The structure and synthesis of trimeric and related dimeric Proteracacinidins from *Acacia hereroensis.*

Thesis submitted in fulfillment of the requirements for the degree

M. Sc.

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By

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Supervisor: Prof. E. Malan

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I hereby wish to express sincere gratitude to the following people:

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Fellow students for their understanding and companionship;

Friends, outside of the chemistry department, for constant support.

E. Fourie

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ERRATA

ITEM	POSITION	CORRECTION
All e.g. references	whole thesis	italicized
4.1	p.18 bottom line	section 4.1
catechin	p.19 10th ft	epicatechin
eter	p.19 9th ft	ether
tetramethylether	p.22 3rd ft	trimethylether
was	p.25 3rd ft	is
mulriplet	p.36 12th fb	multiplet

ft = from top
fb = from bottom

Literature Survey

1

Introduction

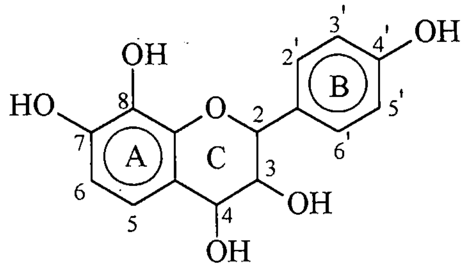
Oligomeric proanthocyanidins represent one of the most widely spread groups of plant phenolics. These compounds have been widely identified in the barks and heartwoods of a variety of tree species, which in some instances have resulted in commercial extraction, to be utilized in the tanning industry.

Despite the industrial use, the chemistry of the proanthocyanidins represents a somewhat neglected area of research, due to the complex composition of the proanthocyanidin extracts and the consequent difficulty in isolation and purification thereof. The lack of a universal method for the synthesis and determination of the stereochemistry at the stereogenic centra and around the interflavanyl linkage and the difficulty experienced due to rotational isomerisation even with the assistance of modern NMR spectral investigations have contributed to the slow development of this particular chemistry[1].

The oligomeric proanthocyanidins are also known for contributing towards the protection of plants from diseases, insects, herbivores and have recently become known for the anti-oxidizing properties, making it useful as pharmaceuticals, wood preservatives and gaining a lot of popularity as a supplement to the human diet. The latter properties have resulted in a dramatic upsurge in worldwide research efforts.

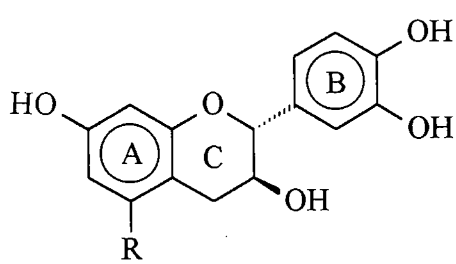
The aim of this study was the structural elucidation and synthesis of proteracacinidin di- and trimers, with a 4', 7, 8 hydroxylation pattern (**1**).

[1] D. Ferreira, J. P. Steynberg, D. G. Roux and E. V. Brand, *Tetrahedron*, 1992, **48**, 1743.



(1)

Research[1,2,3] was done on the synthesis of a number of different proanthocyanidin oligomers based on the phloroglucinol (e.g. catechin **(2)**) and resorcinol (e. g. fisetinidol **(3)**) A-ring flavanoids, but the oligomers with a pyrogallol-type A-ring **(1)**, remained largely unexplored, despite their recent discovery in a number of South African *Acacia* species, e.g. *A. caffra* and *A. galpinii*[3,4,5,6,7,8].



(2) R = OH
(3) R = H

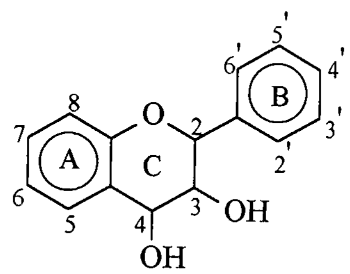
[2] L. J. Poter, *The Flavanoids – Advances in Research since 1980*, ed. J. B. Harborne, Chapman and Hall, London, 1988.
[3] L. J. Porter, *The Flavanoids – Advances in research since 1986*, ed. J. B. Harborne, Chapman and Hall, London, 1994, 23.
[4] D. Ferreira, R. J. J. Nel and R. Bekker, *Comprehensive Natural Products Chemistry*, Vol. 3, Chapter 15, Editors-in-Chief, Sir Derek Barton and K. Nakanishi, Pergamon Press, 1999, **3**, 747.
[5] E. Malan and A. Sireeparsad, *Phytochemistry*, 1995, **38**, 237.
[6] E. Malan, *Phytochemistry*, 1995, **40**, 1519.
[7] E. Malan, A. Sireeparsad, J. F. W. Burger and D. Ferreira, *Tetrahedron Lett.*, 1994, **35**, 7415.
[8] J. W. Clark-Lewis, G. F. Katekar and P. I. Mortimer, *J. Chem. Soc.*, 1961, 499.

2

Leucoanthocyanidins

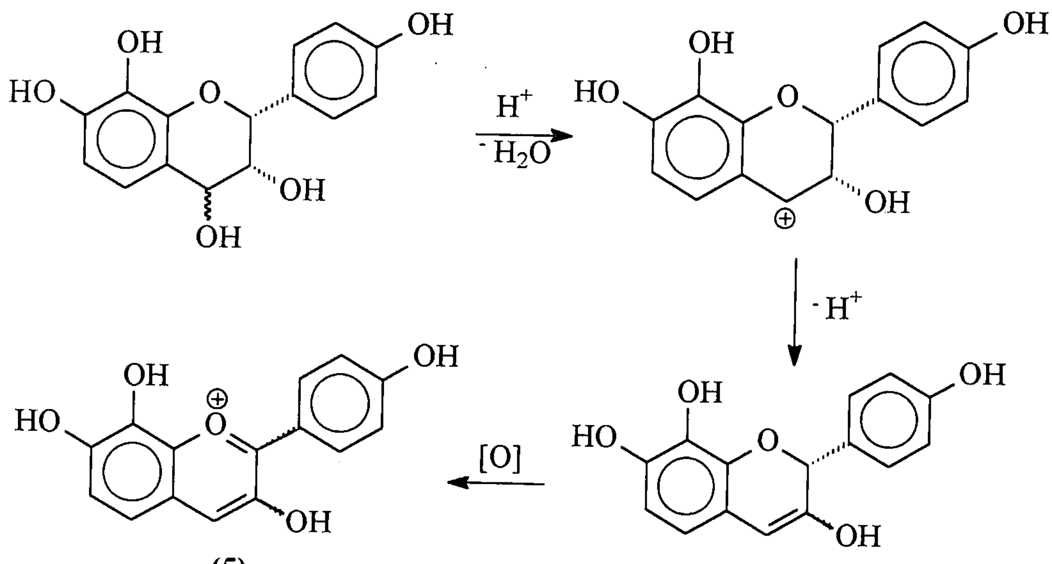
2.1. Introduction and Nomenclature

The flavan-3,4-diol structure **(4)** was accepted during the 1950's as representative for Leucoanthocyanidins[2] and the flavanoid skeleton of these compounds is drawn and numbered as shown in **(4)**.



(4)

Haslam later expanded this definition of leucoanthocyanidins to include all monomeric proanthocyanidins, which were defined to produce anthocyanidins **(5)** upon cleavage of a C-O bond on heating with a mineral acid, as shown in **Scheme 2.1**[2].



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Scheme 2.1

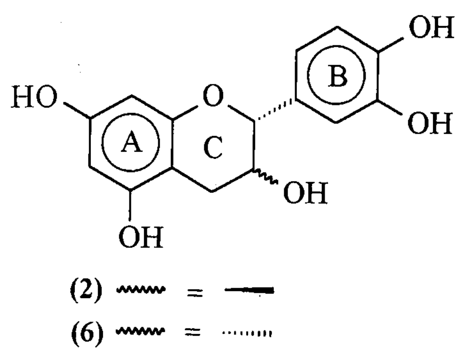
A summary of the most predominant leucoanthocyanidin monomers, with their hydroxylation patterns, is given in **Table 2.1**[3].

Table 2.1:

Leucoanthocyanidin	**Hydroxylation pattern**
Leucoguibourtinidin	3, 7, 4'
Leucofisetinidin	3, 7, 3', 4'
Leucoteracacinidin	3, 7, 8, 4'
Leucomelacacinidin	3, 7, 8, 3', 4'
Leucorobinetinidin	3, 7, 3', 4', 5'
Leucopelargonidin	3, 5, 7, 4'
Leucocyanidin	3, 5, 7, 3', 4'

2.2. Flavan-3-ols

Flavan-3-ols are by far the largest class of monomeric flavanoids, with catechin **(2)** and epicatechin **(6)** among the most widespread flavanoids known. Naturally occurring flavan-3-ols and their derivatives, e.g. simple esters and O-glycosides, as well as their general properties and chemistry have been thoroughly reviewed[2,3,9,10,11].



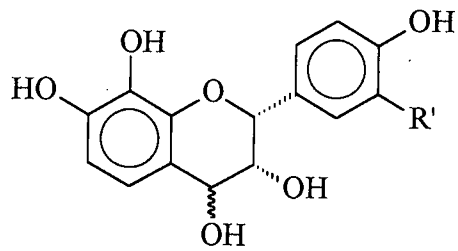
The most important features concerning the chemistry of proanthocyanidins, are the nucleophilicity of the A-rings, the aptitude of the heterocyclic rings to cleave and subsequent rearrangement, the susceptibility of analogues with pyrocatechol- or pyrogallol-type B-rings to phenol oxidative coupling and the conformational mobility of the pyran rings.

2.3. Flavan-3,4-diols

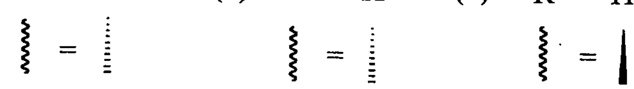
Flavan-3,4-diol moieties form very important units in oligomeric proanthocyanidins. The first of these isolated by King and Bottomley[12] was melacacidin **(7)** (epimesquitol-4α-ol), and was followed by the isolation of several other flavan-3,4-diols, with different hydroxylation patterns, as reported[2,3]. Among these are epioritin-4α-ol **(8)**, epioritin-4β-

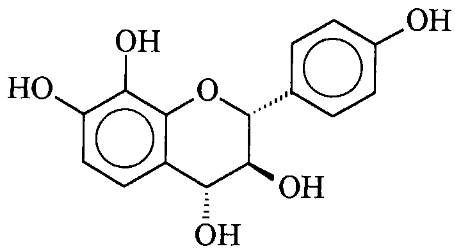
[9] K. Freudenberg and K. Weinges, *The Chemstry of Flavonoid Compounds*, ed. T. A. Geissman, Pergamon Press, Oxford, 1962, 197.
[10] R. W. Hemingway, *Natural Products of Woody Plants I*, ed. J. W. Rowe, Springer-Verlag, New York, 1989, 571.
[11] D. Ferreira and R. Bekker, *Nat. Prod. Rep.*, 1996, **13**, 411.
[12] F. E. King and W. Bottomley, *J. Chem. Soc.*, 1954, 1399.

ol **(9)**, oritin-4α-ol **(10)** and *ent*-oritin-4α-ol **(11)**, the basic monomeric units for proteracacinidins.



(7) R' = OH (8) R' = H (9) R' = H





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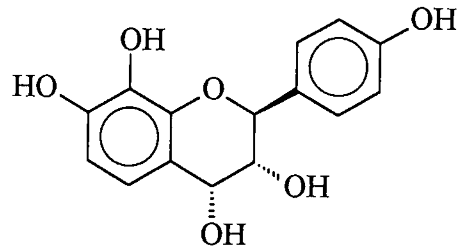
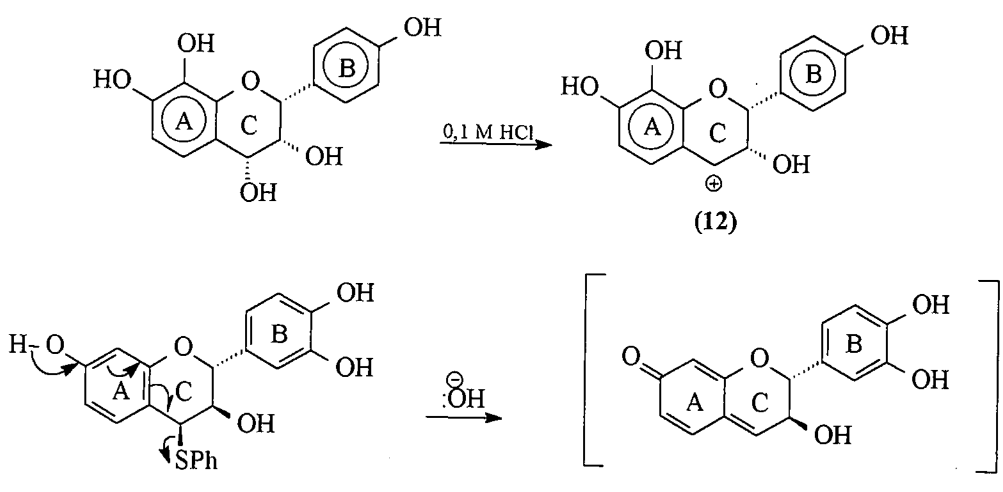


(11)

The predominant feature of the flavan-3,4-diols, relating to the chemistry of oligomeric proanthocyanidins, is their role as precursors to flavan-4-carbocations **(12)** or A-ring quinone methide electrophiles **(13)** [from flavan-4-thioethers **(14)**], as intermediates during interflavanyl bonding reactions[2,3].



(12)

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3

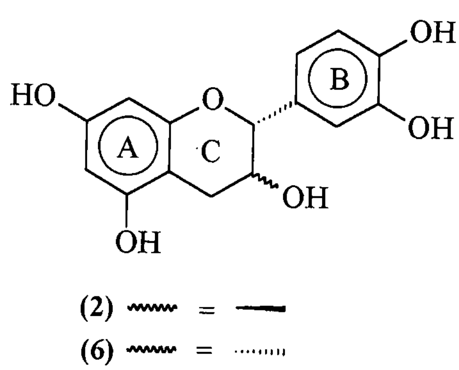
Oligomeric Proanthocyanidins

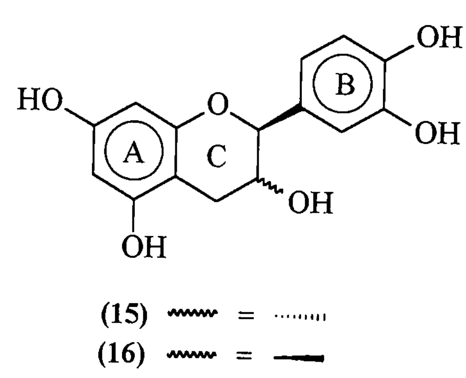
3.1. Introduction and Nomenclature

As a result of the increasing number and conjugate complexity of the flavanoid and proanthocyanidin structures, the need for a more specific system of nomenclature was urgently required.

Hemingway and Porter developed a similar system for the flavanoids, based on the nomenclature used for polysaccharides[2,3,13]. The trivial names of the flavan-3-ols are used to define the monomeric units present in the proanthocyanidins, as shown in **Table 3.1**. The flavan-3-ol structures are drawn and numbered as illustrated in 2.1.

The flavan-3-ols in **Table 3.1** all have a 2R, 3S absolute configuration, e.g. catechin **(2)**. The corresponding 2R, 3R isomers are designated by the "epi" prefix, e.g. epicatechin **(6)**, while the 2S configuration is indicated by the enantio (*ent*) prefix, e.g. *ent*-catechin (2S, 3R) **(15)** and *ent*-epicatechin (2S, 3S) **(16)**.





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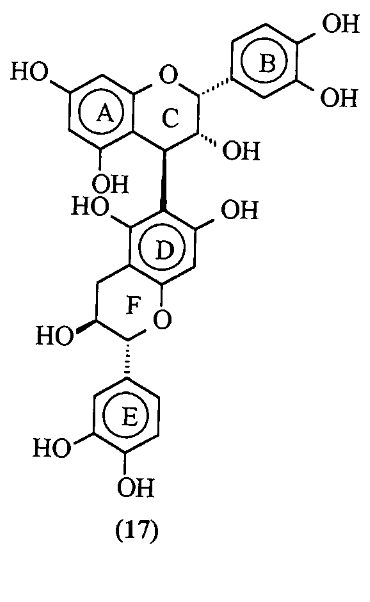
[13] R. W. Hemingway, L. Y. Foo and L. J. Porter, *J. Chem. Soc., Perkin Trans. I*, 1982, 1209.

Table 3.1:

Proanthocyanidin	Monomer	Hydroxylation pattern						
		3	5	7	8	3'	4'	5'
Procassinidin	Cassiaflavan	H	H	OH	H	H	OH	H
Proapigeninidin	Apigeniflavan	H	OH	OH	H	H	OH	H
Proluteolinidin	Luteoliflavan	H	OH	OH	H	OH	OH	H
Protricetinidin	Tricetiflavan	H	OH	OH	H	OH	OH	OH
Prodistenidin	Distenin	OH	OH	OH	H	H	H	H
Propelargonidin	Afzelechin	OH	OH	OH	H	H	OH	H
Procyanidin	Catechin	OH	OH	OH	H	OH	OH	H
Prodelphinidin	Gallocatechin	OH	OH	OH	H	OH	OH	OH
Proguibourtinidin	Guibourtinidol	OH	H	OH	H	H	OH	H
Profisetinidin	Fisetinidol	OH	H	OH	H	OH	OH	H
Prorobinetinidin	Robinetinidol	OH	H	OH	H	OH	OH	OH
Proteracacinidin	Oritin	OH	H	OH	OH	H	OH	H
Promelacacinidin	Mesquitol	OH	H	OH	OH	OH	OH	H

According to IUPAC rules and the system of Hemingway and Porter[3,10,11], the location of the interflavanyl bond in oligomers is given in parentheses and the configuration of the interflavanoid bond at C-4 is indicated by the appropriate α or β, for example epicatechin-(4$\beta\rightarrow$6)-catechin **(17)**.

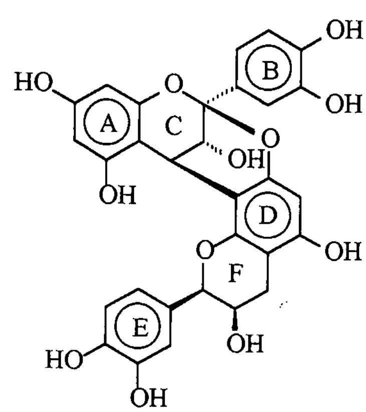


(17)

3.2. A – Type proanthocyanidin dimers

A-type proanthocyanidins **(18)** represent an interesting class of compounds, having an ether linkage from the D-ring of the bottom flavanyl unit to C-2 of the C-ring and a carbon-carbon bond from C-4 of the C-ring to variable positions on the D-ring. This is in contrast to B-type proanthocyanidins, where the constituent flavanyl units are linked via a single carbon-carbon bond. The double bond introduce a high degree of conformational stability with consequent high-quality NMR spectra, free of the effects of dynamic rotational isomerism. It is also the only class of proanthocyanidins suitable for X-ray diffraction analysis[1,3].

Proantocyanidin-A2 **(18)** (epicatechin-(2β→7, 4β→8)-epicatechin), was the first A-type proanthocyanidin to be isolated from the seed shells of *Aesculus hippocastanum* (horse chestnut)[14].



(18)

3.3. B – Type proanthocyanidin dimers

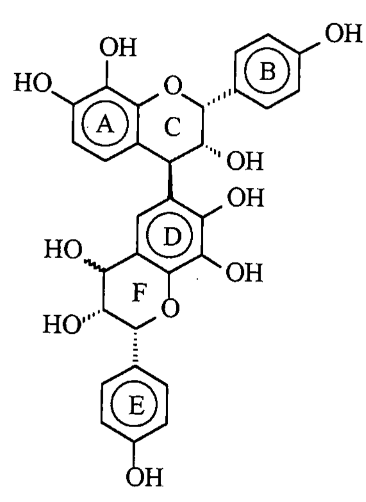
3.3.1. Introduction

B-Type proanthocyanidins are characterised by a single carbon-carbon bond linking the flavanyl units, in contrast to the A-type proanthocyanidins. Bonding usually occurs between the benzylic C-4 of the chain extender unit and C-6 or C-8 of the chain-terminating unit **(17)**.

3.3.2. Proteracacinidins

Although the proteracacinidin monomers are present in a number of *Acacia* species, the corresponding proteracacinidin oligomers are more sparsely populated. The position of the hydroxyl function at C-8 presumably renders the aromatic A-ring less able to react as nucleophile for condensation with C-4 carbocations. Alternatively, 8-hydroxylation might counteract electron release from the 7-hydroxyl group, thus reducing the tendency of flavan-3,4-diols **(8)**, **(9)**, **(10)** and **(11)** to form C-4 carbocations, which are considered essential for initiating condensation. These considerations culminated in suggestions that, based on electronic grounds, oligomers comprised of pyrogallol-type A-ring moieties are unlikely to exist[1, 15, 16].

The discovery of proteracacinidin oligomers in *Acacia caffra*[5] and *Acacia galpinii*[6] have since proven otherwise with the first of the proteracacinidins comprising of epioritin-(4β→6)-epioritin-4α-ol **(19)**[5], epioritin-(4β→6)-epioritin-4β-ol **(20)**[5], *ent*-oritin-(4β→5)-

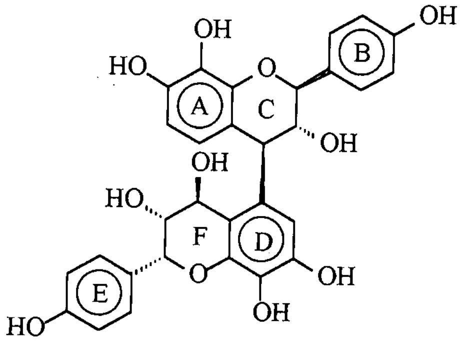


(19) ~~~~ = ...

(20) ~~~~ = ——

[14] D. Jacques, E. Haslam, G. R. Bedford and D. Greatbanks, *J. Chem. Soc., Perkin Trans. I*, 1974, 2663.
[15] J. J. Botha, D. Ferreira and D. G. Roux, *J. Chem. Soc., Chem. Commun.*, 1978, 700.
[16] J. J. Botha, D. Ferreira and D. G. Roux, *J. Chem. Soc. Perkin Trans. I*, 1981, 1235.

epioritin-4β-ol **(21)**[6] and the doubly linked *ent*-oritin-(4β→7, 5→6)-epioritin-4α-ol **(22)**[7].



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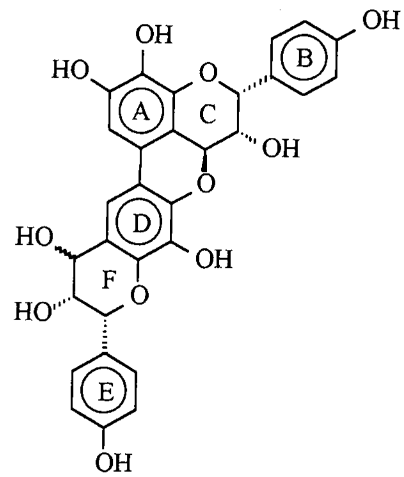
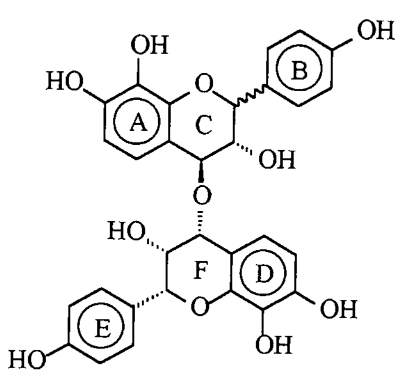


(22)

Except for the A-type proanthocyanidins **(18)**, ether-linked compounds were limited to the doubly ether-linked dioxane-type profisetinidin dimers, which were found in *Acacia mearnsii*[2,3]. Proanthocyanidins possessing a single ether-type interflavanyl linkage are very rare and recently Coetzee[17, 18, 19] isolated two (C_4-O-C_4)-linked compounds **(23)** and **(24)**, as well as the first two (C_4-O-C_3)-linked compounds **(25)** and **(26)** from *A. galpinii*.



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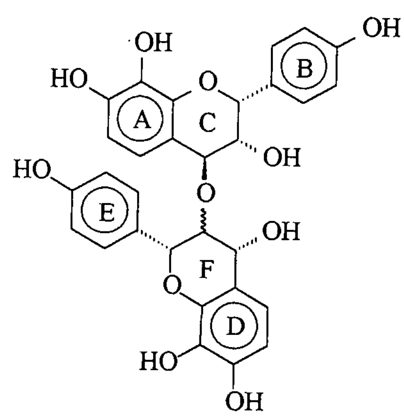

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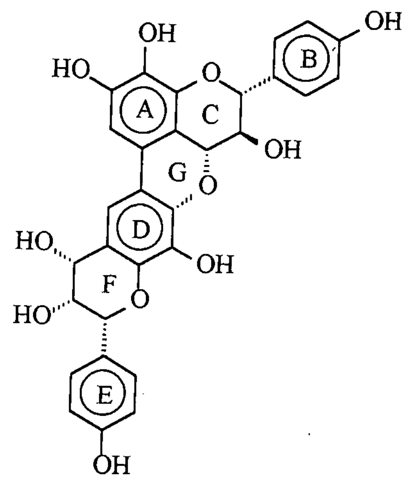
(26) =

[17] J. Coetzee, E. Malan and D. Ferreira, *J. Chem. Res.*, 1998, (S) 526, (M) 2287.
[18] J. Coetzee, E. Malan and D. Ferreira, *Tetrahedron*, 1998, **54**, 9153.
[19] J. Coetzee, E. Malan and D. Ferreira, *Tetrahedron*, 1999, **55**, 9999.

The series of doubly-linked proteracacinidin dimers **(22)**[7] were more recently extended by Bennie[20], with the identification of four new (4→7, 5→6) analogues, e.g. oritin-(4α→7, 5→6)-epioritin-4α-ol **(27)**, oritin-(4β→7, 5→6)-epioritin-4α-ol **(28)**, epioritin-(4β→7, 5→6)-epioritin-4α-ol **(29)** and epioritin-(4β→7, 5→6)-oritin-4α-ol **(30)**, from *A. galpinii* and *A. caffra*. The same study also yielded the first analogue with both a (4→5) C-C bond and a unique (3-O-4) ether linkage, epioritin-(4β→5, 3→4)-oritin-4α-ol **(31)**, from *A. caffra*.



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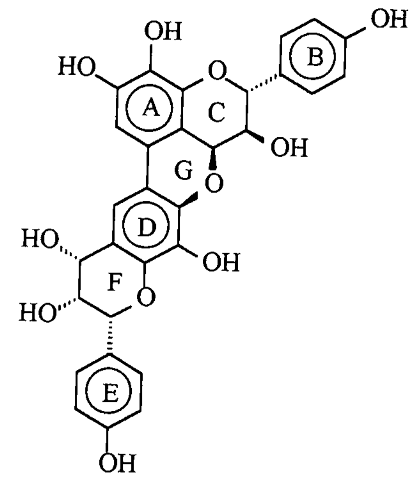

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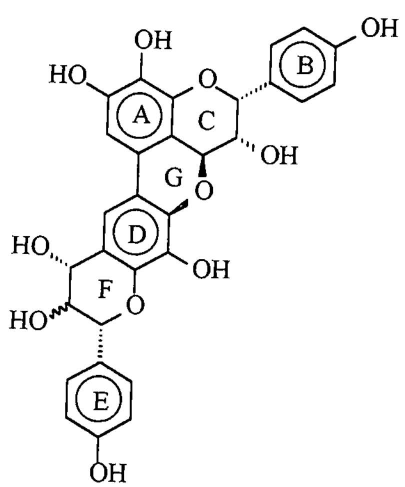

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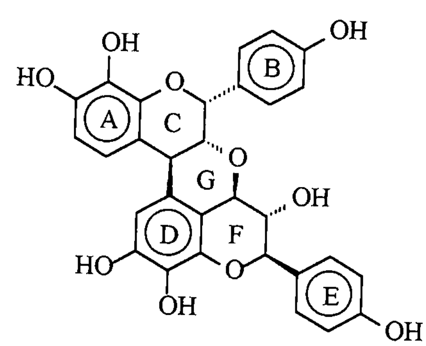

(31)

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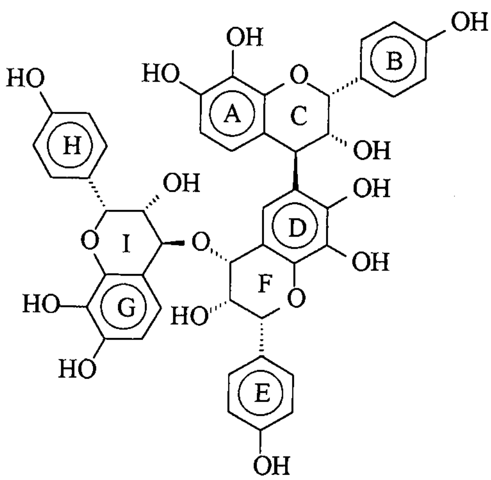
[20] L. Bennie, J. Coetzee, E. Malan, J. R. Woolfrey and D. Ferreira, *Tetrahedron*, 2001, **57**, 661.

Bennie[21] also isolated the first trimeric proteracacinidins with both a carbon-carbon and an ether (C-O-C) interflavanyl bond from *Acacia caffra*. Epioritin-(4β→6)-epioritin-(4α→4)-epioritin-4β-ol **(32)** and epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol **(33)** was identified, as well as the mixed proteracacinidin-promelacacinidin trimer epioritin-(4β→3)-epioritin-(4β→6)-epimesquitol-4α-ol **(34)**.



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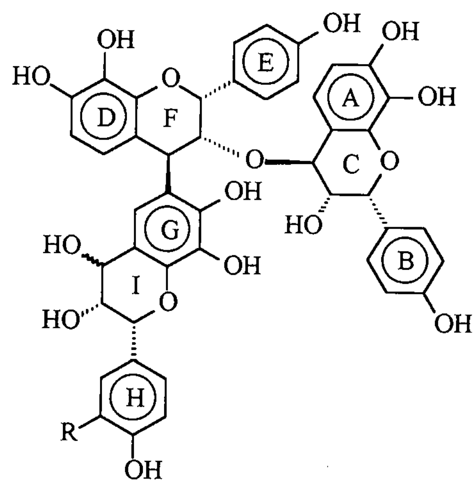


(33) ∿ = — ; R = H

(34) ∿ = ⋯ ; R = OH

The co-occurrence of the ether linked, as well as some carbon-carbon bonded proteracacinidins is further evidence of the much reduced nucleophilicity of the pyrogallol A-ring, which permits other centers to participate in interflavanyl bonding.

[21] L. Bennie, J. Coetzee, E. Malan and D. Ferreira, *Phytochemistry*, 2001, **57**, 1023.

4

Synthesis of Oligoflavanoids

4.1. Flavan-3,4-diols as potential electrophiles

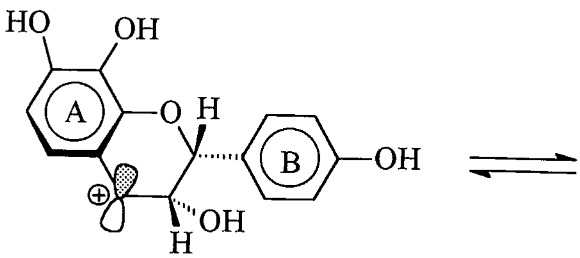
Flavan-3,4-diols represent structural units capable of generating C-4 carbocations **(35)**, under mild acidic conditions. These may be trapped by compounds with nucleophilic centers, e.g. flavan-3-ols or flavan-3,4-diols, leading to chain extension. The interflavanyl linkage usually occurs at C-8 or C-6, in accordance with basic chemical concepts[1,2,3].

The stability of C-4 carbocations is dependent on a number of factors, amongst others the degree of delocalization of the positive charge over the A-ring. Such delocalization will be most effective for C-4 carbocations derived from flavan-3,4-diols with phloroglucinol-type A-rings, intermediate in efficiency for resorcinol-type compounds and even less effective for pyrogallol-type compounds e.g. melacacinidins **(7)** and teracacinidins **(8)**[1,4,22].

The potential of the B-ring to contribute towards the stabilization of the C-4 carbocation was first suggested by Brown[23] and confirmed by Ferreira and co-workers[22,24,25,26]. The B-ring stabilizes the C-4 carbocation **(36)**, *via* an A-conformation, formally designated by Porter[27]. The A-conformation represents a half chair / sofa conformation for the heterocyclic C-ring, where the B-ring occupies an axial position **(36)**, in contrast with the

[22] J. A. Steenkamp, J. C. S. Malan and D. Ferreira, *J. Chem. Soc., Perkin I*, 1988, 2179.
[23] B. R. Brown and M. R. Shaw, *J. Chem. Soc., Perkin I*, 1974, 2036.
[24] J. P. Steynberg, J. F. W. Burger, D. A. Young, E. V. Brandt, J. A. Steenkamp and D. Ferreira, *J. Chem. Soc., Chem. Commun.*, 1988, 1055.
[25] J. P. Steynberg, J. F. W. Burger, D. A. Young, E. V. Brandt and D. Ferreira, *Heterocycles*, 1989, **28**, 923.
[26] J. P. Steynberg, E. V. Brandt and D. Ferreira, *J. Chem. Soc., Perkin Trans. II*, 1991, 1569.

conventional equatorial orientation **(35)**. Hence pyrogallol-type B-rings are more effective in stabilization of the C-4 carbocation followed by doubly substituted catechol-type B-rings, and singly substituted phenol-type B-rings the least effective, such as teracacinidins.



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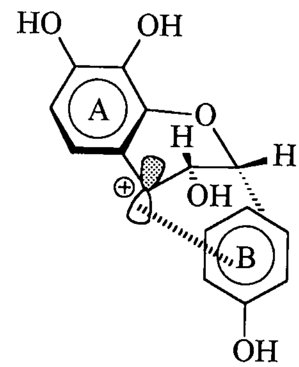
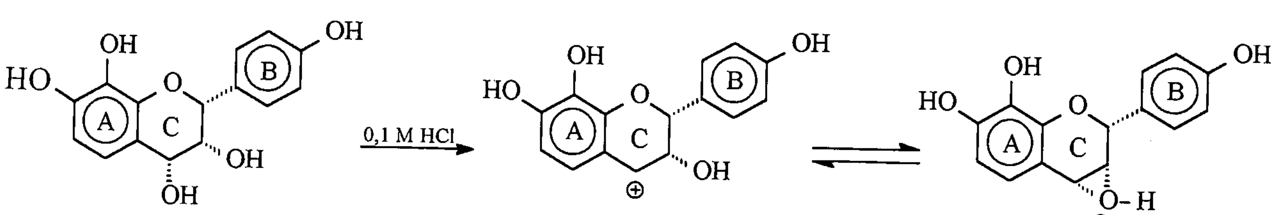


(36)

The stereochemistry at C-3 and C-4 also influences the reactivity of flavan-3,4-diols. Analogues possessing 4-axial hydroxyl groups are susceptible to facile ethanolysis under mild acidic conditions, while those with 4-equatorial hydroxyl functions are less prone to solvolytic reactions[28]. Such differences can be explained in terms of the enhanced leaving group ability of the C-4 hydroxyl group due to overlapping of the developing p-orbital with the π–system of the A-ring[2,28,29]. Axial C-3 hydroxyl groups may further stabilize C-4 carbocations by the so-called neighboring group effect, viz. formation of a protonated epoxide intermediate **(37)**.



(8) **(37)**

The inductive effect of the 4-hydroxyl function of flavan-3,4-diols or of the C-4 carbocation, resulting from its protonation, reduces the nucleophilicity of the A-ring, and

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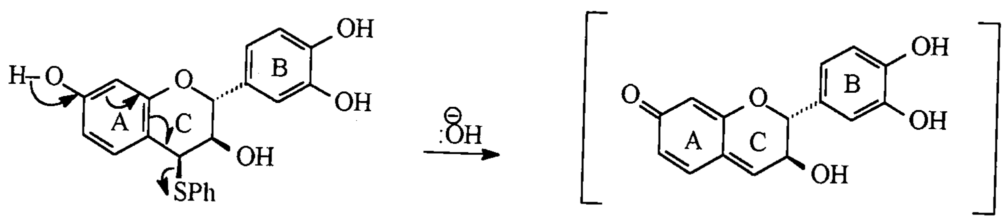
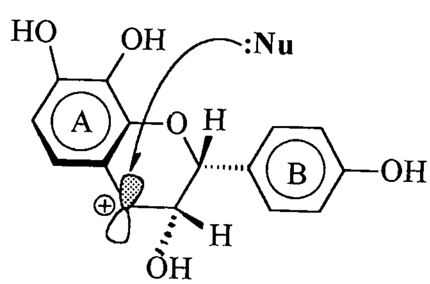
[27] L. J. Porter, R. Y. Wong, M. Benson, B. G. Chan, V. N. Vishwanadhan, R. D. Gandour, W. L. Mattice, *J. Chem. Res.*, 1986, (S), 86, (M), 830.
[28] J. W. Clark-Lewis and P. I. Mortimer, *J. Chem Soc.*, 1960, 4106.
[29] L. Y. Foo and H. Wong, *Phytochemistry*, 1986, **25**, 1961.

thus reduces its tendency for self-condensation. This problem was overcome by Hemingway and Foo, by first synthesizing the flavan-4-thioether **(14)** and then adding the flavan-3-ol as the acting nucleophile. The thio-ether presumably acts as a precursor to an A-ring quinone-methide **(13)**, which is trapped *via* interaction with the A-ring of the added flavan-3-ol.

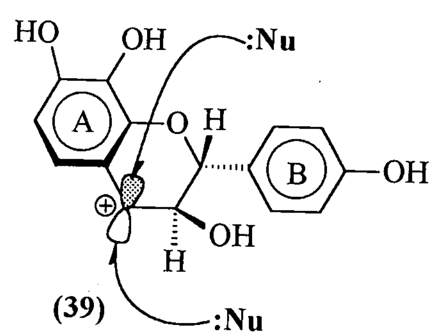


(14) **(13)**

Assuming that the carbocation intermediate possess a sofa conformation, nucleophilic attack on the ion with a 2,3-*cis* configuration (2R, 3R) **(38)** proceeds from the less hindered 'upper' side, presumably with the neighboring group participation of the axial 3-hydroxyl in an E-conformation and by the 2-axial B-ring in an A-conformation. This occurs with complete stereoselectivity. Reaction with a 2,3-*trans* carbocation (2R, 3S) **(39)** is directed preferentially from the less hindered 'lower' side, thus the reaction proceeds with a moderate degree of stereoselectivity[1,2].



