STRUCTURE

AND

SYNTHESIS

OF

FLAVAN OLIGOMERS FROM

CASSIA PETERSIANA

Submitted in fulfilment of the requirements for the degree

MASTER OF SCIENCE

in the Department of Chemistry,
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by

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Dr. J.C. Coetzee for his kind assistance, encouraging spirit and for kindly recording ^{1}H NMR spectra and CD curves.

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My sisters, Vuyokazi, Linda and Nosphiwo, brother Phumlani for their moral support and love.

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Above all I thank God for the gift of life He has given me.

L.P. Mciteka

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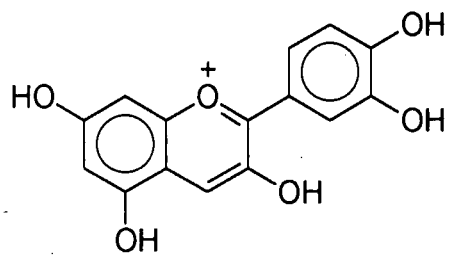
LITERATURE SURVEY

CHAPTER 1

LEUCOANTHOCYANIDINS

1.1 INTRODUCTION

The reddening of plant tissues on treatment with mineral acid, was first observed by Robert Boyle[1] in 1884. Willstater and Tswett have done a substantial amount of work on this phenomena, but Rosenheim first isolated the actual substance (cyanidin **1**) responsible for this reaction from *Vitis vinifera* and the term leucoanthocyanins was assigned to this class of compounds[1].

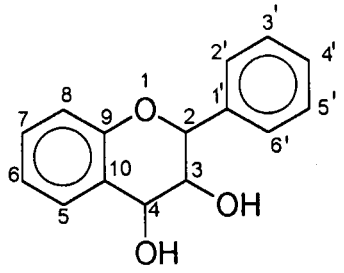


1

The Robinsons[2] noted the presence of leucoanthocyanins in higher plants and this finding was greatly broadened by Bate-Smith[1] who showed the profound existence of these compounds in plants with a woody habit of growth.

Bate-Smith and Swain[1] revealed the close similarity in distribution between leucoanthocyanins and tannins. They managed to show that reactions such as, precipitation of gelatin and alkaloids, astringent taste and the formation of amorphous polymeric phlobaphens with acid were a result of leucoanthocyanins[1] and not tannins as previously thought.

During the1950s it was discovered[3] that leucoanthocyanins did not all contain sugar residues as previously thought and hence the nomenclature was changed to accommodate these compounds to be named leucoanthocyanidins. However, the original belief that these compounds contained a flavan-3,4-diol type of structure **2** remained unchallenged[3].

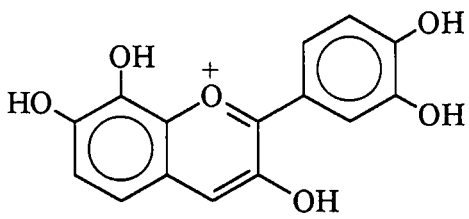


2

When the major chemical advance occurred in this field, Freudenberg and Weinges[3] introduced the term proanthocyanidin for this class of compounds. Researchers such as Weinges[3], Marini-Bettolo[3] and Roux[4] have since made meaningful contributions to the knowledge of proanthocyanidins.

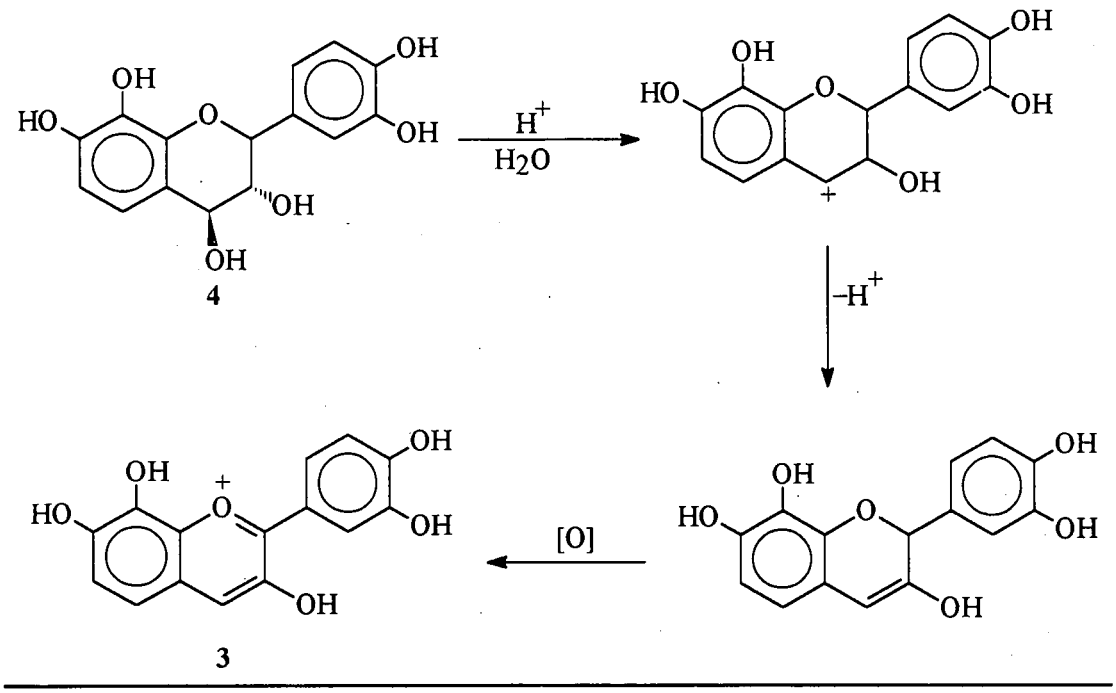
1.2 NOMENCLATURE

Due to an amazing increase in the number of novel proanthocyanidins, a need evolved to review the nomenclature of this group[5] . Weinges, Freudenberg, and Haslam[6] defined leucoanthocyanidins as monomeric proanthocyanidins and this definition was promoted to include all monomeric flavonoids which produced anthocyanidins e.g. **3** by cleavage of a C-O bond on heating with mineral acid as shown in **Scheme 1.1**.

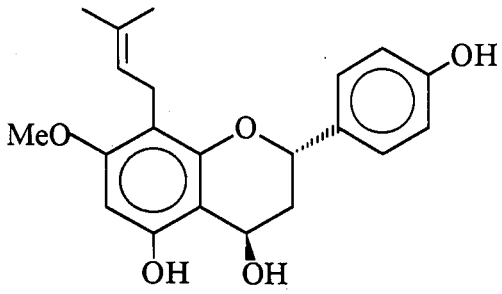


3

SCHEME 1.1



This definition now included not only the flavan-3,4-diols e.g., epiprosopin-4β-ol **4**[7] but also compounds with small variations in the structure e.g., tephrawatsonin A **5**[8] and unusual metabolites e.g., cyanomaclurin **6**[9].



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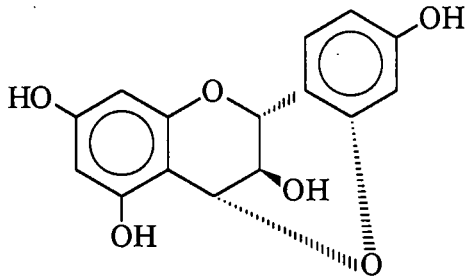


6

A system that employs trivial names was adopted to define the flavan-3,4-diols, possessing more than one name e.g., (+)-mollisacacidin is called fisetinidol-4α-ol and (-)-melacacidin is called epiprosopin-4α-ol, etc.

Table 1 shows the structural types and various hydroxylation patterns

TABLE 1 Structural types of some leucoanthocyanidins

Structural Type	OH-Pattern	Example
Leucoguibourtinidin	7,4'	**guibourtinidol-4α-ol** **guibourtinidol-4β-ol**
Leucofisetinidin	7,3',4'	**fisetinidol-4α-ol** **fisetinidol-4β-ol**
Leucoteracacinidin	7,8,4'	**oritin-4α-ol** **oritin-4β-ol**
Leucomelacacinidin	7,8,3',4'	**prosopin-4α-ol** **epiprosopin-4β-ol**
Leucorobinetinidin	7,3',4',5'	**robinetinidol-4α-ol**

1.3 STRUCTURE AND DISTRIBUTION

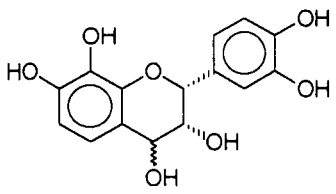
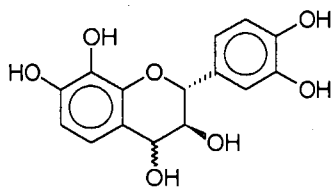
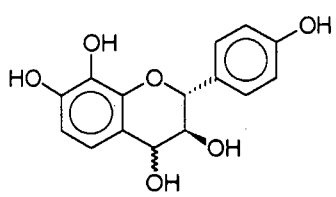
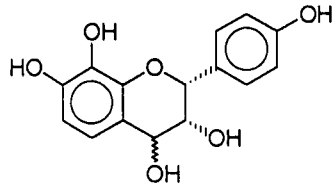
1.3.1 Natural leucoanthocyanidins:flavan-3,4-diols

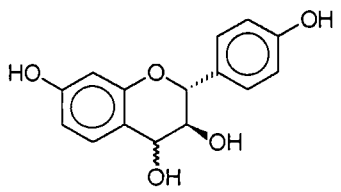
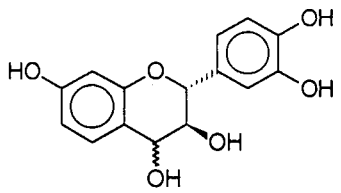
The heartwoods and barks of many *Acacia* species are known[10] to contain various leucoanthocyanidins. Melacacidin **7** which is illustrated in **Table 2** with leucomelacacinidin, isomelacacidin[11] **4** and leucoteracacinidin diastereomers mentioned below were the first flavan-3,4-diols isolated from *Acacia melanoxylon*[12].

The heartwood of *Acacia galpinii* was the first South African *Acacia* known to contain the leucoteracacinidins[13] followed by *Acacia burkei* Benth (a closely related species) [13]. The major component of the heartwood of *Acacia galpinii* was (-)-7,8,4'-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol [(-)-teracacinidin[7]] **11** followed by three diastereomers, (-)-2,3-*cis*-3,4-*trans* **12**, (+)-2,3-*trans*-3,4-*trans* **9** and (+)-2,3-*trans*-3,4-*cis* **10** as well as by (-)-melacacidin [(-)-7,8,3',4'-tetrahydroxy-2,3-*cis*-flavan-3,4-*cis*-diol[14]] **7**.

A complete list of naturally occurring flavan-3,4-diol was compiled by Porter[15].

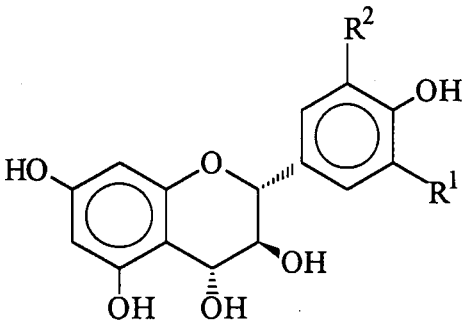
TABLE 2 Structural types of some leucoanthocyanidins

Structure	Structural Type Compound	Source
 7 ~~~ = ····· 4 ~~~ = ▬	**LEUCOMELACACINIDINS** **(2R,3R,4R)-epiprosopin-4α-ol 7** **[(-)-melacacidin] 7** **(2R,3R,4S)-epiprosopin-4β-ol 4** **[(-)-isomelacacidin] 4**	*Acacia calamiformes* *Acacia melanoxylon* *Acacia pungentes*[1] *Acacia plurinerves* *Acacia juliflorae* *Acacia uninerves*[1] *Acacia exelsa*[12] *Acacia harpophylla*[12]
 8 ~~~ = ▬	**(2R,3S,4S)-prosopin-4β-ol 8**	*Acacia pungentes*[1] *Acacia plurinerves*[1] *Acacia juliflorae*[11] *Prosopis glandulosa*[3]
 9 ~~~ = ····· 10 ~~~ = ▬	**LEUCOTERACACINIDINS** **(2R,3S,4R)-oritin-4α-ol 9** **(2R,3S,4S)-oritin-4β-ol 10**	*Acacia galpinii*[13] *Acacia auriculiformis*[2] *Acacia caffra*
 11 ~~~ = ····· 12 ~~~ = ▬	**(2R,3R,4R)-epioritin-4α-ol 11** **[(-)-teracacidin] 11** **(2R,3R,4S)-epioritin 12** **[(-)-isoteracacidin] 12**	*Acacia pungentes* *Acacia uninerves*[1] *Acacia plurinerves* *Acacia luliflorae*[1]

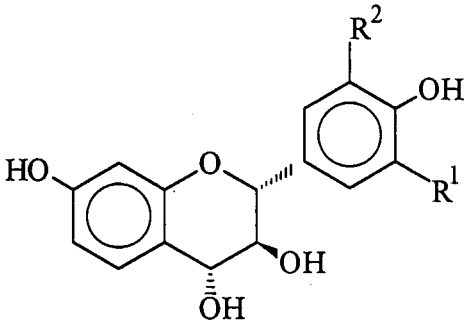
 13 ~~~ = ····· 14 ~~~ = ▬	**LEUCOGUIBOURTINIDINS** **(2R,3S,4R)-guibourtinidol-4α-ol 13** **(2R,3S,4S)-guibourtinidol-4β-ol 14**	*Acacia cultriformis* *Acacia leuderitzii* *Acacia uninerves*[1] *Guibourtia coleosperma* *Acacia brumioides* *Acacia meissueri*
 15 ~~~ = ·····	**LEUCOFISETINIDIN** **(2R,3S,4S)-fisetinidol-4α-ol 15**	*Acacia baileyana* *Acacia plurinerves* *Acacia uninerves*[1]

1.3.2 FLAVAN-3,4-DIOLS AS INCIPIENT ELECTROPHILES

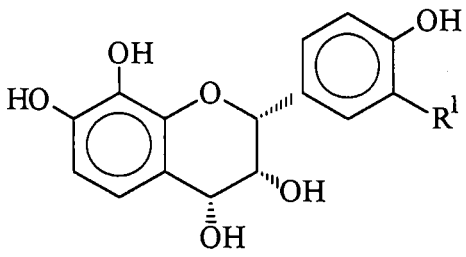
Flavan-3,4-diols served as source of the chain-extender units in the formation of oligomeric flavanoids[16]. The degree of delocalisation of the positive charge over the A-ring determined the stability of the C-4 carbocations[16] and it was established that delocalisation for flavan-3,4-diols with phloroglucinol-type-A-rings **16-18** was most effective compared to intermediate efficiency for resorcinol-type- compounds **15,19** and **13**, with the least effectiveness for pyrogallol-type compounds 7 and **11**.



16 R^1= H, R^2= OH
17 R^1= R^2 = OH
18 R^1= R^2 = H

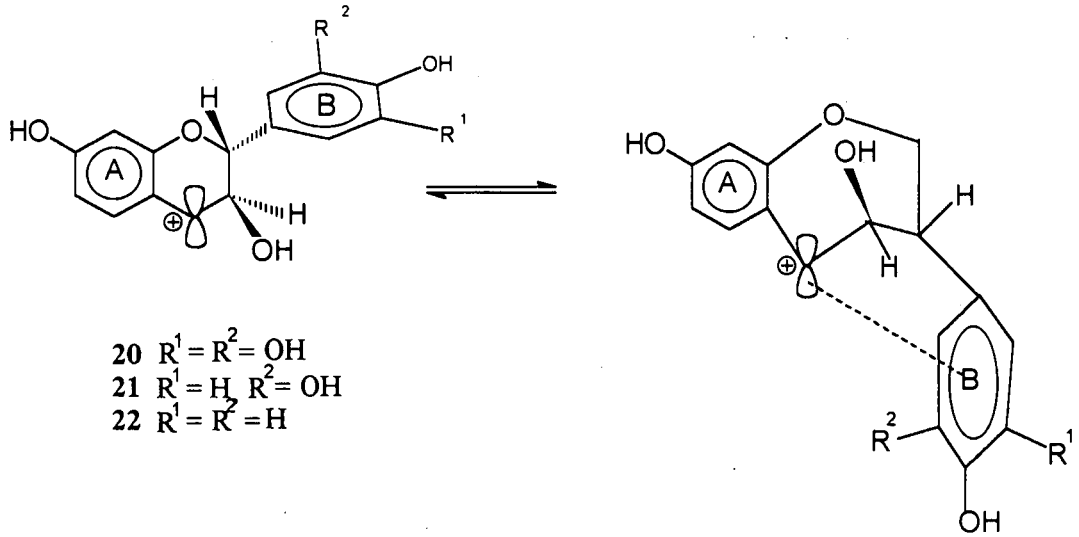


15 R^1= H, R^2= OH
19 R^1= R^2= OH
13 R^1= R^2= H



7 $R^1 = OH$
11 $R^1 = H$

It was Brown[17] who first proposed the ability of the B-ring to contribute towards stabilising C-4 carbocations supported by Ferreira and co-workers[18,19]. The B-ring offered a stabilising effect to C-4 carbocations of type **21** *via* an A-conformation **24**. This A-conformer was represented by a half-chair/sofa conformation for the pyran ring where the 2-aryl group occupied an axial position as shown in **23** in contrast to the equatorial orientation in the E-conformer **21**.



20 $R^1 = R^2 = OH$
21 $R^1 = H$, $R^2 = OH$
22 $R^1 = R^2 = H$

23 $R^1 = R^2 = OH$
24 $R^1 = H$, $R^2 = OH$
25 $R^1 = R^2 = H$

Stabilisation of the C-4 carbocations *via* an A-conformation was proven by the different rates of condensation observed for (+)-leucorobinetinidin[20] **19**, (+)-mollisacacidin[20] **15**, and (+)-guibourtacacidin[21] **13**. Due to the conformational mobility of the pyran

heterocycle, benzylic carbocations **23-25** were additionally stabilized by charge donation from the B-ring . The more electron-rich pyrogallol function in the (+)-leucorobinetinidin carbocations **20↔23** were found to be more effective than the pyrocatechol functionality in (+)-mollisacacidin analogues **21↔24**[13] and the mono-oxygenated moiety in the (+)-guibourtacacidin ions **22↔25**, hence leading to condensation rates decreasing in the order **19>15>13**.

1.4 STRUCTURE ELUCIDATION

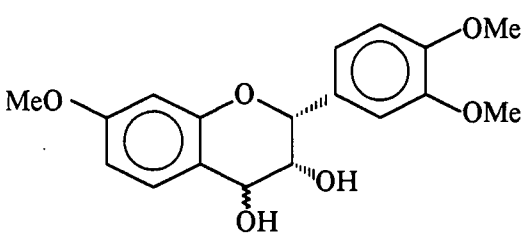
1.4.1 SPECTROSCOPIC METHODS

1.4.1.1 Proton Nuclear Magnetic Resonance Spectroscopy(^{1}H NMR)

Nuclear magnetic resonance spectroscopy is a very powerful analytical tool which is employed in elucidating the structure of flavonoid compounds and in determining the relative stereochemistry of the heterocyclic ring in flavan derivatives, e.g. catechin, flavan-3,4-diol, etc. The relative configuration at positions 2,-3-, and 4- in flavan-3,4-diols could rapidly be determined from their nuclear magnetic resonance data.

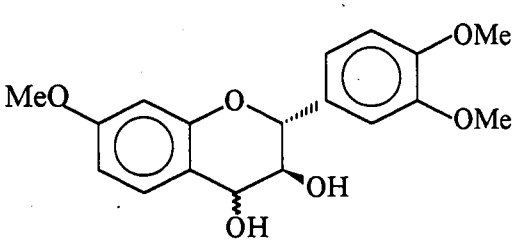
1.4.1.1.1 Heterocyclic proton

Clark-Lewis and co-workers[22] characterized the relative stereochemistry of the four diastereomeric 5,7,3',4'-tetramethoxy diols **26-29**. ^{1}H NMR coupling constant data on the diols **26-29** and their diacetates (shown in **Table 3**) led to a definition of the relative stereochemistry at positions 2-,3- and 4- on the heterocyclic ring. The relative stereochemistry was defined by the coupling constants 2 to 5,5Hz indicating a *cis*- and 5 to 12Hz representing a *trans*-configuration.



26 ~~~ = ·····

27 ~~~ = ◄



28 ~~~ = ·····

29 ~~~ = ◄

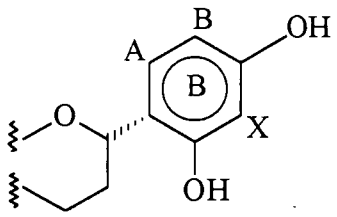
TABLE 3 Coupling constants for the heterocyclic protons of compounds **26-29** and their diacetates

Relative configuration	$J_{2,3}$	$J_{3,4}$
2,3-*cis*-3,4-*cis* **26**	1.0	4.8
2,3-*cis*-3,4-*cis* diacetate	1.6	5.4
2,3-*cis*-3,4-*trans* **27**	0.9	2.5
2,3-*cis*-3,4-*trans* diacetate	1.4	2.6
2,3-*trans*-3,4-*trans* **28**	10.1	7.3
2,3-*trans*-3,4-*trans* diacetate	7.4	7.2
2,3-*trans*-3,4-*cis* **29**	10.1	4.1
2,3-*trans*-3,4-*cis* diacetate	11.1	3.5

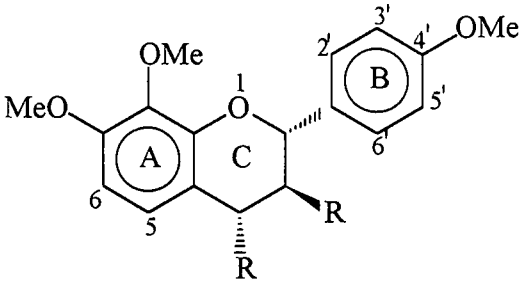
1.4.1.1.2 **Aromatic protons**

^{1}H NMR data have shown that aromatic protons have chemical shifts ranging from 6.0 to 8.0 ppm with *J*-values of 8-, 2-, and 1.0 Hz, corresponding to the *ortho*-, *meta*-, and *para*-coupled protons respectively.

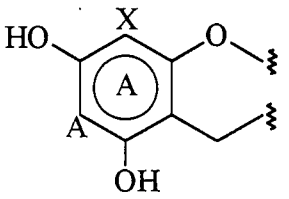
The aromatic protons of flavan-3,4-diols are no exception to these coupling patterns with combinations of ABX **30**; AB for A-ring of **31**; AX **32** and AA'BB' for B-ring of **31**.



30



31 R = OH
33 R = OAc



32

The AB system of the A-ring in 7,8,4'-trimethoxy-2,3-*trans*-3,4-flavandiol **31** gave a set of *ortho*-coupled protons between 7.0 to 9.0ppm. The H-3',5' doublet of the B-ring of **31** which is part of an AA'BB' system, resonated upfield relative to the H-2',6' doublet due to the deshielding effect imposed by the adjacent oxygen of the heterocyclic ring[23]. The ^{1}H NMR spectrum of compound **31** and that of its diacetate **33** were shown to have coupling constants $J_{2,3}$=9.0Hz and $J_{3,4}$=6.3Hz[13] indicating a 2,3-*trans*-3,4-*trans* configuration which were in agreement with previous work on other flavan-3,4-diols[24].

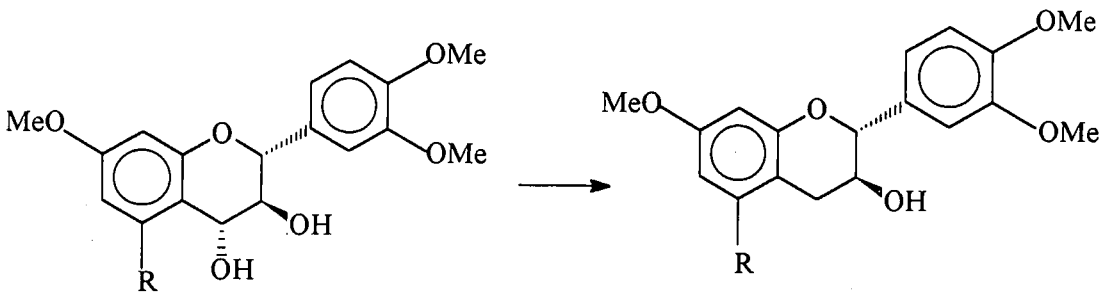
1.4.2.1.3 **Methoxyl and Acetoxyl protons**

The aromatic methoxyl protons occur in the region of 3.5 to 4.1 ppm while the aromatic acetoxyl protons appeared in the region of 2.25 to 2.50ppm[24]. Aliphatic acetoxyl protons absorbed at a higher field in the region of 1.8ppm.

1.4.2.2 **Circular dichroism (CD)**

The determination of the absolute configuration of the flavan-3,4-diols was based on the molecular rotation or ORD comparisons of the corresponding flavan-3-ol obtained by hydrogenolysis of the oxygen function at C-4 position with that of the known catechin or epicatechin.

The reaction of (+)-mollisacacidin trimethyl ether **33** gave (–)-fisetinidol trimethyl ether **34**, and by comparison its molecular rotation showed to be an analogue of (+)-catechin tetramethyl ether **35** and was assigned the (2R,3S) configuration[25].



33

34 R = H

35 R = OMe

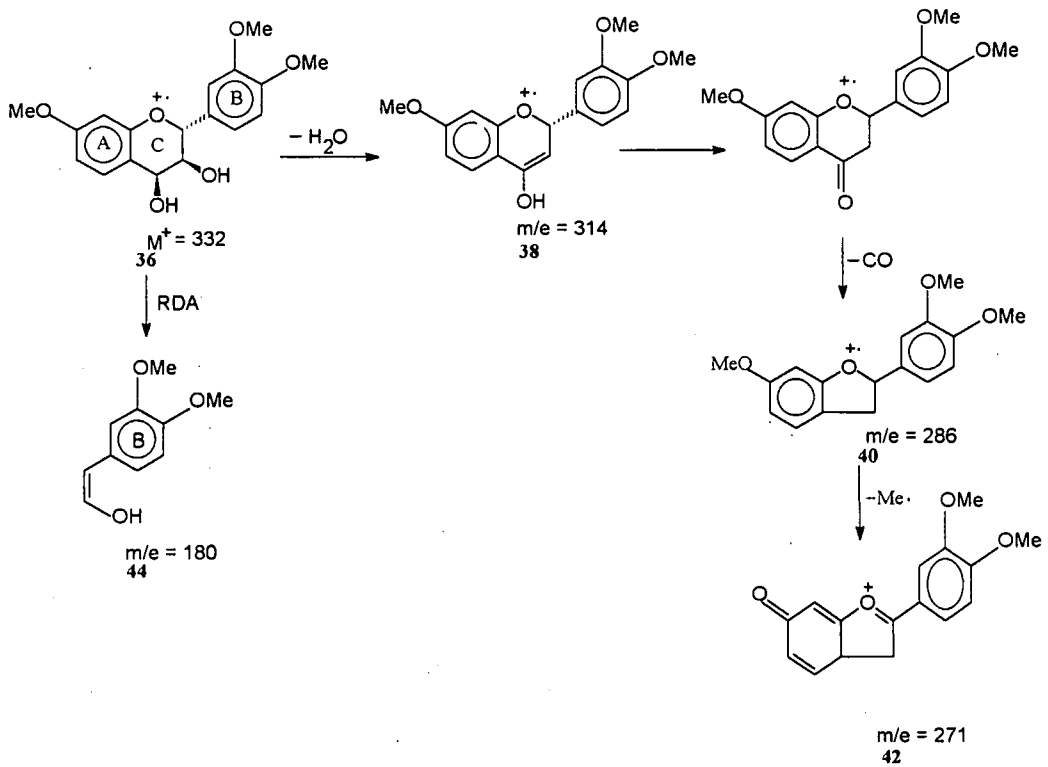
^{1}H NMR spectrum indicated a *trans-trans* relative configuration for (+)-mollisacacidin and in conjunction with the ORD information was assigned a 2R,3S,4R absolute configuration.

1.4.1.3 **Mass Spectrometry**

The retro-Diels-Alder fission of the central ring and a fission accompanied by transfer of hydrogen to ring A during cleavage of the C(3)-C(4) bond have been suggested to be generally the two major modes of fragmentation in the mass spectrometry of derivatives of flavonoids[26]. The methyl ethers of leucofisetinidin **36** and melacacidin **37** showed a common[26] fragmentation pattern, with the loss of 18(H_2O) mass units from the molecular ions to give ions **38** and **39**, respectively, followed by carbon monoxide expulsion to give ions **40** and **41,** respectively, and finally a methyl group loss to give ions **42** and **43**, respectively, as shown in **Schemes 1.3a** and **1.3b**.

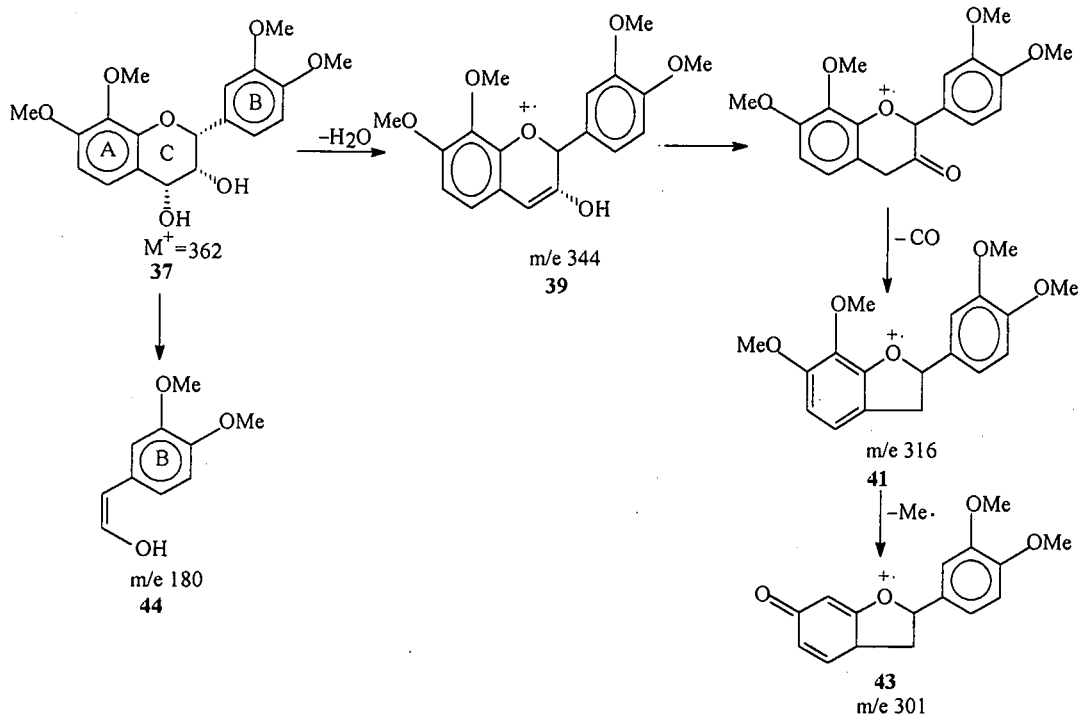
Clark-Lewis[27] formulated the dehydration of molecular ion **36** as the loss of the benzylic 4-hydroxyl group and the C-3-hydrogen atom to give the enol of the 3-oxoflavan (ion **39**, m/e 344 as shown in **Scheme 1.3b)** rather than a 4-oxoflavan ion **38**, as proposed by Drewes[26].

Scheme 1.3a



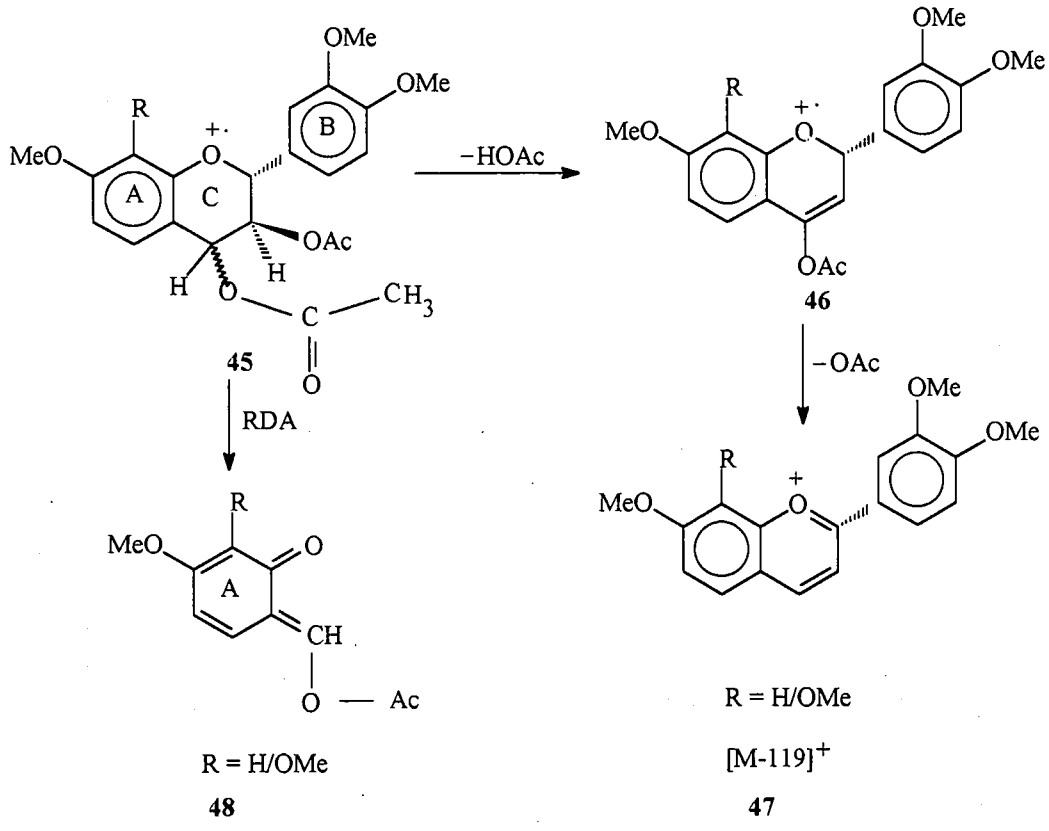
Methyl ether diacetates of ions **36** and **37** showed an initial rapid elimination of 60 mass units (acetic acid) followed by the loss of 59 mass units due to the residual acetyl function derived from the original diol[27]. In the resorcinol flavan-3,4-diols loss of these two groups yielded the most intense peak of the spectrum. Fragments derived from ring-B due to RDA fission also gave prominent peaks. The two pyrogallol-based flavan-3,4-diol acetates presented a somewhat different pattern[26]. The $[M-119]^+$ ion was still fairly intense but very intense peaks arose from ring-A as a result of fission of the central ring (**48**, **Scheme 1.4**).

Scheme 1.3b



The loss of acetic acid from the 3,4-diol acetates occurred by a six-stage elimination reaction of the McLafferty type rearrangement as shown in **Scheme 1.4**[26].

SCHEME 1.4

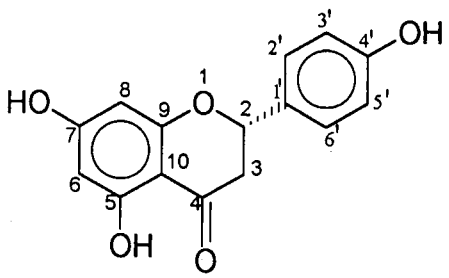

OMe
OMe
R
MeO
+·
O
B
A
C
OAc
H
H
O
C
CH3
O
45
−HOAc
OMe
OMe
R
+·
MeO
O
OAc
46
−OAc
RDA
OMe
OMe
R
MeO
+
O
R
MeO
O
A
CH
O
Ac
R = H/OMe
48
R = H/OMe
[M-119]+
47

CHAPTER 2

FLAVANONES

2.1 INTRODUCTION

Very few flavanones are known to occur in nature, either in the free phenolic state or glycosylated[28]. Flavanones seemed to be more closely associated with heartwoods, barks and roots, and less so with leaves and petals. The most abundant flavanone is (2S)-flavanone (naringenin **49**) isolated for the first time from *Ferreirea spectabilis*[28] formed by the stereospecific action of chalcone isomerase[29] on 4,2',4',6'-tetrahydroxychalcone **50** as shown in **Scheme 2.1**



49

Flavanones appeared to be direct precursors[29] leading to the formation of isoflavones **51,** flavones **52**, and dihydroflavonols **53** by 2,3-aryl shift, oxidation, and hydroxylation, respectively as shown in **Scheme 2.1**.

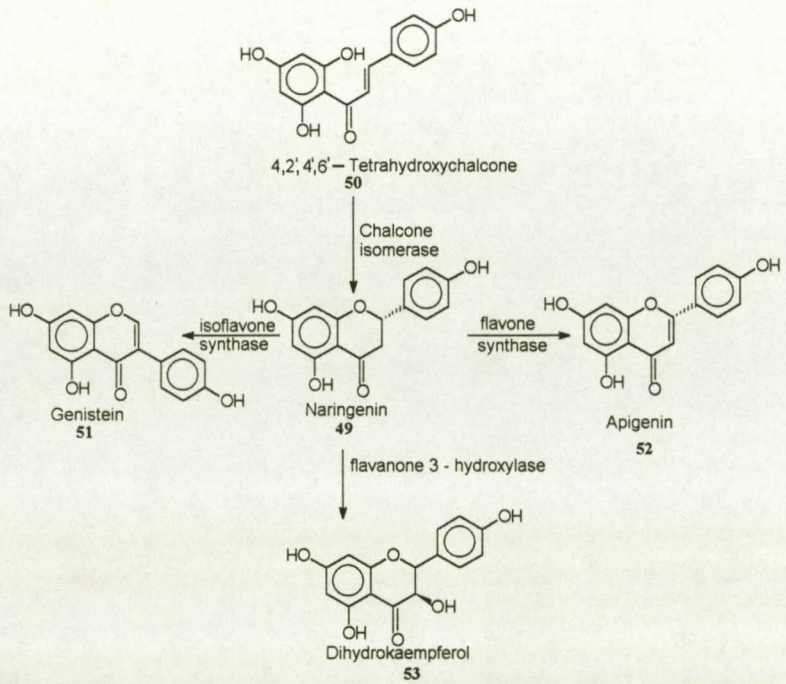
The enzymes responsible are isoflavone synthase, a dioxygenase and a mixed-function mono-oxygenase, and flavanone 3-hydroxylase, respectively[30].

Flavanones were found to have fungistic or fungitoxic properties[30]. Naringenin **49** has been established as a growth inhibitor in dormant peach flowers[30].

2.2 NOMENCLATURE

Flavanones possess the basic structure of 2-phenyl-benzo-pyran-4-one[30] with the numbering system of their nucleus the same as that of most flavanoid compounds. The stereogenic centre at C-2 results in the phenyl substituents either in the 2S or 2R configuration[31]. The 2S configuration was established to be the most abundant form of the naturally occurring flavanones[31].

SCHEME 2.1

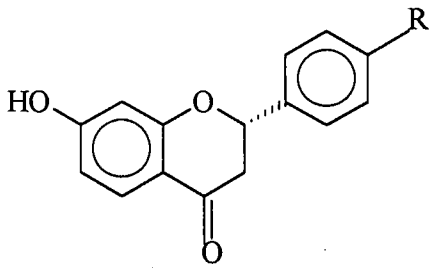


2.3 STRUCTURE AND NATURAL OCCURRENCE

Lists of all reported natural occurrences of flavanones were compiled by Bohm[32,33,34]. These included the free phenolic, the glycosylated as well as the prenylated flavanones. In the following sections, some of the naturally occurring free phenolic flavanones will be discussed with respect to their B - ring hydroxylation pattern.

Flavanones lacking B-ring oxygenation

The 7-hydroxyflavanone **54** is the simplest known flavanone discovered in members of Leguminosae[32] and in a member of Compositae[15]. It was found to be a variable constituent of the profile of *Acacia neovermicosa*[33].

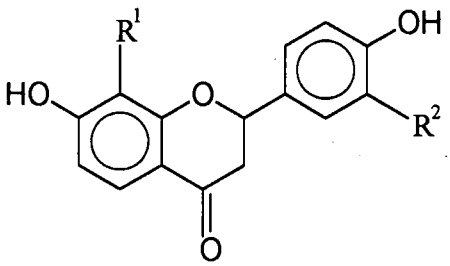


54 R = H

55 R = OH

Flavanones having one B-ring oxygenation

The simplest member of this group is liquiritigenin[35] **55** and is found amongst the member of Leguminosae. Prenylated flavanones e.g. sophoranone[35] and bavachinin[35] were isolated from the roots of *Sophora*[35] species and the seeds of *Psoralea corylifolia*[35], respectively. The 7,8,4'-Trihydroxy substituted flavanone **56** which is a very rare compound was first isolated by Tindale[36] followed by Roux[36], Clark-Lewis[35] and Porter[37] from the heartwood bark extracts of *Acacia* species. Malan and Roux[13] reported *Acacia galpinii* as the first South African source[37] of 7,8,4'-analogues. *Acacia burkei* and *Acacia nigrescens* were added as other sources of 7,8,4'-trihydroxyflavanone **56**.



56 R^1 = OH, R^2 = H

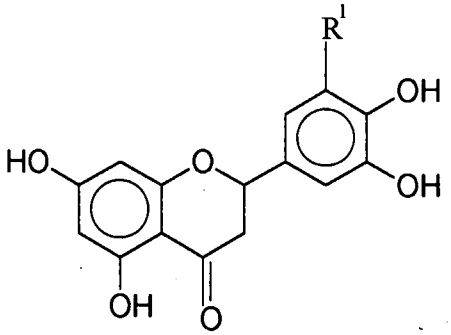
57 R^1 = H, R^2 = OH

58 R^1 = R^2 = OH

Flavanones having two B-ring oxygenations

Butin, 7,3',4'-trihydroxyflavanone **57**, was isolated in its free phenolic form in *Butea*[32], *Mangifera*[32], *Machaerium*[32], and various *Acacia*[36] species. The 7,8,3',4'-tetrahydroxyflavanone **58** was isolated by Fourie and co-workers[38] from *Acacia*

nigrescens[36] an indigenous tree to South Africa. Eriodictyol, 5,7,3',4'-tetrahydroxyflavanone[36] **59** enjoys a fairly wide distribution as either in the free phenolic or in a variety of glycosidic forms, and was found in Hydrangeaceae[36], Gramineae[36], Myoporaceae[36] families. Eriodictyol **59** tested active against the growth of larva of *Heliothis zea*[39].



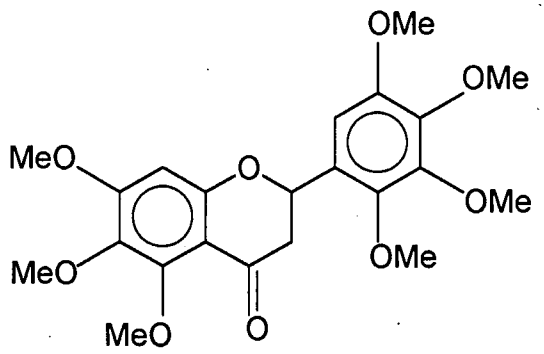
59 $R^1 = H$
60 $R^1 = OH$

Flavanones having three B-ring oxygenations

5,7,3',4',5'-Pentahydroxyflavanone **60** was isolated from the bracts of *Helichrysumbracteatum*[40] where it co-existed with naringenin **49** and eriodictyol **59** and from the flowers of *Verbena hybrida*[40] where it co-existed with other flavanones.

Flavanones having four B-ring oxygenations

Tetrasubstituted B-rings are very rare and 5,6,7,2',3',4',5'-heptamethoxyflavanone **61** was recently extracted from the whole-plant extracts of *Polygonium nepaleuse*[41].

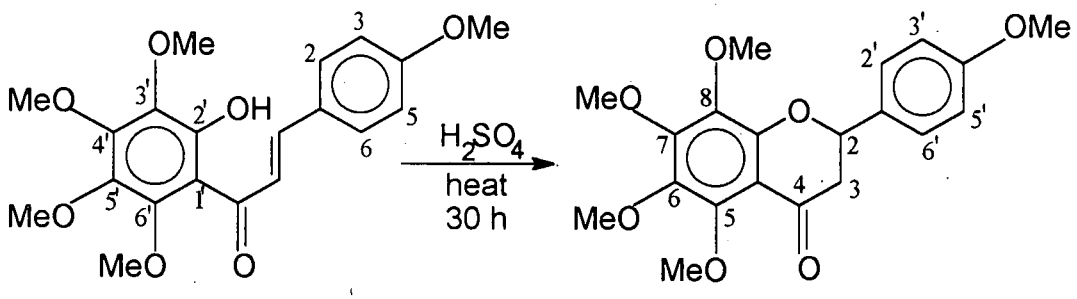


61

2.4 STRUCTURAL ELUCIDATION

2.4.1 SYNTHETIC METHODS

Acid or alkali catalyzed ring closure of chalcones was employed to obtain the corresponding flavanones[42]. Chalcones bearing a 2'-OH and phloroglucinol-type-A-ring favoured this ring closure[42]. The isomerization[42] of 2-hydroxy-3,4,5,6,4'-pentamethoxychalcone **62** to yield 5,6,7,8,4'-pentamethoxyflavanone **63** serves as an example.



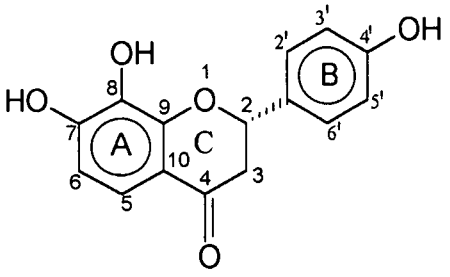
62 **63**

It was further discovered that in most instances isomerization required 1-2% acid or alkali with or without heating[42].

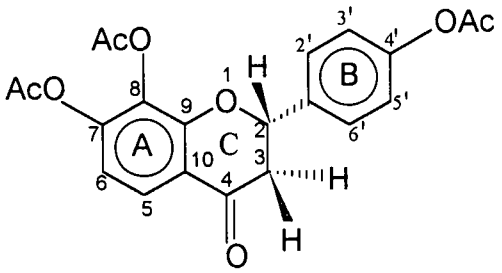
2.4.2 SPECTROSCOPIC METHODS

2.4.2.1 Proton Nuclear Magnetic Resonance (^{1}H NMR) Spectroscopy

In an attempt to isolate some known metabolites from the heartwood of *Acacia nigrescens* Malan[37] came across the rare 7,8,4'-trihydroxyflavanone **56**. The 7,8,4'-trihydroxyflavanone was derivatized to the 7,8,4'-triacetoxyflavanone **64** to obtain the n.m.r. data.



56



64

2.4.2.1.1 **Heterocyclic protons**

The C-ring substitution pattern was described by three doublet of doublets which appeared at δ5.50 (1H, H-2), δ3.04 (1H, H-3ax) and δ2.88 (1H, H-3eq)[37].

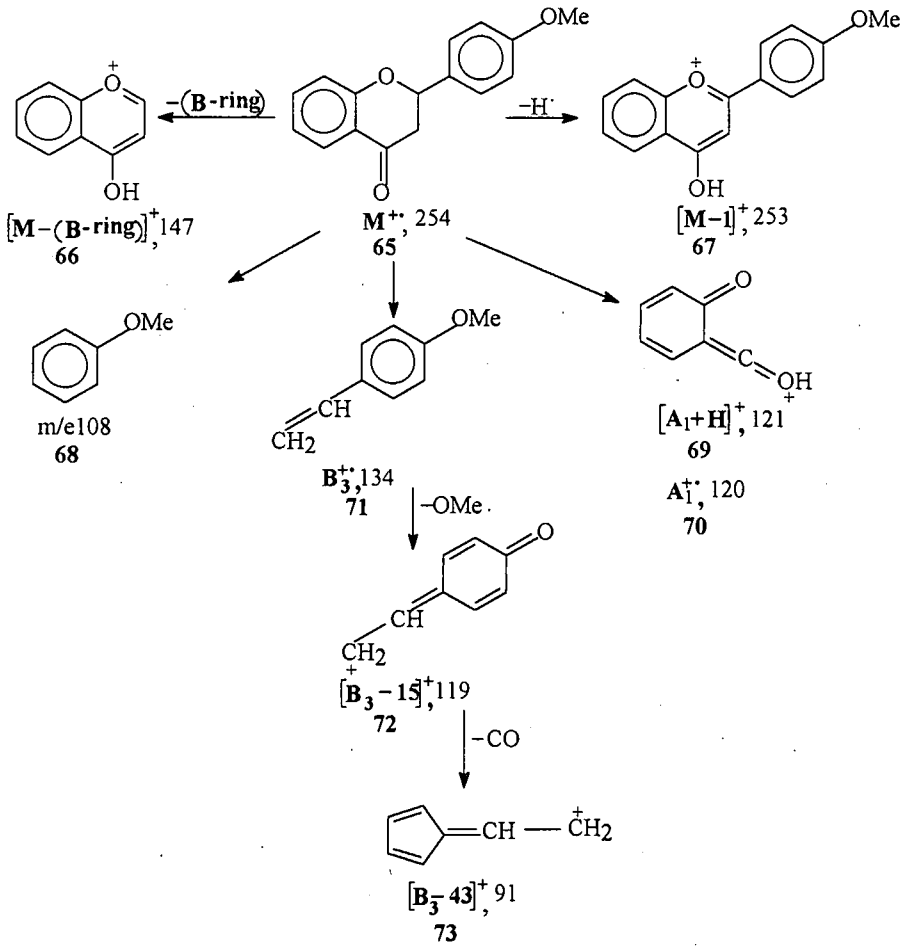
2.4.2.1.2 **Aromatic protons**

The two *ortho*-coupled doublets in the aromatic region at δ7.84 (1H, H-5) and δ6.89 (1H, H-6) were evidence of the AB-spin system describing the A-ring substitution pattern[37]. The B-ring substitution pattern was identified from the appearance of two doublet of doublets at δ7.43 (2H, H-2' and H-6') and δ7.13 (2H, H-3' and H-5') representing an AA'BB'-spin system[37].

2.4.2.2 **Mass Spectroscopy**

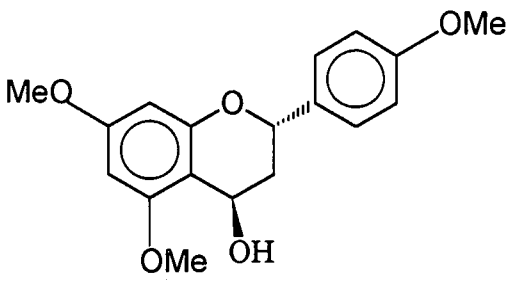
The retro-Diels-Alder[43] fission was found to be the mode of fragmentation for flavanones resulting in ions which corresponded to $A^{+\bullet}{}_1$ **70** and $[A_1 + H]^+$ **69** ions as shown in **Scheme 2.2**. The most important B-ring ion contained an ethylene group, $B^{+\bullet}{}_3$ **71**. This ion was always present with other B-ring fragments even if the B-ring would appear in the quinoid form, e.g.$[B_3\text{-}15]^+$ **72** ion. Like all other flavonoids, the intensities of the A-and B-ring fragments were dependent on the substitution patterns of the two rings[43]. The $B^{+\bullet}{}_3$ **71** ion was found to be the base peak for 4-methoxyflavanone whereas the $A^+{}_1$ **70** ion together with the $[A_1 + H]^+$ **69** fragment had relative intensities of only 3.5 and 30%, respectively.

SCHEME 2.2



2.5 FLAVANONES TO FLAVAN-4-OLS

Very few flavan-4-ols are known with 4',5,7-trimethoxy-2,4-*trans*-flavan-4-ol **65** the first isolated by Lam and Wrang from *Dahlia tenuicaulis*[44] and it remained the only one until the 1980s when other examples isolated from *Tephrosia watsonia* and *Marshallia obovata* were reported[44].

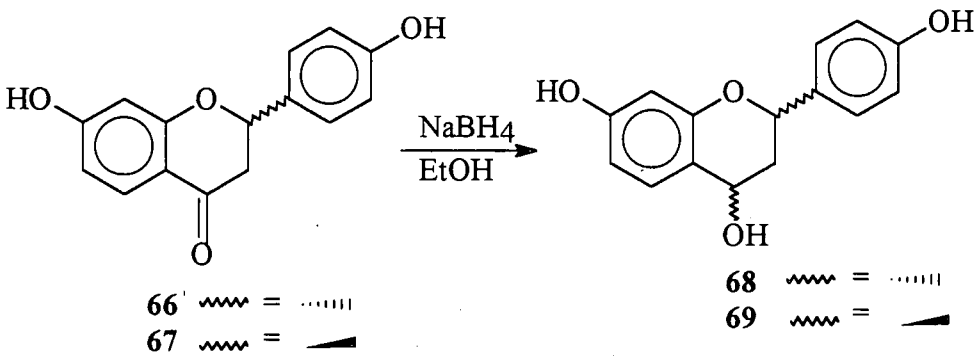


4,5,7'-Trimethoxy-2,4-*trans*-flavan-4-ol

65

Flavan-4-ols were synthesized directly from flavanones by a single reduction step[45] as shown in **Scheme 2.3**.

SCHEME 2.3

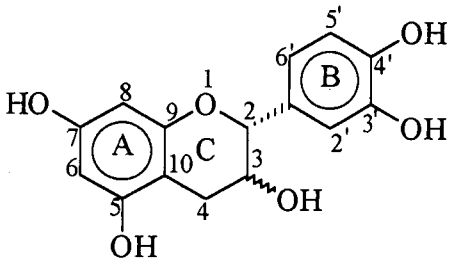


CHAPTER 3

FLAVAN-3-OLS

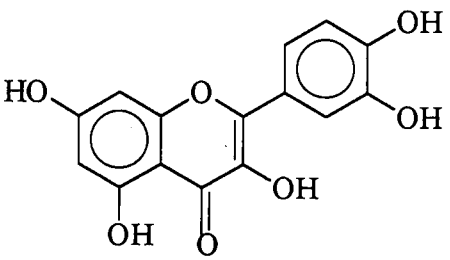
3.1 INTRODUCTION

The flavan-3-ols are the largest class of monomeric flavans. Two compounds, catechin **70** and epicatechin **71**, are the most common flavonoids known, sharing a distribution in the Monocotyledoneae almost as widespread as quercetin **72** in the Dicotyledoneae.[5]



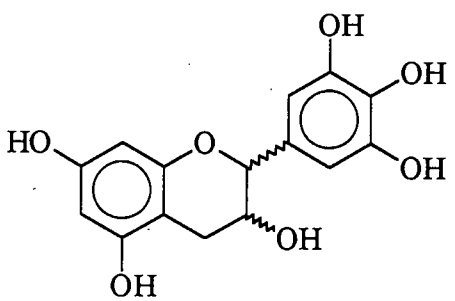
70 ~~~ = ▬

71 ~~~ = ·····



73

Likewise the 3',4',5'-trihydroxy B-ring flavan-3-ols, gallocatechin **74** and epigallocatechin **75** are dominant in primitive plants (the Coniferae being outstanding)[5].

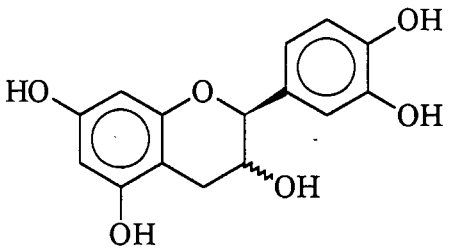


74 ~~~ = ▬

75 ~~~ = ·····

ent-Catechin **76** and *ent*-epicatechin **77** on the contrary are fairly rare, the latter is widespread and occurring as a natural product in several Palm species[5] and was also

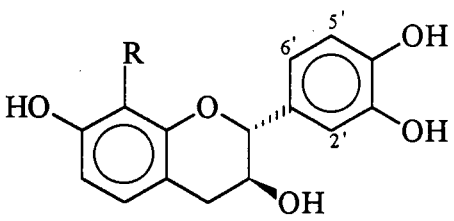
recently isolated from *Polygonum multiflorum*[5]. *ent*-Catechin **76** was recently isolated from *Rhaphiolepis umbellata*[5] and *ent*-epicatechin **77** also co-exists with epicatechin **71** and catechin **70** respectively, suggesting that **76** and **77** result from the action of a C-2 epimerase enzyme[45].



76 ~~~ = ·····

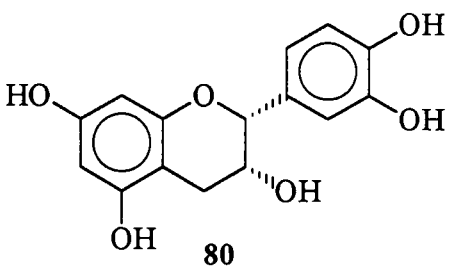
77 ~~~ = ▬

The occurrence of flavan-3-ols with a resorcinol A-ring [5](i.e. fisetinidol **78**, etc) were confined to the Leguminosae and Anacardiaceae, however, fisetinidol **78** was recently isolated from heartwood of two *Virora* (Myristicaceae) species. The latest hydroxyflavan-3-ols reported are, prosopin **79** isolated from *Prosopis glandulosa*[46] with an (2R,3S) absolute stereochemistry and (2R,3R)-3,5,7,3',5'-pentahydroxyflavan **80** from *Humboltia lauriflora*[5].



