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**EPIMESQUITOL-FLAVONE DIMERS AND RELATED
OLIGOMERS FROM *ACACIA NIGRESCENS*. SYNTHESIS OF
3',4',7,8-SUBSTITUTED FLAVONOID MONOMERS.**

Thesis submitted in fulfillment of the requirements for the degree

M.Sc.

in the

Department of Chemistry
Faculty of Natural Sciences

at the

*University of the Free State
Bloemfontein*

by

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November 2001

Universiteit van die
Oranje-Vrystaat
BLOEMFONTEIN
- 9 MAY 2002
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ACKNOWLEDGEMENTS

I hereby wish to praise and thank our heavenly Father for this opportunity and I wish to express sincere gratitude to the following people:

Prof. Malan as supervisor for all the assistance and guidance;

my family and friends for their interest and support during the preparation of this thesis;

Elize Steijn, soon becoming Elize Howell, for all your love and support during my studies;

My father Hiten Howell, my mother Anita and my brother Evan, to whom I dedicate this thesis as a token of my appreciation for a lifetime of love, guidance and motivation, without which completion of this thesis would have been impossible.



Hiten Howell

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SUMMARY

OPSOMMING

LITERATURE SURVEY

CHAPTER 1:

CHEMICAL ANALYSIS

1.1 Introduction:

Acacia nigrescens is commonly known as "Knoppiesdoring" or knobwood and indigenous to the Kruger National Park¹. It derives its vernacular name from the rough black bark, which is often covered with raised knobs terminating in sharp spines. The tree attains a height of up to 17 m and a stem diameter of 20-50 cm. Its habitat is mainly sub-tropical to tropical with a distribution covering parts of Mpumalanga, Kwazulu Natal, Swaziland, Mozambique, Botswana, Malawi and Tanzania

A.nigrescens is a deciduous tree that blooms, during August and September, before the young leaves appear. During this time the tree is covered in creamy-white flower clusters. Hooked thorns occur in pairs while the fruit are black, thin pods that are up to 7-10 cm in length and 1.3 cm wide. The leaves and pods are valuable fodder for a variety of browsers such as elephants and giraffe.

Due to *A.nigrescens* resistance to wood-decaying fungi and woodborers it is commercially used for mine roof-props, fencing posts and to make furniture.

1.2 Previous analysis:

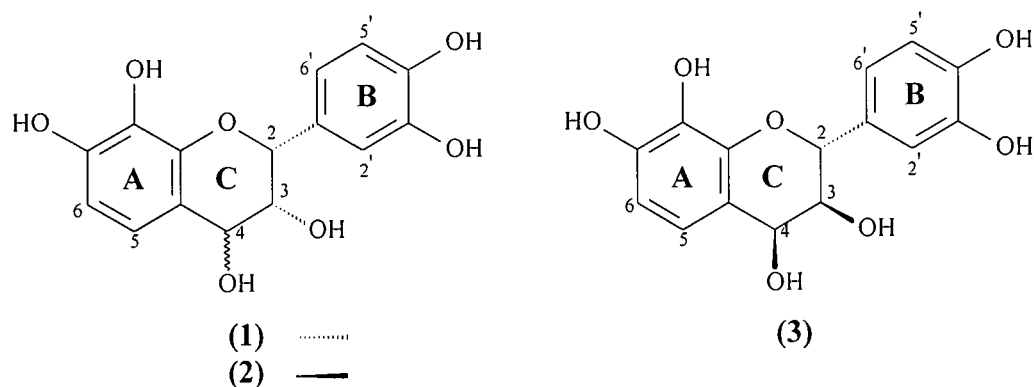
Complete analysis of the dense brown-black heartwood of *A. nigrescens* by Fourie and co-workers², showed, that with the exception of protocatechuic acid, all compounds were 3',4',7,8-tetrahydroxyflavonoids and are similar to those found in a number of Australian *Acacia spp.*³

¹ L. E. D. Codd, *Trees and Shrubs of the Kruger National Park*, Department of Agriculture Botanical Survey, Memoir No.26, Government Printer, Pretoria, 1951, 47.

