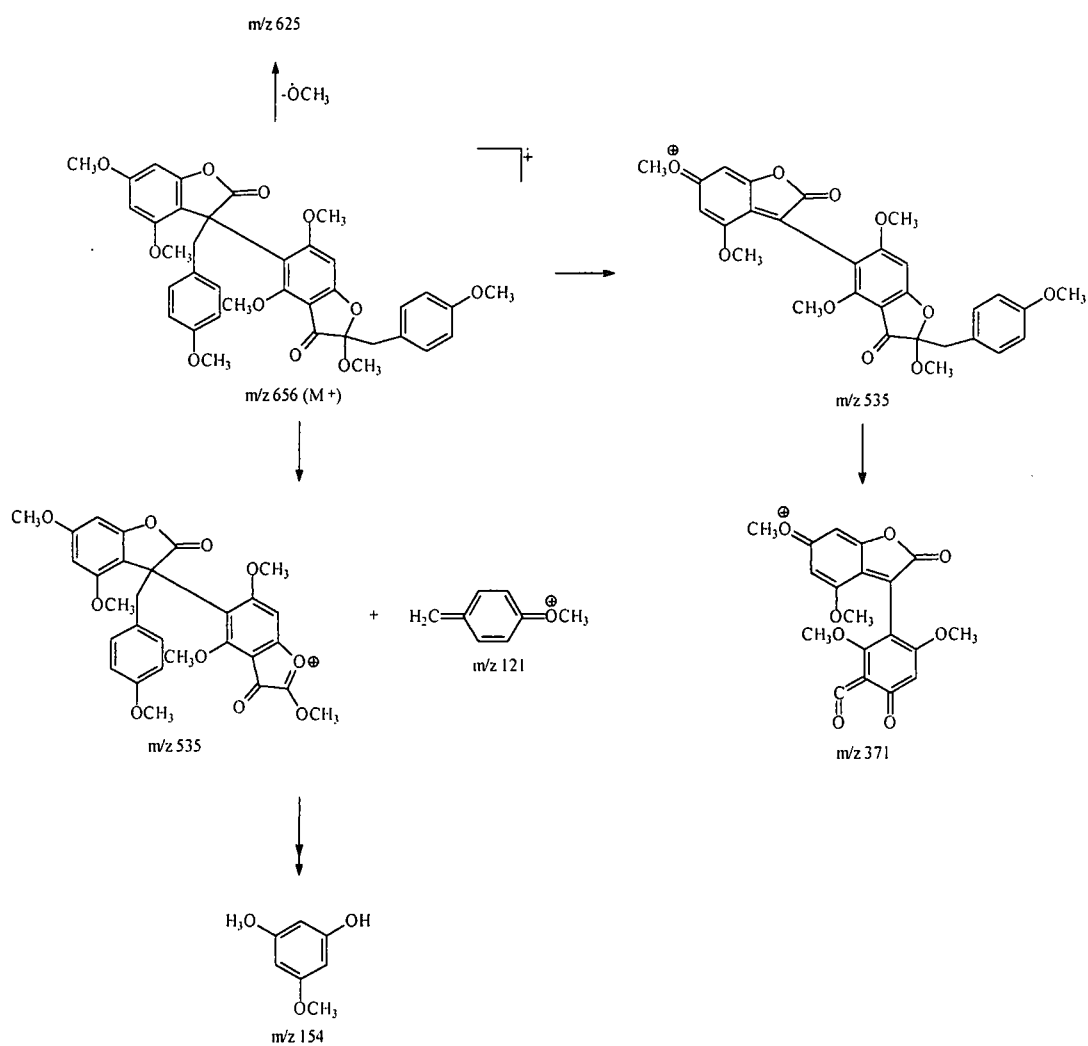
Scheme 6: Fragmentation pattern for 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin (**219**) and 4,6-dimethoxy-3-(4-methoxybenzyl)-benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin (**221**).