78 R = H

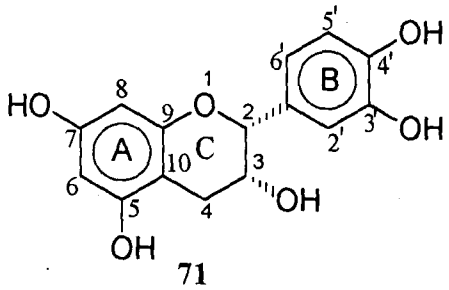
79 R = OH



80

3.2 NOMENCLATURE

A system that employs trivial names was adopted to define flavan-3-ols as shown in **Table 3.1**. All flavan-3-ols named in **Table 3.1** are of the (2R,3S) configuration and those with a (2R,3R) configuration are prefixed with 'epi', e.g. epicatechin[5]. The flavan-3-ol units with a 2S configuration are distinguished by the enantio (*ent*) prefix[5]. The flavanoid skeleton is drawn and numbered as shown below[5].



71

Table 3.1: (2R,3S) monomers

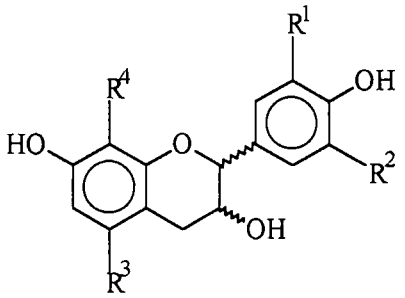
Substitution pattern

Monomer	3	5	7	8	3'	4'	5'
Afzelechin	OH	OH	OH	H	H	OH	H
Catechin	OH	OH	OH	H	OH	OH	H
Gallocatechin	OH	OH	OH	H	OH	OH	OH
Guibourtinidol	OH	H	OH	H	H	OH	H
Fisetinidol	OH	H	OH	H	OH	OH	H
Prosopin	OH	H	OH	OH	OH	OH	H
Oritin	OH	H	OH	OH	H	OH	H

3.3 STRUCTURE AND NATURAL DISTRIBUTION

A list of naturally occurring flavan-3-ols and their sources is given in **Table 3.2**.

TABLE 3.2 Naturally occurring flavan-3-ols and their sources.



Structure	Name	Source
R^1=R^2=R^4=H,R^3=OH (2R,3S)	Afzelechin	*Nothofagus fusca* (heartwood)[5] *Saxifraga ligulata* (root)[5] *Cassia abbreviata*[47]
R^1=R^2=R^4=H,R^3=OH (2R,3R)	Epiafzelechin	*Afzelia* sp (heartwood)[5] *Cassia sieberana* (root)[5]
R^1=R^2=R^4=H,R^3=OH (2R,3R)	*ent*-Epiafzelchin	*Livinstona chinensis* (leaf)[5] *Crateava religiosa* (leaf)[5]
R^1=R^4=H, R^2=R^3=OH (2R,3S)	Catechin	Widespread[5]
R^1=R^4=H, R^2=R^3=OH (2S,3R)	*ent*-Catechin	*Polygonum multiflorum* (root)[5] *Rhaphiolepsis umbellata* (bark)[5]
R^1=R^4=H, R^2=R^3=OH (2R,3R)	Epicatechin	Widespread[5]
R^1=R^4=H, R^2=R^3=OH (2S,3S)	*ent*-Epicatechin	*Polygonum multiflorum* (root)[5] Palmae fruit (leaf)[5]
R^1=R^3=R^4=H,R^2=OH (2R,3S)	Fisetinidol	*Acacia mearnsii* (heartwood)[5] *Colosphospermum mopane* (heartwood)[5]
R^1=R^3=R^4=H,R^2=OH (2S,3R)	*ent*-Fisetinidol	*Afzelia xylocarpa* (wood)[5]
R^1=R^3=R^4=H,R^2=OH (2S,3S)	*ent*-Epifisetinidol	*Colophospermum mopane* (heartwood)[5]
R^1=R^2=R^3=OH,R^4=H (2R,3S)	Gallocatechin	Widespread[5]
R^1=R^2=R^3=OH,R^4=H (2R,3R)	Epigallocatechin	Widespread[5]
R^1=R^3=H,R^2=R^4=OH (2R,3S)	Prosopin	*Prosopis glandulosa* (heartwood)[48]
R^1=R^2=R^3=R^4=H (2R,3S)	Guibourtinidol	*Cassia abbreviata*[49]

3.4 STRUCTURAL ELUCIDATION

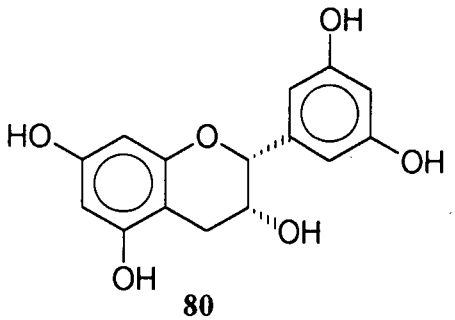
3.4.1 FLAVAN-3-OLS AS NUCLEOPHILES

In contrast to flavonoids bearing C-4 carbonyl functions which exhibit reduced nucleophilicities of their aromatic A-rings[50] and the inductive pull of the 4-hydroxyl function of flavan-3,4-diols or of the C-4 carbocation resulting from its protonation which precludes their innate tendency for self - condensation[49,51], flavan-3-ols such as catechin **70** and epicatechin **71** are capable of a $C_4(sp^3)$-$C_{6/8}(sp^2)$ interflavanyl linkage. Flavan-3-ols with phloroglucinol A-rings e.g (+)-catechin **70** and (+)-gallocatechin **74**, are stronger nucleophilic substrates than their resorcinol counterparts, e.g.(-)-fisetinidol **78**[16] but this will be discussed in detail in **Chapter 4**.

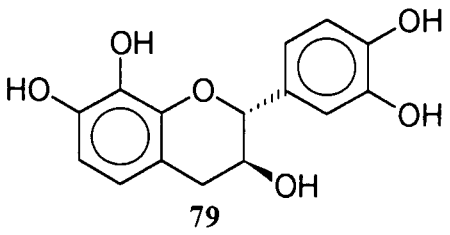
3.4.2 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

CD correlation of prosopin **79** from *Prosopis glandulosa* with an authentic sample of catechin **70** enabled Roux to assign a (2R,3S) absolute streochemistry[5]. A (2R,3R) absolute stereochemistry was assigned to 3,5,7,3',5'-pentahydroxyflavan **80** from *Humboltia laurifolia,* the absolute stereochemistry was verified[5] from the sign and magnitude of the specific rotation similar to that of epicatechin **71**. The proposal for a 3',5'-dihydroxy B-ring is based on the *meta* couplings observed for 2'-H and 6'-H with 5'-H in the tetramethyl ether.

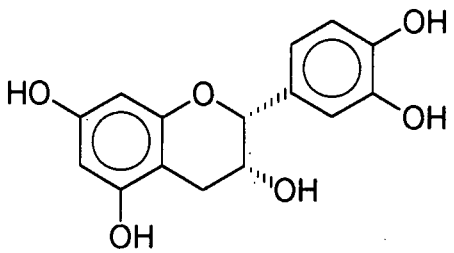
For the structure to be accepted finally ^{13}C NMR spectroscopy should give un-equivocal empirical evidence[5]. Therefore, this shows that the ^{1}H NMR and ^{13}C NMR spectroscopy remain the cornerstone for structural elucidation of flavans[5].



80



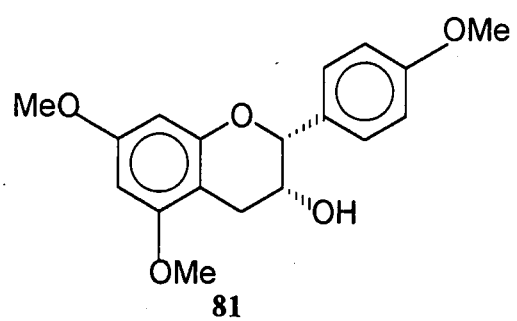
79



71

3.4.3 **Mass Spectrometry**

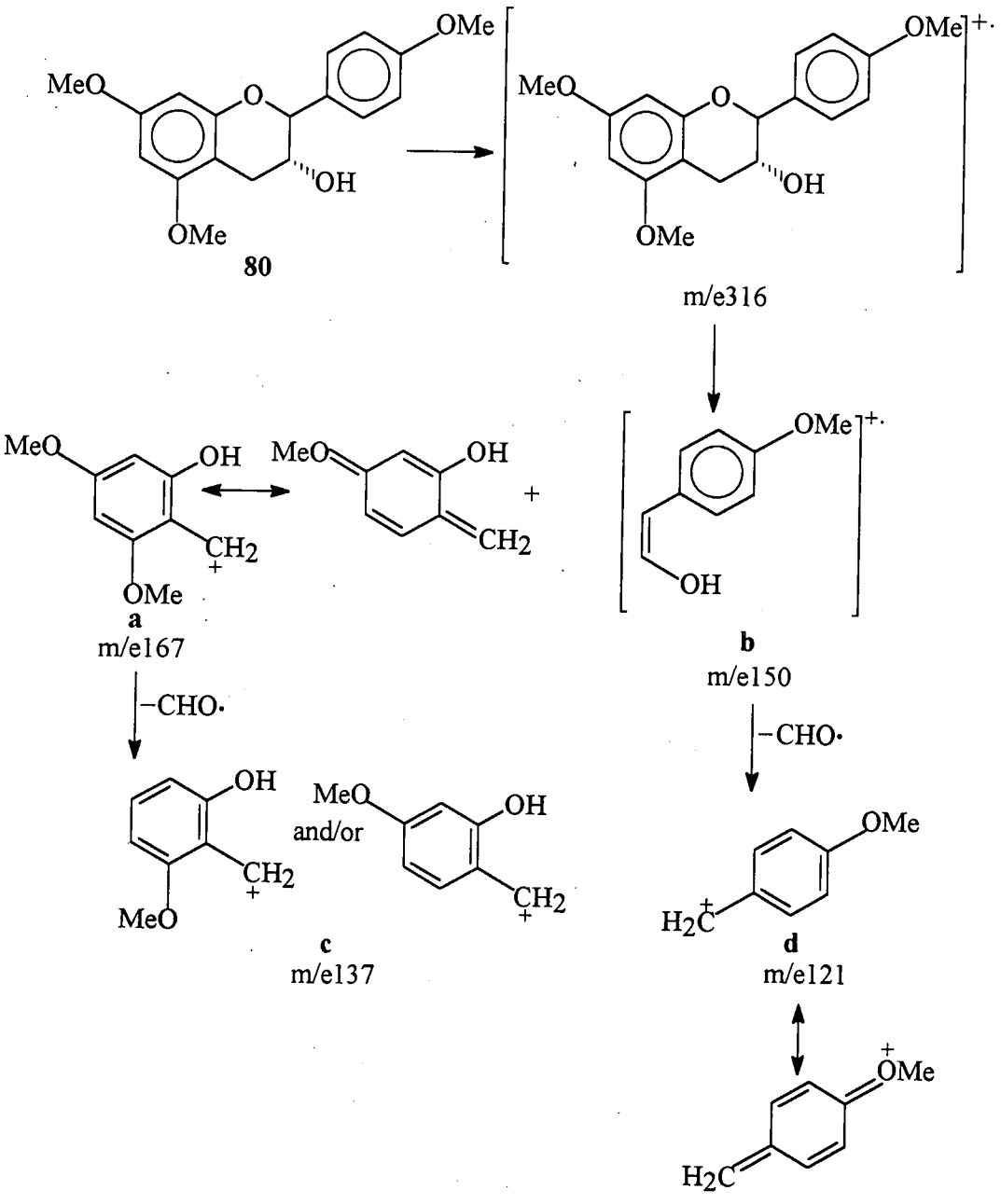
Fragmentation of the molecular ion occurred by retro-Diels-Alder fission of the heterocyclic ring accompanied by hydrogen transfer as with 3-hydroxyflavanones as shown in **Scheme 3.1**. Flavan-3-ols yielded the *o*-hydroxybenzyl cation (or equivalent ion) **167** as the base peak in each case as shown in **Scheme 3.1**. The loss of water and a hydrogen atom from the molecular ion to give the flavylium cation occurred to a minor extent only with all the compounds[27]. Fragmentation of (-)-epiafzelechin trimethyl ether **81** is presented in **Scheme 3.1** as an example for flavan-3-ols[27].



81

The fragment ion **a**, m/e 167(base peak) lost formaldehyde to give the ion **c** with m/e 137, subsequent loss of carbon monoxide yielded m/e 109. The ion **b**, m/e 150 lost a formyl radical to yield ion **d** with m/e 121.

SCHEME 3.1



DISCUSSION

CHAPTER 4

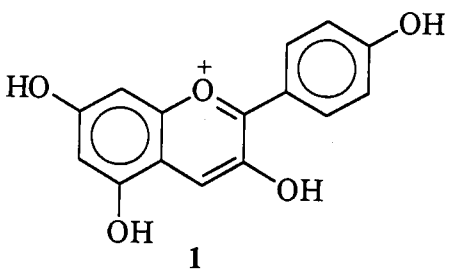
PROANTHOCYANIDINS

4.1 INTRODUCTION

Condensed tannins are widely distributed[52] in nature but the complexity of their extract composition and the consequent difficulty of their isolation and purification as well as the lack of a universal method of both synthesis and of assessing the absolute configuration have been a challenge to scientists for many decades.

4.2 NOMENCLATURE

Recently the term proanthocyanidin was redefined to include all compounds which produced anthocyanidins e.g. **1** by cleavage of a C=O bond on heating with mineral acid[5].

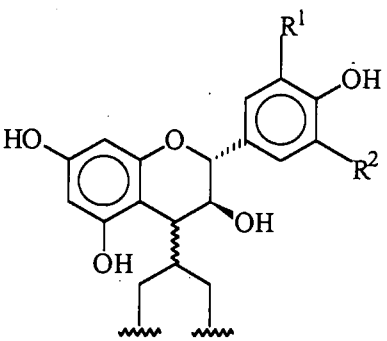


1

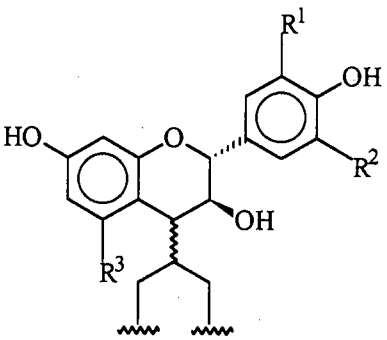
Hemingway[45] and co-workers developed the present system for naming proanthocyanidins based on the system employed for polysaccharides. The position, direction, and configuration of the interflavanoid bond are contained in brackets e.g. (4α→8). The trivial names of monomeric flavan-3-ols are of use in defining the proanthocyanidin monomer units. Examples of proanthocyanidins with 2R,3S monomer units with phloroglucinol A-rings, resorcinol A-rings, and pyrogallol A-rings are listed in **Table 4.1**[5].

Table 4.1

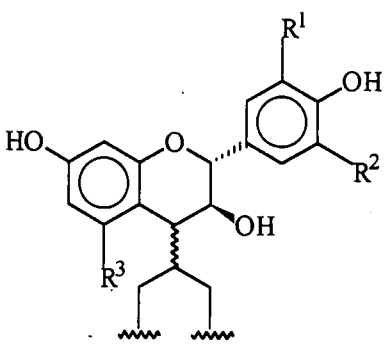
Proanthocyanidin Class	Monomer Unit
Propelargonidin	Afzelechin **82**
Procyanidin	Catechin **83**
Prodelphinidin	Gallocatechin **84**
Proguibourtinidin	Guibourtinidol **85**
Profisetinidin	Fisetinidol **86**
Prorobinetidin	Robinetidol **87**
Proteracacinidin	Oritin **88**
Promelacacinidin	Prosopin **89**



82 $R^1 = R^2 = H$
83 $R^1 = OH$, $R^2 = H$
84 $R^1 = R^2 = OH$

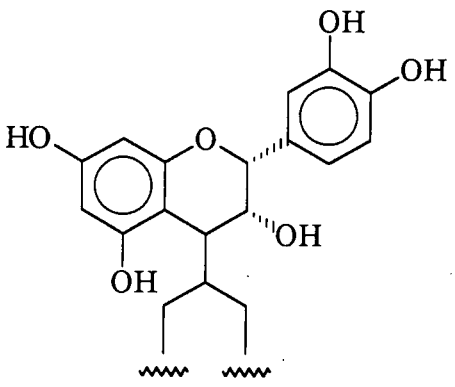


85 $R^1 = R^2 = R^3 = H$
86 $R^1 = OH$, $R^2 = R^3 = H$
87 $R^1 = R^2 = OH$, $R^3 = H$

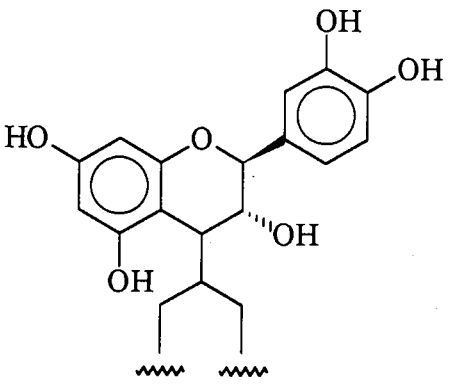


88 $R^1 = R^2 = R^3 = H$
89 $R^1 = OH$, $R^2 = R^3 = H$

The (2R,3R) top unit of structure **90** in the list are prefixed with 'epi', e.g. epicatechin, etc. The top unit of **91** with a (2S,3R) configuration are distinguished by the enantio (*ent*) prefix, e.g. *ent*-epicatechin[5].

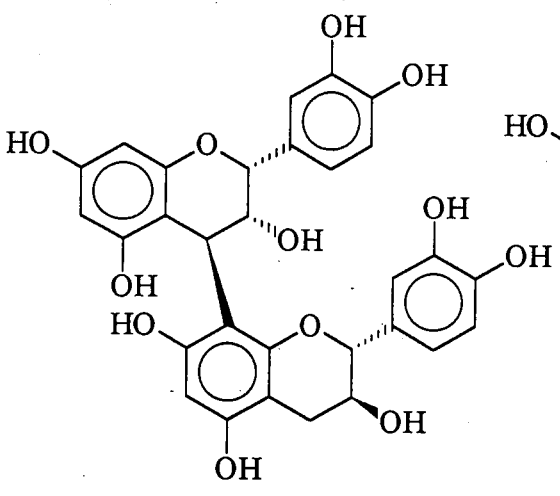


90

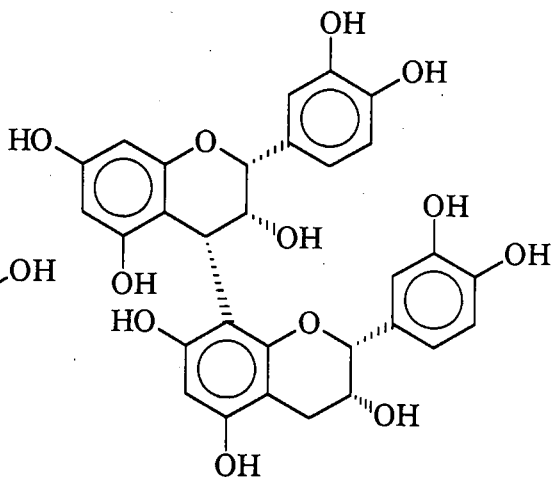


91

Epicatechin-(4β→8)-catechin **92** and epicatechin-(4α→8)-epicatechin **93** are illustrated to give a complete understanding of the nomenclature of proanthocyanidins[5].

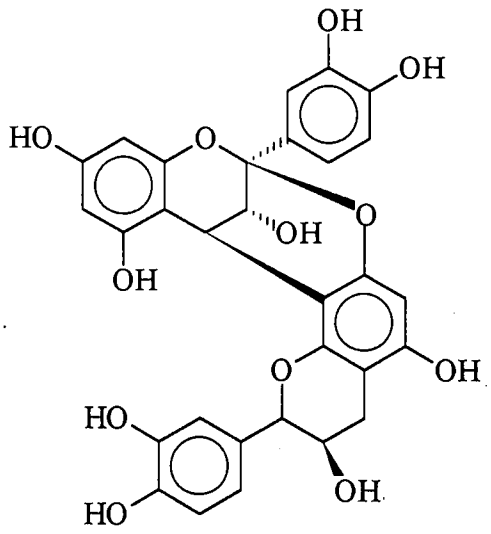


92



93

A considerable number of doubly linked (A-type) proanthocyanidins[44] exist with the proanthocyanidin A2 posing as an example where two epicatechin units fused together through a normal (4β→8) linkage and also through C-2 to O-7 of the neighbouring epicatechin unit. This compound was named, epicatechin-(2β→7,4β→8)-epicatechin[5] **94**.



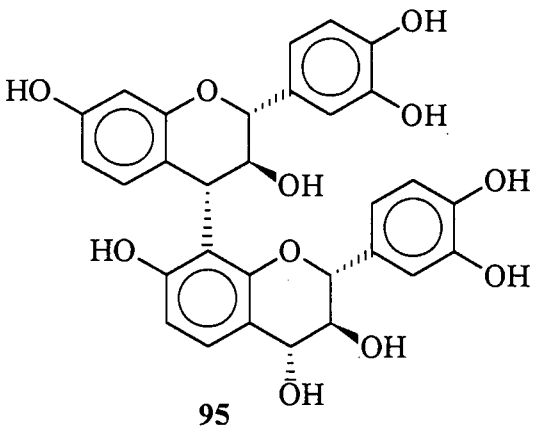
94

4.3 STRUCTURE AND NATURAL DISTRIBUTION

A list of proanthocyanidin oligomers with their respective sources is given by Porter[5]. These oligomers are grouped as the bis-flavan-3,4-diol type, the flavan-3-ol plus flavan-3-ol type, the bis-flavan-3-ol type, and the ether-linked type dimers. In the following section these groups of oligomers are discussed.

4.3.1 Flavan-3,4-diol dimers

Although the link in the biflavanoid is most likely 4,8 (**95**) with the C-4 either having an α or β configuration, it was established that where (+)-catechin or related strongly nucleophilic flavanoids of the phloroglucinol type are absent as in the case of the heartwood of black wattle (*Acacia mearnsii*), but where the predominant flavan-3,4-diol, (+)-leucofisetinidin can act both as electrophile at (C-4) and nucleophile at (C-6), self-condensation of the flavan-3,4-diol occurred to form a variety of 4,6-linked biflavanoids[53] (**96**) , triflavanoids[54], and higher condensates.



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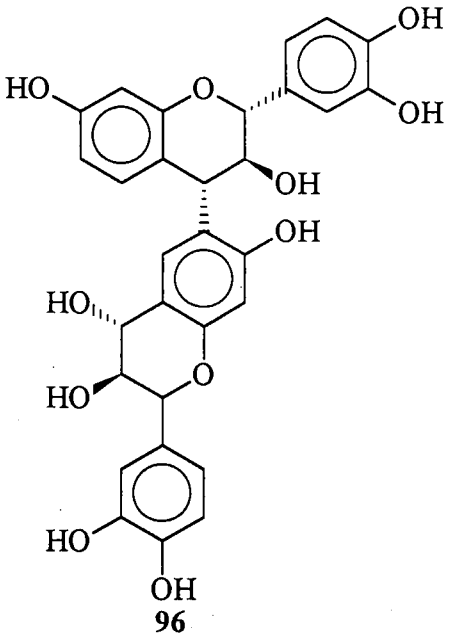
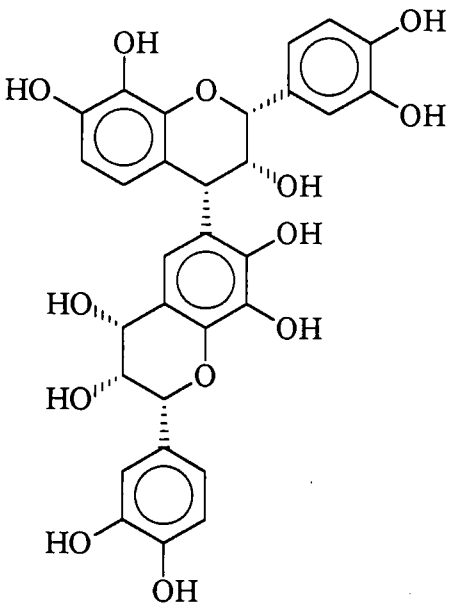


96

There was a strong belief that oligomeric flavanoids with a pyrogallol A-ring i.e. 7,8-dihydroxysubstituted proanthocyanidins were rare due to the fact that the 4-carbonium ions which could presumably originate from them, would be less adequately stabilized by delocalization of the charge (through resonance) and be incapable of undergoing self-condensation such that compounds **97** and **98** were never thought to exist.

The isolation of the natural proanthocyanidin dimer, (+)-2,3-*cis*-3,3',4',7,8-pentahydroxyflavan-(4α→6)-isomelacacinidin from *Acacia melanoxylo*n by Foo[55] and the discovery of dimeric proteracacinidins in *Acacia galpinii*[56] countered the above conception.



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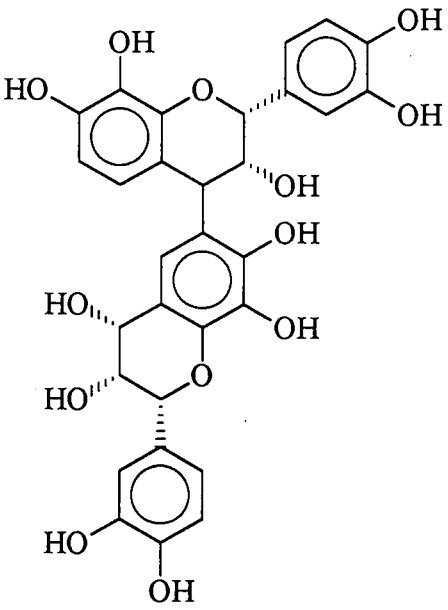
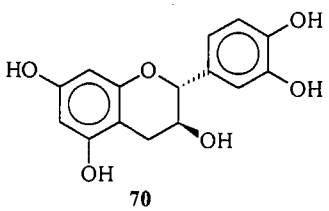


98

4.3.2 **Flavan-3-ol + Flavan-3,4-diol dimers**

The isolated (+)-catechin [(+)-5,7,3',4'-tetrahydroxy-2,3-*trans*-flavan-3-ol] **70** and (+)-leucofisetinidin [(+)-7,3',4'-trihydroxy-2,3-*trans*-flavan-3,4-*trans*-diol] **15** were used in biomimetic synthesis of (+)-leucofisetinidin-(+)-catechin **99**. The [4,8]-biflavanoid thus formed was before isolated from the bark of *Acacia mearnsii*[57] and later from the wood of *Colophospermum mopane*[58].

Catechin **70** provided exceptionally strong nucleophilic centres at position 6 and 8, due to its *meta*-oxygenated substituted pattern. The 8-position was a more favoured site for electrophilic substitution by a resonance stabilized 4-carbonium ion emanating from the flavan-3,4-diol, (+)-leucofisetinidin[58] **15**.



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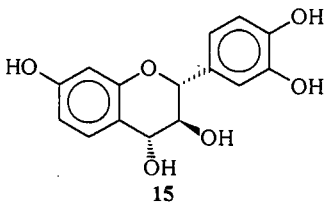

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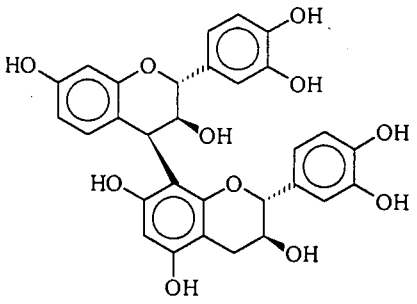
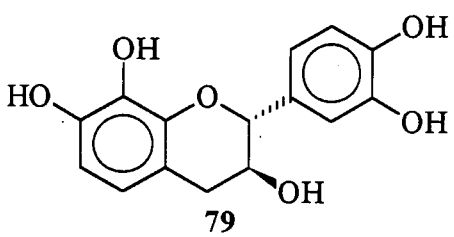


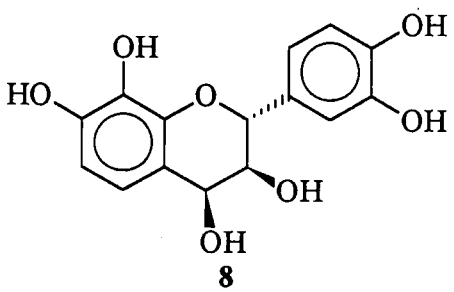
99

The presence of a variety of dimeric flavanoid analogues based on 3',4',7,8-tetrahydroxyphenolic substitution patterns was further emphasized by the isolation of the following biflavanoids **100-103** from the heartwood of *Prosopis glandulosa*(mesquite)[59].

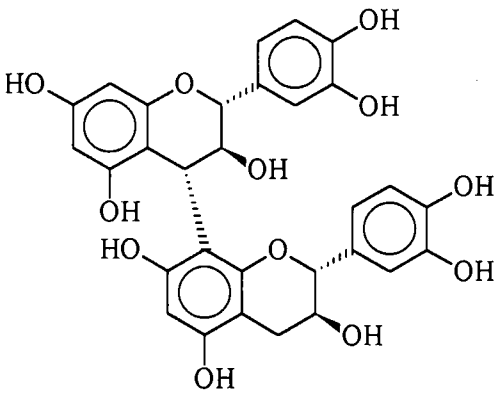
The novel (2R,3S)-2,3-*trans*-3',4',7,8-tetrahydroxyflavan-3-ol **79** which predominates in the heartwood of mesquite and the flavan-3,4-diol **8** were used as putative precursors for a variety of polyflavanoid oligomers[46].



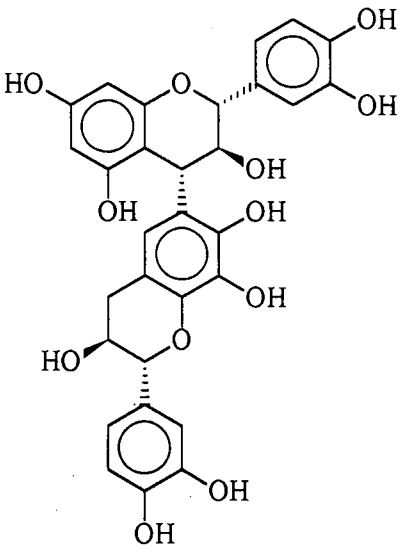
79



8



100



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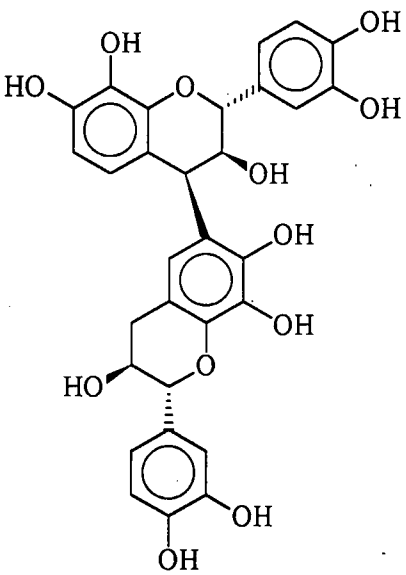

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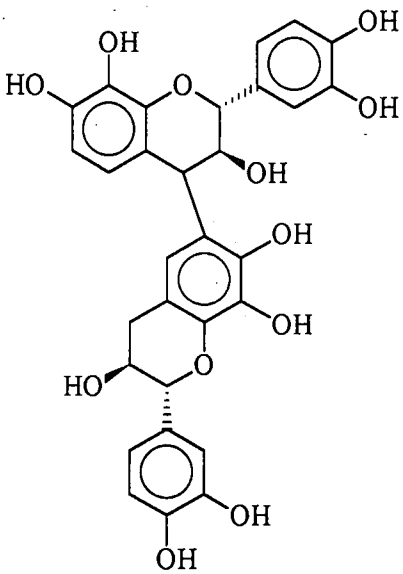
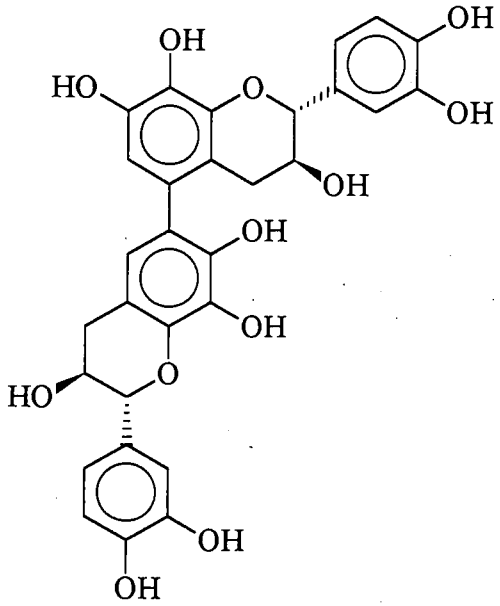


103

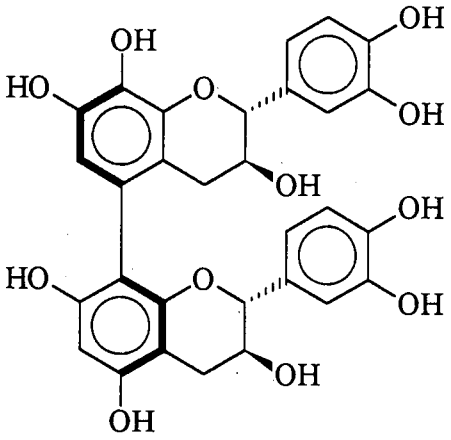
4.3.3 **Flavan-3-ol dimers**

The isolation of a [5,6]-dimer **104** and [5,8]-(+)-mesquitol-(+)-catechins **105** and **106** from the heartwood of *Prosopis glandulosa* demonstrated an alternative method of tannin formation *via* oxidative phenol coupling as opposed to the normal (4→8) and (4→6) dimers[59].

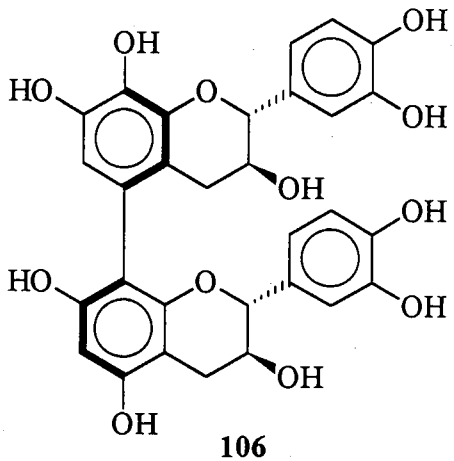
S and R absolute configuration at the $C(sp^2)$-$C(sp^2)$ were assigned to compounds **105** and **106**, respectively. **105** and **106** were synthesized from the corresponding precursors, **79** and **70**



104



105



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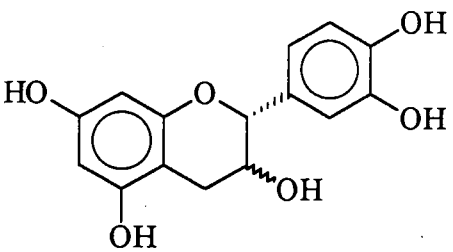
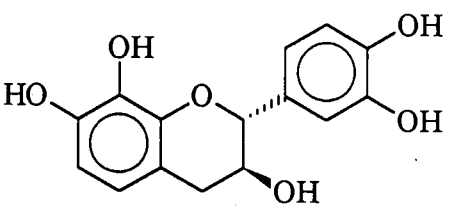


70 ~~~ = ▬

71 ~~~ = ·····

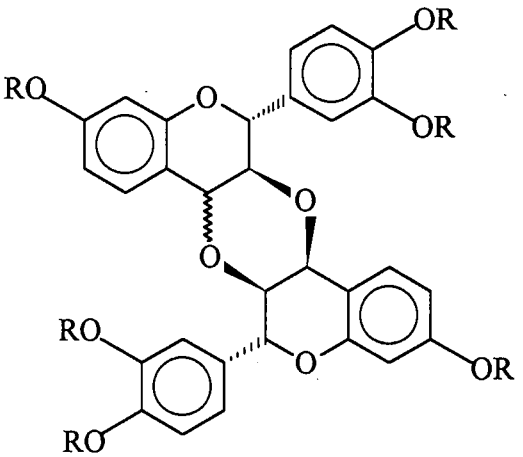


79

4.3.4 Ether-Linked dimers

With the exception of A-group biflavanoid procyanidins **94** bearing an ether linkage between C-2→C-7 in addition to the C-4 to C-8' carbon-carbon linkage, the occurrence of natural ether- linked condensed tannins are atypical[57].

The doubly ether-linked dioxane-type biflavanoid **107** isolated by Drewes and Isley from the heartwood of *Acacia mearnsii* serves as an example[60].



107 R = H , ~~~ = ▬

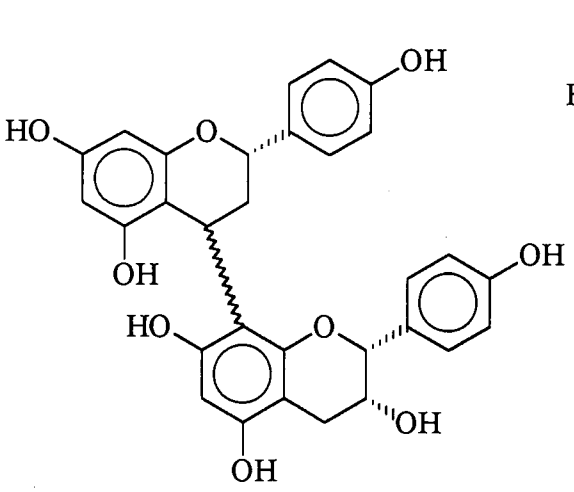
108 R = H , ~~~ = ·····

109 R = Me , ~~~ = ·····

A stereochemically asymmetrical 2,3-trans-3,4-*trans*:2',3'-*trans*-3',4'-*cis* **108** diastereomer of 2,3-*trans*-3,4-*cis*:2',3'-*trans*-3',4'-*cis* **107**, was later isolated by Young and co-workers from the same source and this was derivatised as hexamethyl ether **109** to allow structural characterisation[61].

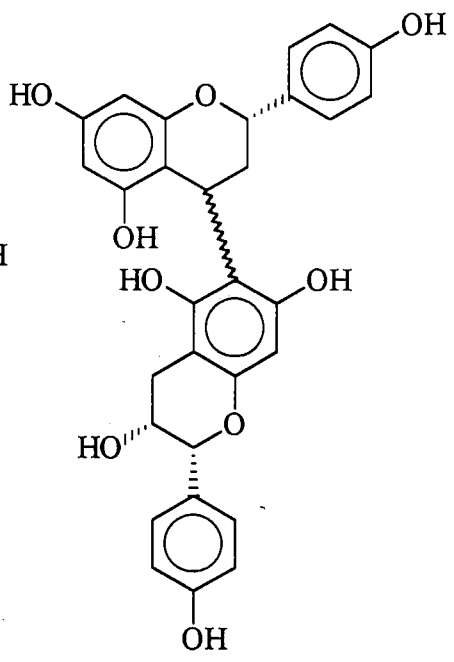
4.3.5 Novel-Flavanone Biflavanoids

The occurrence of four possible 4α/β diastereomeric pairs of cassiaflavan linked to epiafzelechin **110**, **111**, **112** and **113** in the leaves of *Cassia fistula* served as the first examples of a new class of proanthocyanidins called procassinidins[62].



110 ~~~ = ◢

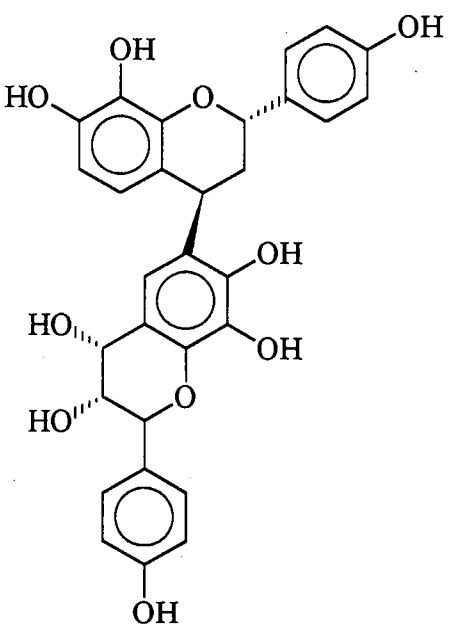
111 ~~~ = ⋯⋯



112 ~~~ = ◢

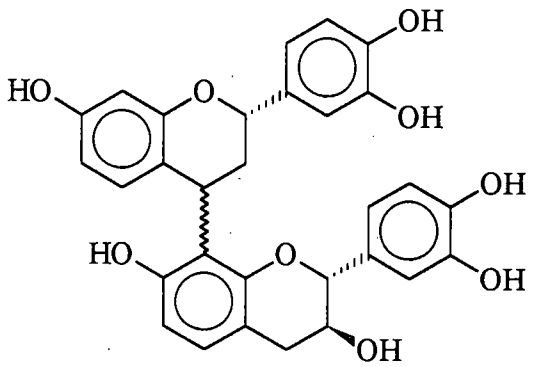
113 ~~~ = ⋯⋯

The first (2S)-7,8,4'-trihydroxyflavan-epioritin-4α-ol **114** dimer was isolated from the heartwood of *Acacia caffra*[63].



114

Very recently, two new flavan dimers from *Cassia nomame*[64] were isolated and synthesized. The dimers were identified as (2S)-3',4',7'-trihydroxyflavan-(4β→8)-catechin **115** and (2S)-3',4',7-trihydroxyflavan-(4α→8)-catechin **116** with the latter showing potent lipase inhibitory effect.



115 ~~~ = ▬

116 ~~~ = ·····

4.4 STRUCTURE ELUCIDATION

4.4.1 Synthetic Methods

4.4.1.1 Biomimetic Synthesis

Acid-catalyzed reaction (**Scheme 4.1**) to produce flavan-4-carbocations **117** from flavan-3,4-diols **15** that react with the A-ring of flavan-3-ols **70** to produce proanthocyanidins have been successfully employed and is referred to as biomimetic syntheses[65].

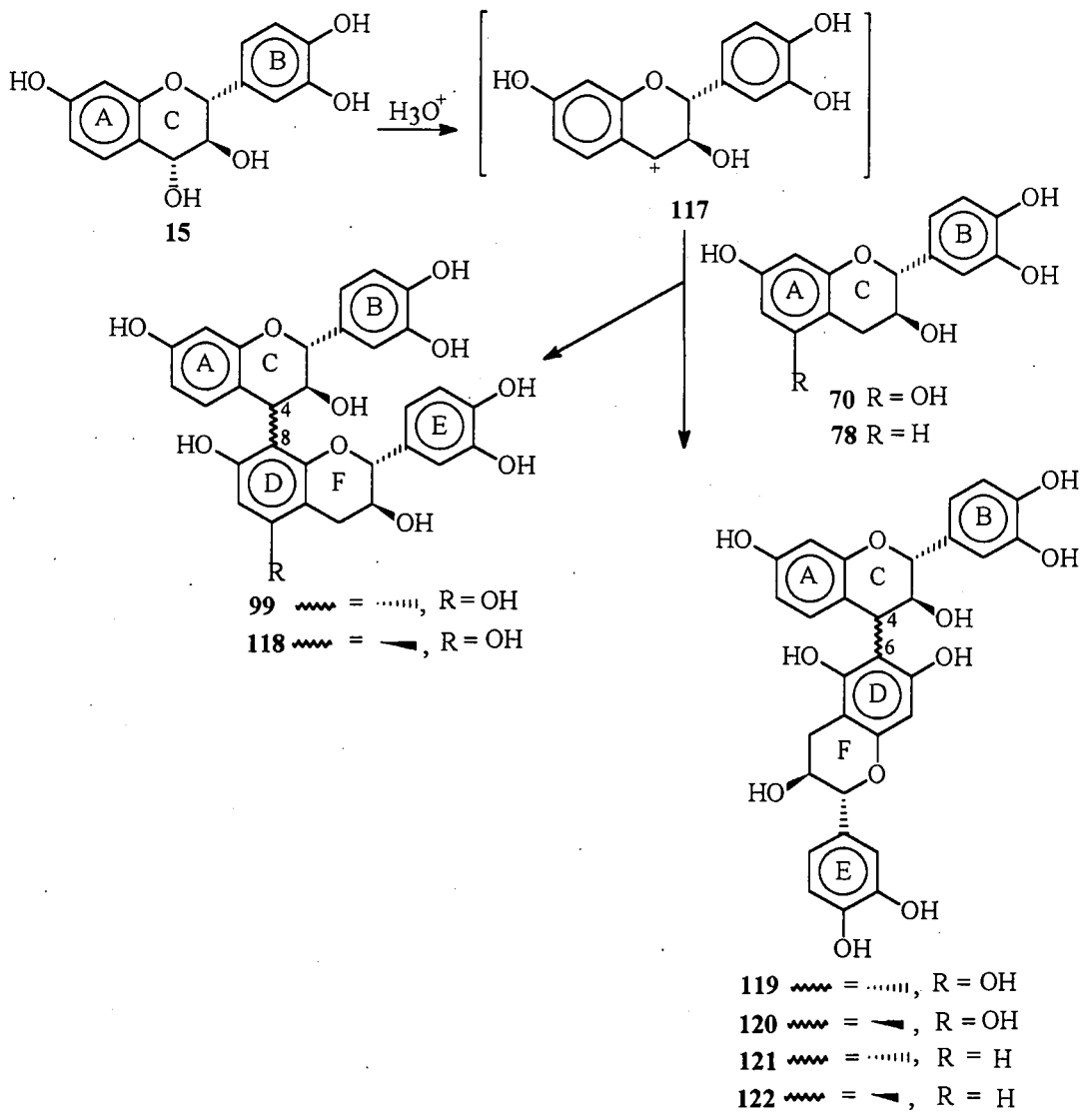
The C-4 carbocations **117** were trapped[66] with the potent nucleophilic flavan-3-ols **70** with phloroglucinol A-rings to yield the [4α→8]- and [4β→8]-biflavanoids **99** and **118** as the major products and also the [4α→6]- and [4β→8]-biflavanoids **119** and **120** as the minor products.

The selectivity as well as the steric considerations of the relatively stable C-4 carbocations **117** derived from diols favoured substitution at C-8 on catechins[67] **70**.

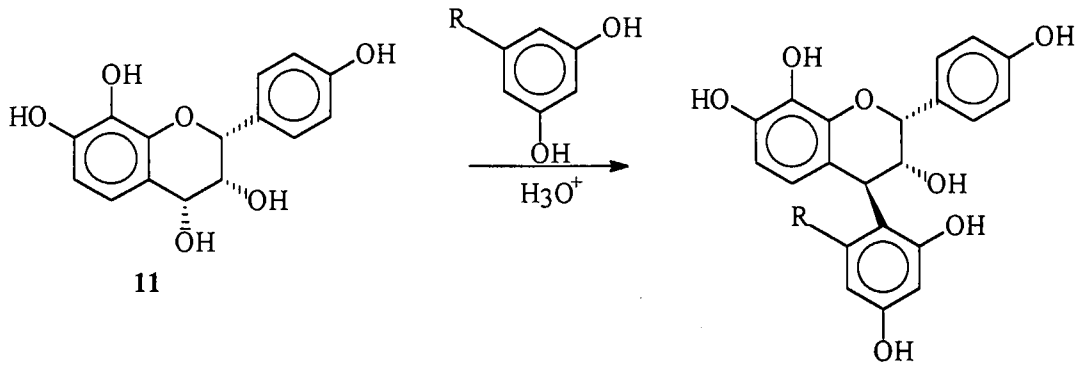
Condensation of (−)-fisetinidol **78** with (+)-mollisacacidin **15** yielded only the [4→6]-biflavanoids **121** and **122** as shown in **Scheme 4.1**[17]. These products indicated a selective condensation sequence due to steric constraints[68].

The stereoselective condensation[69,70] of (2R,3R,4R)-epioritin-4α-ol **11** with phloroglucinol and resorcinol to yield 2,3-*cis*-3,4-*trans*-4-aryl-flavan-3-ols **123** and**124** occured with an inversion of configuration at C-4 as shown in **Scheme 4.2**.

SCHEME 4.1



SCHEME 4.2



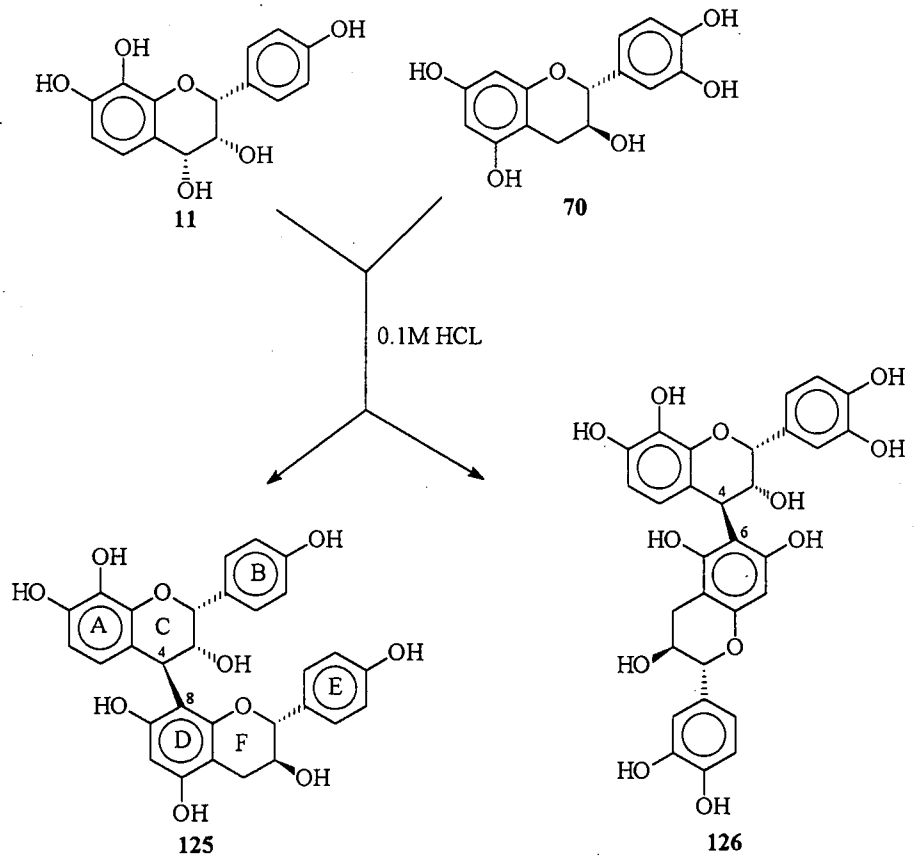
11

123 R = OH

124 R = H

The acid catalyzed reaction of epioritin-4α-ol **11** and catechin **70** occurred stereospecifically[50] to yield both the (4→8) and the (4→6)-2,3-*cis*-3,4-*trans* isomers **125**and **126** as shown in **Scheme 4.3**.

SCHEME 4.3



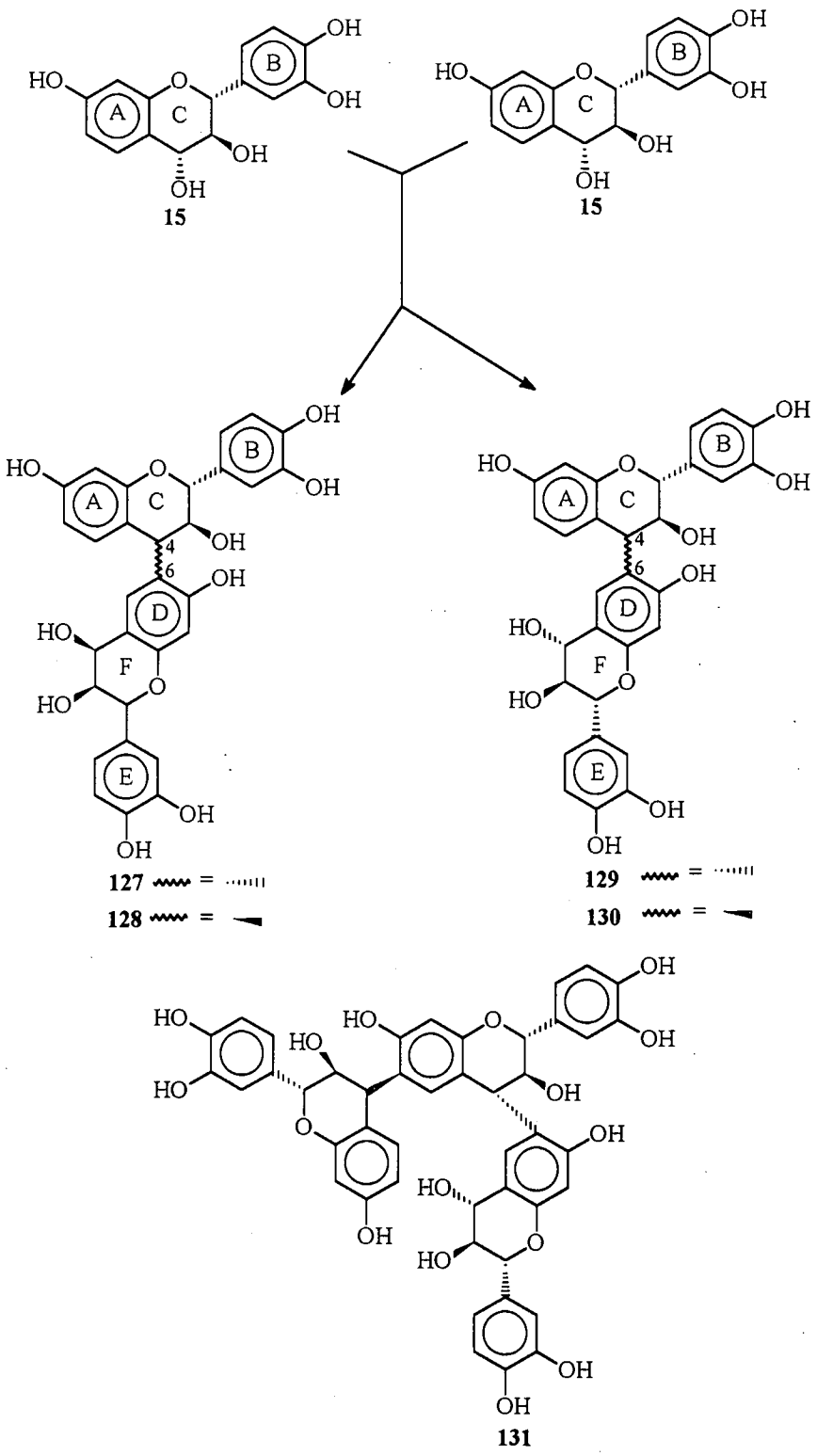
11 **70**

125 **126**

Acid-induced self-condensation of (+)-mollisacacidin **15** yielded biflavanoids **128** and **130** and the triflavanoid **131** formed in very low yields together with high molecular condensates as shown in **Scheme 4.4**. Conditions for self condensation of flavan-3,4-diol **15** were noted to be generally more drastic[48] compared to the facile condensation of the flavan-3,4-diol **15** with its flavan-3-ol analogue, (-)-fisetinidol **78**[68,71,72].

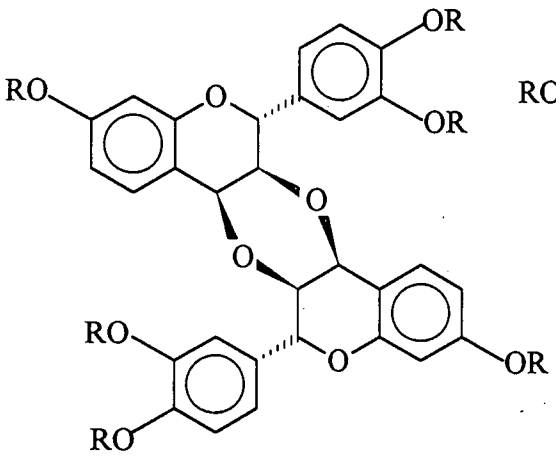
The more prolonged or drastic condition required for initial dimerization of flavan-3,4-diol **15** to biflavanoids **127-130** would result in a preferred and accelerated condensation with the top ABC units of products to form higher condensates. This suggestion was supported by the formation of high condensates rather than oligomers of intermediate mass due to the uncontrollable nature of the flavan-3,4-diol self-condensation.

SCHEME 4.4

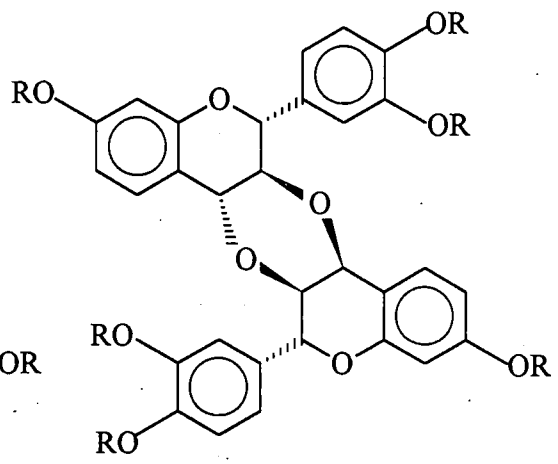


4.4.1.2 **Synthesis of Ether linked compounds**

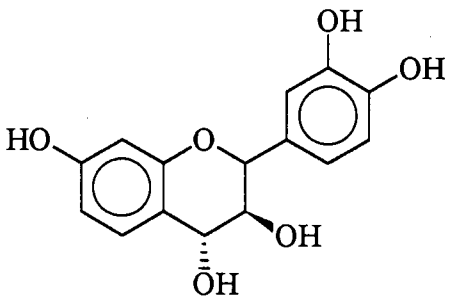
The synthesis of the natural doubly ether-linked profisetinidins **132** and **134** posed a problem in that the acid-induced self condensation of the free phenolic form of (+)-mollisacacidin **15** in protic media induced C-C bonding which led to low yields of [4,6]-linked biflavanoids and a triflavanoid as well as an abundance of high condensates[61].