(38)



(39)

The question regarding the intermediate of a C-4 carbocation **(35)** or an A-ring quinone methide **(13)**, in the condensation of flavan-3,4-diols with nucleophiles, is irrelevant to the stereochemical course of the coupling step, since C-4 is in both cases sp^2 hybridized with similar heterocyclic ring geometry. The formation of A-ring quinone methide intermediates nevertheless constitutes a viable mechanism for the condensation of 4-substituted flavans over a wide range of pH values[30,31,32].

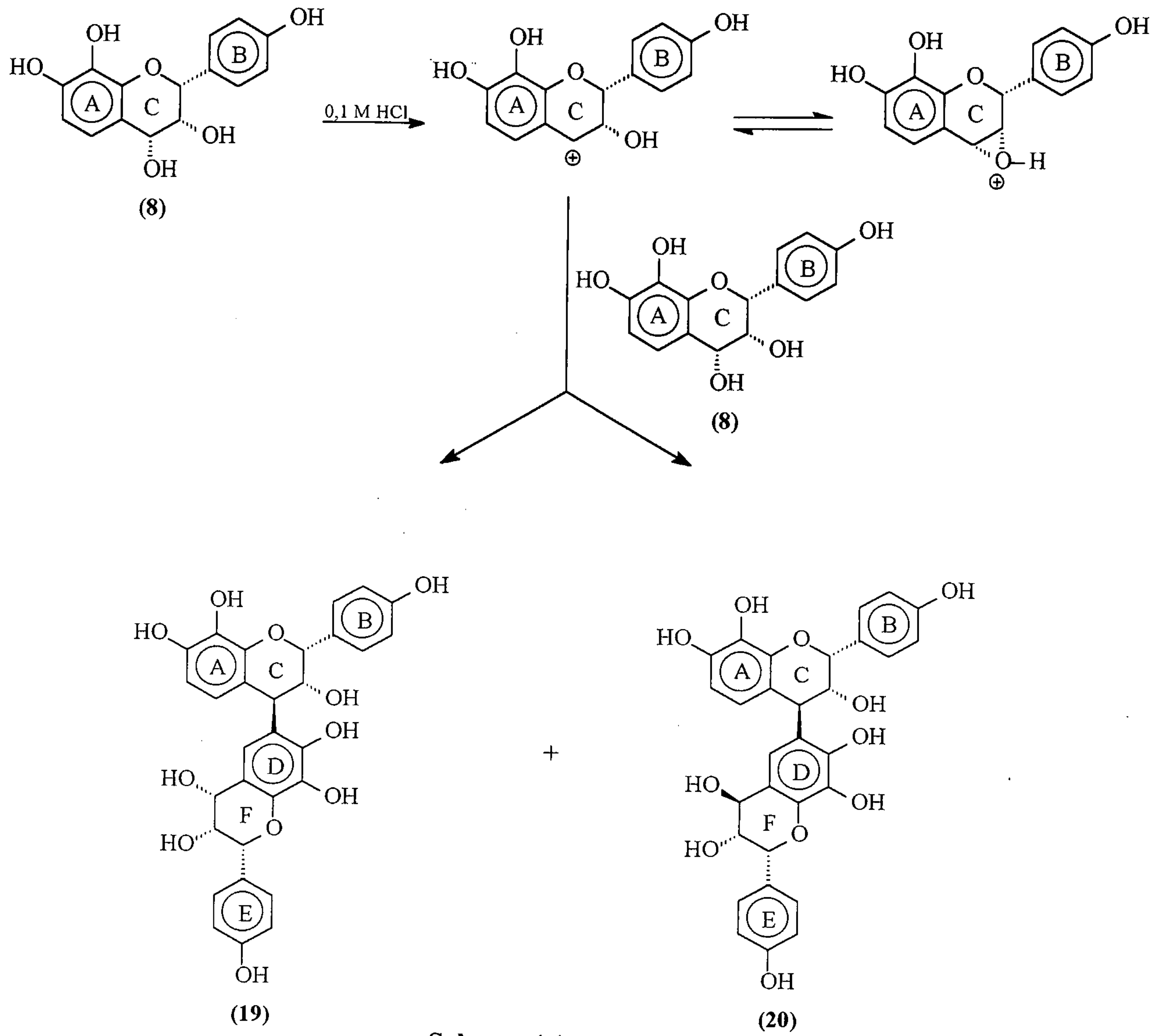
4.2. Acid catalyzed condensation reactions

Biomimetic synthesis is a convenient method used to confirm the structures of novel oligomers, by synthesizing the oligomer from the corresponding monomeric precursor. Acid catalyzed reactions, to produce C-4 carbocations or A-ring quinone methides, from flavan-3,4-diols have been thoroughly reviewed[1,2,3,4].

The structures of proteracacinidin dimers **(19)** and **(20)** were confirmed by the acid catalyzed self condensation of their biogenetic flavan-3,4-diol precursor, epioritin-4α-ol **(8)**, which occur in the heartwood of *A. galpinii* and *A. caffra*[5,6].

The flavan-3,4-diols are converted to an intermediate C-4 carbocation under mild acidic conditions, and stabilized by the neighboring hydroxyl group, as shown in **Scheme 4.1**.The unprotonated epioritin-4α-ol then acts as the nucleophile and couples *via* the C-6 position to the carbocation, to stereoselectively form the 4β- dimers **(19)** and **(20)**.

30 R. W. Hemingway and L. Y. Foo, *J. Chem. Soc., Chem. Commun.*, 1983, 1035.
31 M. R. Atwood, B. R. Brown, S. G. Lisseteer, C. L. Torrero and P. M.Weaver, *J. Chem. Soc., Chem. Commun.*, 1984, 177.
32 L. Y. Foo and R. W. Hemingway, *J. Chem. Soc., Chem. Commun.*, 1984, 85.



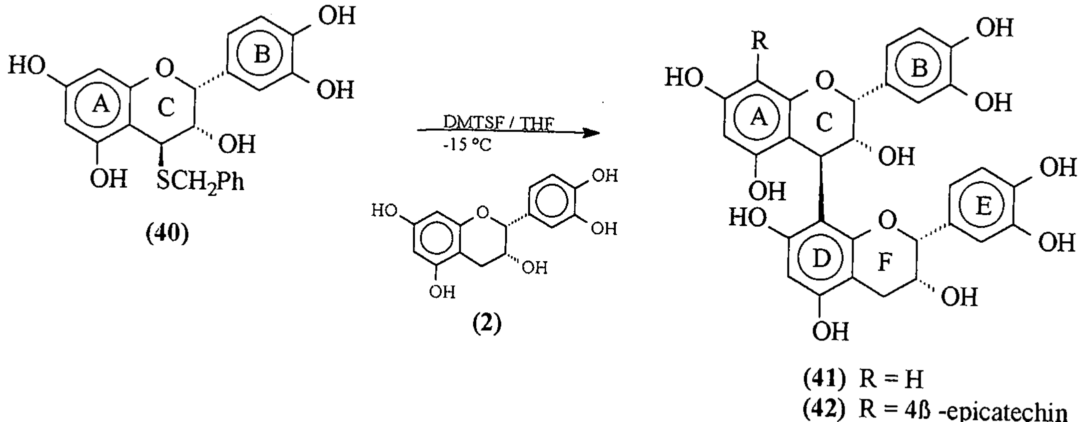
Scheme 4.1

4.3. C4 – Thiobenzylethers as electrophiles

A different approach towards the synthesis of oligomeric proanthocyanidins was used by Hemingway and Foo[30,32]. It utilized 4-thiobenzylethers as electrophiles under mild basic conditions (pH 9). Proanthocyanidin synthesis via quinone methide route has yielded dimers and related derivatives more cleanly and efficiently than the acid-catalyzed reactions[30]. In this approach an A-ring quinone methide is formed from the 4-thiobenzylethers, as shown in 4.1 **(13)**, and is trapped by the appropriate nucleophile.

The existing synthetic methods involve coupling of the electrophilic C-4 substituted flavan-3-ols under either acidic or basic conditions[30,32,33]. Under these conditions, the interflavanyl bonds are labile which invariably leads to an equilibrium between substrates and products[34]. The effectiveness of the thiophilic Lewis acids, dimethyl(methylthio)-sulfonium tetrafluoroborate (DMTSF) and silver tetrafluoroborate ($AgBF_4$), to activate the C_4-S bond in the 4-thioethers of flavan-3-ols towards carbon nucleophiles, and hence to generate the interflavanyl bond of proanthocyanidins under neutral conditions, were investigated by Ferreira and co-workers[1,33,34].

A typical procedure comprises a mixture of epicatechin-4β-thiobenzyleter **(40)**, an excess of catechin **(2)** as nucleophile and DMTSF or $AgBF_4$ in THF. This yielded procyanidin B1 **(41)**, as well as the analogous trimeric procyanidin **(42)**, as shown in scheme 4.2, with the difference in that the $AgBF_4$ yielded more of the dimeric compounds, and the DMTSF more of the trimeric and higher oligomeric compounds. This protocol compares favorably with the classical acid catalyzed condensation of catechin-4α-ol and catechin, which yielded a mixture of procyanidins and trimeric compounds[30,33,34].



Scheme 4.2.

[33] J. A. Delcour, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. I*, 1983, 1711.
[34] P. J. Steynberg, R. J. J. Nel, H. van Rensburg, B. C. B. Bezuidenhoudt and D. Ferreira, *Tetrahedron*, 1998, **54**, 8153.

Discussion

5

Leucoanthocyanidins from *Acacia hereroensis*

5.1. Introduction

Acacia hereroensis[35], also known as the Mountain Thorn, is a small to medium-sized single-stemmed tree with an irregular rounded crown, that can grow to a height of 11 m, or a small multi-stemmed shrub. The bark on the main stem is dark grey to greyish-brown and longitudinally fissured. The new season's shoots are sparsely hairy and green to reddish brown. The paired prickles are slightly recurved and sparsely covered with hairs when young. The leaves are borne at the nodes, singly or up to 3 leaves per node.

[35] N. Smit, Guide to the *Acacias* of South Africa, Briza Publications, 1999.

Light yellow to cream coloured flowering spikes are borne singly or in pairs at the nodes on hairy peduncles. The dehiscent pods are flat and straight with occasional constrictions between the seeds.


A. hereroensis can be found in open savanna and dry grassland, notably on rocky hillsides and along shallow watercourses. It may grow on a variety of soil types, but is often associated with dolomite formations, and it is fairly tolerant to cold.

The tree has been collected near Klerksdorp, in the North-West province of South Africa, and it has been classified as:

Family	:	Leguminosae (pod-bearing family)
Sub Family	:	Mimosiadae
Genus	:	*Acacia*
Species	:	*Hereroensis*

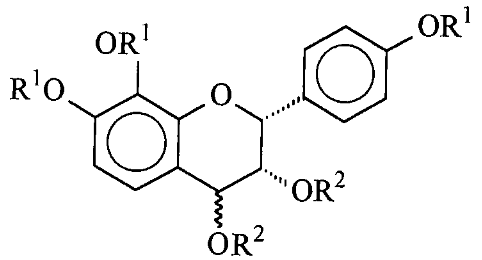
5.2. Flavan-3,4-diols from *Acacia hereroensis*

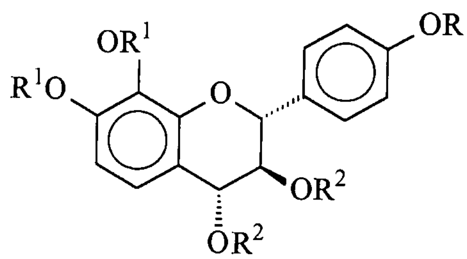
Acacia galpinii and *Acacia caffra* represent the first two South African species, which contain flavanoid analogues with a 7,8,4'-trihydroxy phenolic substitution pattern[5,36].

Flavanoids with a 7,8,4'-hydroxylation pattern, correlating with those in *A. galpinii* and *A. caffra*, have now also been isolated from *Acacia hereroensis*. The monomer epioritin-

4α-ol **(8)** dominates in the heartwood (12.0 %, w/w), while the diastereomers, epioritin-4β-ol **(9)** (3.3%, w/w) and oritin-4α-ol **(10)** (1.3%, w/w) are also present.

The structure and absolute configurations of the teracacinidin tetramethylether diacetate derivatives **(43)**, **(44)** and **(45)** were confirmed by comparison of their ^{1}H NMR [$CDCl_3$, 296 K] data **(Table 5.1, Plate 1 – 3)** with those of the corresponding monomers from *A. galpinii*[6] and *A. caffra*[5].





(8) ∿∿∿ = ⋯⋯ , $R^1 = R^2 = H$
(43) ∿∿∿ = ⋯⋯ , R^1 = Me, R^2 = Ac
(9) ∿∿∿ = ▬ , $R^1 = R^2 = H$
(44) ∿∿∿ = ▬ , R^1 = Me, R^2 = Ac

(10) $R^1 = R^2 = H$
(45) R^1 = Me, R^2 = Ac

^{1}H NMR data **(Table 5.1, Plate 1 – 3)** of the methylether acetate derivatives **(43)**, **(44)** and **(45)** indicated an AA'BB'- and an AB- system in the aromatic region. An AMX-system in the heterocyclic region together with three methoxy and two acetoxy signals confirmed the flavan-3,4-diol nature of the three analogues.

[36] E. Malan and D. G. Roux, *Phytochemistry*, 1975, **14**, 1835.

Table 5.1: ^1H NMR peaks (δ_H) of the flavan-3,4-diol derivatives **(43)**, **(44)** and **(45)** at 300 MHz (296 K). Splitting patterns and J values (Hz) are given in parentheses.

Ring	Proton	**(43)** $CDCl_3$	**(44)** $CDCl_3$	**(45)** $CDCl_3$
A	5	6.92 (d, 9.0)	7.19 (d, 9.0)	6.89 (d, 9.0)
	6	6.61 (d, 9.0)	6.62 (d, 9.0)	6.63 (d, 9.0)
B	2', 6'	7.40 (d, 9.0)	7.43 (d, 9.0)	7.36 (d, 9.0)
	3', 5'	6.91 (d, 9.0)	6.94 (d, 9.0)	6.91 (d, 9.0)
C	2	5.34 (s, 1.0)	5.34 (s, 1.5)	5.17 (d 8.5)
	3	5.62 (dd, 1.0, 4.5)	5.23 (dd, 1.5, 3.0)	5.50 (dd, 8.5, 7.0)
	4	6.31 (d, 4.5)	5.90 (d, 3.0)	6.21 (d, 7.0)
OMe		3.90, 3.88, 3.81 (all s)	3.93, 3.90, 3.84 (all s)	3.89, 3.87, 3.83 (all s)
OAc		2.11, 1.92 (all s)	2.15, 1.90 (all s)	1.99, 1.90 (all s)

The small $^3J_{2,3}$ values in the ^1H NMR spectra **(Plate 1 and 2)** of **(43)** and **(44)** together with a broad singlet for both **(43)** and **(44)** are reminiscent of a 2,3-*cis* relative configuration for both. Comparison of the $^3J_{3,4}$ values of 4.5 and 3.0 Hz for **(43)** and **(44)** respectively with those of authentic samples confirmed the 3,4-*cis* and 3,4-*trans* relative configuration for **(43)** and **(44)** respectively. The large heterocyclic coupling constant ($^3J_{2,3}$, 8.5 Hz) **(Plate 3)** of **(45)** is characteristic of a 2,3-*trans* relative configuration, as well as the large $^3J_{3,4}$ value of 7.0, reminiscent of a 3,4-*trans* relative configuration, after comparison with those of an authentic sample[5,6].

6

Dimeric Proanthocyanidins from *Acacia Hereroensis*

6.1. Introduction

The very recent discovery of proanthocyanidins with a pyrogallol A-ring was limited to the heartwoods of *Acacia galpinii*[6] and *Acacia caffra*[5] for proteracacinidins.

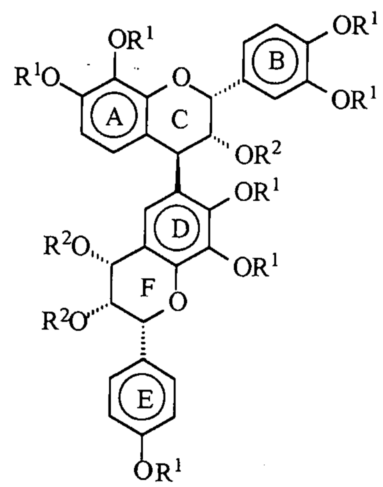
The notable variety of types and positions of interflavanyl bonds, eg. carbon-carbon and ether-linked, at C-3, C-4, C-5 and C-6 positions, present in the flavanoid compounds isolated from *A. galpinii* and *A. caffra* to date, are a manifestation of the relatively reduced nucleophilicity of the pyrogallol A-ring.

The present investigation of the methanol extract of the *Acacia hereroensis* heartwood revealed the occurrence of three novel C-4(C-ring) → C-6(D-ring) linked proteracacinidin dimers and one novel C-4(C-ring) → C-6(D-ring) linked promelacacinidin / proteracacinidin dimer.

6.2. C-4(C-ring) → C-6(D-ring) Proanthocyanidins

6.2.1. Epimesquitol-(4β→6)-epioritin-4α-ol (46)

The ^1H NMR data **(table 6.1, plate 10)** of the heptamethylether triacetate derivative **(47)** showed two heterocyclic AMX-systems belonging to a teracacidin and melacacidin unit present in the dimer.



(46) $R^1 = R^2 = H$
(47) $R^1 = Me, R^2 = Ac$

The presence of an AB- and an ABX-system of the melacacidin unit, and an AA'BB'-system and a singlet assigned to the teracacidin unit in the aromatic resonance region, was identified from the COSY experiment. The residual proton at δ 6.27 was indicative of the lower teracacidin moiety **(47)**.

The respective proton-systems belonging to the top and bottom units were evident from the COSY $^4J_{HH}$ couplings between 2-H (C, δ 5.13), 2-H (F, δ 5.33) and the 2',6'-protons of the ABX- and AA'BB'-systems respectively. $^3J_{HH}$ interaction between 4-H (C, δ 5.47) and 5-H at δ 6.75 identified the AB-system of the A-ring.

Phase sensitive NOESY experiment showed associations from 5-H (D, δ 6.27) to 4-H (F) and 4-H (C) suggesting a (4→6) bond between the two moieties.

Coupling ($^4J_{HH}$) of the residual singlet 5-H (D) at δ 6.27 to both 4-H (F, δ 6.20) and 4-H (C, δ 4.44) established the (4→6) interflavanyl linkage.

HMQC, HMBC and ^{13}C data **(table 6.2)** confirmed the top unit of **(47)** as a melacacidin moiety and the terminal unit as a teracacidin flavan-3,4-diol. The 4-C (C) at δ_C 41.7 is in accordance with a phenyl substituent at this carbon[37]. Long-range HMBC correlations between H-4 (C, δ 4.44) and 6-C (D, δ 128.5, $^{2}J_{CH}$) from H-5 (D, δ 6.27) to 5-C (D, 2JCH) and 3-H (C, δ 5.47) to 5-C (D, $^{3}J_{CH}$) confirmed the 4-C (A) to 6-C (D) linkage.

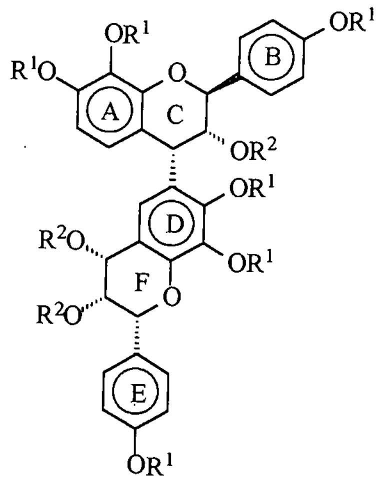
The positive Cotton effect (**plate 3**) of $[\theta]_{242.5}$ 1532 is characteristic of a 4β C-ring substituent[38]. The AMX heterocyclic system of the C-ring showed coupling constants ($J_{2,3}$ = 1.5 Hz and $J_{3,4}$ 3.0 Hz) typical of a 2,3-*cis*-3,4-*trans* stereochemistry[5,6]. The F-ring exhibited coupling constants ($J_{2,3}$ 1.0 Hz and $J_{3,4}$ 4.5 Hz) indicative of a 2,3-*cis*-3,4-*cis* relative stereochemistry[5,6], the latter was confirmed by n.O.e. association between 2-H (F) and 4-H (F) suggesting the two protons to be cofacial. From the chiroptical data (4β, C-ring) in conjunction with relative stereochemistry of *cis-trans* the absolute stereochemistry of the C-ring was assigned as 2R,3R,4R. The abundant presence of epioritin-4α-ol in the heartwood makes it the logical precursor for the bottom unit and an absolute stereochemistry of 2R,3R,4R was assigned to the F-ring.

6.2.2. *ent*-Oritin-(4α→6)-epioritin-4α-ol (48)

The ^{1}H NMR data **(table 6.1, plate 11)** of the hexamethyl triacetate derivative **(49)** showed the presence of an AB- and two AA'BB'-spin systems and one residual singulet (δ 6.54) for the aromatic protons. Two AMX-systems were present for the heterocyclic protons (C- and F-rings) with the characteristic lower field 4-H (F) present, which had been diagnostic for a flavan-3,4-diol terminating unit[39]. The respective B- and E-ring systems were identified by $^{4}J_{HH}$ coupling between 2-H (C) and the 2',6'-protons. The $^{4}J_{HH}$ coupling (benzylic) between 4-H (C) and 5-H (A) established the AB-system of the A-ring. The interflavanyl linkage 4-C (C) → 6-C (D) was established by $^{4}J_{HH}$ coupling between 5-H (D, δ 6.54) and 4-H (F, δ 6.25) and to 4-H (C, δ 4.67) respectively; n.O.e. interaction between the 5-H (D) and the 4-H (F) and 4-H (C) protons; HMBC

[37] L. Y. Foo, *J. Chem. Soc., Chem. Comm.*, 1985, 1273.
[38] J. H. van der Westhuizen, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin I*, 1981, 1220.
[39] P. M. Viviers, D. A. Young, J. J. Botha, D. Ferreira, D. G. Roux and W. E. Hull, *J. Chem. Soc., Perkin I*, 1982, 535.

correlations between 4-H (C) and 6-C (D, δ_C 126.2, $^2J_{CH}$), 3-H (C) to 6-C (D, $^3J_{CH}$) and 5-H (D) to 6-C (D, $^2J_{CH}$), all which confirmed the position of the interflavanyl linkage.



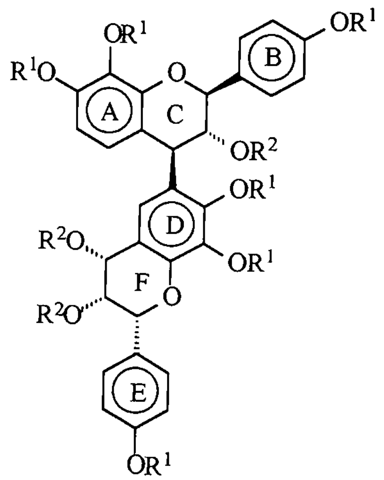
(48) $R^1 = R^2 = H$
(49) $R^1 = Me, R^2 = Ac$

The ^{13}C resonance appearance **(table 6.2)** of the 4-C (C) at δ_C 36.1 is diagnostic for a phenyl-substituent at the benzylic carbon of the heterocyclic ring, thus confirming the upper position of interflavanyl linkage[37]. No n.O.e. association between 2-H (C) and 4-H (C) could be detected. The deviation in coupling constants shown by **(49)** ($J_{2,3} = 6.5$ Hz; $J_{3,4} = 4.5$ Hz.) are well acounted for, arising from results obtained from similar compounds with a 2,3-*trans*-3,4-*cis* stereochemistry[5,6], attributed to a quasi-axial conformation of the 4-C (C) substituent.

The negative Cotton effect (**plate 4**) of -14 300 is reminiscent of a 4α C-ring substituent, which in conjunction with the 2,3-*trans*-3,4-*cis* stereochemistry supported the 2S,3R,4S absolute configuration for the top unit of **(49)**[38]. The relative and absolute stereochemistry (2R,3R,4R) of the F-ring were assigned from the coupling constants and the fact that epioritin-4α-ol are abundantly present in the heartwood and thus would logically form the the terminal flavan-3,4-diol unit.

6.2.3. *ent*-Oritin-(4β→6)-epioritin-4α-ol (50)

The ^{1}H NMR data **(table 6.1, plate 12)** of the hexamethyl triacetate derivative **(51)** showed the presence of an AB- and two AA'BB'-spin systems and one residual singulet (δ 6.91) for the aromatic protons. Two AMX-systems were present for the heterocyclic protons (C- and F-rings) with the characteristic lower field 4-H (F) present, which had been diagnostic for a flavan-3,4-diol terminating unit[39]. The respective B- and E-ring systems were identified by $^{4}J_{HH}$ coupling between 2-H (C) and the 2',6'-protons. The $^{4}J_{HH}$ coupling (benzylic) between 4-H (C) and 5-H (A) established the AB-system of the A-ring. The interflavanyl linkage 4-C (C) → 6-C (D) was established by $^{4}J_{HH}$ coupling between 5-H (D, δ 6.91) and 4-H (F, δ 6.32) and to 4-H (C, δ 4.45) respectively; N.O.e. interaction between the 5-H (D) to the 4-H (F) and 4-H (C) protons, the HMBC correlation between 4-H (C) and 6-C (D, δ_C 126.8, $^{2}J_{CH}$), 3-H (C) to 6-C (D, $^{3}J_{CH}$), 5-H (D) to 6-C (D, $^{2}J_{CH}$) confirmed the position of the interflavanyl linkage.



(50) $R^1 = R^2 = H$
(51) $R^1 = Me, R^2 = Ac$

The ^{13}C NMR resonance appearance **(table 6.2)** of the 4-C (C) at δ_C 45.5 is diagnostic for a phenyl-substituent at the benzylic carbon of the heterocyclic ring, thus confirming the upper position of interflavanyl linkage[37]. N.O.e. association between 2-H (C, δ 5.01) and 4-H (C, δ 4.45) was very prominent, supporting the assigned relative stereochemistry.

The coupling constants ($J^{2,3} = 10.0$; $J^{3,4} = 10.0$) between the heterocyclic protons of the C-ring are representative of a *trans-trans* relative configuration[5,6].

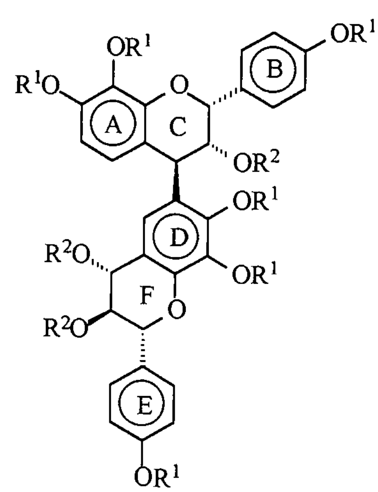
The positive Cotton effect (**plate 5**) of 12 850 is reminiscent of a 4β C-ring substituent, which in cunjunction with the 2,3-*trans*-3,4-*trans* stereochemistry supported the 2S,3R,4R absolute configuration for the top unit of **(51)**[38]. The relative and absolute stereochemistry (2R,3R,4R) of the F-ring were assigned from the coupling constants and the fact that epioritin-4α-ol are abundantly present in the heartwood and thus would logically form the the terminal flavan-3,4-diol unit.

Table 6.1: ^{1}H NMR peaks (δ_C) for **(47)**, **(49)**, **(51)** and **(53)** at 300 MHz (296 K). Splitting patterns and J values (Hz) are given in parentheses.

Ring	Carbon	(47) $CDCl_3$	(49) $CDCl_3$	(51) Ac-d_6	(53) $CDCl_3$
A	5	6.75(d, 8.5)	6.66(d, 8.5)	6.44(d, 9.0)	6.68(d, 9.0)
	6	6.59(d, 8.5)	6.52(d, 8.5)	6.58(d, 9.0)	6.57(d, 9.0)
B	2',6'		7.33(d, 9.0)	7.47(d, 9.0)	7.31(d, 9.0)
	3',5'		6.89(d, 9.0)	6.96(d, 9.0)	6.88(d, 9.0)
	2'	7.00(d, 2.0)			
	5'	6.82(d, 8.5)			
	6'	6.88(dd, 2.0, 8.5)			
C	2	5.13(br.s, 1.5)	5.34(d, 6.5)	5.01(d, 10.0)	5.14(br.s, 1.5)
	3	5.47(dd, 1.5, 3.0)	5.43(dd, 6.5, 4.5)	5.77(dd, 10.0, 10.0)	5.39(dd, 1.5, 3.0)
	4	4.44(d, 3.0)	4.67(d, 4.5)	4.45(br.d, 10.0)	4.43(d, 3.0)
D	5	6.27(br.s)	6.54(br.s)	6.91 (br.s)	6.20(br.s)
E	2',6'	7.40(d, 8.5)	7.41(d, 9.0)	7.53(d, 9.0)	7.37(d, 9.0)
	3',5'	6.92(d, 8.5)	6.93(d, 9.0)	6.98(d, 9.0)	6.92(d, 9.0)
F	2	5.33(br.s, 1.0)	5.32(br.s, 1.0)	5.63(br.s, 1.0)	5.08(d, 10.0)
	3	5.58(dd, 1.0, 4.5)	5.59(dd, 1.0, 4.5)	5.59(dd, 1.0, 4.5)	5.46(dd, 10.0, 8.0)
	4	6.20(d, 4.5)	6.25(d, 4.5)	6.32(br.d, 4.5)	6.16(d, 8.0)
OMe		4.01, 3.99, 3.98, 3.89, 3.88, 3.87, 3.83 (all s)	3.76, 3.82, 3.84, 3.88, 3.93, 3.96 (all s)	3.63, 3.76, 3.79, 3.82, 3.82, 3.85 (all s)	4.00, 3.99, 3.90, 3.88, 3.84, 3.82 (all s)
OAc		1.95, 1.92, 1.87 (all s)	1.87, 1.94, 1.96 (all s)	1.63, 1.92, 2.05 (all s)	1.92, 1.82, 1.81 (all s)

6.2.4. Epioritin-(4β→6)-oritin-4α-ol (52)

The ^{1}H NMR data **(table 6.1, plate 13)** of the hexamethyl triacetate derivative **(53)** showed the presence of an AB- and two AA'BB'-spin systems and one residual singulet (δ 6.20) for the aromatic protons. Two AMX-systems were present for the heterocyclic protons (C- and F-rings) with the characteristic lower field 4-H (F) representing the diagnostic flavan-3,4-diol terminating unit[39]. The respective B- and E-ring systems were identified by $^4J_{HH}$ coupling between 2-H (C) and the 2',6'-protons. The $^4J_{HH}$ coupling (benzylic) between 4-H (C) and 5-H (A) established the AB-system of the A-ring. The interflavanyl linkage 4-C (C) → 6-C (D) was established by $^4J_{HH}$ coupling between 5-H (D, δ 6.20) and 4-H (F, δ 6.16) and to 4-H (C, δ 4.43) respectively. N.O.e. interaction between the 5-H (D) and the 4-H (F) and 4-H (C) protons, together with HMBC correlations between 4-H (C) and 6-C (D, δ_C 129.4, $^2J_{CH}$), 3-H (C) to 6-C (D, $^3J_{CH}$), 5-H (D) to 6-C (D, $^2J_{CH}$) confirmed the position of the interflavanyl linkage.



(52) $R^1 = R^2 = H$
(53) $R^1 = Me, R^2 = Ac$

The ^{13}C NMR **(table 6.2)** resonance appearance of the 4-C (C) at δ_C 41.4 is diagnostic for a phenyl-substituent at the benzylic carbon of the heterocyclic ring, thus confirming the upper position of interflavanyl linkage[37]. No n.O.e. association between 2-H (C) and 4-H

(C) could be detected. The coupling constants ($J^{2,3}$ = 1.5; $J^{3,4}$ = 3.0) between the heterocyclic protons of the C-ring is reminiscent of of a *cis-trans* relative configuration[5,6].

The positive Cotton effect (**plate 6**) of 5 463 is a confirmed indication of a 4β C-ring substituent, which in conjunction with the 2,3-*cis*-3,4-*trans* stereochemistry supported the 2R,3R,4R absolute configuration for the top unit of **(53)**[37]. The relative and absolute stereochemistry (2R,3S,4R) of the F-ring were assigned from the coupling constants and the fact that oritin-4α-ol are also abundantly present in the heartwood and thus would logically form the the terminal flavan-3,4-diol unit.

<u>Table 6.2:</u> ^{13}C NMR peaks (δ_C) for **(47)**, **(49)**, **(51)** and **(53)**.

Ring	Carbon	**(47)** $CDCl_3$	**(49)** $CDCl_3$	**(51)** Ac-d_6	**(53)** $CDCl_3$
A	5	125.5	124.5	123.3	125.3
	6	105.4	105.0	106.0	105.6
B	2’	110.2	127.9	129.3	128.0
	3’	148.9	114.2	113.9	113.9
	5’	111.0	114.2	113.9	113.9
	6’	119.1	127.9	129.3	128.0
C	2	73.6	76.1	80.5	73.6
	3	72.1	71.6	72.3	72.2
	4	41.7	36.1	45.5	41.4
D	5	123.4	123.5	122.9	124.3
	6	128.5	126.2	126.8	129.4
E	2’	127.9	128.0	128.2	129.0
	3’	114.2	114.3	113.8	114.2
	5’	114.2	114.3	114.2	113.8
	6’	127.9	128.0	128.2	129.0
F	2	77.4	77.4	77.2	79.4
	3	66.9	67.0	67.3	71.4
	4	67.1	67.0	66.9	70.7

7

Ether-linked dimeric Proanthocyanidin from *Acacia Hereroensis*

7.1. Introduction

Proanthocyanidins possesing ether-type interflavanyl linkages are extremely rare, except for the A-type oligomers, which contain the conventional C_4 (C-ring) → C_6/C_8 (D-ring) bond, as well as an additional ether linkage connecting C_2 (C-ring) and C_5/C_7 (D-ring)[2,3,11]. Analogues which posses exclusive ether bonds are hitherto restricted to the 1,4-dioxane-type profisetinidins from *Acacia mearnsii*[40,41], the recently reported (4→7:5→6) doubly linked proteracacinidin from *A. caffra*[7], two (C_4-O-C_3)- and two (C_4-O-C_4)-linked proteracacinidins from *A. galpinii*[17,18], as well as two (C_4-O-C_4)-promelacacinidins from *A. melanoxylon*[42].

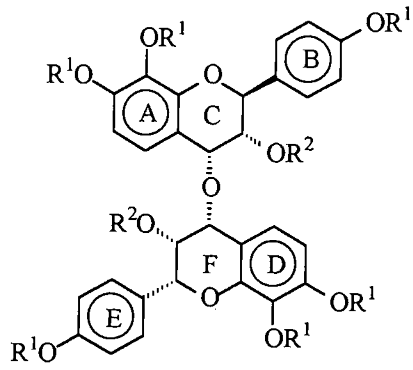
7.2. *ent*-Oritin-(4α→4)-epioritin-4α-ol (54)

Owing to the complexity of the phenolic mixture, the dimer **(54)** was identified as the hexamethyl ether diacetate derivative **(55)**, which enabled additional chromatographic steps to purify the compound.

The ^{1}H NMR data **(table 7.1, plate 14)** of the derivative **(55)** indicated the presence of two AB- and two AA'BB'-spin systems for aromatic protons as well as two AMX-systems for protons of the heterocyclic rings, hence indicating the dimeric nature of the compound. Differentiation of the spin systems and the connectivities between aromatic

[40] S. E. Drewes and A. H. Ilsley, *J. Chem. Soc. (C)*,1969, 897.
[41] D. A. Young, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin I*, 1983, 2031.

and heterocyclic protons were effected with 2D COSY experiments. The presence of six O-methyl and two O-acetyl resonances, as well as the FAB-MS data, showed a molecular ion at m/z 730 and suggested a molecular formula of $C_{40}H_{42}O_{13}$ for the compound, which proposed an ether-type interflavanyl linkage.



(54) $R^1 = R^2 = H$
(55) $R^1 = Me$; $R^2 = Ac$

Application of the shielding phenomenon observed for 4-H(C) of the ABC chain extender unit of oligomeric proanthocyanidins relative to the chemical shifts of the same proton in the 3,4-di-O-acetyl derivative of the flavan-3,4-diol precursor[5,15,42], indicated a C_4-O-C_4 ether bond [4-H(C), δ 4.91 and 4-H(F), δ 5.12]. The chemical shifts of the 3-H resonances of both the C- and F-rings of the derivative **(55)** are reminiscent of methine hydrogens of an O-acetyl substituted carbon, hence supporting the ether linkage involving C-4(C) of both flavanyl constituent units.

Prominent $^4J_{HH}$ couplings, evident in the 2D COSY spectrum of **(55)**, between 2-H(C, δ 5.65) and 2'6'-H(B, δ 7.37), as well as between 2-H(F, δ 5.29) and 2'6'-H(E, δ 7.42) differentiated the AA'BB' spin systems of the constituent flavanyl units. The A/C- and D/F-ring junctions were respectively connected *via* the observed benzylic coupling of 5-H(A, δ 6.98) with 4-H(C, δ 4.91) and of 5-H(D, δ 6.84) with 4-H(F, δ 5.12).

The coupling constants of the heterocyclic ring system ($J_{2,3(C)} = 10.5$, $J_{3,4(C)} = 2.5$ Hz and $J_{2,3(F)} = 1.0$, $J_{3,4(F)} = 4.0$ Hz) indicated a 2,3-*trans*-3,4-*cis* (C); 2,3-*cis*-3,4-*cis* (F) relative

[42] L. Y. Foo, *J. Chem. Soc., Chem. Comm.*, 1989, **20**, 1505.

configuration for the proteracacinidin derivative **(55)**[5,6]. A strong NOE association was observed between 2- and 4-H(F), which confirmed the 2,4-*cis* relative configuration of the DEF constituent unit. The conspicuous absence of NOE association between 2- and 4-H(C) was interpreted as confirmation of the 2,4-*trans* relative configuration of the ABC moiety.