Fragments (m/z)	(223) Relative intensity (%)	(225) Relative intensity (%)
656	77.9	63.2
625	22.6	21.1
535	91.9	100.0
371	18.6	19.9
154	51.1	28.3
121	100.0	97.1

Table B7: Fragments of 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin **(223)** and 4,6-dimethoxy-3-(4-methoxybenzyl)-benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin **(225)**.
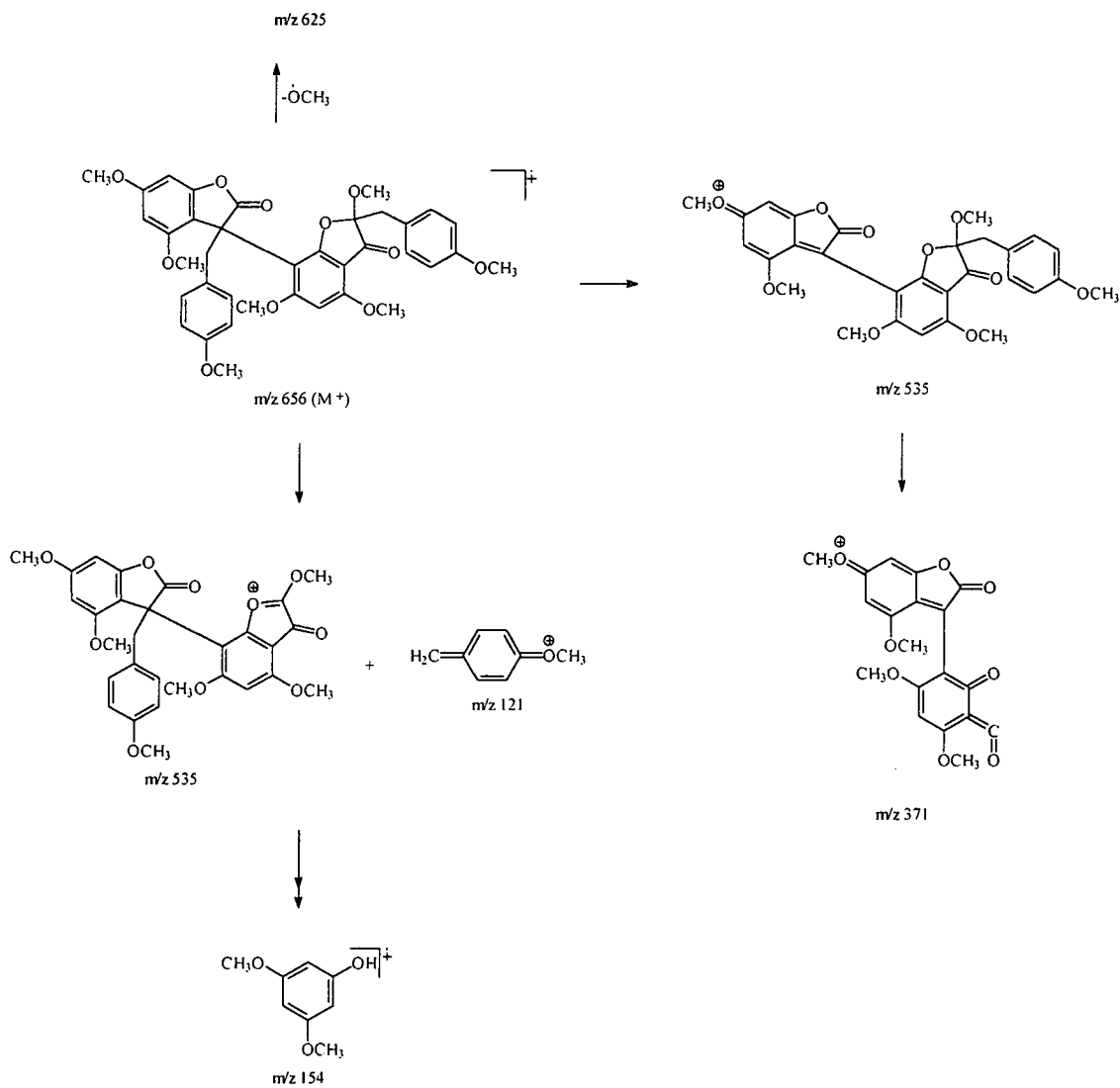
Scheme 7: Fragmentation pattern of 4,6-dimethoxy-3-(4-methoxybenzyl)benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**223**) and 4,6-dimethoxy-3-(4-methoxybenzyl)-benzo[*b*]furan-2(3H)-one-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (**225**).