132 R = H
133 R = Me



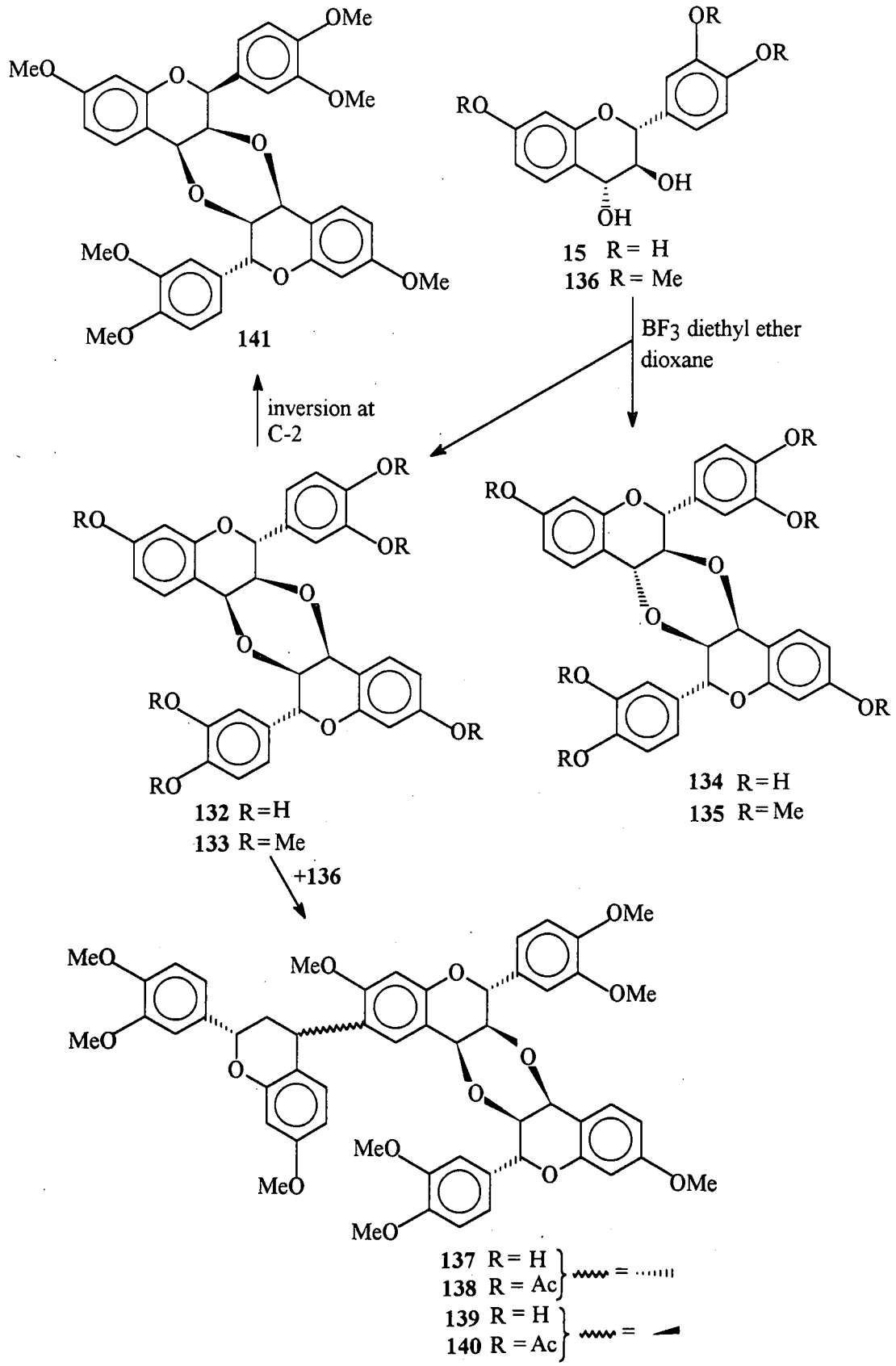
134 R = H
135 R = Me



15

Clark-Lewis[61] repeated application of acid-induced (HOAc) ethanolic conditions to 4',7,8-trimethoxy-2,3-*trans*-flavan-3,4-*trans*-diol in an attempt to promote solvolysis at C-4. This method produced (*ca.*10% yield) the bis-4',7,8-trimethoxy analogue of compound **133** as shown in **Scheme 4.5** as the sole product. These limitations were to an extent overcome by use of boron trifluoride-diethyl ether as catalyst in effecting the desired self condensation of (+)-mollisacacidin **15**[61]. BF_3 is known for promoting the formation of ether rather than C-C links[70].

SCHEME 4.5

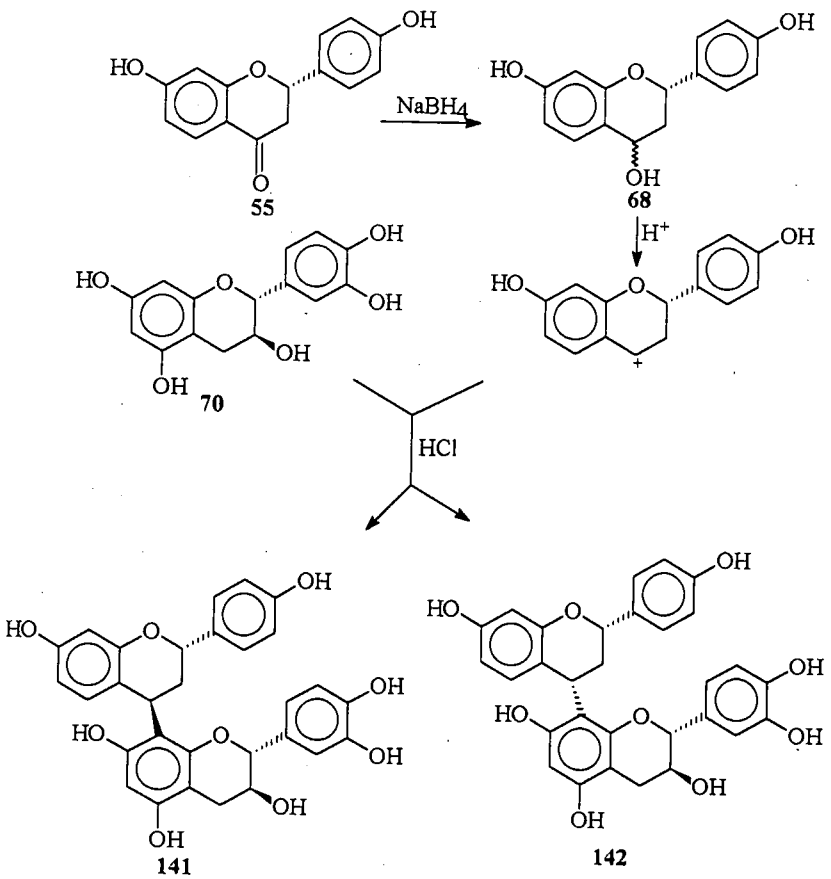

141
15 R = H
136 R = Me
BF3 diethyl ether
dioxane
inversion at
C-2
132 R=H
133 R=Me
134 R=H
135 R=Me
+136
137 R = H
138 R = Ac
139 R = H
140 R = Ac

4.4.1.3 Synthesis of Flavanone-biflavonoids

Clark-Lewis noticed that most flavan-4-ols occurred together with the corresponding flavanones:an observation that suggested that these compounds have the same 2S configuration and could be useful as precursors in the synthesis of dimers[44].

(2S)-Liquiritigenin **55** was used as a starting material to synthesize compounds **141** and **142**. (2S)-Liquiritigenin **55** was treated with $NaBH_4$ to yield (2S)-7,4'-dihydroxyflavan-4-ol **68**. A C-4 carbocation which retained its configuration at C-2[70] was generated on treatment with acid. Condensation of (2R,3S)-catechin **70** with this carbocation yielded (2S)-4',7-dihydroxyflavan-(4β→8)-catechin **141** and (2S)-4',7-dihydroxyflavan-(4α→8)-catechin **142** as shown in **Scheme 4.6**.

SCHEME 4.6



4.4.2 Spectroscopic Methods

4.4.2.1 Proton Magnetic Resonance Spectroscopy(^{1}H NMR)

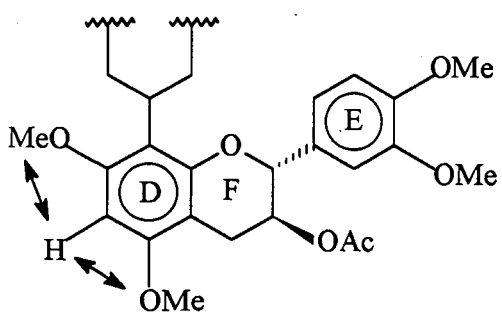
Ferreira and Brandt[73] summarized the modern ^{1}H NMR advances responsible for the progress made in the study of proanthocyanidins over the past decade. These ^{1}H NMR experiments included techniques such as NOE difference spectroscopy, 2-D COSY and chemical shift correlation methods, together with ^{13}C and heteronuclear analysis.

At ambient temperature the ^{1}H NMR spectra of the free phenolic proanthocyanidins resulted in a complicated spin pattern due to dynamic rotational isomerism about the C4-C6/8 bond. Derivatisation to phenolic permethyl ether acetate derivatives indicated two major rotameric forms in the ratio 2:1 on 300 MHz spectra at room temperature and these duplicated signals were carefully coalesced at elevated temperatures[74].

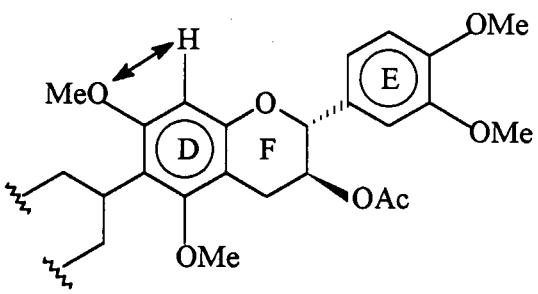
Selective bromination and debromination reactions were used to obtain diagnostic chemical shifts which resulted in a method for differentiation between the 4(C)→6(D)and 4(C)→8(D) interflavanoid links[75,76] when the bottom unit was a substituted catechin or epicatechin.

Viviers and co-workers allocated bonding positions based on chemical shift differences between the residual 6-(δ6.10 to δ6.22) and 8-protons (δ6.32 to δ6.47) of the methyl ether acetates of **143** and **144** in $CDCl_3$ at 100°C respectively.

NOE difference spectroscopy was elegantly applied to differentiate between the C-8 of **143** and C-6 of **144** coupled moieties of condensed tannins[59]. This was accomplished by examining the association of the residual proton with either two methoxy groups (H-6 with 5-OMe and 7-OMe as in **143**) or one methoxy group (H-8 with 7-OMe as in **144**).



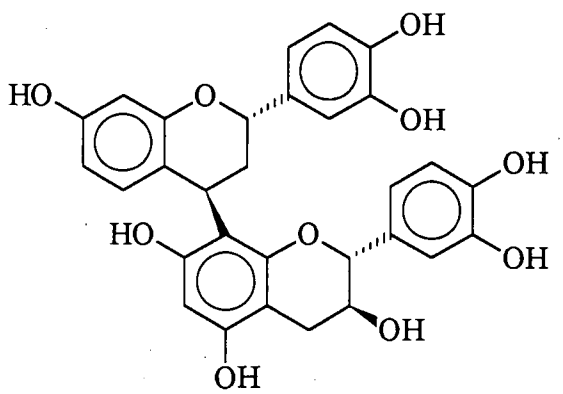
143



144

4.4.2.2 **Flavanone-biflavonoid ^{1}H NMR**

The ^{1}H NMR spectrum of **145** showed broad signals instead of duplication of signals. Signals of nine protons out of ten aromatic protons in the spectrum of **145** formed three sets of ABX -spin systems of 1,3,4-trisubstituted B- and E- rings{δ6.23-6.26[2H, *m*; H-8 of upper unit(U) and H-6(U)],6.62-6.66[3H, *m*; H-5(U), H-6'(U) and H-6'of lower unit(L)], 6.69, 6.74[1H each, *d*, J=8 Hz; H-5'(U) and H-5(L)], 6.83[2H, *d*,J=2 Hz; H-2'(U) and H-2'(L)]}. Two of the three tri-substituted benzene rings were attributed to B-and E-rings of dimeric flavan structure and the other an A-ring lacking a hydroxyl group at C-5. The remaining aromatic proton at δ6.08 is a singlet and was ascribed to the D-ring[64].



145

Signals of the eight aliphatic protons formed two sets of 4-spin systems{δ5.24[1H, *m*; H-2(U)], 2.17[1H, H-3(U)],2.45[1H, br *m*; H-3(U)]and 4.47[1H, t, J=6 Hz; H-4(U)]} and were assigned to the upper flavan unit. These protons indicated that the upper flavan unit lacked a hydroxyl group at C-3.

The remaining four protons also formed a 4-spin system{δ4.42[1H, br; H-2(L)]4.02[1H, *m*; H-3(L)], 2.57[1H, *dd*, J=8 Hz,16 Hz; H-4(L)]and 2.92[1H, *dd*, J=5.5,16 Hz; H-4(L)]}[64].

The broadening of signals of **145** was attributed to restricted rotation around the interflavan bond suggesting a 4→8 connection rather than a 4→6 interflavan bond[64].

4.4.2.3 Circular Dichroism(CD) Spectroscopy

Botha and co-workers[70] developed the first method for assignment of absolute configuration at C-4 of 4- aryl-flavan-3-ol chromophores by means of circular dichroism. They showed that the absolute configuration of the interflavanoid bond could be correlated to the CD curve near 230nm, i.e. a positive sign indicated a 4β while a negative sign indicated a 4α configuration.

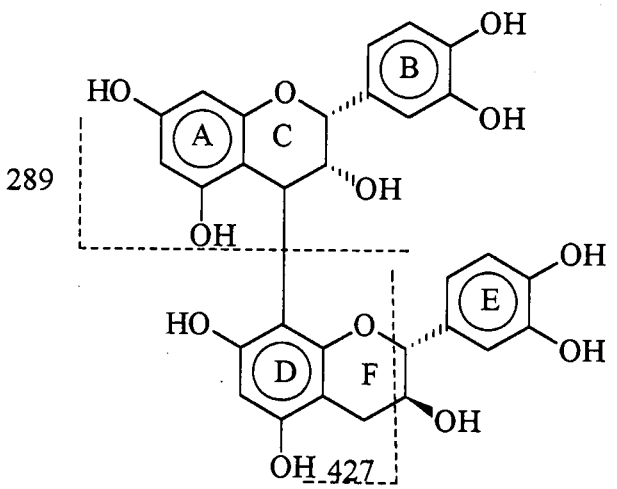
4.4.2.4 Mass Spectrometry(MS)

Fast Atomic Bombardment Mass Spectrometry (FAM-MS) is widely used to determine the mass of flavans and proanthocyanidins.

Both the positive and negative mass spectra produced by this method displayed abundant molecular ions $(M+H)^+$ and $(M-H)^-$.

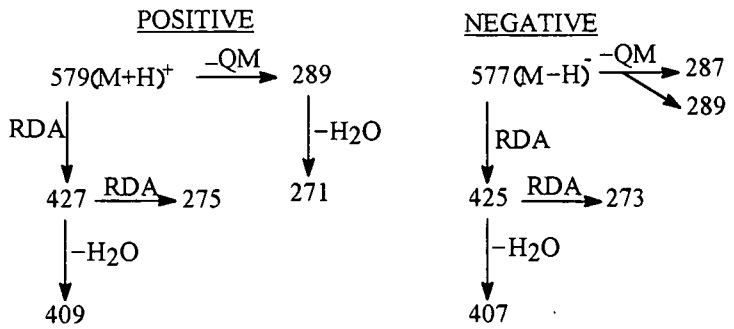
The sequence of the monomeric units was indicated by the ions produced on fragmentation. These ions resulted from the cleavage of the interflavanoid bond *via* a quinone methide mechanism in addition to ions that resulted from one or two stages of RDA fission[77,78].

In a typical analysis Karchesy and co-workers[77] proposed positive and negative ion fragmentation pathways for the dimer **146** as shown in **Scheme 4.7**.

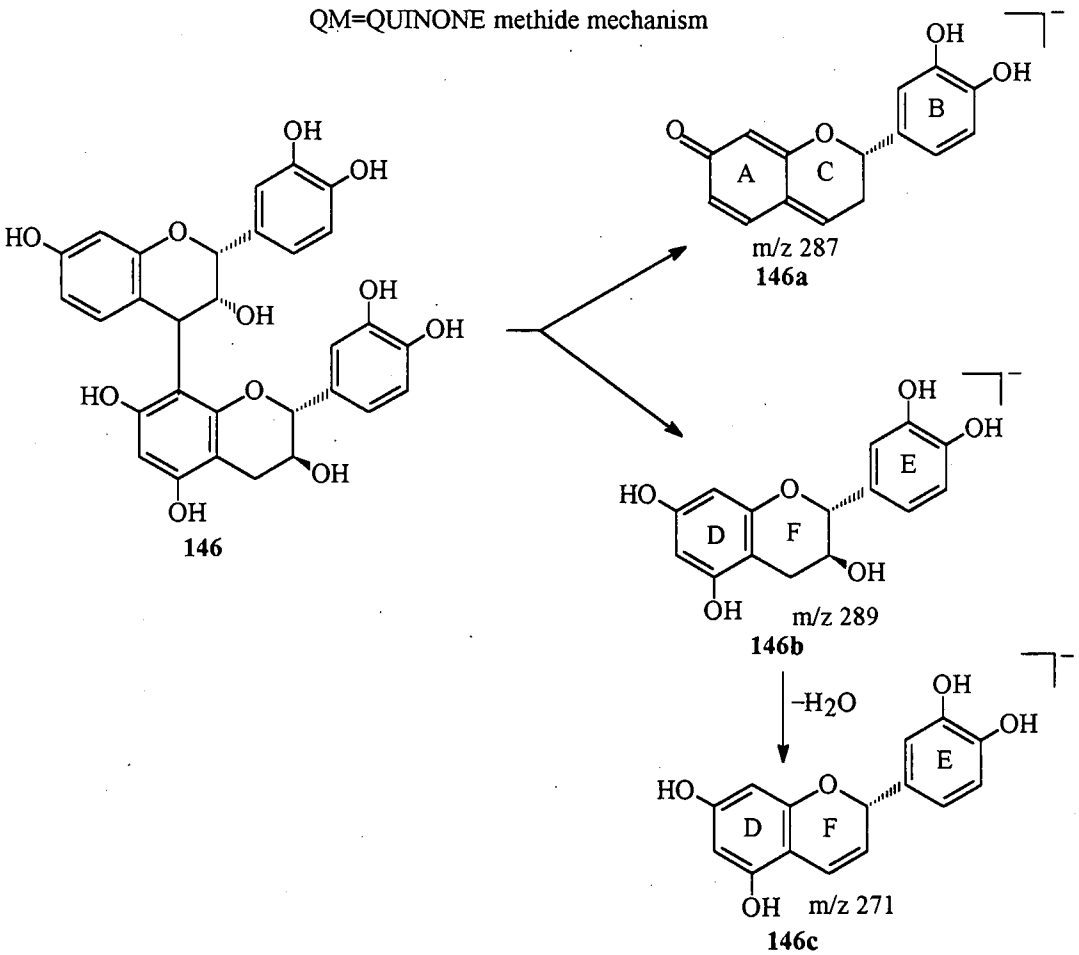


146 $(M+H)^+$ 579

SCHEME 4.7



QM=QUINONE methide mechanism



CHAPTER 5

5.1 INTRODUCTION

Cassia petersiana, commonly known as the Monkey pod has long and slender pods which are often slightly constricted between the seeds, is a shrub, or small to medium sized tree (12m) occurring most frequently along rivers and streams in riverine fringe thicket. The bark is brown, rough and fissured[79].

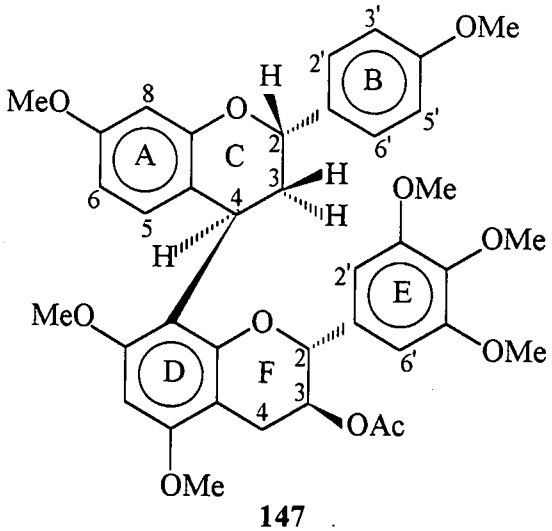
Various parts of the tree are used in African medicine as purgative and to treat fevers, gonorrhoea and skin infections. The root bark is given in soup or milk to a dog injured by the horns of an antelope, or to make a lazy hunting dog lean and hungry and so more eager for the chase[79].

The tree has been classified as:
Family: Caesalpinideae
Genus: *Cassia*
Species: *petersiana*
The present investigation of the acetone extract of the bark of *Cassia petersiana* revealed the known flavan-3-ols, (+)-catechin, (-)-epicatechin, (+)-gallocatechin and epigallocatechin. They were identified by comparison of the ^{1}H NMR and CD data of the permethylaryl ether acetate derivatives with those of authentic samples. The flavan-3-ols were accompanied by a variety of dimeric compounds of which the procassinidins **147**, **148** and **149** as well as the probutinidins (see Chapter 6) will be discussed.

5.2 PROCASSINIDINS

5.2.1 Cassiaflavan-(4β→8)-gallocatechin

The procassinidin derivative **147** was obtained after methylation and subsequent acetylation of the fraction C_3 from the acetone extract of *Cassia petersiana* as discussed in Chapter 8.



147

Due to rotational isomerism the optimum conditions with regard to solvent and temperature had to be found to obtain a workable spectrum to distinguish between the rotamers. The signals in the 300MHz ^1H NMR spectrum (C_6D_6,343K) of the heptamethyl ether acetate derivative **147** (table 1, plate 1) exhibited an ABX-, AA'BB'-, AA'- coupled systems, and one singlet in the aromatic region. The AB_2X- and ABMX- spin systems in the 2-6ppm region define the heterocyclic protons of the C-and F-rings[63,64].

The 2D COSY experiment showed connectivity of the 2-H(C)[δ5.67] with 3-H_{ax}(C)[δ2.80] and 3-H_{eq}(C)[δ2.55] resulting in a doublet of doublets and the 4-H(C)[δ5.01] also showing couplings to the same 3-H_{ax}(C)[δ2.80] and 3-H_{eq}(C)[δ2.55]. Two doublet of doublets resulted when each of the 3-H(C) protons coupled with each other and in turn showed coupling with both 2-H(C) and 4-H(C) respectively. These systems are typical of a flavan top unit bonded at C-4[63,64].

NOE experiments showed a lack of couplings between 2-H(C) and 4-H(C) suggesting 2,4-*trans* relative configuration of the C-ring. Associations between 2-H(C)[δ5.67] and 2',6'-H(B)[δ7.41] and 5-H(A)[δ7.02] with 4-H(C)[δ5.01] were confirmed by NOESY experiments. This data defined the ABC top unit.

Table 1 ^{1}H NMR (300MHz) data of compounds **147**,**148** and **149**

*Signals of the minor rotamer.

Ring	H	**147**(C_6D_6,343K)	**148**(C_6D_6,343K)	**149**(C_6D_6,313K)
A	5	7.02(d,8.5)	7.04(d,8.5)	7.20,7.19*(d,8.5)
	6	6.56(dd,8.5,2.5)	6.55(dd,8.5,2.5)	6.66,6.73*(dd,8.5,2.5)
	8	6.63(d,2.5)	6.63(d,2.5)	6.77,6.91*(d,2.5)
B	2'	7.41(d,9.0)	7.45(d,9.0)	7.27,7.46*(d,9.0)
	3'	6.85(d,9.0)	6.85(d,9.0)	6.80,6.93*(d,9.0)
	5'	6.85(d,9.0)	6.85(d,9.0)	6.80,6.93*(d,9.0)
	6'	7.41(d,9.0)	7.45(d,9.0)	7.27,7.46*(d,9.0)
C	2	5.67(dd,8.0,3.5)	5.78(dd,6.5,3.5)	5.26,5.16*(dd,12.0,2.5)
	3	2.80(ddd,13.5,6.5,3.5)	2.94(ddd,13.5,7.5,6.0)	3.32,2.13*(m)
	3	2.55(ddd,13.5,7.0,6.5)	2.56(ddd,13.5,7.5,6.0)	2.29,2.11*(m)
	4	5.01(t,6.0)	5.06(t,6.5)	5.51,5.43*(dd,11.0,5.5)
D	6	6.12(s)	6.12(s)	6.19,6.05*(s)
E	2'	6.67(s)	7.45(s)	6.46,6.90*(s)
	6'	6.67(s)	7.45(s)	6.46,6.90*(s)
F	2	4.72(d,7.5)	4.60(br.s)	4.65,5.02*(br.s)
	3	5.85(m)	5.59(m)	5.51,5.78*(m)
	4	3.40(dd,17.0,6.0)	3.06(dd,18.0,5.0)	3.18,3.06*(18.0,4.5)
	4	2.99(dd,17.0,7.5)	3.33(dd,18.0,2.5)	3.45,3.31*(m)
	OMe	3.45,3.46,3.52,3.53(×s), 3.62(×2),3.91	3.43,3.47,3.49,3.53, 3.93(6×s),3.68(×2)	3.36,3.43*,3.48,3.49*,3.50 3.55,3.62*(2×s),3.62 3.70(2×s),3.92,3.96*
	OAc	1.64(s)	1.64(s)	-

A COSY experiment showed coupling of 2-H(F)[δ4.72] and 3-H(F)[δ5.85] resulting in a doublet. The 3-H(F) proton also showed a multiplet-coupling with 2-H(F), 4-H_{ax}(F)[δ3.40] and 4-H_{eq}(F)[δ2.99]. Two doublet of doublets resulted when each of the 4-H(F) protons coupled with 3-H(F). Long distance association between 2-H(F) and 2',6'-H(E)[δ7.41] was confirmed by NOE experiment.

Both the A- and B-ring substitution patterns of the top-unit were defined by an ABX and AA'BB' patterns respectively (δ7.02, d, J=8.5Hz, 5-H; δ6.56, dd, J=2.5 and 8.5Hz, 6-H; δ6.63, d, J=2.5Hz, 8-H; δ7.41, 2prd, J=9.0Hz, 2',6'-H; δ6.85, 2prd, J=9.0Hz, 3',5'-H). The 7-OMe substitution of the A-ring was confirmed by a strong NOE association with 8-H(A).

The bottom-unit aromatic substitution was defined by the appearance of an AA' pattern (δ6.67, 2prs, 2',6'-H(E)) assigned to the E-ring protons. The appearance of a one-proton singlet (δ6.12) in the aromatic region showed a strong NOE association with both 5- and 7-OMe of the D-ring and was identified as 6-H(D)[59]. This information now implied that the bottom unit was coupled at C-8 and confirmed the $C_4 \rightarrow C_8$[59] coupling and the dimeric structure of compound **147**. This was supported by FAB-MS (m/z 686.2725) which confirmed a molecular formula of $C_{39}H_{42}O_{11}$ for **147**.

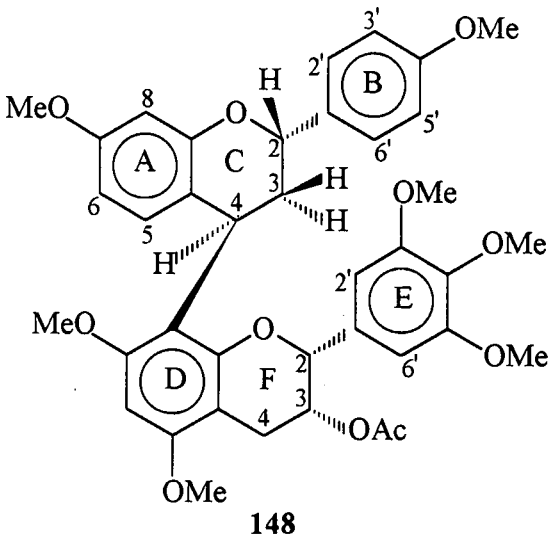
The abscence of NOE association between 2-H(F) and 3-H(F) together with a $^3J_{2,3(F)}$-value of 7.5Hz suggested 2,3-*trans* relative stereochemistry[82] of the F-ring. This information in conjunction with the D- and E-ring substitution pattern was reminiscent of the coupling pattern [2-H(d), 3-H(m)] of the monomeric gallocatechin **168**.

The CD spectrum of the dimer **147** showed a high amplitude positive Cotton effect $[\theta]_{238.9}$+14440 which confirmed the interflavanyl bond[80,81] as 4β and consequently the absolute configuration of the top unit to be 2R,4S.

The absolute stereochemistry of the bottom unit and hence of the dimer **147** was established by synthesis.

A mixture of flavan-4-ol diastereomers **162** as an electrophile was coupled with penta-*O*-methylgallocatechin **168** (**Scheme:**not included but the same as **5.1**) of known absolute configuration (2R,3S) (nucleophile) using titanium tetrachloride in dichloromethane as Lewis acid[84] afforded a mixture (difficult to separate) of dimeric compounds wich after separation yielded a dimer identical to the natural product **147** by comparison of ^{1}H NMR and CD data.

5.2.2 Cassiaflavan-(4β→8)-epigallocatechin



148

The procassinidin derivative **148** was obtained after methylation and subsequent acetylation of the fraction C_3 from the acetone extract of *Cassia petersiana* as discussed in Chapter 8.

The optimum conditions for a workable 300MHz ^{1}H NMR spectrum (C_6D_6,343K) of the heptamethyl ether acetate derivative **148** (table 1, plate 2) exhibited an ABX-, AA'BB'-, AA'-coupled systems, and one singlet in the aromatic region. The AB_2X- and ABMX-spin systems in the 2-6ppm region define the heterocyclic protons of the C- and F-rings[63,64].

The 2D COSY experiment showed couplings of the 2-H(C)[δ4.60] with 3-H_{ax}(C)[δ2.94] and 3-H_{eq}(C)[δ2.56] resulting in a doublet of doublets and the 4-H(C)[δ5.06] also showing couplings to the same 3-H_{ax}(C)[δ2.94] and 3-H_{eq}(C)[δ2.56]. Two doublet of doublets resulted when each of the 3-H(C) protons coupled with each other and in turn showed coupling with both 2-H(C) and 4-H(C) respectively. These systems are typical of a flavan top unit bonded at C-4[63,64].

A COSY experiment showed $^4J_{HH}$ long range coupling between 2-H(C)[δ4.60] and 2',6'-H(B)[δ7.45] and a benzylic coupling between 5-H(A)[δ7.04] and 4-H(C)[δ5.06]. This data defined the ABC top unit. NOE experiment showed a lack of coupling between 2-H(C) and 4-(H) suggesting 2,4-*trans* relative configuration of the C-ring. Associations between 2-H(C) and 2',6'-H(B) and 5-H(A) with 4-H(C) were confirmed by NOESY experiments.

A COSY experiment showed coupling of 2-H(F)[δ4.60] and 3-H(F)[δ5.59] as a result of which 2-H(F) appeared as a broad singlet. The 3-H(F) proton also showed a multiplet-coupling with 2-H(F), 4-H_{ax}(F)[δ3.06] and 4-H_{eq}(F)[δ3.33]. Two doublet of doublets resulted when each of the 4-H(F) protons showed coupling with 3-H(F). Long distance association between 2-H(F) and 2',6'-H(E)[δ7.45] was confirmed by NOE experiment.

Both the A- and B-ring substitution patterns of the top-unit were defined by an ABX and AA'BB' patterns respectively (δ7.04, d, J=8.5Hz, 5-H; δ6.55, dd, J=2.5 and 8.5Hz, 6-H; δ6.63, d, J=2.5Hz, 8-H; δ7.45, 2prd, J=9.0Hz, 2',6'-H; δ6.85, 2prd, J=9.0Hz, 3',5'-H). The 7-OMe substitution of the A-ring was confirmed by a strong NOE association with 8-H(A).

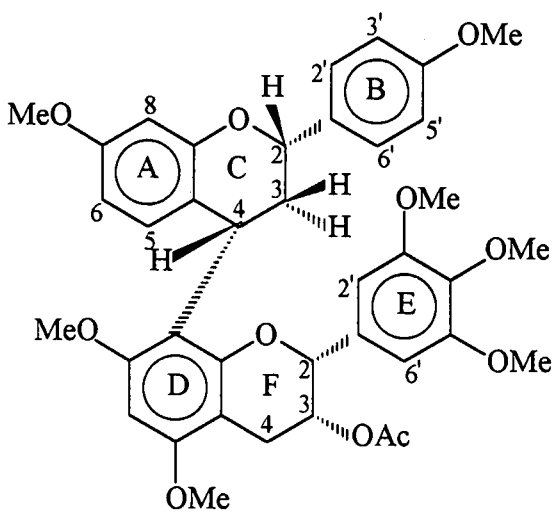
The bottom-unit aromatic substitution was defined by the appearance of an AA' pattern (δ7.45, 2prs, 2',6'-H(E)) assigned to the E-ring protons. The appearance of a one-proton singlet (δ6.12) in the aromatic region showed a strong NOE association with both 5- and 7-OMe of the D-ring and was identified as 6-H(D)[59].

This information now implied that the bottom unit was coupled at C-8 and confirmed the $C_4 \rightarrow C_8$[59] coupling and the dimeric structure of compound **148**. This was supported by FAB-MS (m/z 686.2724) which confirmed a molecular formula of $C_{39}H_{42}O_{11}$ for **148**.

The prominent association between 2-H(F) and 3-H(F) together with $^3J_{2,3(F)}$-value of 1.0Hz suggested 2,3-*cis* relative stereochemistry[82] of the F-ring. This data together with substitution pattern of the D- and E-rings was reminiscent of the coupling pattern [2-H(d), 3-H(m)] of the monomeric epigallocatechin **163**.

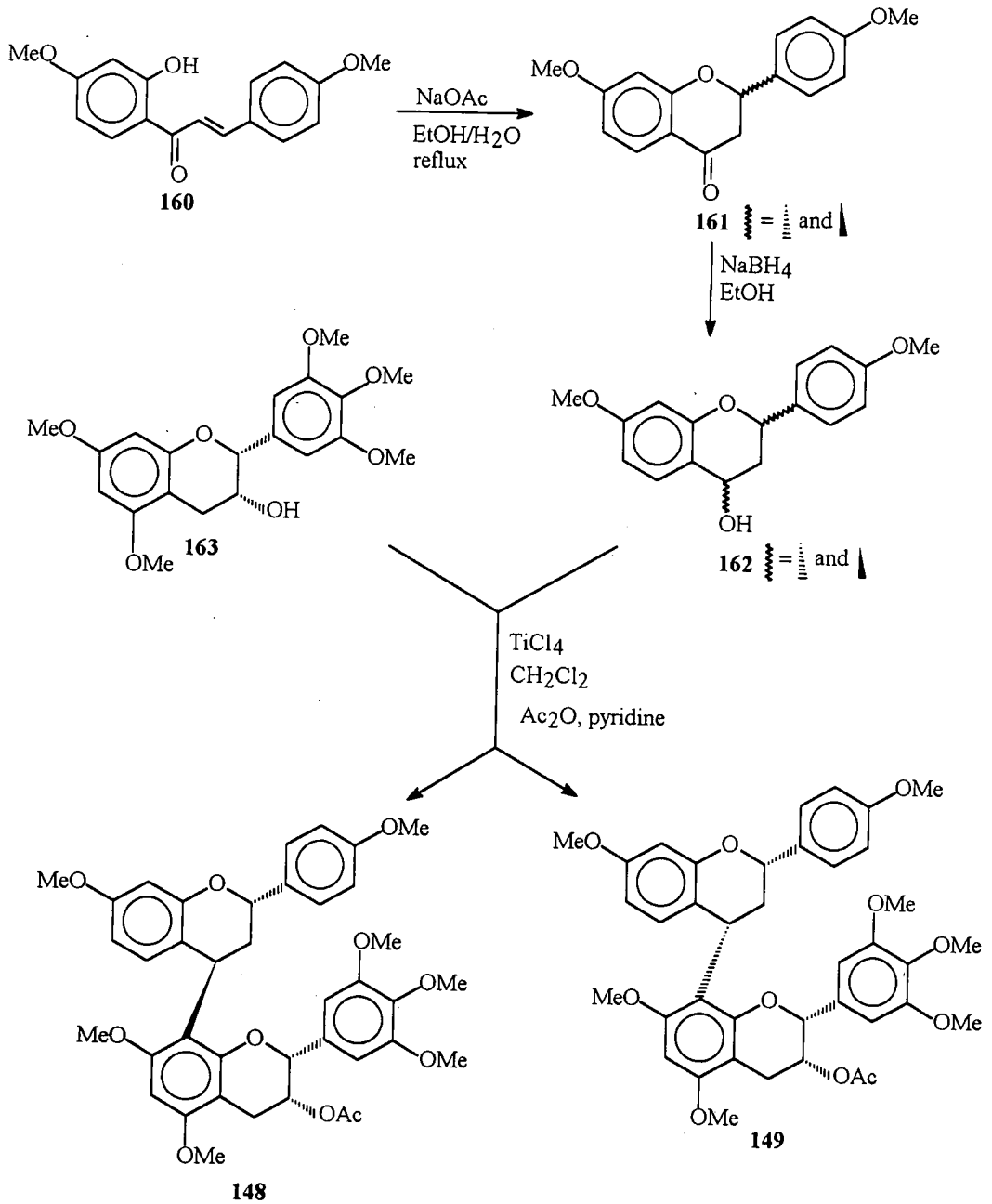
The CD spectrum of the dimer **148** showed a high amplitude positive Cotton effect $[\theta]_{248.1}$+12180 which confirmed the interflavanyl bond[80,81] to be 4β and consequently the absolute configuration of the top unit to be 2R,4S. The absolute stereochemistry of the bottom unit and hence of the dimer **148** was established by synthesis. A mixture of diastereomeric pairs of flavan-4-ol **162** as an electrophile was coupled with penta-*O*-methylepigallocatechin **163** (**Scheme 5.1**) of known configuration (2R,3R) (nucleophile) using titanium tetrachloride in dichloromethane as Lewis acid[84] afforded a mixture of dimeric compounds which after separation yielded the two dimers identical to the natural products **148** and **149** by comparison of their ^{1}H NMR and CD data.

5.2.3 Cassiaflavan-(4α→8)-epigallocatechin



149

SCHEME 5.1


MeO
OH
OMe
O
160
NaOAc
EtOH/H2O
reflux
MeO
OMe
O
161 = and
NaBH4
EtOH
MeO
OMe
OH
162 = and
MeO
OMe
OMe
OMe
OH
OMe
163
TiCl4
CH2Cl2
Ac2O, pyridine
MeO
OMe
OMe
OMe
OAc
OMe
148
MeO
OMe
OMe
OMe
OAc
OMe
149

The procassinidin derivative **149** was obtained after methylation and subsequent acetylation of the fraction C_4 from the acetone extract of *Cassia petersiana* as discussed in Chapter 8.

The signals in the 300MHz ^1H NMR optimised spectrum (C_6D_6, 313K) of the heptamethyl ether acetate derivative **149** (table 1, plate 3) exhibited an ABX-, AA'BB'-, AA'-coupled systems, and one singlet in the aromatic region. The AB_2X- and ABMX-spin systems in the 2-6ppm region define the heterocyclic protons of the C- and F-rings[63,64]. The two rotamers were well resolved in a ratio of 2:1*.

The 2D COSY experiment showed connectivities of the 2-H(C)[δ5.26, 5.16*] with 3-H_{ax}(C)[δ3.32, 3.13*] and 3-H_{eq}(C)[δ2.29, 2.11*] resulting in a doublet of doublets and the 4-H(C)[δ5.51, 5.43*] also showing couplings to the same 3-H_{ax}(C)[δ3.32, 3.13*] and 3-H_{eq}(C)[δ2.29, 2.11*]. Two multiplets resulted when each of the 3-H(C) protons coupled with each other and in turn showed coupling with both 2-H(C) and 4-H(C) respectively. These systems are reminiscent of a flavan top unit bonded at C-4[63,64].

The COSY experiment showed $^4J_{HH}$ long range coupling between 2-H(C)[δ5.26, 5.16*] and 2',6'-H(B)[δ7.27, 7.46*] and strong benzylic coupling between 5-H(A)[δ7.20, 7.19*] and 4-H(C)[δ5.51,5.43*]. NOE experiment showed coupling of 2-H(C) with 4-H(C) suggesting 2,4-*cis* relative configuration of the C-ring. Associations between 2-HC)[δ5.26, 5.16*] and 2',6'-H(B)[δ7.27, 7.46*] and 5-H(A)[δ7.20, 7.19*] with 4-H(C)[δ5.51, 5.43*] were confirmed by NOESY experiment. This data defined the ABC top unit.

A COSY experiment showed coupling of 2-H(F)[δ4.65, 5.02*] and *3*-H(F)[δ5.51, 5.78*] resulting in a broad singlet. The 3-H(F) proton also showed a multiplet-coupling with 2-H(F), 4-H(F)[δ3.18, 3.06*] and 4-H(F)[δ3.45, 3.31*] protons. A doublet of doublets and a multiplet resulted when each 4-H(F) proton coupled with 3-H(F). Long distance coupling between 2-H(F) and 2',6'-H(E)[δ6.46, 6.90*] was confirmed by NOE experiment.

The A- and B-ring substitution patterns of the top-unit were defined by an ABX and AA'BB' patterns respectively (δ7.20, 7.19*, d, J=8.5Hz, 5-H; δ6.66, 6.73*, dd, J=2.5 and 8.5Hz, 6-H; δ6.77, 6.91*, d, J=2.5Hz, 8-H; δ7.27, 7.46*, 2prd, J=9.0Hz, 2',6'-H; δ6.80, 6.93*, 2prd, J=9.0Hz, 3',5'-H). The 7-OMe substitution of the A-ring was confirmed by a strong NOE association with 8-H(A).

The bottom-unit aromatic substitution was defined by the appearance of an AA' pattern (δ6.46 6.60*, 2prs, 2',6'-H(E)) assigned to the E-ring protons. The appearance of a one-proton singlet (δ6.19, 6.05*) in the aromatic region showed a strong NOE association with both 5- and 7-OMe of the D-ring and was identified as 6-H(D)[59]. This information now implied that the bottom unit was coupled at C-8 and confirmed the $C_4 \rightarrow C_8$[59] coupling and the dimeric structure of compound **149**. This was supported by FAB-MS (m/z 686.2725) which confirmed a molecular formula of $C_{39}H_{42}O_{11}$ for **149**.

NOE association between 2-H(F) and 3-H(F) together with $^3J_{2,3(F)}$-value of 0.0Hz suggested 2,3-*cis* relative stereochemistry[82] of the F-ring. This information in conjunction with the D- and E-ring substitution pattern was reminiscent of the coupling pattern [2-H(br.s), 3-H(m)] of the monomeric epigallocatechin **163**.

The CD spectrum of the dimer **149** showed a high amplitude negative Cotton effect $[\theta]_{243.7}$-11860 which confirmed the interflavanyl bond[80,81] to be 4α and consequently the absolute configuration of the top unit to be 2R,4R. The absolute stereochemistry of the bottom unit and hence of the dimer **149** was established by synthesis as discussed for compound **148** as shown in **Scheme 5.1**.

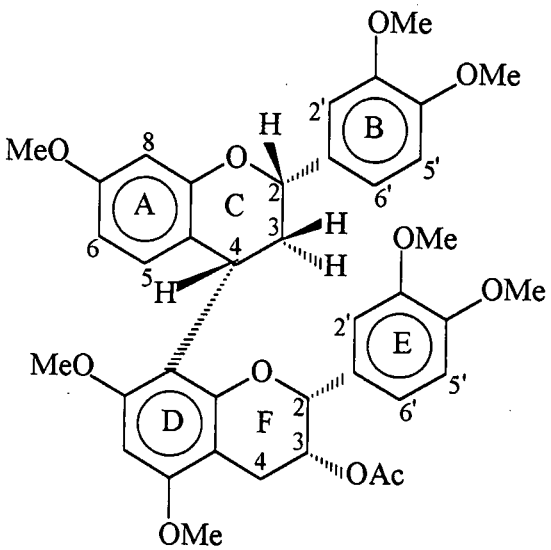
CHAPTER 6

6.1 INTRODUCTION

The probutinidins **150**, **151**, **152** and **153** were also found co-occurring with the flavan-3-ols as mentioned in section 5.1.

6.2 PROBUTINIDINS

6.2.1 Butiniflavan-(4α→8)-epicatechin



150

The probutinidin derivative **150** was obtained after methylation and subsequent acetylation of the fraction C_4 from the acetone extract of *Cassia petersiana* as discussed in Chapter 8.

The signals in the 300MHz ^1H NMR spectrum ($CDCl_3$, 293K) of the heptamethyl ether acetate derivative **150** (table 2, plate 4) exhibited three ABX- and one singlet spin systems in the aromatic region. The AB_2X- and ABMX-spin systems in the 2-6ppm region define the heterocyclic protons of the C- and F-rings [63,64].

The 2D COSY experiment showed couplings of the 2-H(C)[δ5.17] with 3-H_{ax}(C)[δ1.98] and 3-H_{eq}(C)[δ2.79] resulting in a doublet of doublets and the 4-

Table 2 ^{1}H NMR (300MHz) data of compounds **150**,**151** and **152**

Ring	H	**150**($CDCl_3$,293K)	**151**($C_6D_6$343K)	**152**($C_6D_6$343K)
A	5	6.75(d,8.5)	7.07 (d,8.5)	7.05 (d,8.5)
	6	6.41(dd,8.5,2.5)	6.57(dd,8.5,2.5)	6.55(dd,8.5,2.5)
	8	6.51(d,2.5)	6.75(d,2.5)	6.67(d,2.5)
B	2'	6.70(d,2.5)	7.17(d,2.5)	7.17(d,2.5)
	5'	6.77(d,8.5)	6.73(d,8.5)	6.75(d,8.5)
	6'	6.84(dd,8.5,2.5)	7.13(dd,8.5,2.5)	7.13(dd,8.5,2.5)
C	2	5.17(dd,12.0,2.0)	5.81(dd,6.5,3.0)	5.81(dd,6.5,3.0)
	3	1.98(ddd,13.0,5.5,2.0)	2.59(ddd,13.0,7.0,6.0)	2.61(ddd,13.0,7.0,6.0)
	3	2.97(m)	2.97(ddd,13.0,7.0,3.5)	2.97(ddd,13.0,7.0,3.5)
	4	4.94(dd,12.0,5.5)	4.94(t,6.0,)	5.10(t,6.0,)
D	6	6.25(s)	6.12(s)	6.12(s)
E	2'	6.54(d,2.5)	7.06(d,2.5)	7.69(s)
	5'	6.67(d,8.5)	6.77(d,8.5)	-
	6'	6.32(dd,8.5,2.5)	6.84(dd,8.5,2.5)	6.69(s)
F	2	4.89(br.s)	4.68(br.s)	4.60(br.s)
	3	5.30(m)	5.62(m)	5.39(m)
	4	}4.94(m)	3.32(dd,17.0,2.5)	3.33(dd,18.0,2.5)
	4		3.05(dd,17.0,4.5)	3.05(dd,18.0,5.0)
	OMe	3.55,3.75,3.79,3.85,3.86	3.42,3.49,3.53,3.55,3.57	3.44,3.49,3.53,3.56,3.58
		3.89,3.90(7×s)	3.60,3.71(7×s)	3.93(6×s),3.69(2×s)
	OAc	1.77(s)	1.64(s)	1.65(s)

H(C)[δ4.94] also showing coupling to the same 3-H_{eq}(C)[δ2.79]. A doublet of doublets and a multiplet resulted when 3-H_{ax}(C)[1.98] and 3-H_{eq}(C)[δ2.79] coupled with each other and in turn showed coupling with both 2-H(C) and 4-H(C) respectively. These systems are typical of flavan top unit with an interflavanyl linkage bonded at C-4[63,64].

A COSY experiment showed $^4J_{HH}$ long range coupling between 2-H(C)[δ5.17] and 2'-, 6'-H(B)[δ6.70, δ6.84 respectively] and a strong benzylic coupling between 5-H(A)[δ6.75] and 4-H(C)[δ4.94]. This data defined the ABC top unit. NOE experiment showed coupling of 2-H(C) with 4-H(C) suggesting 2,4-*cis* relative configuration of the C-ring. Associations between 2-H(C) and 2'-, 6'-H(B) and 5-H(A) with 4-H(C) by NOESY experiments confirmed the ABC-moiety.

Both the A- and B-ring substitution patterns of the top-unit were defined by an ABX pattern (δ6.75, d, J=8.5Hz, 5-H; δ6.41, dd, J=2.5 and 8.5Hz, 6-H; δ6.51, d, J=2.5Hz, 8-H; δ6.70, d, J=2.5Hz, 2'-H; δ6.77, d, J=8.5Hz, 5'-H; δ6.84, dd, J=2.5 and 8.5Hz, 6'-H) respectively. The 7-OMe position on the A-ring was confirmed by a strong NOE association with 8-H(A).

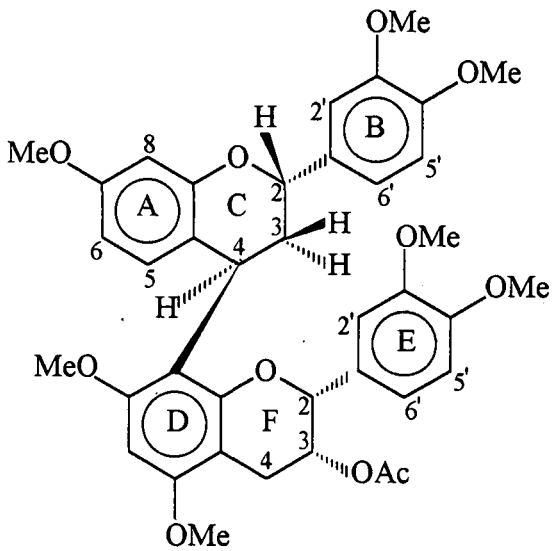
The bottom-unit aromatic substitution was defined by the appearance of an ABX pattern (δ6.54, d, J=2.5Hz, 2'-H; δ6.67, d, J=8.5Hz, 5'-H; δ6.32, dd, J=2.5 and 8.5Hz, 6'-H) assigned to the E-ring protons. The appearance of a one-proton singlet (δ6.25) in the aromatic region showed a strong NOE assocation with both 5- and 7-OMe of the D-ring and was identified as 6-H(D)[59]. This information now implied that the bottom unit was coupled at C-8 and confirmed the $C_4 \rightarrow C_8$[59] coupling and the dimeric structure of compound **150**. This was supported by FAB-MS (m/z 686.2724) which confirmed a molecular formula of $C_{39}H_{42}O_{11}$ for **150**.

The prominent association between 2-H(F) and 3-H(F) together with $^3J_{2,3}$-values *ca* 1.5Hz suggested 2,3-*cis* relative stereochemistry[82] of the F-ring.

This data together with substitution pattern of the D- and E-rings was reminiscent of the coupling pattern [2-H(d), 3-H(m)] of the monomeric epicatechin **167**.

The CD spectrum of the dimer **150** showed a high amplitude negative Cotton effect $[\theta]_{244.7}$-16640 which confirmed the interflavanyl bond[80,81] to be 4α and consequently the absolute configuration of the top unit to be 2R,4R. The absolute stereochemistry of the bottom unit and hence of the dimer **150** was established by synthesis. A mixture of diastereomeric pairs of flavan-4-ol **166** (**Scheme 6.1**) as an electrophile was coupled with tetra-*O*-methylepicatechin **167** of known absolute configuration (2R,3R) (nucleophile) using titanium tetrachloride in dichloromethane as Lewis acid[84] afforded a mixture of dimeric compounds which after separation and purification afforded the three dimers identical to the natural products **150**, **151** and **153** according to the similarity of their ^{1}H NMR and CD data.

6.2.2 **Butiniflavan-(4β→8)-epicatechin**

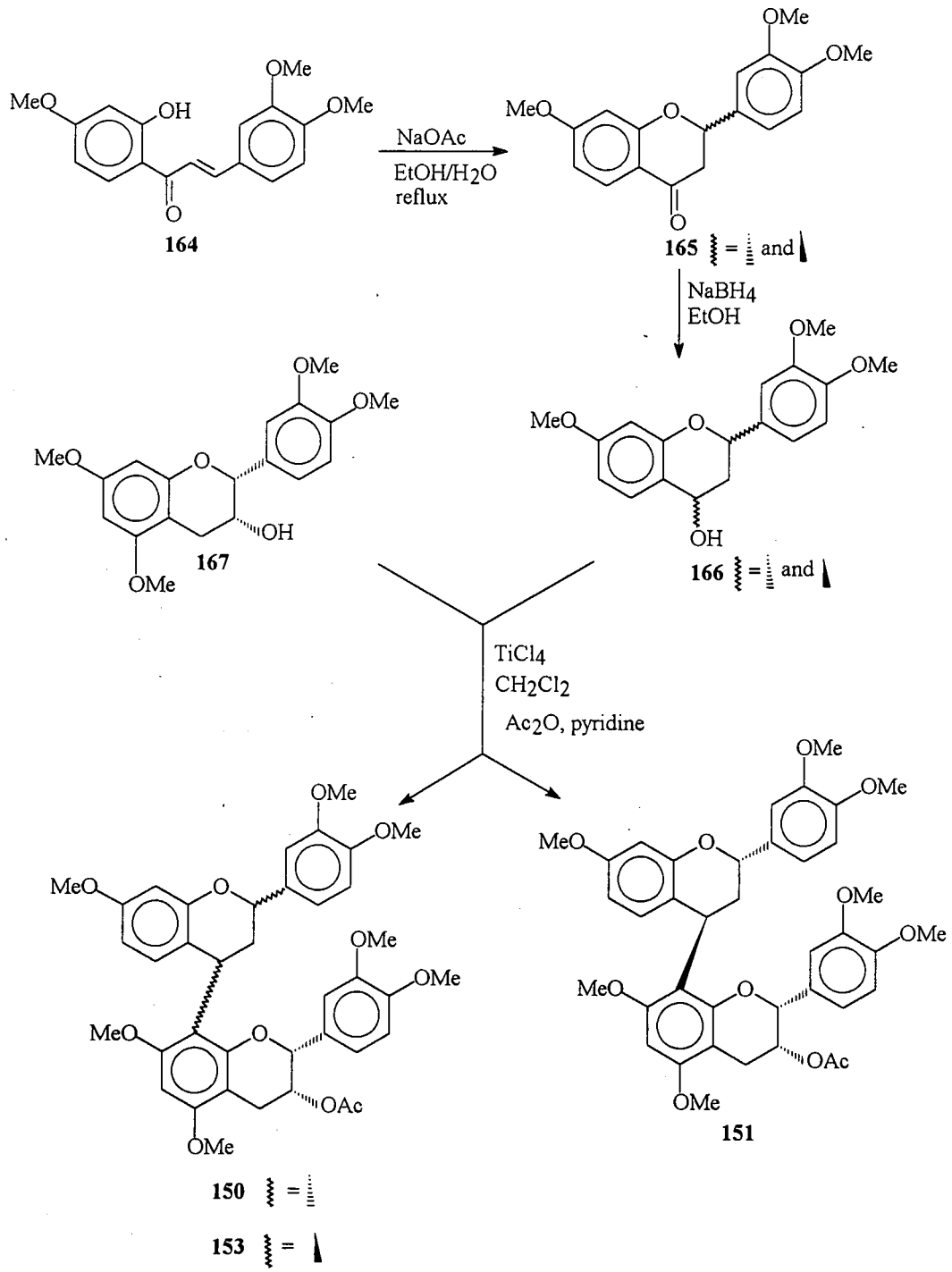


151

The probutinidin derivative **151** was obtained after methylation and subsequent acetylation of the fraction C_3 from the acetone extract of *Cassia petersiana* as discussed in Chapter 8.

Optimisation of the solvent-temperature combination to give the best spectrum of the main rotamer in the 300MHz ^{1}H NMR spectrum ($CDCl_3$, 343K) of the heptamethyl

SCHEME 6.1


MeO
OH
OMe
OMe
O
164
NaOAc
EtOH/H2O
reflux
MeO
OMe
OMe
O
165 = and
NaBH4
EtOH
MeO
OMe
OMe
OH
166 = and
MeO
OMe
OMe
OH
OMe
167
TiCl4
CH2Cl2
Ac2O, pyridine
MeO
OMe
OMe
OMe
OMe
OAc
OMe
150 =
153 =
MeO
OMe
OMe
OMe
OMe
OAc
OMe
151

ether acetate derivative **151** (table 2, plate 5) exhibited three ABX- and one singlet spin systems in the aromatic region. The AB_2X- and ABMX-spin systems in the 2-6ppm region define the heterocyclic protons of the C- and F-rings[63,64].