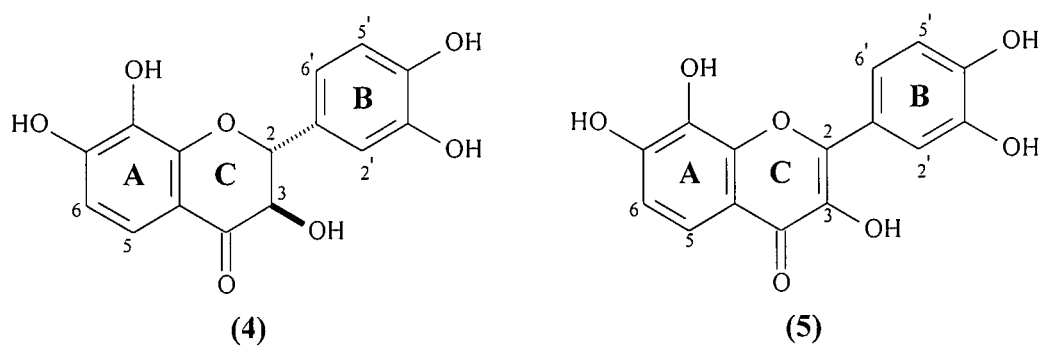
² T. G. Fourie, I. C. du Preez and D. G. Roux, *Phytochemistry*, 1972, 11, 1763.

³ M. D. Tindale and D. G. Roux, *Phytochemistry*, 1969, 8, 1713.

The flavonoids found are classified into flavan-3,4-diols with three stereogenic centra represented by the diastereomers 2,3-*cis*-3,4-*cis*(**1**), 2,3-*cis*-3,4-*trans*(**2**) and 2,3-*trans*-3,4-*cis*-flavan-3,4-diols(**3**). Compounds (**1**) and (**2**) were also isolated from *Acacia caffra* by Malan and Sireeparsad⁴.

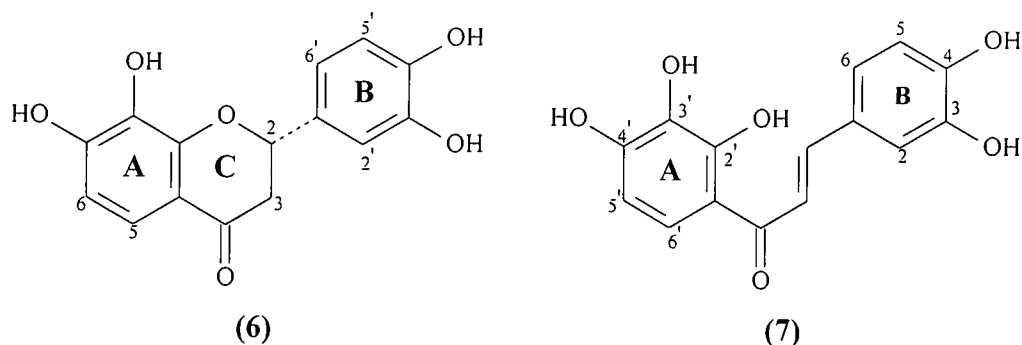


A dihydroflavonol(**4**) was found and has two asymmetric carbons at C-2 and C-3, thus four diastereomers are possible. Dehydrogenation of the dihydroflavonol yields a flavonol(**5**) and by reduction of the carbonyl functional group it affords flavan-3,4-diols.



The flavonol(**5**), was isolated and represents a flavone with a hydroxyl group on the 3-position.

The flavanone or a 2-phenyl-benzopyran-4-one(6) was also present. Flavanones are isomeric with chalcones from which they can be synthetically obtained and from which they arise during the biogenetic process in plants. Flavanones have a stereogenic centre at C-2, thus two enantiomers are possible.



The chalcone is represented by structure(7) and is an open chain flavonoid. The A-ring substitution pattern comprises a pyrogallol system (2', 3', 4' – trihydroxy), which is responsible for the variation of dimeric melacacinidins, as will be discussed later. Numbering of the positions of substitution in the chalcone nucleus is different from that of other flavonoids.

Flavan-3-ols are important chain extender- and chain terminating units of oligomeric proanthocyanidins, which prompted a thorough review^{5,6,7,8,9} of derivatives, chemistry and general properties of naturally occurring flavan-3-ols over the years. Studies on the

⁴ E. Malan and A. Sireeparsad, *Phytochemistry*, 1995, 38, 237.

⁵ K. Freudenberg and K. Weinges, *The Chemistry of flavonoid Compounds*, ed. T.A. Geissman, Pergamon Press, Oxford, 1962, 197.

⁶ K. Weinges, W. Bähr, W. Ebert, K. Görts and H. D. Marx, *Fortschr. Chem. Org. Naturst.*, 1969, 27, 158.