A phase sensitive NOESY experiment of derivative **(55)** showed associations of 5-H(D) with 5-H(A), 2-H(C) and 4-H(C), of 5-H(A) with 3-H(F), 4-H(F) and 5-H(D) and of 4-H(C) with 4-H(F). Collectively these NOE effects are only reconcilable with C_4-O-C_4 interflavanyl linkage for derivative **(55)** of the novel proteracacinidin dimer **(54)**.

The CD spectrum (**plate** 7) of the proteracacinidin derivative **(55)** exhibited a strong Cotton effect near 275 (positive), 240 (negative), 225 (positive) and 220 nm (negative). The aromatic quadrant rule[43] is a powerful probe for establishing the absolute configuration at C-4 of 'conventional' $C_4 \rightarrow C_6/C_8$ coupled dimeric proanthocyanidins[15,16,44], but it cannot be used to the same effect for ether linked analogues. The CD data were only useful in a comparative capacity when derivative **(55)** was also available *via* synthesis using flavan-3,4-diol precursors with established absolute configuration at C-2 and –3.

The 2D COSY spectrum showed no couplings between 4-H(C) and 5-H(A) to 4-H(F) and neither was there any from 5-H(D) to 4-H(C).

The ^{13}C data (**plate 16**) showed the absence of a 4-C(C) resonance at around δ 43.0, which is consistent with an aryl substituent at this position is indirect proof of a C-O-C linkage[37].

The appearance of 4-C(F) and 4-C(C) at δ 69.77 and 72.34 respectively is in accordance with the downfield shift[42] (Δ δ, +3 to +6) of an ether bonded carbon with reference to a -

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[43] G. G. de Angelis and W. C. Wildman, *Tetrahedron*, 1969, **25**, 5099.

C-OAc bonded carbon occuring at approximately δ 66.0, and thus supporting the 4-C(C) to 4-C(F) ether bond.

Table 7.1: ^{1}H and ^{13}C NMR resonances of proteracacinidin derivative **(55)** in CDCl3 at 300 MHz (296 K). Splitting patterns and coupling constants (Hz) are given in parentheses.

Ring	Carbon	δ ^{13}C ($CDCl_3$)	δ ^{1}H ($CDCl_3$)
C	2	74.83	5.65 (d, 10.5)
	3	72.29	5.38 (dd, 2.5, 10.5)
	4	72.34	4.91 (d, 2.5)
A	5	125.27	6.98 (d, 8.5)
	6	104.53	6.58 (d, 8.5)
	7	155.18	
	8	137.92	
	9	148.63	
	10	114.33	
B	1'	129.55	
	2'	129.12	7.37 (d, 9.0)
	3'	114.19	6.89 (d, 9.0)
	4'	160.12	
	5'	114.19	6.89 (d, 9.0)
	6'	129.12	7.37 (d, 9.0)
F	2	77.63	5.29 (br.s, 1.0)
	3	66.58	5.76 (dd, 1.0, 4.0)
	4	69.77	5.12 (d, 4.0)
D	5	122.31	6.84 (d, 9.0)
	6	105.56	6.53 (d, 9.0)
	7	153.34	
	8	136.94	
	9	148.41	
	10	115.63	
E	1'	130.40	
	2'	127.86	7.42 (d, 9.0)
	3'	114.10	6.93 (d, 9.0)
	4'	159.95	
	5'	114.10	6.93 (d, 9.0)
	6'	127.86	7.42 (d, 9.0)
OCH_3		55.64, 55.67, 56.50, 56.50, 61.35, 61.42	3.90, 3.88, 3.87, 3.85, 3.84, 3.82
$COCH_3$		21.12, 21.29	1.97, 1.86
$COCH_3$		170.06, 170.41	

[44] M. W. Barrett, S. Klyne, P. M. Scopes, A. C. Fletcher, L. J. Porter and E. Haslam, *J. Chem. Soc., Perkin I*, 1979, 2375.

7.3. Synthesis of *ent*-Oritin-(4α→4)-epioritin-4α-ol (54)

The absolute structure and stereochemistry of **(55)** was established by employing a biomimetic synthetic procedure, synthesizing **(55)** from the corresponding proteracacinidin monomers **(8)** and **(11)**.

Compound **(55)** has previously been synthesized by Coetzee *et al.*[18], by reacting the appropriate 4-chloroflavan-3-ol derivative with *ent*-oritin-4α-ol **(11)** in the presence of thionyl chloride and dry THF. In this study, however, **(55)** was synthesized under neutral conditions, with DMTSF as catalyst, following the protocol developed by Ferreira and co-workers[1,33,34].

ent-Oritin-4α-ol **(11)** was converted to *ent*-oritin-4α-benzylthioether **(56)**, with toluene-α-thiol and $SnCl_4$, according to the procedure developed by Hemingway and co-workers[45]. The ^{1}H NMR **(plate 6)** spectrum (acetone-d_6, 296 K) showed an AA'BB'-system (δ 7.26 and 6.84, d, J = 8.0 Hz) for the B-ring and an AB-system (δ 6.46 and 6.40, d, J = 8.5 Hz) for the A-ring. The characteristic methylene doublets (δ 4.09 and 3.92, d, J =13.0 Hz) and D-ring mulriplet (δ 7.20 – 7.41) were evidence of the C-4 thiobenzyl substituent[46]. The heterocyclic AMX-system exhibited coupling constants ($J_{2,3}$ = 9.0, $J_{3,4}$ = 4.0 Hz) in accordance with a 2,3-*trans*-3,4-*cis* relative stereochemistry[5,6]. The CD data **(plate 2)** confirming the 4α configuration of the C-ring. When taken in conjunction with the known absolute configuration of the starting material, *ent*-oritin-4α-ol **(11)**, compound **(56)** was identified as (2S,3S,4R)-2,3-*trans*-3,4-*cis*-2,4',7,8,-tetrahydroxy-4-benzylthioflavan.

Compound **(55)** was synthesized stereospecifically by reacting *ent*-oritin-4α-benzylthioether **(56)**, with epioritin-4α-ol **(8)** in the presence of DMTSF, as shown in

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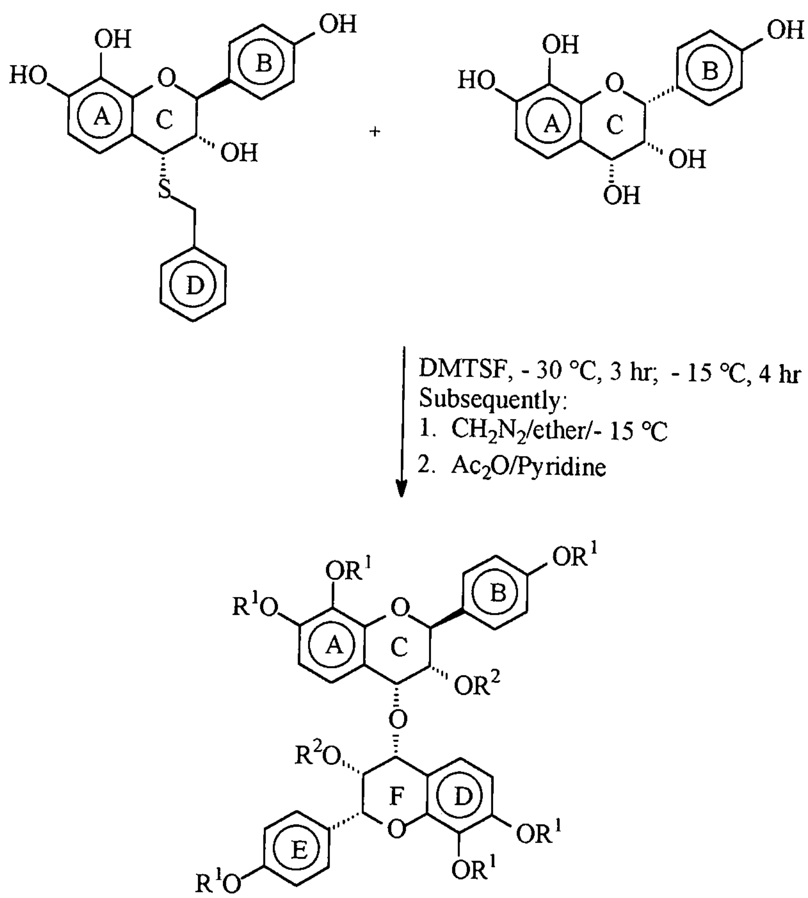
[45] R. W. Hemingway, J. J. Karchezy, G. W. McGraw and R. A. Wielesek, *Phytochemistry*, 1983, **22**, 275.
[46] P. J. Steynberg, J. P. Steynberg, E. V. Brandt, D. Ferreira and R. W. Hemingway, *J. Chem. Soc., Perkin I*, 1997, 1943.

scheme7.1. This was done by adding DMTSF to a mixture of **(8)** and **(56)**, in dry THF and under a N_2-atmosphere. The mixture was stirred at –30 °C for 3 hours and 4 hours at –15 °C, before stopped. After methylation, acetylation and PLC purification, compound **(55)** was identified and compared with the isolated compound **(55)**, thereby confirming the relative and absolute configuration and conformation of compound **(55)**.



(54) $R^1 = R^2 = H$
(55) $R^1 = Me$; $R^2 = Ac$

<u>**Scheme 7.1**</u>

8

Trimeric Proanthocyanidin from *Acacia Hereroensis*

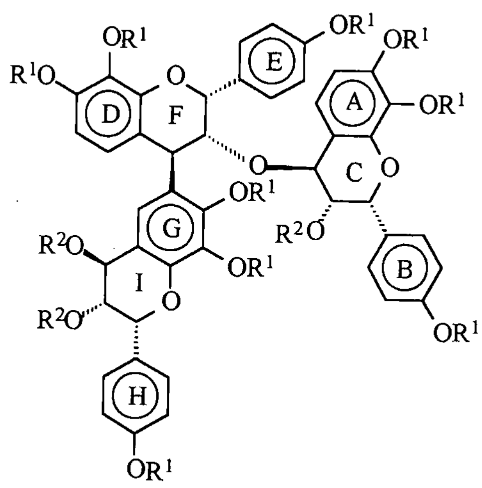
8.1. Introduction

Historically, the oligomeric proanthocyanidins isolated from plant material only involved the presence of carbon-carbon interflavanyl bonds. The first ether-linked dimeric promelacacinidin with a 7,8-dihydroxy A-ring substitution was isolated from *Acacia melanoxylon*[42]. Very recently, quite a number of the ether linked dimeric proteracacinidins were reported from *A. galpinii*[17,18] and *A. caffra*[21]. The latter tree also produced the first triflavanoids with both a C-C and C-O-C interflavanyl bond.

Epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol **(57)**, amongst other compounds was isolated as the nona-O-methyl-ether triacetate derivative **(58)** from the MeOH extract of the heartwood of *Acacia hereroensis.* This action was required due to the complexity of the free phenolic fractions. The structure was elucidated with the use of extensive ^{1}H, ^{13}C and 2D NMR spectroscopic studies and the absolute stereochemistry was finalised by synthesis.

8.2. Epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol (57)

The ^{1}H NMR data **(table 8.1, plate 15)** showed the presence of nine methoxyl and three acetoxyl groups (four acetoxyl groups required for an all carbon-carbon linked trimer) together with three heterocyclic AMX-systems and in the benzenoid resonance range occurred three AA'BB'-, two AB-systems and a residual singlet. FAB-MS analysis showed a molecular ion of m/z 1086 and suggested a molecular formula of $C_{60}H_{62}O_{19}$, which in conjunction with the NMR data confirmed the trimeric character of compound **(58)** and strongly suggested C-C and C-O-C interflavanyl linkages between the units.



(57) $R^1, R^2 = H$
(58) $R^1 = Me$; $R^2 = Ac$

NOESY experiments exhibited interaction between the 2- and 2',6'-protons, and between the 4-C and 5-C protons to confirm the respective ABC, DEF and GHI ring systems. Long range $^4J_{HH}$ (COSY) couplings of 5-H(G) with both 4-H(I) and 4-H(F) established the C-4 (F-ring) to C-6 (G-ring) linkage. The 4-H(F) low field resonance at δ 5.66 confirmed the flavan-3,4-diol[39] as a terminal unit (I-ring) and part of the GHI-system.

Phase sensitive NOESY experiment showed interaction between 3-H(F) with both 4-H(C) and 3-H(C) (no COSY coupling) which confirmed the interflavanyl ether linkage between the C- and F-rings. The 3-C(F) proton is shielded (1.06 ppm) with reference to the to the 3-H(C) of the monomeric epioritin-4α-ol methylether diacetate and this observation supported the C_3-O-C_4 linkage of compound **(58)**[18,42].

Table 8.1: ^{1}H and ^{13}C NMR chemical shift assignments of compound **(58)**. J values (Hz) are given in parentheses.

Ring	Carbon	δ ^{13}C ($CDCl_3$)	δ ^{1}H ($CDCl_3$)
C	2	73.98	4.69 (br.s, 1.0)
	3	69.88	4.59 (dd, 1.0, 2.5)
	4	71.45	4.56 (d, 2.5)
A	5	126.24	6.82 (d, 8.5)
	6	105.85	6.55 (d, 8.5)
	7	153.50	
	8	137.22	
	9	149.50	
	10	113.93	
B	1'	129.35	
	2'	128.33	6.89 (d, 8.5)
	3'	113.69	6.80 (d, 8.5)
	4'	160.01	
	5'	113.69	6.80 (d, 8.5)
	6'	128.33	6.89 (d, 8.5)
F	2	74.31	5.12 (br.s, 1.5)
	3	78.33	4.18 (dd, 1.5, 2.5)
	4	41.13	4.76 (d, 2.5)
D	5	125.26	6.77 (d, 8.5)
	6	105.96	6.59 (d, 8.5)
	7	152.41	
	8	140.77	
	9	149.11	
	10	114.98	
E	1'	131.41	
	2'	128.01	7.43 (d, 8.5)
	3'	113.98	6.91 (d, 8.5)
	4'	159.48	
	5'	113.98	6.91 (d, 8.5)
	6'	128.01	7.43 (d, 8.5)
I	2	74.58	5.29 (br.s, 1.5)
	3	69.04	5.23 (dd, 1.5, 3.0)
	4	66.44	5.66 (d, 3.0)
G	5	127.63	6.59 (s)
	6	129.56	
	7	151.92	
	8	137.60	
	9	148.99	
	10	113.46	
H	1'	128.88	
	2'	128.12	7.42 (d, 8.5)
	3'	114.21	6.94 (d, 8.5)
	4'	159.58	
	5'	114.21	6.94 (d, 8.50)
	6'	128.12	7.42 (d, 8.5)
OCH_3		55.50, 55.60, 55.67, 56.53, 56.53, 61.20, 61.28, 61.38, 61.42	3.77, 3.81, 3.84, 3.86, 3.87, 3.91, 3.94, 3.95, 3.99
$COCH_3$		21.01, 21.01, 21.68	1.82, 1.88, 2.09
$COCH_3$		169.48, 169.59, 169.75	

The heterocyclic ring of the terminal GHI unit recorded coupling constants (**table 8.1**) of $J_{2,3}$ = 1.5 Hz and $J_{3,4}$ = 3.0 Hz which are representative of a 2,3-*cis*-3,4-*trans* relative stereochemistry[5]. The coupling constants $J_{2,3}$ = 1.0 Hz and $J_{3,4}$ = 2.5 Hz for the C-ring, with $J_{2,3}$ = 1.5 Hz and $J_{3,4}$ = 2.5 Hz for the F-ring are indicative of a somewhat distorted sofa conformation, but represent a 2,3-*cis*-3,4-*trans* relative stereochemistry. The absence of any n.O.e. interactions between the 2- and 4-protons of all three rings confirmed that these pairs of heterocyclic protons are not on the same face of the respective rings[5].

The assistance of HMQC and HMBC experiments facilitated the assignment of the ^{13}C resonances (**table 8.1, plate 17**). The HMBC data confirmed the suggested structure of compound **(58)** as indicated in **table 8.2**. The HMBC couplings from 5-H(D), 2-H(F) and 4-H(F) to 6-C(G) supported the 4-C(C)→6-C(G) interflavanyl bond. No coupling could be detected whatsoever from 4-H(F) and 2-H(F) to 4-C(C) or from 5-H(A), 2-H(C) and 3-H(C) to 3-C(F), all of which supported the ether linkage between 4-C(C) and 3-C(F).

Table 8.2: HMBC Correlations of compound **(58)**

Proton	**Carbon**	**Remarks**
5-H(D) 4-H(F)	6-C(G, $^4J_{CH}$) 6-C(G, $^2J_{CH}$)	Confirm C-C linkage to 6-C(G)
4-H(F) 2-H(F)	4-C(C, none) 4-C(C, none)	Ether linkage from 3-C(F) to 4-C(C)
5-H(A) 5-H(A)	4-C(C, $^3J_{CH}$) 3-C(F, none)	Confirm position of ether linkage

The occurrence of 4-C(F) at δ_C 41.13 is characteristic of a phenyl substituent at this position[39]. Comparison of the carbon resonances involved in the ether linkage[42], showed the same direction and magnitude of shift effects (**table 8.3**) when the resonances of similar carbons of the I-ring are used as reference, thus supporting the present allocation.

Table 8.3: ^{13}C NMR Relative chemical shifts* of differently substituted carbons of compound **(58)**

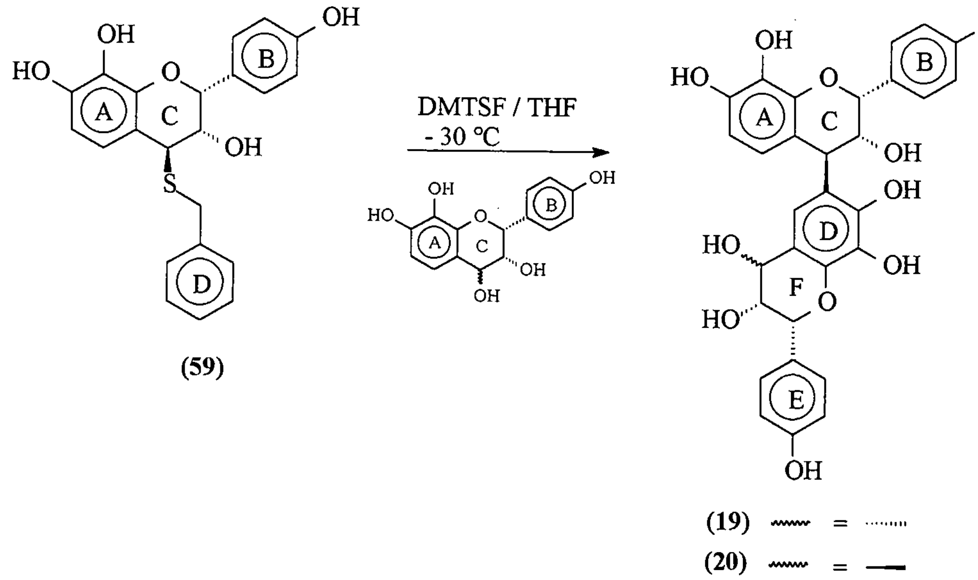
Carbon	**Ring**	**δ_{13}**	**Average $\Delta\delta$ (* - ø)**
3-C-O-flav.*	F	78.33*	
3-C-OAc	C	69.88ø	+ 8.45
3-C-OAc	I	69.04ø	+ 9.29
4-C-flav.	F	41.13	
4-C-O-flav.*	C	71.45*	
4-C-OAc	I	66.44ø	+ 5.01

8.3. Synthesis of Epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol nonamethyl-O-ether triacetate (58)

In an attempt to establish the structure and stereochemistry of compound **(58)**, we employed a biomimetic synthetic procedure, attempting to synthesize compound **(58)** from the appropriate proteracacinidin monomers **(8)** and **(9)**.

Epioritin-4α-ol **(8)** was converted to epioritin-4β-benzylthioether **(59)**, with toluene-α-thiol and $SnCl_4$, in accordance with the protocol used by Hemingway and co-workers[45]. The ^{1}H NMR **(plate 4)** spectrum (acetone-d_6, 296 K) exhibited an AA'BB'-system (δ 7.34 and 6.83, d, J = 9.0 Hz) for the B-ring and an AB-system (δ 6.53 and 6.43, d, J = 9.0 Hz) for the A-ring. The characteristic methylene doublets (δ 4.04 and 3.93, d, J = 13.5 Hz) and D-ring multiplet (δ 7.23 – 7.48) were evidence of the C-4 thiobenzyl substituent[46]. The heterocyclic AMX-system exhibited coupling constants ($J_{2,3}$ = 1.5, $J_{3,4}$ = 2.5 Hz) in accordance with a 2,3-*cis*-3,4-*trans* relative stereochemistry[5,6]. The high amplitude positive Cotton effect, $[\theta]_{244.9} = 1.177 \times 10^4$, in the CD spectrum **(plate 1)** of **(59)** confirmed the 4β configuration. When taken in conjunction with the known absolute configuration of the starting material epioritin-4α-ol **(8)**, compound **(59)** was identified as (2R,3S,4S)-2,3-*cis*,3,4-*trans*-3,4',7,8-tetrahydroxy-4-benzylthioflavan.

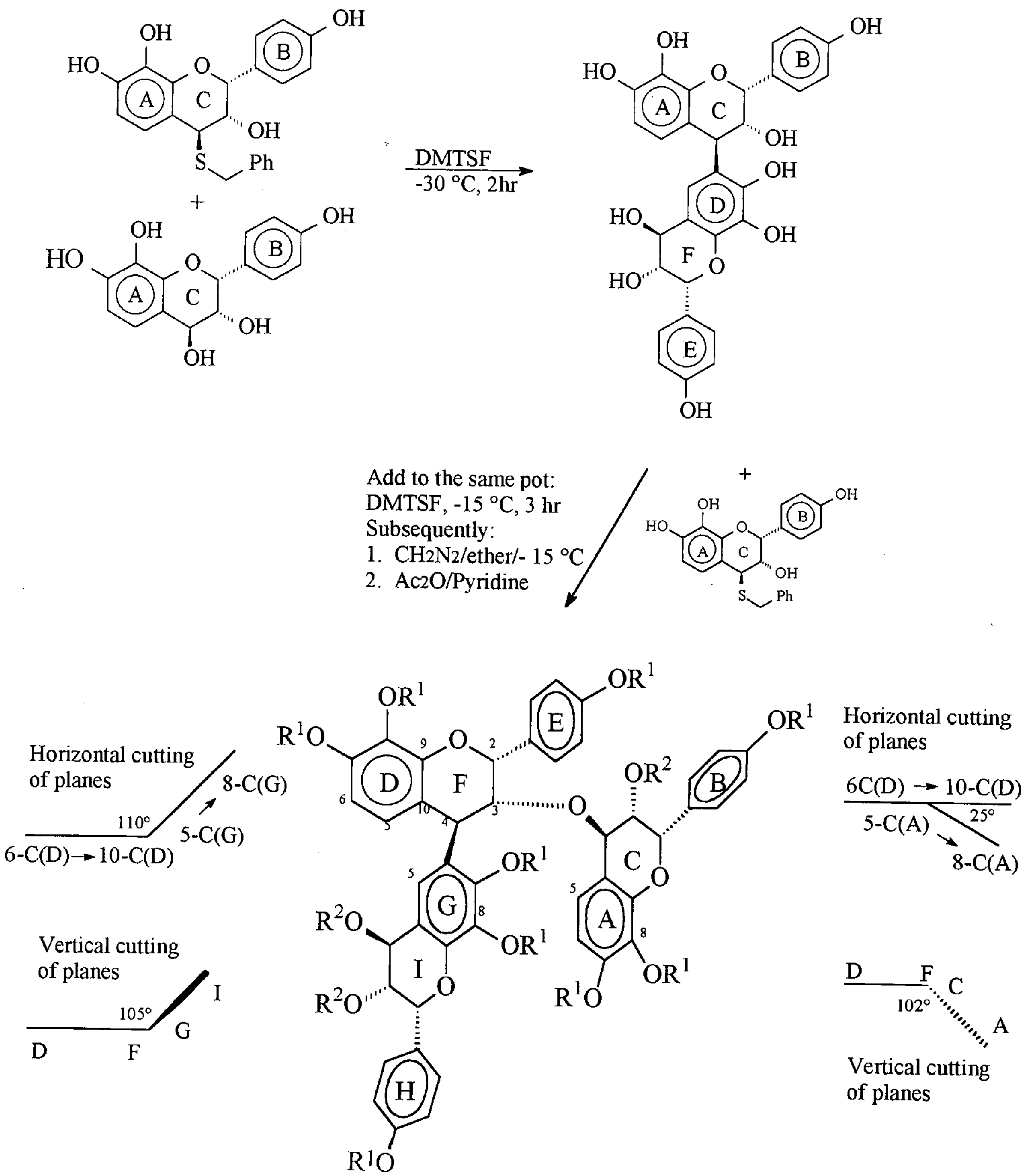
By reacting epioritin-4β-benzylthioether (59) with epioritin-4α-ol (8) or epioritin-4β-ol (9), with DMTSF, or $AgBF_4$ as catalyst in dry THF, it is possible to synthesize the corresponding dimers (19) and (20) stereospecifically, yielding epioritin-(4β→6)-epioritin-4α-ol (19) from the reaction with epioritin-4α-ol (8), and epioritin-(4β→6)-epioritin-4β-ol (20) from the reaction with epioritin-4β-ol (9), as shown in **scheme 8.1**.



Scheme 8.1

Extending this methodology, developed by Ferreira and co-workers[1,33,34], we were able to synthesize compound (58). This was done by adding DMTSF to a mixture of epioritin-4β-ol (9) and epioritin-4β-benzylthioether (59), in dry THF and under a N_2-atmosphere. This mixture was stirred for 2 hours at –30 °C, after which more DMTSF and epioritin-4β-benzylthioether (59) was added, and the mixture stirred for another 3 hours at –15 °C. The reaction yielded (58), after methylation, acetylation and PLC purification, as shown in **scheme 8.2**.

The stereoselective coupling of the 4β-thioether derivative (59) with epioritin-4β-ol (9) to give the trimer (58) with retention of configuration at both interflavanyl bonds, may be explained in terms of a neighbouring group effect, involving intramolecular displacement of the quasi-axial C-4 sulfur nucleofuge by the axial C-3 hydroxyl group to form an intermediate oxirane. This transient protonated epoxide (37) then permits attack by the nucleophile only from the less hindered β-face, resulting in a highly stereoselective coupling step, as discussed in chapter 4.



(54) $R^1 = R^2 = H$

(55) $R^1 = Me$; $R^2 = Ac$

<u>**Scheme 8.**</u>

8.4. Configuration and Conformation of Epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol nonamethyl-O-ether triacetate (58)

From basic NMR and CD (**plate 8**) experiments, as well as biomimetic synthesis, we have established the 2,3-*cis*-3,4-*trans* relative stereochemistry for rings C, F and I, as well as the absolute stereochemistry of the trimer **(58)** as 2R,3R,4S (C-ring), 2R,3R,4R (F-ring) and 2R,3R,4S (I-ring).

However, rings C and F cannot be in the "perfect" sofa conformation to meet with the coupling constants **(table 8.4)** and the distance requirements as reflected in **table 8.5**.

Table 8.4: Coupling constants (Hz)

Ring	$^3J_{2,3}$	$^3J_{3,4}$
C	1.0	2.5
F	1.5	2.5
I	1.5	3.0

Table 8.5: Distances between protons (Å) and NOESY-interactions* in the preferred conformation **(58)**.

3-H(F) to	3-H(C, 2.809)*	4-H(C, 2.261)*
2-H(C) to	2'6'-H(E, 2.770)*	
4-H(F) to	4-H(C, 2.606)*	5-H(A, 2.508)*
5-H(C) to	2-H(F, 2.358)*	3-H(F, 3.342)

The 2D NOESY experimental data was utilized as a basis in the computer modeling exercise [PC Spartan Pro Mechanics Program (PC/x86) 6.0.6] to construct the most likely preferred low energy conformation as depicted in **figure 8.1**.

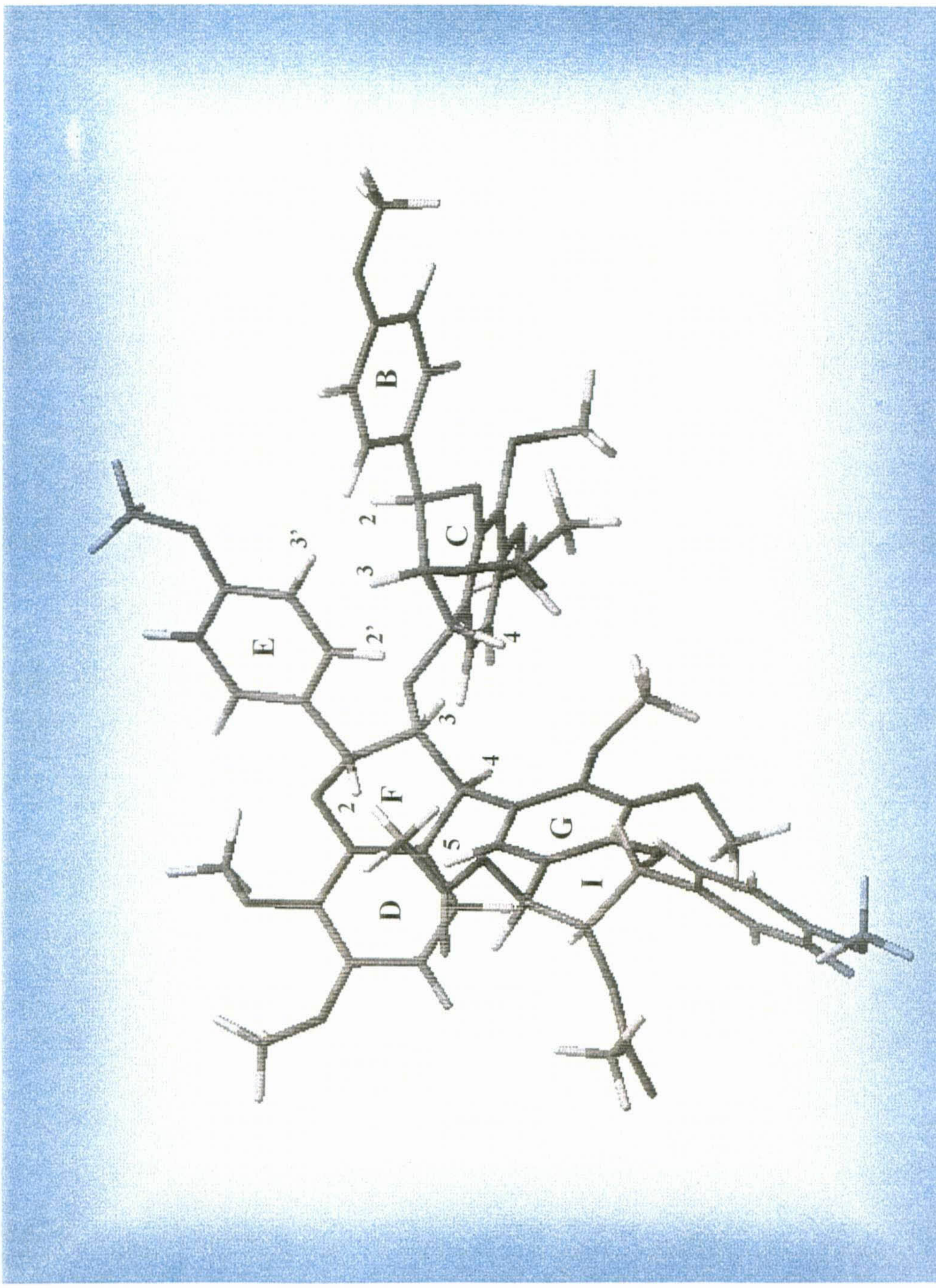


Figure 8.1

When taken that the D/F rings are in the plane of the paper, then the GI plane cuts at an angle of 105 ° from the top. The two planes are not square to each other as expected[6] in that a line drawn through 7-C(D) → 10-C(D) cuts the line through 6-C(D) → 9-C(D) horizontally at an angle of 110 °. The GHI moiety is above the DF-plane.

The angle and the length of the interflavanyl ether bond between 3-C(F) and 4-C(C) in compound **(58)** is somewhat stretched to 118 ° and 2.493 Å as compared with a normal C-O-C angle of 110 ° and 2.309 Å. When the DF-rings are in the plane of the paper, then the A/C cuts at an angle of 102 ° from the bottom. The A/C plane is somewhat twisted and a line drawn through 6-C(D) → 10-C(D) cuts the line connecting 5-C(A) → 8-C(A) horizontally at an angle of 25 °. The ABC-moiety is below the D/F plane.

8.5. Conclusion

In the recent research of Bennie *et al.*[21], the structure of the C-C/C-O-C coupled trimer **(58)** was determined by means of reductive cleavage of the ether bond with sodium cyanoborohydride in trifluoroacetic acid, followed by assignment of the absolute configuration of the ensuing flavanyl units. During the current investigation as to the flavanoids present in the heartwood of *Acacia hereroensis* it was possible to establish the structure and absolute stereochemistry of the trimer (55) as 2R,3R,4S (C-ring) : 2R,3R,4R (F-ring) : 2R,3R,4S (I-ring) by utilizing the biomimetic synthesis as portrayed in **scheme 8.2**.

Experimental

9

Standard Experimental Techniques

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

9.1. Chromatography

9.1.1. Thin Layer Chromatography

Qualitative thin layer chromatography (TLC) was conducted on "Merck TLC-aluminium sheets: Silica Gel F_{254}" (0.2 mm layer) cut into strips of 5 x 10 cm. R_f values recorded were observed from these qualitative TLC assessments.

Preparative scale thin layer chromatography (PLC) was conducted on glass plates (20 x 20 cm) coated with a layer (1.0 mm) of unactivated Merck Kieselgel 60 PF_{254} (100 g Kieselgel in 230 ml distilled water for every 5 plates) and which were dried overnight at room temperature. After development in the appropriate eluent the plates were dried in a rapid air current and the bands identified either by UV (254 nm) or by the appropriate spray reagent. The compounds were extracted with acetone from the silica gel and the acetone removed under reduced pressure. The plates were loaded with 10 – 15 mg of crude product per plate. Small-scale separations were conducted on Merck " Pre-coated (0.25 mm) TLC Plates Silica Gel 60 PF_{254}" with each plate loaded with 3 – 5 mg of semi-pure product.

9.1.2. Column Chromatography

Separations of Sephadex LH-20 were done using 120 x 4 cm columns, at a flow rate of 30 ml / hr using ethanol as eluent. Fractions were collected with an ISCO (model 273) automatic fraction collector.

Flash column chromatography (FCC) was carried out in a glass column (54 x 6.5 cm) charged with Merck Kieselgel 60 (230 – 400 mesh) using benzene – acetone as eluent at a flow rate of 60 ml / min.

9.2. Development of Chromatograms

9.2.1. Formaldehyde-Sulphuric Acid

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

9.2.2. Anisaldehyde-Sulphuric Acid

Thin layer chromatograms were gently sprayed with a solution of anisaldehyde (5 ml) and concentrated sulphuric acid (5 ml) in EtOH (90 ml) and heated in an oven (90 °C) to ensure optimum colour development.

9.3. Anhydrous Solvents

THF and diethyl ether were refluxed over sodium-benzophenone under a N_2-atmosphere until a dark blue colour persisted and was freshly distilled under N_2 prior to use.

DCM and DMF were refluxed over CaH_2 for 12 hours under a N_2-atmosphere with subsequent fresh distillation under N_2 before use.

9.4. Abbreviations

The following abbreviations were used in the descriptions of the solvent systems used during the development of TLC plates:

A	Acetone
B	Benzene
C	Chloroform
DCM	Dichloromethane
E	Diethyl ether
EA	Ethyl acetate
H	Hexane
M	Methanol
MEK	Methyl ethyl ketone
T	Toluene
THF	Tetrahydrofuran

9.5. Chemical Methods

9.5.1. Methylation with Diazomethane

Methylations were performed with an excess of diazomethane, prepared by the reaction of potassium hydroxide [(5 g) in a 95 % (v/v) ethanol solution] with N-methyl-N-nitroso-p-toluene sulphonamide (15 g) in ether and distilled directly into the previously prepared reaction mixture [200mg dry phenolic material dissolved in methanol (50 ml) and cooled to −10 °C]. After 48 hours at −15 °C the excess diazomethane and solvent were evaporated at room temperature.

9.5.2. Acetylation

Dry phenolic material was dissolved in an adequate volume of pyridine and twice the amount of acetic anhydride was added. After 8 hours at ambient temperature the reaction was stopped by adding ice and the excess pyridine removed by repetitive washing with cold water.

9.6. Spectroscopical and Spectrometric Methods

9.6.1. Nuclear Magnetic Resonance Spectroscopy (NMR)

NMR spectra were recorded on an AVANCE DPX_{300} Bruker spectrometer with tetramethylsilane (TMS) as internal standard. The ^{1}H NMR spectra were recorded at 296 K (23 °C).

Abbreviations were used as follows:

s	Singlet
d	Doublet
t	Triplet
dd	Doublet of doublets
m	Multiplet
br.	Broadened
eq.	Equatorial
ax.	Axial

The solvents used were deuteriochloroform ($CDCl_3$, δ 7.24), deuteriobenzene (C_6D_6, δ 7.15) and deuterioacetone [$(CD_3)_2CO$ / acetone-d_6, δ 2.04]. Chemical shifts were expressed in terms of parts per million (ppm) on the δ scale and coupling constants were measured in Hz.

9.6.2. Circular Dichroism (CD)

CD spectra were recorded on a Jasco J-710 spectropolarimeter in spectrophotometric grade methanol. The formula used to calculate the molecular ellipticity [θ] was:

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

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

9.6.3. Fast Atom Bombardment (FAB) Mass Spectrometry

FAB mass spectra were recorded on a VG70-70E double focusing mass spectrometer using a VG11-250J data system and iontech saddlefield FAB gun.