Appendix C

Tables C1 - C2

APPENDIX C

CRYSTAL DATA AND STRUCTURE REFINEMENT

Empirical formula	$C_{37}H_{36}O_{11}$
Molecular mass	656.66
Crystal system	Orthorhombic
Point group	P_{bca}
Unit cell dimensions	$a = 6.193(2)$ Å $\quad \alpha = 90°$
	$b = 25.8080(5) \quad \beta = 90°$
	$c = 27.3020(5) \quad \gamma = 90°$
Final R index	$R_1 = 0.0570$
Temperature	293(2)K
Wavelength	1.54200 Å
Calculated density	1.999 mg/m^3
Absorption coefficient	1.227 mm^{-1}
Theta range for data collection	3.24 to 89.71 deg.
Index limits	0<=h<=8, 0<=k<=26, 0<=l<=22
Collected reflections	1638
Independent reflections	1638 [R(int) = 0.0000]
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1638 / 0 / 442
Extinction coefficient	0.0016(2)

Atom	x	y	z	U(eq)
O(1)	9059(4)	820(1)	3434(2)	41(1)
O(2)	5672(5)	1435(2)	3632(2)	52(1)
O(3)	10044(5)	2214(1)	3977(2)	49(1)
O(4)	11659(6)	3281(2)	3393(2)	70(2)
O(5)	7781(5)	-805(2)	2777(2)	56(1)
O(6)	4162(5)	408(2)	3377(2)	53(1)
O(7)	10574(6)	2896(2)	2422(2)	60(2)
O(8)	7684(5)	1363(2)	2663(2)	49(1)
O(9)	6518(6)	-235(2)	5474(2)	62(1)
O(10)	12323(8)	2499(2)	4216(4)	118(3)
O(11)	4506(11)	3660(3)	4241(3)	124(3)
C(11)	6642(7)	603(2)	3384(2)	38(2)
C(12)	5551(8)	244(2)	3270(2)	40(2)
C(13)	5903(9)	-232(2)	3083(2)	47(2)
C(14)	7371(10)	-354(2)	2986(3)	46(2)
C(15)	8469(8)	-14(2)	3105(2)	43(2)
C(16)	8069(8)	455(2)	3300(2)	38(2)
C(21)	7992(8)	869(2)	4480(2)	40(2)
C(22)	8650(8)	393(2)	4505(3)	52(2)
C(23)	8195(9)	0(2)	4812(3)	51(2)

Table C1: Atomic coordinates ($\text{Å} \times 10^4$) and equivalent isotropic parameters ($\text{Å}^2 \times 10^3$) for 4,4',6-tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methylmaesopsin (211/212). $\underline{U}_{eq} = \frac{1}{3} \sum_i \sum_j a_i a_j (a_i . a_j)$

Atom	x	y	z	U(eq)
C(24)	7019(9)	102(3)	5127(3)	49(2)
C(25)	6322(9)	570(3)	5107(3)	56(2)
C(26)	6816(9)	946(2)	4783(3)	53(2)
C(31)	6654(8)	1132(2)	3542(2)	42(2)
C(32)	8279(7)	1277(2)	3631(3)	44(2)
C(33)	8568(8)	1310(2)	4178(2)	43(2)
C(41)	10164(8)	2544(2)	3203(3)	39(2)
C(42)	9941(8)	2527(2)	2704(3)	45(2)
C(43)	9059(9)	2130(2)	2505(2)	46(2)
C(44)	8523(7)	1763(2)	2829(3)	45(2)
C(45)	8789(7)	1744(2)	3336(3)	42(2)
C(46)	9615(7)	2153(2)	3501(3)	40(2)
C(51)	8951(11)	3211(3)	4337(3)	49(2)
C(52)	8583(13)	3656(3)	4088(3)	59(2)
C(53)	7094(14)	3820(3)	4048(3)	63(2)
C(54)	6086(15)	3523(4)	4278(4)	82(3)
C(55)	6424(17)	3097(4)	4507(5)	111(4)
C(56)	7824(15)	2931(4)	4547(4)	86(3)
C(61)	11029(8)	2880(3)	3497(3)	55(2)
C(62)	11009(9)	2661(2)	4014(3)	62(2)
C(63)	10487(11)	3026(3)	4407(3)	57(2)
C(111)	6695(10)	-1172(2)	2628(3)	62(2)

Table C1:   (Continued)