⁷ D. Ferreira and R. Bekker, *Nat. Prod. Rep.*, 1996, 13, 411.

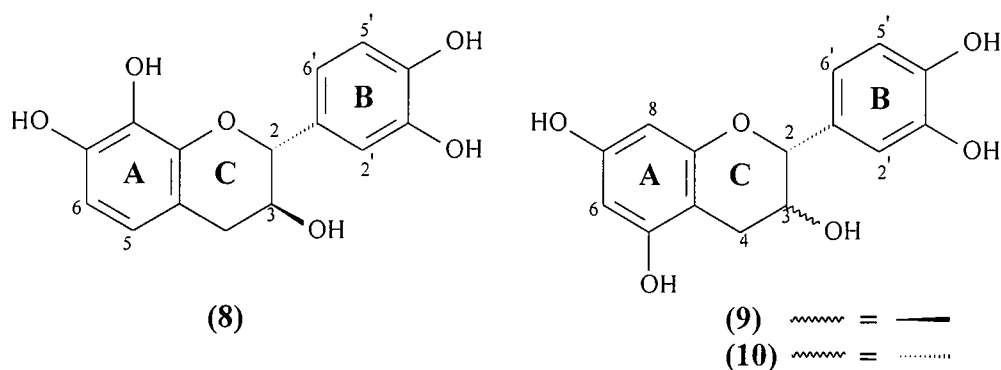
⁸ R. W Hemingway, *Natural Products of Woody Plants 1*, ed. J. W. Rowe, Springer-Verlag, New York, 1989, 571.

⁹ L. J. Porter, *The Flavonoids - Advances in Research since 1986*, ed. J. B. Harborne, Chapman and Hall, London, 1994, p. 23.

biogenetic process in plants suggested that the flavan-3-ols are biosynthesized from flavan-3,4-diols¹⁰.

Fourie noted the absence of the 3',4',7,8-tetrahydroxyflavan-3-ol (mesquitol) and related analogues in *A. nigrescens*. Later it was found that the 2,3-*trans*-flavan-3-ol(**8**) and the 2,3-*trans*-3,4-*cis*-flavan-3,4-diol(**3**) co-exists in *Prosopis glandulosa*¹¹.

Catechin (**9**) and epicatechin (**10**) are two structures representative of flavan-3-ols and they constitute the predominant chain-terminating units of oligomeric proanthocyanidins found in plants¹².



¹⁰ E. Haslam, *Flavanoids*, Ed. J. B. Harborne, T. J. Mabry and H. Mabry, Chapman and Hall, New York, 1975, 551.

¹¹ L. Y. Foo, *J. Chem. Soc., Chem. Commun.*, 1986, 236.

¹² L. J. Porter, *The Flavonoids - Advances in Research since 1986*, ed. J. B. Harborne, Chapman and Hall, London, 1994, 27.

CHAPTER 2: LEUCOANTHOCYANIDINS

2.1 Introduction:

In the 1920's Rosenheim¹³ examined anthocyanin pigments of the young grape vine *Vitis vinifera* and proposed the term leucoanthocyanin for a colourless modification of the pigment, which is convertible into anthocyanidin by HCl. The first definitive structural work was done in the 1950's by King and Bottomley¹⁴ who isolated melacacidin(1) from Australian blackwood, *Acacia melanoxylon*. They determined its structure to be a tetrahydroxyflavan-3,4-diol. They revised the nomenclature and classified the compound as a leucoanthocyanidin, but according to Swain^{15,16} without adequate proof. In the 1960's Freudenberg and Weinges¹⁷ collectively designated all the colourless substances isolated from plants, which form anthocyanidins(11) when heated with acid as proanthocyanidins (**scheme 2.1.**).

The name proanthocyanidin is a chemical and not a biological term and it does not imply any biogenetic relationship. Weinges et al⁶ reserved the term leucoanthocyanidin for the monomeric proanthocyanidins such as the flavan-3,4 -diols and the name condensed proanthocyanidins for the various flavan-3-ol dimers and higher oligomers.