9.7. Freeze Drying

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

10

Isolation of Phenolic Metabolites from *Acacia Hereroensis*

10.1. Extraction of the Heartwood Components

Drillings (6.5 kg) from the heartwood of *A. hereroensis* were first extracted with $(CH_3)_2CO$ (3 x 3.0 l) for 24 h periods at room temperature (25 °C). Secondly the dried drillings were extracted with MeOH (3 x 3.0 l) under the same conditions as above. Subsequently the solid extract was obtained by evaporating the MeOH under reduced pressure at 40 °C (283 g).

10.2. Separation of the Phenolic Components

A Sephadex column, loaded with 22 g of the MeOH extract, was used to separate the compounds. The first 1.5 l of EtOH was discarded and the fractions of two columns were combined as shown in **Table 10.1**.

All the fractions were analyzed by TLC and selectively certain fractions were analyzed by PLC. According to the initial analyses, fractions A_1 up to A_5 consisited mainly of known monomeric flavanoids. Small quantities of these selected fractions were derivatized to identify the teracacinidin flavan-3,4-diols[5,6] for later use in the synthetic study.

Table 10.1:

Fraction	Yield	Fraction	Yield
A_1	1.759 g	A_{17}	0.378 g
A_2	7.241 g	A_{18}	0.512 g
A_3	6.501 g	A_{19}	0.660 g
A_4	4.641 g	A_{20}	0.539 g
A_5	2.192 g	A_{21}	0.411 g
A_6	0.538 g	A_{22}	0.375 g
A_7	0.646 g	A_{23}	0.399 g
A_8	1.219 g	A_{24}	0.429 g
A_9	0.979 g	A_{25}	0.334 g
A_{10}	0.867 g	A_{26}	0.230 g
A_{11}	0.528 g	A_{27}	0.316 g
A_{12}	0.501 g	A_{28}	0.203 g
A_{13}	0.508 g	A_{29}	0.320 g
A_{14}	0.779 g	A_{30}	0.261 g
A_{15}	0.815 g	Residue	0.354 → 35.91 g
A_{16}	0.471 g		

10.3. Analysis of fraction A_6

Methylation of a portion of fraction A_6 (250 mg), followed by PLC (B:A, 8:2, v/v), gave four main bands A6/1 (R_f 0.37, 21 mg), A6/2 (R_f 0.36, 11 mg), A6/3 (R_f 0.31, 31 mg) and A6/4 (R_f 0.30, 25 mg).

10.3.1. Epioritin-4α-ol tri-O-methylether diacetate (43)

Band A6/1, after acetylation and PLC purification (B:A, 9:1, v/v) yielded the title compound (**43**, $R_f = 0.54$, 23 mg) as a white amorphous solid.

^{1}H NMR data : Plate 1; Table 5.1

10.3.2. Epioritin-4β-ol tri-O-methylether diacetate (44)

Band A6/2, after acetylation and PLC purification (B:A, 9:1, v/v) yielded the title compound (**44**, $R_f = 0.58$, 13 mg) as a white amorphous solid.

^{1}H NMR data : Plate 2; Table 5.1

10.3.3. Epioritin-(4β→6)-epioritin-4α-ol hexa-O-methyl triacetate (60)

Band A6/3, after acetylation and PLC purification (B:A, 9:1, v/v) yielded the title compound (**60**, $R_f = 0.53$, 27 mg) as an amorphous solid.

^{1}H NMR data : Plate 8

10.3.4. Epioritin-(4β→6)-oritin-4α-ol hexa-O-methyl triacetate (53)

Band A6/4, after acetylation and further PLC separation (B:A, 9:1, v/v) yielded the title compound (**53**, R_f 0.38, 12 mg) as a white amorphous solid.

Found	:	M^+, 772.2730. $C_{42}H_{44}O_{16}$ requires M^+, 772.2731
^{1}H NMR data	:	Plate 13; Table 6.1
CD data	:	Plate 6

10.4. Analysis of fraction A_{10}

Methylation of a portion of fraction A_{10} (250 mg), followed by PLC (B:A, 8:2, v/v), gave four main bands A10/1 (R_f 0.38, 11 mg), A10/2 (R_f 0.36, 58 mg), A10/3 (R_f 0.34, 23 mg) and A10/4 (R_f 0.32, 21 mg).

10.4.1. Ent-oritin-(4α→6)-epioritin-4α-ol hexa-O-methyl triacetate (49)

Band A10/2, after acetylation and further PLC separation (B:A, 9:1, v/v) yielded two major bands, A10/2/1 (R_f 0.40, 37 mg) and A10/2/2 (R_f 0.38, 16 mg). The latter band yielded the title compound (**49**) as an off-white amorphous solid.

Found	:	M^+, 772.2729. $C_{42}H_{44}O_{16}$ requires M^+, 772.2731
^{1}H NMR data	:	Plate 11; Table 6.1
CD data	:	Plate 4

10.5. Analysis of fraction A_{13}

Methylation of a portion of fraction A_{13} (250 mg), followed by PLC (B:A, 8:2, v/v), gave band A13/1 (R_f 0.48, 10.4 mg).

10.5.1. *ent*-Oritin-(4α→4)-epioritin-4α-ol hexa-O-methylether diacetate (55)

Band A13/1, after acetylation and further PLC separation (B:A, 9:1, v/v) yielded the title compound (**55**) as a white amorphous solid. (R_f 0.65, 2.1 mg)

Found	:	M^+, 730.7728. $C_{40}H_{42}O_{13}$ requires M^+, 730.7729
^{1}H NMR data	:	Plate 14; Table 7.1
^{13}C NMR data	:	Plate.16; Table 7.1
CD data	:	Plate 7

10.6. Analysis of fraction A_{14}

Methylation of a portion of fraction A_{14} (250 mg), followed by PLC (B:A, 8:2, v/v), gave four main bands A14/1 (R_f 0.39, 44 mg), A14/2 (R_f 0.36, 17 mg), A14/3 (R_f 0.34, 37 mg) and A14/4 (R_f 0.32, 9 mg).

10.6.1. Epimesquitol-(4β→6)-epioritin-4α-ol hepta-O-methylether triacetate (47)

Band A14/1, after acetylation and further PLC separation (B:A, 9:1, v/v) yielded the title compound (**47**) as an off-white amorphous solid. (R_f 0.44, 20 mg)

Found	:	M^+, 802.2839. $C_{43}H_{46}O_{15}$ requires M^+, 802.2837
^1H NMR data	:	Plate 10; Table 6.1
CD data	:	Plate 3

10.6.2. *ent*-Oritin-(4β→6)-epioritin-4α-ol hexa-O-methyl triacetate (51)

Band A14/3, after acetylation and further PLC separation (B:A, 9:1, v/v) yielded two major bands, A14/3/1 (R_f 0.31, 16 mg) and A14/3/2 (R_f 0.26, 11 mg). The former band yielded the title compound (**51**) as a white amorphous solid.

Found	:	M^+, 772.2733. $C_{42}H_{44}O_{16}$ requires M^+, 772.2731
^1H NMR data	:	Plate 12; Table 6.1
CD data	:	Plate 5

The remaining band, A10/2/1 was identified as a low molecular mass polymer.

10.7. Analysis of fraction A_{22}

Methylation of a portion of fraction A_{14} (220 mg), followed by PLC (B:A, 8:2, v/v), gave band A22/3 (R_f 0.45, 21.4 mg).

10.7.1. Epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol nona-O-methylether triacetate (58)

Band A22/3, after acetylation and further PLC separation (B:A, 9:1, v/v) yielded band A22/3/4 as the title compound (**58**) as a white amorphous solid. (R_f 0.61, 4.2 mg)

Found	:	M^+, 1087.1515. $C_{60}H_{62}O_{19}$ requires M^+, 1087.1517
^{1}H NMR data	:	Plate 15; Table 8.1
^{13}C NMR data	:	Plate 17; Table 8.1
CD data	:	Plate 8

11

Synthesis of C-C and C-O-C linked Proteracacinidins

11.1. Synthesis of (2R,3S,4S)-2,3-*cis*-3,4-*trans*-3,4',7,8-tetrahydroxy-4-benzylthioflavan (59) [Epioritin-4β-benzylthioether]

Epioritin-4α-ol **(8)** (200 mg) was dissolved in dry THF (10 ml) and cooled to –20 °C. Under a N_2 atmosphere BnSH (0.33 ml, 4 eq.) was added, followed, after 10 min., by $SnCl_4$ (0.34 ml, 0.5 eq.). The mixture was stirred for 2 h at 0 °C, after which H_2O (100 ml) was added and the reaction mixture was extracted with EtOAc (50 ml x 6). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure and the product was purified by PLC (B:A, 8:2, v/v), yielding the title compound (R_f = 0.64, 180 mg, 90 %) as a yellow amorphous solid. A portion of the product (10 mg) was acetylated **(61)** for confirmation of the structure (**plate 5**).

^{1}H NMR data	:	Plates 4 and 5
CD data	:	Plate 1

11.2. Synthesis of (2S,3S,4R)-2,3-*trans*-3,4-*cis*-3,4',7,8-tetrahydroxy-4-benzylthioflavan (59) [*ent*-oritin-4α-benzylthioether]

ent-Oritin-4α-ol **(11)** (200 mg) was dissolved in dry THF (10 ml) and cooled to –20 °C. Under a N_2 atmosphere BnSH (0.33 ml, 4 eq.) was added, followed, after 10 min., by

$SnCl_4$ (0.34 ml, 0.5 eq.). The mixture was stirred for 2 h at 0 °C, after which H_2O (100 ml) was added and the reaction mixture was extracted with EtOAc (50 ml x 6). The combined organic layers were dried (Na_2SO_4) and evaporated under reduced pressure and the product was purified by PLC (B:A, 8:2, v/v), yielding the title compound (R_f = 0.59, 170 mg, 85 %) as a yellow amorphous solid. A portion of the product (10 mg) was acetylated **(62)** for confirmation of the structure (**plate 7**).

^{1}H NMR data	:	Plates 6 and 7
CD data	:	Plate 2

11.3. Synthesis of C-O-C linked dimeric Proteracacinidin

11.3.1. Optimization of Reaction Conditions

a) A total of fourteen reactions with DMTSF as catalyst were conducted to optimize the reaction conditions. It was found that the reaction was sensitive to all the parameters, e.g. starting and final temperatures as well as the reaction times.

b) The $AgBF_4$ assisted reactions, of which twelve were carried out, showed less sensitivity and the temperature played the more important role.

11.3.2. Synthesis of *ent*-Oritin-(4α→4)-epioritin-4α-ol hexa-O-methylether diacetate (55)

ent-Oritin-4α-ol **(11)** (100 mg) and epioritin-4α-ol **(8)** (200mg, 2 eq.) was dissolved in dry THF (15 ml). Solid DMTSF (60 mg, 1 eq.) was added, under a N_2-atmosphere, and the mixture was stirred at –30 °C, for 3 hours. The temperature was raised to –15 °C and the mixture was stirred for a further 4 hours, after which the reaction was stopped by adding a KH_2PO_4/Na_2HPO_4 buffer (pH = 6.8, 100 ml). The mixture was extracted with

EtOAc (50 ml x 6) and the combined organic layers dried (Na_2SO_4) and the solvent evaporated under reduced pressure.

Methylation and PLC separation (B:A, 8:2, v/v) yielded two main bands at $R_f = 0.73$ (20 mg) and $R_f = 0.48$ (26 mg). Acetylation and PLC purification (B:A, 9:1, v/v) of band $R_f = 0.44$, yielded the title compound **(55)** ($R_f = 0.65$, 4.2 mg, 4.20 %) as a white amorphous solid. The $R_f = 0.73$ band contained unreacted epioritin-4α-ol **(8)**.

^{1}H NMR data	:	Plate 14
^{13}C NMR data	:	Plate 16
CD data	:	Plate 7

11.4. Synthesis of C-C and C-O-C linked trimeric Proteracacinidin

11.4.1. Synthesis of Proteracacinidin dimers with $AgBF_4$

Epioritin-4β-benzylthioether **(59)** (50 mg) and epioritin-4α-ol **(8)** (100 mg, 2 eq.) were dissolved in dry THF (15 ml) and solid $AgBF_4$ (70 mg, 2.5 eq.) was added. The mixture was stirred, under a N_2-atmosphere, for 90 min. at 0 °C. H_2O (100 ml) was added and the mixture extracted with EtOAc (50 ml x 6). The combined organic layers were dried with Na_2SO_4 and the solvent evaporated under reduced pressure.

Methylation and PLC separation (B:A, 8:2, v/v) yielded two main bands at $R_f = 0.73$ (31 mg) and $R_f = 0.30$ (29 mg). Acetylation and PLC purification (B:A, 9:1, v/v) of band $R_f = 0.30$, yielded the two products, epioritin-(4β→6)-epioritin-4α-ol hexa-O-methylether triacetate **(60)** and epioritin-(4β→6)-epioritin-4β-ol hexa-O-methylether triacetate **(63)** ($R_f = 0.39$, 4.0 mg, 8.00 %, $R_f = 0.37$, 8.2 mg, 16.40 %) as white amorphous solids. The $R_f = 0.73$ band contained unreacted epioritin-4α-ol **(8)**.

^{1}H NMR data of **(60)** : Plate 8

^{1}H NMR data of **(63)** : Plate 9

11.4.2. Synthesis of Proteracacinidin dimers with DMTSF

Epioritin-4β-benzylthioether **(59)** (100 mg) and epioritin-4α-ol **(8)** (200 mg, 2 eq.) were dissolved in dry THF (15 ml). Solid DMTSF (60 mg, 1 eq.) was added and the mixture stirred, under a N_2-atmosphere, at –30 °C for 2 hours. The temperature was raised to –15 °C and stirred for a further 4 hours, after which the reaction was stopped by adding a KH_2PO_4/Na_2HPO_4 buffer (pH = 6.8, 100 ml). The mixture was extracted with EtOAc (50 ml x 6). The combined organic layers were dried (Na_2SO_4) and the solvent evaporated under reduced pressure.

Methylation and PLC separation (B:A, 8:2, v/v) yielded two main bands at R_f = 0.73 (38 mg) and R_f = 0.30 (27 mg). Acetylation and PLC purification (B:A, 9:1, v/v) of band R_f = 0.30, yielded the desired product, epioritin-(4β→6)-epioritin-4α-ol hexa-O-methylether triacetate **(60)** (R_f = 0.39, 20 mg, 20.00 %) as a white amorphous solid. The R_f = 0.73 band contained unreacted epioritin-4α-ol **(8)**.

^{1}H NMR data : Plate 8

11.4.3. Synthesis of Epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol nona-O-methylether triacetate (58)

Epioritin-4β-benzylthioether **(59)** (200 mg) and epioritin-4β-ol **(9)** (350 mg, 1.75 eq.) were dissolved in dry THF (30 ml). Solid DMTSF (120 mg, 1 eq.) was added and the mixture stirred, under a N_2-atmosphere, at –30 °C for 2 hours, after which more DMTSF (120 mg, 1 eq.) and epioritin-4β-benzylthioether **(59)** (200 mg) was added, and the mixture stirred for another 3 hours at –15 °C. The reaction was stopped by adding a KH_2PO_4/Na_2HPO_4 buffer (pH = 6.8, 200 ml). The mixture was extracted with EtOAc (50 ml x 6). The combined organic layers were dried (Na_2SO_4) and the solvent evaporated under reduced pressure.

Methylation and PLC separation (B:A, 8:2, v/v) yielded three main bands at $R_f = 0.71$ (50 mg), $R_f = 0.45$ (16 mg) and $R_f = 0.31$ (25 mg). Acetylation and PLC purification (B:A, 9:1, v/v) of band $R_f = 0.45$, yielded the product **(58)** ($R_f = 0.61$, 5 mg, 2.50 %) as a white amorphous solid. The $R_f = 0.73$ band contained unreacted epioritin-4α-ol **(8)**, and the band $R_f = 0.31$ band contained the corresponding dimer **(63)**.

^{1}H NMR data	:	Plate 15
^{13}C NMR data	:	Plate 17
CD data	:	Plate 8

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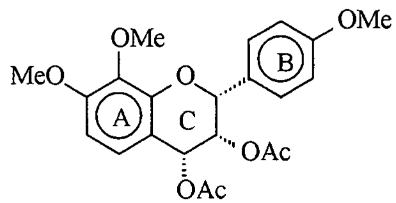
Appendix A: NMR Spectra

1. Epioritin-4α-ol **(43)**
2. Epioritin-4β-ol **(44)**
3. Oritin-4α-ol **(45)**
4. Epioritin-4β-benzylthioether **(59)**
5. Epioritin-4β-benzylthioether tetra-acetate **(61)**
6. *ent*-Oritin-4α-benzylthioether **(56)**
7. *ent*-Oritin-4α-benzylthioether tetra-acetate **(62)**
8. Epioritin-(4β→6)-epioritin-4α-ol hexa-O-metylether triacetate **(60)**
9. Epioritin-(4β→6)-epioritin-4β-ol hexa-O-methylether triacetate **(63)**
10. Epimesquitol-(4β→6)-epioritin-4α-ol hepta-O-methylether triacetate **(47)**
11. *ent*-Oritin-(4α→6)-epioritin-4α-ol hexa-O-methylether triacetate **(49)**
12. *ent*-Oritin-(4β→6)-epioritin-4α-ol hexa-O-methylether triacetate **(51)**
13. Epioritin-(4β→6)-oritin-4α-ol hexa-O-methylether triacetate **(53)**
14. *ent*-Oritin-(4α→4)-epioritin-4α-ol hexa-O-methylether diacetate **(55)**
15. Epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol nonamethyl-O-ether triacetate **(58)**
16. *ent*-Oritin-(4α→4)-epioritin-4α-ol hexa-O-methylether diacetate **(55)** (^{13}C NMR)
17. Epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol nonamethyl-O-ether triacetate **(58)** (^{13}C NMR)

Plate 1 ($CDCl_3$, 296 K)



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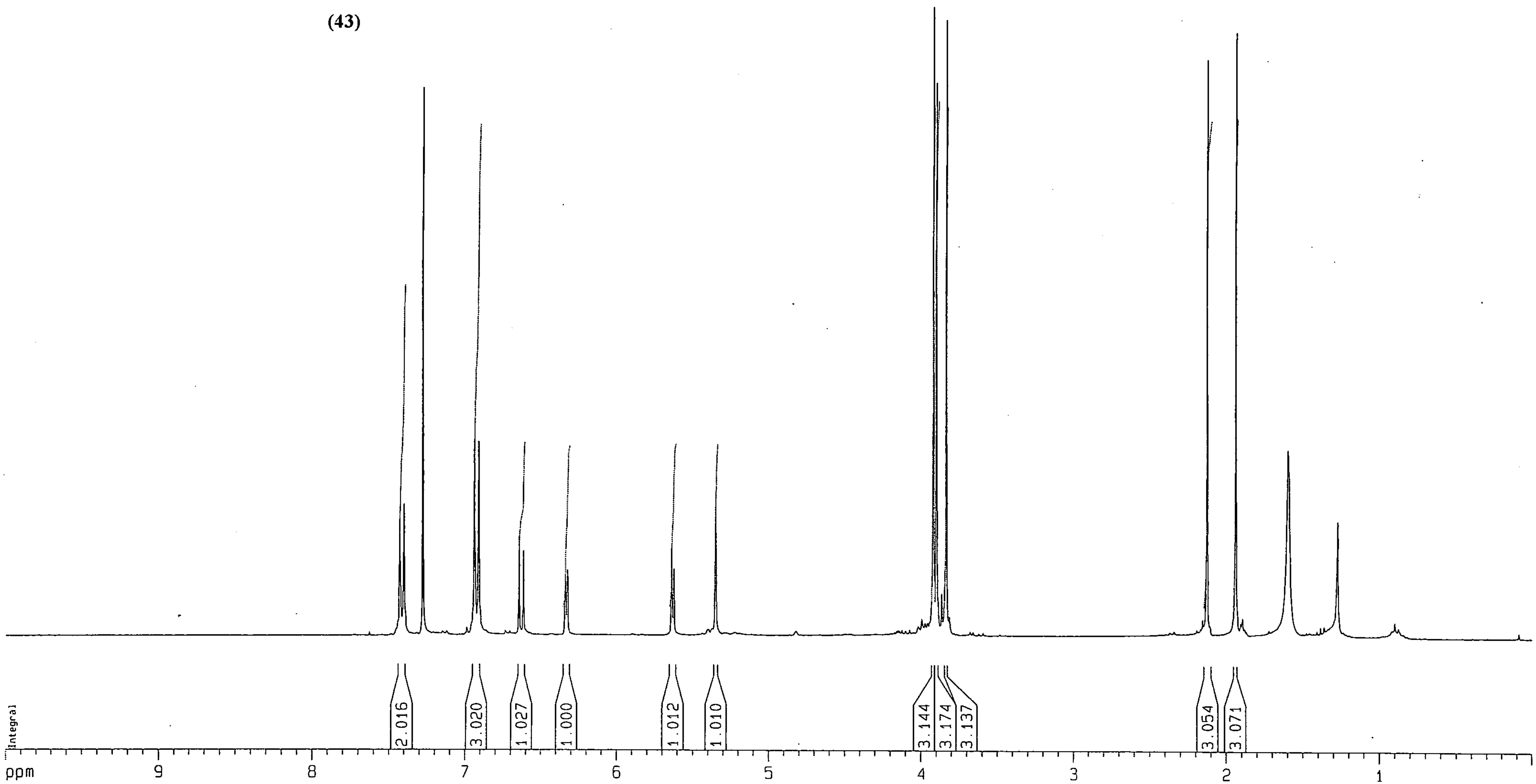

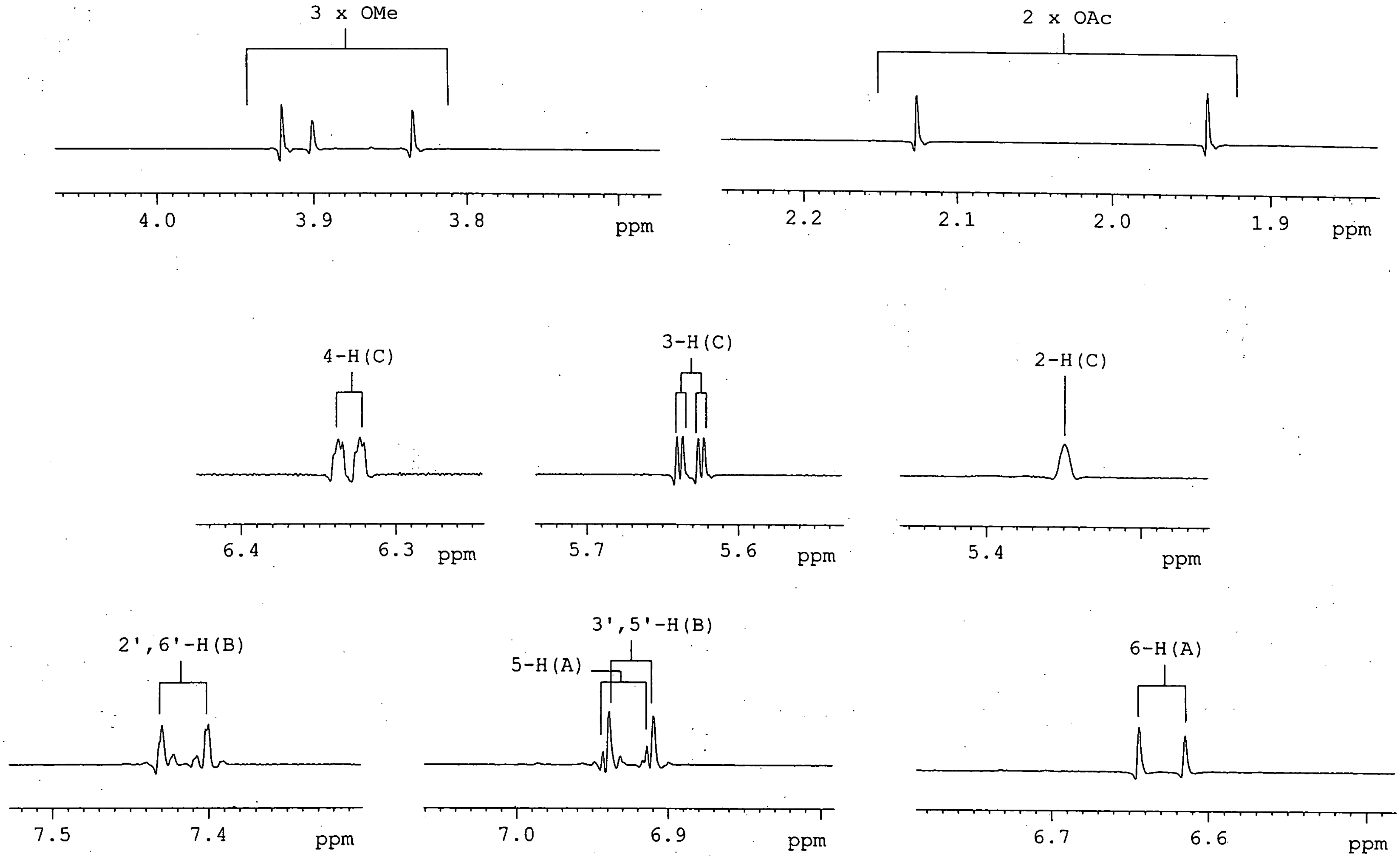

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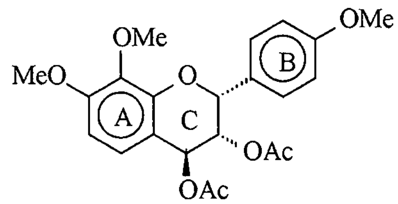
3.9
3.8
ppm
2 x OAc
2.2
2.1
2.0
1.9
ppm
4-H(C)
6.4
6.3
ppm
3-H(C)
5.7
5.6
ppm
2-H(C)
5.4
ppm
2',6'-H(B)
7.5
7.4
ppm
3',5'-H(B)
5-H(A)
7.0
6.9
ppm
6-H(A)
6.7
6.6
ppm

Plate 2 (CDCl$_3$, 296 K)



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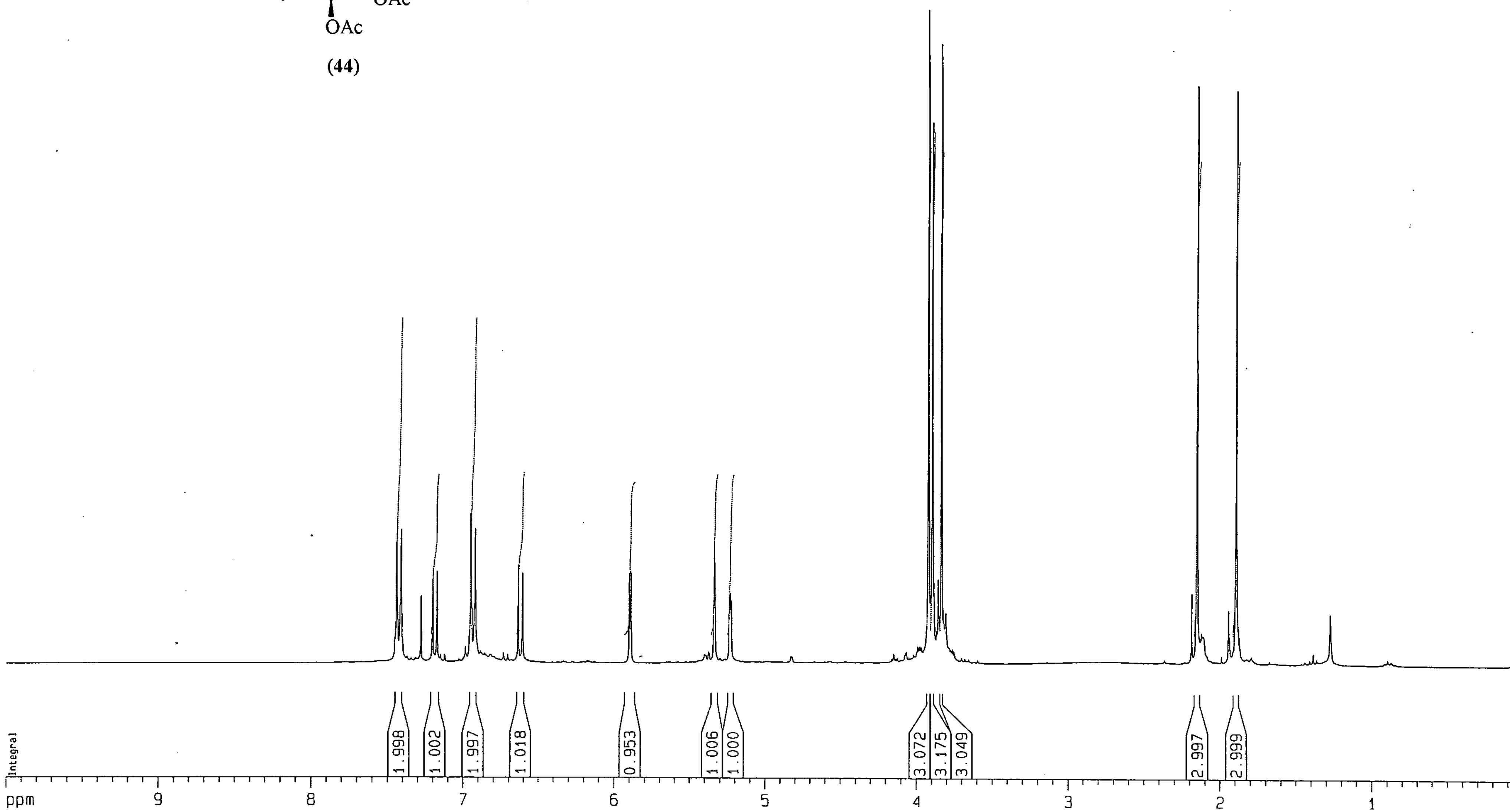

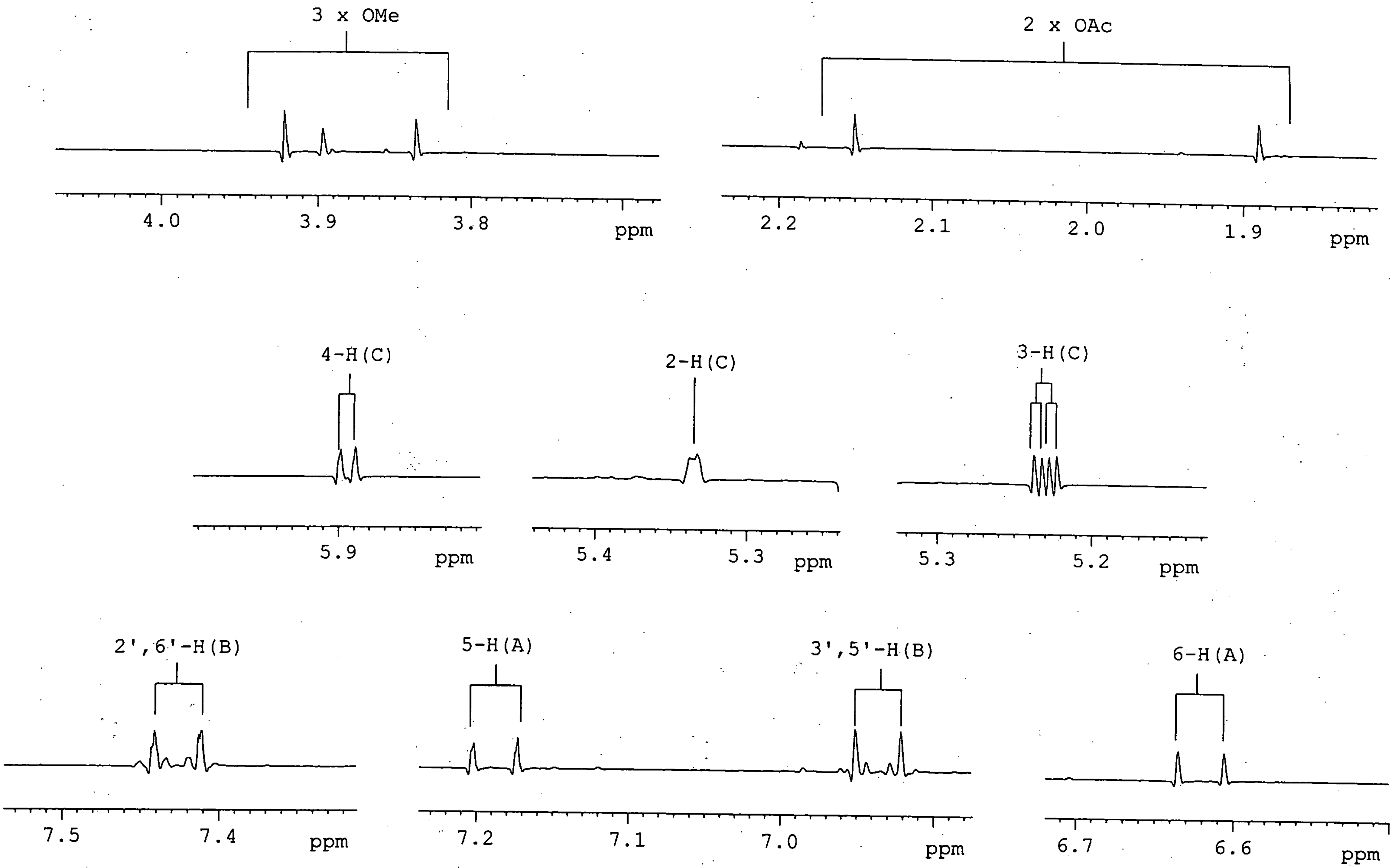
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3.9
3.8
ppm
2 x OAc
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2.1
2.0
1.9
ppm
4-H(C)
5.9
ppm
2-H(C)
5.4
5.3
ppm
3-H(C)
5.3
5.2
ppm
2',6'-H(B)
7.5
7.4
ppm
5-H(A)
3',5'-H(B)
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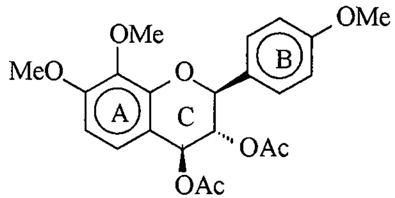
7.1
7.0
ppm
6-H(A)
6.7
6.6
ppm

Plate 3 ($CDCl_3$, 296 K)



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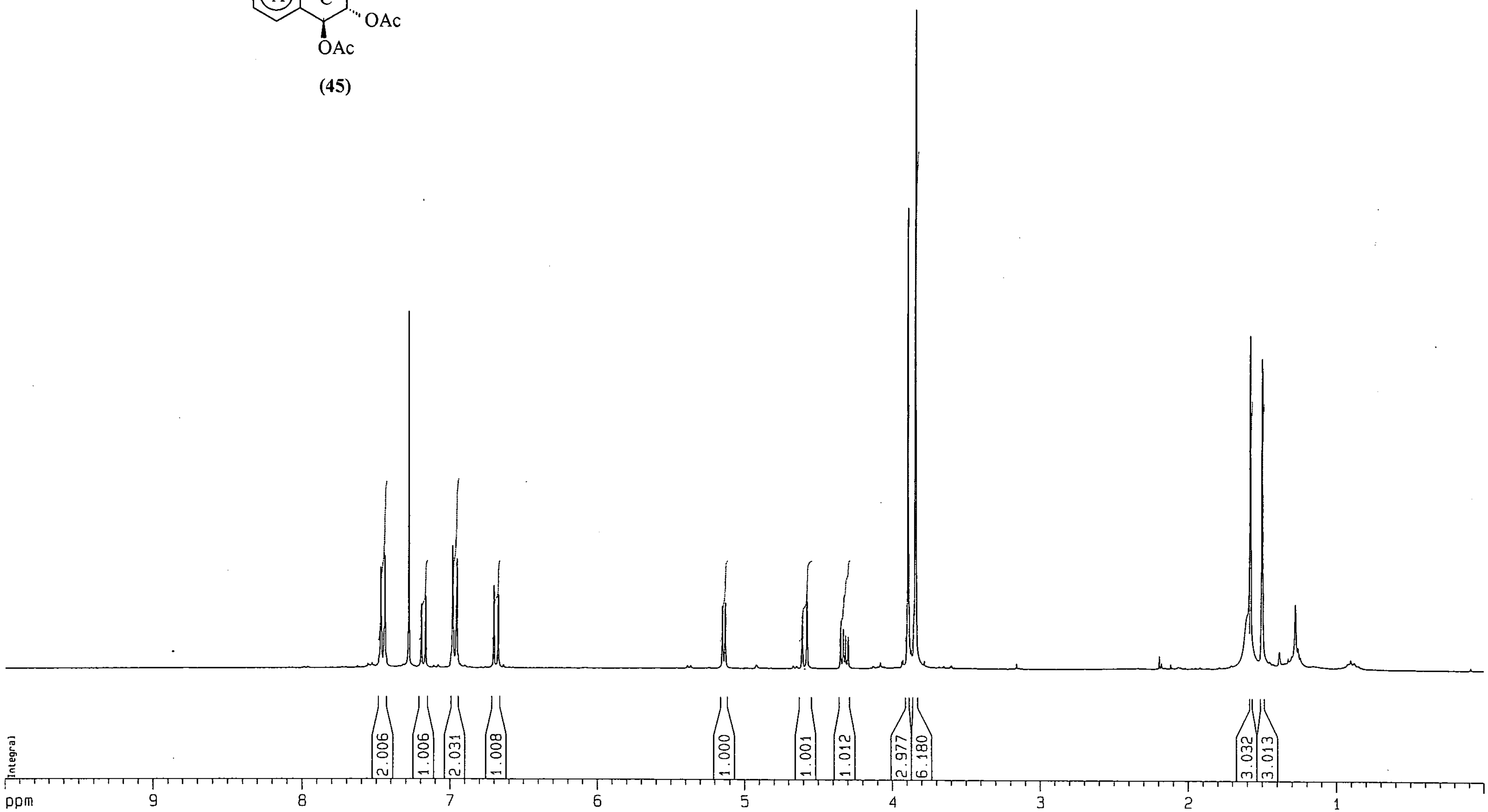