Atom	x	y	z	U(eq)
C(112)	2990(9)	40(3)	3333(3)	69(2)
C(211)	7106(10)	-748(3)	5488(3)	71(2)
C(411)	7337(9)	1331(3)	2151(3)	55(2)
C(412)	10481(11)	2865(3)	1898(3)	66(2)
C(511)	4143(14)	4066(3)	3977(4)	101(3)
C(611)	13067(12)	2132(4)	4022(5)	120(4)

Table C1: (Continued)

O(1)-C(16)	1.183(6)	O(1)-C(32)	1.384(6)
O(1)-C(11)	1.605(5)	O(1)-C(31)	1.719(6)
O(2)-C(31)	1.021(6)	O(2)-C(32)	1.665(6)
O(3)-C(62)	1.303(7)	O(3)-C(46)	1.335(8)
O(3)-O(10)	1.721(6)	O(4)-C(61)	1.143(7)
O(5)-C(111)	1.232(7)	O(5)-C(14)	1.320(7)
O(6)-C(12)	1.002(6)	O(6)-C(112)	1.202(7)
O(6)-C(11)	1.616(6)	O(7)-C(42)	1.286(8)
O(7)-C(412)	1.432(8)	O(8)-C(44)	1.240(7)
O(8)-C(411)	1.416(8)	O(9)-C(24)	1.324(8)
O(9)-C(211)	1.375(8)	O(10)-C(62)	1.070(8)
O(10)-C(611)	1.179(10)	O(11)-C(54)	1.045(10)
O(11)-C(511)	1.292(10)	O(11)-C(53)	1.737(11)
C(11)-C(16)	0.989(6)	C(11)-C(12)	1.186(7)
C(11)-C(31)	1.434(8)	C(12)-C(13)	1.350(8)
C(12)-C(16)	1.653(7)	C(12)-C(15)	1.979(8)
C(13)-C(14)	0.997(8)	C(13)-C(15)	1.687(7)
C(14)-C(15)	1.156(8)	C(15)-C(16)	1.345(8)
C(21)-C(26)	1.121(8)	C(21)-C(22)	1.296(8)
C(21)-C(33)	1.450(8)	C(22)-C(23)	1.347(9)
C(22)-C(26)	1.976(9)	C(23)-C(24)	1.156(9)
C(24)-C(25)	1.285(9)	C(25)-C(26)	1.348(9)
C(31)-C(32)	1.101(7)	C(32)-C(45)	1.482(8)
C(32)-C(33)	1.507(9)	C(41)-C(61)	1.298(9)
C(41)-C(46)	1.341(8)	C(41)-C(42)	1.368(9)
C(42)-C(43)	1.282(8)	C(43)-C(44)	1.339(8)
C(44)-C(45)	1.395(10)	C(45)-C(46)	1.256(8)
C(51)-C(63)	1.081(9)	C(51)-C(56)	1.156(10)

Table C2: Bond lengths (Å) and angles (degrees) for compound (**211/212**).