The 2D COSY experiment showed connectivities of the 2-H(C)[δ5.81] with 3-H_{ax}(C)[δ2.59] and 3-H_{eq}(C)[δ3.00] resulting in a doublet of doublets and the 4-H(C)[δ5.11] also showing coupling to the same 3-H_{ax}(C)[δ2.59] and 3-H_{eq}(C)[δ3.00]. A doublet of doublets and another doublet of doublets resulted when 3-H_{ax}(C)[2.59] and 3-H_{eq}(C)[δ3.00] coupled with each other and in turn showed coupling with both 2-H(C) and 4-H(C) respectively. These systems are typical of flavan top unit with an interflavanyl linkage bonded at C-4[63,64].

A COSY experiment showed $^4J_{HH}$ long range coupling between 2-H(C)[δ5.81] and 2'-, 6'-H(B)[δ7.17, δ7.13 respectively] and a benzylic coupling between 5-H(A)[δ7.07] and 4-H(C)[δ5.11]. This data defined the ABC top unit. NOE experiment showed a lack of coupling between 2-H(C) and 4-H(C) suggesting 2,4-*trans* relative configuration of the C-ring. Associations between 2-H(C) and 2'-, 6'-H(B) and 5-H(A) with 4-H(C) were confirmed by NOESY experiments.

Both the A- and B-ring substitution patterns of the top-unit were defined by an ABX pattern (δ7.07, d, J=8.5Hz, 5-H; δ6.57, dd, J=2.5 and 8.5Hz, 6-H; δ6.75, d, J=2.5Hz, 8-H; δ7.17, d, J=2.5Hz, 2'-H; δ6.73, d, J=8.5Hz, 5'-H; δ7.13, dd, J=2.5 and 8.5Hz, 6'-H) respectively. The 7-OMe position on the A-ring was confirmed by a strong NOE association with 8-H(A).

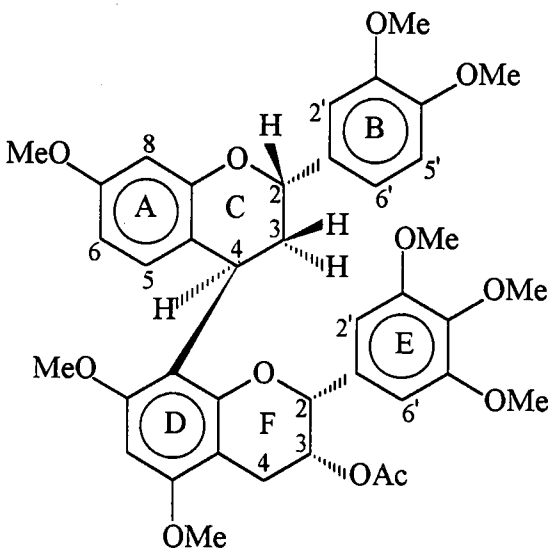
The bottom-unit aromatic substitution was defined by the appearance of an ABX pattern (δ7.06, d, J=2.5Hz, 2'-H; δ6.677, d, J=8.5Hz, 5'-H; δ6.84, dd, J=2.5 and 8.5Hz, 6'-H) assigned to the E-ring protons. The appearance of a one-proton singlet (δ6.12) in the aromatic region showed a strong NOE assocation with both 5- and 7-OMe of the D-ring and was identified as 6-H(D)[59].

This information now implied that the bottom unit was coupled at C-8 and confirmed the $C_4 \rightarrow C_8$[59] coupling and the dimeric structure of compound **151**. This was supported by FAB-MS (m/z 686.2725) which confirmed a molecular formula of $C_{39}H_{42}O_{11}$ for **151**.

The prominent association between 2-H(F) and 3-H(F) together with $^3J_{2,3}$-values *ca* 1.5Hz suggested 2,3-*cis* relative stereochemistry[82] of the F-ring. This data together with substitution pattern of the D- and E-rings was reminiscent of the coupling pattern [2-H(d), 3-H(m)] of the monomeric epicatechin **167**.

The CD spectrum of the dimer **151** showed a high amplitude positive Cotton effect $[\theta]_{244.6}$+14050 which confirmed the interflavanyl bond[80,81] to be 4β and consequently the absolute configuration of the top unit to be 2R,4S. The absolute stereochemistry of the bottom unit and hence of the dimer **151** was established by synthesis as discussed for compound **150** (**Scheme 6.1**).

6.2.3 **Butiniflavan-(4β→8)-epigallocatechin**



152

The probutinidin derivative **152** was obtained after methylation and subsequent acetylation of the fraction C_5 from the acetone extract of *Cassia petersiana* as discussed in Chapter 8.

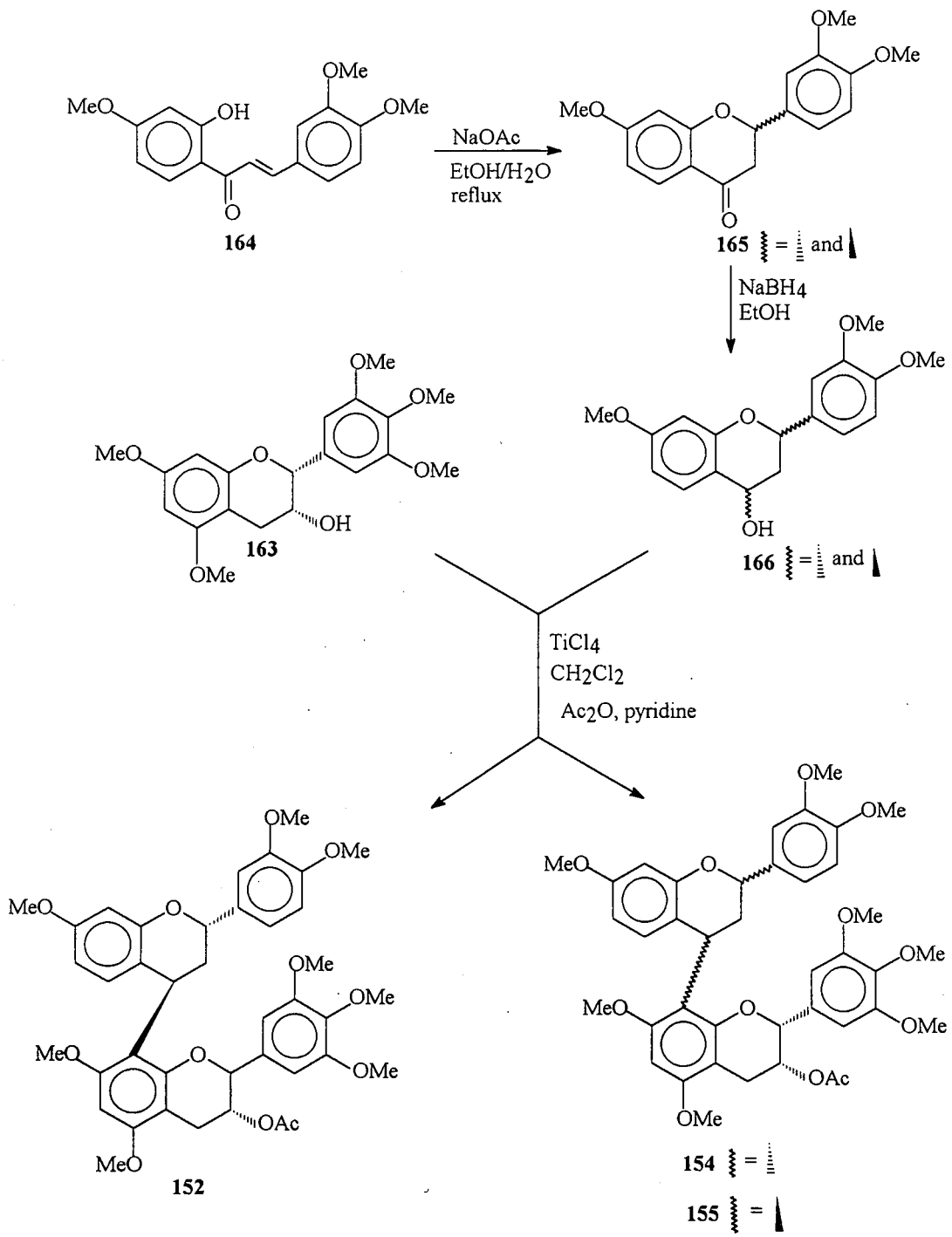
The signals in the 300MHz ^1H NMR optimised spectrum (C_6D_6, 343K) of the main rotamer of heptamethyl ether acetate derivative **152** (table 2, plate 6) exhibited two ABX-, AA'-coupled spin systems, and one singlet spin systems in the aromatic region. The AB_2X- and ABMX-spin systems in the 2-6ppm region define the heterocyclic protons of the C- and F-rings[63,64].

The 2D COSY experiment showed couplings of the 2-H(C)[δ5.81] with 3-H_{ax}(C)[δ2.61] and 3-H_{eq}(C)[δ2.97] resulting in a doublet of doublets and the 4-H(C)[δ5.10] also showing connectivities to the same 3-H_{ax}(C)[δ2.61] and 3-H_{eq}(C)[δ2.97]. Two doublet of doublets resulted when 3-H_{ax}(C)[2.59] and 3-H_{eq}(C)[δ3.00] coupled with each other and in turn showed coupling with both 2-H(C) and 4-H(C) respectively. These systems are typical of flavan top unit with an interflavanyl linkage at C-4[63,64].

A COSY experiment showed $^4J_{HH}$ long range coupling between 2-H(C)[δ5.81] and 2'-, 6'-H(B)[δ7.17, δ7.13 respectively] and a benzylic coupling between 5-H(A)[δ67.05] and 4-H(C)[δ5.10]. This data defined the ABC top unit. NOE experiment showed a lack of coupling between 2-H(C) with 4-H(C) suggesting 2,4-*trans* relative configuration of the C-ring. Associations between 2-H(C) and 2'-, 6'-H(B) and 5-H(A) with 4-H(C) by NOESY experiments confirmed the ABC-moiety.

Both the A- and B-ring substitution patterns of the top-unit were defined by an ABX pattern (δ7.05, d, J=8.5Hz, 5-H; δ6.55, dd, J=2.5 and 8.5Hz, 6-H; δ6.67, d, J=2.5Hz, 8-H; δ7.17, d, J=2.5Hz, 2'-H; δ6.75, d, J=8.5Hz, 5'-H; δ7.13, dd, J=2.5 and 8.5Hz,6'-H) respectively.

SCHEME 6.2


MeO
OH
OMe
OMe
O
164
NaOAc
EtOH/H2O
reflux
165 and
NaBH4
EtOH
OH
166 and
163
TiCl4
CH2Cl2
Ac2O, pyridine
OAc
152
154
155

The 7-OMe position on the A-ring was confirmed by a strong NOE association with 8-H(A).

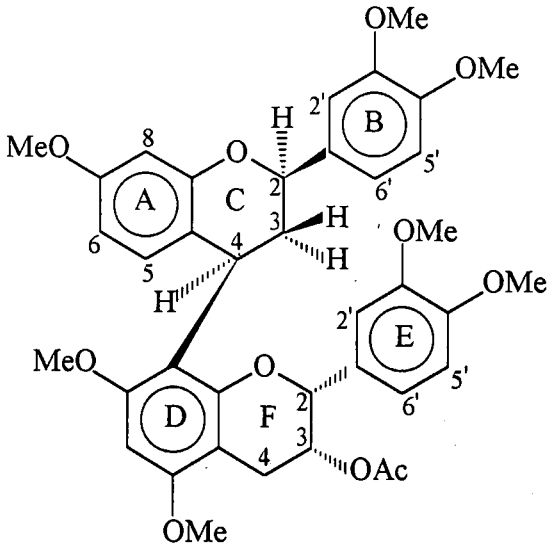
The bottom-unit aromatic substitution was defined by the appearance of an AA' pattern (δ6.69, 2prs, 2',6'-H) assigned to the E-ring protons. The appearance of a one-proton singlet (δ6.12) in the aromatic region showed a strong NOE assocation with both 5- and 7-OMe of the D-ring and was identified as 6-H(D)[59]. This information now implied that the bottom unit was coupled at C-8 and confirmed the $C_4 \rightarrow C_8$[59] coupling and the dimeric structure of compound **152**. This was supported by FAB-MS (m/z 716.2831) which confirmed a molecular formula of $C_{40}H_{44}O_{12}$ for **152**.

The prominent association between 2-H(F) and 3-H(F) together with $^3J_{2,3}$-values of *ca* 1.5Hz suggested 2,3-*cis* relative stereochemistry[82] of the F-ring. This data together with substitution pattern of the D- and E-rings was reminiscent of the coupling pattern [2-H(d), 3-H(m)] of the monomeric epigallocatechin **163**.

The CD spectrum of the dimer **152** showed a high amplitude positive Cotton effect $[\theta]_{244.6}$+47980 which confirmed the interflavanyl bond[80,81] to be 4β and consequently the absolute configuration of the top unit to be 2R,4S. The absolute stereochemistry of the bottom unit and hence of the dimer **152** was established by synthesis. A mixture of diastereomeric pairs of flavan-4-ol **166** (**Scheme 6.2**) as an elctrophile was coupled with penta-*O*-methylepigallocatechin **163** of known configuration (2R,3R) (nucleophile) using titanium tetrachloride in dichloromethane as Lewis acid[77] afforded a mixture of dimeric compounds **154**, **155** and one identical to the natural product **152** by comparison of ^{1}H NMR and CD data.

6.2.4 ***ent*-Butiniflavan-(4β→8)-epicatechin**

The probutinidin derivative **153** was obtained after methylation and subsequent acetylation of the fraction C_5 from the acetone extract of *Cassia petersiana* as discussed in Chapter 8.



153

Optimisation of the solvent-temperature conditions resulted in two rotamers in a ratio of 4:3* in the 300MHz ^{1}H NMR spectrum ($CDCl_3$, 293K) of the heptamethyl ether acetate derivatives **153** (table3, plate7) exhibited three ABX-, and one singlet spin systems in the aromatic region. The AB_2X- and ABMX-spin systems in the 2-6ppm region define the heterocyclic protons of the C- and F-rings for both rotamers[63,64].

The 2D COSY experiment showed couplings of the 2-H(C)[δ5.15, 5.08*] with 3-H_{ax}(C)[δ2.22, 2.09*] and 3-H_{eq}(C)[δ2.82, 2.84*] resulting in a doublet of doublets and the 4-H(C)[δ4.94, 5.01*] also showing associations to the same 3-H_{ax}(C)[δ2.22, 2.09*] and 3-H_{eq}(C)[δ2.82, 2.84*]. A doublet of doublets and one multiplet resulted when 3-H_{ax}(C)[δ2.22,2.09*] and3-H_{eq}(C)[2.82, 2.84*] coupled with each other and in turn showed coupling with both 2-H(C) and 4-H(C) respectively. These systems are reminiscent of a flavan top unit with an interflavanyl linkage at C-4[63,64].

The COSY experiment showed $^4J_{HH}$ long range coupling between 2-H(C)[δ5.15, 5.08*] and 2'-, 6'-H(B)[δ6.69, 6.91*, δ7.05, 6.89* respectively] and a strong benzylic coupling between 5-H(A)[δ6.64, 6.82*] and 4-H(C)[δ4.94, 5.01*]. NOE experiment showed coupling of 2-H(C) and 4-H(C) suggesting 2,4-*cis* relative configuration of the C-

Table 3 ^{1}H NMR (300MHz) data of compounds **153**,**154** and **155**

*Signals of the minor rotamer.

Ring	H	**153**($CDCl_3$,296K	**154**($CDCl_3$,293K)	**155**($CDCl_3$,293K)
A	5	6.64,6.82*(d,8.5)	6.70,6.61*(d,8.5)	6.61,6.81*(d,8.5)
	6	6.25,6.40*(dd,8.5,2.5)	6.38,6.35*(dd,8.5,2.5)	6.23,6.37*(dd,8.5,2.5)
	8	6.20,6.52*(d,8.5,2.5)	6.43,6.49*(d,2.5)	6.12,6.50*(d,2.5)
B	2'	6.69,6.91*(d,8.5)	6.73,7.06*(d,2.5)	7.02,7.03*(d,2.5)
	5'	7.01,6.90*(d,8.5)	6.77,6.91*(d,8.5)	6.89,7.94*(d,8.5)
	6'	7.05,6.89*(dd,8.5,2.5)	6.84,7.02*(dd,8.5,2.5)	7.05,6.91*(dd,8.5,2.5)
C	2	5.15,5.08*(dd,12.0, 5.0)	5.20,5.09*(dd,11.5,2.0)	5.15,5.09*(dd,12.0,1.5)
	3	2.22,2.09*(m)	1.97,2.22*(ddd,13.0,5.0,2.5)	2.31,2.10*(ddd,13.0,6.0,2.0)
	3	2.82,2.84*(m)	2.78,2.91*(m)	2.75,2.85*(m)
	4	4.94,5.01*(dd,12.0,5.0)	4.95,5.52*(dd,12.5,5.0)	4.93,5.01*(dd,12.5,6.0)
D	6	6.26,6.15*(s)	6.27,6.10*(s)	6.27,6.15*(s)
E	2'	6.74,7.02*(d,2.5)	6.26,6.69*(s)	6.36,6.69*(s)
	5'	6.77,6.87*(d,8.5)	-	-
	6'	6.70,7.02*(dd,8.5,2.5)	6.26,7.69*(s)	6.36,7.69*(s)
F	2	5.13,4.49*(br.s)	4.84,5.11*(br.s)	4.39,5.12*(br.s)
	3	5.46,5.50*(m)	5.27,5.59*(m)	5.37,5.51*(m)
	4	3.05,3.05*(m)	2.99,3.08*(dd,18.0,4.5)	3.01,3.09*(dd,18.0,2.5)
	4	2.98,2.87*(dd,18.0,5.0)	2.95,2.95*(m)	2.87,2.98*(dd,18.0,4.5)
	OMe	3.55,3.58,3.78,3.84,3.85	3.52*,3.57,3.74,3.76(×2)	3.56*,3.57*,3.75(×2)
		3.87,3.88,3.91,3.92,3.93	3.77*,3.82,3.83*,3.84*,3.85	3.76(×2),3.77*,3.84,3.85*
		3.78(×2),3.90(×2)(s)	3.87*,3.89,3.90*,3.91,3.92*	3.86,3.86,3.88*3.89*,3.90
			3.95*(s)	3.91,3.92*,3.93*(s)
	OAc	1.87,1.95(s)	1.81,1.93(s)	1.96,1.89*(s)

ring. Associations between 2-H(C)[δ5.15, 5.08*] and 2'-,6'-H(B)[δ6.69, 6.91*, δ7.05, 6.89*] and 5-H(A)[δ6.64, 6.82*] with 4-H(C)[δ4.94, 5.01*] by NOESY experiment confirmed the ABC-moiety.

A COSY experiment showed coupling of 2-H(F)[δ5.13, 4.49*] and 3-H(F)[δ5.46, 5.50*] as a result of which 2-H(F) appeared as a broad singlet. The 3-H(F) proton also showed a multiplet-coupling with 2-H(F), 4-H_{ax}(F)[δ3.05, 3.05*] and 4-H_{eq}(F)[δ2.98, 2.87*] protons. A multiplet and a doublet of doublets resulted when 4-H_{ax}(F) and 4-H_{eq}(F) coupled with 3-H(F) respectively. Long distance association between 2-H(F) and 2'-,6'-H(E)[δ6.26, 6.69*] was confirmed by NOE experiment.

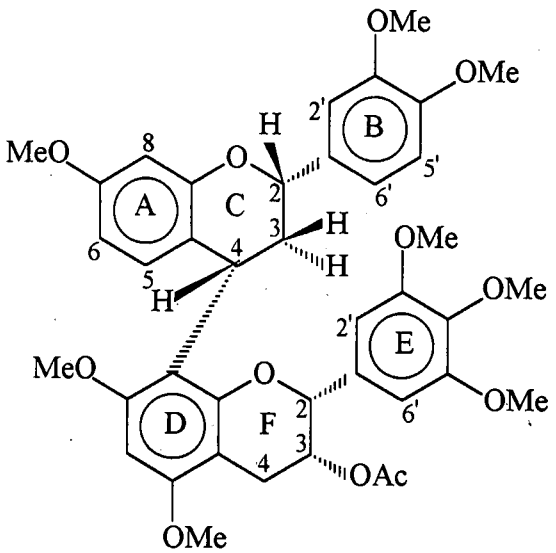
Both the A- and B-ring substitution patterns of the top-unit were defined by an ABX pattern (δ6.64, 6.82*, d, J=8.5Hz, 5-H; δ6.25, 6.40*, dd, J=2.5 and 8.5Hz, 6-H; δ6.20, 6.52*, d, J=2.5Hz, 8-H; δ6.69, 6.91*, d, J=2.5Hz, 2'-H; δ7.01, 6.90*, d, J=8.5Hz, 5'-H, δ7.05, 6.89*, dd, J=2.5 and 8.5Hz, 6'-H) respectively. The 7-OMe position on the A-ring was confirmed by a strong NOE association with 8-H(A).

The bottom-unit aromatic substitution was defined by the appearance of an ABX pattern (δ6.74 7.02*, d, J=2.5Hz, 2'-H; δ6.77, 6.87*, d, J=8.5Hz, 5'-H; δ6.70, 7.02*, dd, J=2.5 and 8.5Hz, 6'-H) assigned to the E-ring protons. The appearance of a one-proton singlet (δ6.26, 6.15*) in the aromatic region showed a strong NOE association with both 5- and 7-OMe of the D-ring and was identified as 6-H(D)[59]. This information now implied that the bottom unit was coupled at C-8 and confirmed the $C_4 \rightarrow C_8$[59] coupling and the dimeric structure of compound **153**. This was supported by FAB-MS (m/z 686.2727) which confirmed a molecular formula of $C_{39}H_{42}O_{11}$ for **153**.

NOE association between 2-H(F) and 3-H(F) together with $^3J_{2,3(F)}$-values of *ca* 1.5Hz suggested 2,3-*cis* relative stereochemistry[82] of the F-ring. This information in conjunction with the D- and E-ring substitution pattern was reminiscent of the coupling pattern [2-H(br.s), 3-H(m)] of the monomeric epicatechin **167**.

The CD spectrum of the dimer **153** showed a high amplitude positive Cotton effect $[\theta]_{244.6}$-47980 which confirmed the interflavanyl bond[80,81] to be 4β and consequently the absolute configuration of the top unit to be 2S,4S. The absolute stereochemistry of the bottom unit and hence of the dimer **153** was established by synthesis as discussed for compound **153** (**Scheme 6.1**).

6.2.5 **Butiniflavan-(4α→8)-epigallocatechin**



154

The probutinidin derivatives **154** and **155** were obtained after methylation and subsequent acetylation of the diastereomeric mixture that resulted from the coupling of flavan-4-ol **163** and **166**. The natural product analogues of derivatives **154** and **155** have not yet been isolated from natural sources (**Scheme 6.2**).

The signals in the 300MHz ^{1}H NMR spectrum ($CDCl_3$, 293K) of the two rotamers heptamethyl ether acetate derivative **154** (table 3, plate 8) exhibited two ABX-, AA'-coupled systems and one singlet spin systems in the aromatic region. The AB_2X- and ABMX-spin systems in the 2-6ppm region define the heterocyclic protons of the C- and F-rings[63,64].

The 2D COSY experiment showed couplings of the 2-H(C)[δ5.20, 5.09*] with 3-H_{ax}(C)[δ1.97, 2.22*] and 3-H_{eq}(C)[δ2.78, 2.91*] resulting in a doublet of doublets and the 4-H(C)[δ4.95, 5.52*] also showing couplings to the same 3-H_{ax}(C)[δ1.97, 2.22*] and 3-H_{eq}(C)[δ2.78, 2.91*]. A doublet of doublets and one multiplet resulted when 3-H_{ax}(C)[δ1.97,2.22*] and3-H_{eq}(C)[2.78, 2.91*] coupled with each other and in turn showed coupling with both 2-H(C) and 4-H(C) respectively. These systems are reminiscent of flavan top unit with an interflavanyl linkage at C-4[63,64].

A COSY experiment showed $^4J_{HH}$ long range coupling between 2-H(C)[δ5.20 5.09*] and 2',6'-H(B)[δ6.26, 6.69*] and a benzylic coupling between 5-H(A)[δ6.70, 6.61*] and 4-H(C)[δ4.95, 5.52*].

NOE experiment showed coupling between 2-H(C) with 4-H(C) suggesting 2,4-*cis* relative configuration of the C-ring. Associations between 2-H(C)[δ5.20, 5.09*] and 2'-,6'-H(B)[δ6.26, 6.69*] and 5-H(A)[δ6.70, 6.61*] with 4-H(C)[δ4.95, 5.52] by NOESY experiment confirmed the ABC-moiety.

A COSY experiment showed coupling of 2-H(F)[δ4.84, 5.11*] and *3*-H(F)[δ5.27, 5.59*] resulting in a broad singlet. The 3-H(F) proton also showed a multiplet-coupling with 2-H(F), 4-H_{ax}(F)[δ2.99, 3.08*] and 4-H_{eq}(F)[δ2.95, 2.95*] protons. A doublet of doublets and a multiplet resulted when 4-H_{ax}(F) and 4-H_{eq}(F) coupled with 3-H(F) respectively. Long distance association between 2-H(F) and 2',6'-H(E)[δ6.26, 6.69*] was confirmed by NOE experiment.

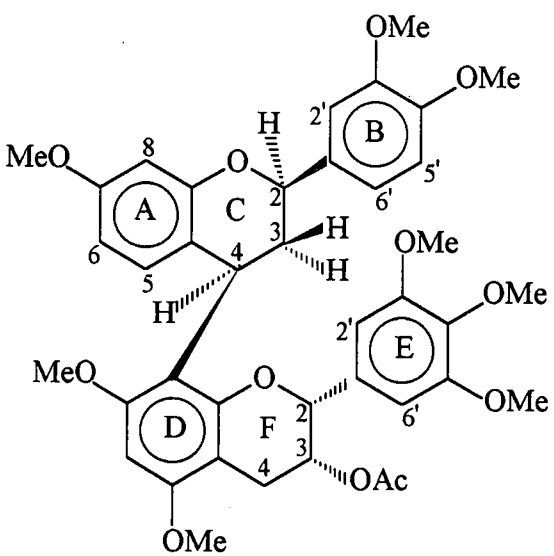
Both the A- and B-ring substitution patterns of the top-unit were defined by an ABX pattern (δ6.70, 6.61*, d, J=8.5Hz, 5-H; δ6.38, 6.35*, dd, J=2.5 and 8.5Hz, 6-H; δ6.43, 6.49*, d, J=2.5Hz, 8-H; δ6.73, 7.06*, d, J=2.5Hz, 2'-H; δ6.77, 6.91*, d, J=8.5Hz, 5'-H; δ6.84, 7.02*, dd, J=2.5 and 8.5Hz, 6'-H) respectively. The 7-OMe position on the A-ring was confirmed by a strong NOE association with 8-H(A).

The bottom-unit aromatic substitution was defined by the appearance of an AA' pattern (δ6.26 6.69*, 2prs, 2',6'-H) assigned to the E-ring protons. The appearance of a one-proton singlet (δ6.27, 6.10*) in the aromatic region showed a strong NOE association with both 5- and 7-OMe of the D-ring and was identified as 6-H(D)[59]. This information now implied that the bottom unit was coupled at C-8 and confirmed the $C_4 \rightarrow C_8$[59] coupling and the dimeric structure of compound **154**. This was supported by FAB-MS (m/z 716.2830) which confirmed a molecular formula of $C_{40}H_{44}O_{12}$ for **154**.

NOE association between 2-H(F) and 3-H(F) together with $^3J_{2,3(F)}$-values of *ca* 1.5Hz suggested 2,3-*cis* relative stereochemistry[82] of the F-ring. This information in conjunction with the D- and E-ring substitution pattern was reminiscent of the coupling pattern [2-H(br.s), 3-H(m)] of the monomeric epigallocatechin **163**.

The CD spectrum of the dimer **154** showed a high amplitude positive Cotton effect $[\theta]_{243.7}$-21880 which confirmed the interflavanyl bond[80,81] to be 4α and consequently the absolute configuration of the top unit to be 2R,4R.

6.2.6 ***ent*-Butiniflavan-(4β→8)-epigallocatechin**



155

The signals in the 300MHz ^1H NMR spectrum of the two rotamers ($CDCl_3$, 293K) of the heptamethyl ether acetate derivatives **155** (table 3, plate 9) exhibited two ABX-, AA'-coupled systems and one singlet spin systems in the aromatic region. The AB_2X- and ABMX-spin systems in the 2-6ppm region define the heterocyclic protons of the C- and F-rings [63,64].

The 2D COSY experiment showed couplings of the 2-H(C)[δ5.15, 5.09*] with 3-H_{ax}(C)[δ2.31, 2.10*] and 3-H_{eq}(C)[δ2.75, 2.85*] resulting in a doublet of doublets and the 4-H(C)[δ4.93, 5.01*] also showing couplings to the same 3-H_{ax}(C)[δ2.31, 2.10*] and 3-H_{eq}(C)[δ2.75, 2.85*].

A doublet of doublets and one multiplet resulted when 3-H_{ax}(C)[δ2.31,2.10*] and 3-H_{eq}(C)[2.75, 2.85*] coupled with each other and in turn showed coupling with both 2-H(C) and 4-H(C) respectively. These systems are reminiscent of flavan top unit with an interflavanyl linkage at C-4[63,64].

A COSY experiment showed $^4J_{HH}$ long range coupling between 2-H(C)[δ5.15 5.09*] and 2'-,6'-H(B)[δ6.36, 6.69*] and a benzylic coupling between 5-H(A)[δ6.61, 6.81*] and 4-H(C)[δ4.93, 5.01*]. NOE experiment showed coupling between 2-H(C) with 4-H(C) suggesting 2,4-*cis* relative configuration of the C-ring. Associations between 2-H(C)[δ5.15, 5.09*] and 2'-,6'-H(B)[δ6.36, 6.69*] and 5-H(A)[δ6.61, 6.81*] with 4-H(C)[δ4.93, 5.01*] by NOESY experiment confirmed the ABC-moiety.

A COSY experiment showed coupling of 2-H(F)[δ4.39, 5.12*] and 3-H(F)[δ5.37, 5.51*] as a result of which 2-H(F) appeared as a broad singlet. The 3-H(F) proton also showed a multiplet-coupling with 2-H(F), 4-H_{ax}(F)[δ3.01, 3.09*] and 4-H_{eq}(F)[δ2.87, 2.98*] protons. Two doublet of doublets resulted when 4-H_{ax}(F) and 4-H_{eq}(F) coupled with 3-H(F) respectively. Long distance association between 2-H(F) and 2',6'-H(E)[δ6.36, 6.69*] was confirmed by NOE experiment.

Both the A- and B-ring substitution patterns of the top-unit were defined by an ABX pattern (δ6.61, 6.81*, d, J=8.5Hz, 5-H; δ6.23, 6.37*, dd, J=2.5 and 8.5Hz, 6-H; δ6.12, 6.50*, d, J=2.5Hz, 8-H; δ7.02, 7.03*, d, J=2.5Hz, 2'-H; δ6.89, 6.94*, d, J=8.5Hz, 5'-H; δ7.05, 6.91*, dd, J=2.5 and 8.5Hz, 6'-H) respectively. The 7-OMe substitution of the A-ring was confirmed by a strong NOE association with 8-H(A).

The bottom-unit aromatic substitution was defined by the appearance of an AA' pattern (δ6.36 6.69*, 2prs, 2',6'-H) assigned to the E-ring protons. The appearance of a one-proton singlet (δ6.27, 6.15*) in the aromatic region showed a strong NOE association with both 5- and 7-OMe of the D-ring and was identified as 6-H(D)[59]. This information now implied that the bottom unit was coupled at C-8 and confirmed the $C_4 \rightarrow C_8$[59] coupling and the dimeric structure of compound **155**. This was supported by FAB-MS (m/z 716.2830) which confirmed a molecular formula of $C_{40}H_{44}O_{12}$ for **155**.

NOE association between 2-H(F) and 3-H(F) together with $^3J_{2,3(F)}$-values of *ca* 1.5Hz suggested 2,3-*cis* relative stereochemistry[82] of the F-ring. This information in conjunction with the D- and E-ring substitution pattern was reminiscent of the coupling pattern [2-H(br.s), 3-H(m)] of the monomeric epigallocatechin **163**.

The CD spectrum of the dimer **155** showed a high amplitude positive Cotton effect $[\theta]_{246.5}$-9881 which confirmed the interflavanyl bond[80,81] to be 4β and consequently the absolute configuration of the top unit to be 2S,4S.

EXPERIMENTAL

CHAPTER 7

STANDARD EXPERIMENTAL PROCEDURE

The following standard experimental techniques (details below) were used in this study and will be referred to in subsequent chapters.

7.1 CHROMATOGRAPHIC METHODS

7.1.1 COLUMN CHROMATOGRAPHY

Glass columns of dimensions, 22 × 900, 50 × 300 and 50 × 1200mm designated small(S), medium(M) and large(L) respectively, were used.

7.1.1.1 SILICA GEL AS ADSORBENT

The slurry used to pack the column was prepared by mixing Merck Kieselgel Art 773 (170-230 mesh) with the eluting solvent. To ensure dense, efficient packing without air bubbles, the column was vibrated with the tap open.

The material to be separated was first absorbed onto a small quantity of silica gel before being loaded onto the column. The ratio of material to be separated to silica gel was 1:2. Fractions were collected in test tubes.

7.1.1.2 LH-20.AS ADSORBENT

Ethanol was used to prepare a slurry of Sephadex LH-20 which was then left to stand for 24 hours. The slurry was subsequently poured into the column with the tap left open while mild vibration was used to ensure compact packing. The ratio of material to be separated to LH-20 was 1:25. The material was loaded with a minimum of ethanol. Fractions were collected in test tubes.

7.1.2 THIN LAYER CHROMATOGRAPHY

Two types of plates, preparative thin layer chromatography (PLC) plates and aluminium backed silica gel (TLC) plates were used.

PLC plates were prepared by uniformly spreading a slurry of 200g of Merk Kieselgel (Art 7747) silica gel in 475 cm^3 of water, over 200 ×200mm glass plates. The plates were air dried and then subjected to 80 $^{\circ}C$ for 24 hours before being used. The material to be separated was loaded with a maximum of 25mg per plate (minimum 10mg). A separating tank was set up with the appropriate solvent and the chromatogram developed therein.

After development, the plates were dried and examined under ultraviolet light (254nm and 360nm). The compounds which formed prominent bands were marked and scraped off. Acetone was used to extract the compounds from the scraped silica gel. The acetone was removed under pressure and the residue dried in a vacuum oven. Aluminium backed silica gel plates (Merk Art 5554) were used for refined separations by the same method as described before. The loading was from 3 to 5mg per plate.

7.1.3 SPRAY REAGENT

Thin layer chromatograms were lightly sprayed with a mixture of 50% p-anisaldehyde, 5% conc. H_2SO_4 and 90% ethanol, (V/V). The colour was developed in an oven at 110°C. The plate was removed when the maximum colour developed.

7.2 SPECTROSCOPIC METHODS

7.2.1 PROTON MAGNETIC RESONANCE SPECTROSCOPY (^{1}H NMR)

A 300MHz Bruker and a 300MHz Varian spectrometer were used to record the ^{1}H NMR, NOE, COSY, HOMODEC and ^{3}C experiments were executed in either $CDCl_3$, acetone-d_6 or benzene-d_6. Chemical shifts are given in parts per million (ppm) on the delta (δ) scale and coupling constants (J) are accurate to 0,1Hz. The abbreviations s,d,dd,t,q,m and br are used to denote singlet, doublet, doublet of doublets, triplet, quarted, multiplet and broad respectively.

7.2.2 MASS SPECTROMETRY

Fast Atom Bombardment Mass Spectrometry (FAB-MS) data was recorded on a VG 70-70E spectrometer fitted with an ion tech. B11N saddle field gun. Xenon was the bombardment gas used in a glycerol matrix. Accurate masses and Electron Impact Mass Spectrometry (EI-MS) data were recorded on VG 70-70E when it was tuned in for EI-MS.

7.2.3 CIRCULAR DICHROISM (CD)

A Jasco J-710 spectropolarimeter was used and the recordings were made in methanol. The formula used to calculate the molecular ellipticity [θ] was:

$$[\theta] = \frac{L \times (\text{scale}) \times [\text{molecular weight (g/mol)}] \times 100}{[\text{lenght of tube (cm)}] \times [\text{concentration}]}$$

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

7.3 CHEMICAL METHODS

7.3.1 ACETYLATION WITH ACETIC ANHYDRIDE[11]

To the completely dried material a minimum amount of pyridine was added. An excess of acetic anhydride was then added to the dissolved material. The resulting solution was heated at about 60°C for 2 hours after which it was poured over crushed ice and

shaken vigorously. This resulted in the precipitation of the acetate derivative. The precipitate was filtered, washed with ice water to remove the excess pyridine and acetic anhydride, dried in a vacuum oven.

7.3.2 METHYLATION WITH DIAZOMETHANE[83]

Dry methanol ($50cm^3$) was used to dissolve the dried phenolic material (150-200mg) in an erlenmeyer flask. The solution was cooled to - 10°C in an ice-salt bath. The reaction KOH(8g) in ethanol ($48cm^3$) and water (2 cm^3) with N-methyl-N-nitroso-p-toluene sulphonamide (diazald, 10g) in ether under mild reflux, generated diazomethane (CH_2N_2). This was directly transferred (by distillation) into the pre-prepared phenolic solution. The mixture was left in a deep freeze at -10°C for 48 hours. Excess CH_2N_2 was evaporated in a fume cupboard at room temperature.

ABBREVIATIONS

The following abbreviations were used in describing the solvent systems and protective groups used in this study

A = acetone

B = benzene

EtAC = ethyl acetate

MeOH = methanol

EtOH = ethanol

Me = methyl

Ac = acetyl

CHAPTER 8

ISOLATION OF METABOLITES FROM *CASSIA PETERSIANA*

8.1 EXTRACTION OF BARK

Milled bark of 6.3kg was separated and repeatedly extracted with acetone (3×7.5L) for 48 hr periods at 25°C. The acetone was removed under vacuum at 35°C and the residue was dissolved in water and freeze dried to give a brown powder (370g).

8.2 SEPARATION

The combined column fractions were grouped as ;

A = comprising of phytosteroids and related compounds

B = free phenolic monomeric flavonoids

C = oligomeric free phenolic compounds

8.2.1 The extract (two batches of 25g each) was subjected to CC on Sephadex LH-20 in EtOH (6×180cm column, 0.5 ml/min flow rate, 32 min fractions) to give the following fractions : B_1 (tubes 225-264, 82mg), B_2 (265-279, 62mg), B_3 (280-285, 10mg), B_4 (286-319), B_5 (320-354), B_6 (355-364, 29mg), B_7 (365-399, 260mg) and B_8 (400-414, 117mg).

8.2.2 Methylation of fraction B_1 (82mg) followed by subsequent separation by preparative TLC in benzene-acetone (95:5) afforded six bands at R_f 0.77 (0.2mg), 0.67 (0.1mg), 0.20 (4.1mg), 0.19 (20.3mg), 0.17 (3.0mg) and 0.16 (4.2mg).

8.2.2.1 (2R,3R)-2,3-*cis* -3-Acetoxy-3,4,5,7-tetramethoxyflavan **156**.

[Epicatechin acetate] 156.

Acetylation of R_f 0.19 band and separation by preparative TLC in benzene-acetone (9:1, ×2) to yield compounds **156** (R_f 0.33, 2.1mg) and **157** (R_f 0.21, 5.7mg) respectively.

8.2.2.2 (2R,3S)-2,3-*trans*-3-Acetoxy-3,4,5,7-tetramethoxyflavan **157**.

[Catechin acetate] 157.

8.2.3 Methylation of a portion (200mg) of fraction B_7 and the separation by preparative TLC in benzene-acetone-methanol (90:8:2) afforded eight bands at R_f 0.61 (16mg), 0.50 (9.9mg), 0.39 (9.4mg), 0.30 (16mg), 0.26 (19.2mg), 0.21 (23.2mg) and 0.07 (5.6mg).

8.2.3.1 (2R,3R)-2,3-*cis*-3-Acetoxy-3,4,5,5,7-pentamethoxyflavan **158**.

[Epigallocatechin acetate] 158.

Acetylation of R_f 0.5 band followed by separation by preparative TLC in benzene-acetone (9:1, ×2) to yield compounds **158** (R_f 0.56, 0.9mg) and **159** (R_f 0.50, 7.0mg).

8.2.3.2 (2R,3S)-2,3-*trans*-3-Acetoxy-3',4',5',5,7-pentamethoxyflavan **159**

[Gallocatechin acetate] 159.

8.2.3.3 The remaining fractions contained related mixtures of monomeric flavans.

8.2.4 Two portions (2×25g) were subjected to CC on Sephadex LH-20 in EtOH (6×180cm column, 0.5 ml/min flow rate, 32 min fractions) to give the following fractions: C_1(tubes 21-27, 1.571g), C_2 (28-33, 1,293g), C_3 (34-42, 0.61g), C_4 (90-109, 2.394g), C_5 (110-160, 1.186g), C_6(162-281, 1.989g), C_7 (388-421, 1.980g), C_8 (422-469, 1.504), C_9 (470-505, 1.207g), C_{10} (506-579, 3.144g) and C_{11} (580-683, 1.464g)

8.2.4.1 Methylation of a portion (200mg) of fraction C_3 followed by preparative TLC in benzene-acetone (8:2) gave three bands at R_f 0.65 (47.3mg), 0.48 (52.2mg), and 0.32 (44.8mg).

8.2.4.1.1 (2R,4S)-2,4-*trans*-[(2R,3S)-2,3-*trans*-3-Acetoxy-3',4',5',5,7-pentamethoxyflavan-8-yl]-4',7-dimethoxyflavan **147**.

[Cassiaflavan-(4β→8)-gallocatechin] 147.

Acetylation of the R_f 0.65 band followed by preparative TLC in benzene-acetone (96:4, ×2) gave two bands at R_f 0.61 bands which were further purified by preparative TLC in hexane-acetone-ethyl acetate (60: 25: 15, ×2) to yield compounds **147** (R_f 0.51, 5.2mg) and **148** (R_f 0.46, 5.8) respectively.

^{1}H NMR data : plate1,table1

CD data : plate1

MS (FAB) data: m/z 686

8.2.4.1.2 (2R,4S)-2,4-*trans*-4-[(2R,3R)-2,3-*cis*-3-Acetoxy-3',4',5',5,7-pentamethoxy-flavan-8-yl]-4',7-dimethoxyflavan **148**.

[Cassiaflavan-(4β→8)-epigallocatechin] 148

^{1}H NMR data : plate2,table2

CD data : plate2

MS (FAB) data : m/z 686

8.2.4.1.3 (2R,4S)-2,4-*trans*-4[(2R,3R)-2,3-*cis*-Acetoxy-3',4',5,7-tetramethoxyflavan-8-yl]-3',4',7-trimethoxyflavan **153**.

***ent*-[Butiniflavan-(4β→8)-epicatechin] 153**.

Acetylation of the R_f 0.48 band followed and by preparative TLC in benzene-acetone (8:2) to give three bands at R_f 0.61 (9.2mg), 0.64 (12.2mg) and 0.54 (26.7mg). The R_f 0.64 gave compound **153**.

^1H NMR data : plate7,table7

CD data : plate7

MS (FAB) data : m/z 686

8.2.4.1.4 (2S,4S)-2,4-*cis*-4-[(2R,3R)-2,3-*cis*-3-Acetoxy-3',4',5,7-tetramethoxyflavan-8-yl]-3',4',7-trimethoxyflavan **151**.

[*ent*-Butiniflavan-(4β→8)-epicatechin] 151.

The R_f 0.50 band was further purified by preparative TLC in benzene-EtOAc-Me_2CO (21:3:1, ×2) to yield compound **151** (R_f 0.29, 3.9mg) as a light-brown amorphous solid.

^1H NMR data : plate5,table5

CD data : plate5

MS (FAB) data : m/z 686

8.2.4.1.5 The R_f 0.32 band was acetylated and separated by preparative TLC in benzene-acetone (8:2) to give a band at R_f 0.47 (11.0mg) which was further separated by preparative TLC in benzene-ethyl acetate-acetone (21:3:1, ×2) to give compound **151** as above.

8.2.4.1.6 The remaining bands contained mixtures of related proanthocyanidins which could not be separated.

8.2.5 A portion (200mg) of fraction C_4 was methylated and the mixture was separated by preparative TLC in benzene-acetone (8:2) to give five bands at R_f 0.64 (21.8mg), 0.60 (17.3mg), 0.51 (10.5mg), 0.45 (25.2mg) and 0.36 (13.7mg).

8.2.5.1 (2R,4R)-2,4-*cis*-4-[(2R,3R)-2,3-*cis*-3-Acetoxy-3',4',5',5,7-pentamethoxyflavan-8-yl]-4',7-dimethoxyflavan **149**.

[Cassiaflavan-(4α→8)-epigallocatechin] 149

The R_f 0.64 band was acetylated and separated by preparative TLC in dichloroethane-acetone (95:5, ×2) to give compound **149** (0.54, 5.9mg).

^{1}H NMR data : plate3,table3

CD data :plate3

MS (FAB) data : m/z 686

8.2.5.2 (2R,4R)-2,4-*cis*-4-[(2R,3R)-2,3-*cis*-3-Acetoxy-3',4',5,7-tetramethoxyflavan-8-yl]-3' 4',7-trimethoxyflavan **150**.

[Butiniflavan-(4α→8)-epicatechin] 150

Acetylation of R_f 0.51 band followed by preparative TLC in dichloroethane-acetone (95:2, ×2) gave compound **150** (R_f 0.39, 5.2mg) as a brown amorphous solid.

^1H NMR data : plate4,table4

CD data : plate4

MS (FAB) : m/z 686

8.2.5.3 The remaining bands contained mixtures of related proanthocyanidin-type compounds which were difficult to separate.

8.2.6 Methylation of a portion (200mg) of fraction C_5 followed by preparative TLC in benzene-acetone (8:2) gave three bands at R_f 0.61 (26.5mg), 0.50 (24.9mg) and 0.36 (18.8mg).

8.2.6.1 (2R,4R)-2,4-*trans*-4-[(2R,3R)-2,3-*cis*-3-Acetoxy-3',4',5,7-tetramethoxyflavan-8-yl]-3',4',7-trimethoxyflavan **152**.

[Butiniflavan-(4β→8)-epigallocatechin]152

Acetylation of R_f 0.50 band followed by preparative TLC in toluene-2-butanone (9:1) gave compound **152** (R_f 0.21, 5.3mg) as a rustic-brown amorphous solid.

^1H NMR data : plate6,table6

CD data : plate6

MS (FAB) data : m/z 716

8.2.6.2 The remaining bands contained mixtures of related proanthcyanidin-type compounds which were difficult to separate.

8.2.7 The probutinidins **154** and **155** were obtained as described in Chapter 10 (sections 10.5.2 and 10.5.1) respectively.

CHAPTER 9

BIOMIMETIC SYNTHESIS OF PROCASSINIDIN DIMERS

9.1 INTRODUCTION

The procassinidin dimers were grouped based on epigallocatechin as chain terminating unit and synthesized *via* the condensation of a mixture of two diastereomeric pairs of flavan-4-ol **162** (electrophile) with penta-*O*-methylepigallocatechin **163** (nucleophile) using titanium tetrachloride in dichloromethane as Lewis acid[84] to give a mixture of dimeric compounds as shown in **Scheme 9.1**.

The nucleophile **163** was isolated as discussed in section 8.2.3.1. The electrophile **162** was not obtained from the natural source but synthesized *via* base-catalyzed cyclization[85] of the (E)-chalcone **160** to afford the racemic flavanone **161** which was reduced by $NaBH_4$ to give flavan-4-ol **162** as a mixture of two diastereomeric pairs.

9.2 CYCLIZATION OF THE (E)-CHALCONE 160

The (E)-chalcone **160** (300mg) was dissolved in a mixture of ethanol:H_2O (10:1, 10ml) to which NaOAc (179.4mg) was added and the mixture refluxed for 12hrs.

H_2O:Et_2O (1:1, 20ml) was added to this mixture followed by Et_2O (20ml, ×3) extraction. After drying (Et_2O, Na_2SO_4) the Et_2O was removed under reduced pressure.

9.2.1 4',7-Dimethoxyflavanone 161

The reaction product was purified by preparative TLC in hexane-benzene-Me_2CO (5:4:1) to yield a product at R_f 0.57 (210mg).

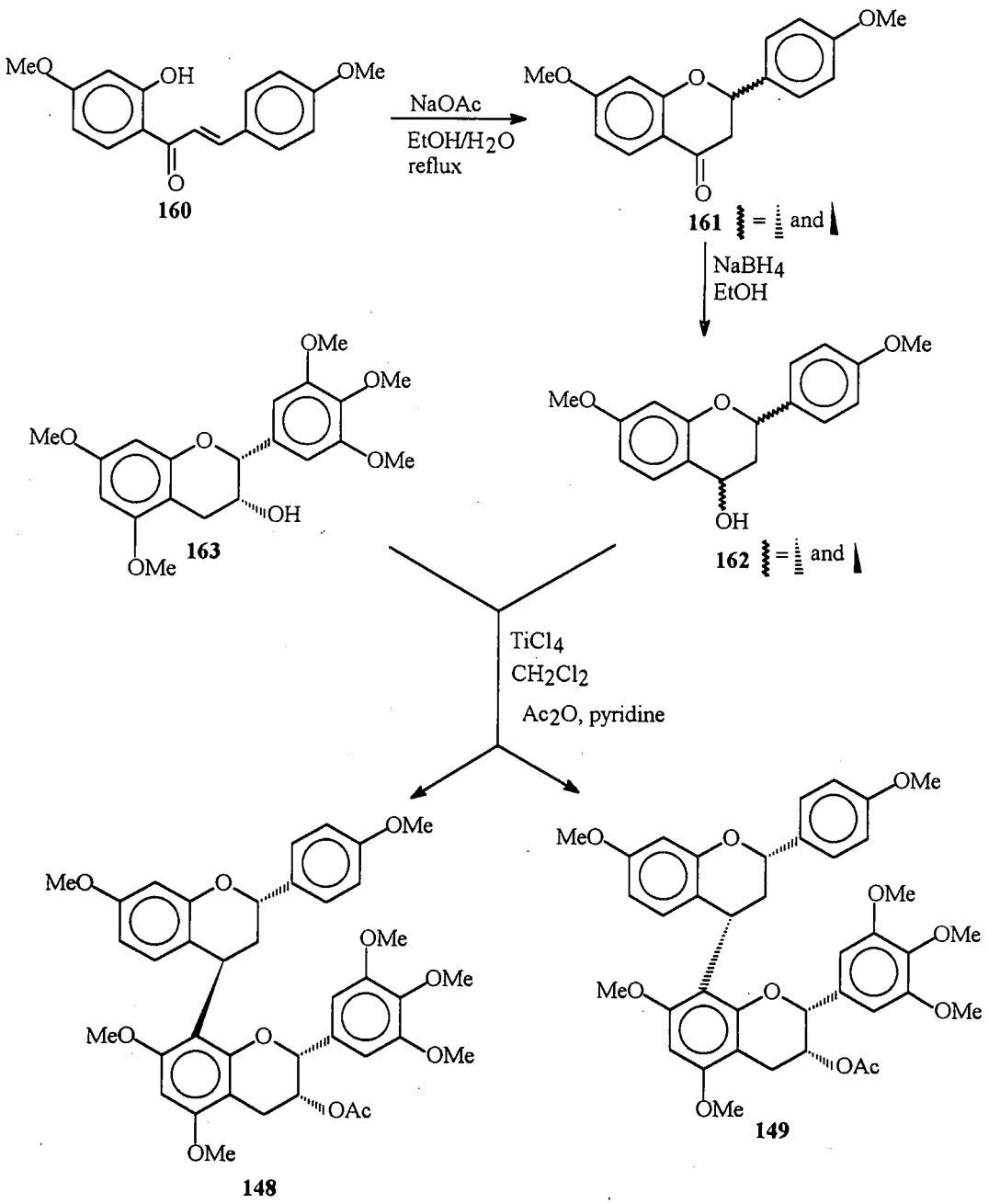
Table 4 ^{1}H NMR (300MHz) data of compounds **161** and **162**

Ring	H	**161**($CDCl_3$,293K)	**162**($CDCl_3$,293K)
A	5	7.88(d,8.5)	7.41(d,8.5)
	6	6.63(dd,8.5,2.5)	6.58(dd,8.5,2.5)
	8	6.50(d,2.5)	6.44(d,2.5)
B	2'	7.42(d,8.5)	7.38(d,8.5)
	3'	6.97(d,8.5)	6.95(d,8.5)
	5'	6.97(d,8.5)	6.95(d,8.5)
	6'	7.42(d,8.5)	7.38(d,8.5)
C	2	5.43(dd,13.0,3.0)	5.12(dd,12.0,2.0)
	3	3.08(dd,13.0,12.0)	2.48(ddd,13.0,6.0,2.0)
	3	2.81(dd,12.0,3.0)	2.14(ddd,13.0,12.0,12.0)
	4	-	5.05(br.m)
	OMe	3.84,3.85(2×s)	3.78,3.84(2×s)

^{1}H NMR data: plate10,table 4

CD data: plate10

SCHEME 9.1



9.3 REDUCTION OF THE FLAVANONE 161

A quantity of (6.9mg) $NaBH_4$ was dissolved in methanol (12ml) with continuous stirring. The flavanone **161** (210mg) was dissolved in (84ml) ethanol and dropwise added to the borohydride solution over a 10 minute period. The reaction mixture was allowed to stir at room temperature for 12hrs. H_2O (70ml) was added to this mixture and a few drops of $HCl:H_2O$ (1:1) were used to destroy the excess $NaBH_4$. The ethanol was removed under reduced pressure at 55^0C and the resulting aqueous solution was extracted with Et_2O (3×70ml). The Et_2O was removed under reduced pressure.

9.3.1 4',7-Dimethoxyflavan-4-ol 162

^1H NMR data: plate11,table 4

CD data: plate11

9.4 CONDENSATION OF FLAVAN-4-OL 175 AND PERMETHYL ETHER OF EPIGALLOCATECHIN 163

To a dry solution of 4',7-dimethoxyflavan-4-ol **162** (56.0mg) in CH_2Cl_2 (20ml) was added the permethyl ether **163** of epigallocatechin (200mg) and $TiCl_4$ (0.02ml, 1.2equiv.). The mixture was stirred at 0^0C under N_2 for 60 minutes and the temperature was allowed to rise to 40^0 for a further 6hrs. An excess of cold H_2O (40ml) was added and the mixture extracted with Et_2O (3×20ml).

After drying (Na_2SO_4) the ether was removed under vacuum and the mixture was resolved by preparative TLC in hexane-benzene-Me_2CO (5:3:2, ×3) to give two bands at R_f 0.53 (12.5mg) and 0.37(22.2mg). The R_f 0.53 band yielded starting material **180**.

Acetylation of the R_f 0.37 band followed by preparative TLC in hexane-benzene-Me_2CO (5:3:2, ×2) gave two bands at R_f 0.54 (5.2mg) and 0.49 (12.4mg).

9.4.1 **Cassiaflavan-(4β→8)-epigallocatechin 148**

The R_f 0.49 band was further separated by preparative TLC in hexane-benzene-Me_2CO (5:4:1, ×2) to give a band at R_f 0.51 (9.8mg) with ^1H NMR, CD and MS data identical to those of the natural product derivative **148**.

The R_f 0.54 band was further separated by preparative TLC in hexane-benzene-Me_2CO (5:4:1, ×4) to give two bands at R_f 0.37 (0.9mg) and 0.31 (1.0mg).

9.4.2 **Cassiaflavan-(4α→8)-epigallocatechin 149**

The R_f 0.37 band yielded starting material **163** and the R_f 0.31 band yielded a compound with ^1H NMR, CD and MS data identical to those of the natural product derivative **149**.

CHAPTER 10

BIOMIMETIC SYNTHESIS OF PROBUTINIDIN DIMERS

10.1 INTRODUCTION

The probutinidin dimers were grouped based on epicatechin and epigallocatechin as chain terminating units and synthesized *via* the condensation of a mixture of two diastereomeric pairs of flavan-4-ol **166** (electrophile) with tetra-*O*-methylepicatechin **167** and penta-*O*-methylepigallocatechin **163** (nucleophile) using titanium tetrachloride in dichloromethane as Lewis acid[84] to give a mixture of dimeric compounds as shown in **Schemes 10.1** and **10.2** respectively.

The nucleophile **167** was also isolated as discussed in section 6.2.1.3. The electrophile **166** was not obtained from the natural source but synthesized *via* base-catalyzed cyclization[85] of the (E)-chalcone **164** to afford the racemic flavanone **165** which was reduced by $NaBH_4$ to give flavan-4-ol **166** as a mixture of two diastereomeric pairs.

10.2 CYCLIZATION OF THE (E)-CHALCONE 164

The (E)-chalcone **164** (300mg) was dissolved in a mixture of ethanol:H_2O (10:1, 6.0ml) to which NaOAc (754mg) was added to the mixture and refluxed for 12hrs.

H_2O:Et_2O (1:1, 12ml) was used to this mixture followed by Et_2O(20ml, ×3) extraction. After drying (Et_2O, 20ml; Na_2SO_4) the Et_2O was refluxed under reduced pressure.