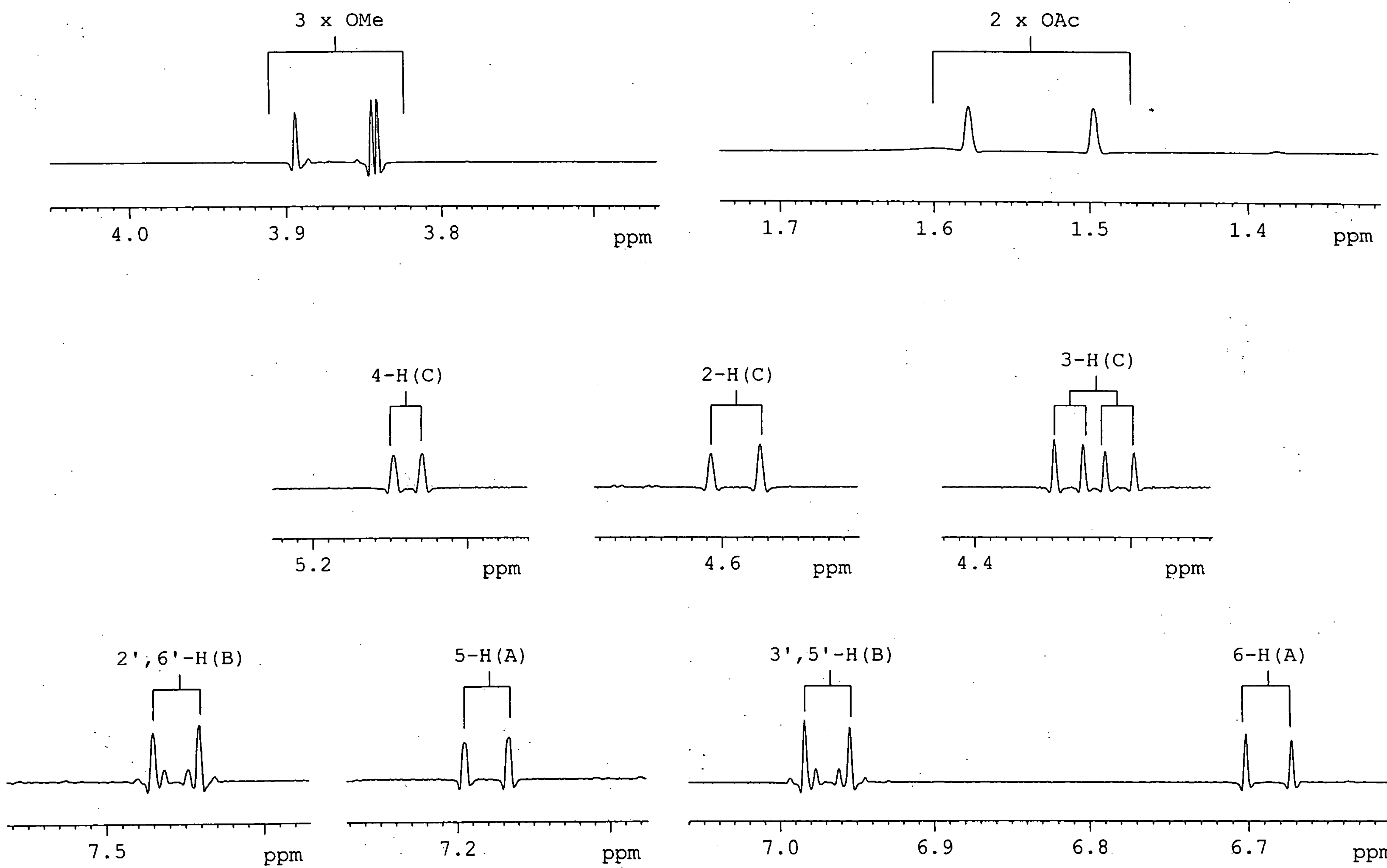
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ppm
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1.5
1.4
ppm
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ppm
2-H(C)
4.6
ppm
3-H(C)
4.4
ppm
2',6'-H(B)
7.5
ppm
5-H(A)
7.2
ppm
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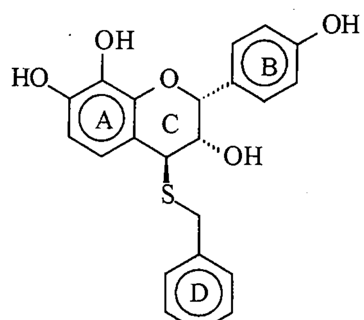
6-H(A)
7.0
6.9
6.8
6.7
ppm

Plate 4 ($CDCl_3$, 296 K)



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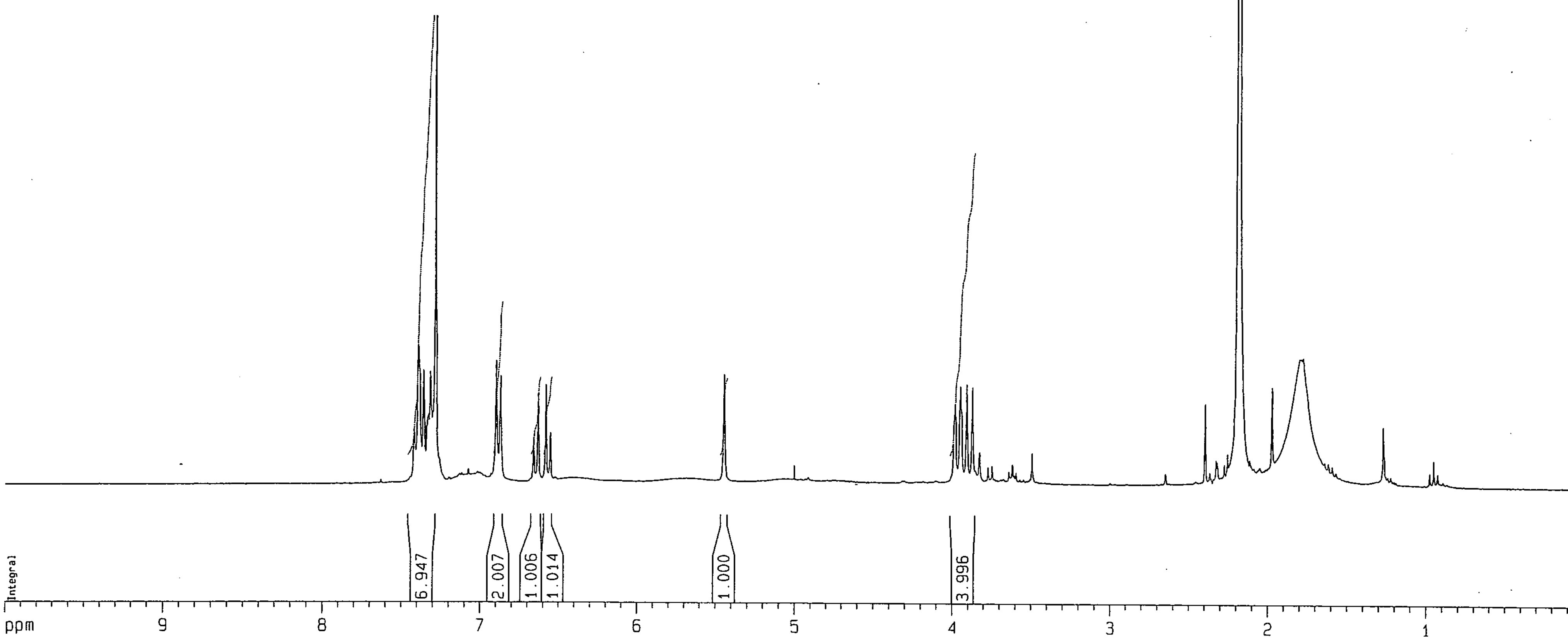

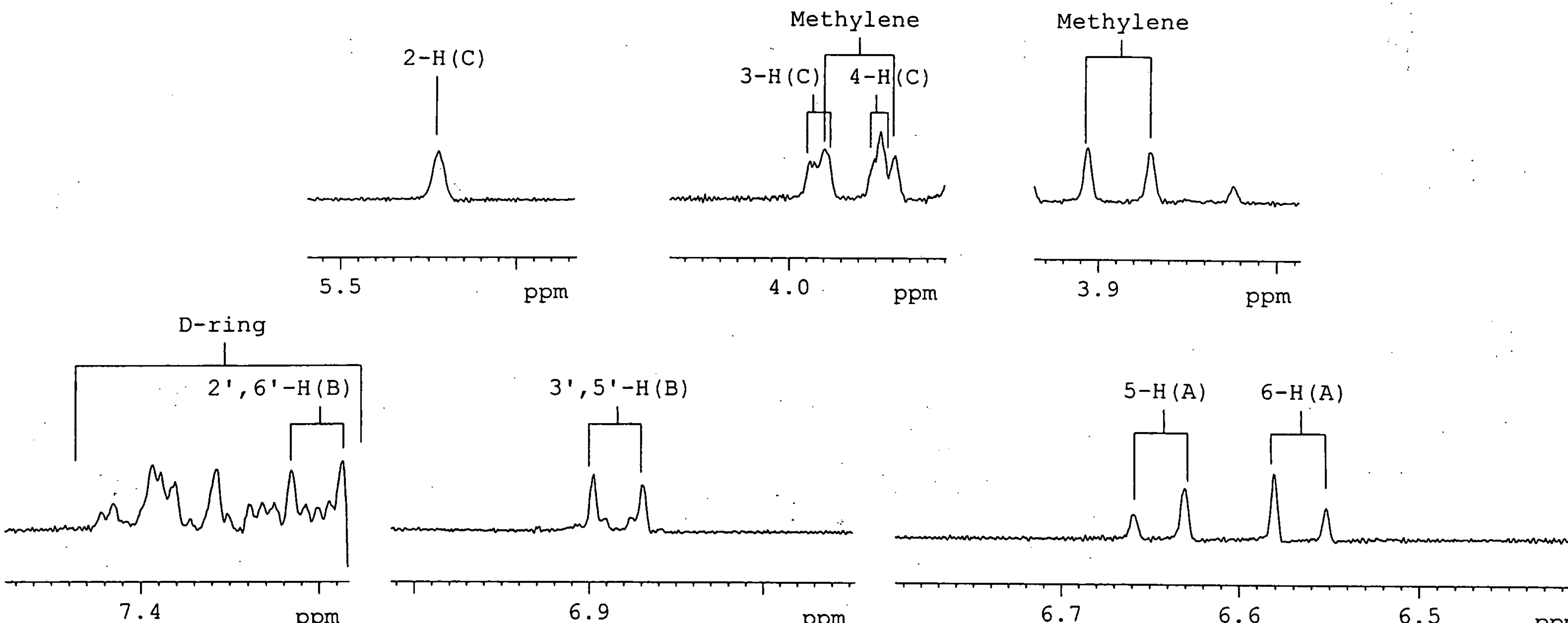
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ppm
Methylene
3-H(C)
4-H(C)
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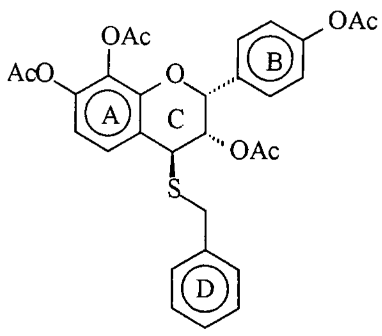
ppm
Methylene
3.9
ppm
D-ring
2',6'-H(B)
7.4
ppm
3',5'-H(B)
6.9
ppm
5-H(A)
6-H(A)
6.7
6.6
6.5
ppm

Plate 5 ($CDCl_3$, 296 K)



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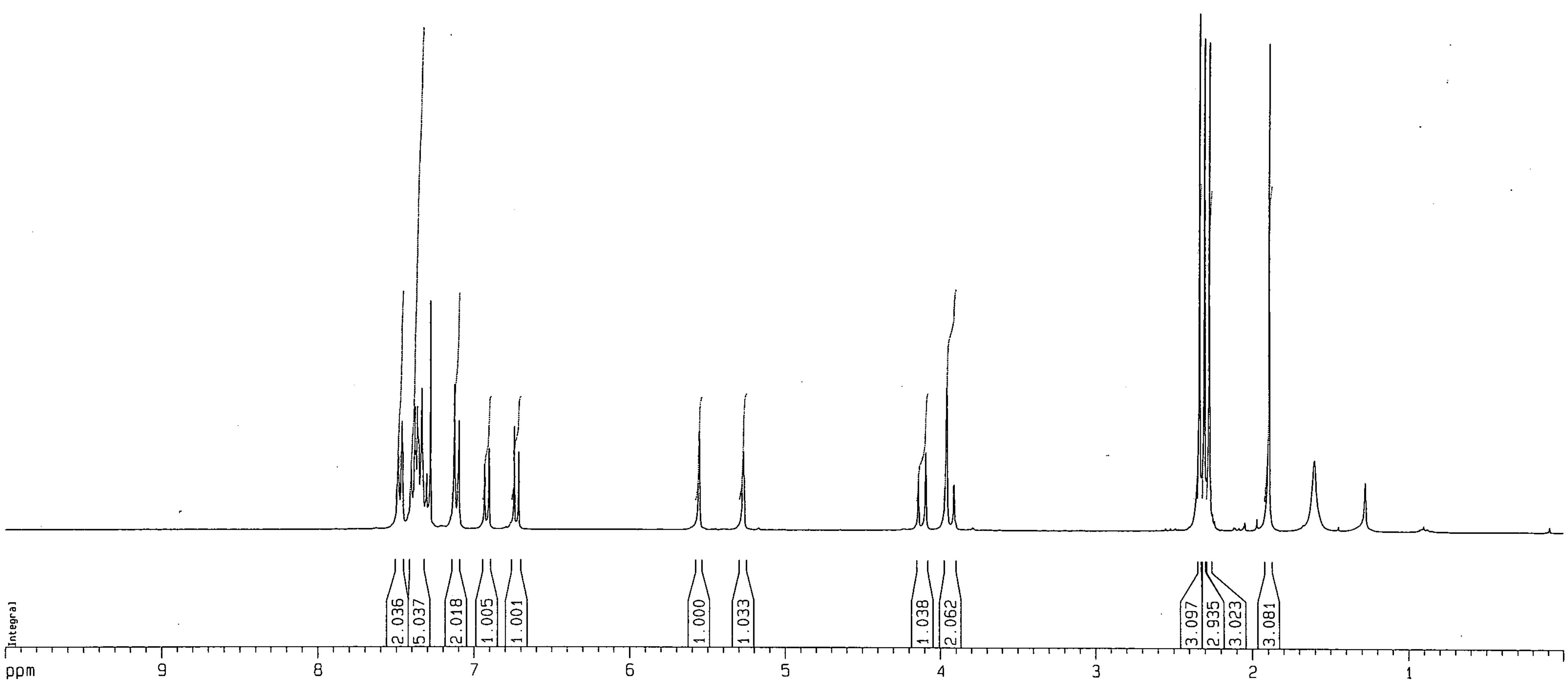

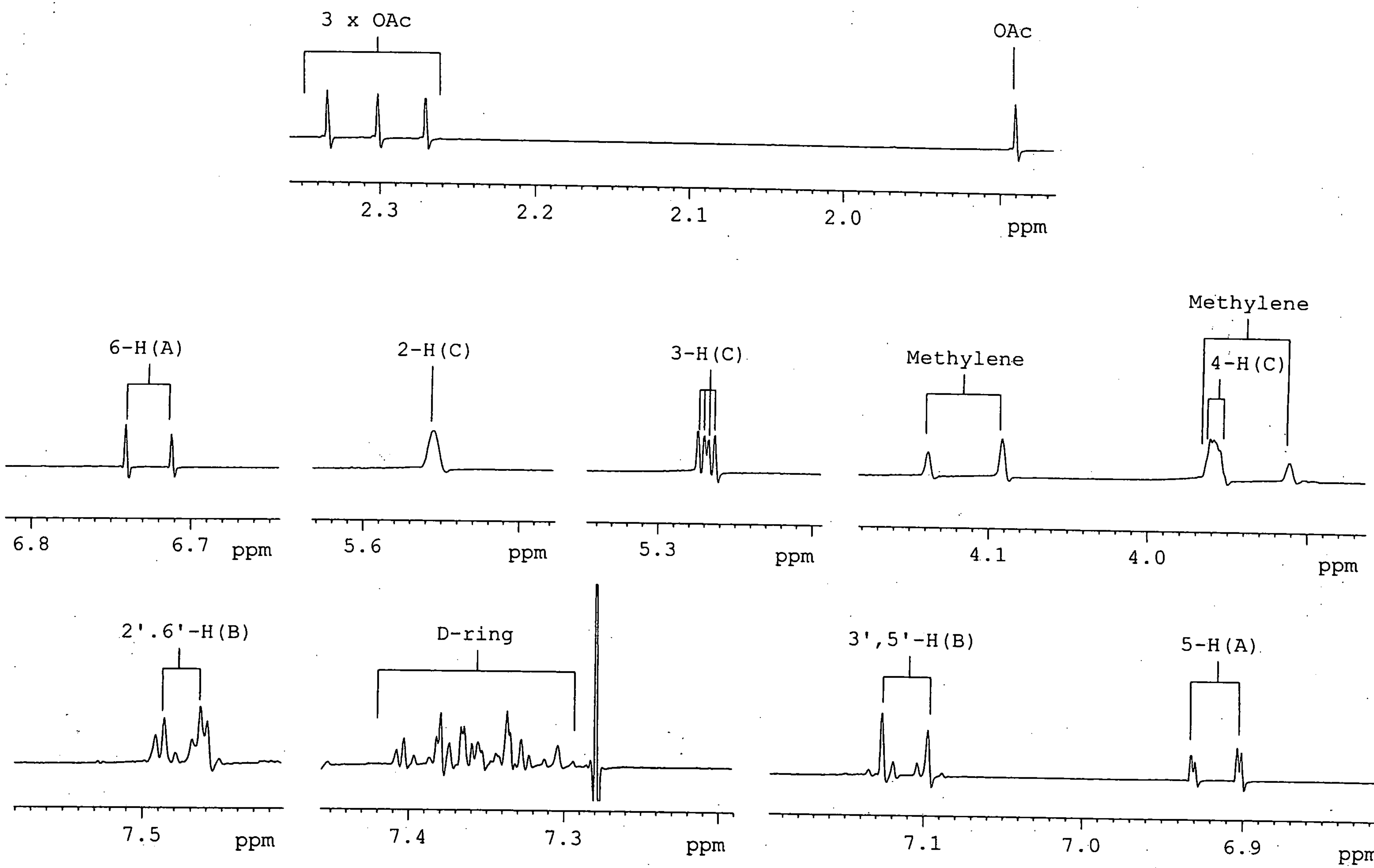
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ppm
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5.6
ppm
3-H(C)
5.3
ppm
Methylene
Methylene
4-H(C)
4.1
4.0
ppm
2'.6'-H(B)
7.5
ppm
D-ring
7.4
7.3
ppm
3',5'-H(B)
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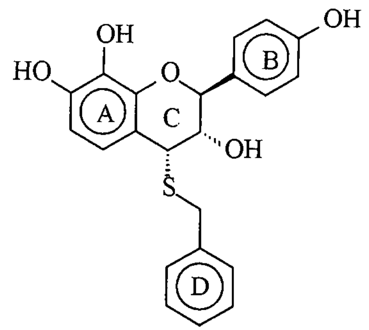
7.0
6.9
ppm

Plate 6 ($CDCl_3$, 296 K)



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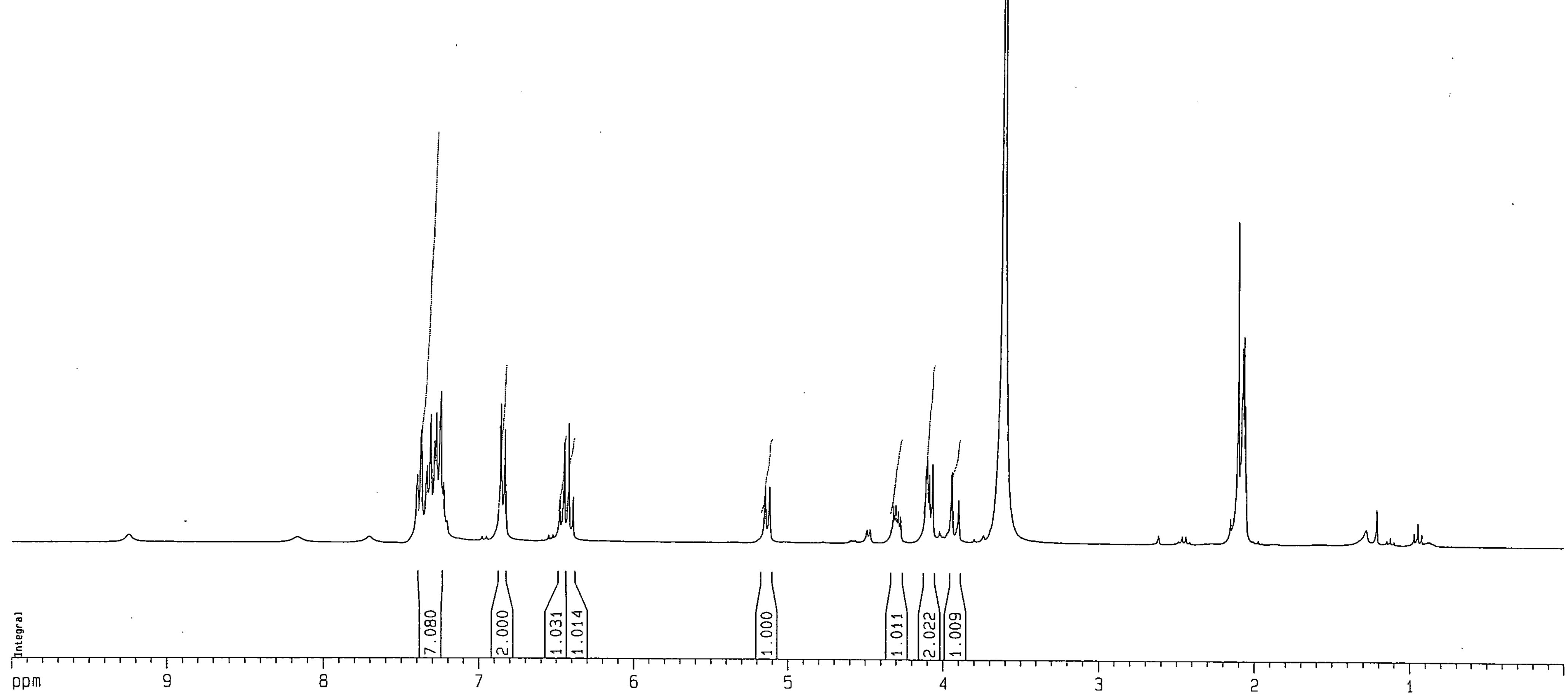

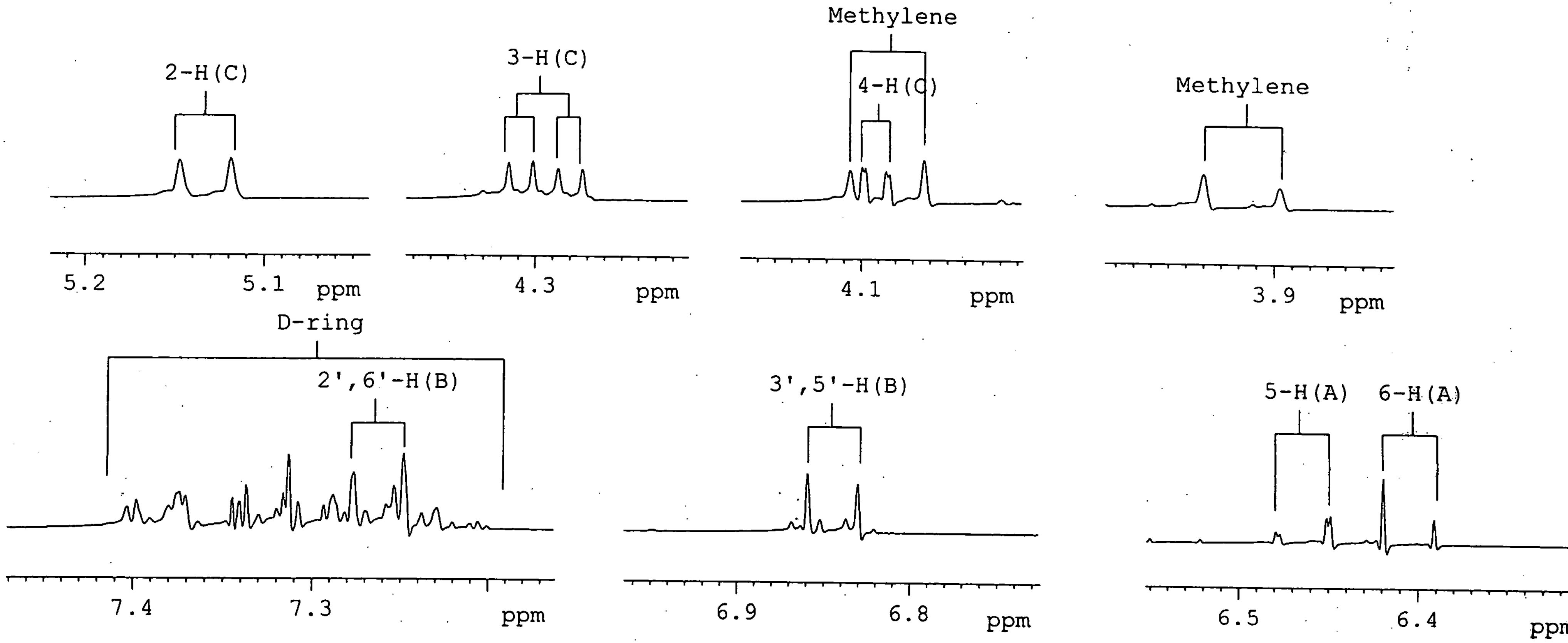
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3-H(C)
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ppm
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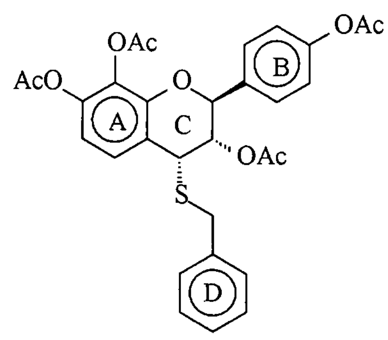
ppm
Methylene
3.9
ppm
D-ring
2',6'-H(B)
7.4
7.3
ppm
3',5'-H(B)
6.9
6.8
ppm
5-H(A)
6-H(A)
6.5
6.4
ppm

Plate 7 ($CDCl_3$, 296 K)



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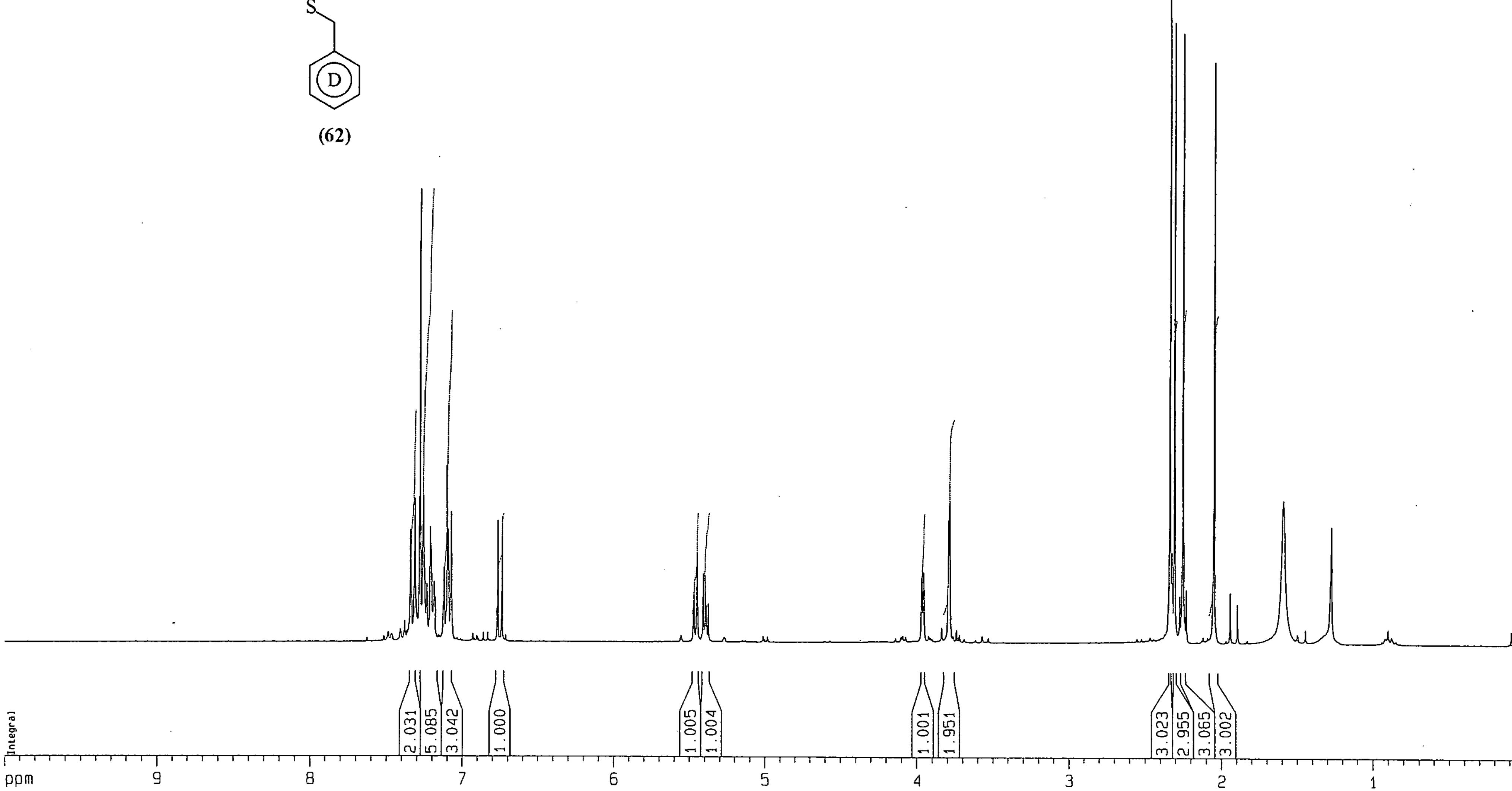

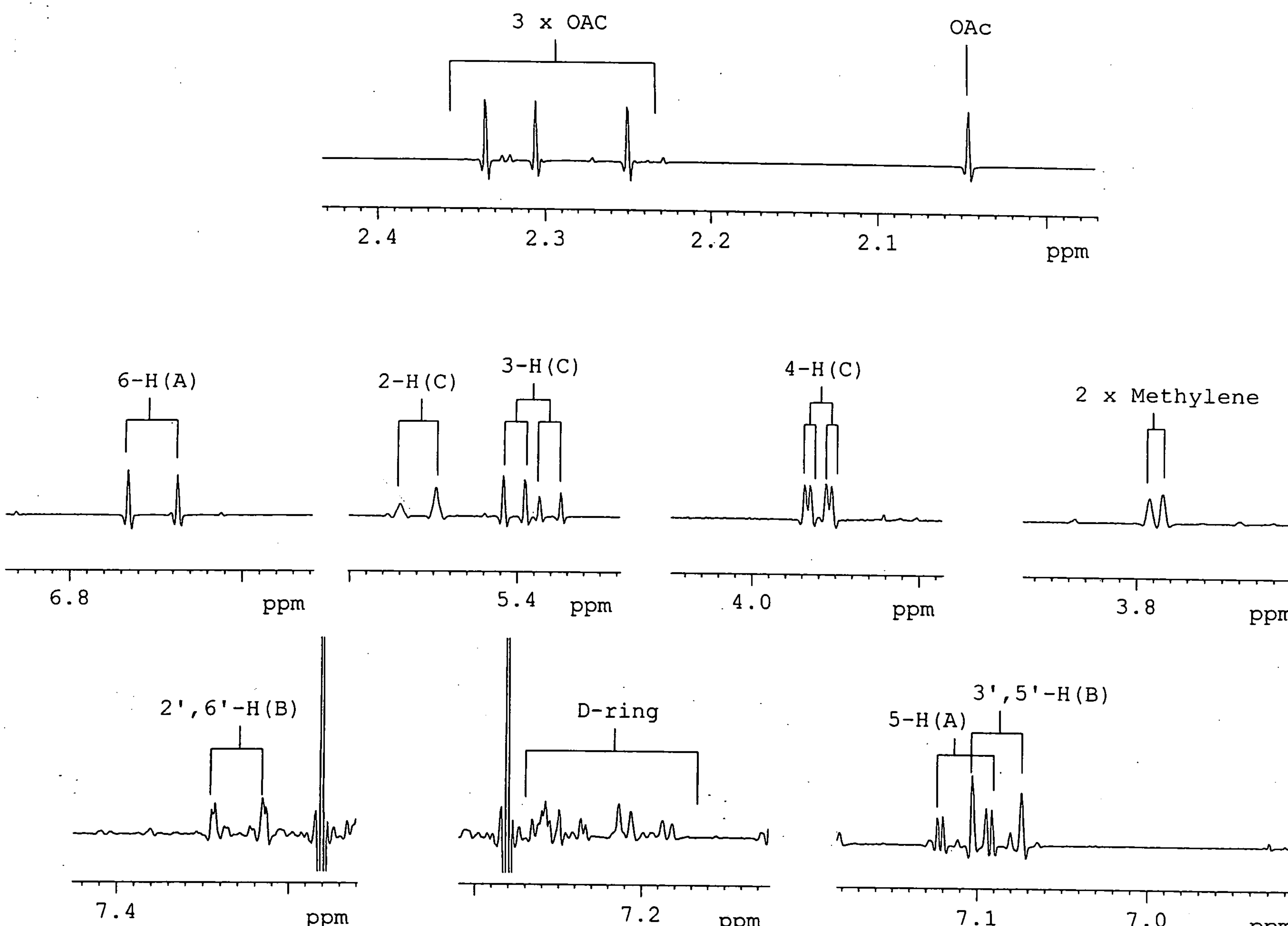
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OAc
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2.3
2.2
2.1
ppm
6-H(A)
6.8
ppm
2-H(C)
3-H(C)
5.4
ppm
4-H(C)
4.0
ppm
2 x Methylene
3.8
ppm
2',6'-H(B)
7.4
ppm
D-ring
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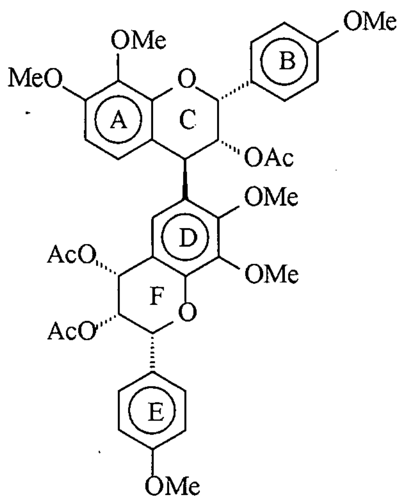
ppm
5-H(A)
3',5'-H(B)
7.1
7.0
ppm

Plate 8 ($CDCl_3$, 296 K)



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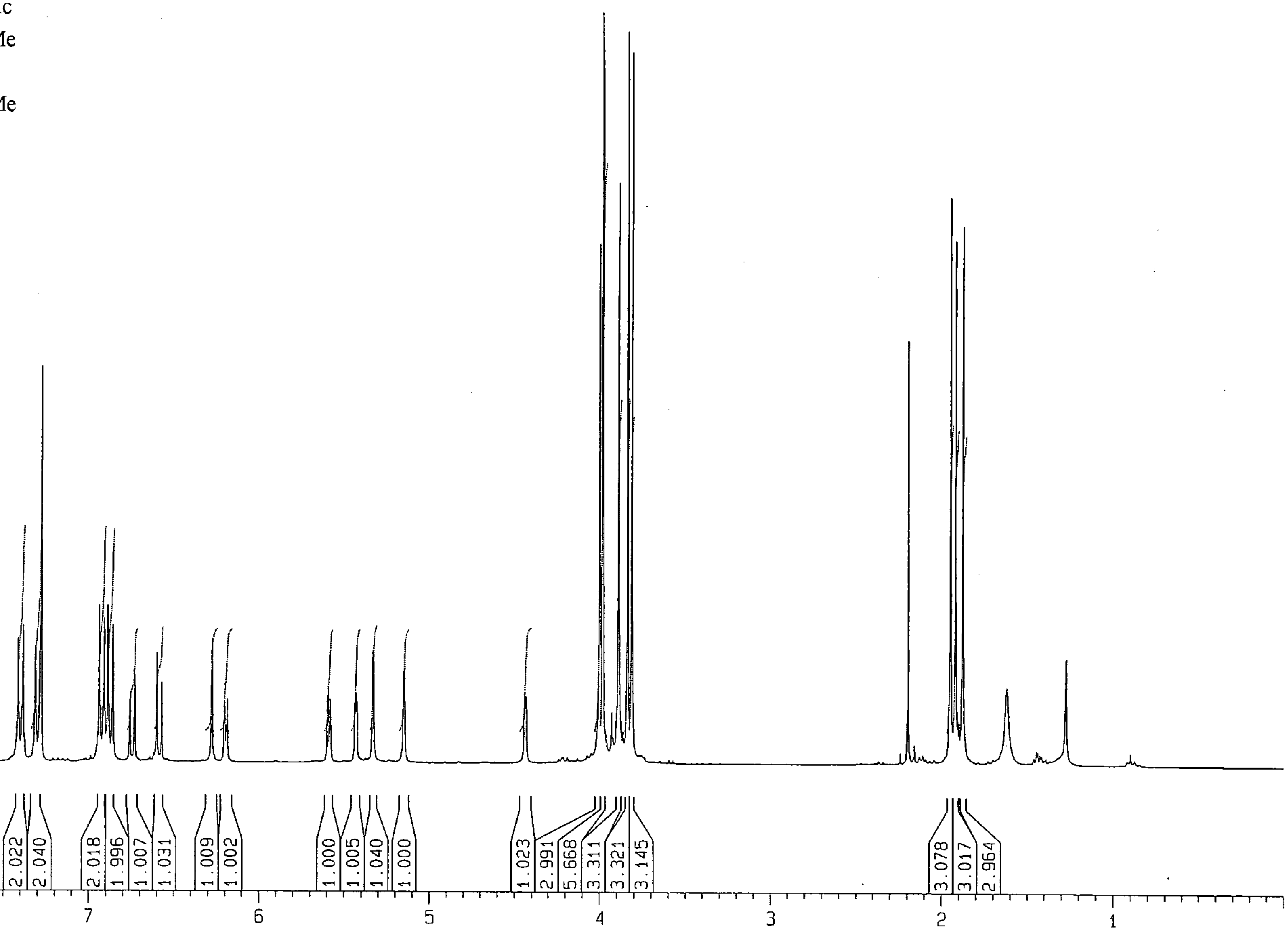