C(51)-C(52)	1.355(10)	C(51)-C(55)	1.659(11)
C(51)-C(54)	1.955(12)	C(52)-C(53)	1.020(9)
C(52)-C(54)	1.667(11)	C(53)-C(54)	1.171(11)
C(54)-C(55)	1.281(13)	C(54)-C(56)	2.007(14)
C(55)-C(56)	0.973(11)	C(61)-C(62)	1.518(10)
C(62)-C(63)	1.465(10)	C(16)-O(1)-C(32)	128.2(4)
C(16)-O(1)-C(11)	37.9(3)	C(32)-O(1)-C(11)	90.3(3)
C(16)-O(1)-C(31)	88.7(4)	C(32)-O(1)-C(31)	39.7(3)
C(11)-O(1)-C(31)	51.0(3)	C(31)-O(2)-C(32)	40.0(4)
C(62)-O(3)-C(46)	105.7(6)	C(62)-O(3)-O(10)	38.4(3)
C(46)-O(3)-O(10)	125.7(5)	C(111)-O(5)-C(14)	135.7(5)
C(12)-O(6)-C(112)	99.0(5)	C(12)-O(6)-C(11)	47.0(4)
C(112)-O(6)-C(11)	145.2(5)	C(42)-O(7)-C(412)	123.0(6)
C(44)-O(8)-C(411)	118.3(5)	C(24)-O(9)-C(211)	126.1(6)
C(62)-O(10)-C(611)	112.2(10)	C(62)-O(10)-O(3)	49.2(4)
C(611)-O(10)-O(3)	78.8(5)	C(54)-O(11)-C(511)	119.4(9)
C(54)-O(11)-C(53)	41.1(7)	C(511)-O(11)-C(53)	78.4(6)
C(16)-C(11)-C(12)	98.5(6)	C(16)-C(11)-C(31)	115.6(5)
C(12)-C(11)-C(31)	145.6(5)	C(16)-C(11)-O(1)	47.3(4)
C(12)-C(11)-O(1)	145.7(5)	C(31)-C(11)-O(1)	68.6(3)
C(16)-C(11)-O(6)	136.6(6)	C(12)-C(11)-O(6)	38.1(3)
C(31)-C(11)-O(6)	107.7(4)	O(1)-C(11)-O(6)	175.1(5)
O(6)-C(12)-C(11)	94.9(6)	O(6)-C(12)-C(13)	129.3(6)
C(11)-C(12)-C(13)	135.8(5)	O(6)-C(12)-C(16)	131.1(6)
C(11)-C(12)-C(16)	36.3(3)	C(13)-C(12)-C(16)	99.5(4)
O(6)-C(12)-C(15)	173.2(6)	C(11)-C(12)-C(15)	78.6(4)
C(13)-C(12)-C(15)	57.2(3)	C(16)-C(12)-C(15)	42.3(3)
C(14)-C(13)-C(12)	122.3(7)	C(14)-C(13)-C(15)	41.9(5)
C(12)-C(13)-C(15)	80.5(4)	C(13)-C(14)-C(15)	102.9(7)
C(13)-C(14)-O(5)	124.5(7)	C(15)-C(14)-O(5)	132.6(6)
C(14)-C(15)-C(16)	133.2(6)	C(14)-C(15)-C(13)	35.2(4)
C(16)-C(15)-C(13)	98.1(4)	C(14)-C(15)-C(12)	77.4(4)
C(16)-C(15)-C(12)	55.8(3)	C(13)-C(15)-C(12)	42.3(3)
C(11)-C(16)-O(1)	94.8(6)	C(11)-C(16)-C(15)	127.1(6)
O(1)-C(16)-C(15)	138.1(5)	C(11)-C(16)-C(12)	45.2(4)
O(1)-C(16)-C(12)	140.0(5)	C(15)-C(16)-C(12)	81.9(4)
C(26)-C(21)-C(22)	109.5(7)	C(26)-C(21)-C(33)	116.2(6)
C(22)-C(21)-C(33)	134.2(6)	C(21)-C(22)-C(23)	132.9(7)
C(21)-C(22)-C(26)	32.3(4)	C(23)-C(22)-C(26)	100.6(5)
C(24)-C(23)-C(22)	115.0(6)	C(23)-C(24)-C(25)	113.3(7)
C(23)-C(24)-O(9)	122.0(7)	C(25)-C(24)-O(9)	124.7(7)
C(24)-C(25)-C(26)	128.9(7)	C(21)-C(26)-C(25)	120.4(7)
C(21)-C(26)-C(22)	38.2(4)	C(25)-C(26)-C(22)	82.2(4)
O(2)-C(31)-C(32)	103.3(6)	O(2)-C(31)-C(11)	143.1(5)

Table C2: (Continued)