10.1 3',4',7-Trimethoxyflavanone 165

The reaction product was purified by preparative TLC in hexane-benzene-Me_2CO (5:4:1) to yield a product at R_f 0.57 (240mg).

^1H NMR data: plate12,table 5

CD data: plate12

10.3 REDUCTION OF THE FLAVANONE 165

A quantity of (49.3mg) $NaBH_4$ was dissolved in methanol (15ml) with continuous stirring. The flavanone **165** (240mg) was dissolved in (96ml) ethanol and dropwise added to the borohydride solution over a 10 minute period. The reaction mixture was allowed to stir at room temperature for 12hrs. H_2O (175ml) was added to this mixture and a few drops of $HCl:H_2O$ (1:1) were used to destroy the excess $NaBH_4$. The ethanol was removed under reduced pressure at 55^0C and the resulting aqueous solution was extracted with Et_2O (3×175ml). The Et_2O was removed under reduced pressure.

10.3.1 3',4',7-Trimethoxyflavan-4-ol 166

^1H NMR data: plate13,table 5

CD data: plate13

10.4 CONDENSATION OF FLAVAN-4-OL 166 AND PERMETHYL ETHER OF EPICATECHIN 167

To a dry solution of 3',4',7-trimethoxyflavan-4-ol **166** (90.0mg) in CH_2Cl_2 (20ml) was added the permethyl ether **167** of epicatechin (296mg) and $TiCl_4$ (0.04ml, 1.2equiv.). The mixture was stirred at 0^0C under N_2 for 60 minutes and the temperature was allowed to rise to 40^0 for a further 6hrs. An excess of cold H_2O (40ml) was added and the mixture extracted with Et_2O (3×20ml). After drying (Na_2SO_4) the ether was removed under vacuum and the mixture was resolved by preparative TLC in hexane-

Table 5 ^{1}H NMR (300MHz) data of compounds **165** and **166**

Ring	H	**165**($CDCl_3$,293K)	**166**($CDCl_3$,293K)
A	5	7.88(d,8.5)	7.43,8.5)
	6	6.63(dd,8.5,2.5)	6.60dd,8.5,2.5)
	8	6.51(d,2.5)	6.46,2.5)
B	2'	7.03(d,2.5)	7.00d,2.5)
	3'	-	-
	5'	6.92(d,8.5)	6.91(d,8.5)
	6'	7.02(dd,8.5,2.5)	7.01(dd,8.5,2.5)
C	2	5.42(dd,13.5,3.0)	5.13(dd,12.0,2.0)
	3	3.08(dd,13.0,12.0)	2.51(ddd,13.0,6.0,2.0)
	3	2.82(dd,12.0,2.5)	2.16(ddd,13.0,12.0,12.0)
	4	-	5.07(br.m,12.0,6.0)
	OMe	3.85,3.92,3.94(3×s)	3.79,3.91,3.93(3×s)

benzene-Me_2CO (5:2:3) to give three bands at R_f 0.36 (128.0mg), 0.31 (48.9mg) and 0.25 (22.1mg). The R_f 0.36 band yielded starting material **167**.

Acetylation of the R_f 0.31 band followed by preparative TLC in methanol-benzene-Me_2CO (5:2:3) gave an R_f 0.43 band (36.9mg) which was further purified by preparative TLC in benzene-EtOAc-Me_2CO (21:3:1, ×2) to give bands at R_f 0.65 (16.1mg) and 0.51 (14.5mg).

10.4.1 **Butiniflavan-(4α→8)-epicatechin 150**

The R_f 0.51 band yielded a compound with ^1H NMR, CD and MS data identical to those of the natural product derivative **150**.

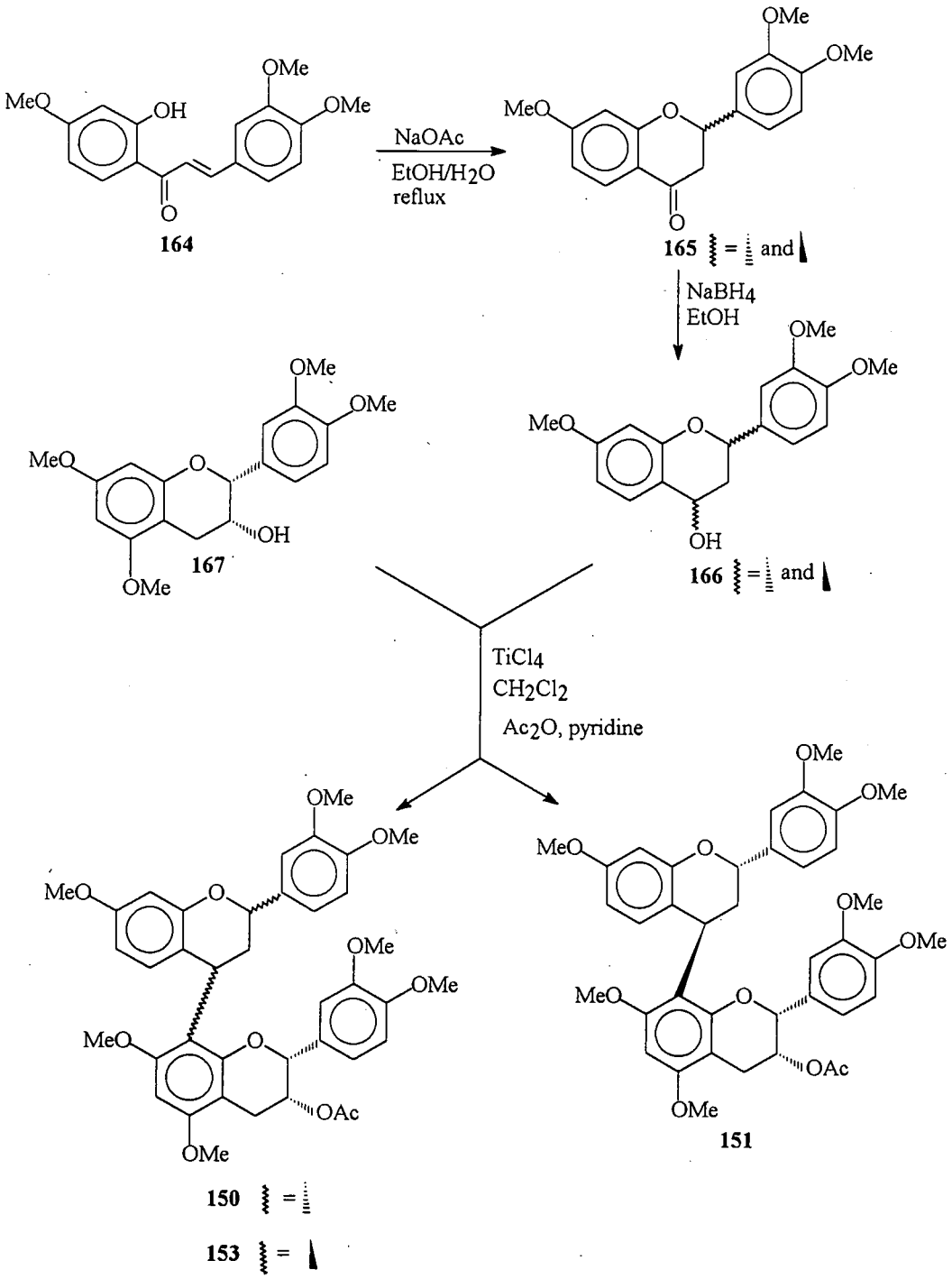
10.4.2 ***ent*-Butiniflavan-(4β→8)-epicatechin 153**

The R_f 0.65 band also gave a compound with ^1H NMR, CD and MS data identical to those of the natural derivative **153**.

10.4.3 **Butiniflavan-(4β→8)-epicatechin 151**

Acetylation of the R_f 0.25 band followed by preparative TLC in benzene-EtOAc (13:7, ×4) gave a product identical to the natural product derivative **151** with respect to ^1H NMR, CD and MS data.

SCHEME 10.1


MeO
OH
OMe
OMe
O
164
NaOAc
EtOH/H2O
reflux
165 and
NaBH4
EtOH
166 and
167
TiCl4
CH2Cl2
Ac2O, pyridine
OAc
150
153
151

10.5 CONDENSATION OF FLAVAN-4-OL 166 AND PERMETHYL ETHER OF EPIGALLOCATECHIN 163

To a dry solution of 3',4',7-trimethoxyflavan-4-ol **166** (44.0mg) in CH_2Cl_2 (10ml) was added the permethyl ether **163** of epigallocatechin (157.0mg) and $TiCl_4$ (0.02ml, 1.2equiv.). The mixture was stirred at 0^0C under N_2 for 60 minutes and the temperature was allowed to rise to 40^0 for a further 6hrs. An excess of cold H_2O (20ml) was added and the mixture extracted with Et_2O (3×10ml). After drying (Na_2SO_4) the ether was removed under vacuum and the mixture was resolved by preparative TLC in benzene-Me_2CO (9:1, ×2) to give three bands at R_f 0.61 (129.0mg), 0.54 (22.0mg) and 0.41 (12.3mg). Acetylation of the R_f 0.54 band followed by preparative TLC in benzene-Me_2CO (9:1, ×2) gave two bands at R_f 0.52 (6.6mg) and 0.43 (4.5mg).

10.5.1 *ent*-Butiniflavan-(4β→8)-epigallocatechin 155

Both the R_f 0.52 and 0.43 bands yielded compounds **155** and **154** respectively and were both not previously isolated from natural source.

H NMR data : plate9,table3

CD data : plate9

MS(FAB) data : m/z 716

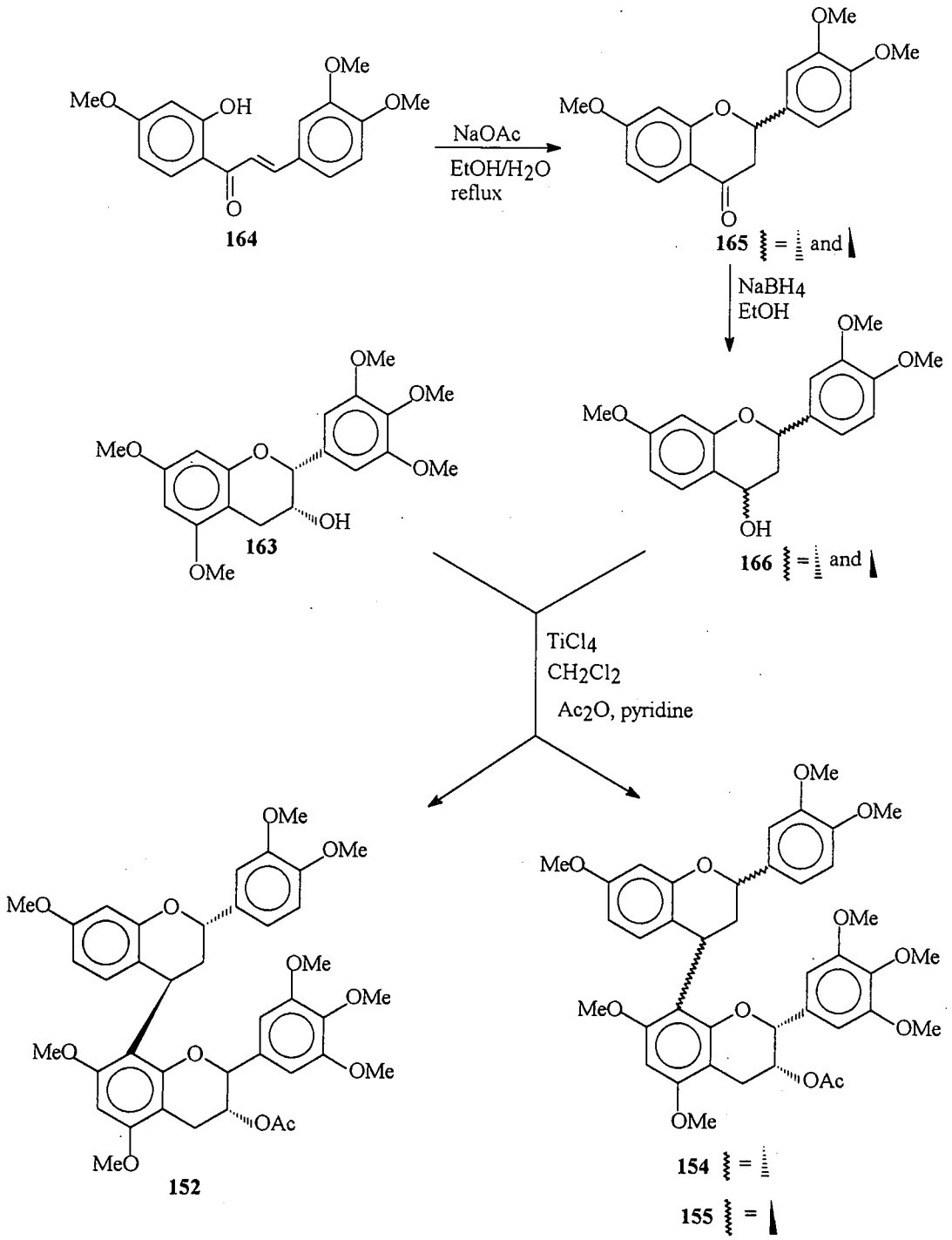
10.5.2 Butiniflavan-(4α→8)-epicatechin 154

H NMR data : plate8,table3

CD data : plate8

MS(FAB) data : m/z 716

SCHEME 10.2



CIRCULAR DICHROISM

INDEX-CIRCULAR DICHROISM

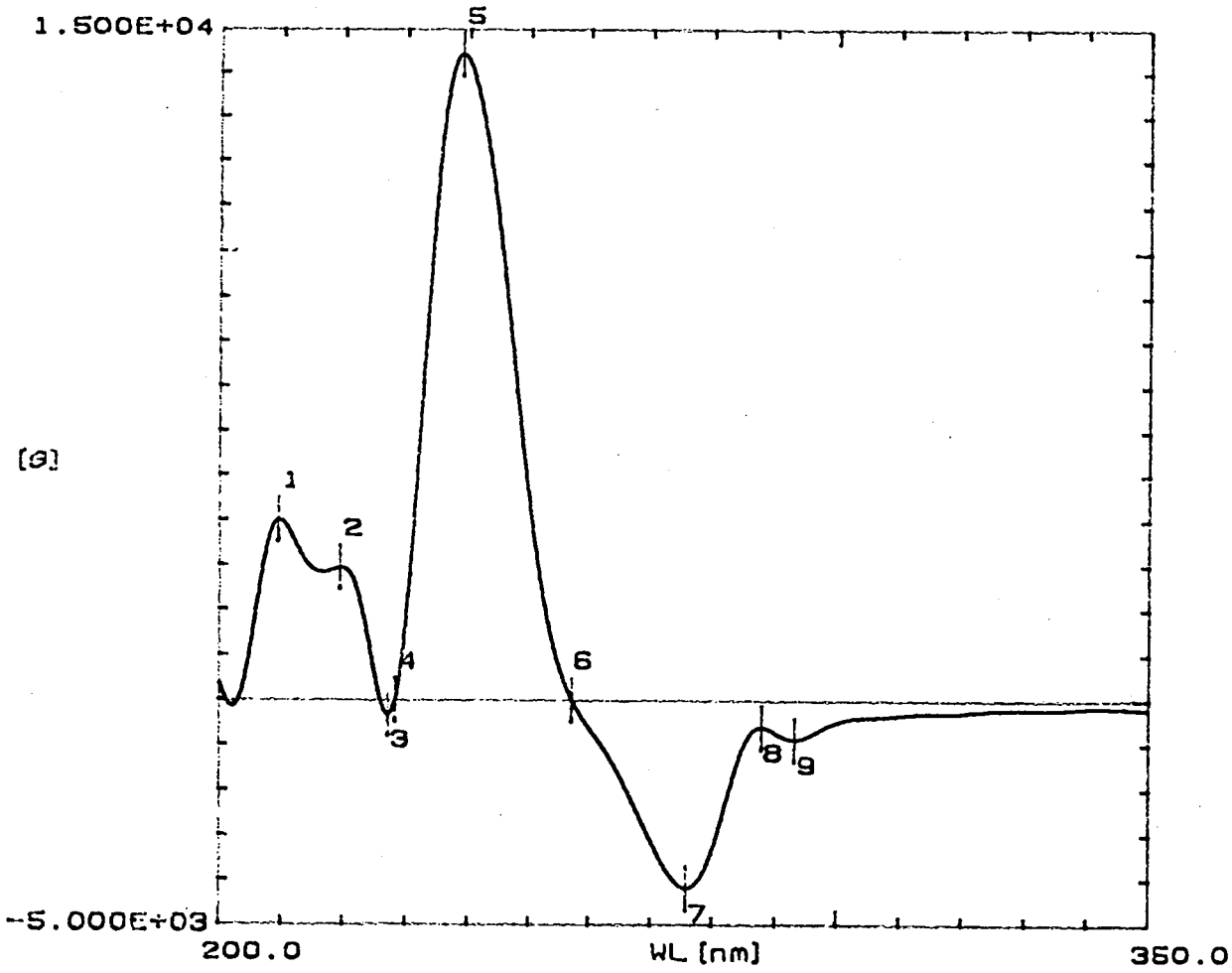
PLATE	COMPOUND
1.	Cassiaflavan-(4β→8)-gallocatechin permethyl ether acetate **147**
2.	Cassiaflavan-(4β→8)-epigallocatechin permethyl ether acetate **148**
3.	Cassiaflavan-(4α→8)-epigallocatechin permethyl ether acetate **149**
4.	Butiniflavan-(4α→8)-epicatechin permethyl ether acetate **150**
5.	Butiniflavan-(4β→8)-epicatechin permethyl ether acetate **151**
6.	Butiniflavan-(4β→8)-epigallocatechin permethyl ether acetate **152**
7.	*ent*-Butiniflavan-(4β→8)-epicatechin permethyl ether acetate **153**
8.	Butiniflavan-(4α→8)-epigallocatechin permethyl ether acetate **154**
9.	*ent*-Butiniflavan-(4β→8)-epigallocatechin permethyl ether acetate **155**

10. 4',7-Dimethoxyflavanone **161**

11. 4',7-Dimethoxyflavan-4-ol **162**

12. 3',4',7-Trimethoxyflavanone **165**

13. 3',4',7-Trimethoxyflavan-4-ol **166**



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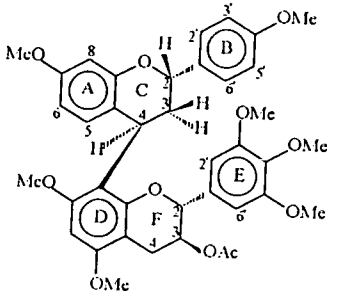
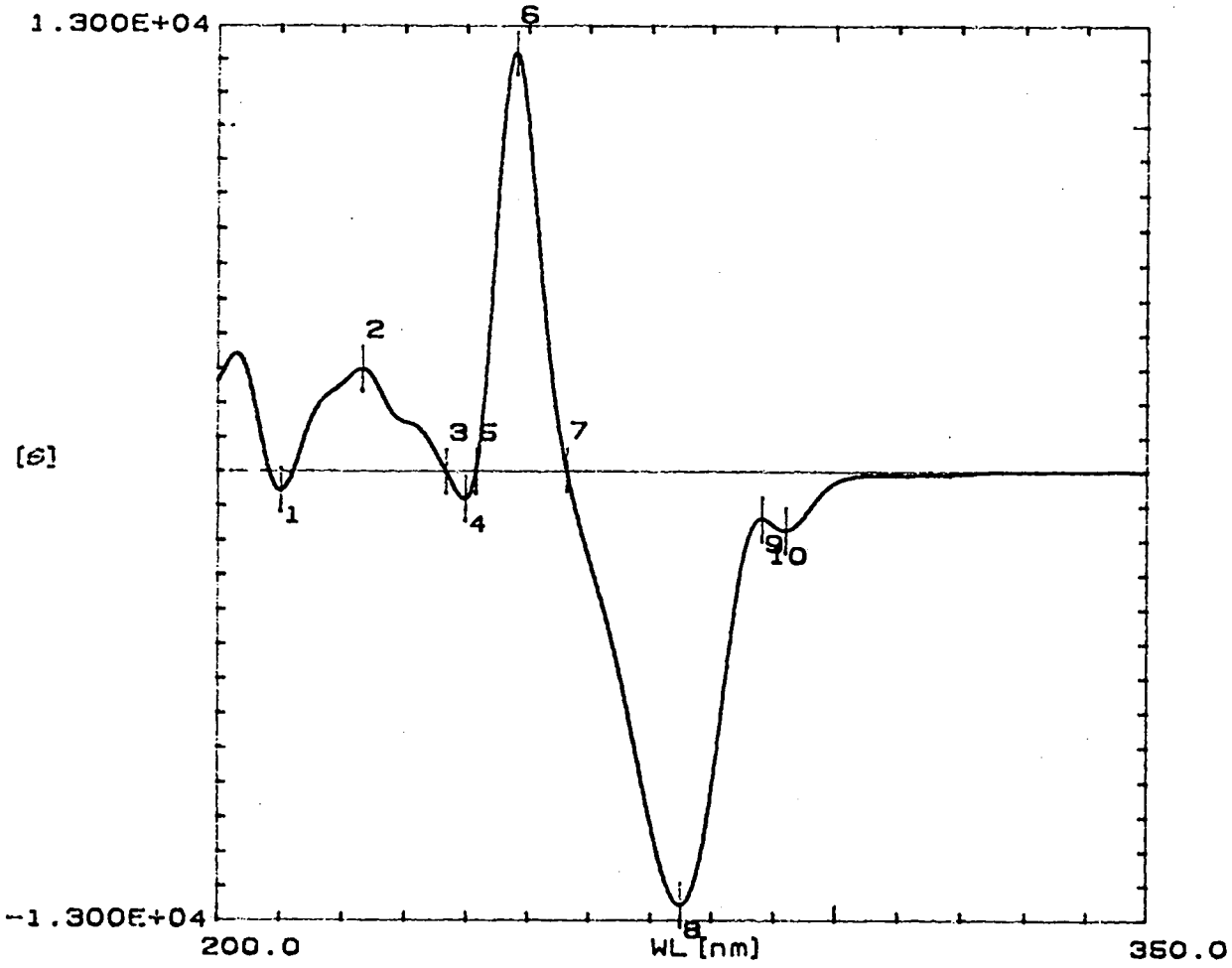
4	228.30 nm	1.429E+01
5	238.90 nm	1.444E+04
6	257.10 nm	1.580E+01
7	275.80 nm	-4.174E+03
8	287.90 nm	-5.956E+02
9	293.20 nm	-8.729E+02



147

Plate1(147)



No.	Wavelength	Value
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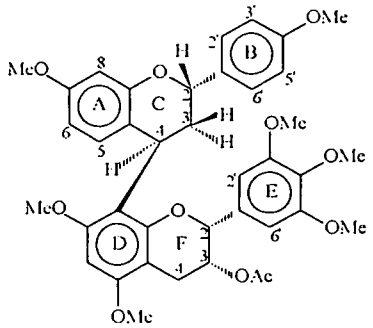
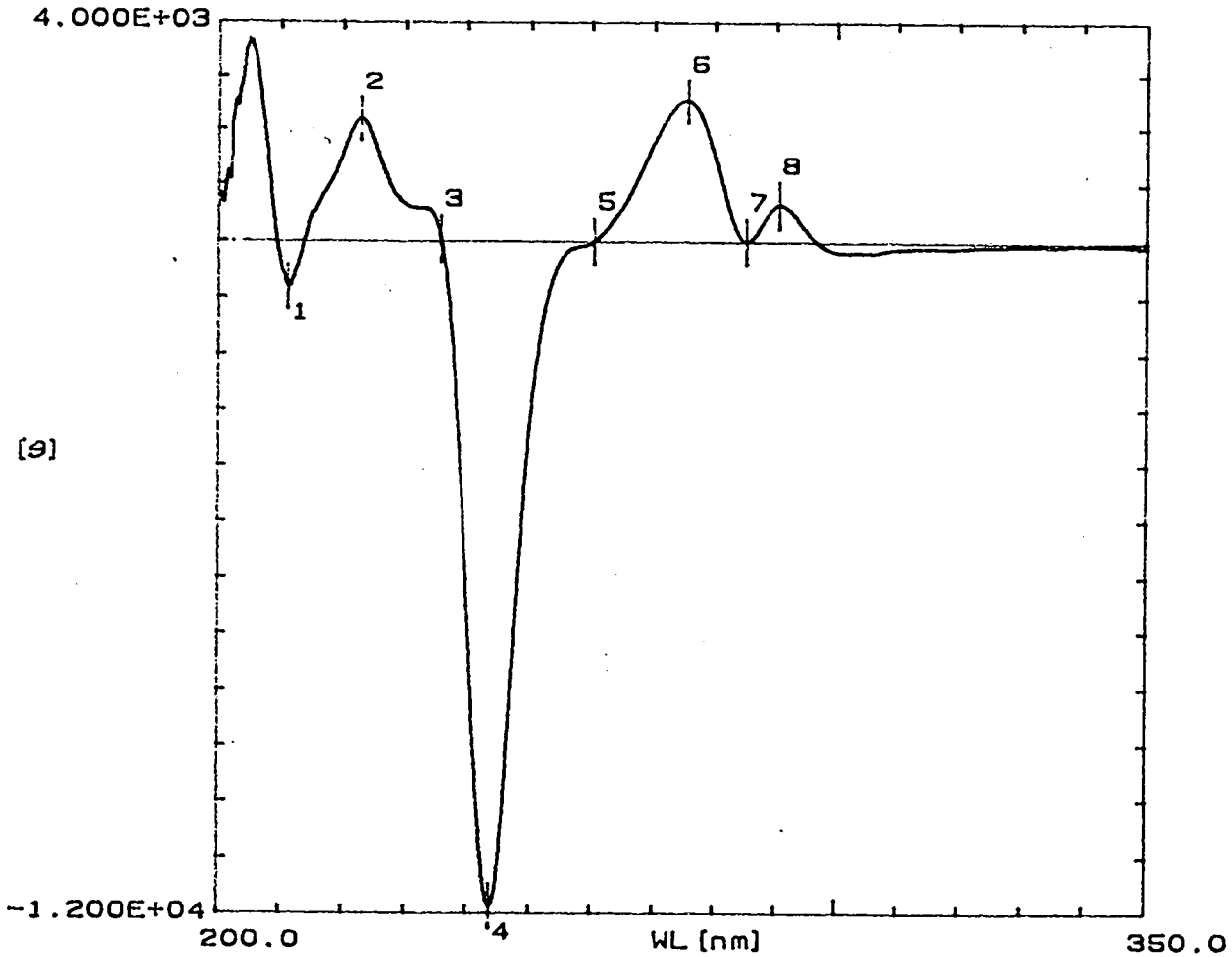
4	239.70 nm	-8.042E+02
5	241.50 nm	5.402E-03
6	248.10 nm	1.218E+04
7	256.30 nm	5.151E+01
8	275.00 nm	-1.254E+04
9	288.10 nm	-1.366E+03
10	291.90 nm	-1.706E+03



148

Plate2(148)



No.	Wavelength	Value
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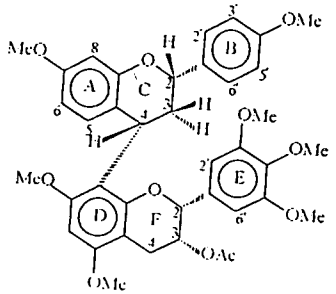
3	235.70 nm	4.542E+01
4	243.70 nm	-1.186E+04
5	260.30 nm	4.239E+00
6	276.50 nm	2.535E+03
7	285.10 nm	4.886E+00
8	290.50 nm	6.452E+02



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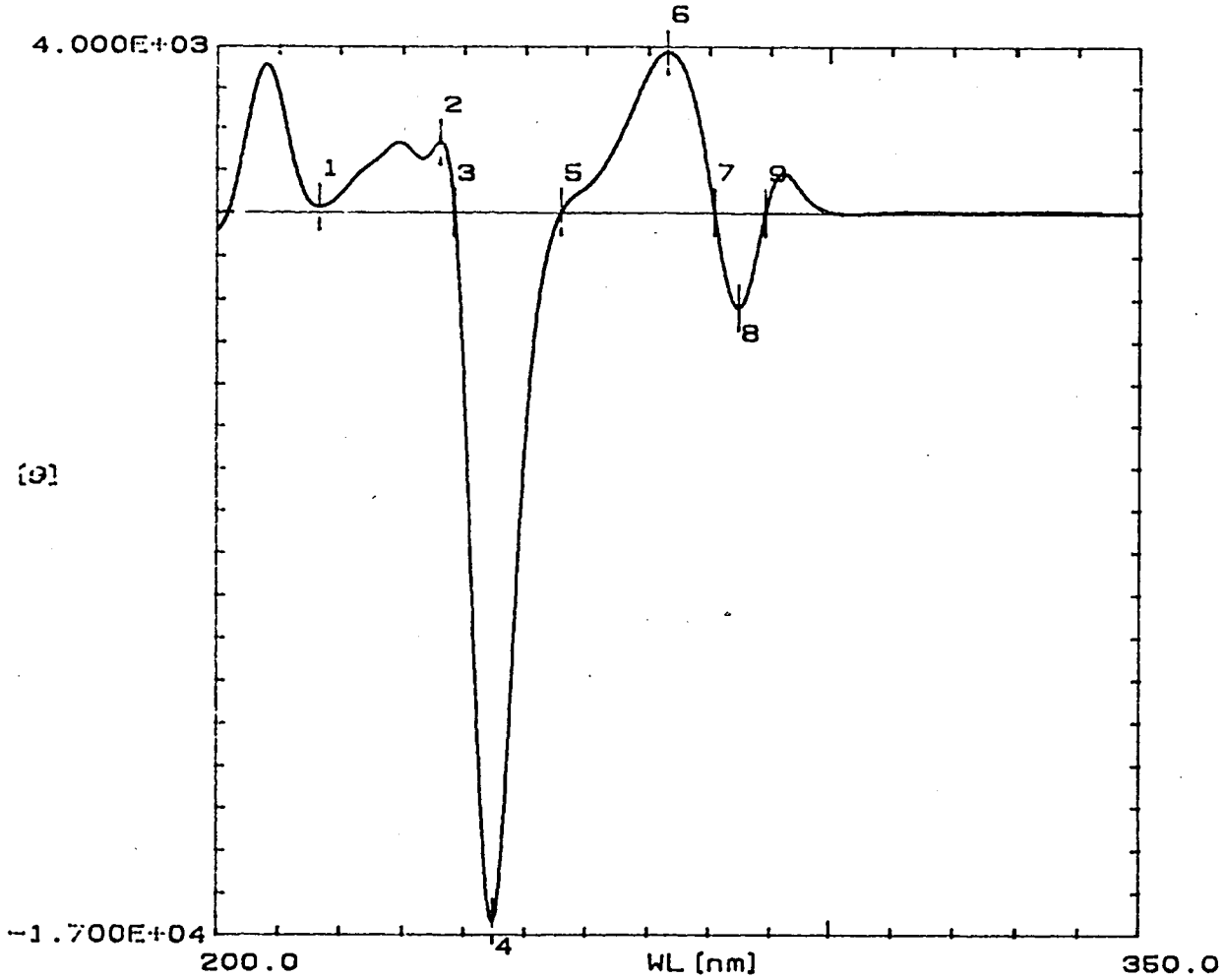

No.	Wavelength	Value
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5	255.80 nm	2.477E+01
6	273.10 nm	3.877E+03
7	280.80 nm	2.728E+01
8	284.80 nm	-2.190E+03
9	289.20 nm	1.434E+01

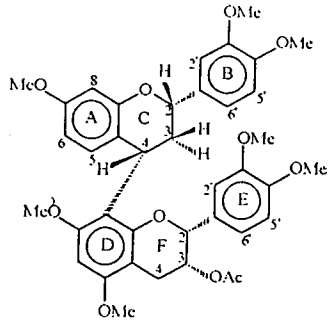

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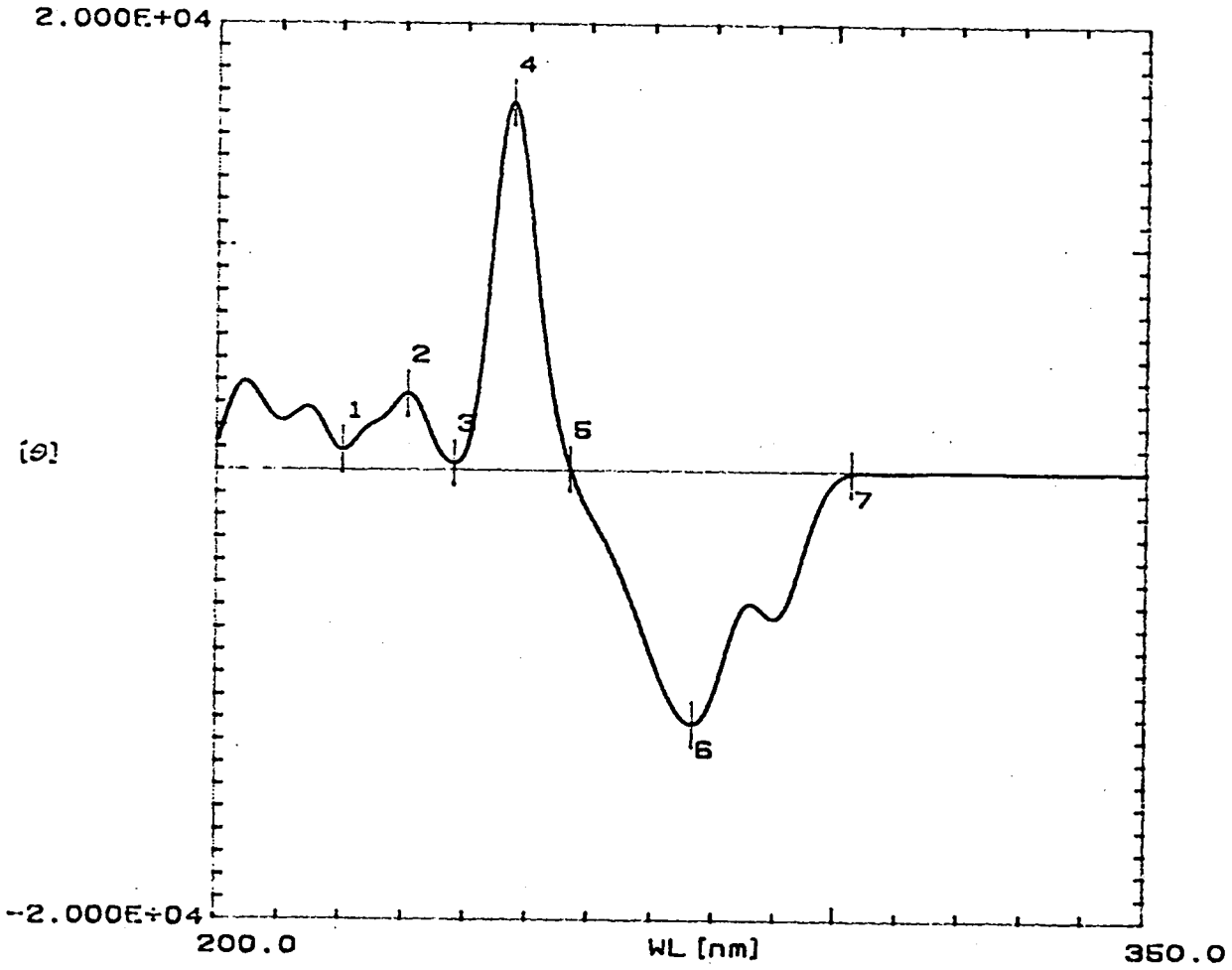
Plate4(150)



No.	Wavelength	Value
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2	230.70 nm	3.408E+03
3	238.20 nm	3.128E+02
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6	276.70 nm	-1.126E+04
7	302.60 nm	-6.334E+01

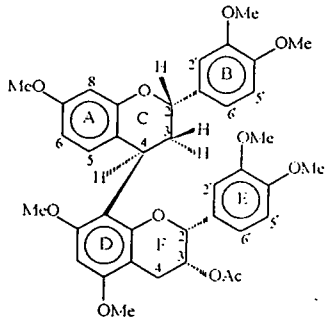

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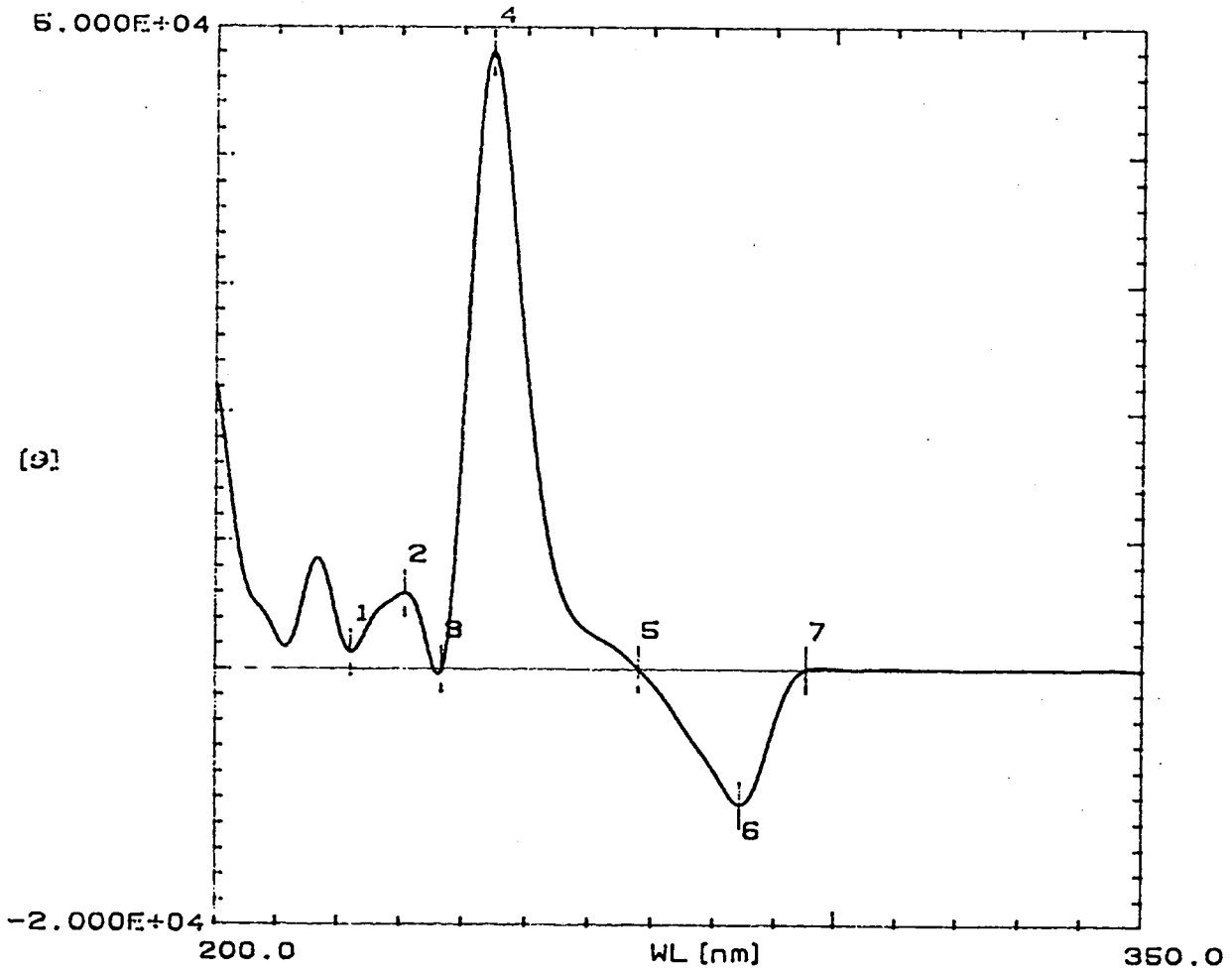
Plate5(151)



No.	Wavelength	Value
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2	230.80 nm	5.902E+03
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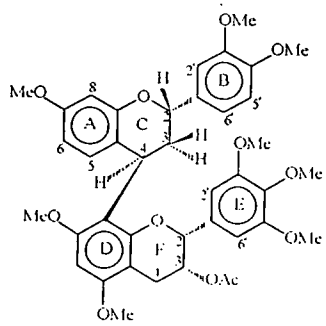
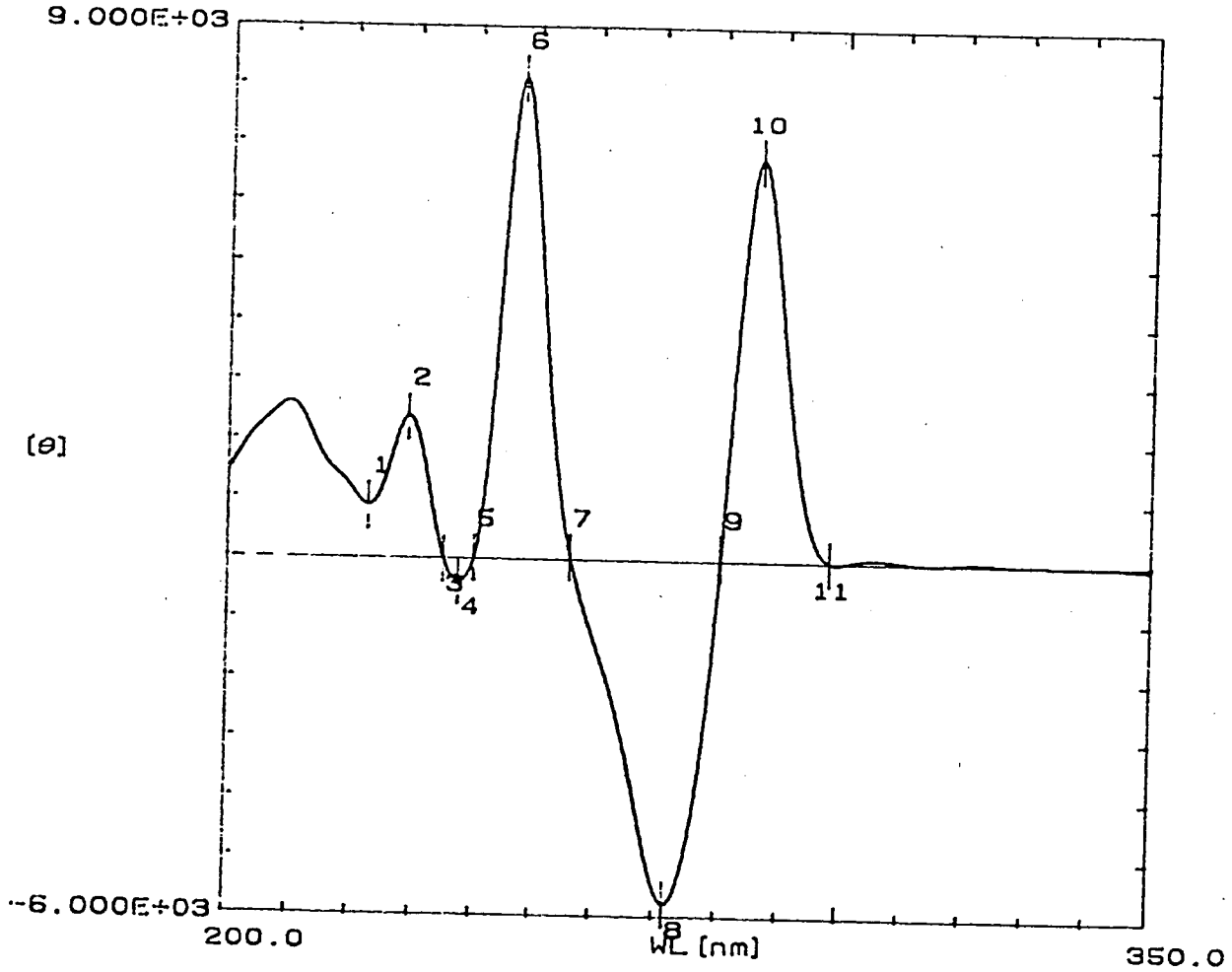
4	244.60 nm	4.798E+04
5	267.90 nm	2.482E+01
6	284.40 nm	-1.053E+04
7	295.30 nm	1.214E+00



152

Plate6(152)



No.	Wavelength	Value
1	222.70 nm	8.754E+02
2	228.90 nm	2.375E+03
3	234.80 nm	-1.678E+01
4	237.30 nm	-3.829E+02
5	239.80 nm	1.341E+01
6	247.40 nm	8.102E+03
7	256.00 nm	3.569E+01
8	271.80 nm	-5.765E+03
9	280.10 nm	5.021E+01
10	286.10 nm	6.729E+03
11	298.20 nm	-1.476E+01

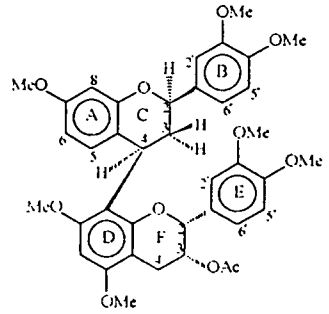


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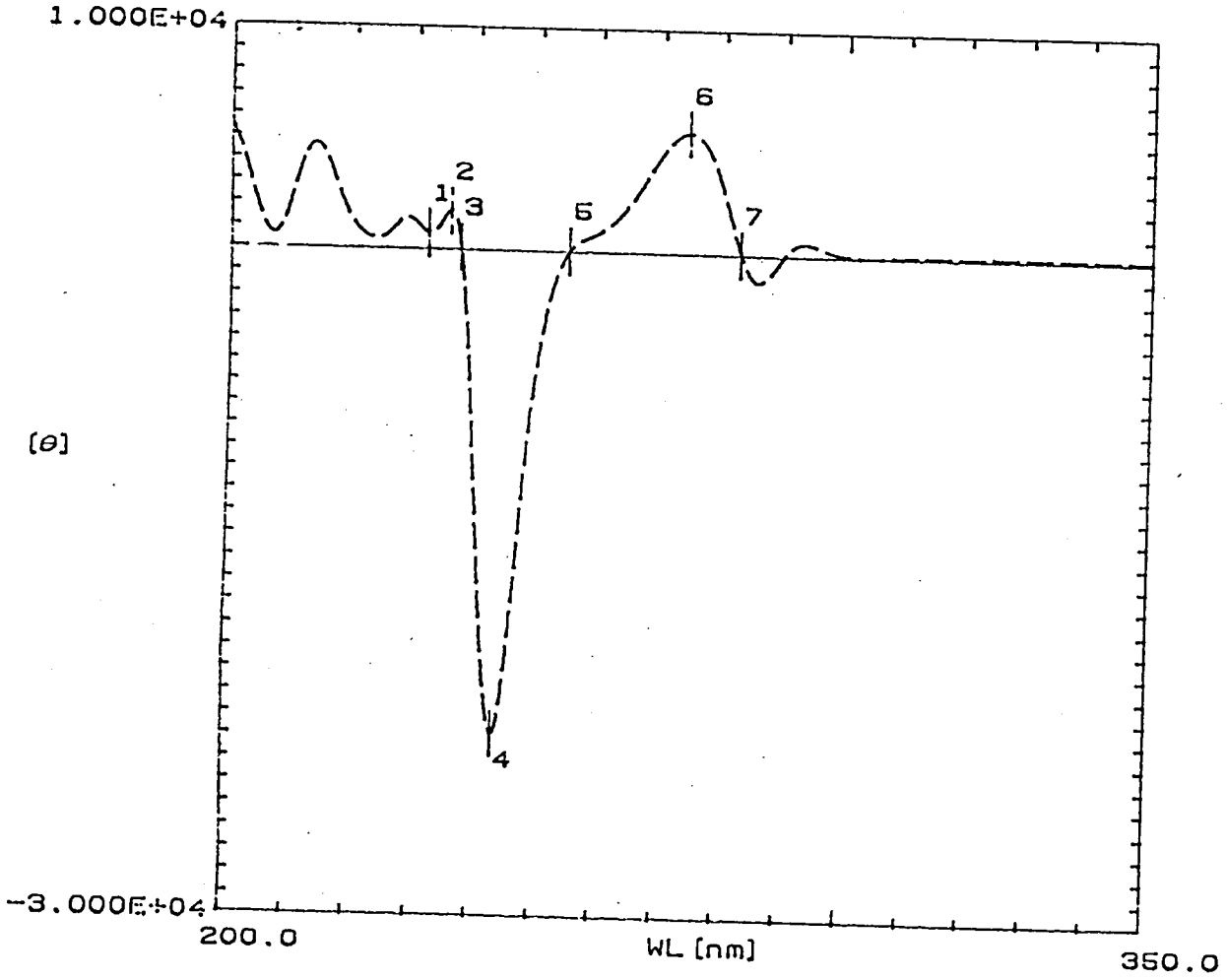
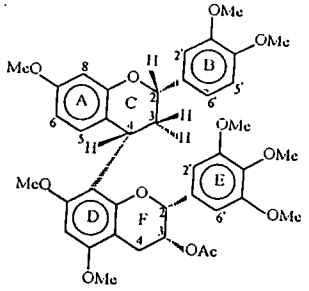
Plate7(153)

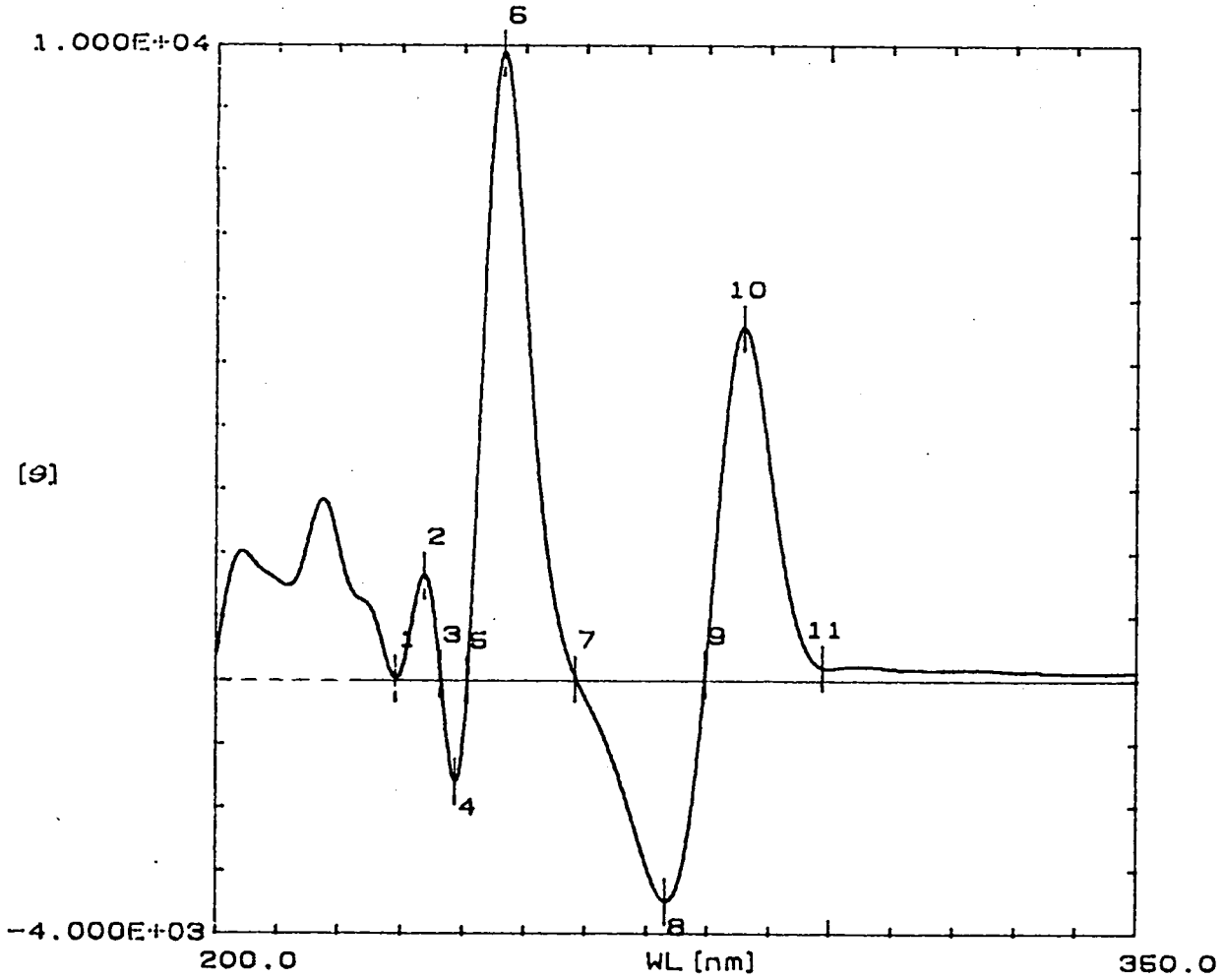


No.	Wavelength	Value
1	232.00 nm	6.986E+02
2	235.60 nm	1.671E+03
3	237.30 nm	1.034E+02
4	243.70 nm	-2.188E+04
5	255.10 nm	2.703E+01
6	274.30 nm	5.366E+03
7	282.90 nm	2.223E+01



154

Plate8(154)



No.	Wavelength	Value
1	229.20 nm	2.846E+01
2	233.70 nm	1.638E+03
3	236.30 nm	9.969E+01
4	238.80 nm	-1.595E+03
5	240.70 nm	7.447E+00
6	246.50 nm	9.881E+03
7	258.40 nm	2.177E+01
8	273.10 nm	-3.481E+03
9	279.60 nm	9.773E+01
10	285.90 nm	5.653E+03
11	298.90 nm	2.011E+02

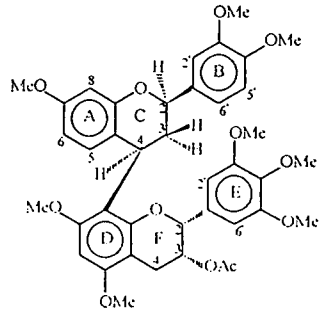


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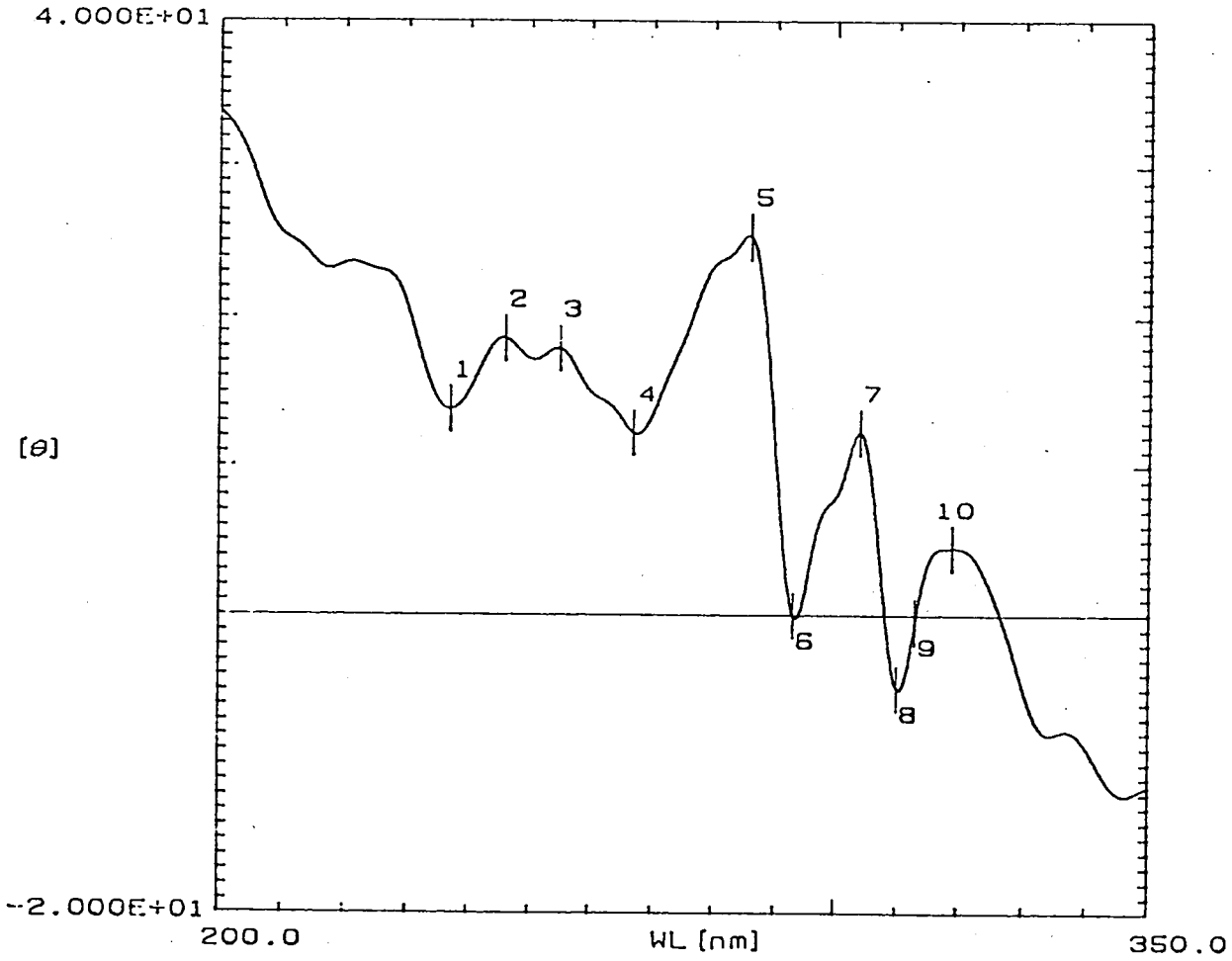
Plate9(155)