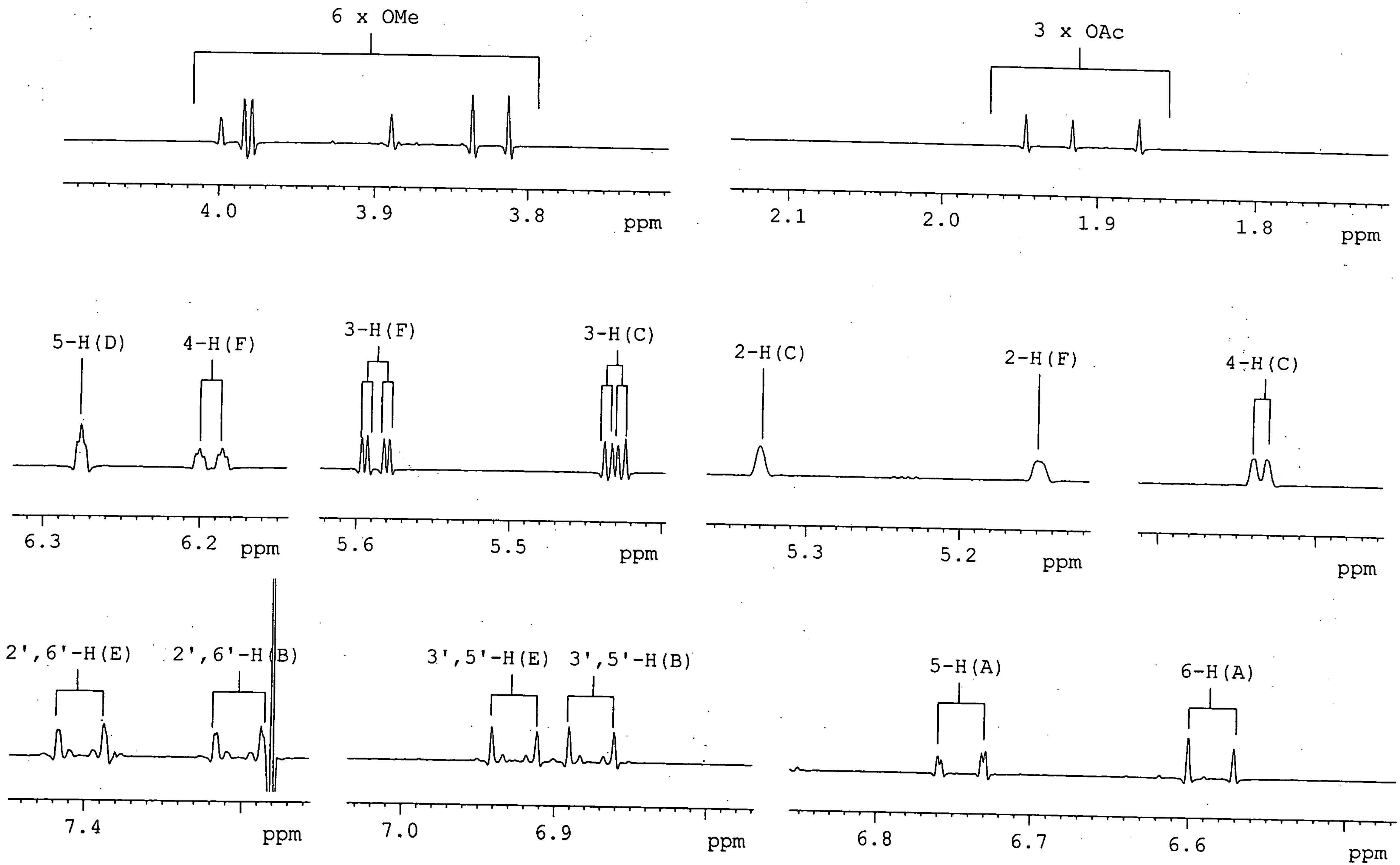
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3.9
3.8
ppm
3 x OAc
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2.0
1.9
1.8
ppm
5-H(D)
4-H(F)
6.3
6.2
ppm
3-H(F)
3-H(C)
5.6
5.5
ppm
2-H(C)
2-H(F)
5.3
5.2
ppm
4-H(C)
ppm
2',6'-H(E)
2',6'-H(B)
7.4
ppm
3',5'-H(E)
3',5'-H(B)
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ppm
5-H(A)
6-H(A)
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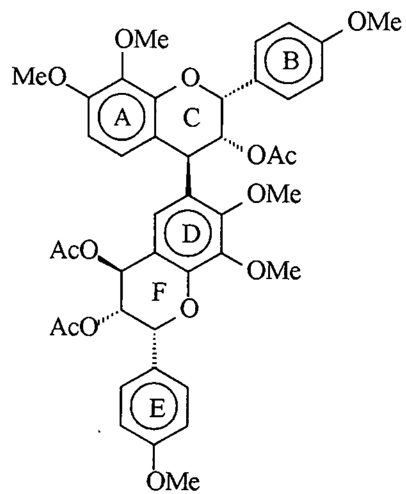
6.7
6.6
ppm

Plate 9 ($CDCl_3$, 296 K)



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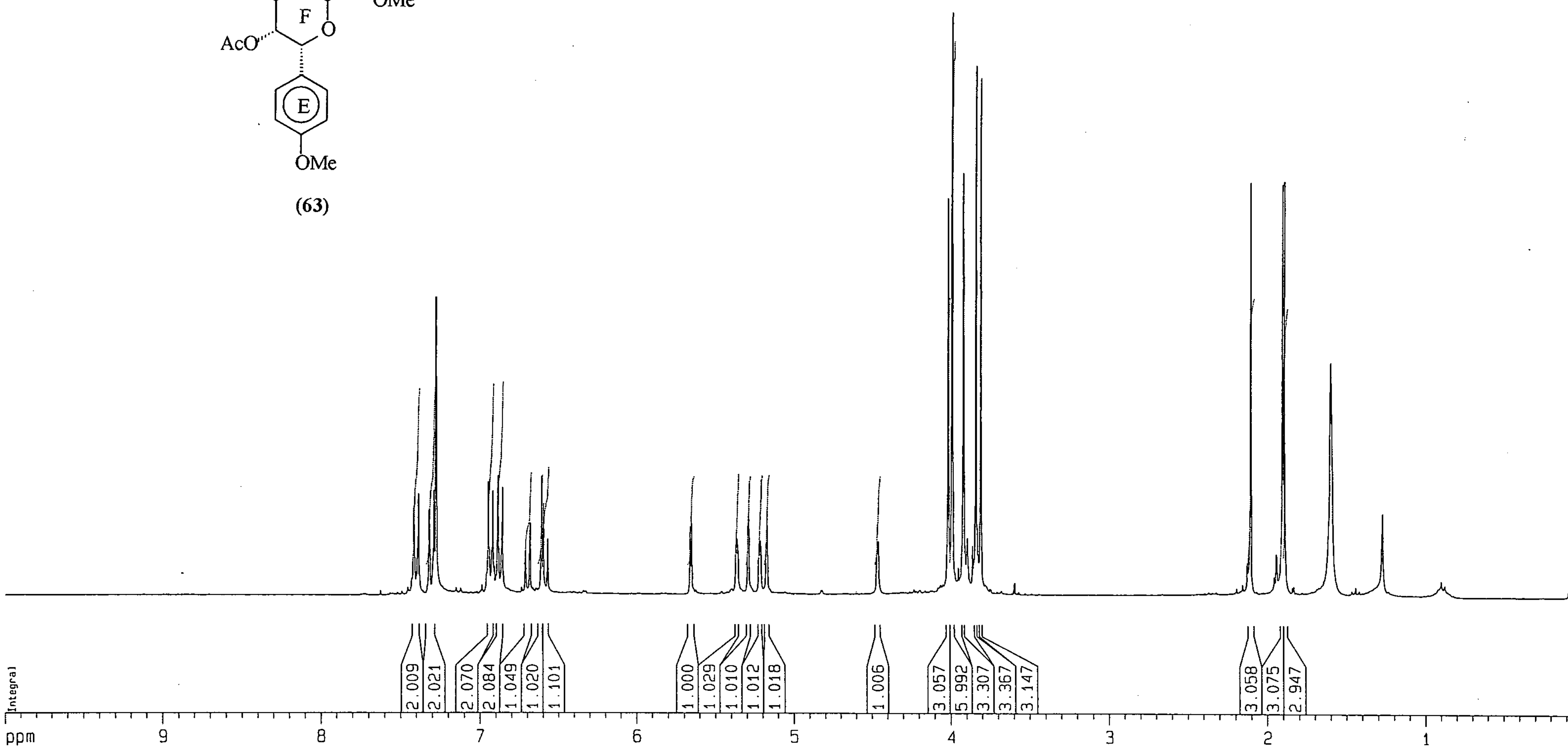

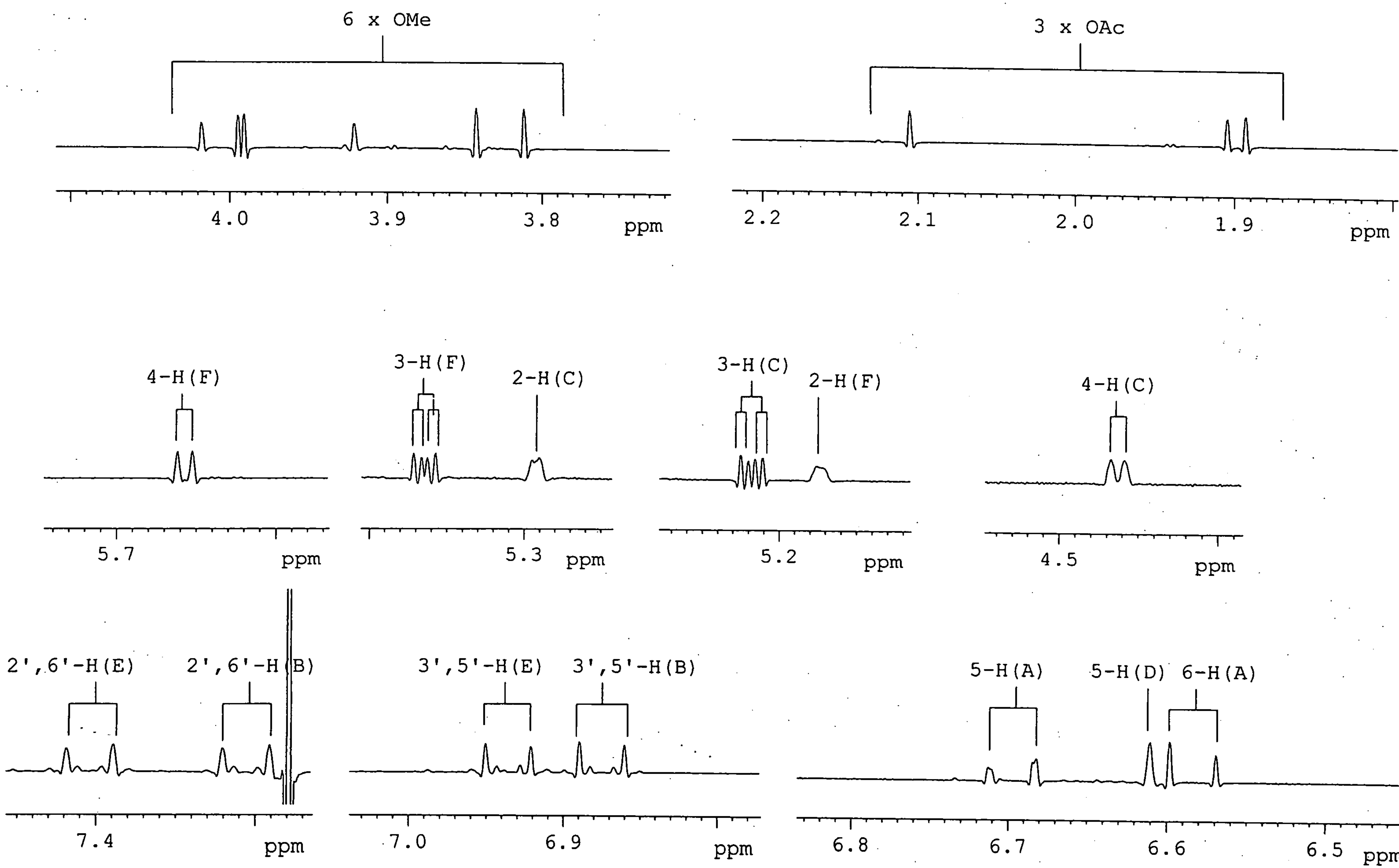
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ppm
3 x OAc
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2.1
2.0
1.9
ppm
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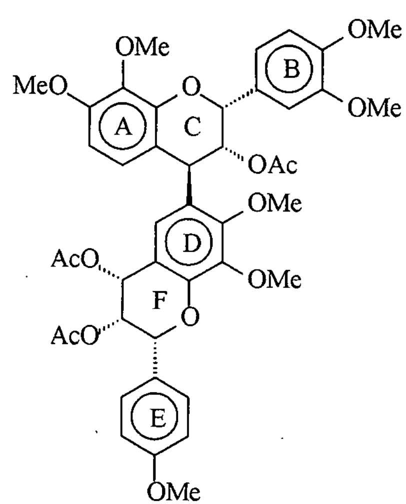
5.7
ppm
3-H(F)
2-H(C)
5.3
ppm
3-H(C)
2-H(F)
5.2
ppm
4-H(C)
4.5
ppm
2',6'-H(E)
2',6'-H(B)
7.4
ppm
3',5'-H(E)
3',5'-H(B)
7.0
6.9
ppm
5-H(A)
5-H(D)
6-H(A)
6.8
6.7
6.6
6.5
ppm

Plate 10 ($CDCl_3$, 296 K)



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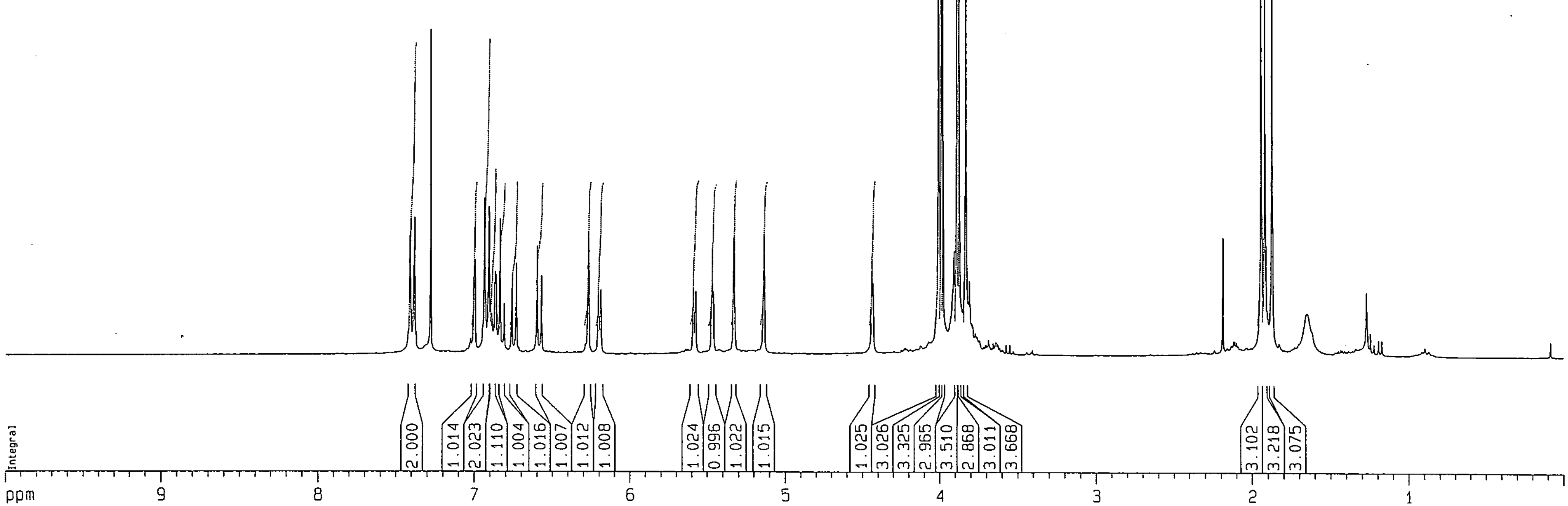

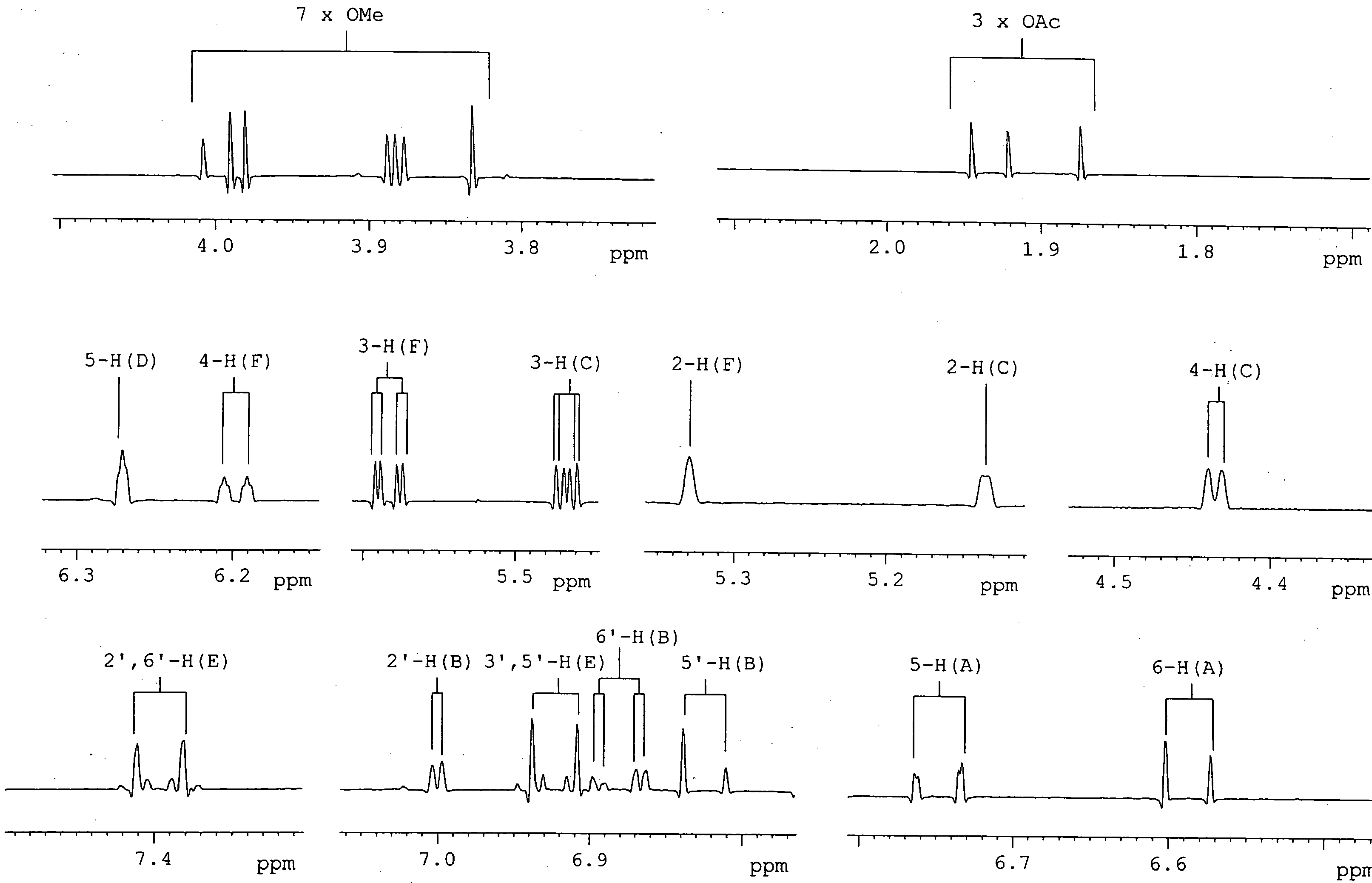
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ppm
3 x OAc
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1.9
1.8
ppm
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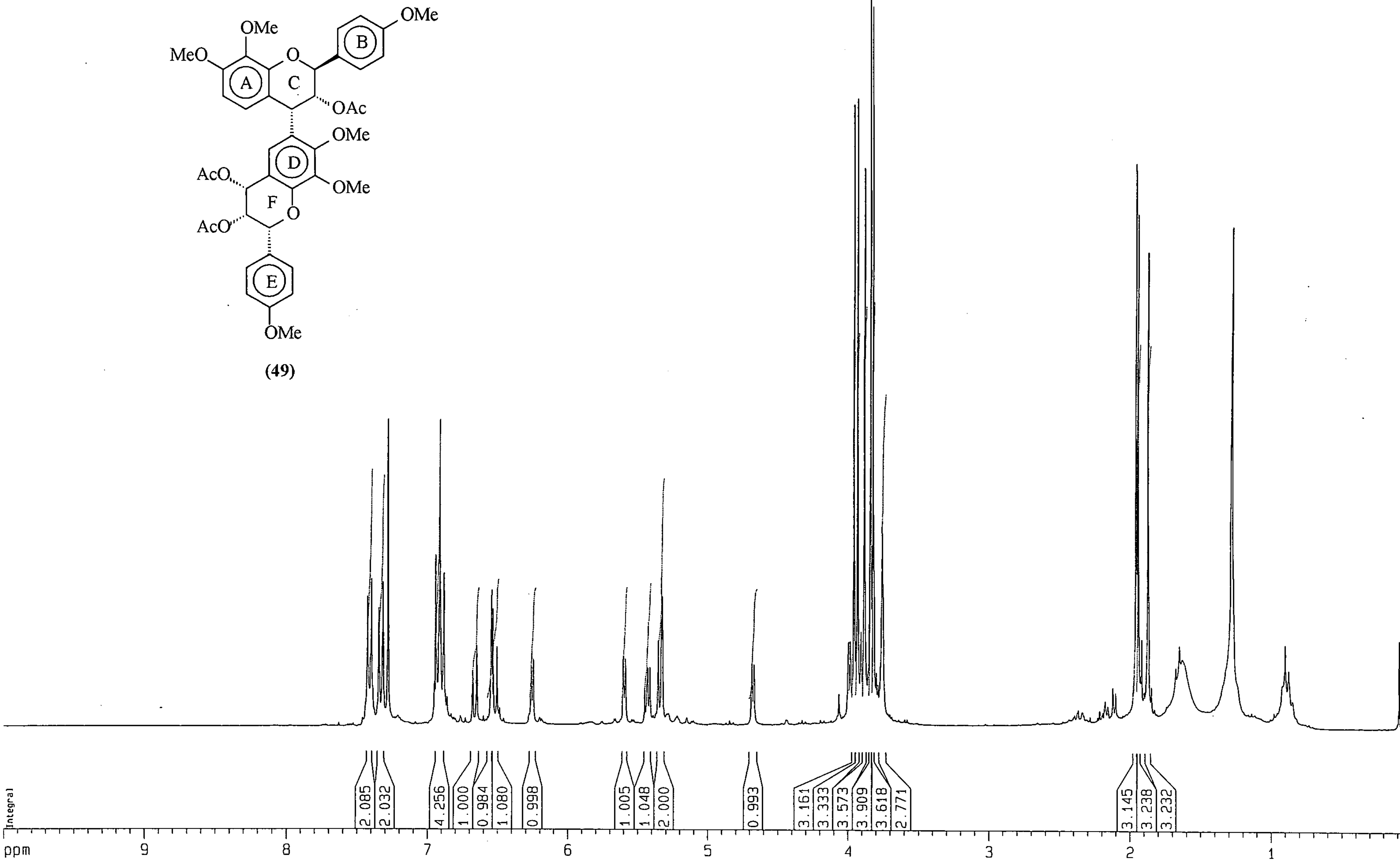
4-H(F)
6.3
6.2
ppm
3-H(F)
3-H(C)
5.5
ppm
2-H(F)
2-H(C)
5.3
5.2
ppm
4-H(C)
4.5
4.4
ppm
2',6'-H(E)
7.4
ppm
2'-H(B)
3',5'-H(E)
6'-H(B)
5'-H(B)
7.0
6.9
ppm
5-H(A)
6-H(A)
6.7
6.6
ppm

Plate 11 ($CDCl_3$, 296 K)



(49)

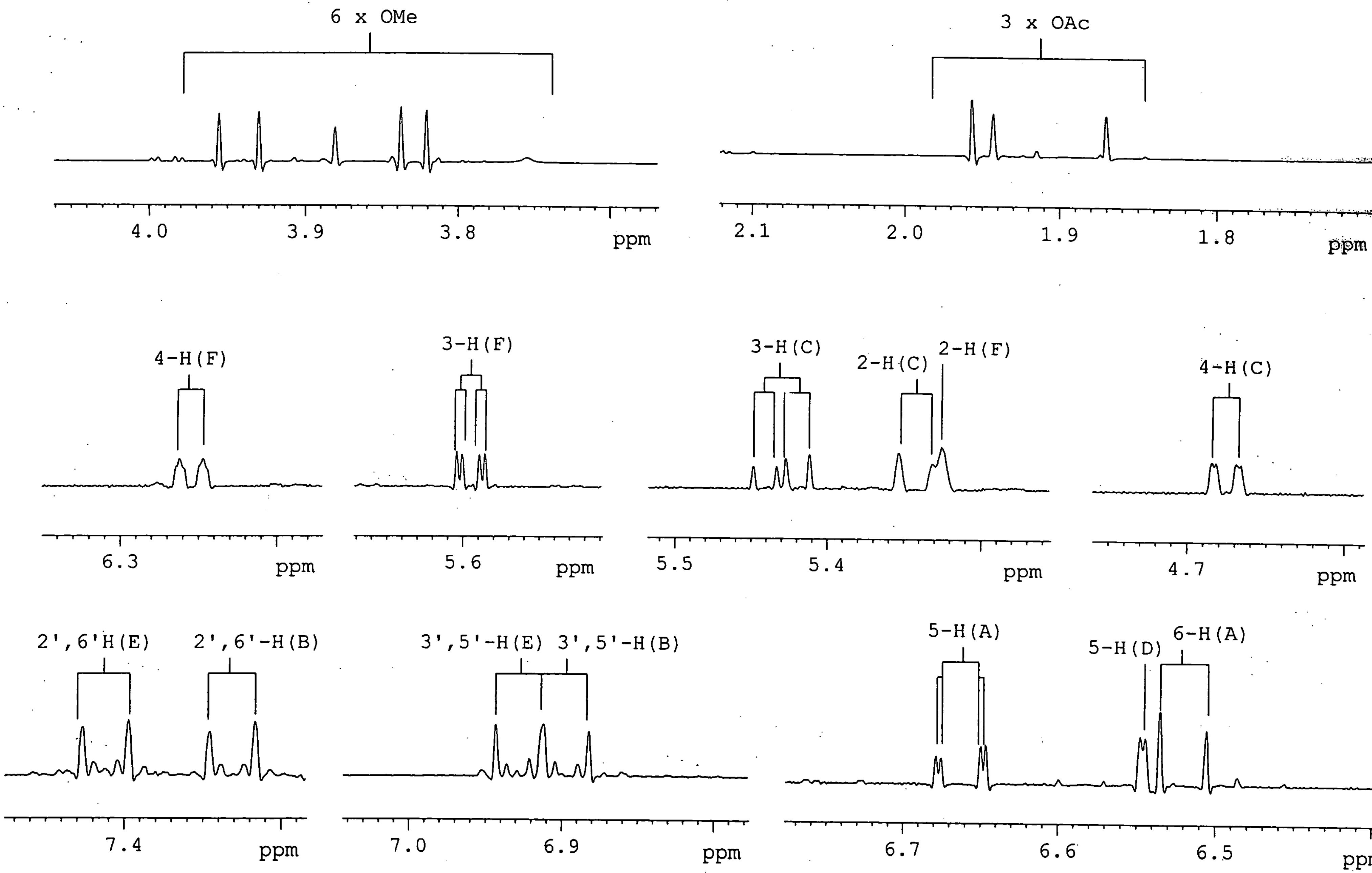
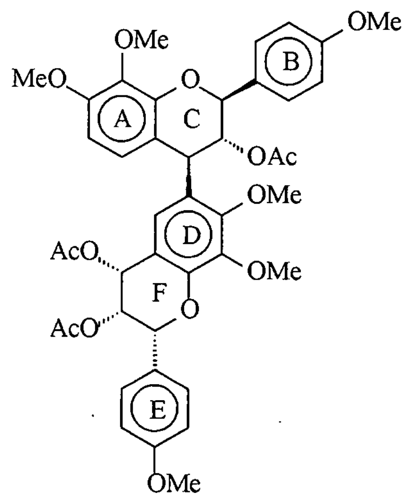
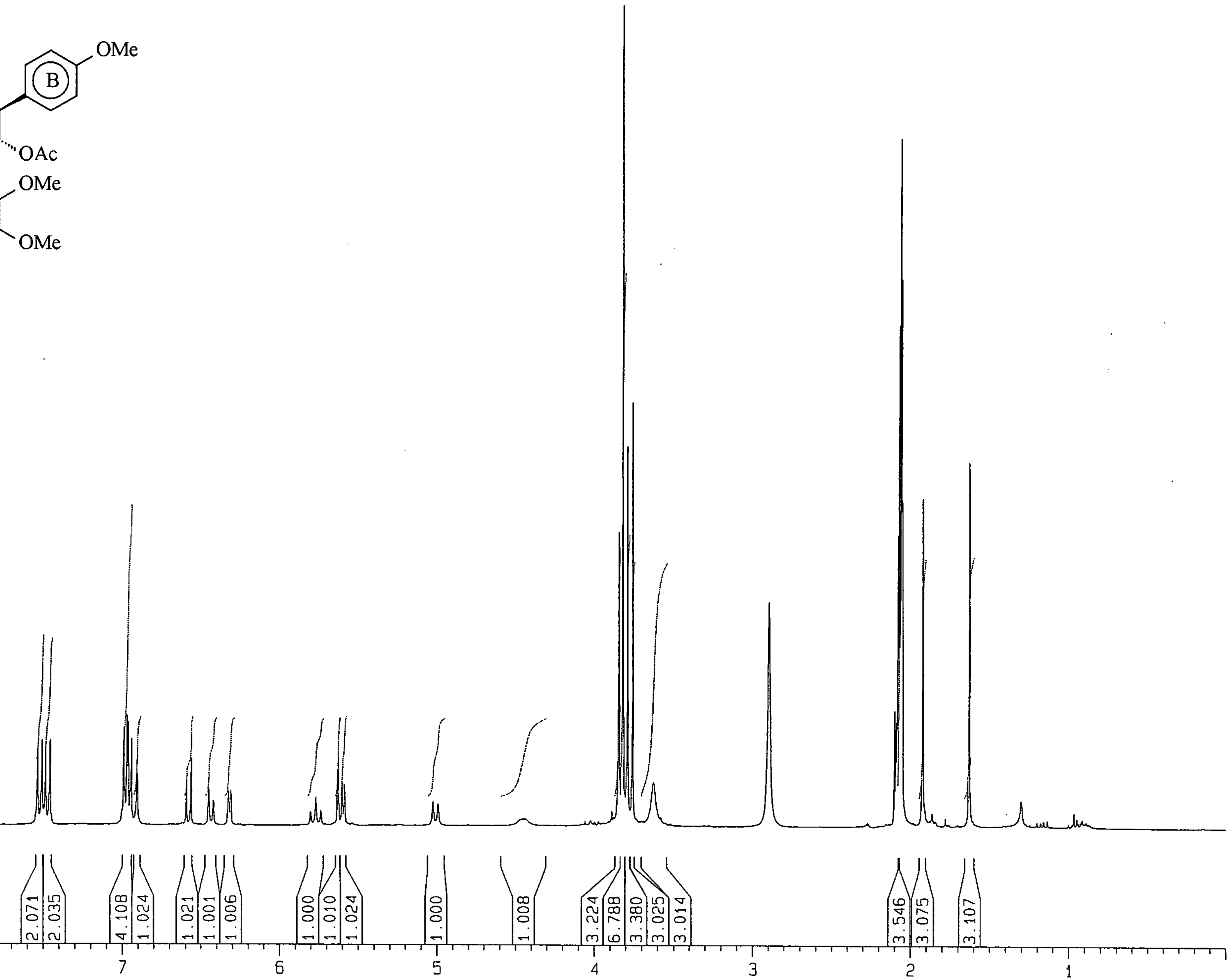

6 x OMe
4.0
3.9
3.8
ppm
3 x OAc
2.1
2.0
1.9
1.8
ppm
4-H(F)
6.3
ppm
3-H(F)
5.6
ppm
3-H(C)
2-H(C)
2-H(F)
5.5
5.4
ppm
4-H(C)
4.7
ppm
2',6'H(E)
2',6'-H(B)
7.4
ppm
3',5'-H(E)
3',5'-H(B)
7.0
6.9
ppm
5-H(A)
5-H(D)
6-H(A)
6.7
6.6
6.5
ppm

Plate 12 ($CDCl_3$, 296 K)



(51)