C(32)-C(31)-C(11)	113.3(6)	O(2)-C(31)-O(1)	156.4(5)
C(32)-C(31)-O(1)	53.5(4)	C(11)-C(31)-O(1)	60.4(3)
C(31)-C(32)-O(1)	86.7(5)	C(31)-C(32)-C(45)	110.6(6)
O(1)-C(32)-C(45)	114.0(5)	C(31)-C(32)-C(33)	110.3(6)
O(1)-C(32)-C(33)	113.0(5)	C(45)-C(32)-C(33)	117.9(5)
C(31)-C(32)-O(2)	36.6(4)	O(1)-C(32)-O(2)	123.2(4)
C(45)-C(32)-O(2)	90.5(4)	C(33)-C(32)-O(2)	95.7(4)
C(21)-C(33)-C(32)	119.4(5)	C(61)-C(41)-C(46)	103.4(6)
C(61)-C(41)-C(42)	132.8(6)	C(46)-C(41)-C(42)	123.6(6)
C(43)-C(42)-O(7)	117.8(8)	C(43)-C(42)-C(41)	119.4(6)
O(7)-C(42)-C(41)	122.8(6)	C(42)-C(43)-C(44)	113.1(7)
O(8)-C(44)-C(43)	116.8(7)	O(8)-C(44)-C(45)	112.6(6)
C(43)-C(44)-C(45)	130.6(6)	C(46)-C(45)-C(44)	112.0(6)
C(46)-C(45)-C(32)	125.1(7)	C(44)-C(45)-C(32)	122.8(5)
C(45)-C(46)-O(3)	121.9(6)	C(45)-C(46)-C(41)	121.3(7)
O(3)-C(46)-C(41)	116.8(5)	C(63)-C(51)-C(56)	99.7(9)
C(63)-C(51)-C(52)	127.7(8)	C(56)-C(51)-C(52)	132.5(9)
C(63)-C(51)-C(55)	134.7(8)	C(56)-C(51)-C(55)	35.0(6)
C(52)-C(51)-C(55)	97.5(7)	C(63)-C(51)-C(54)	174.1(8)
C(56)-C(51)-C(54)	75.5(7)	C(52)-C(51)-C(54)	57.1(5)
C(55)-C(51)-C(54)	40.5(4)	C(53)-C(52)-C(51)	123.9(9)
C(53)-C(52)-C(54)	44.0(7)	C(51)-C(52)-C(54)	79.9(6)
C(52)-C(53)-C(54)	98.8(9)	C(52)-C(53)-O(11)	134.7(9)
C(54)-C(53)-O(11)	35.9(6)	O(11)-C(54)-C(53)	103.0(11)
O(11)-C(54)-C(55)	119.3(11)	C(53)-C(54)-C(55)	137.4(10)
O(11)-C(54)-C(52)	140.2(11)	C(53)-C(54)-C(52)	37.2(5)
C(55)-C(54)-C(52)	100.2(8)	O(11)-C(54)-C(51)	175.4(10)
C(53)-C(54)-C(51)	80.2(7)	C(55)-C(54)-C(51)	57.2(6)
C(52)-C(54)-C(51)	43.0(4)	O(11)-C(54)-C(56)	142.6(10)
C(53)-C(54)-C(56)	114.1(8)	C(55)-C(54)-C(56)	23.3(5)
C(52)-C(54)-C(56)	76.9(5)	C(51)-C(54)-C(56)	33.9(3)
C(56)-C(55)-C(54)	125.3(11)	C(56)-C(55)-C(51)	43.0(7)
C(54)-C(55)-C(51)	82.3(7)	C(55)-C(56)-C(51)	102.0(11)
C(55)-C(56)-C(54)	31.4(7)	C(51)-C(56)-C(54)	70.6(7)
O(4)-C(61)-C(41)	126.3(8)	O(4)-C(61)-C(62)	124.8(7)
C(41)-C(61)-C(62)	108.9(6)	O(10)-C(62)-O(3)	92.5(6)
O(10)-C(62)-C(63)	92.3(8)	O(3)-C(62)-C(63)	121.7(6)
O(10)-C(62)-C(61)	128.3(7)	O(3)-C(62)-C(61)	105.1(6)
C(63)-C(62)-C(61)	116.3(5)	C(51)-C(63)-C(62)	110.4(7)

Table C2:   (Continued)

Summary/

Opsomming

SUMMARY

Keywords: *Berchemia zeyheri*, diastereomeric, benzofuranone biflavonoids, absolute configuration, asymmetric synthesis of benzofuranoids

Berchemia zeyheri is known for its unique red heartwood, a property that was probably responsible for the first phytochemical investigation into the flavonoid content of this tree. The heartwood contains a unique series of biflavonoids with one of more benzofuranoid moieties. These are usually found in diastereomeric mixtures, the biogenetic origin and stereochemistry of which have hitherto been unknown. This investigation thus represents a renewed effort to solve some of the intricate problems associated with these compounds.

The high concentration of maesopsin in the heartwood made extensive enrichment and fractionation by the use of Craig countercurrent distribution techniques and Sephadex LH-20 gelchromatography necessary.