No.	Wavelength	Value
1	237.00 nm	1.381E+01
2	246.00 nm	1.857E+01
3	255.00 nm	1.789E+01
4	267.00 nm	1.224E+01
5	286.00 nm	2.538E+01
6	293.00 nm	-3.888E-02
7	304.00 nm	1.230E+01
8	310.00 nm	-4.926E+00
9	313.00 nm	-4.242E-01
10	319.00 nm	4.519E+00

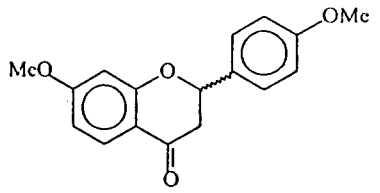


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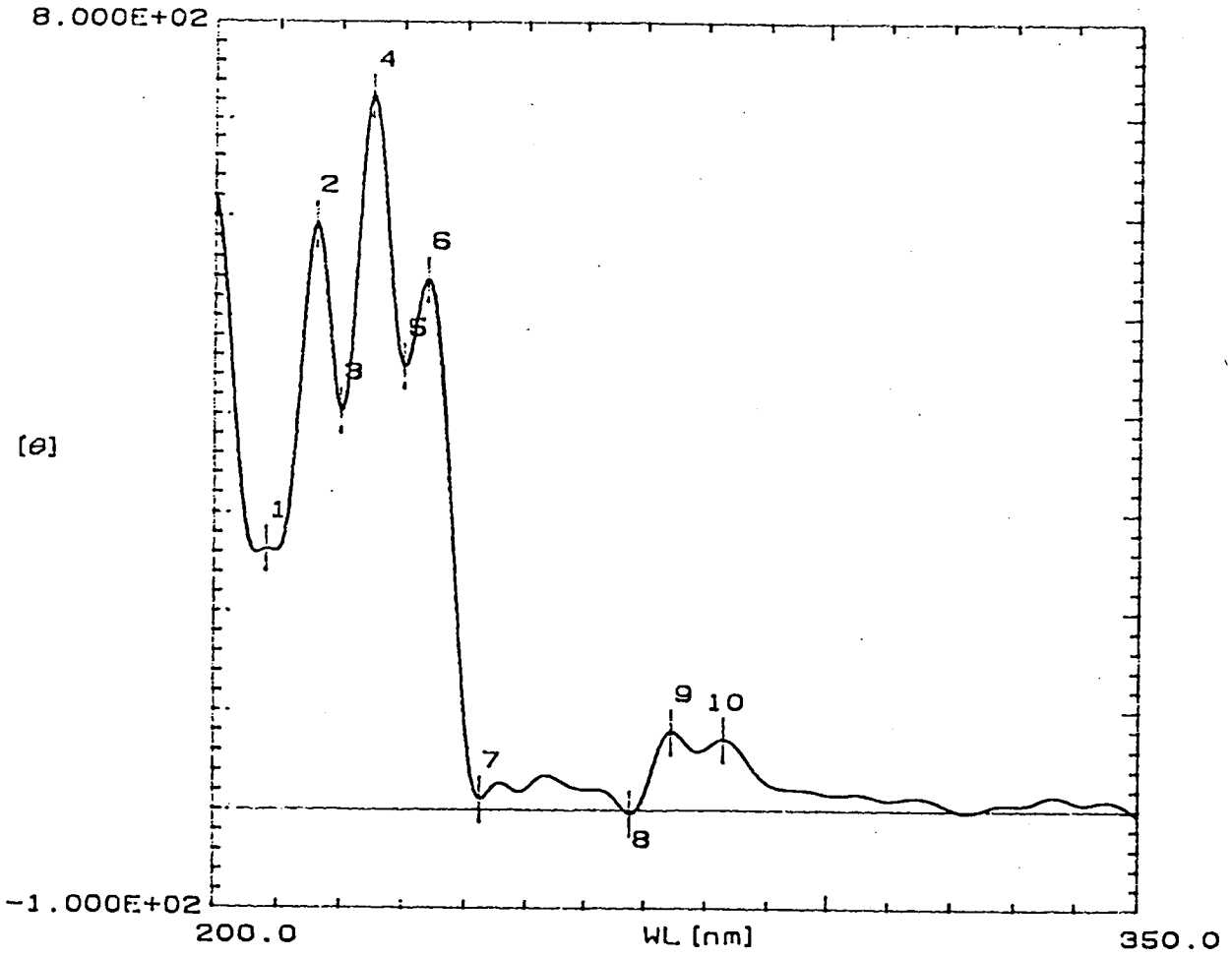
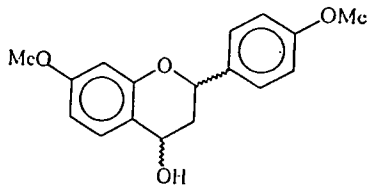
Plate10(161)

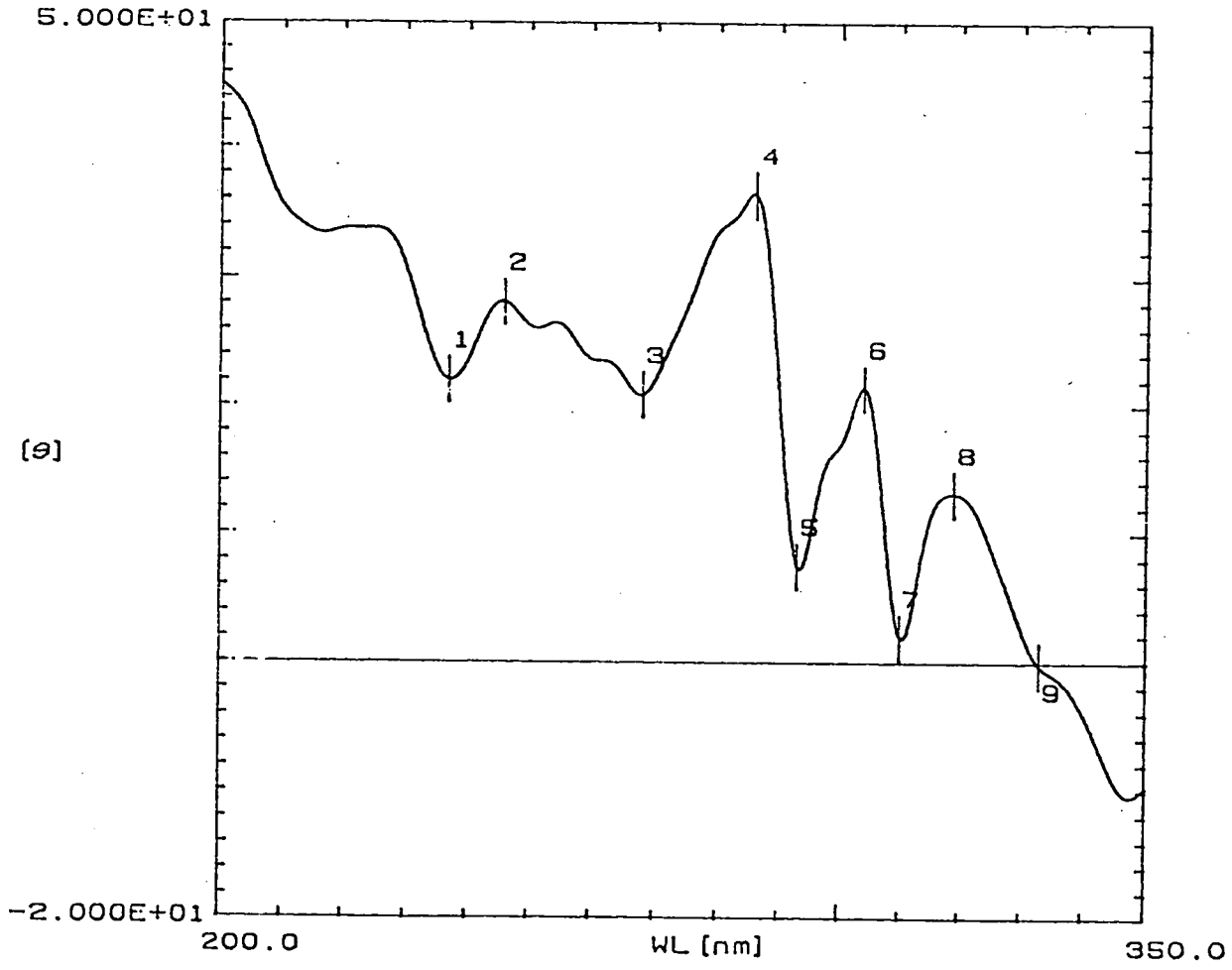


No.	Wavelength	Value
1	208.30 nm	2.629E+02
2	216.00 nm	5.923E+02
3	220.00 nm	4.039E+02
4	225.00 nm	7.236E+02
5	230.20 nm	4.486E+02
6	233.80 nm	5.365E+02
7	242.60 nm	1.093E+01
8	267.70 nm	-3.368E+00
9	274.50 nm	7.919E+01
10	282.90 nm	7.140E+01



162

Plate11(162)



No.	Wavelength	Value
1	237.00 nm	2.203E+01
2	246.00 nm	2.809E+01
3	268.00 nm	2.091E+01
4	286.00 nm	3.634E+01
5	293.00 nm	7.475E+00
6	304.00 nm	2.142E+01
7	310.00 nm	1.939E+00
8	319.00 nm	1.315E+01
9	333.00 nm	-2.205E-01

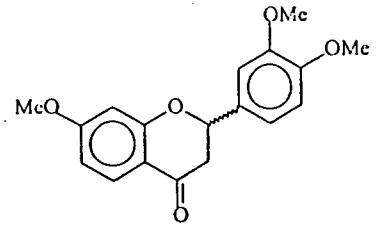


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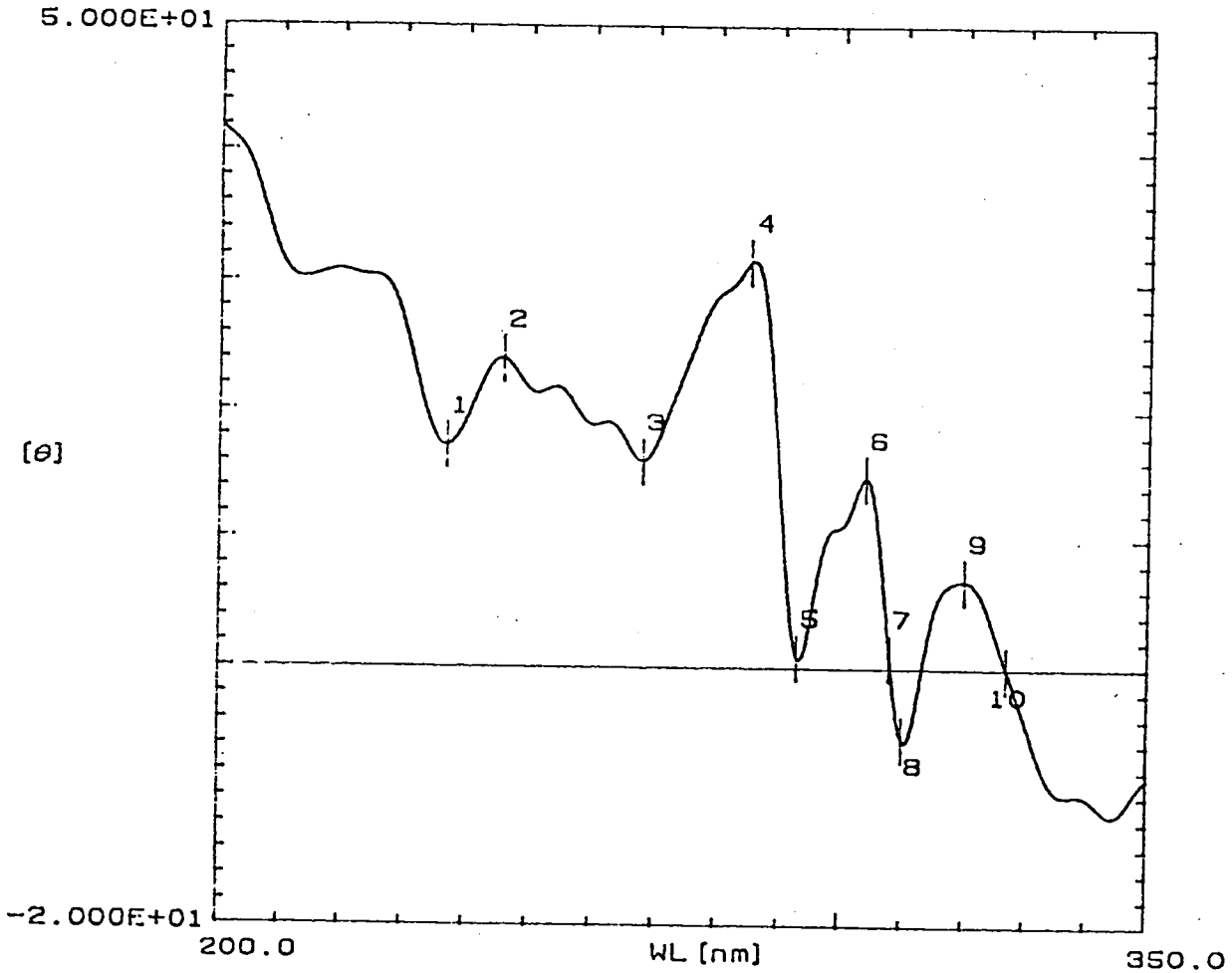
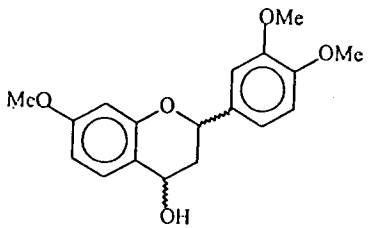
Plate12(165)



No.	Wavelength	Value
1	237.00 nm	1.725E+01
2	246.00 nm	2.398E+01
3	268.00 nm	1.601E+01
4	285.00 nm	3.160E+01
5	293.00 nm	8.233E-01
6	304.00 nm	1.474E+01
7	308.00 nm	8.286E-01
8	310.00 nm	-5.522E+00
9	320.00 nm	6.794E+00
10	327.00 nm	-1.053E-01



166

Plate13(166)

NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

INDEX-NMR SPECTRA

^{1}H NMR:

9. *ent*-Butiniflavan-(4β→8)-epigallocatechin permethyl ether acetate **155**

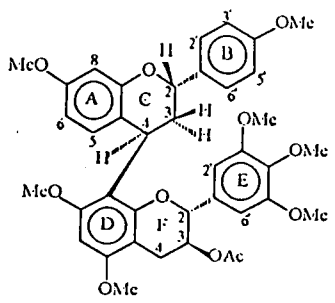
10. 4',7-Dimethoxyflavanone **161**

11. 4',7-Dimethoxyflavan-4-ol **162**

12. 3',4',7-Trimethoxyflavanone **165**

13. 3',4',7-Trimethoxyflavan-4-ol **166**

Plate1(C_6D_6,343K)



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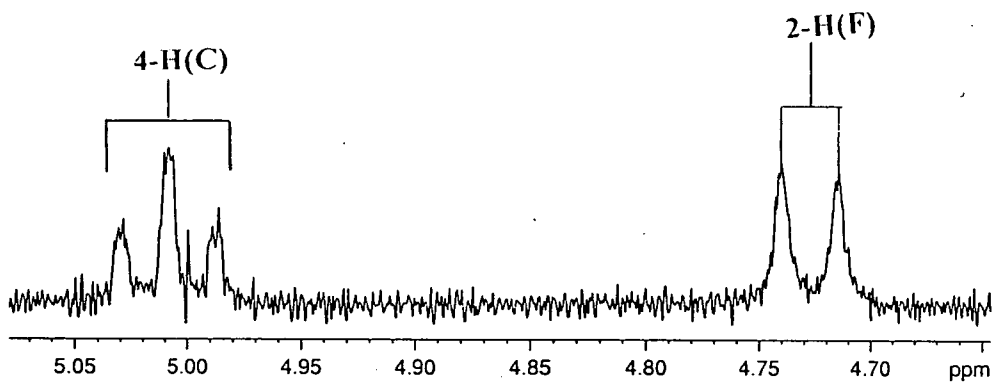
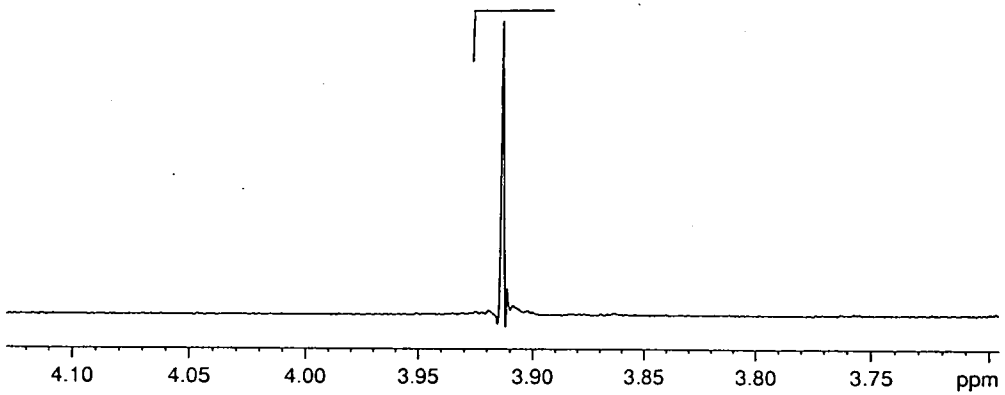
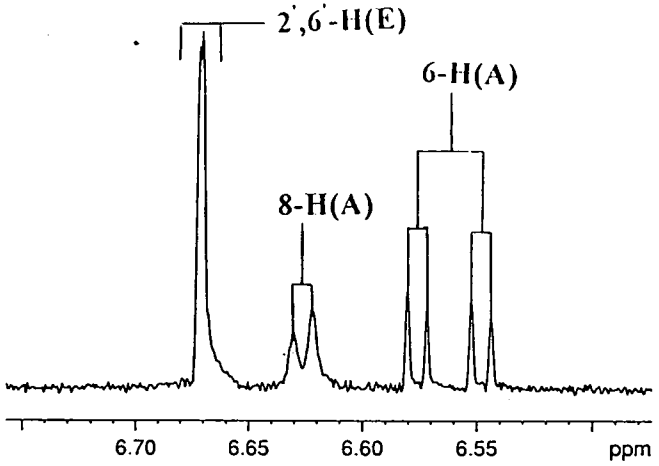
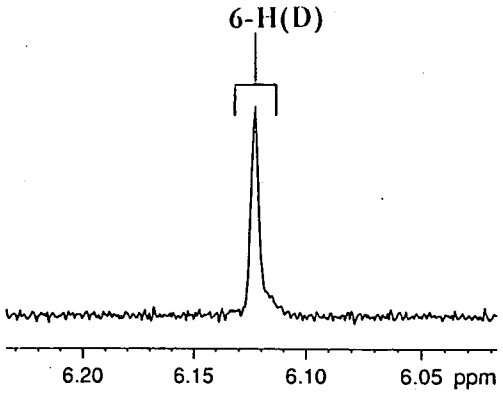
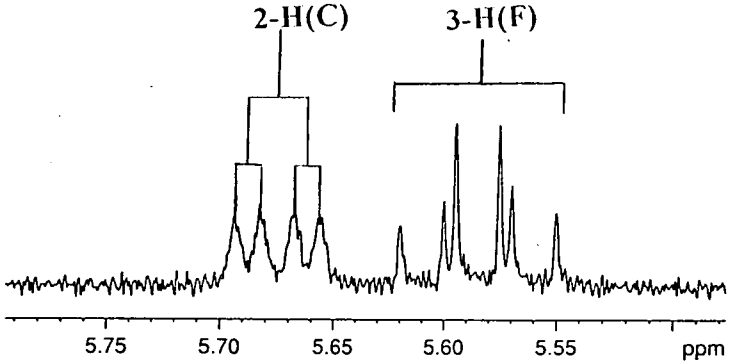
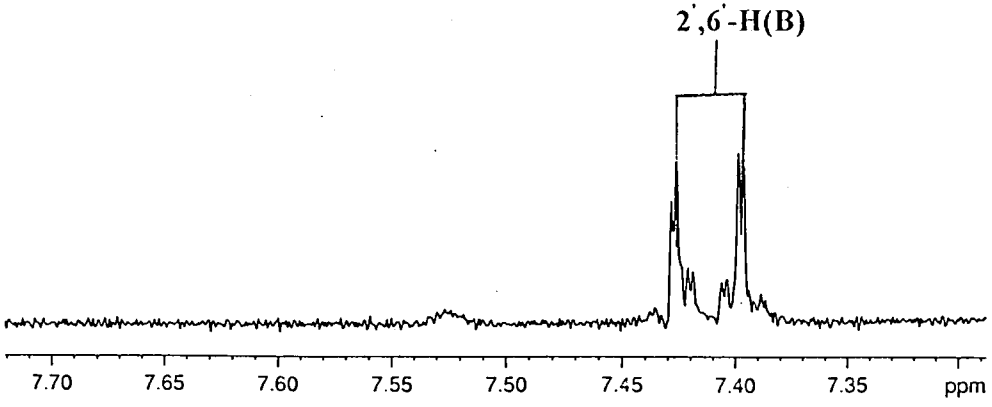

4-H(C)
2-H(F)
5.05
5.00
4.95
4.90
4.85
4.80
4.75
4.70
ppm

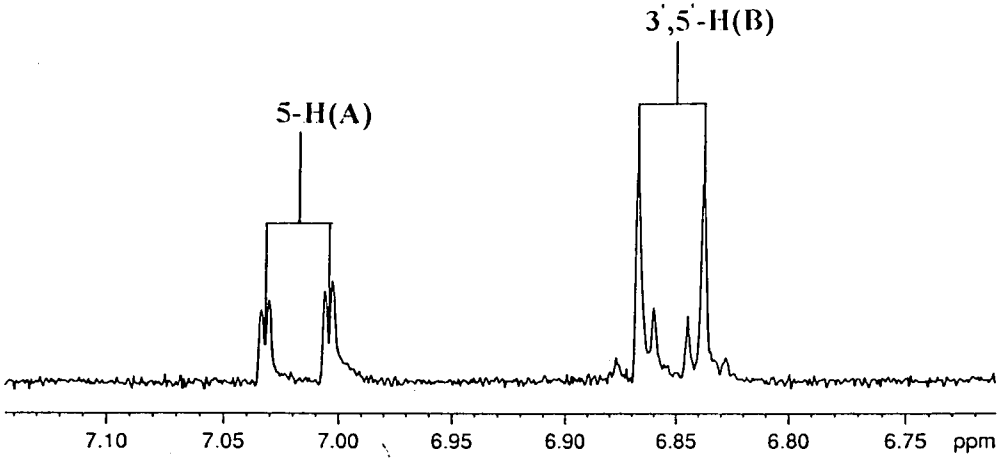

4.10
4.05
4.00
3.95
3.90
3.85
3.80
3.75
ppm


2',6'-H(E)
6-H(A)
8-H(A)
6.70
6.65
6.60
6.55
ppm


6-H(D)
6.20
6.15
6.10
6.05 ppm


2-H(C)
3-H(F)
5.75
5.70
5.65
5.60
5.55
ppm


2',6'-H(B)
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
ppm


3',5'-H(B)
5-H(A)
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
ppm

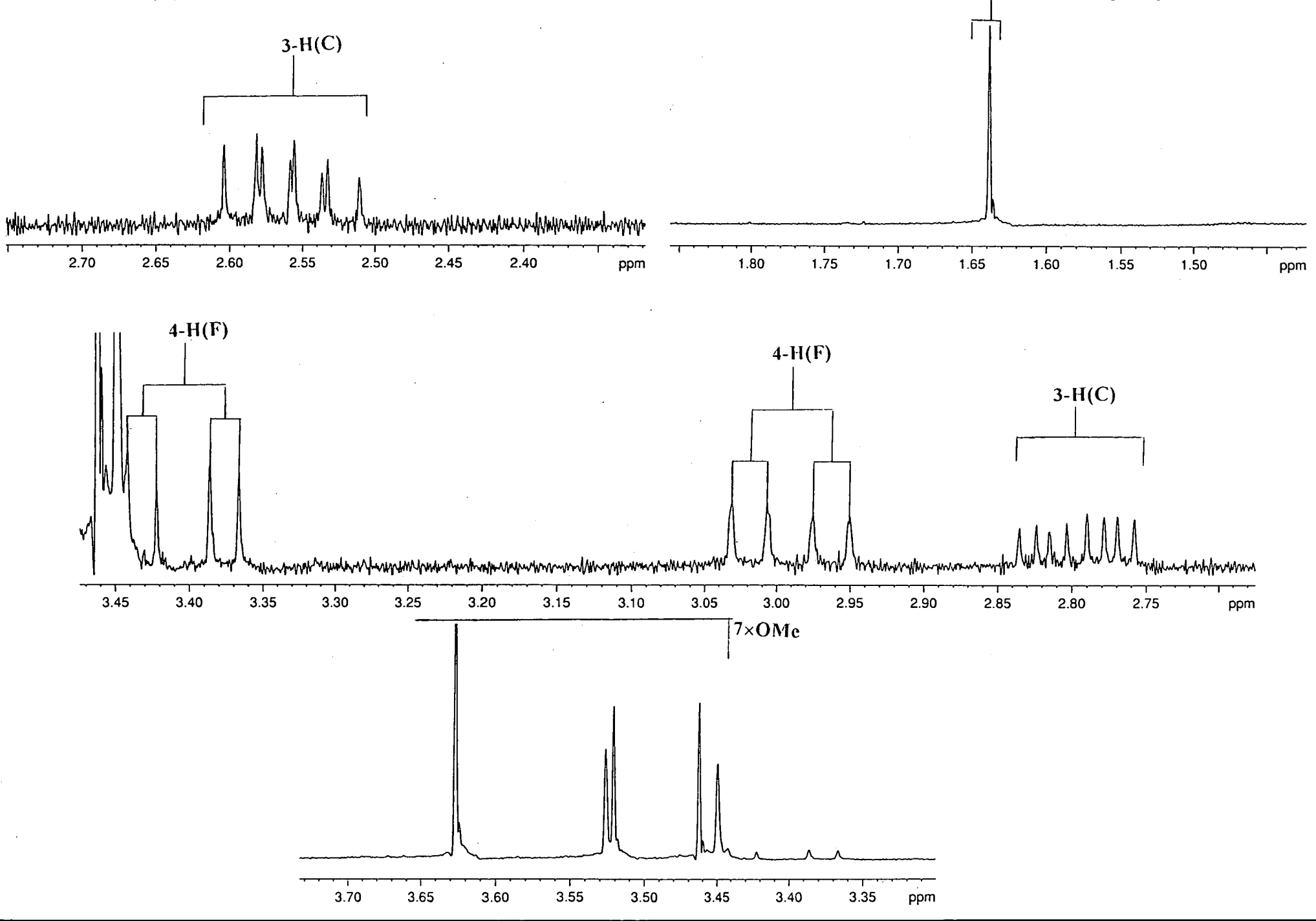
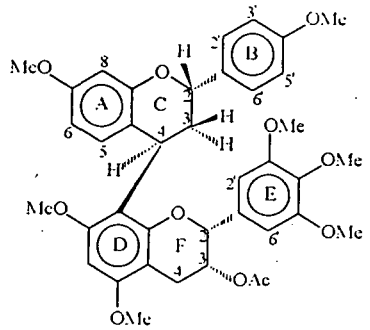
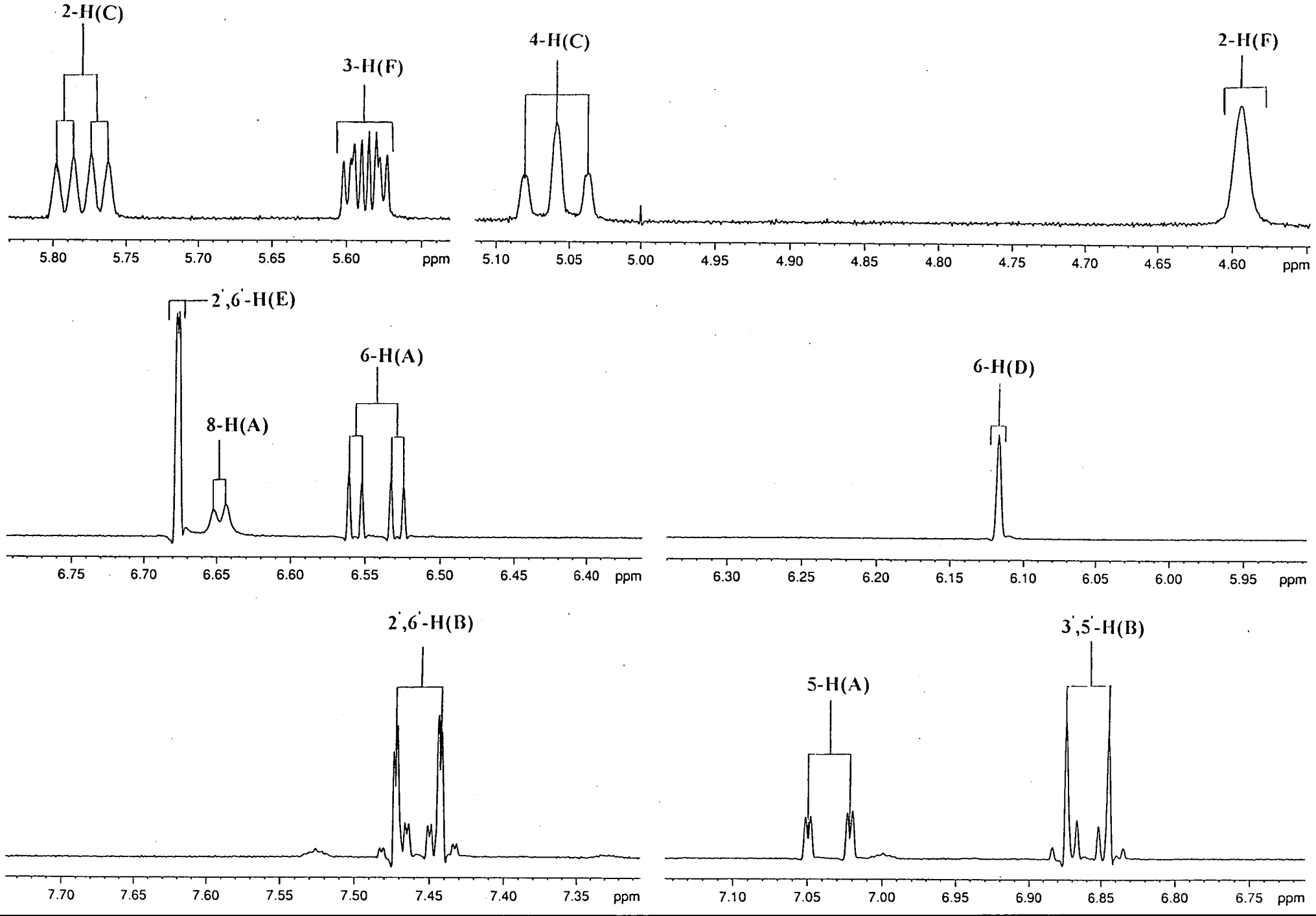
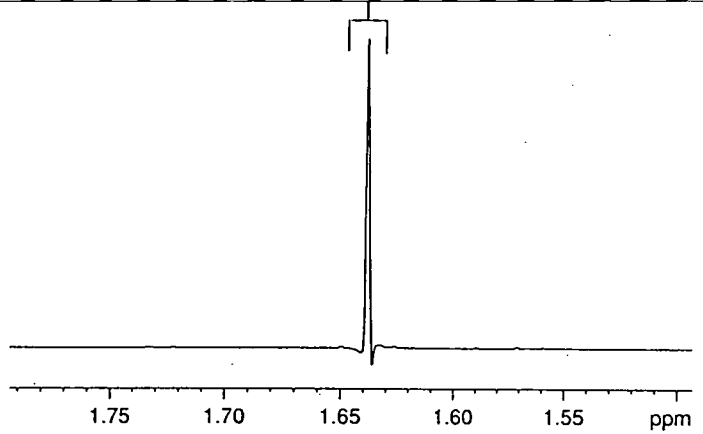

3-H(C)
2.70
2.65
2.60
2.55
2.50
2.45
2.40
ppm
1.80
1.75
1.70
1.65
1.60
1.55
1.50
ppm
4-H(F)
4-H(F)
3-H(C)
3.45
3.40
3.35
3.30
3.25
3.20
3.15
3.10
3.05
3.00
2.95
2.90
2.85
2.80
2.75
ppm
7×OMe
3.70
3.65
3.60
3.55
3.50
3.45
3.40
3.35
ppm

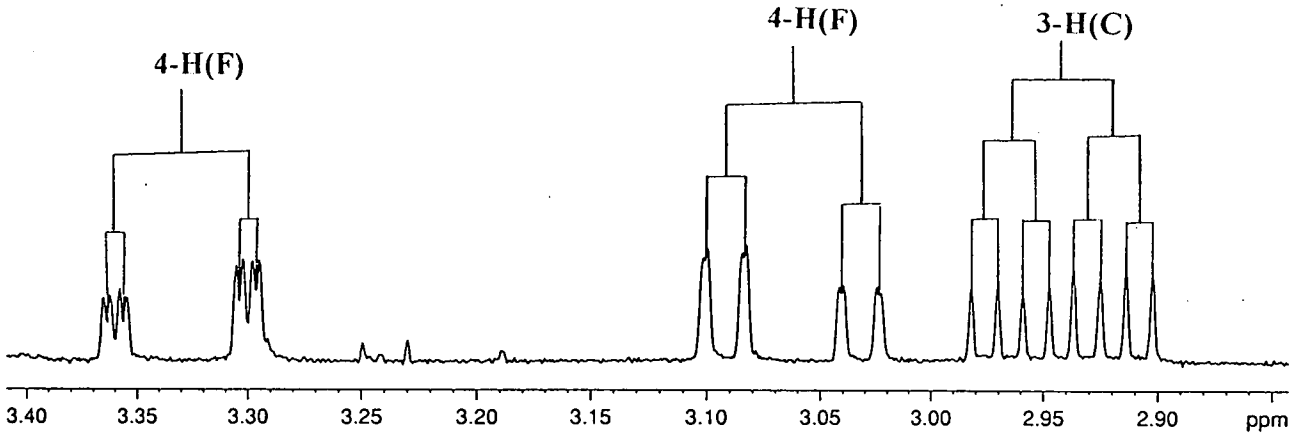
Plate2(C_6D_6,343K)

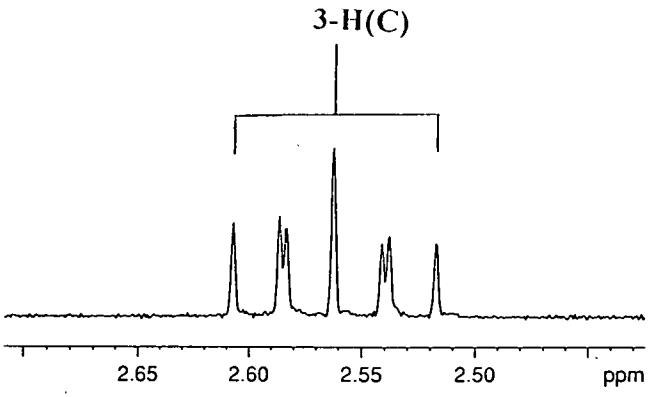


148


2-H(C)
3-H(F)
5.80
5.75
5.70
5.65
5.60
ppm
4-H(C)
2-H(F)
5.10
5.05
5.00
4.95
4.90
4.85
4.80
4.75
4.70
4.65
4.60
ppm
2',6'-H(E)
6-H(A)
8-H(A)
6.75
6.70
6.65
6.60
6.55
6.50
6.45
6.40
ppm
6-H(D)
6.30
6.25
6.20
6.15
6.10
6.05
6.00
5.95
ppm
2',6'-H(B)
7.70
7.65
7.60
7.55
7.50
7.45
7.40
7.35
ppm
3',5'-H(B)
5-H(A)
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
ppm


1.75
1.70
1.65
1.60
1.55
ppm


4-H(F)
4-H(F)
3-H(C)
3.40
3.35
3.30
3.25
3.20
3.15
3.10
3.05
3.00
2.95
2.90
ppm


3-H(C)
2.65
2.60
2.55
2.50
ppm

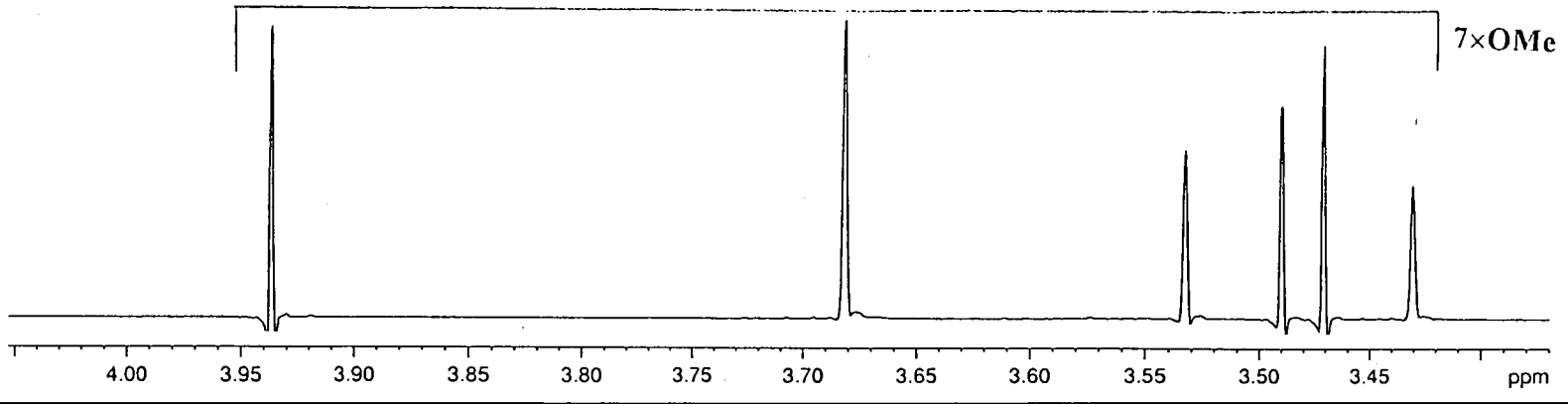
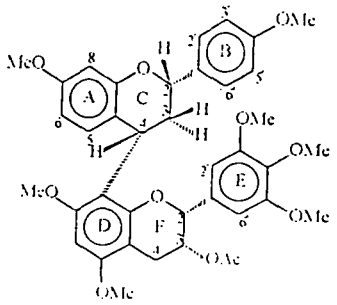
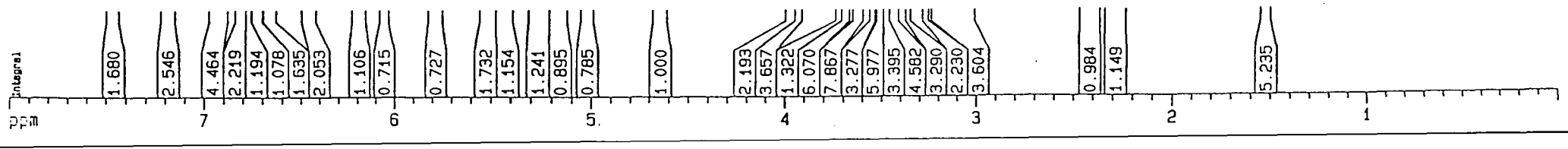
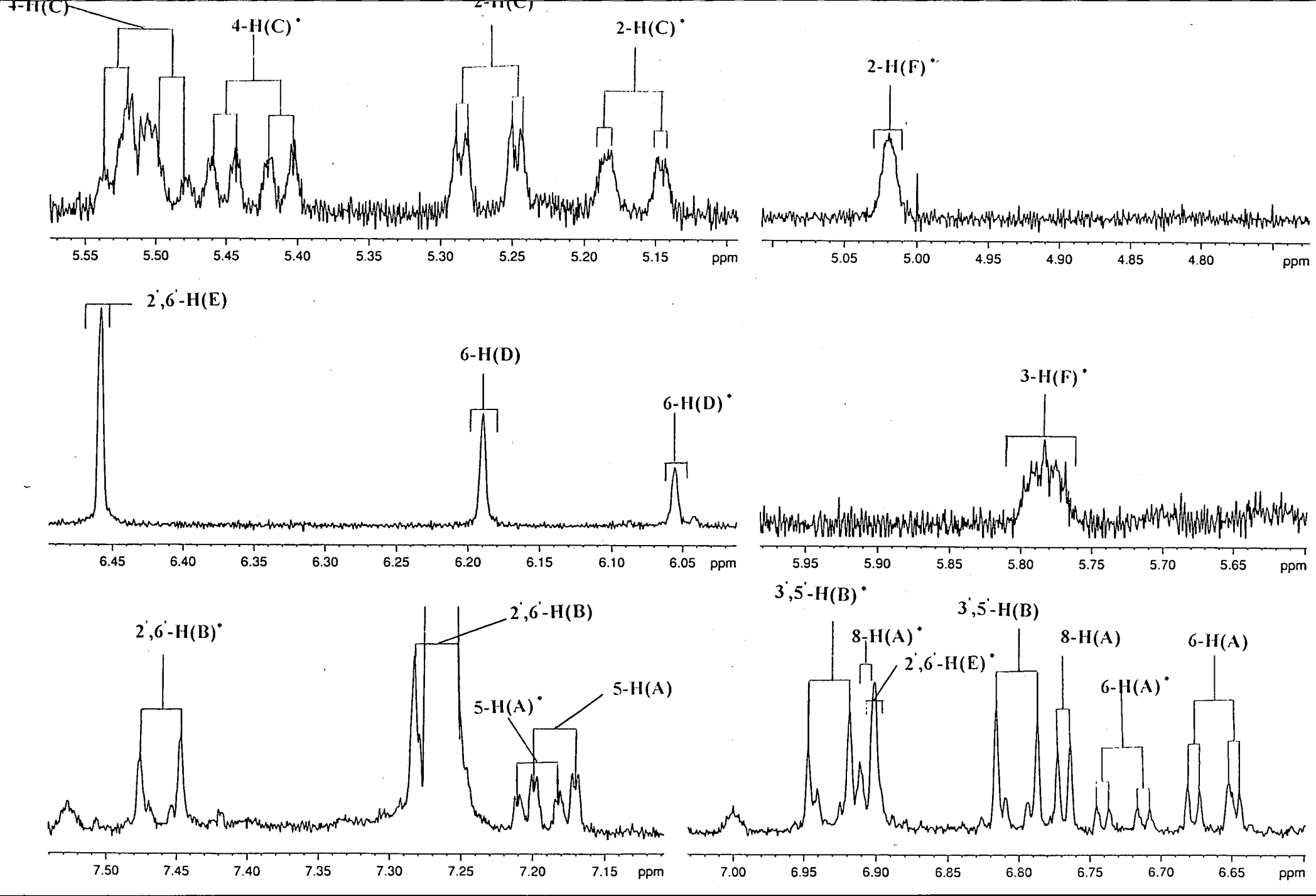

7×OMe
4.00
3.95
3.90
3.85
3.80
3.75
3.70
3.65
3.60
3.55
3.50
3.45
ppm

Plate3(C_6D_6,313K)



149




4-H(C)
4-H(C)*
2-H(C)*
5.55
5.50
5.45
5.40
5.35
5.30
5.25
5.20
5.15
ppm
2-H(F)*
5.05
5.00
4.95
4.90
4.85
4.80
ppm
2',6'-H(E)
6-H(D)
6-H(D)*
6.45
6.40
6.35
6.30
6.25
6.20
6.15
6.10
6.05
ppm
3-H(F)*
5.95
5.90
5.85
5.80
5.75
5.70
5.65
ppm
2',6'-H(B)*
2',6'-H(B)
5-H(A)*
5-H(A)
7.50
7.45
7.40
7.35
7.30
7.25
7.20
7.15
ppm
3',5'-H(B)*
8-H(A)*
2',6'-H(E)*
3',5'-H(B)
8-H(A)
6-H(A)*
6-H(A)
7.00
6.95
6.90
6.85
6.80
6.75
6.70
6.65
ppm

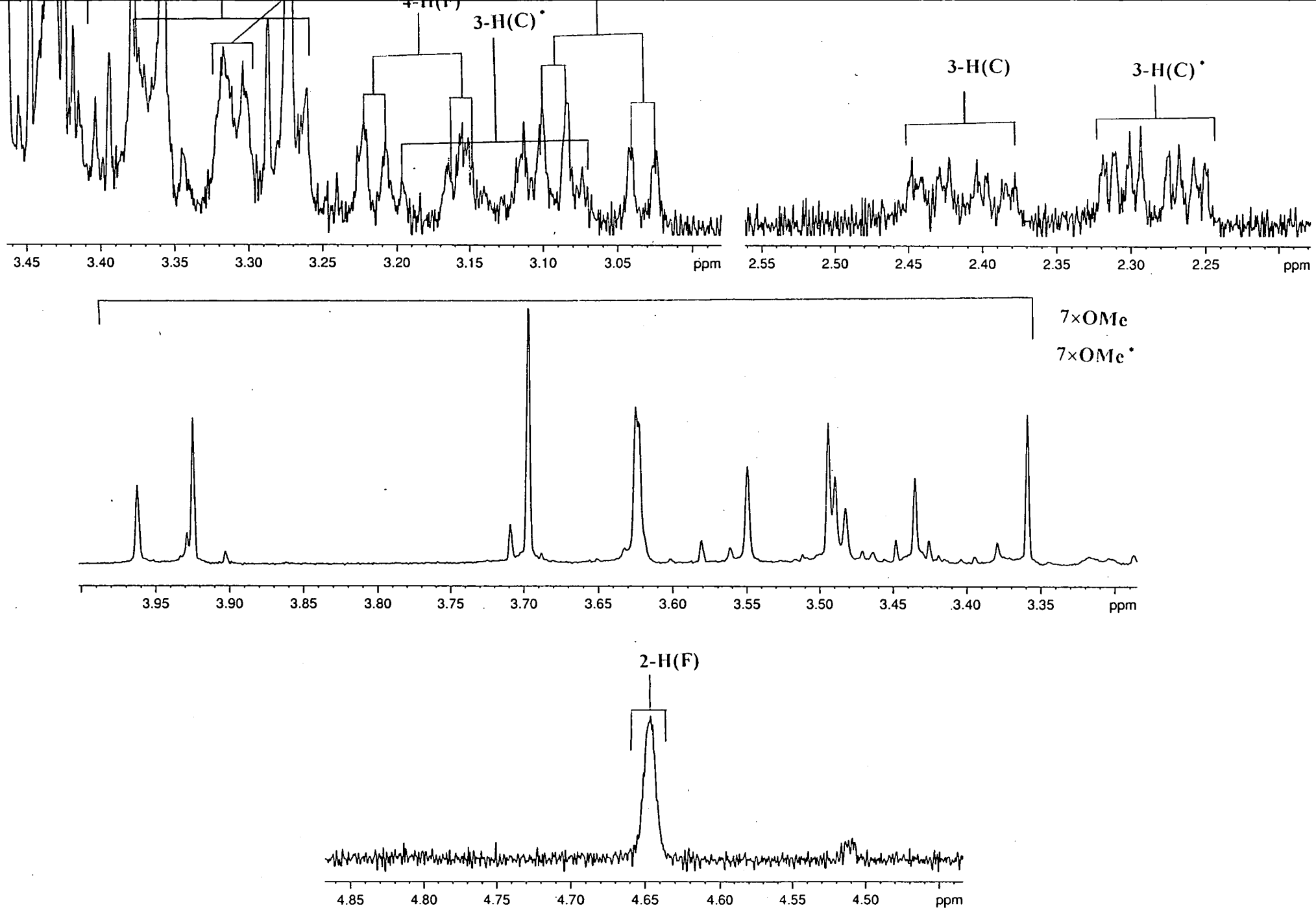
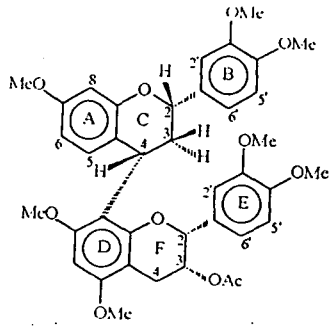
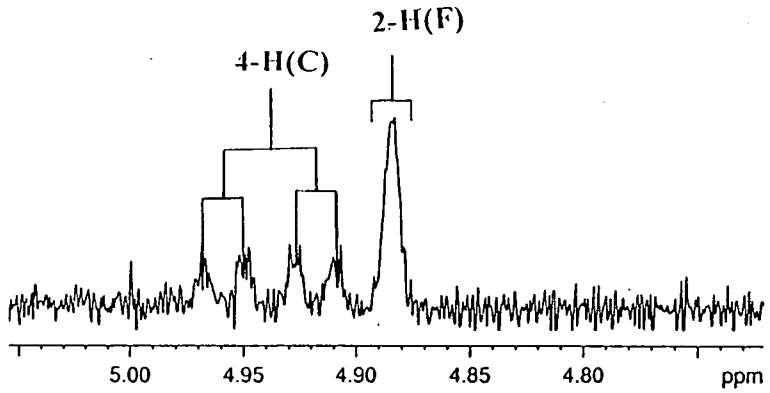
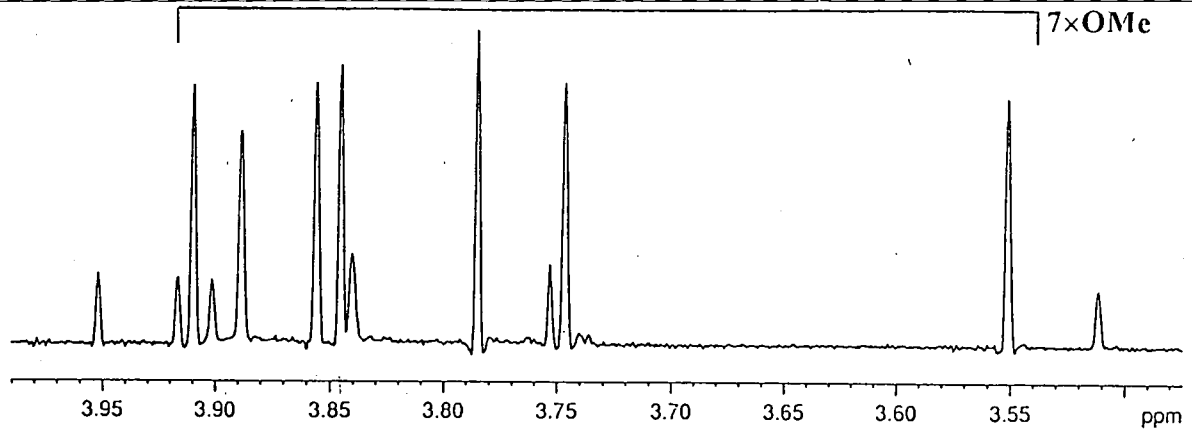

3-H(C)*
3-H(C)
3-H(C)*
3.45 3.40 3.35 3.30 3.25 3.20 3.15 3.10 3.05 ppm
2.55 2.50 2.45 2.40 2.35 2.30 2.25 ppm
7×OMe
7×OMe*
3.95 3.90 3.85 3.80 3.75 3.70 3.65 3.60 3.55 3.50 3.45 3.40 3.35 ppm
2-H(F)
4.85 4.80 4.75 4.70 4.65 4.60 4.55 4.50 ppm

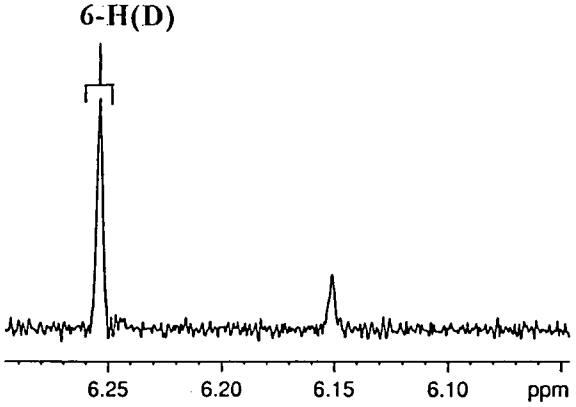
Plate4(CDCl$_3$,293K)

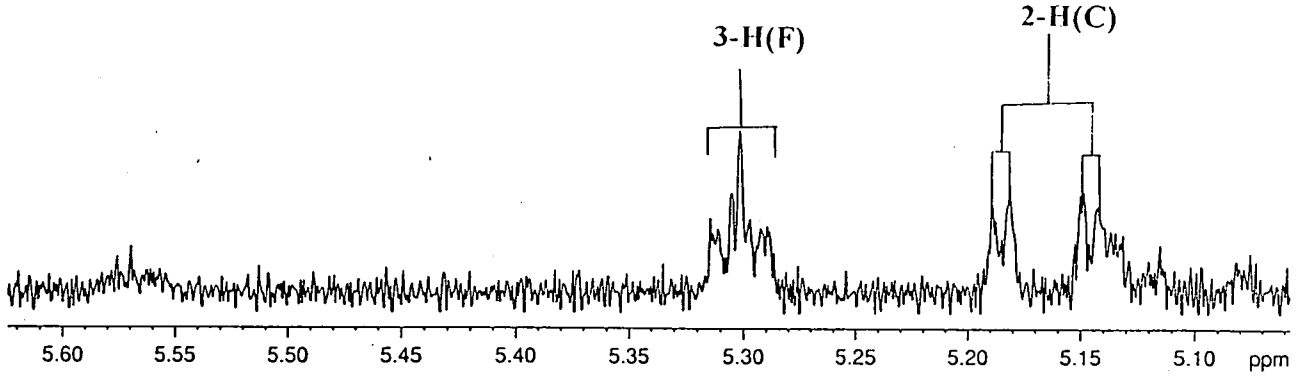


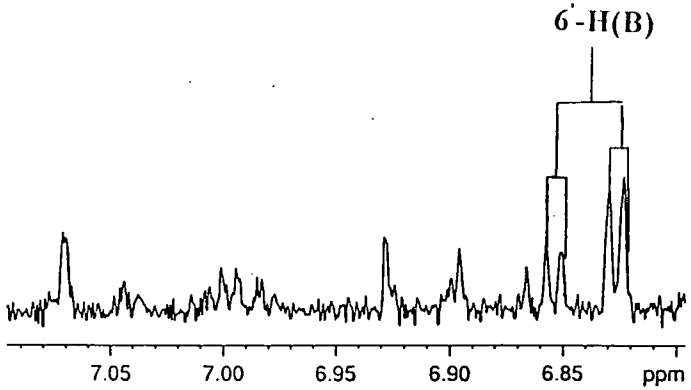
150

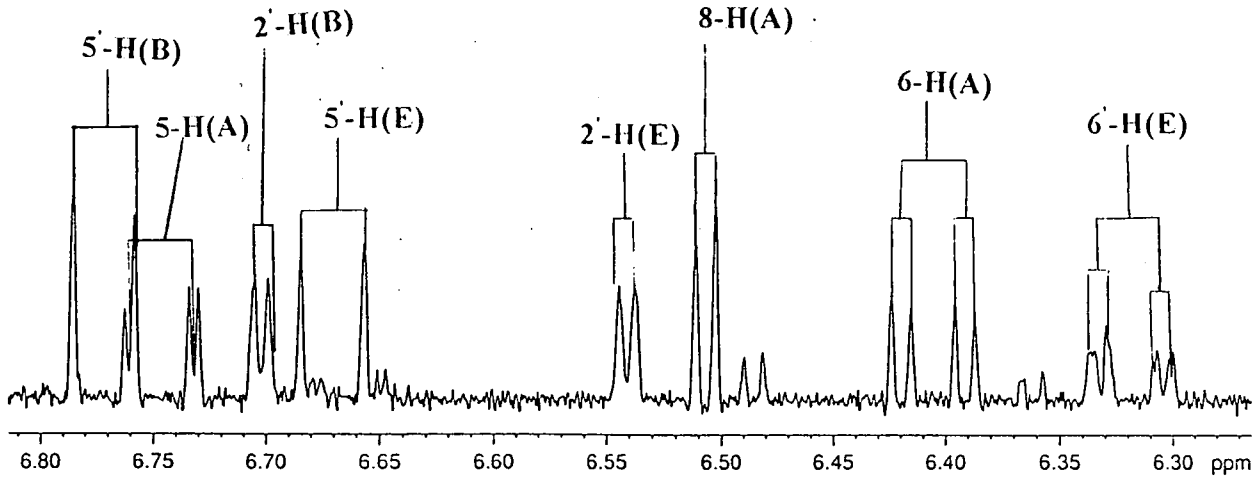

2-H(F)
4-H(C)
5.00
4.95
4.90
4.85
4.80
ppm

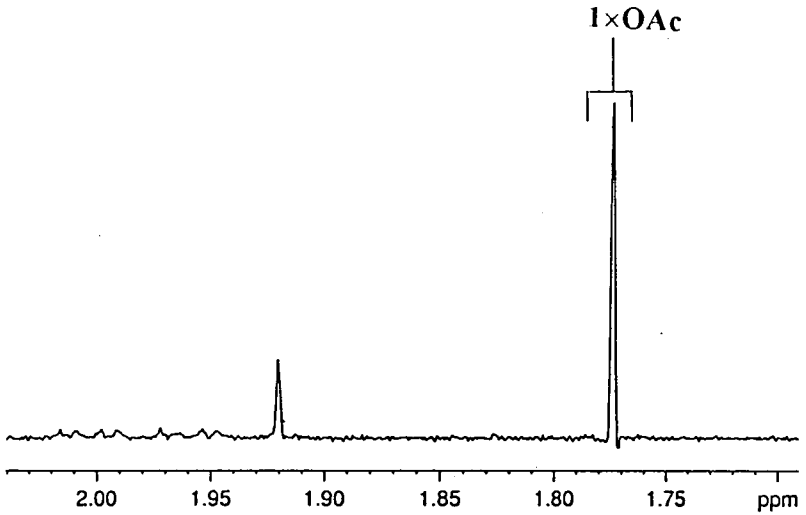

7×OMe
3.95
3.90
3.85
3.80
3.75
3.70
3.65
3.60
3.55
ppm

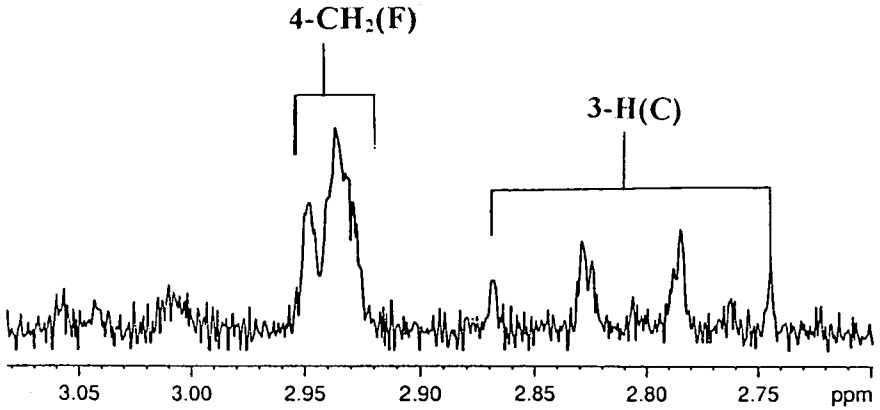

6-H(D)
6.25
6.20
6.15
6.10
ppm


3-H(F)
2-H(C)
5.60
5.55
5.50
5.45
5.40
5.35
5.30
5.25
5.20
5.15
5.10
ppm


6'-H(B)
7.05
7.00
6.95
6.90
6.85
ppm


5'-H(B)
2'-H(B)
8-H(A)
5-H(A)
5'-H(E)
2'-H(E)
6-H(A)
6'-H(E)
6.80
6.75
6.70
6.65
6.60
6.55
6.50
6.45
6.40
6.35
6.30
ppm


1×OAc
2.00
1.95
1.90
1.85
1.80
1.75
ppm


4-CH₂(F)
3-H(C)
3.05
3.00
2.95
2.90
2.85
2.80
2.75
ppm

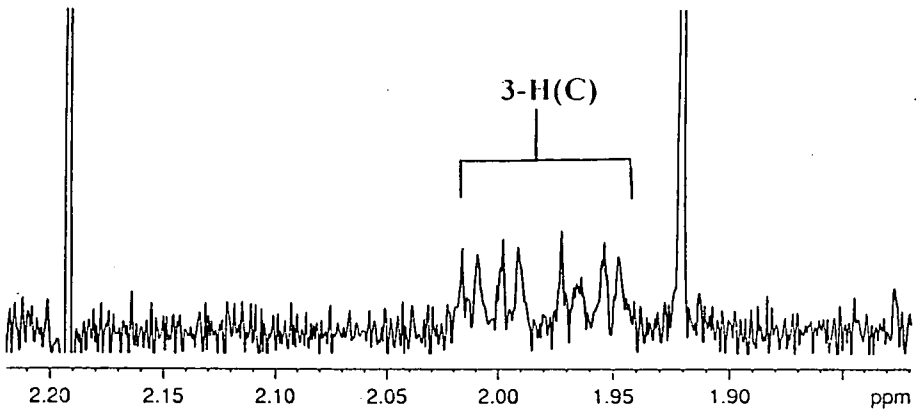
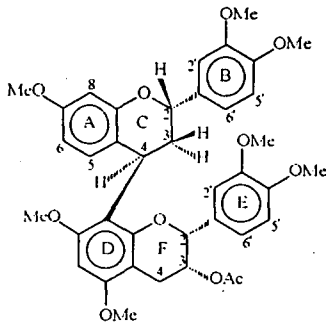