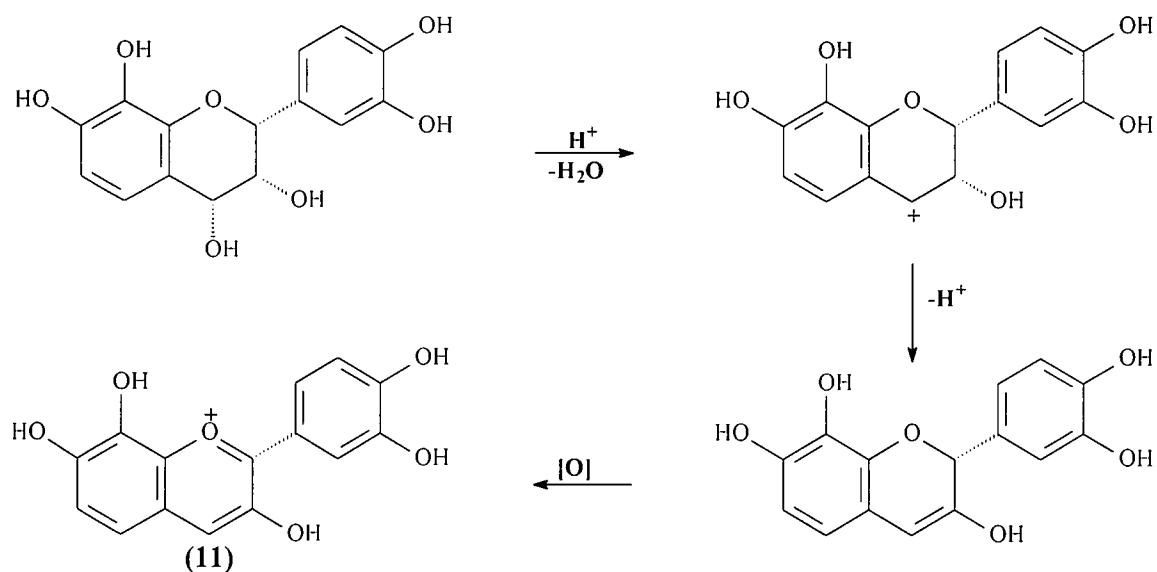
¹³ O. Rosenheim, *Biochemical J.*, 1920, 14, 278.

¹⁴ F. E. King and W. Bottomley, *J. Chem. Soc.*, 1954, 1399.

¹⁵ T. Swain, *The Chemistry of Flavonoid Compounds*, ed. T. A. Geissman, Pergamon Press, Oxford, 1962, 513.

¹⁶ J. L. Goldstein and T. Swain, *Phytochemistry*, 1963, 2, 371.

¹⁷ K. Freudenberg and K. Weinges, *Tetrahedron*, 1960, 8, 336.



Scheme 2.1

Table 1 contains a list of predominant flavan-3-ol chain-extender units with their hydroxylation patterns.

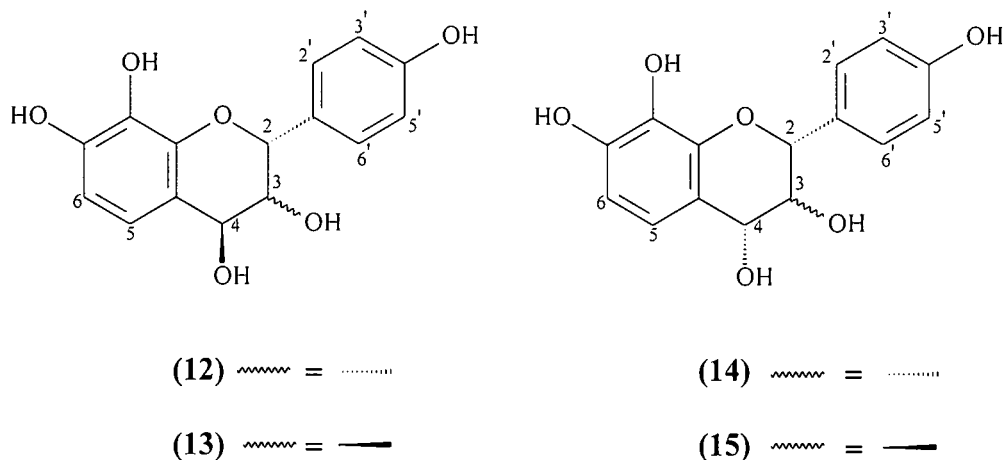
Table 1:

Unit	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'

2.2 A-ring with a 7,8 – hydroxylation pattern:

A. galpinii represents the first South African plant source known to contain flavonoid analogues with the 7,8,4'-trihydroxyphenolic substitution pattern. The formerly named

(-)-7,8,4'-trihydroxy-2,3-*cis*-flavan-3,4-*cis*-diol(**14**) [(-)-teracacidin] predominates and is accompanied by three diastereoisomers, (-)-2,3-*cis*-3,4-*trans*(**12**), (+)-2,3-*trans*-3,4-*cis*(**13**) and (+)-2,3-*trans*-3,4-*trans*(**15**). Small quantities of (-)-7,8,3',4'-tetrahydroxy-2,3-*cis*-flavan-3,4-*cis*-diol(**1**) [(-)-melacacidin] was also found¹⁸.