The two diastereomers of 4',5,7-tri-*O*-methylnaringenin-(3α→7)-2,4,4',6-tetra-*O*-methylmaesopsin were, for the first time, successfully isolated and separated. Reduction of these diastereomers with $Na(CN)BH_3$ gave two enantiomeric pure fragments. The conformations of the heterocyclic rings of these fragments were established by molecular mechanics (MMX and GMMX) and semi-empirical methods (AM1). These results allowed the absolute configuration of the fragments to be deduced from CD-curves of the compounds by application of Snatzke's rule for α,β-unsaturated five-membered cyclic rings. A n.O.e. correlation observed for one of the diastereomers only, correlates the stereocenter of the maesopsin moiety, of known absolute configuration, with a specific configuration of the naringenin unit, thus defining the absolute configuration of the dimer. These results also allowed the determination of the absolute stereochemistry of two regio-isomers of the above dimers, 4',5,7-tri-*O*-methylnaringenin-(3α→5)-2,4,4',6-tetra-*O*-methylmaesopsin and its epimer. The ^{13}C NMR spectra of these related dimers were also studied and fully elucidated by means of HMQC and HMBC experiments. The structure and stereochemistry of two novel isoflavanone-benzofuranone biflavonoids, 4',5,7-tri-*O*-

methyldihydrogenistein-(2α→7)-2,4,4',6-tetra-*O*-methylmaesopsin and its epimer, were similarly determined.

Resolution of maesopsin, the main metabolite in the heartwood, by means of HPLC using a chiral column, for the first time gave access to the two enantiomers of this benzofuranoid.

4,4',6-Tri-*O*-methyl-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-methyl-maesopsin and its epimer, consist of two benzofuranoid constituent units. An X-ray crystal structure was obtained for the one diastereomer, but due to the presence of a symmetric P_{bca} point group, only the relative configuration could be determined. After the racemic nature of each of the diastereomers was determined, each epimer was resolved with HPLC into its constituent enantiomers. The information obtained from the CD curves and crystal structure allowed the determination of the absolute stereochemistry of each of the enantiomers.

Four further epimeric biflavonoids were isolated as the hepta-*O*-methyl ethers. Evidence obtained from ^{13}C NMR data suggested the presence of a γ-lactone functionality in the upper benzofuranoid moiety, identifying the dimers as the epimers of 4,6-dimethoxy-3-(4-methoxy-benzyl)benzo[*b*]furan-2(3H)-one-(2→5)-2,4,4',6-tetra-*O*-methylmaesopsin and the (2→7)-coupled regio-isomer.

In order to supplement the above data, an asymmetric synthesis of maesopsin was attempted. The first attempt involved the oxidation of 2-(4-methoxybenzyl)-4,6-dimethoxybenzo[*b*]furan-3(2H)-one, obtained by reduction of the corresponding aurone, with AD-mix-α, a stereoselective catalyst, or chiral oxaziridine. The former afforded the desired product in low yield but no stereoselectivity while the latter method realized a much-improved yield, but still with no selectivity. This lack of selectivity is attributed to equilibrium of the product with the α-diketone. Attempts to prevent the formation of this equilibrium product were unsuccessful. A second synthetic attempt involved benzylation of 2,4,6-trimethoxybenzo[*b*]furan-3-(2H)-one with (-)-sparteine as chiral auxiliary, but again resulted in high yields but no stereoselectivity.

OPSOMMING

Berchemia zeyheri is bekend vir 'n unieke rooi kernhout, 'n eienskap wat aanvanklik verantwoordelik was vir die eerste fitochemiese ondersoek op hierdie houtsoort. Die kernhout bevat o.a. 'n unieke reeks biflavonoïede met een of meer bensofuranoïedeenhede wat kenmerkend as diastereomeriese mengsels aangetref word. Die oorsprong van die diastereomeriese aard van hierdie mengsels, asook die stereochemie van die verbindings, kon tot op hede egter nog nie bepaal word nie.

Na aanleiding hiervan is 'n herondersoek na die flavonoïedinhoud van *Berchemia zeyheri* aangepak. Die hoë konsentrasie maesopsin in die kernhout het uitgebreide verryking en fraksionering deur benutting van o.a. Craig teenstroomverdelingstegnieke en Sephadex LH-20 jelchromatografie genoodsaak.