3-H(C)
2.20
2.15
2.10
2.05
2.00
1.95
1.90
ppm

Plate5($CDCl_3$,293K)



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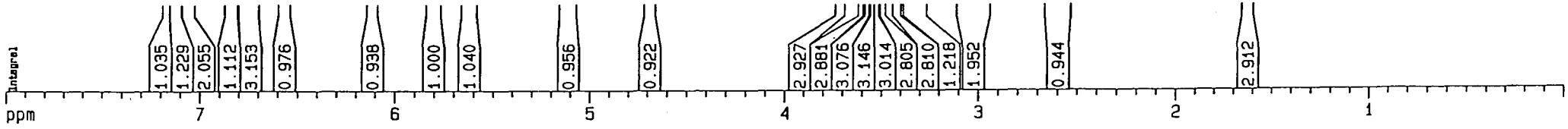
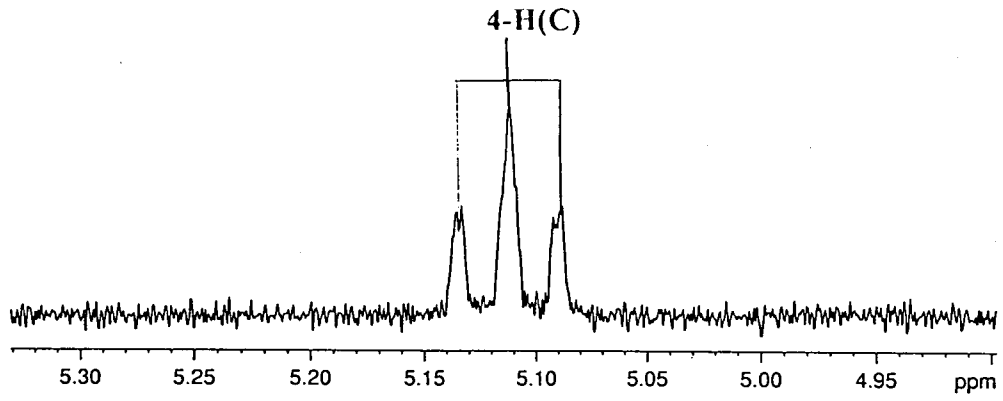
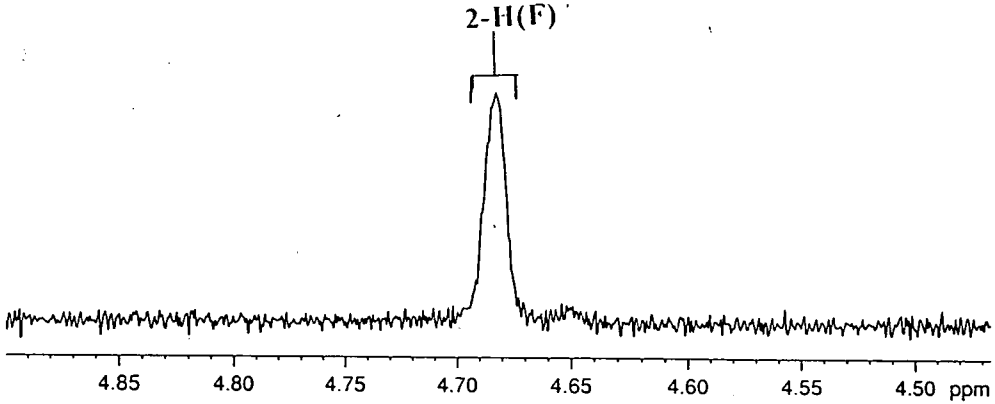
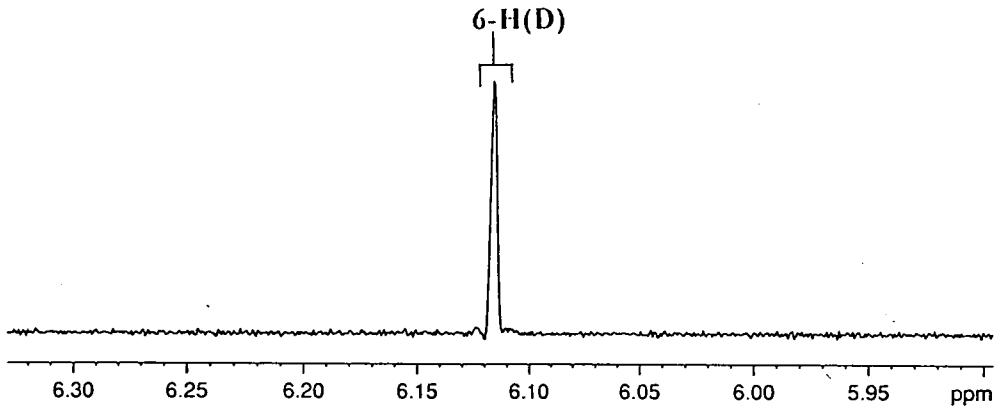
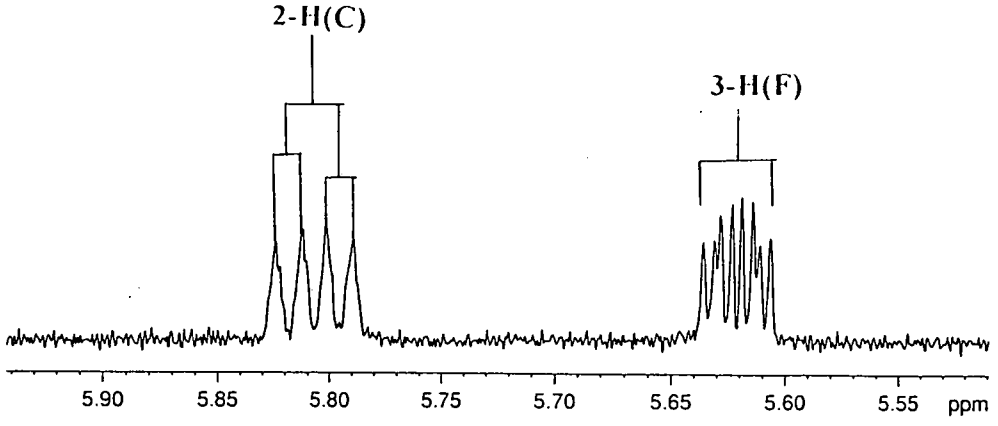
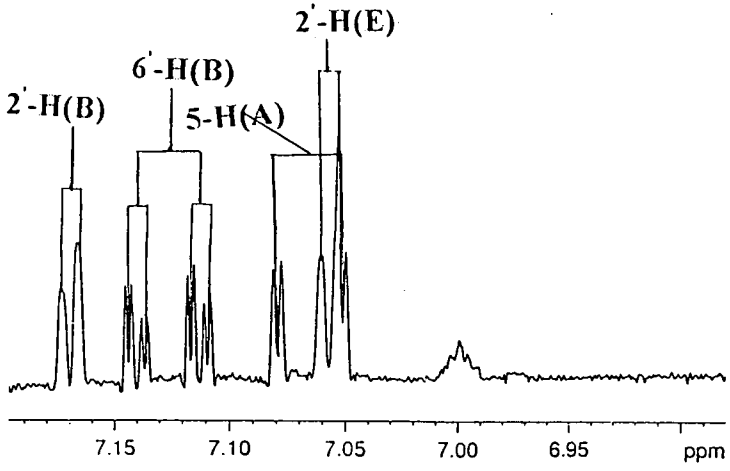


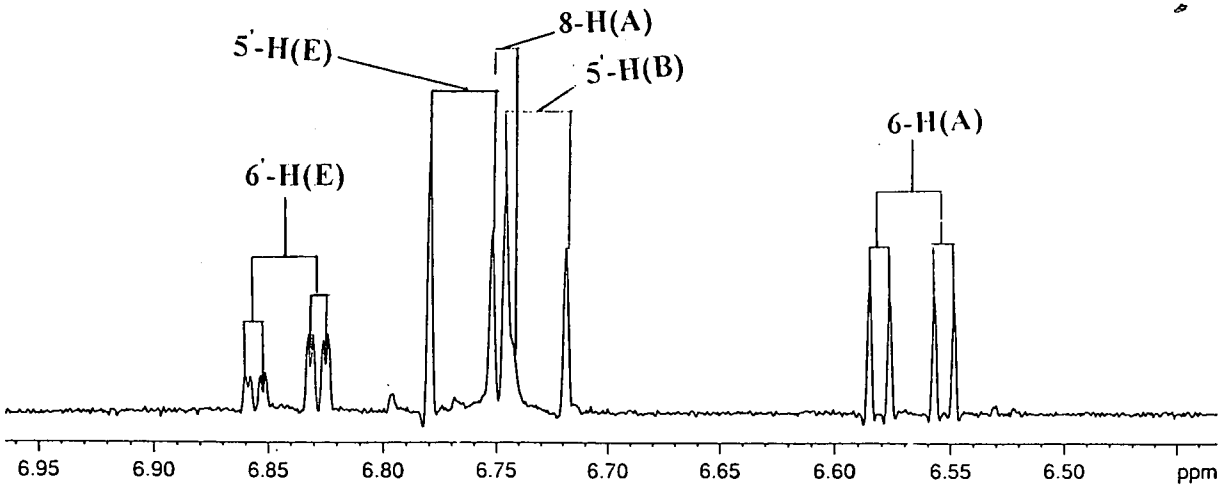

4-H(C)
5.30
5.25
5.20
5.15
5.10
5.05
5.00
4.95
ppm

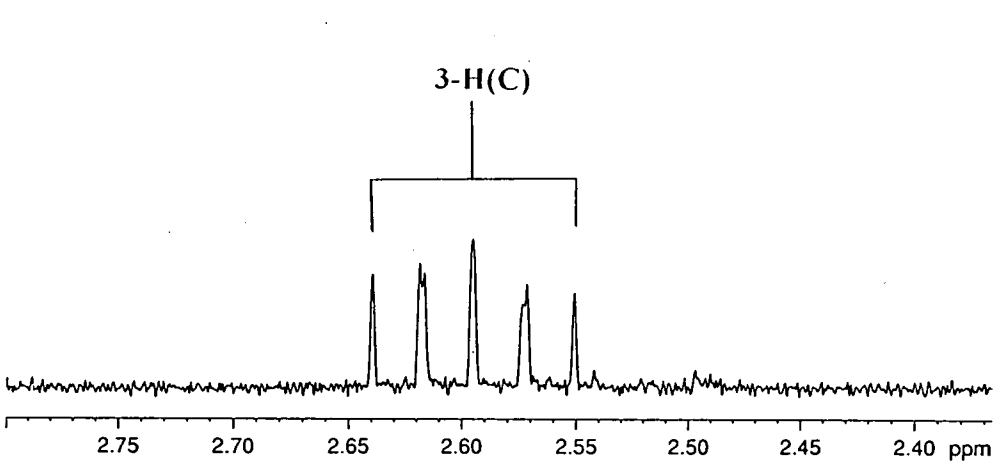

2-H(F)
4.85
4.80
4.75
4.70
4.65
4.60
4.55
4.50
ppm

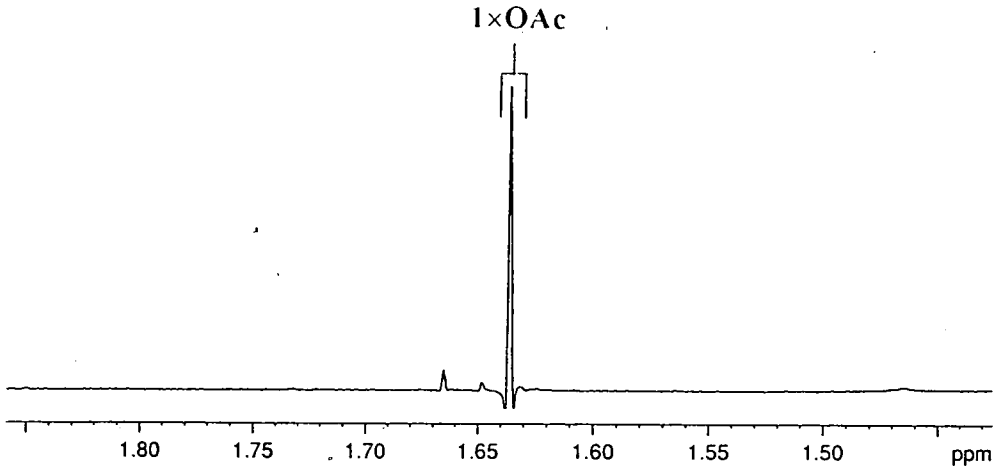

6-H(D)
6.30
6.25
6.20
6.15
6.10
6.05
6.00
5.95
ppm

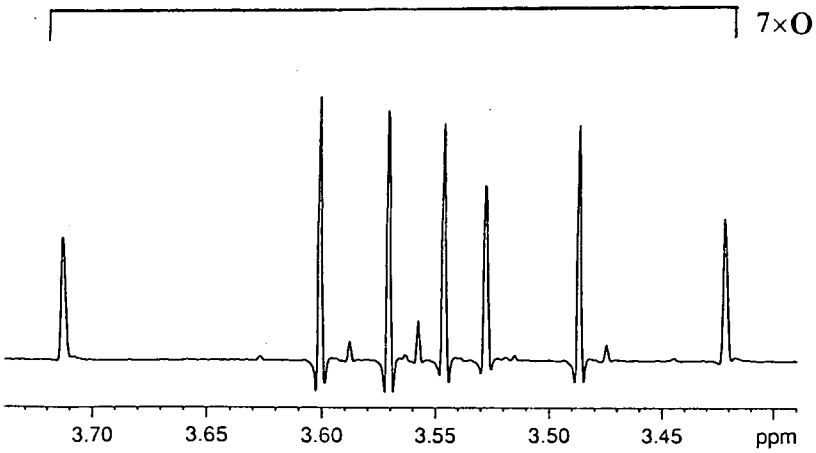

2-H(C)
3-H(F)
5.90
5.85
5.80
5.75
5.70
5.65
5.60
5.55
ppm


2'-H(E)
6'-H(B)
2'-H(B)
5-H(A)
7.15
7.10
7.05
7.00
6.95
ppm


8-H(A)
5'-H(E)
5'-H(B)
6-H(A)
6'-H(E)
6.95
6.90
6.85
6.80
6.75
6.70
6.65
6.60
6.55
6.50
ppm


3-H(C)
2.75
2.70
2.65
2.60
2.55
2.50
2.45
2.40 ppm


1×OAc
1.80
1.75
1.70
1.65
1.60
1.55
1.50
ppm


7×OMe
3.70
3.65
3.60
3.55
3.50
3.45
ppm

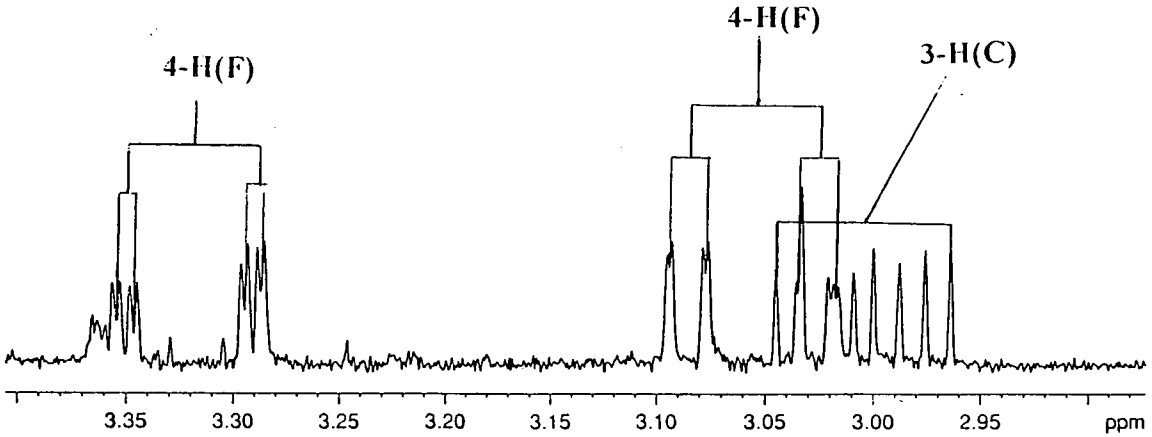
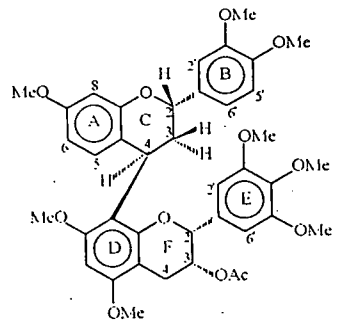

4-H(F)
4-H(F)
3-H(C)
3.35
3.30
3.25
3.20
3.15
3.10
3.05
3.00
2.95
ppm

Plate6(C_6D_6,343K)



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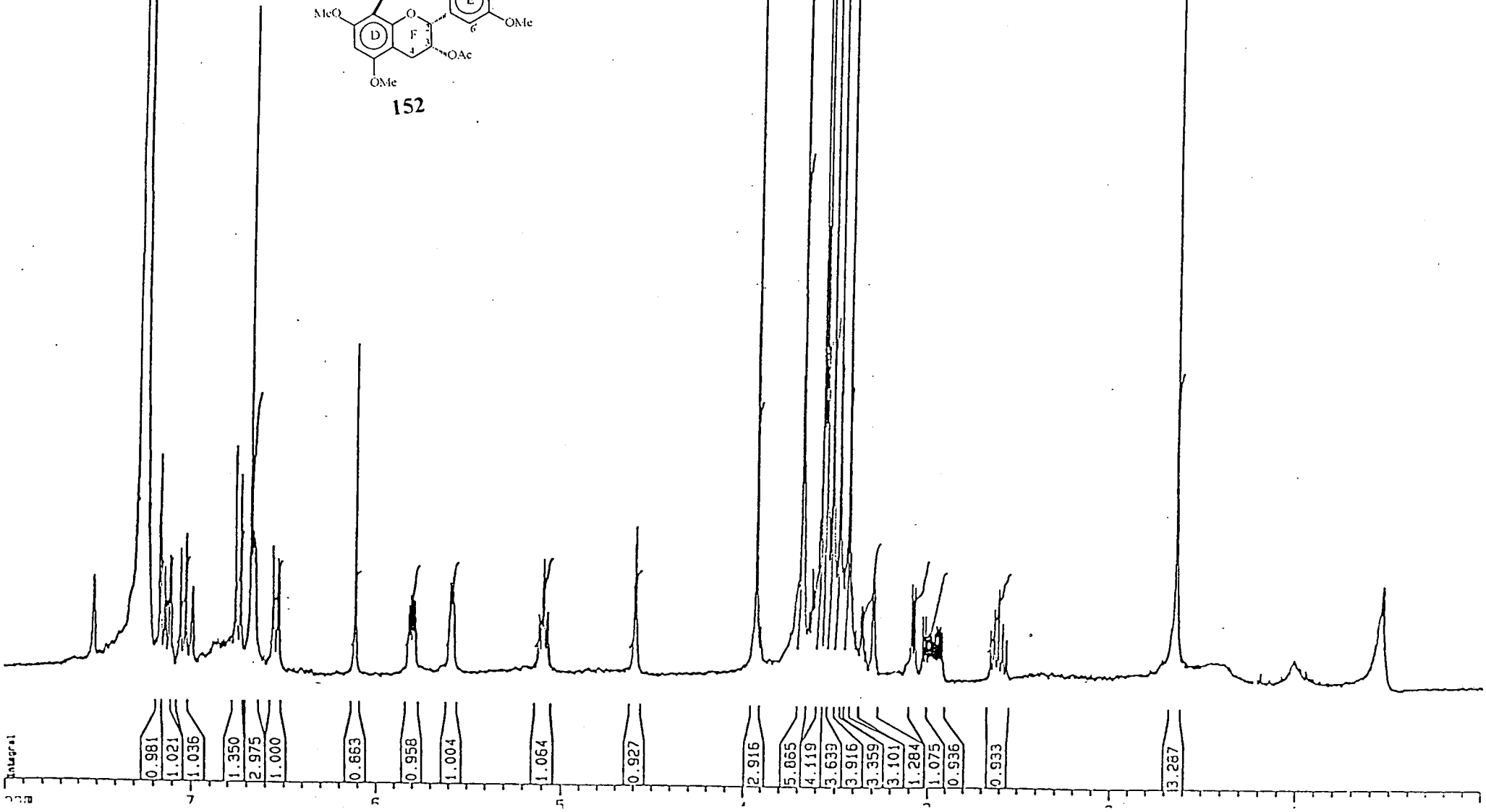
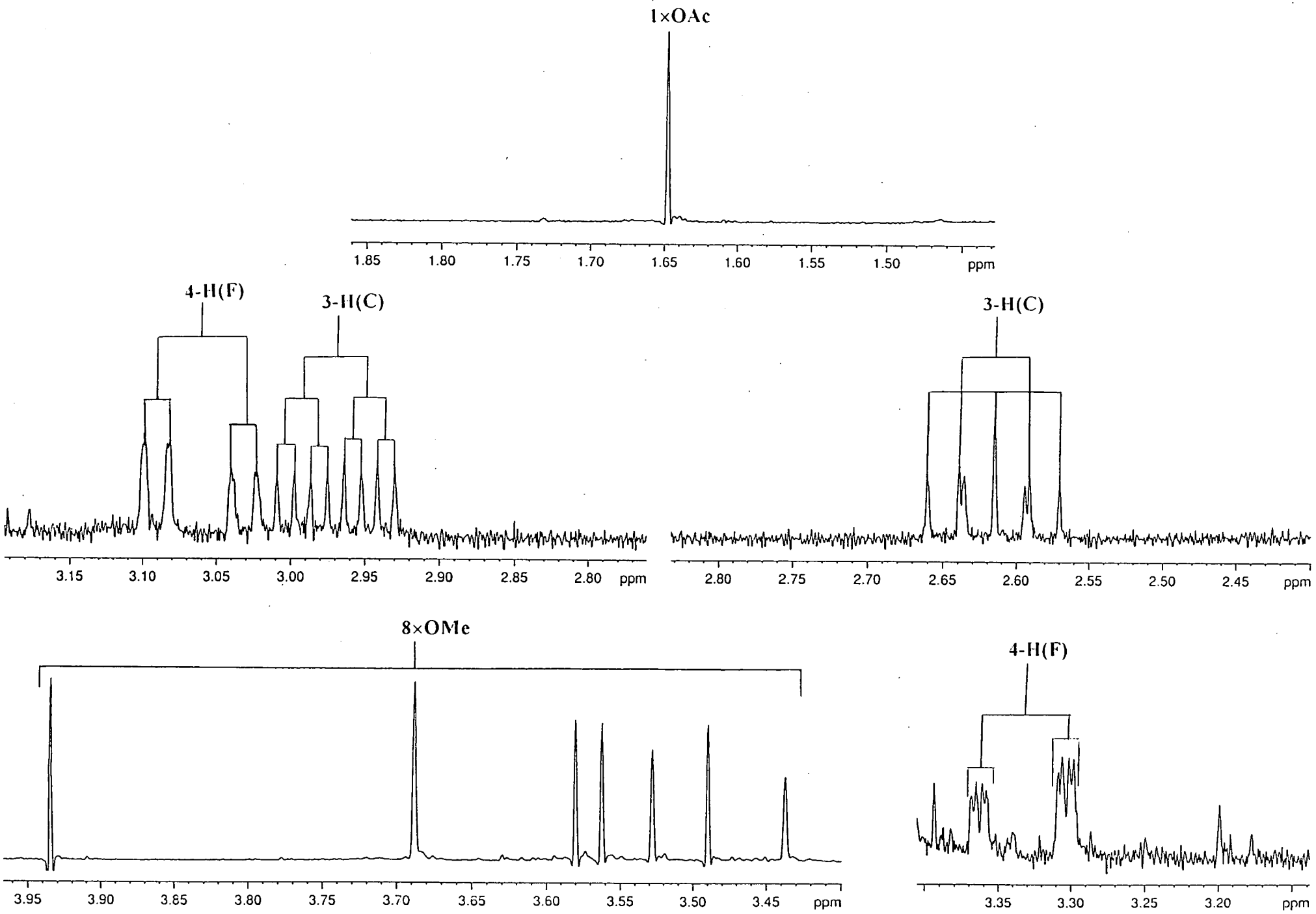
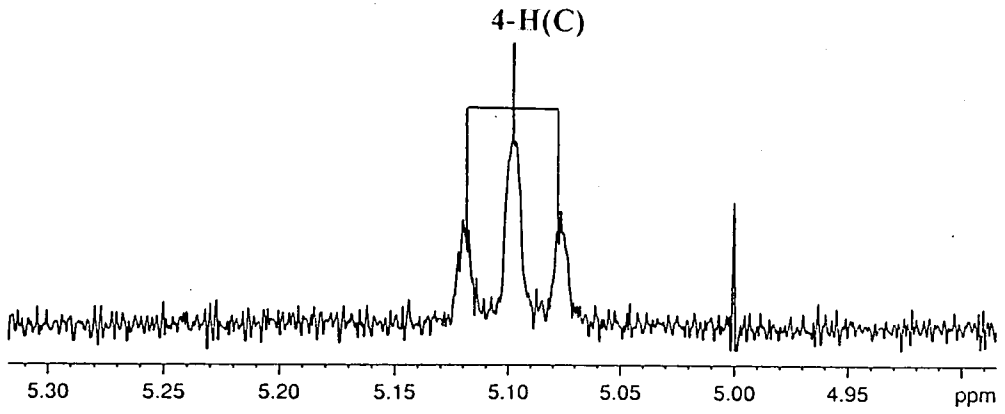
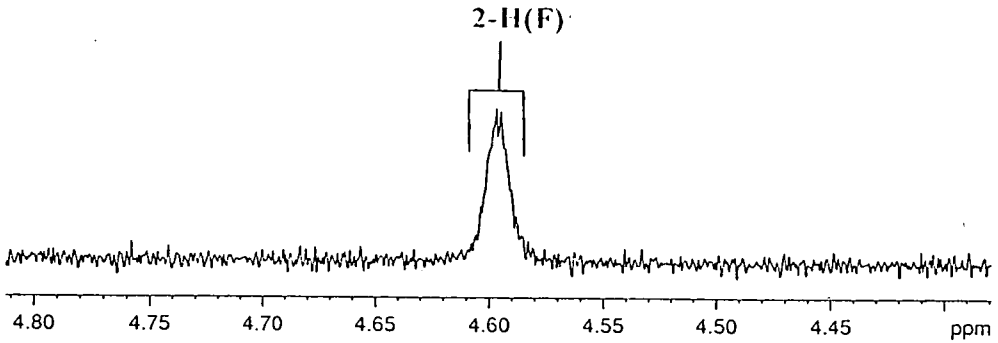
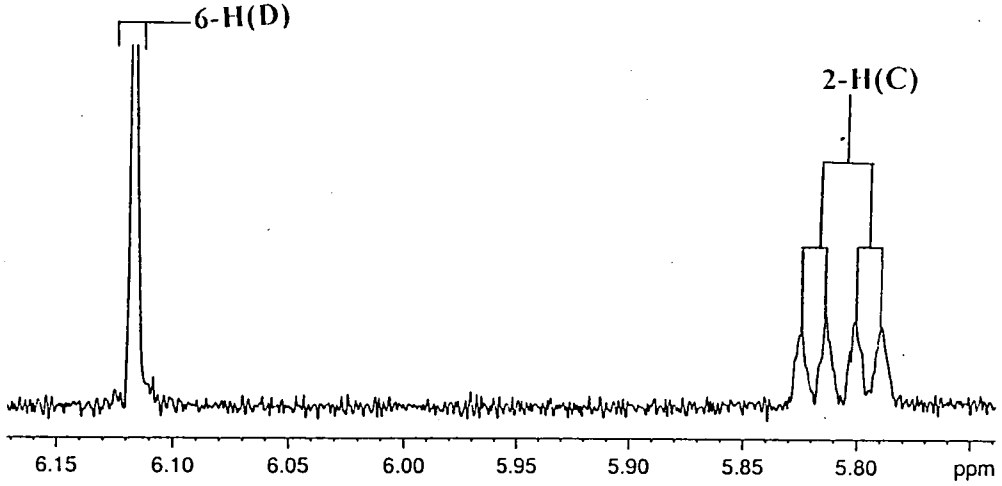
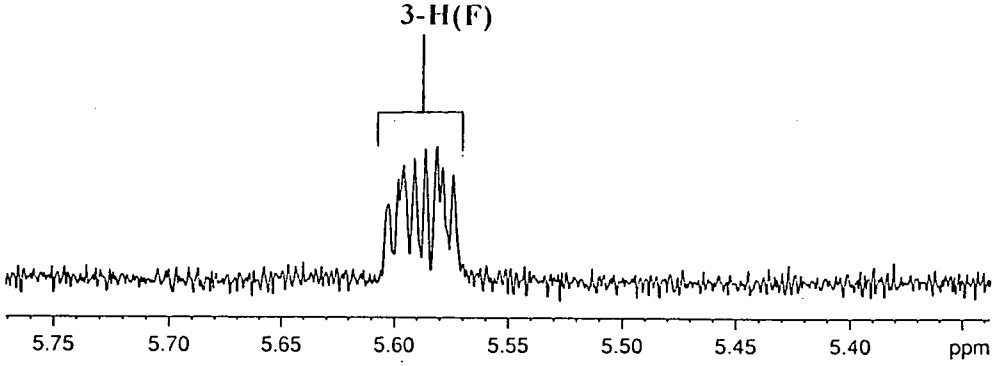


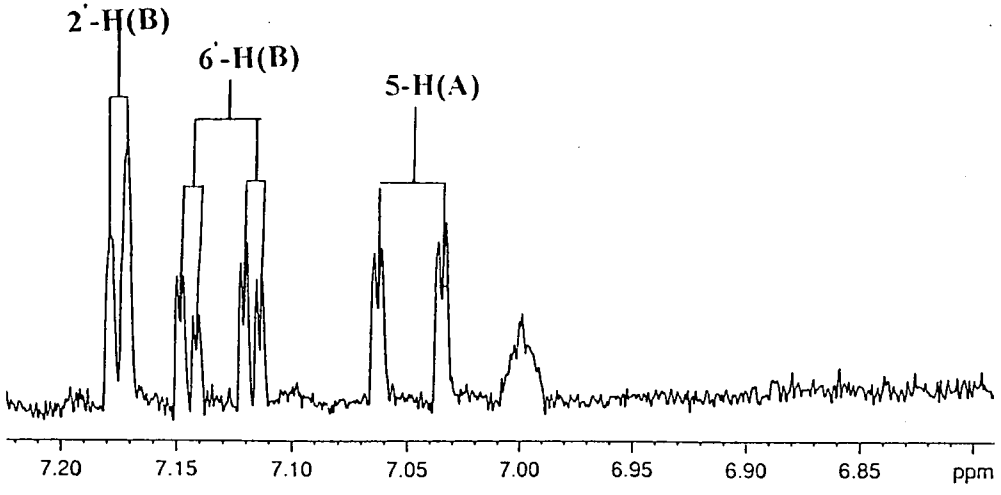

1×OAc
1.85
1.80
1.75
1.70
1.65
1.60
1.55
1.50
ppm
4-H(F)
3-H(C)
3.15
3.10
3.05
3.00
2.95
2.90
2.85
2.80
ppm
3-H(C)
2.80
2.75
2.70
2.65
2.60
2.55
2.50
2.45
ppm
8×OMe
3.95
3.90
3.85
3.80
3.75
3.70
3.65
3.60
3.55
3.50
3.45
ppm
4-H(F)
3.35
3.30
3.25
3.20
ppm


4-H(C)
5.30
5.25
5.20
5.15
5.10
5.05
5.00
4.95
ppm


2-H(F)
4.80
4.75
4.70
4.65
4.60
4.55
4.50
4.45
ppm


6-H(D)
2-H(C)
6.15
6.10
6.05
6.00
5.95
5.90
5.85
5.80
ppm


3-H(F)
5.75
5.70
5.65
5.60
5.55
5.50
5.45
5.40
ppm


2'-H(B)
6'-H(B)
5-H(A)
7.20
7.15
7.10
7.05
7.00
6.95
6.90
6.85
ppm

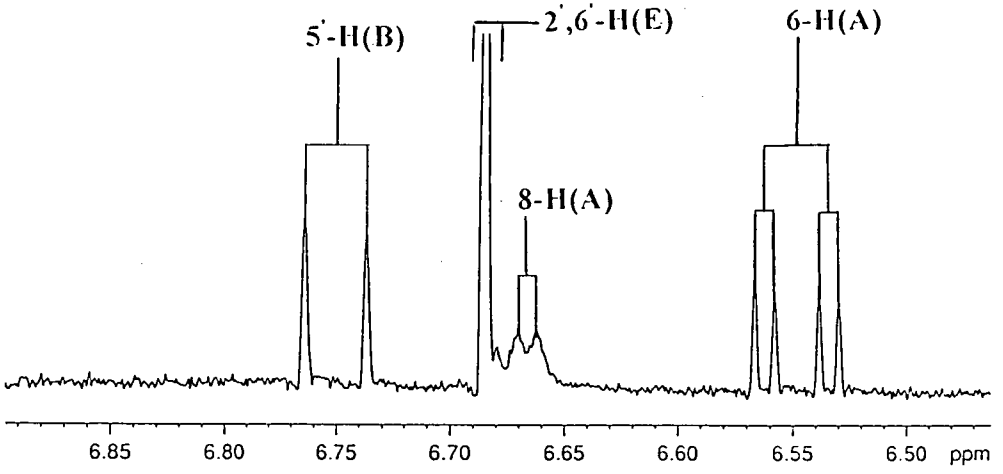
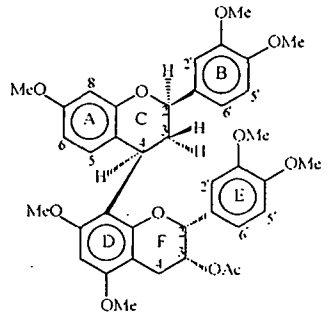

5'-H(B)
2',6'-H(E)
6-H(A)
8-H(A)
6.85
6.80
6.75
6.70
6.65
6.60
6.55
6.50
ppm

Plate7($CDCl_3$,293K)



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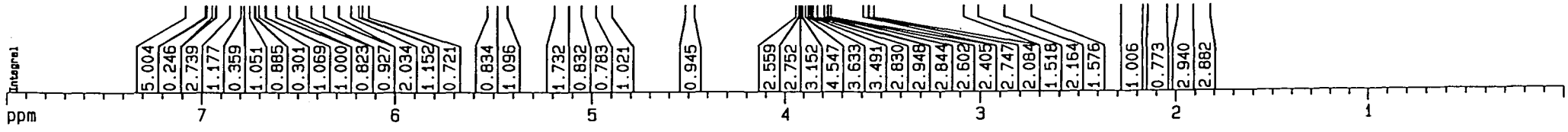
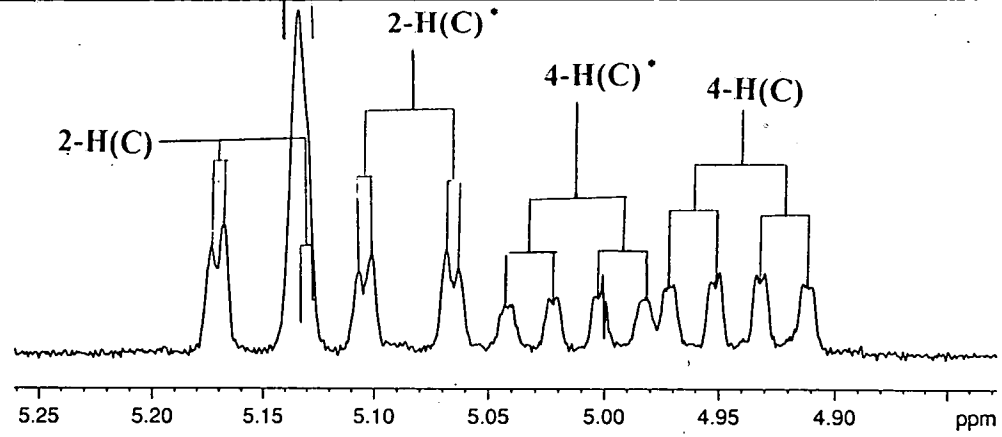
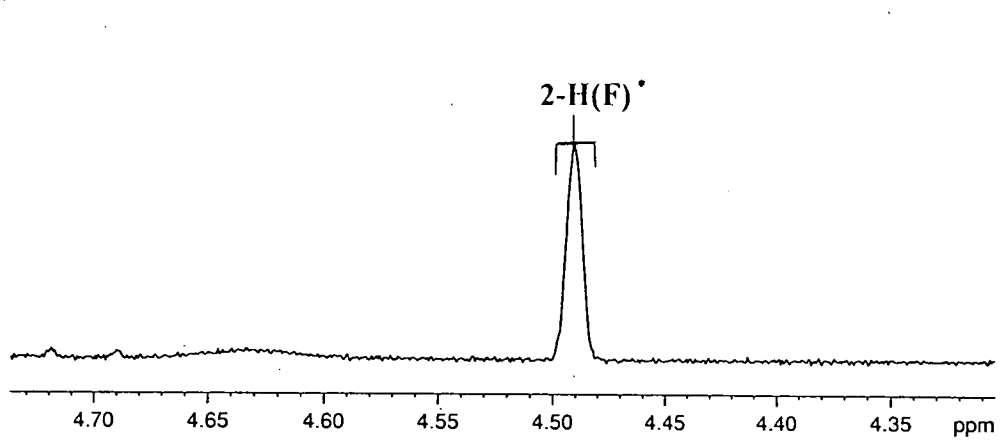
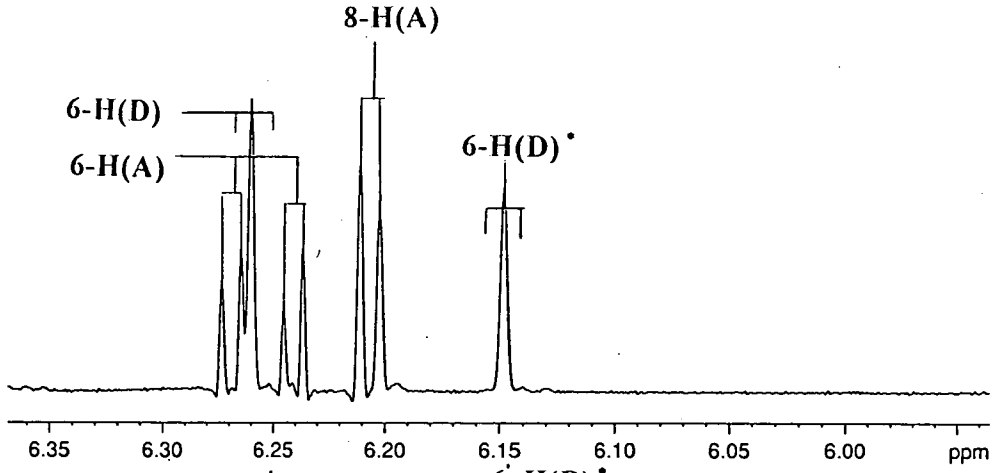
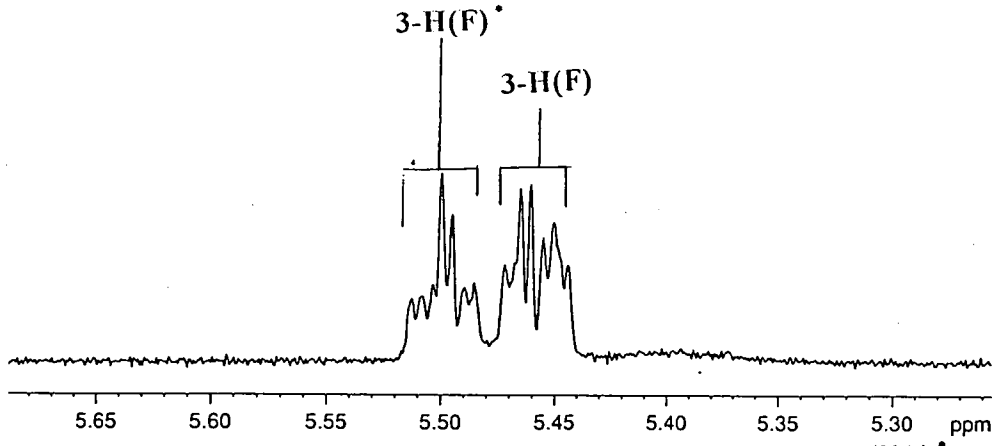
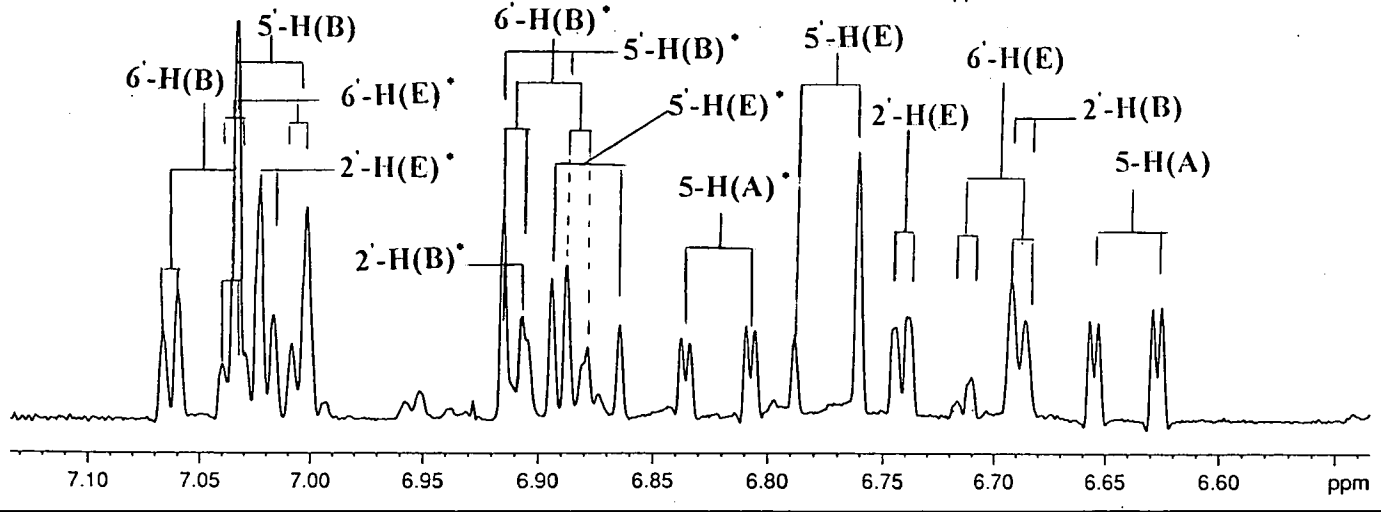


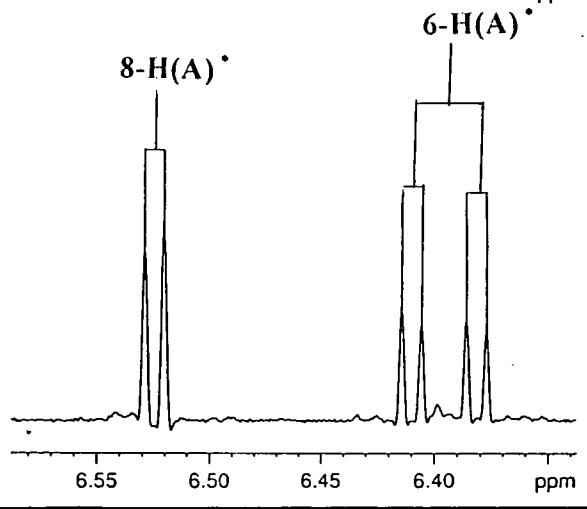

2-H(C)*
4-H(C)*
4-H(C)
2-H(C)
5.25
5.20
5.15
5.10
5.05
5.00
4.95
4.90
ppm

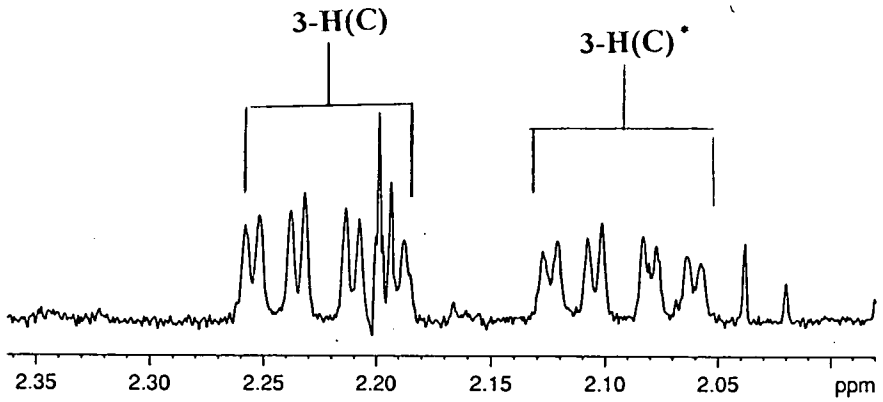

2-H(F)*
4.70
4.65
4.60
4.55
4.50
4.45
4.40
4.35
ppm

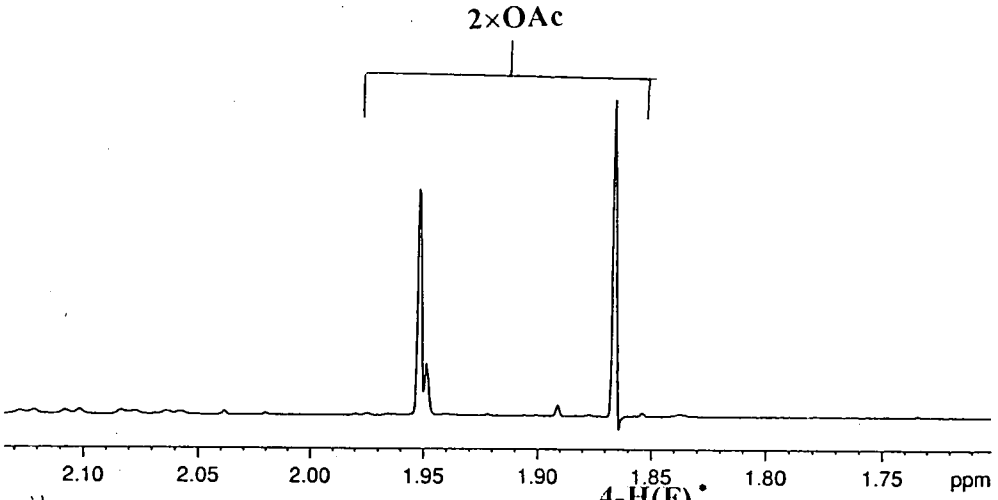

8-H(A)
6-H(D)
6-H(A)
6-H(D)*
6.35
6.30
6.25
6.20
6.15
6.10
6.05
6.00
ppm

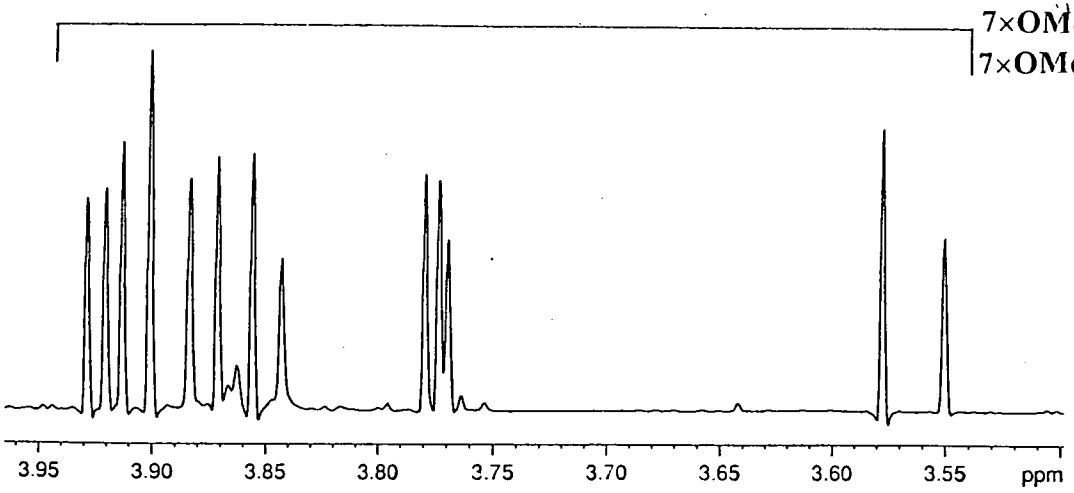

3-H(F)*
3-H(F)
5.65
5.60
5.55
5.50
5.45
5.40
5.35
5.30
ppm


5'-H(B)
6'-H(B)
6'-H(E)*
2'-H(E)*
6'-H(B)*
5'-H(B)*
5'-H(E)*
2'-H(B)*
5-H(A)*
5'-H(E)
2'-H(E)
6'-H(E)
2'-H(B)
5-H(A)
7.10
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70
6.65
6.60
ppm


8-H(A)*
6-H(A)*
6.55
6.50
6.45
6.40
ppm


3-H(C)
3-H(C)*
2.35
2.30
2.25
2.20
2.15
2.10
2.05
ppm


2×OAc
2.10
2.05
2.00
1.95
1.90
1.85
1.80
1.75
ppm


7×OMe
7×OMe*
3.95
3.90
3.85
3.80
3.75
3.70
3.65
3.60
3.55
ppm

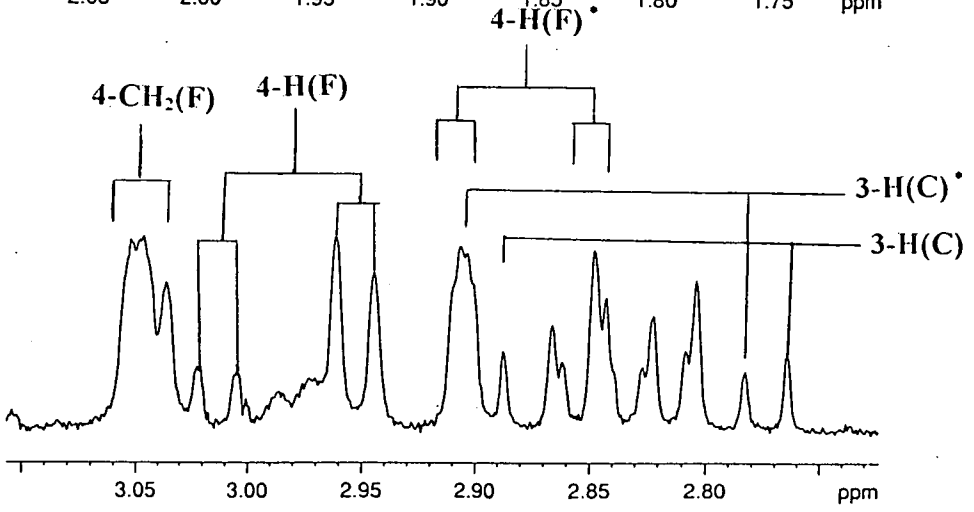
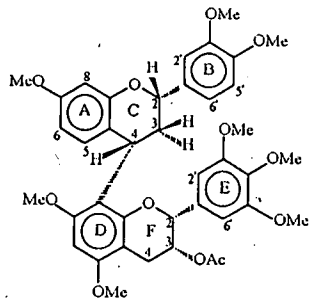

4-H(F)*
4-CH2(F)
4-H(F)
3-H(C)*
3-H(C)
3.05
3.00
2.95
2.90
2.85
2.80
ppm

Plate8(CDCl3,293K)