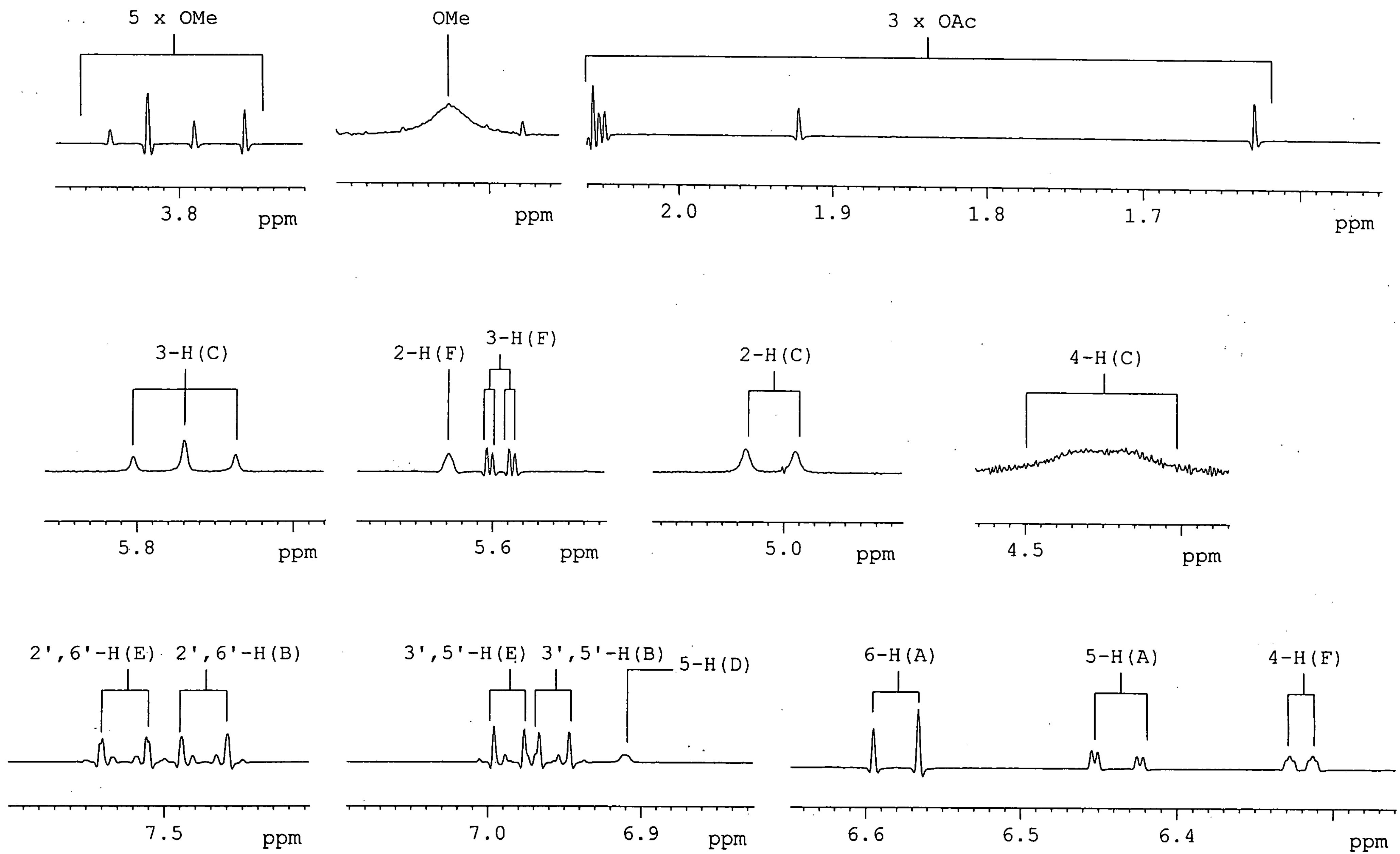
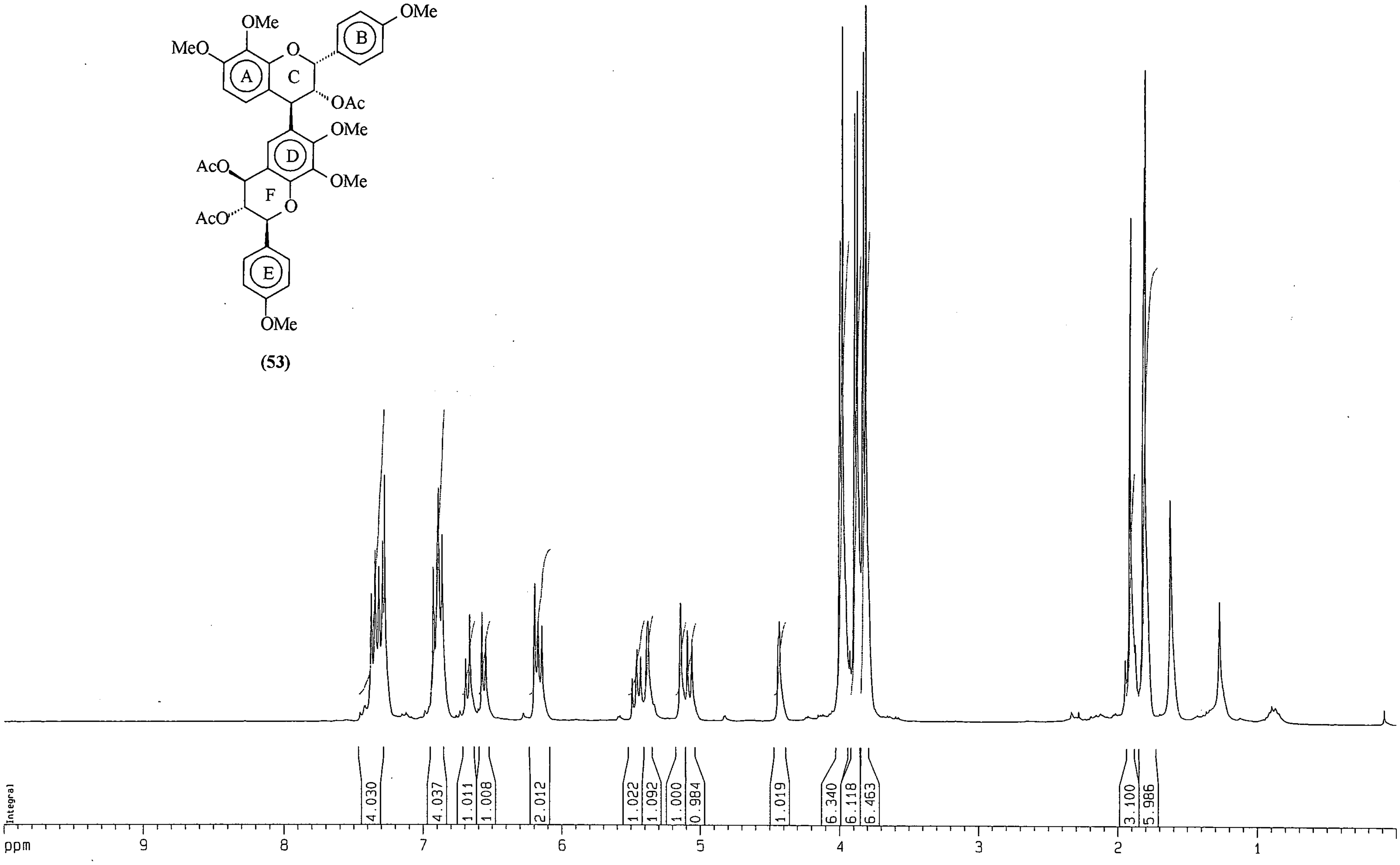
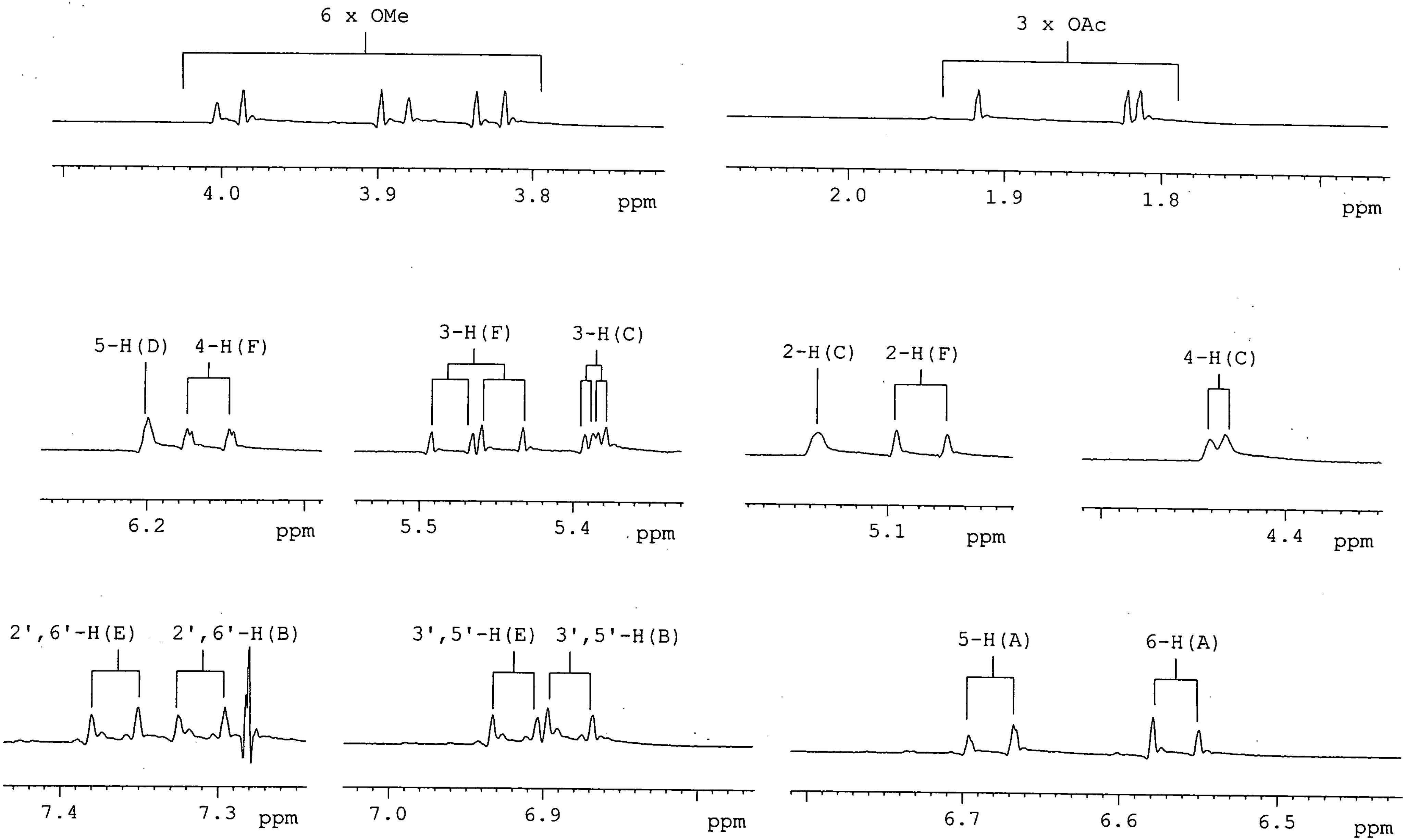

5 x OMe
OMe
3 x OAc
3.8
ppm
ppm
2.0
1.9
1.8
1.7
ppm
3-H(C)
2-H(F)
3-H(F)
2-H(C)
4-H(C)
5.8
ppm
5.6
ppm
5.0
ppm
4.5
ppm
2',6'-H(E)
2',6'-H(B)
3',5'-H(E)
3',5'-H(B)
5-H(D)
6-H(A)
5-H(A)
4-H(F)
7.5
ppm
7.0
6.9
ppm
6.6
6.5
6.4
ppm

Plate 13 ($CDCl_3$, 296 K)

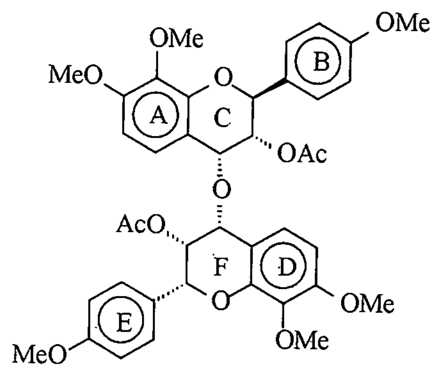


(53)

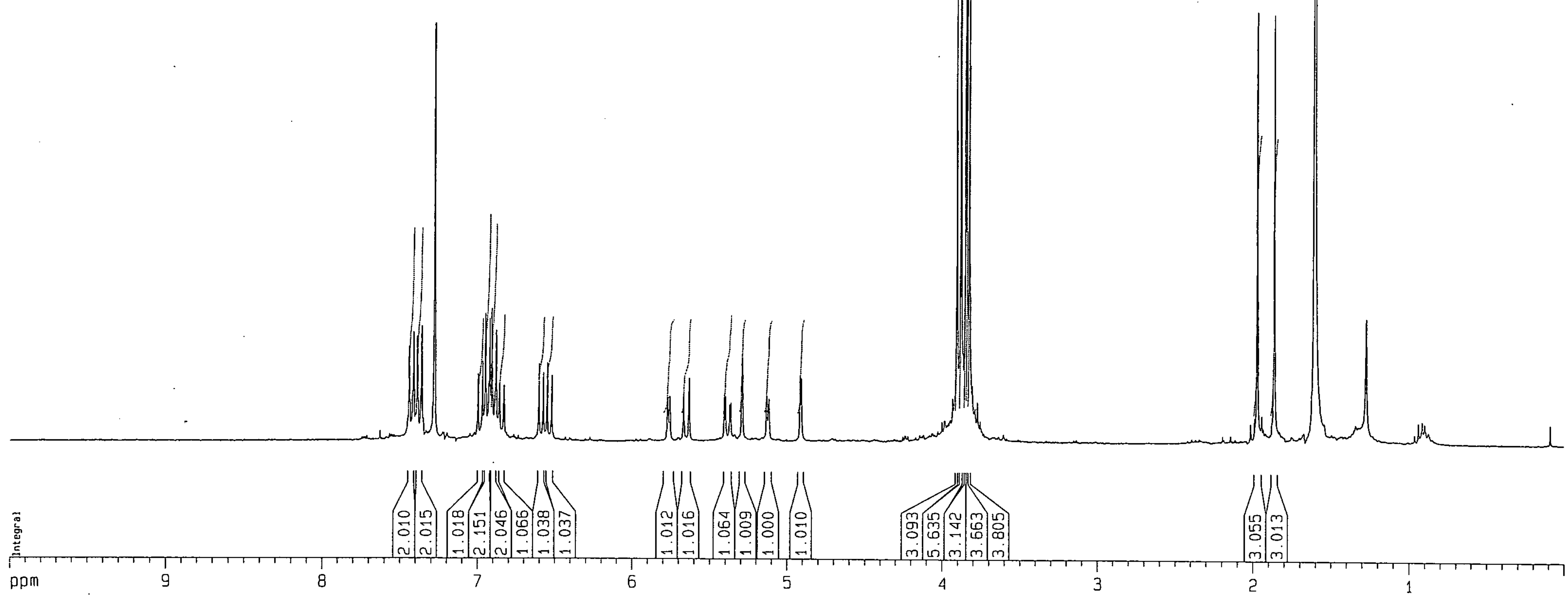


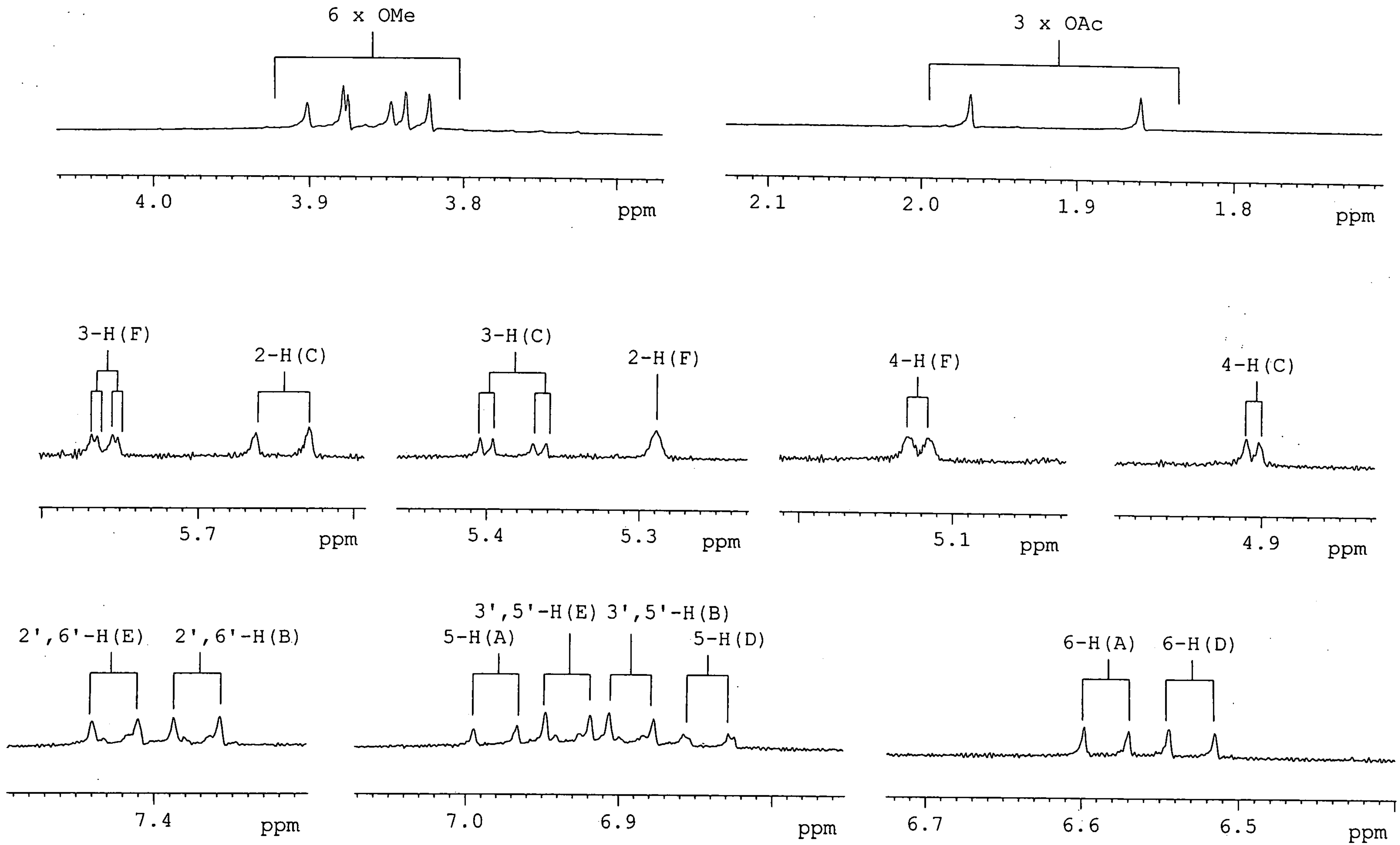
6 x OMe
4.0
3.9
3.8
ppm
3 x OAc
2.0
1.9
1.8
ppm
5-H(D)
4-H(F)
6.2
ppm
3-H(F)
3-H(C)
5.5
5.4
ppm
2-H(C)
2-H(F)
5.1
ppm
4-H(C)
4.4
ppm
2',6'-H(E)
2',6'-H(B)
7.4
7.3
ppm
3',5'-H(E)
3',5'-H(B)
7.0
6.9
ppm
5-H(A)
6-H(A)
6.7
6.6
6.5
ppm

Plate 14 ($CDCl_3$, 296 K)



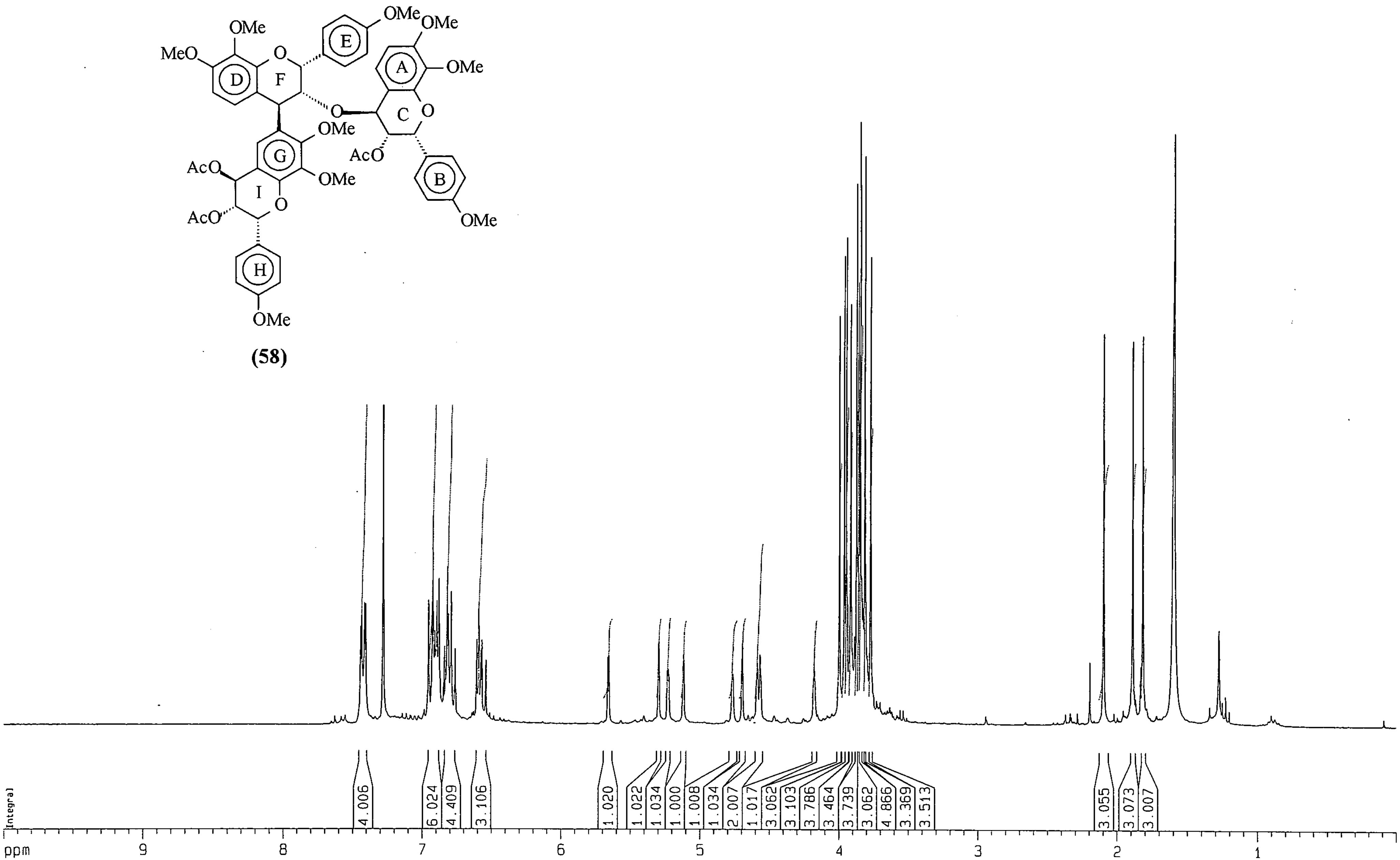
(55)





6 x OMe
4.0
3.9
3.8
ppm
3 x OAc
2.1
2.0
1.9
1.8
ppm
3-H(F)
2-H(C)
5.7
ppm
3-H(C)
2-H(F)
5.4
5.3
ppm
4-H(F)
5.1
ppm
4-H(C)
4.9
ppm
2',6'-H(E)
2',6'-H(B)
7.4
ppm
5-H(A)
3',5'-H(E)
3',5'-H(B)
5-H(D)
7.0
6.9
ppm
6-H(A)
6-H(D)
6.7
6.6
6.5
ppm

Plate 15 ($CDCl_3$, 296 K)



(58)

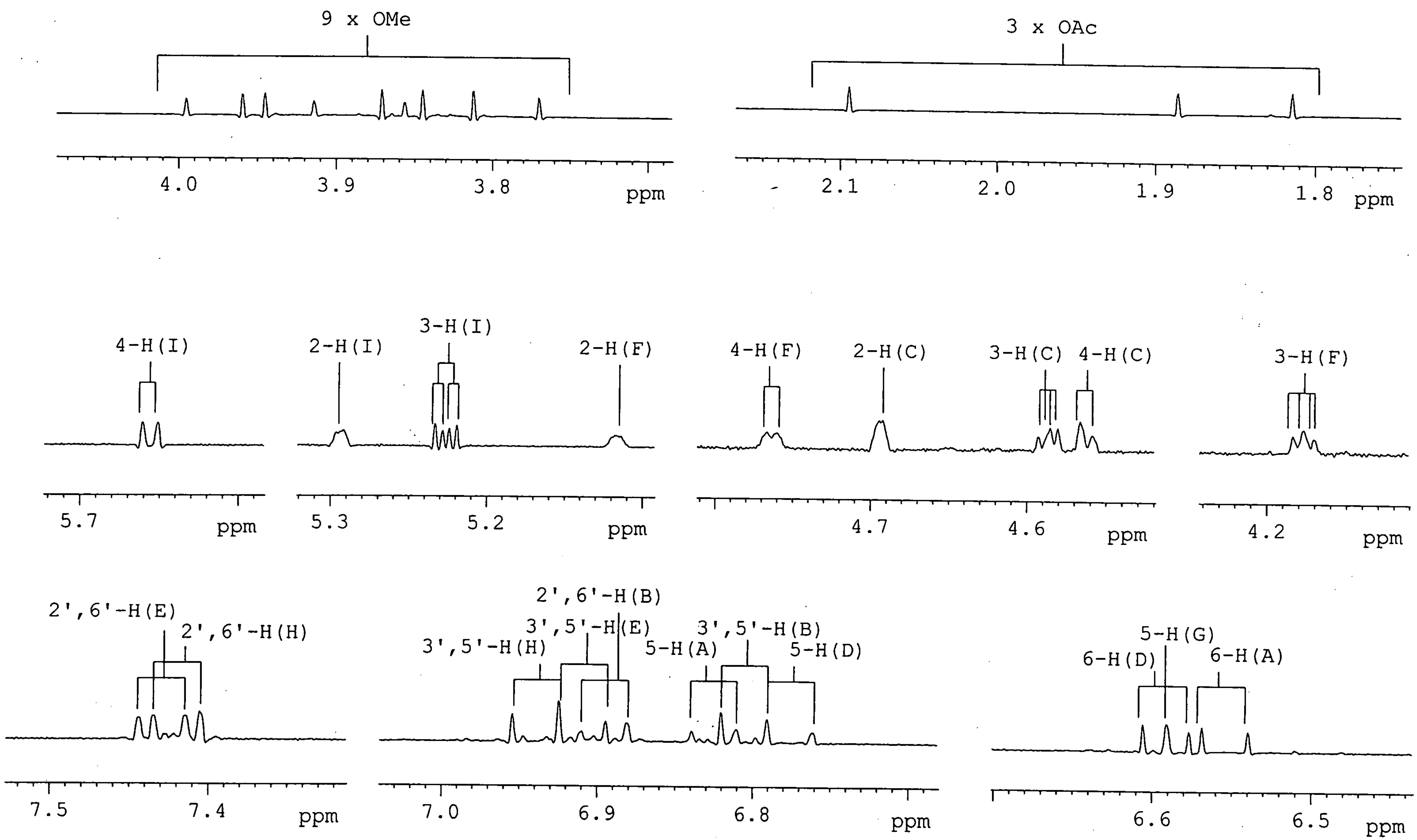
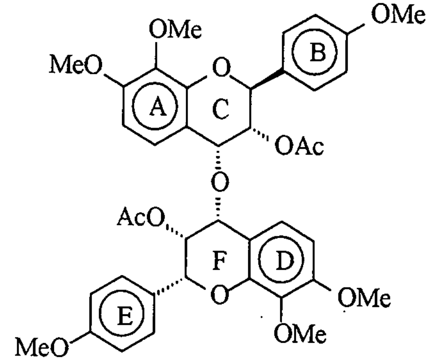
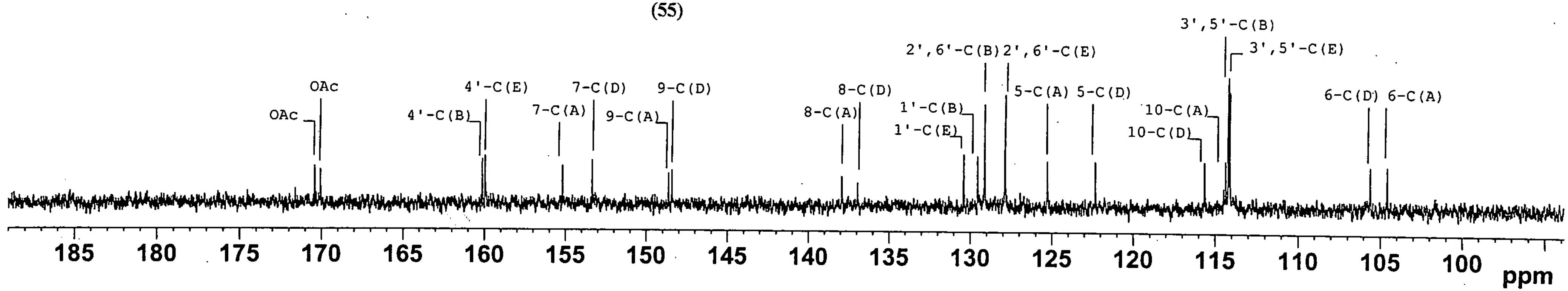

9 x OMe
4.0
3.9
3.8
ppm
3 x OAc
2.1
2.0
1.9
1.8
ppm
4-H(I)
5.7
ppm
2-H(I)
3-H(I)
2-H(F)
5.3
5.2
ppm
4-H(F)
2-H(C)
3-H(C)
4-H(C)
4.7
4.6
ppm
3-H(F)
4.2
ppm
2',6'-H(E)
2',6'-H(H)
7.5
7.4
ppm
2',6'-H(B)
3',5'-H(E)
3',5'-H(H)
5-H(A)
3',5'-H(B)
5-H(D)
7.0
6.9
6.8
ppm
5-H(G)
6-H(D)
6-H(A)
6.6
6.5
ppm

Plate 16 (^{13}C, $CDCl_3$, 296 K)



(55)



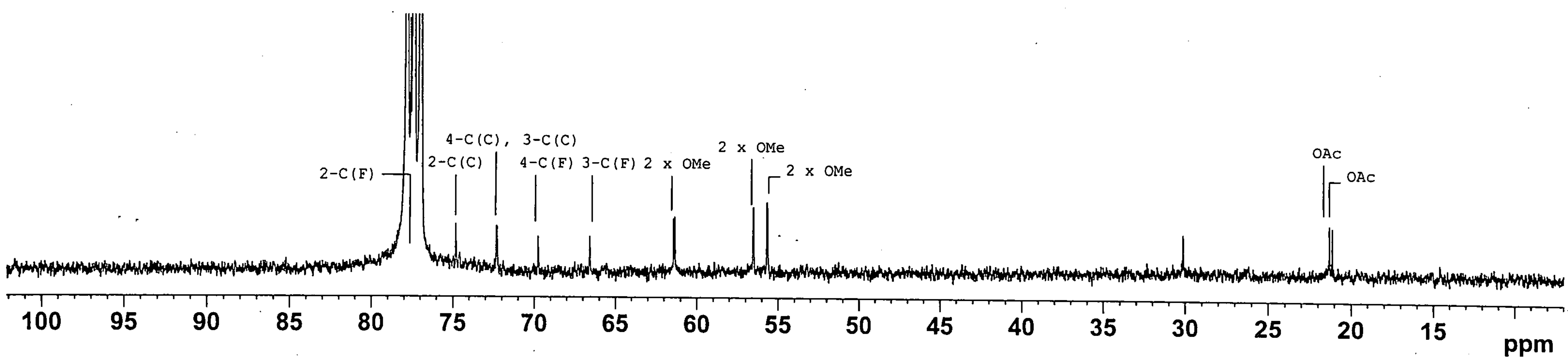
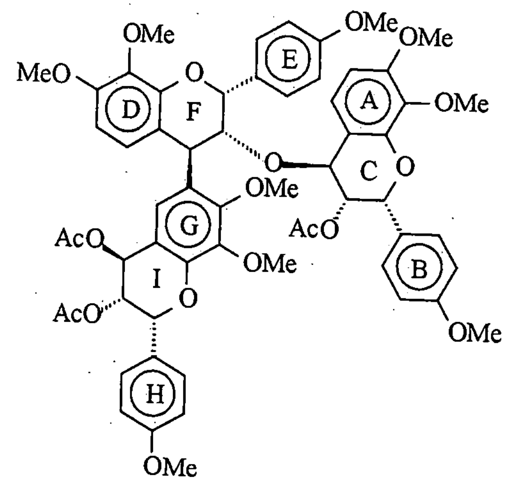
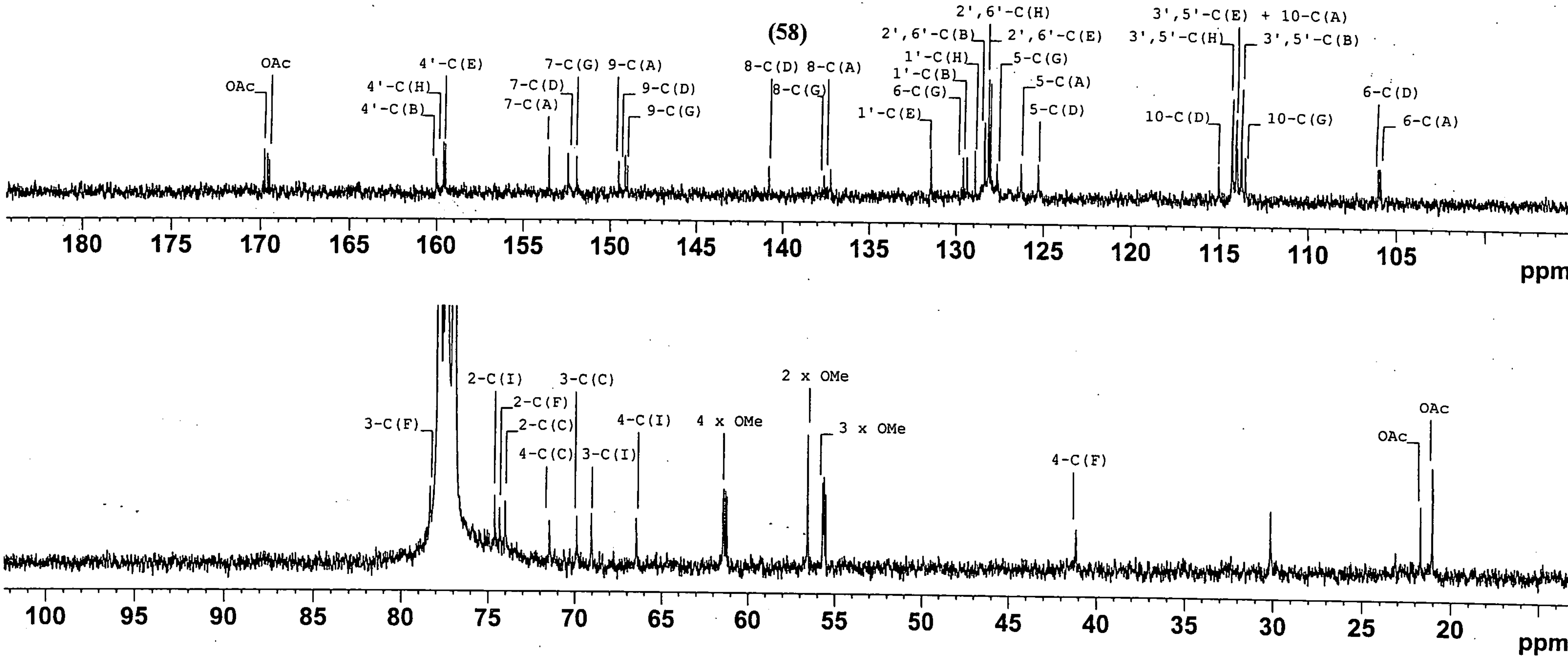


Plate 17 (^{13}C, $CDCl_3$, 296 K)

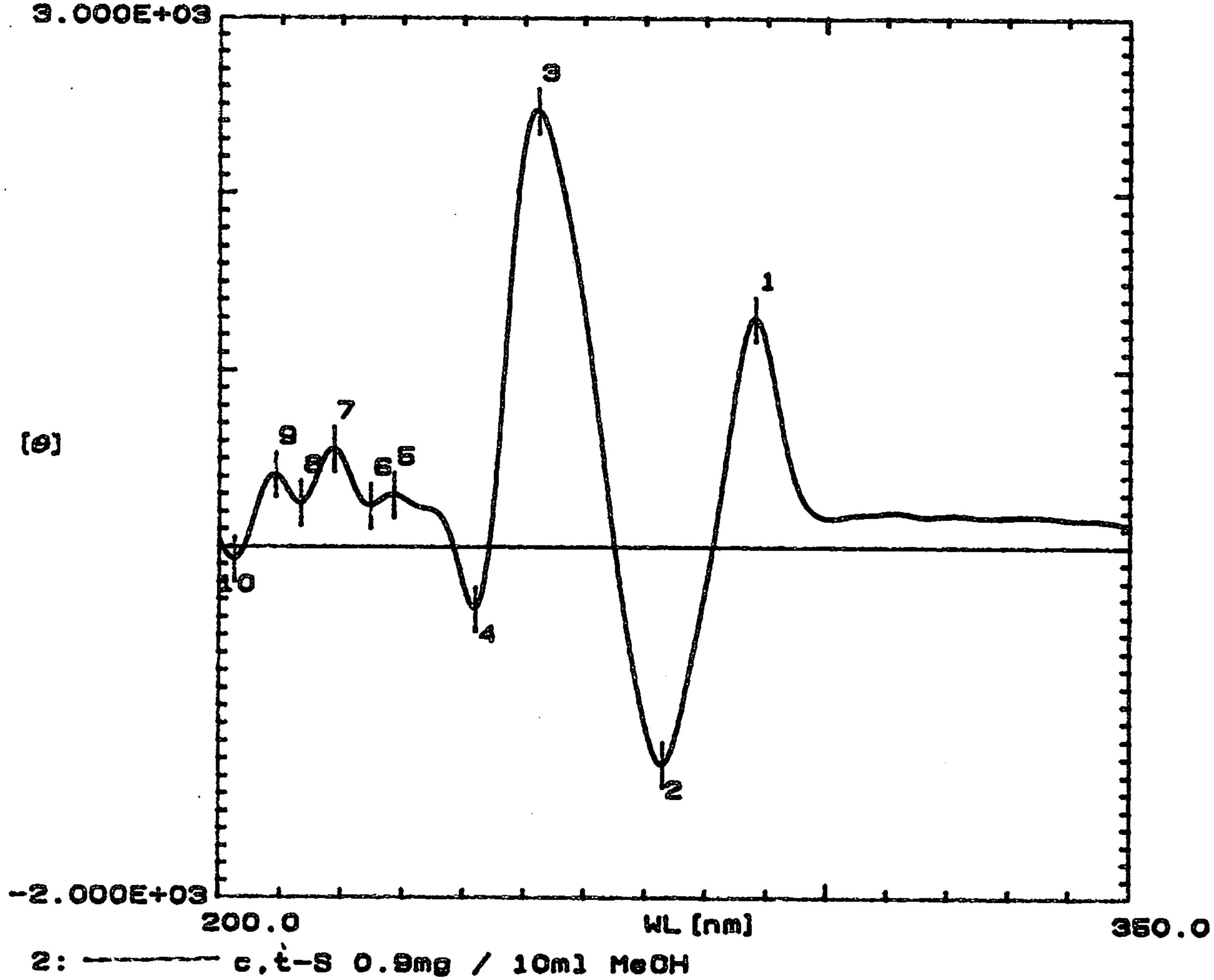


(58)



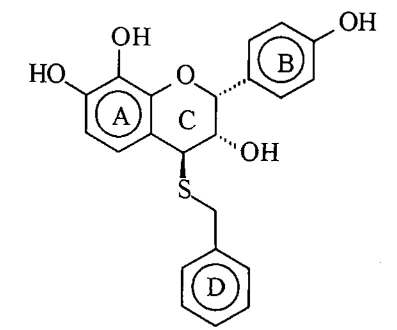
Appendix B: CD Spectra

1. Epioritin-4β-benzylthioether **(59)**
2. *ent*-Oritin-4α-benzylthioether **(56)**
3. Epimesquitol-(4β→6)-epioritin-4α-ol hepta-O-methylether triacetate **(47)**
4. *ent*-Oritin-(4α→6)-epioritin-4α-ol hexa-O-methylether triacetate **(49)**
5. *ent*-Oritin-(4β→6)-epioritin-4α-ol hexa-O-methylether triacetate **(51)**
6. Epioritin-(4β→6)-oritin-4α-ol hexa-O-methylether triacetate **(53)**
7. *ent*-Oritin-(4α→4)-epioritin-4α-ol hexa-O-methylether diacetate **(55)**
8. Epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol nonamethyl-O-ether triacetate **(58)**