Die twee diastereomere van 4',5,7-tri-*O*-metielnaringenin-(3α→7)-2,4,4',6-tetra-*O*-metielmaesopsin is vir die eerste keer suksesvol geïsoleer en geskei. Reduksie van hierdie diastereomere met $Na(CN)BH_3$ gee aanleiding tot twee suiwer enantiomeriese brokstukke. Die konformasie van die heterosikliese ring van hierdie brokstukke is m.b.v. molekulêre meganika (MMX en GMMX) en semi-empiriese metodes (AM1) bepaal. Na aanleiding van hierdie resultate kon die absolute konfigurasie van die fragmente m.b.v. van Snatzke se reël vir α,β-onversadigde vyfledige sikliese ketone vir die eerste keer uit die SD-kurwes bepaal word. 'n N.O.e. korrelasie wat slegs by een diastereomeer waargeneem is, korreleer die stereosentrum van die maesopsineenheid met 'n bepaalde konfigurasie van die naringenineenheid en gee toegang tot die absolute konfigurasie van die dimere. Hierdie resultate laat ook die bepaling van die absolute stereochemie van 4',5,7-tri-*O*-metielnaringenin-(3α→5)-2,4,4',6-tetra-*O*-metielmaesopsin en sy epimeer, twee regio-isomere van die bogenoemde dimere, toe. Die ^{13}C KMR spektra van hierdie dimere is ook bestudeer m.b.v. HMQC en HMBC eksperimente. Die stuktuur en stereochemie van twee unieke isoflavanoon-bensofuranoon biflavonoïede, 4',5,7-tri-*O*-metieldihydrogenistein-(2α→7)-2,4,4',6-tetra-*O*-metielmaesopsin en sy epimeer, is ook bepaal.

Resolusie van maesopsin, die hoof metaboliet in die kernhout, deur middel van HPLC met 'n chirale kolom gee vir die eerste keer toegang tot die twee enantiomere van hierdie benzofuranoïed.

4,4',6-Tri-*O*-metiel-2-deoxymaesopsin-(2→7)-2,4,4',6-tetra-*O*-metiel-maesopsin en sy epimeer bestaan uit twee bensofuranoïedeenhede. Ten spyte van die feit dat 'n X-straal kristalstruktuur van die een diastereomeer verkry is, kon slegs die relatiewe konfigurasie van hierdie verbinding bepaal word aangesien die P_{bca} ruimtegroep waarin die kristalle gekristalliseer het, inherent simmetries is. Nadat vasgestel is dat elk van die epimere rasemies is, is hulle m.b.v. HPLC geskei om vier enantiomere te gee. Die inligting verkry vanaf die SD-kurwes en kristalstruktuur het die bepaling van die absolute konfigurasie van elk van die enantiomere moontlik gemaak.

Vier verdere epimeriese dimere is ook as die hepta-*O*-metiel eters geïsoleer. Inligting vanaf die ^{13}C KMR data van hierdie verbindings dui op die teenwoordigheid van 'n γ-laktoon funksionaliteit in die boonste bensofuranoïedeenheid. Die dimere is geïdentifiseer as die epimere van 4,6-dimetoksi-3-(4-metoksibensiel)benso[*b*]furan-2(3H)-oon-(2→5)-2,4,4',6-tetra-*O*-metielmaesopsin en die (2→7) gekoppelde regio-isomere.

Ten einde die bogenoemde inligting aan te vul, is 'n asimmetriese sintese van maesopsin aangepak. Die eerste poging behels die oksidasie van 2-(4-metoksibensiel)-4,6-dimetoksibenso[*b*]furan-3(2H)-oon, wat verkry is deur die reduksie van die geskikte auroon, deur gebruik te maak van AD-mengsel-α, 'n stereoselektiewe katalis, en chirale oksaziridien. Die eerste van hierdie metodes het die vereiste produk in lae opbrengs verskaf, maar sonder enige stereoselektiwiteit terwyl die tweede metode die produk in hoë opbrengs gee, maar weereens sonder selektiwiteit. Hierdie gebrek aan seleksie kan waarskynlik toegeskryf word aan die ewewig wat tussen die produk en die α-diketoon bestaan. Pogings om die vorming van die α-diketoon te beperk was onsuksesvol. 'n Tweede poging behels die bensilering van 2,4,6-trimetoksibenso[*b*]furan-3(2H)-oon met (-)-sparteine as chirale hulpmiddel. Weereens is maesopsin in hoë opbrengs gevorm, maar sonder enige stereoselektiwiteit.

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