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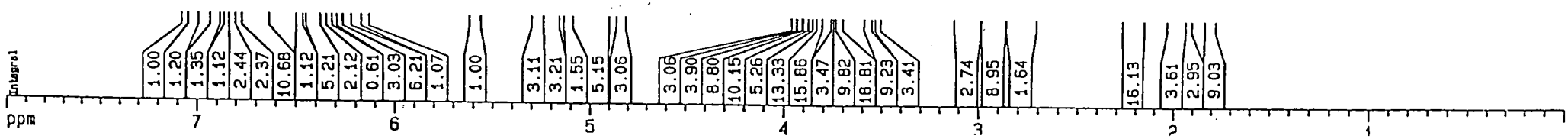
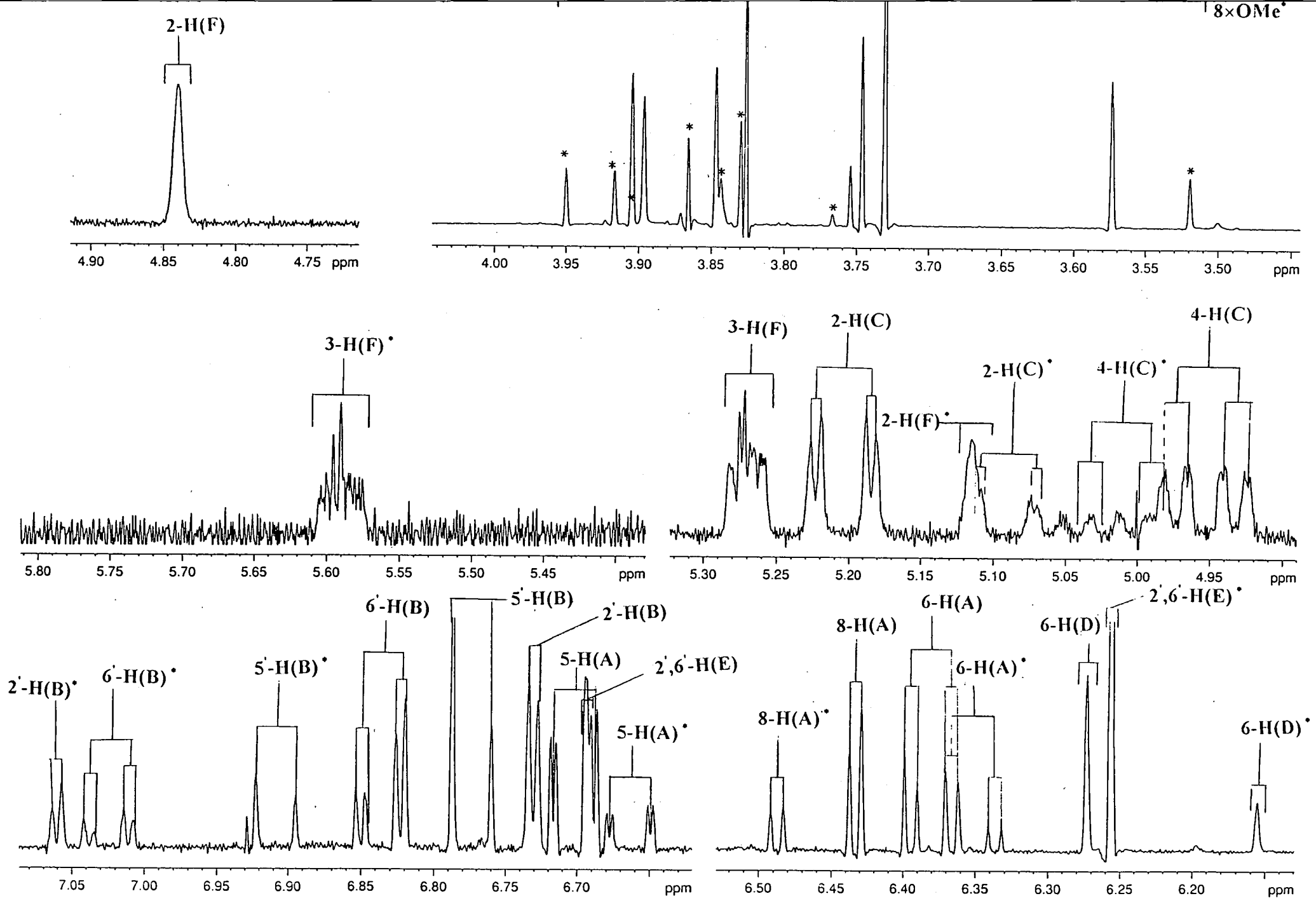
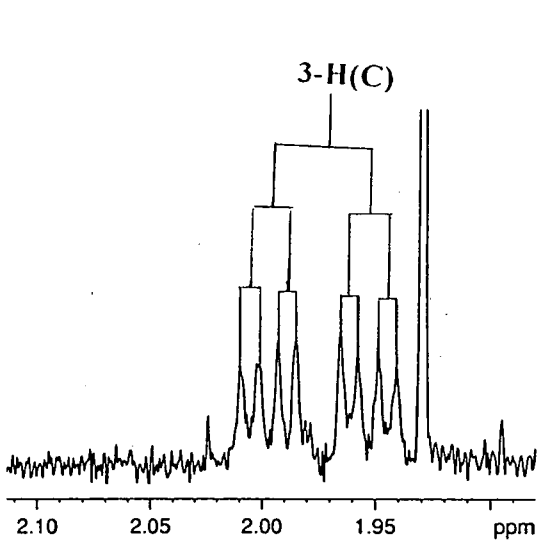
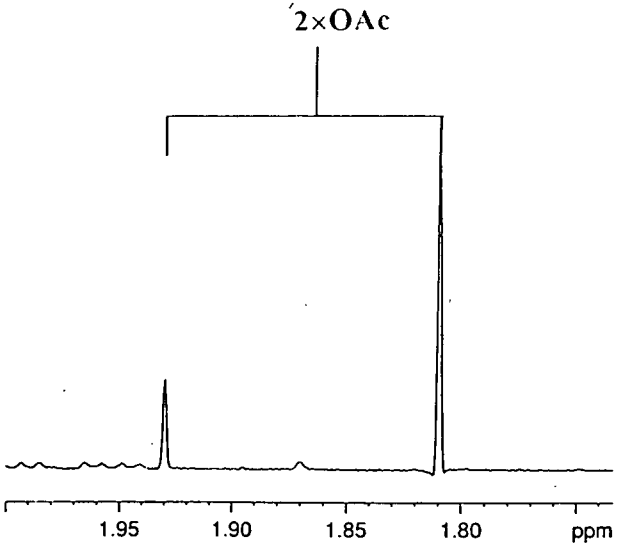
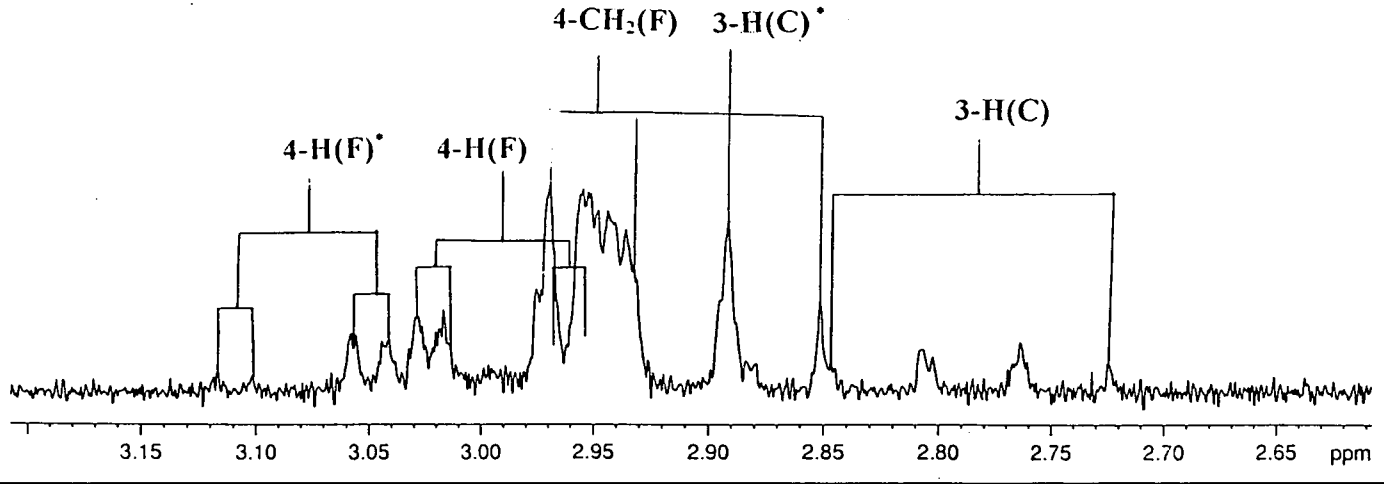



2-H(F)
8×OMe
4.90
4.85
4.80
4.75 ppm
4.00
3.95
3.90
3.85
3.80
3.75
3.70
3.65
3.60
3.55
3.50 ppm
3-H(F)
3-H(F)
2-H(C)
2-H(C)
4-H(C)
4-H(C)
2-H(F)
5.80
5.75
5.70
5.65
5.60
5.55
5.50
5.45 ppm
5.30
5.25
5.20
5.15
5.10
5.05
5.00
4.95 ppm
6'-H(B)
5'-H(B)
2'-H(B)
2'-H(B)
6'-H(B)
5'-H(B)
5-H(A)
2',6'-H(E)
5-H(A)
8-H(A)
6-H(A)
2',6'-H(E)
6-H(D)
8-H(A)
6-H(A)
6-H(D)
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70 ppm
6.50
6.45
6.40
6.35
6.30
6.25
6.20 ppm


3-H(C)
2.10
2.05
2.00
1.95
ppm


2×OAc
1.95
1.90
1.85
1.80
ppm


4-CH2(F)
3-H(C)*
3-H(C)
4-H(F)*
4-H(F)
3.15
3.10
3.05
3.00
2.95
2.90
2.85
2.80
2.75
2.70
2.65
ppm

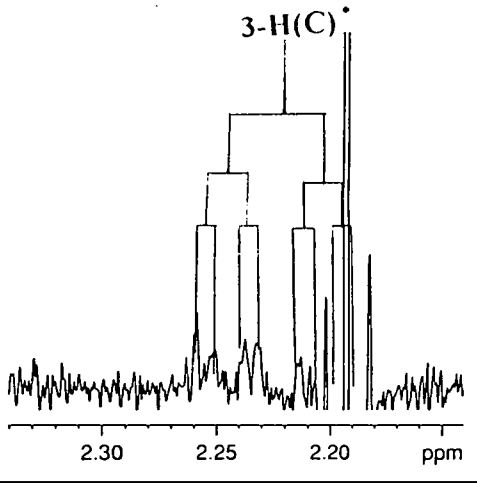
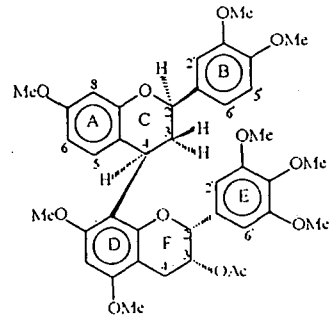
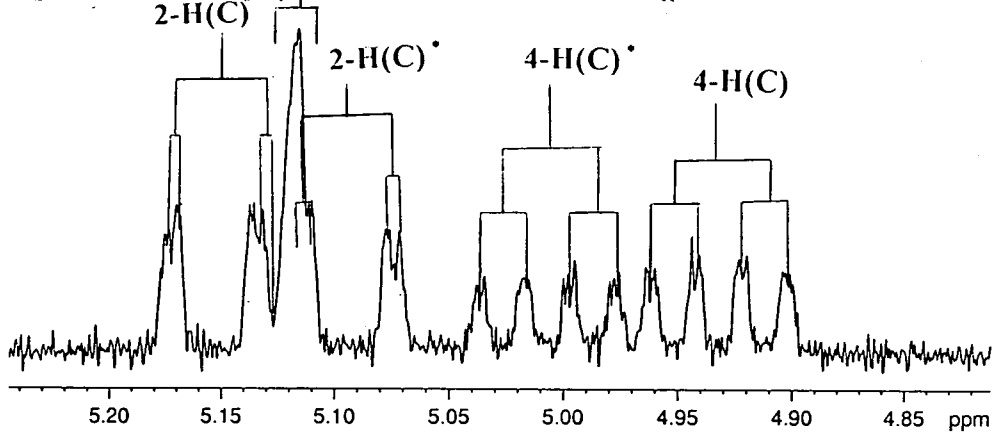
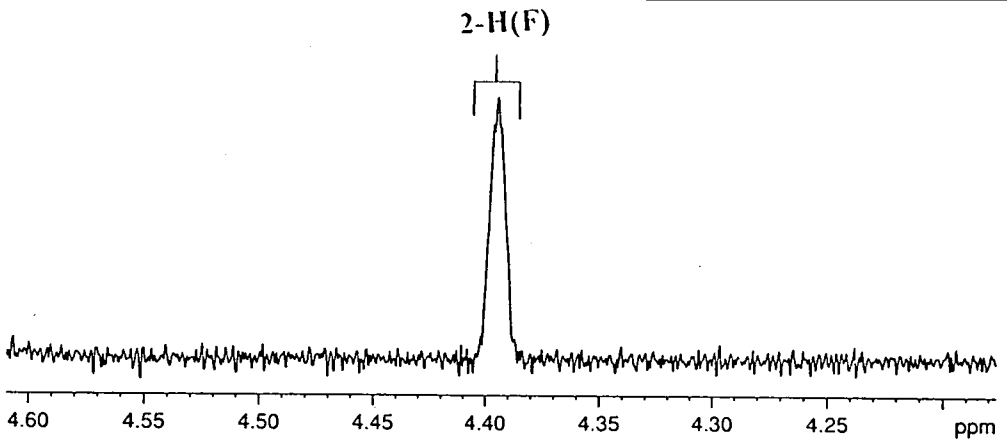

3-H(C)*
2.30
2.25
2.20
ppm

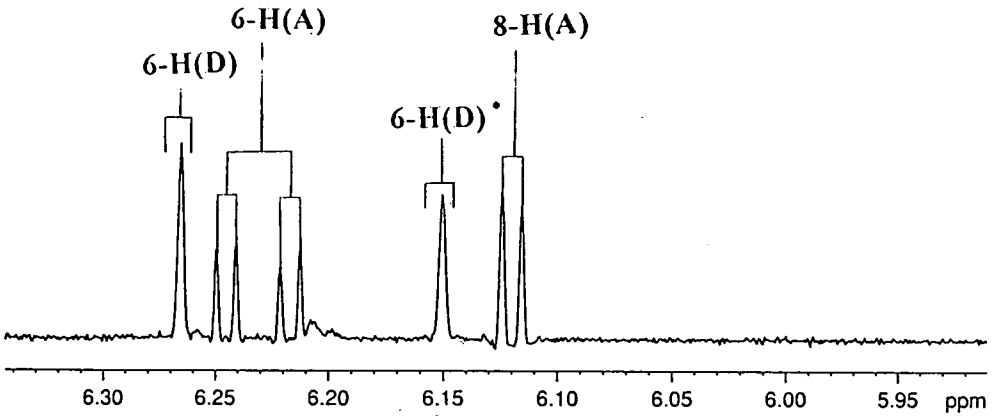
Plate9($CDCl_3$,293K)

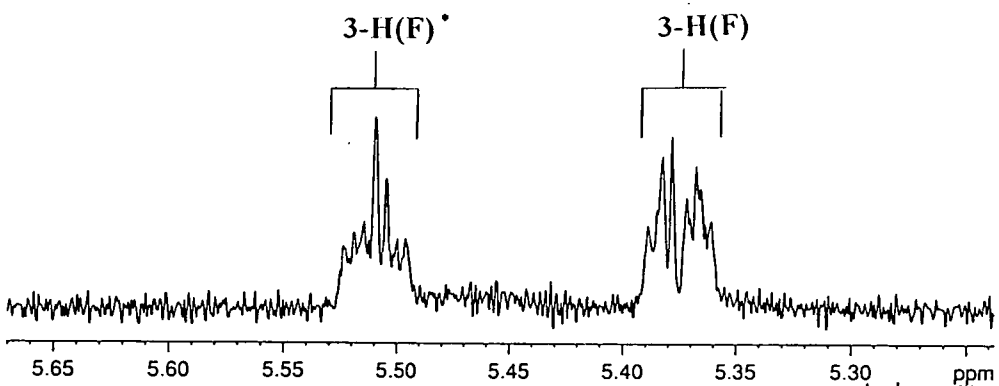


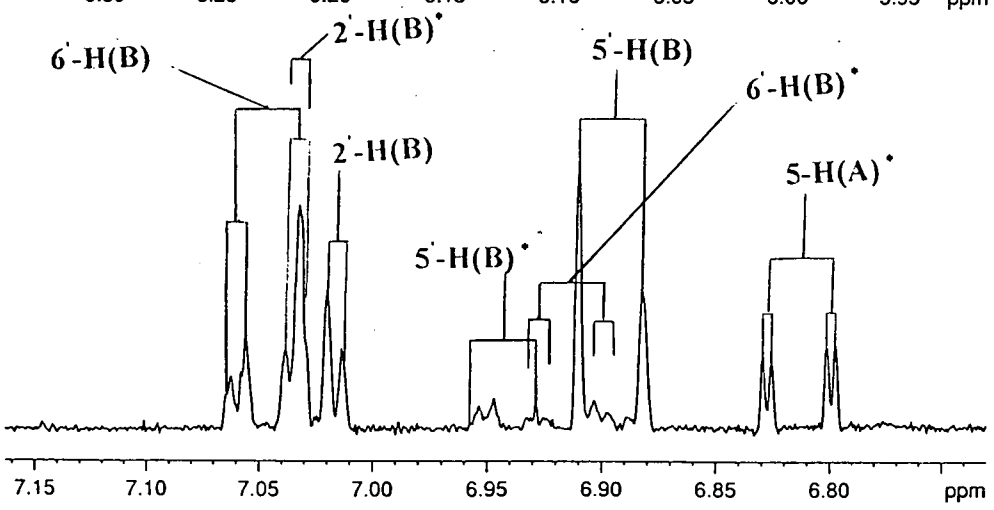
155

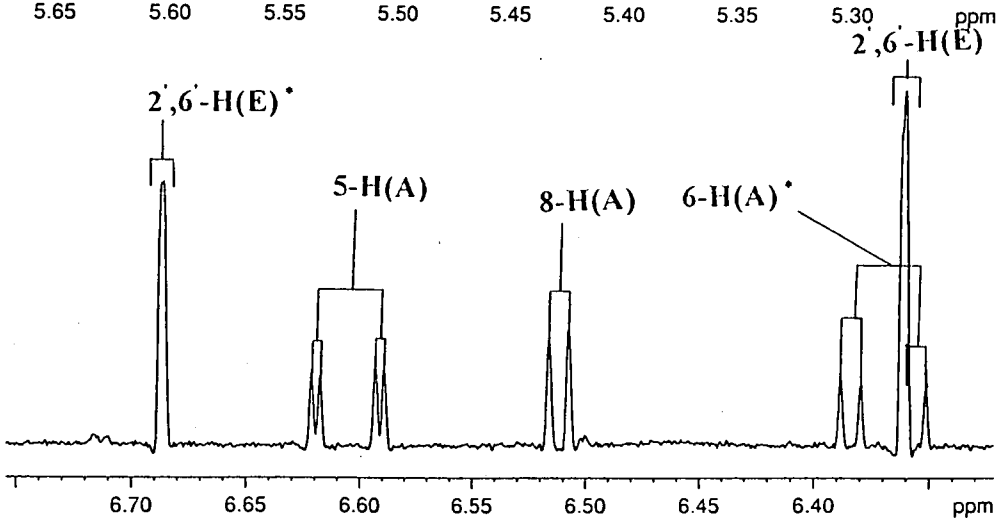

2-H(C)
2-H(C)*
4-H(C)*
4-H(C)
5.20
5.15
5.10
5.05
5.00
4.95
4.90
4.85
ppm


2-H(F)
4.60
4.55
4.50
4.45
4.40
4.35
4.30
4.25
ppm


6-H(A)
6-H(D)
6-H(D)*
8-H(A)
6.30
6.25
6.20
6.15
6.10
6.05
6.00
5.95
ppm


3-H(F)*
3-H(F)
5.65
5.60
5.55
5.50
5.45
5.40
5.35
5.30
ppm


6'-H(B)
2'-H(B)*
2'-H(B)
5'-H(B)
6'-H(B)*
5-H(A)*
5'-H(B)*
7.15
7.10
7.05
7.00
6.95
6.90
6.85
6.80
ppm


2',6'-H(E)
2',6'-H(E)*
5-H(A)
8-H(A)
6-H(A)*
6.70
6.65
6.60
6.55
6.50
6.45
6.40
ppm

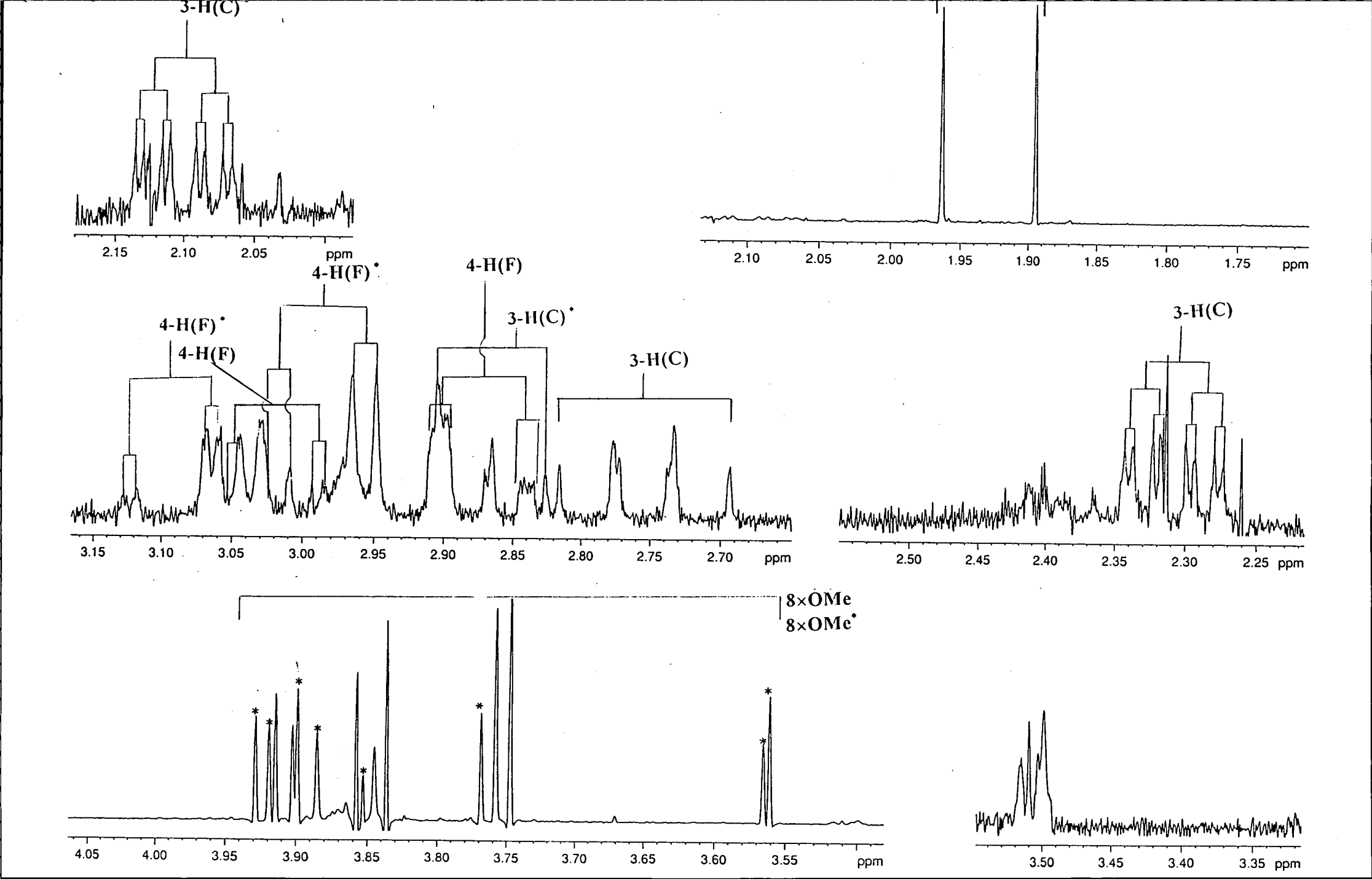
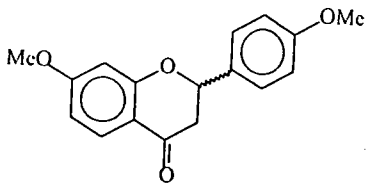
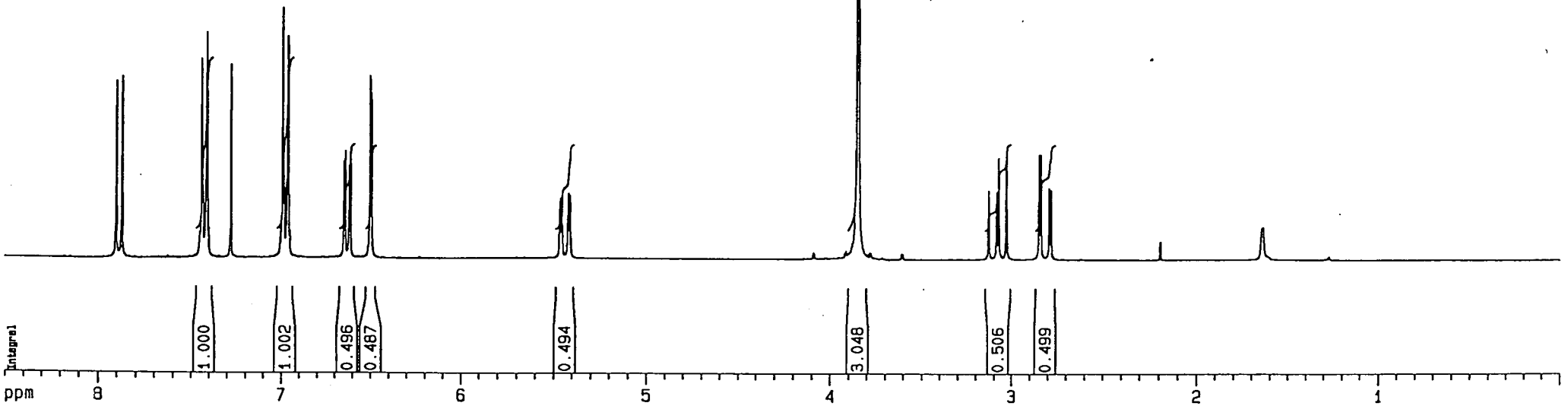

3-H(C)
2.15
2.10
2.05
ppm
2.10
2.05
2.00
1.95
1.90
1.85
1.80
1.75
ppm
4-H(F)*
4-H(F)
4-H(F)*
3-H(C)*
3-H(C)
3.15
3.10
3.05
3.00
2.95
2.90
2.85
2.80
2.75
2.70
ppm
3-H(C)
2.50
2.45
2.40
2.35
2.30
2.25
ppm
8×OMe
8×OMe*
4.05
4.00
3.95
3.90
3.85
3.80
3.75
3.70
3.65
3.60
3.55
ppm
3.50
3.45
3.40
3.35
ppm

Plate10($CDCl_3$,293K)



161



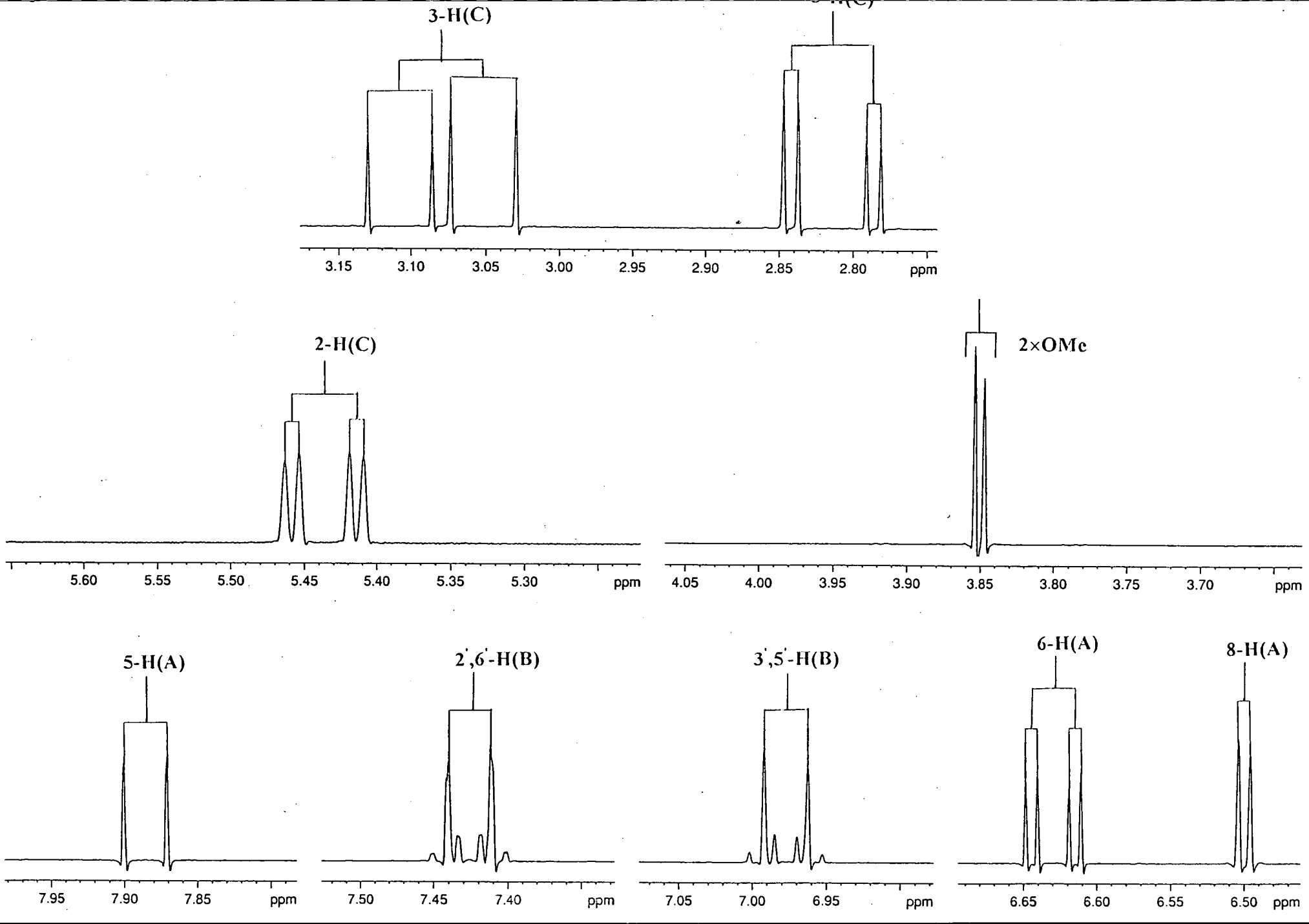
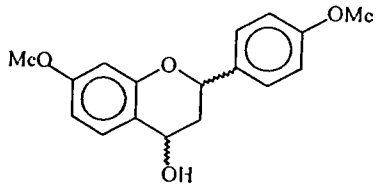
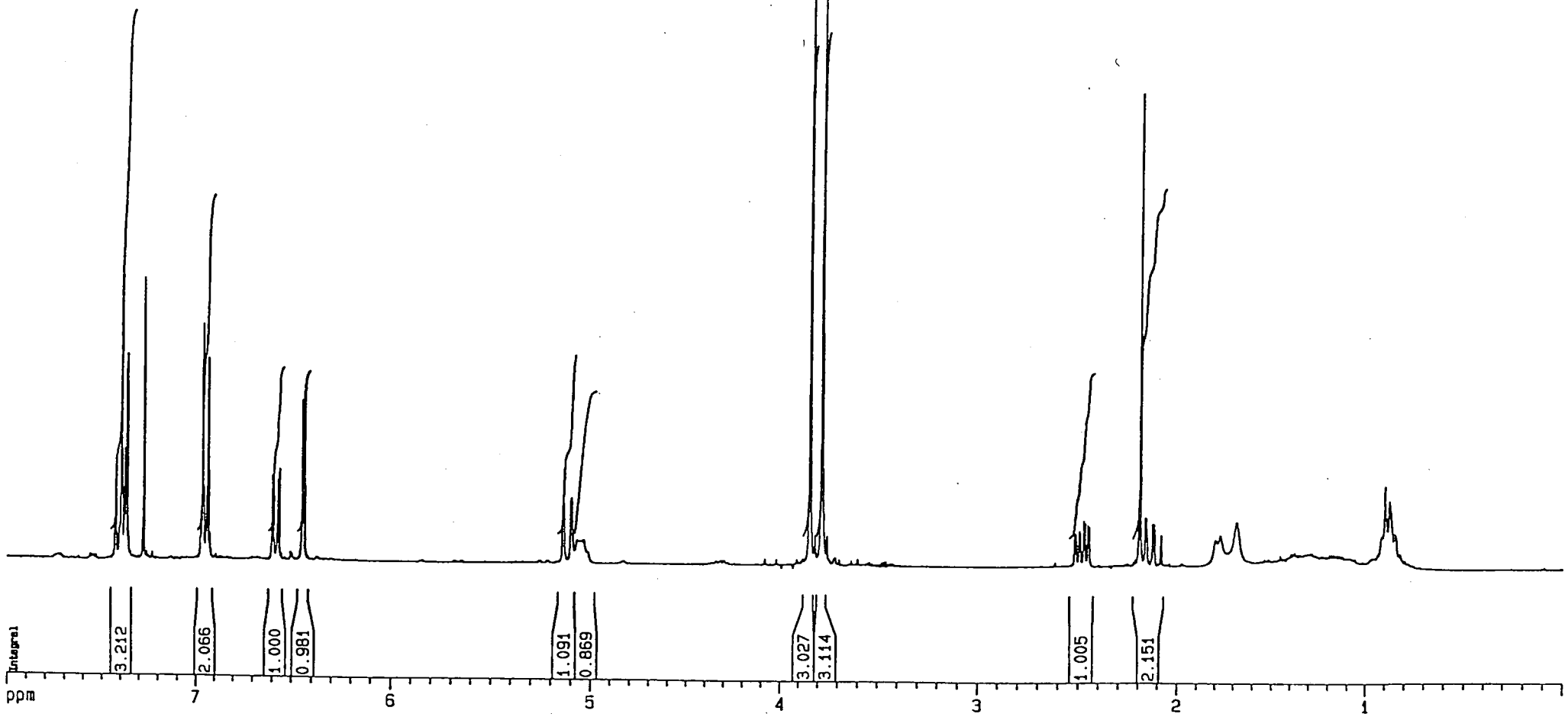

3-H(C)
3.15
3.10
3.05
3.00
2.95
2.90
2.85
2.80
ppm
2-H(C)
5.60
5.55
5.50
5.45
5.40
5.35
5.30
ppm
2×OMe
4.05
4.00
3.95
3.90
3.85
3.80
3.75
3.70
ppm
5-H(A)
7.95
7.90
7.85
ppm
2',6'-H(B)
7.50
7.45
7.40
ppm
3',5'-H(B)
7.05
7.00
6.95
ppm
6-H(A)
8-H(A)
6.65
6.60
6.55
6.50
ppm

Plate11(CDCl$_3$,293K)



162



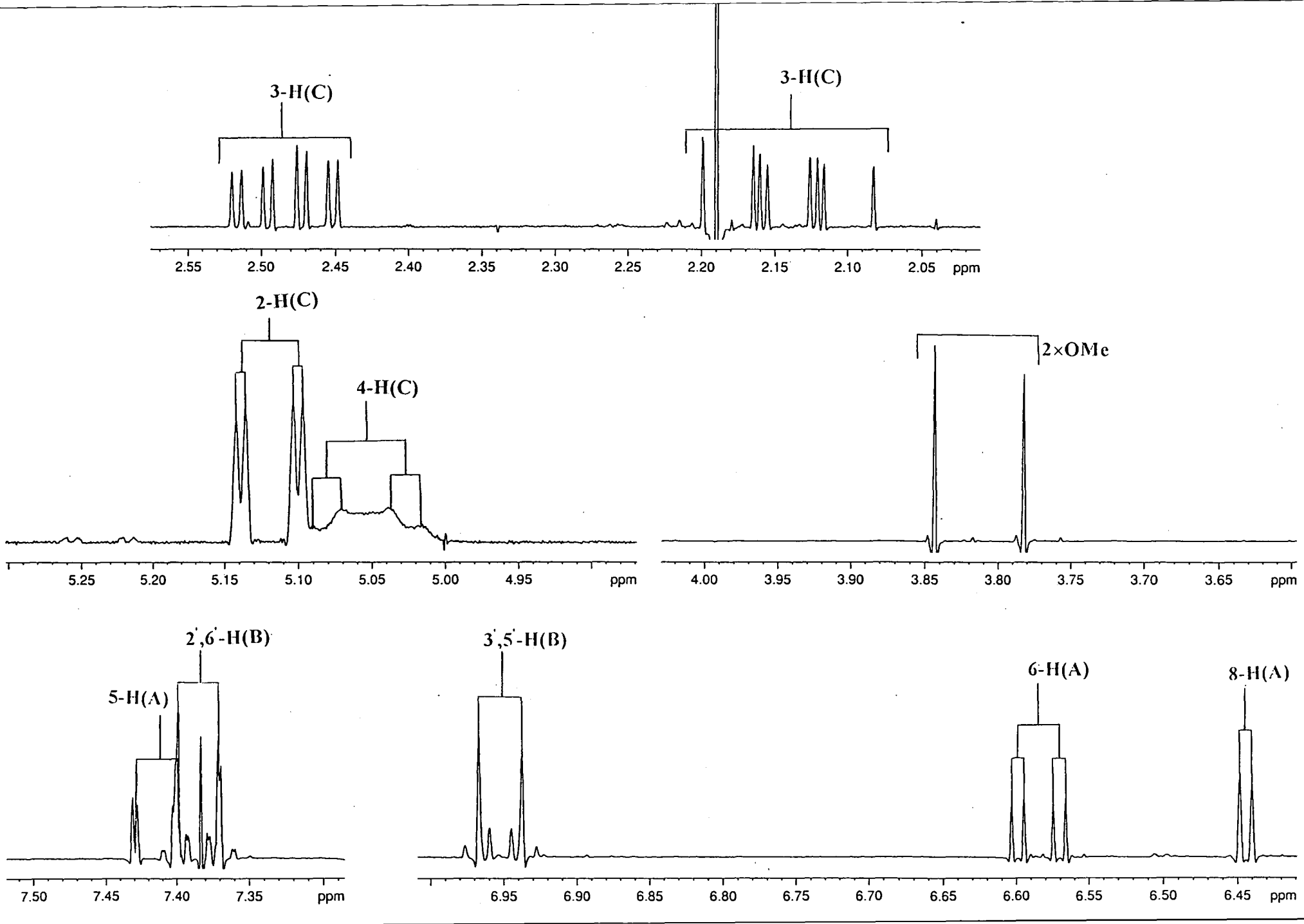
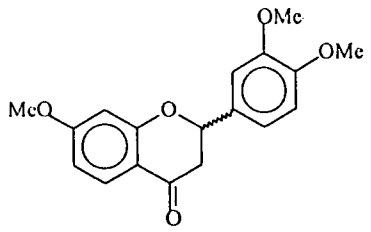

3-H(C)
3-H(C)
2.55 2.50 2.45 2.40 2.35 2.30 2.25 2.20 2.15 2.10 2.05 ppm
2-H(C)
4-H(C)
5.25 5.20 5.15 5.10 5.05 5.00 4.95 ppm
2×OMe
4.00 3.95 3.90 3.85 3.80 3.75 3.70 3.65 ppm
2',6'-H(B)
5-H(A)
7.50 7.45 7.40 7.35 ppm
3',5'-H(B)
6-H(A)
8-H(A)
6.95 6.90 6.85 6.80 6.75 6.70 6.65 6.60 6.55 6.50 6.45 ppm

Plate12($CDCl_3$,293K)



165

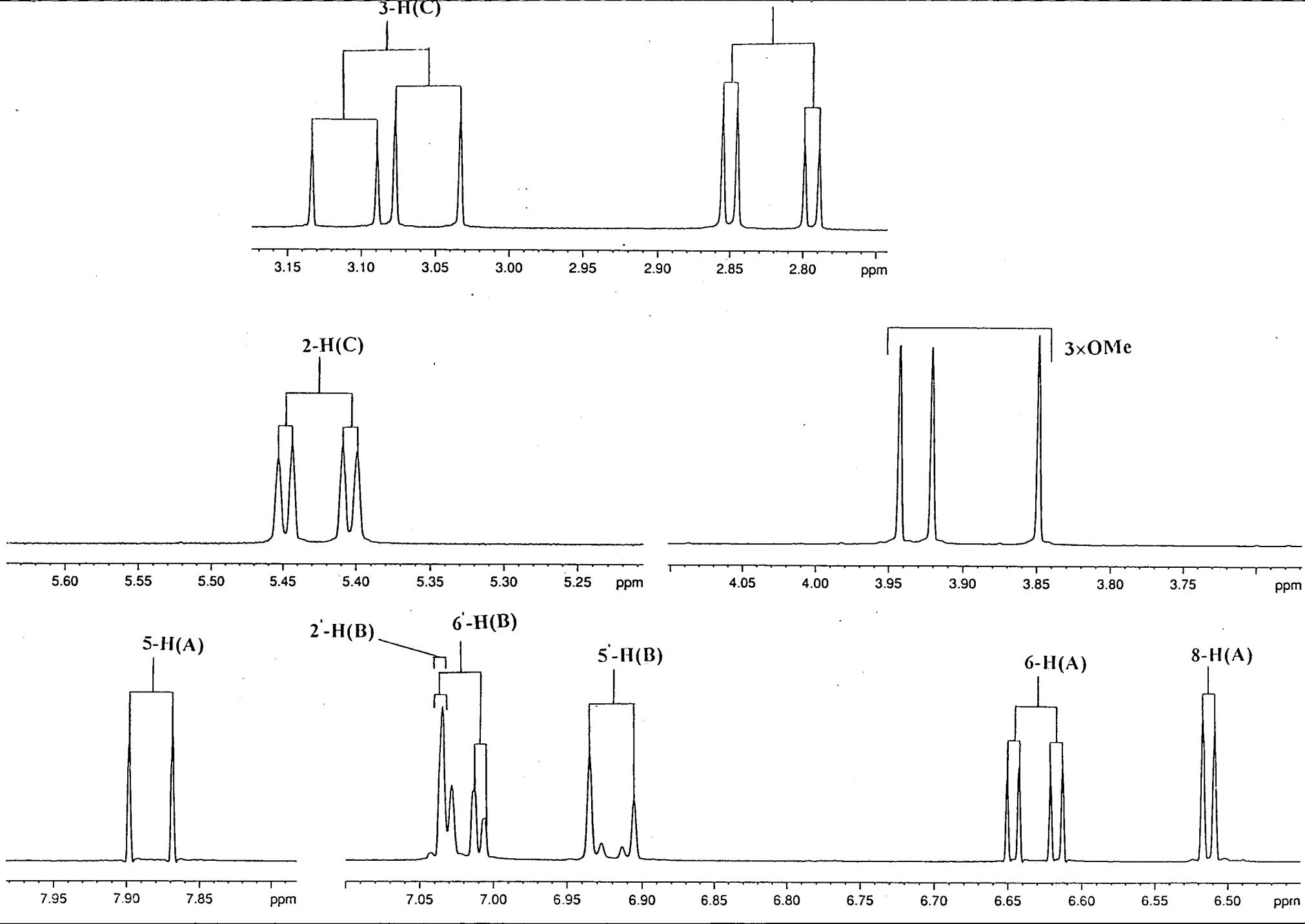
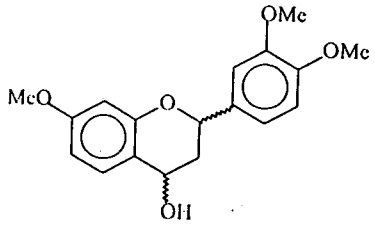
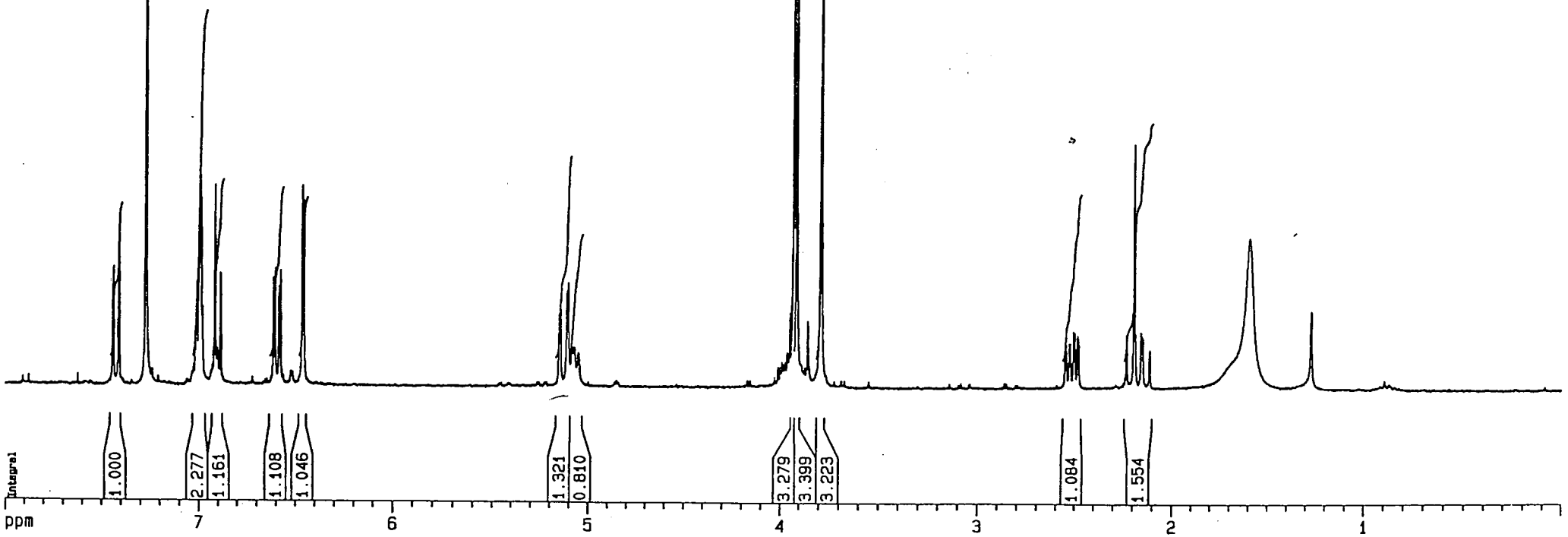
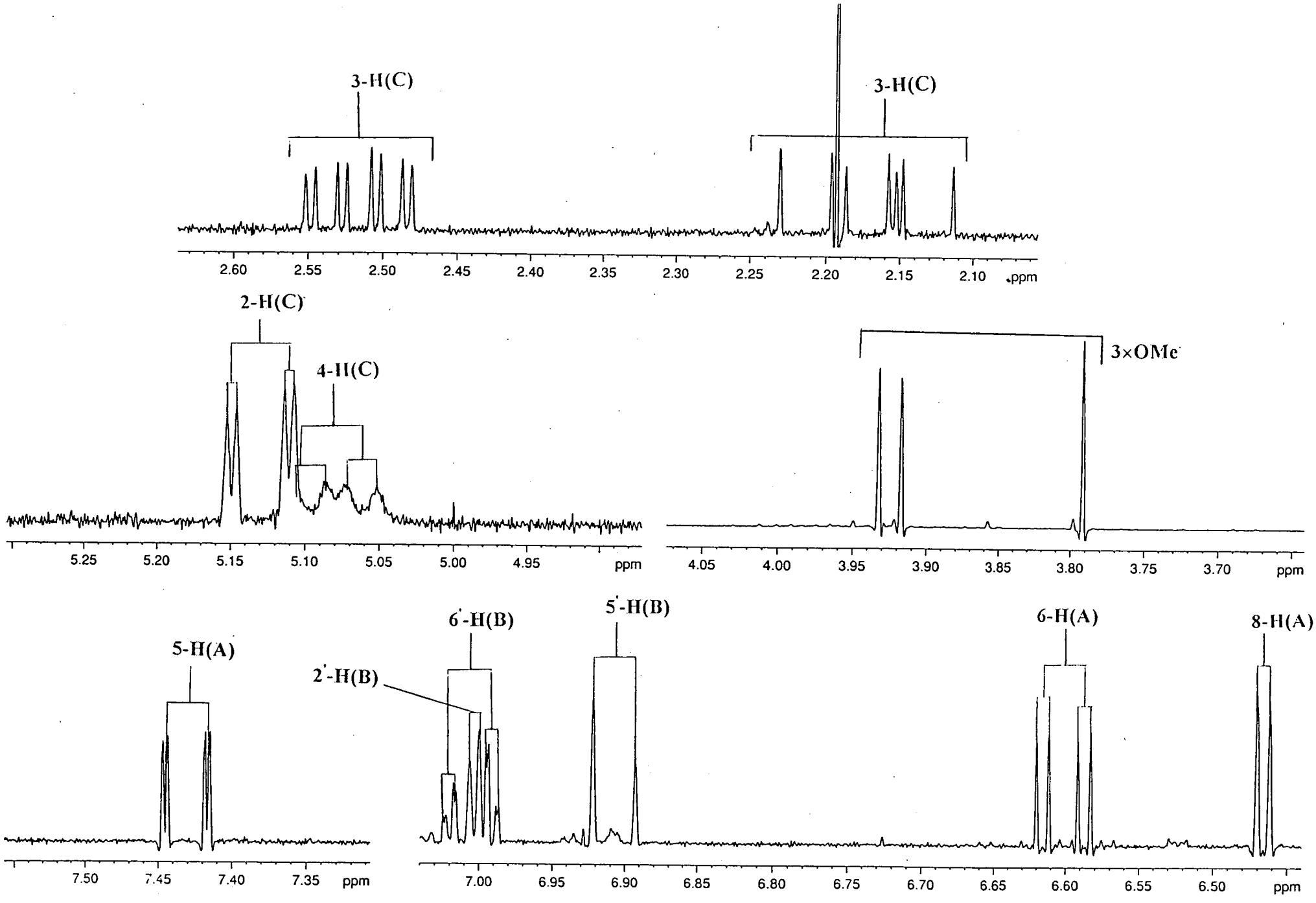

3-H(C)
3.15
3.10
3.05
3.00
2.95
2.90
2.85
2.80
ppm
2-H(C)
5.60
5.55
5.50
5.45
5.40
5.35
5.30
5.25
ppm
3×OMe
4.05
4.00
3.95
3.90
3.85
3.80
3.75
ppm
5-H(A)
7.95
7.90
7.85
ppm
2'-H(B)
6'-H(B)
5'-H(B)
6-H(A)
8-H(A)
7.05
7.00
6.95
6.90
6.85
6.80
6.75
6.70
6.65
6.60
6.55
6.50
ppm

Plate13($CDCl_3$,293K)



166




3-H(C)
3-H(C)
2.60 2.55 2.50 2.45 2.40 2.35 2.30 2.25 2.20 2.15 2.10 ppm
2-H(C)
4-H(C)
5.25 5.20 5.15 5.10 5.05 5.00 4.95 ppm
3×OMe
4.05 4.00 3.95 3.90 3.85 3.80 3.75 3.70 ppm
5-H(A)
7.50 7.45 7.40 7.35 ppm
6'-H(B)
5'-H(B)
2'-H(B)
6-H(A)
8-H(A)
7.00 6.95 6.90 6.85 6.80 6.75 6.70 6.65 6.60 6.55 6.50 ppm

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ABSTRACT

Keywords:*Cassia petersiana*; Leguminosae; flavanoids; leucoanthocyanidins; flavanones; flavan-flavan-3-ol dimers; proanthocyanidins, procassinidins; probutinidins; biomimetic synthesis

In the present study the acetone extract of the bark of *Cassia petersiana* was investigated.

The bark of *Cassia petersiana* afforded the known flavan-3-ols (+)-catechin, (-)-epicatechin, (+)-gallocatechin and epigallocatechin which co-occured with three new procassinidins namely **cassiaflavan-(4β→8)-gallocatechin**, **cassiaflavan-(4β→8)-epigallocatechin** and **cassiaflavan-(4α→8)-epigallocatechin** as well as four novel probutinidins namely **butiniflavan-(4α→8)-epicatechin**, **butiniflavan-(4β→8)-epicatechin**, **butiniflavan-(4β→8)-epigallocatechin** and ***ent*-butiniflavan-(4β→8)-epicatechin**.

A combination of solvent extraction , column (LH20) and thin layer chromatography procedures were used to isolate and purify the compounds mentioned.

Structure elucidation was done using high resolution ^{1}H NMR spectroscopy which included NOE and COSY experiments in conjunction with FAB-MS. Due to a high degree of rotational isomerism structural assignments of the following compounds **cassiaflavan-(4β→8)-epigallocatechin**, **cassiaflavan-(4α→8)-epigallocatechin**, **butiniflavan-(4α→8)-epicatechin**, **butiniflavan-(4β→8)-epicatechin**, **butiniflavan-(4β→8)-epigallocatechin** and ***ent*-butiniflavan-(4β→8)-epicatechin** were obtained by tedious NMR experiments and CD data hence recourse to synthesis was absolutely essential to confirm the proposed structures beyond any doubt.

Biomimetic synthesis of the procassinidin dimers *via* reduction of the racemic flavanones, (±)-4',7-di-*O*-methylcassinidin to the diastereomeric flavan-4-ols and condensation with 3',4',5',5,7-penta-*O*-methylepigallocatechin using titanium tetrachloride as Lewis acid was used to confirm the structures.

A similar biomimetic synthesis of probutinidins *via* reduction of the racemic flavanones, (±)-3',4',7-tri-*O*-methylbutin to the diastereomeric flavan-4-ols followed by condensation with 3',4',5,7-tetra-*O*-methylepicatechin and 3',4',5',5,7-penta-*O*-methylepigallocatechin using titanium tetrachloride as Lewis acid was also employed to confirm the proposed structures.

The biomimetic synthesis of probutinidins yielded two other novel compounds which were not obtained from natural source viz. **butiniflavan-(4α→8)-epigallocatechin** and ***ent*-butiniflavan-(4β→8)-epigallocatechin**.

The isolation and identification of the three new procassinidins and four probutinidins from *Cassia petersiana* represent the first report of dimeric compounds in this rare class of the proanthocyanidins.

This study also represents the first report of the synthesis of the new dimers **cassiaflavan-(4β→8)-epigallocatechin**, **cassiaflavan-(4α→8)-epigallocatechin**, **butiniflavan-(4α→8)-epicatechin**, **butiniflavan-(4β→8)-epicatechin**, **butiniflavan-(4β→8)-epigallocatechin** and ***ent*-butiniflavan-(4β→8)-epicatechin** as well as **butiniflavan-(4α→8)-epigallocatechin** and ***ent*-butiniflavan-(4β→8)-epigallocatechin**.

OPSOMMING

Tydens die huidige studie is die asetoonekstrak van die bas van *Cassia petersiana* ondersoek.

Die bas van *Cassia petersiana* het die bekende flavan-3-ole (+)-katesjien, (-)-epi katesjien, (+)-gallokatesjien en epigallokatesjien gelewer wat saam met drie nuwe procassinidiene voorkom, naamlik **cassiaflavan-(4β→8)-gallokatesjien**, **cassiaflavan-(4β→8)-epigallokatesjien** and **cassiaflavan-(4α→8)-epigallokatesjien** en ook vier on bekende probutinidiene, naamlik **butiniflavan-(4α→8)-epikatesjien**, **butiniflavan-(4β→8)-epikatesjien**, **butiniflavan-(4β→8)-epigallokatesjien** en ***ent*-butiniflavan-(4β→8)-epikatesjien**.

'n Kombinasie van oplosmiddelekstrahering, kolom (LH20) en dun laag chromatografie metodes is gebruik om die gemelde verbindings te isoleer en te suiwer.

Struktuuropklarings is gedoen met hoë resolusie ^{1}H KMR spektroskopie wat NOE en COSY eksperimente ingesluit het te same met FAB-MS. Te wyte aan die hoë mate van rotasieisomerie is die struktuuropklaring van die volgende verbindings, nl.**cassiaflavan-(4β→8)-epigallokatesjien**, **cassiaflavan-(4α→8)-epigallokatesjien**, **butiniflavan-(4α→8)-epikatesjien**, **butiniflavan-(4β→8)-epikatesjien**, **butiniflavan-(4β→8)-epigallokatesjien** en ***ent*-butiniflavan-(4β→8)-epikatesjien** baie bemoeilik en nieteenstaande uitgebreide KMR eksperimente en SD data moes toevlug geneem word tot sintese om die voorgestelde strukture sonder enige twyfel te bevestig.

Biomimetiese sintese van die procassinidien dimere *via* reduksie van die rasemiese flavanoon, (±)-4',7-di-*O*-metielcassinidien na die diastereomeriese flavan-4-ole en kondensasie met 3',4',5',5,7-penta-*O*-metielepigallokatesjien is met titaniumtetrachloried as Lewis suur gedoen om die strukture te bevestig.

Soortgelyke biomimetiese sintese van probutinidiene *via* reduksie van die rasemiese flavanoon, (±)-3',4',7-tri-*O*-metielbutin na die diastereomeriese flavan-4-ole en kondensasie met 3',4',5,7-tetra-*O*-metielepikatesjien en 3',4',5',5,7-penta-*O*-metielepigallokatesjien is ook met titaniumtetrachloried as Lewis suur gedoen om weereens die voorgestelde strukture te bevestig.

Die biomimetiese sintese van probutinidiene het twee ander onbekende verbindings gelewer wat nie in die huidige natuurbron voorkom nie, naamlik, **butiniflavan-(4α→8)-epigallokatesjien** en ***ent*-butiniflavan-(4β→8)-epigallokatesjien**.

Die isolasie en identifikasie van die drie nuwe procassinidiene en vier probutinidiene vanuit *Cassia petersiana* is die eerste van die dimeriese verbindings in hierdie skaars klas van proanthocyanidiene.

Hierdie studie behels ook die eerste verslag van die sintese van die nuwe dimere **cassiaflavan-(4β→8)-epigallokatesjien**, **cassiaflavan-(4α→8)-epigallokatesjien**, **butiniflavan-(4α→8)-epikatesjien**, **butiniflavan-(4β→8)-epikatesjien**, **butiniflavan-(4β→8)-epigallokatesjien** en ***ent*-butiniflavan-(4β→8)-epikatesjien** asook van **butiniflavan-(4α→8)-epigallokatesjien** en ***ent*-butiniflavan-(4β→8)-epigallokatesjien**.