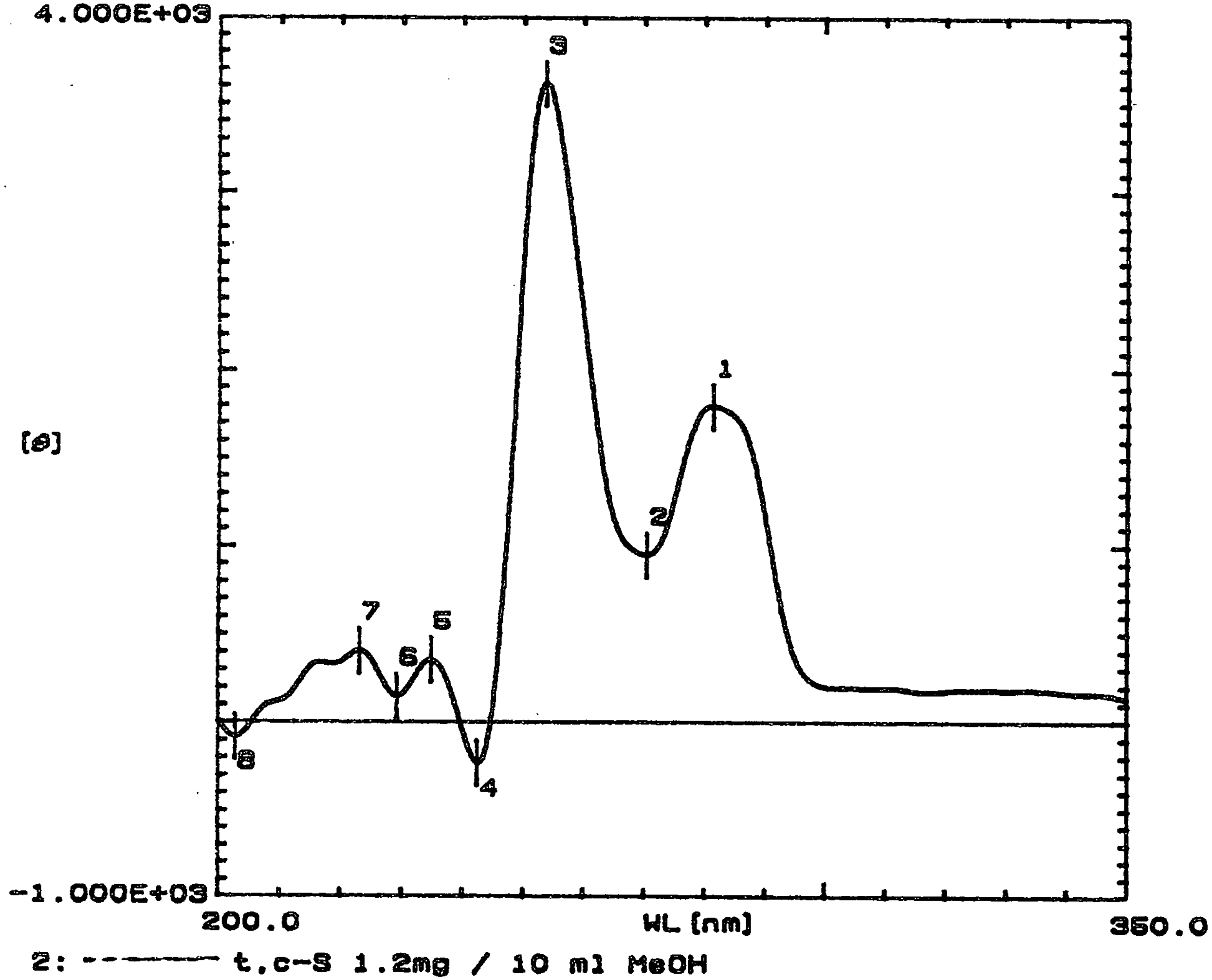


No.	Wavelength	Value
1	288.00 nm	1.294E+03
2	272.70 nm	-1.236E+03
3	252.10 nm	2.463E+03
4	241.80 nm	-3.537E+02
5	228.30 nm	2.934E+02
6	224.50 nm	2.312E+02
7	218.50 nm	5.545E+02
8	213.10 nm	2.466E+02
9	208.90 nm	4.083E+02
10	202.30 nm	-7.411E+01

Plate 1

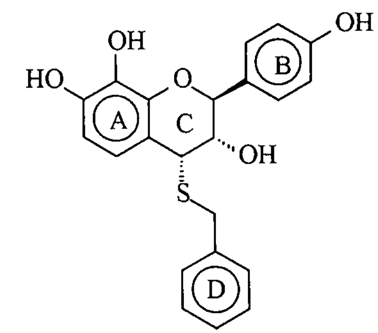


(59)

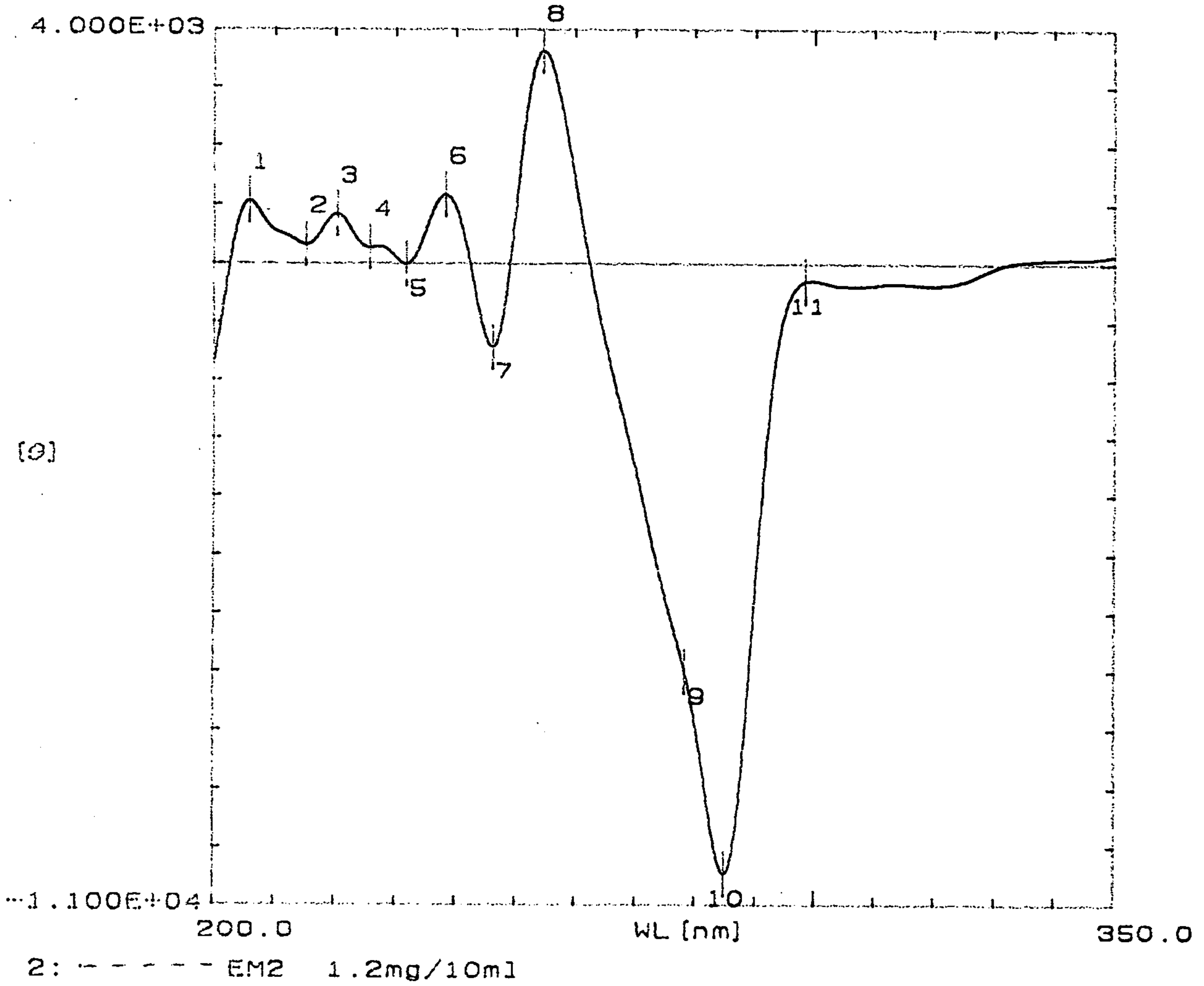


No.	Wavelength	Value
1	281.40 nm	1.803E+03
2	270.50 nm	9.537E+02
3	253.70 nm	3.622E+03
4	242.60 nm	-2.265E+02
5	234.90 nm	3.579E+02
6	229.30 nm	1.510E+02
7	223.20 nm	4.097E+02
8	202.80 nm	-7.712E+01

Plate 2

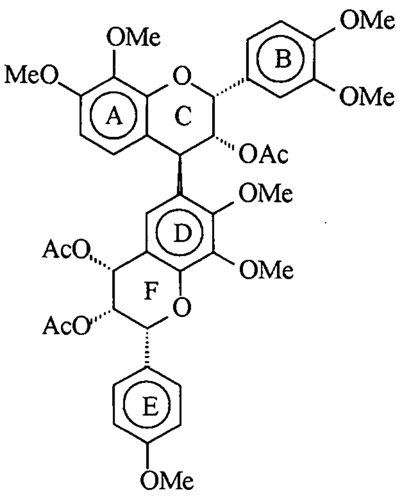


(56)

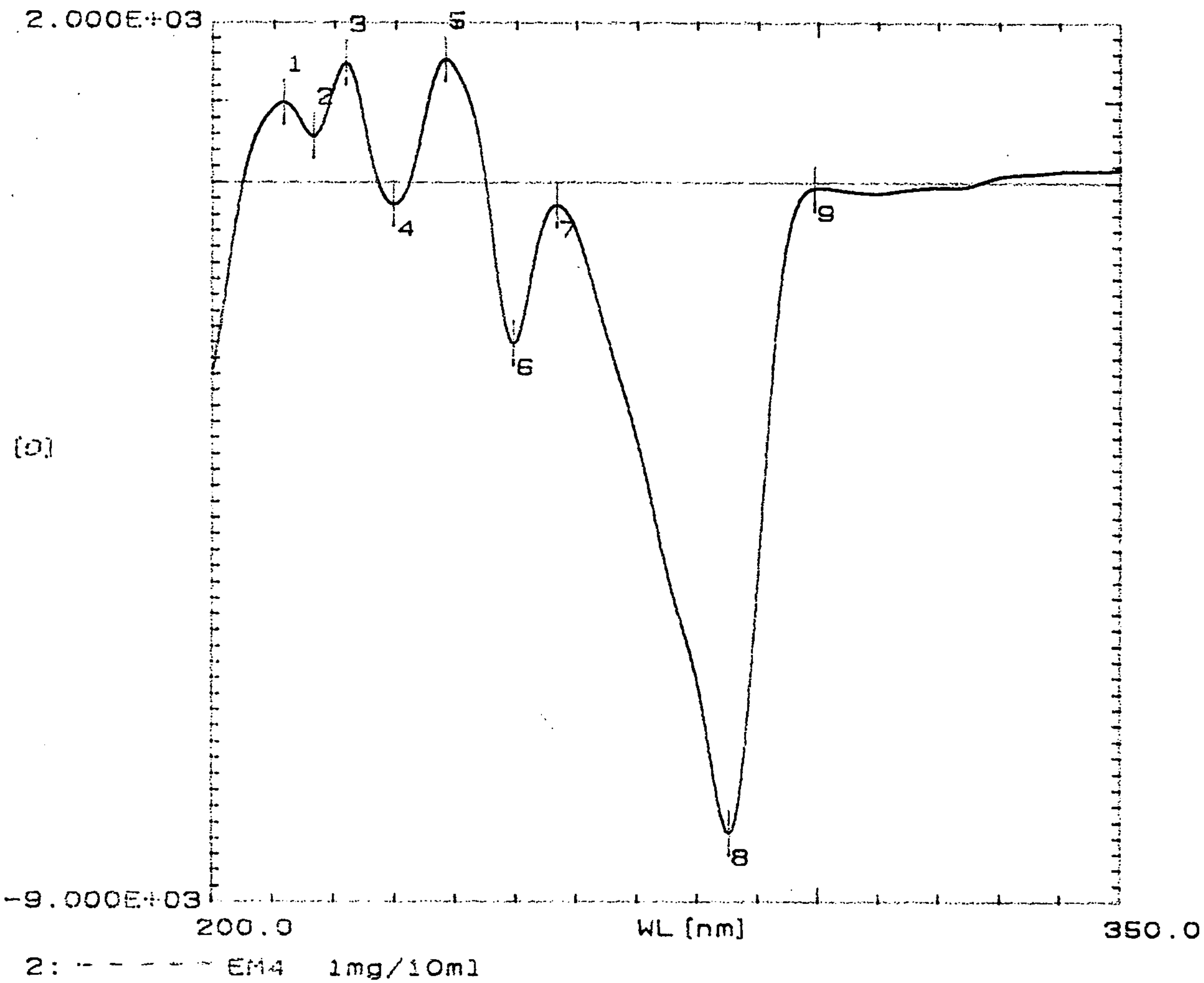


No.	Wavelength	Value
1	205.70 nm	1.072E+03
2	215.20 nm	3.249E+02
3	220.30 nm	8.400E+02
4	225.80 nm	2.755E+02
5	231.70 nm	-6.711E+00
6	238.30 nm	1.175E+03
7	246.10 nm	-1.430E+03
8	254.60 nm	3.625E+03
9	278.30 nm	-6.995E+03
10	284.90 nm	-1.046E+04
11	298.40 nm	-2.922E+02

Plate 3

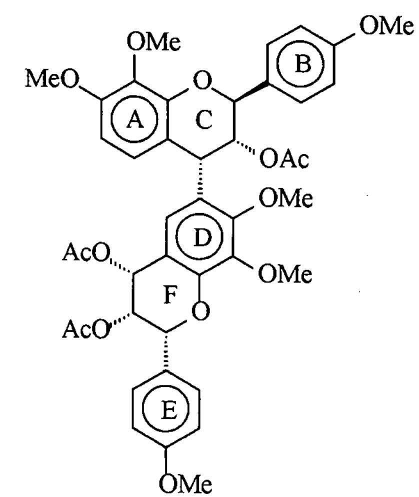


(47)

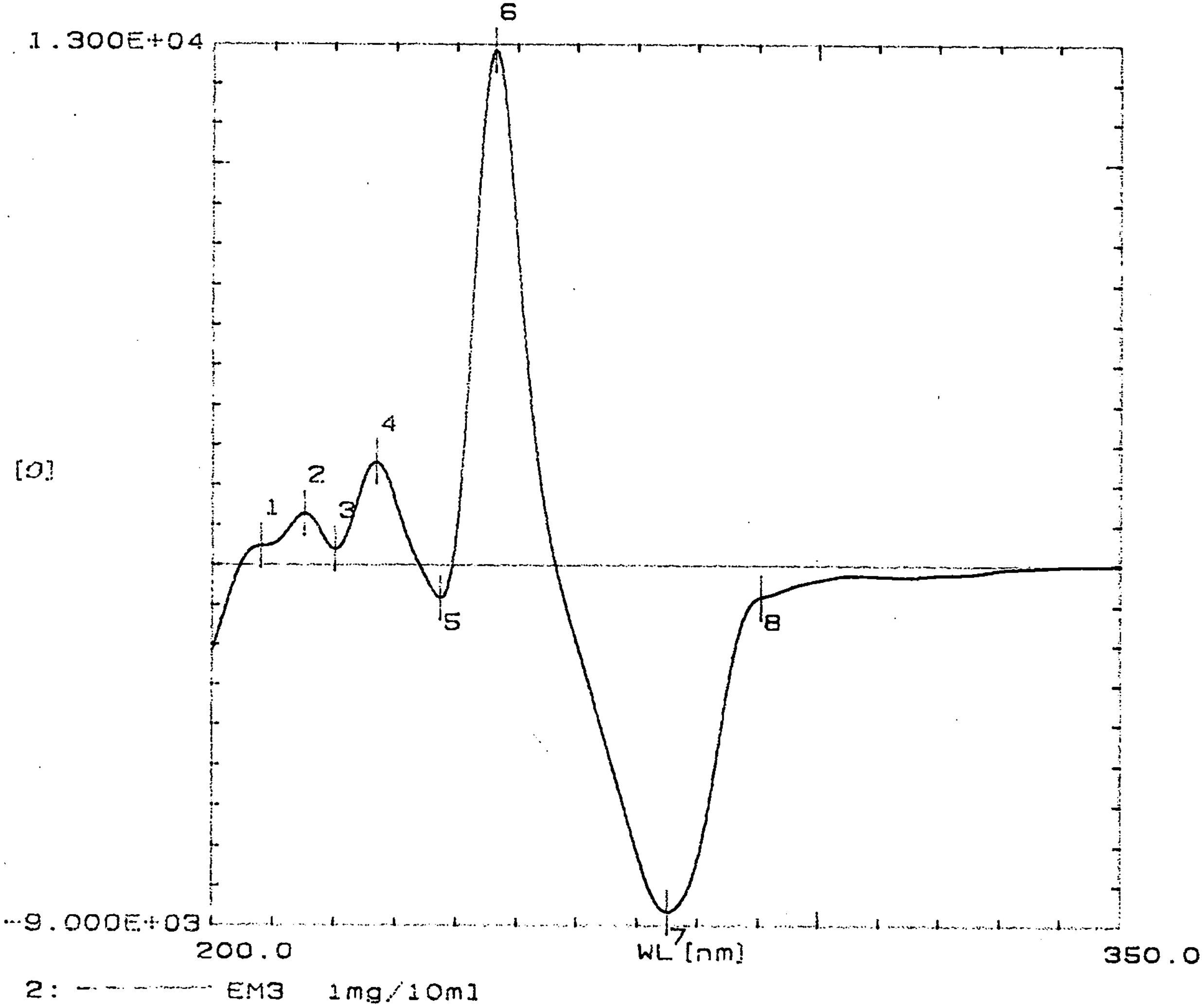


No.	Wavelength	Value
1	211.60 nm	9.921E+02
2	216.50 nm	5.720E+02
3	221.80 nm	1.485E+03
4	229.70 nm	-2.673E+02
5	238.10 nm	1.532E+03
6	249.40 nm	-2.008E+03
7	256.60 nm	-2.785E+02
8	285.20 nm	-8.128E+03
9	299.30 nm	-6.906E+01

Plate 4

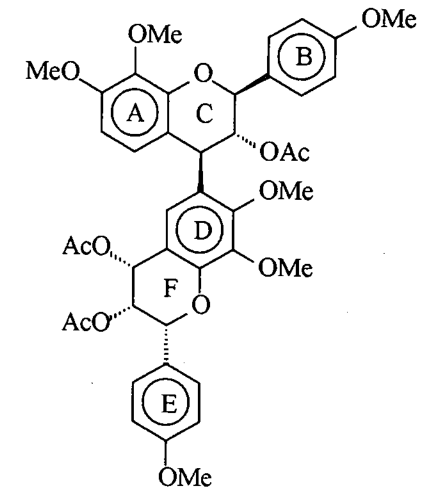


(49)

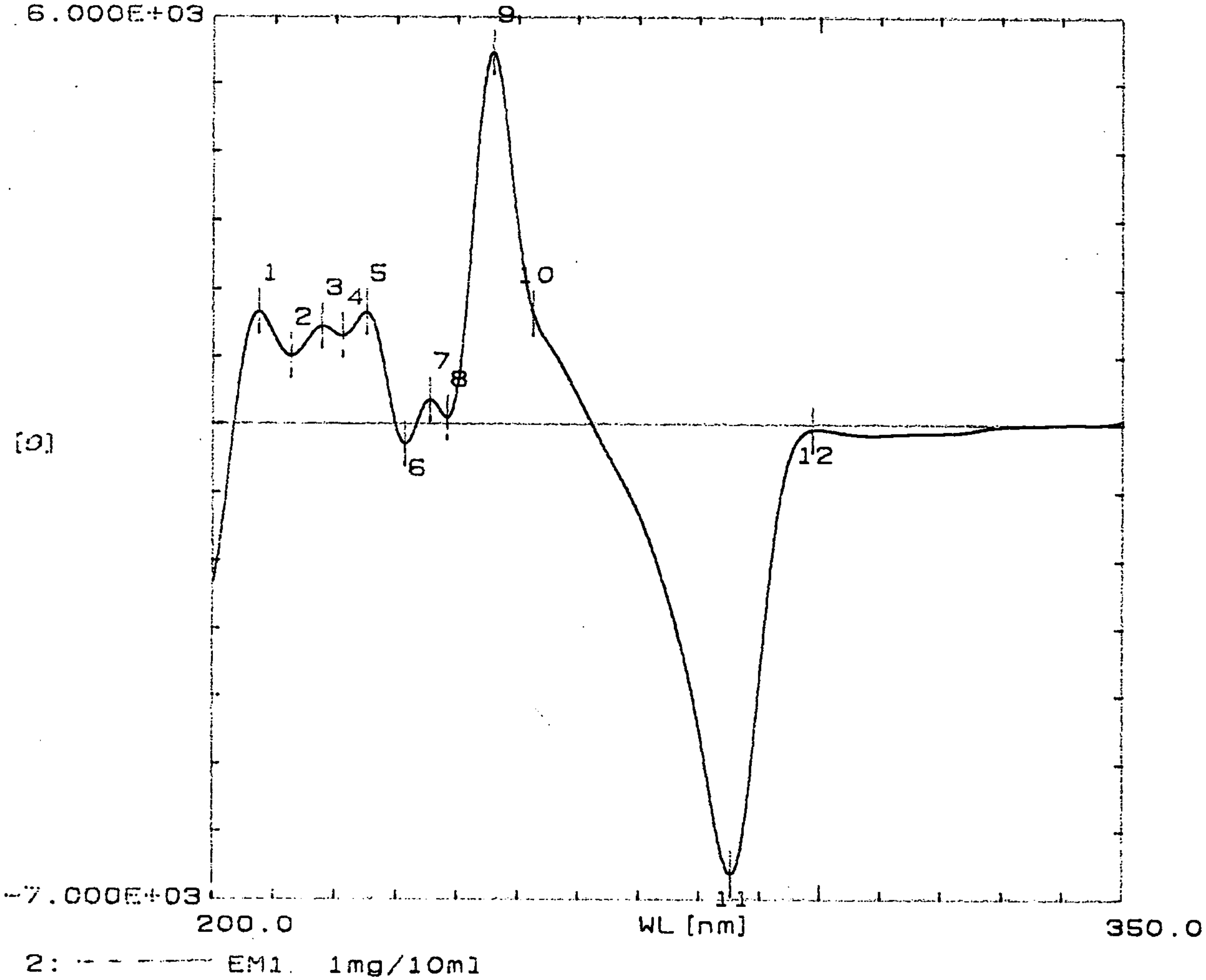


No.	Wavelength	Value
1	207.90 nm	4.897E+02
2	215.10 nm	1.296E+03
3	220.10 nm	4.047E+02
4	226.80 nm	2.586E+03
5	237.40 nm	-8.167E+02
6	246.40 nm	1.285E+04
7	275.10 nm	-8.636E+03
8	290.50 nm	-7.896E+02

Plate 5

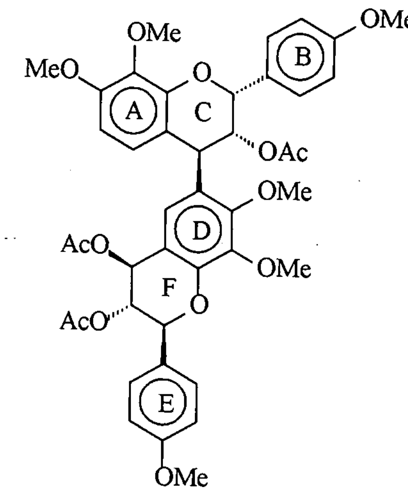


(51)

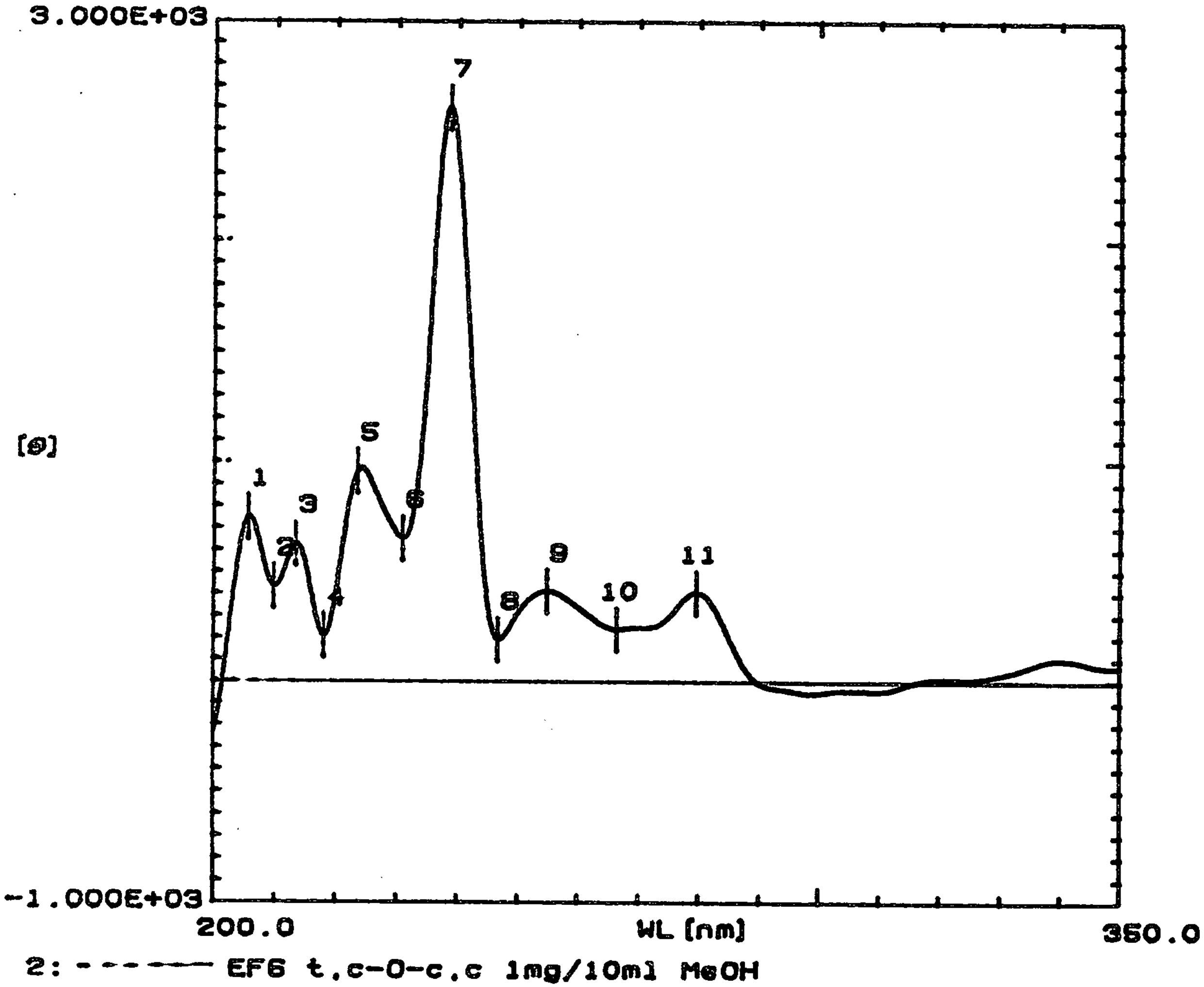


No.	Wavelength	Value
1	207.40 nm	1.664E+03
2	212.70 nm	1.010E+03
3	217.80 nm	1.451E+03
4	221.10 nm	1.308E+03
5	225.00 nm	1.654E+03
6	231.40 nm	-2.885E+02
7	235.50 nm	3.614E+02
8	238.30 nm	9.853E+01
9	245.90 nm	5.463E+03
10	252.50 nm	1.641E+03
11	285.30 nm	-6.625E+03
12	298.80 nm	-8.002E+01

Plate 6

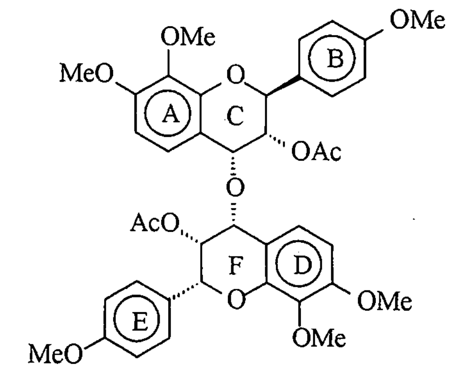


(53)

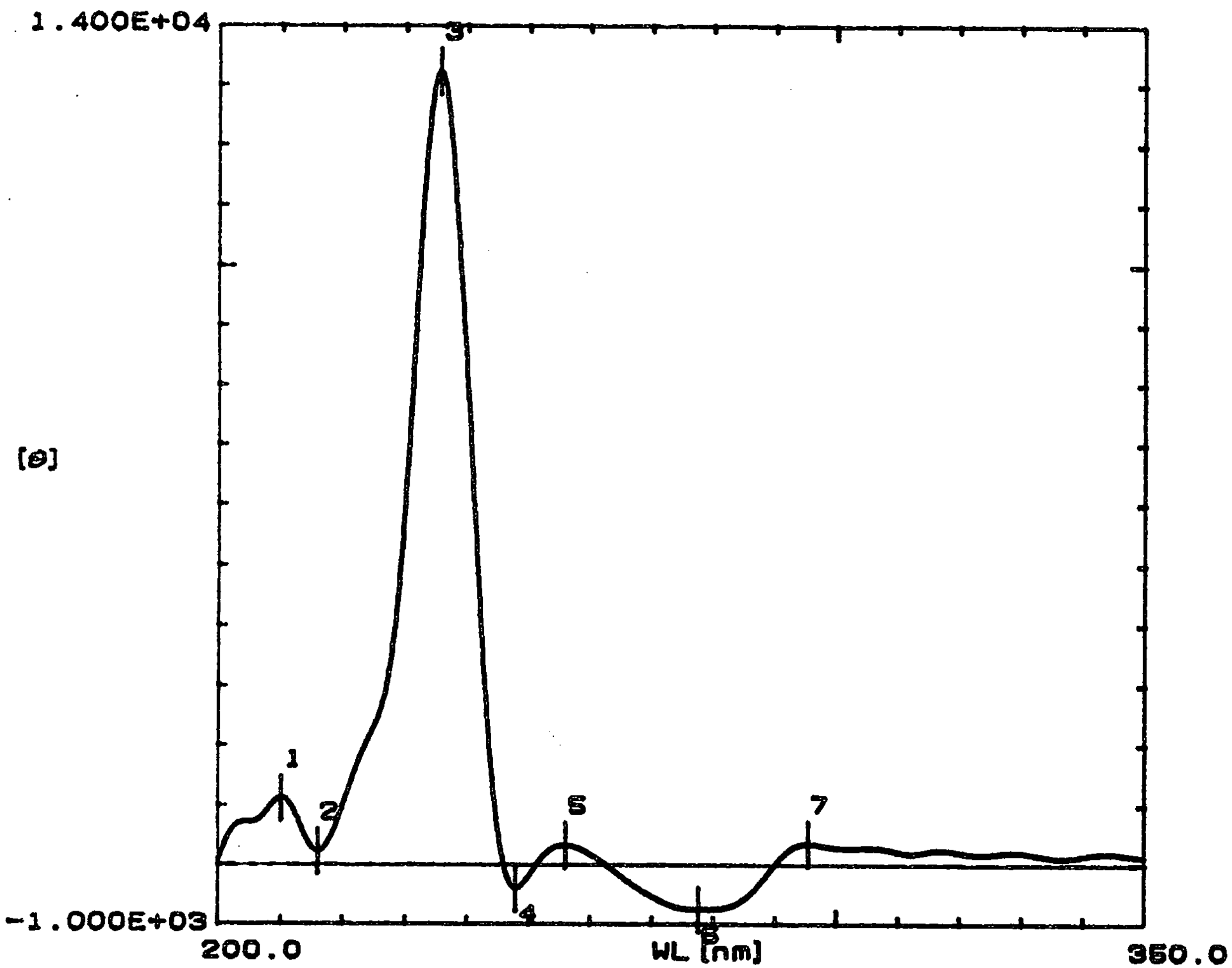


No.	Wavelength	Value
1	205.70 nm	7.507E+02
2	209.80 nm	4.334E+02
3	213.50 nm	6.266E+02
4	218.10 nm	2.083E+02
5	223.50 nm	9.580E+02
6	231.00 nm	6.523E+02
7	238.70 nm	2.603E+03
8	246.90 nm	1.903E+02
9	254.80 nm	4.124E+02
10	266.60 nm	2.377E+02
11	279.70 nm	4.070E+02

Plate 7



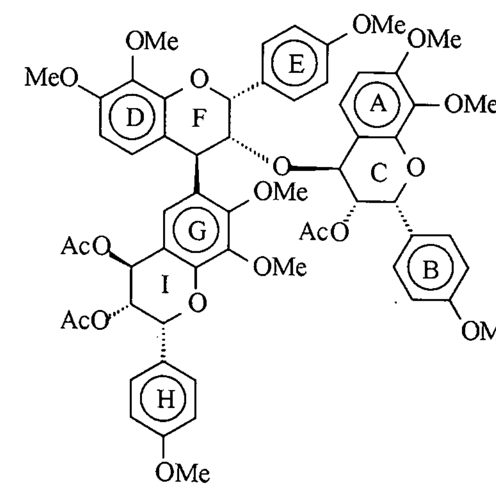
(55)



2: ——— EF7 c,t-c,t-O-c,t 1mg/10 ml MeOH

No.	Wavelength	Value
1	210.20 nm	1.125E+03
2	216.00 nm	2.334E+02
3	235.60 nm	1.323E+04
4	248.00 nm	-3.904E+02
5	256.00 nm	3.374E+02
6	277.60 nm	-7.445E+02
7	295.40 nm	3.612E+02

Plate 8



(58)

Summary

The presence of 4',7,8-trihydroxyl substituted flavanoids in plant material is limited to *Acacia galpinii* and *Acacia caffra* in Southern Africa. The existence of pyrogallol-type A-ring oligomers in these trees was for a long time believed not to be possible. During this investigation the presence of a number of dimeric and trimeric proteracacinidins in the heartwood of *Acacia hereroensis* were manifested and synthesized.

Column separations of the extract resulted in the enriched complex phenolic fractions which subsequently were derivatized and the compounds isolated as the methylether acetate derivatives. Extensive ^{1}H and ^{13}C (300 MHz) work, which entailed COSY, NOESY, HMQC and HMBC experiments were conducted to enable structure elucidations.

The monomeric leucoanthocyanidins epioritin-4α-ol, epioritin-4β-ol and oritin-4α-ol, are present in large quantities in the heartwood of A. hereroensis and were also accompanied by a variety of dimeric and trimeric proteracacinidins in lower concentrations. The two, by now well-known dimeric C-4 (C-ring) → C-6 (D-ring) linked compounds, e.g. epioritin-(4β→6)-epioritin-4α-ol and epioritin-(4β→6)-epioritin-4β-ol, were isolated.

The rare group of proteracacinidins with a C-4 (C-ring) → C-6 (D-ring) interflavanyl bond was extended with the isolation of *ent*-oritin-(4α→6)-epioritin-4α-ol, *ent*-oritin-(4β→6)-epioritin-4α-ol, epioritin-(4β→6)-oritin-4α-ol and the novel promela-/proteracacinidin dimer *viz.* epimesquitol-(4β→6)-epioritin-4α-ol. The C-4 (C-ring) → C-4 (F-ring) ether linked dimer *ent*-oritin-(4α→4)-epioritin-4α-ol was isolated, together with the unique C-C and C-O-C linked trimer epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol.

The difficult and sensitive synthetic approach during which the 4β-benzylthioether derivatives, epioritin-4β-benzylthioether and *ent*-oritin-4α-benzylthioether together with

their corresponding underivatized monomers, e.g. epioritin-4β-ol and epioritin-4α-ol as nucleophiles in the presence of DMTSF as thiophilic Lewis acid were used to synthesize *ent*-oritin-(4α→4)-epioritin-4α-ol and epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol, using different reaction conditions. The use of DMTSF and $AgBF_4$ as thiophilic Lewis acid catalysts were tested with the successful synthesis of the two dimers, epioritin-(4β→6)-epioritin-4α-ol and epioritin-(4β→6)-epioritin-4β-ol. Extensive use of these reactions were utilized to optimise the reaction conditions required during the other procedures.

Opsomming

Die natuurlike voorkoms van 4',7,8-trihidroksie gesubstitueerde flavanoïede, in die Suid Afrikaanse konteks, is beperk tot *Acacia galpinii* en *Acacia caffra*. Daar is egter getwyfel oor die bestaan van oligomere met 'n pirogallol-tipe A-ring. Tydens hierdie ondersoek is 'n verskeidenheid dimeriese en trimeriese proteracacinidiene uit *Acacia hereroensis* geïsoleer en gesintetiseer.

Kolom skeidings van die verrykte kernhout ekstrak het steeds onskeibare komplekse fenoliese mengsels gelewer en gevolglik is die metieleter asetaat derivate berei. Struktuur opklaring van die derivate is gedoen deur van massaspektrometrie en ^{1}H KMR spektrometrie (300 MHz), ^{13}C, COSY, NOESY, HMQC and HMBC eksperimente gebruik te maak.

Afgesien van die monomeriese epioritin-4α-ol, epioritin-4β-ol en oritin-4α-ol, het die kernhout van *Acacia hereroensis* ook 'n verskeidenheid dimeriese en trimeriese proteracacinidiene gelewer. Twee bekende C-4 (C-ring) → C-6 (D-ring) gebonde dimere, epioritin-(4β→6)-epioritin-4α-ol en epioritin-(4β→6)-epioritin-4β-ol, is geïsoleer.

Die unieke groep proanthosianidiene met 'n C-4 (C-ring) → C-6 (D-ring) interflavaniel binding, is uitgebrei deur die isolasie van *ent*-oritin-(4α→6)-epioritin-4α-ol, *ent*-oritin-(4β→6)-epioritin-4α-ol, epioritin-(4β→6)-oritin-4α-ol en die unieke promela-/proteracacinidien dimeer, epimesquitol-(4β→6)-epioritin-4α-ol. Die C-4 (C-ring) → C-4 (F-ring) eter gebonde dimeer *ent*-oritin-(4α→4)-epioritin-4α-ol is ook geïsoleer, asook die C-C en C-O-C gebonde trimeer, epioritin-(4β→3)-epioritin-(4β→6)-epioritin-4β-ol.

Die moeilike en delikate sintetiese benadering waartydens die 4β-bensieltioeter derivate, nl. epioritin-4β-bensieltioeter en *ent*-oritin-4α-bensieltioeter saam met die ooreenstemmende monomere, nl. epioritin-4β-ol en epioritin-4α-ol as nukleofiel en DMTSF as tiofiliese Lewissuur gebruik is, het tot die sintese van *ent*-oritin-(4α→4)-

epioritin-4α-ol en epioritin-(4$\beta\rightarrow$3)-epioritin-(4$\beta\rightarrow$6)-epioritin-4β-ol gelei. Die sukses van DMTSF en $AgBF_4$ as tiofiliese Lewissure is ook getoets, met die suksesvolle sintese van die bekende dimere, epioritin-(4$\beta\rightarrow$6)-epioritin-4α-ol en epioritin-(4$\beta\rightarrow$6)-epioritin-4β-ol, en die optimisering van reaksie kondisies.