In contrast to the strong nucleophilic sites in resorcinol and phloroglucinol A-ring containing flavonoids, the 7,8-dihydroxy substituted pyrogallol type A-ring leads to a general distribution of electron density across the unsubstituted 5- and 6-positions. The additional hydroxyl function at C-8 presumably counteracts electron release from the 7-hydroxyl group, thus reducing the tendency of flavan-3,4-diols(**1**), (**12**), (**13**), (**14**) and (**15**) to form C-4 carbocations or A-ring quinone methides, which are essential for initiating condensation^{2,18} reactions. This implies that structures of type (**1**), (**12**), (**13**), (**14**) and (**15**) are poorer nucleophiles and permits other centers to participate in the formation of interflavanyl bonds. For the same reason the 4-carbonium ions, which could presumably also originate from them, will be less adequately stabilized by delocalization of the charge.

The B-ring contributes towards stabilizing the C-4 carbocation via an A-conformation, which will be discussed at a later stage, as well as the fact that the stereochemistry at C-3 and C-4 also influences the reactivity of flavan-3,4-diols as incipient electrophiles.

¹⁸ E. Malan, D. G. Roux, *Phytochemistry*, 14, 1975, 1835.

CHAPTER 3:

PROANTHOCYANIDIN DIMERS

3.1 Introduction:

Proanthocyanidins are found in fairly high concentrations in the bark and heartwood of various tree species, this has resulted in their commercial extraction to be used primarily in the leather industry¹⁹. Proanthocyanidins also play a role in the protection of plants from microorganisms and insects^{20,21}.

3.2 Proteracacinidins:

Until the isolation of proteracacinidin dimers from the heartwood of *A. galpinii*⁴ the occurrence of condensed tannins with a pyrogallol A-ring was limited to the heartwoods of *Prosopis glandulosa*^{22,23} and *A. melanoxylon*²⁴. This is a good reason why note should be taken of the work done on proteracacinidins.

The first dimeric proteracacinidins were isolated from *A. galpinii*²⁵ and *A. caffra*²⁶ and comprises of ent-oritin-(4 β \rightarrow 5)-epioritin-4 β -ol(**16**), epioritin-(4 β \rightarrow 6)-epioritin-4 α -ol(**17**), epioritin-(4 β \rightarrow 6)epioritin-4 β -ol(**18**) and the doubly-linked epioritin-(4 β \rightarrow 7,5 \rightarrow 6)-epioritin-4 α -ol(**19**).

¹⁹ D. G. Roux and D. Ferreira, *Pure and Appl. Chem.*, 1982, 54, 2465.

²⁰ J. A. Klocke and B. C. J. Chan, *J. Insect Physiol.*, 1982, 28, 911.

²¹ W. V. Zucker, *Am. Nat.*, 1983, 121, 335.

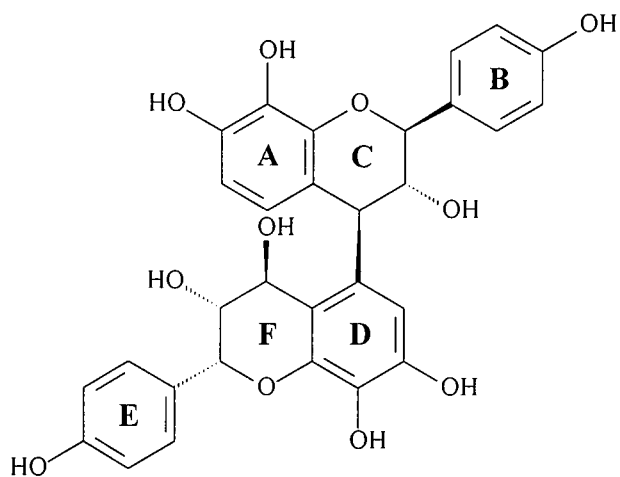
²² E. Jacobs, D. Ferreira and D. G. Roux, *Tetrahedron Lett.*, 1983, 24, 4627.

²³ E. Young, E. V. Brandt, D. A. Young, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1986, 1737.

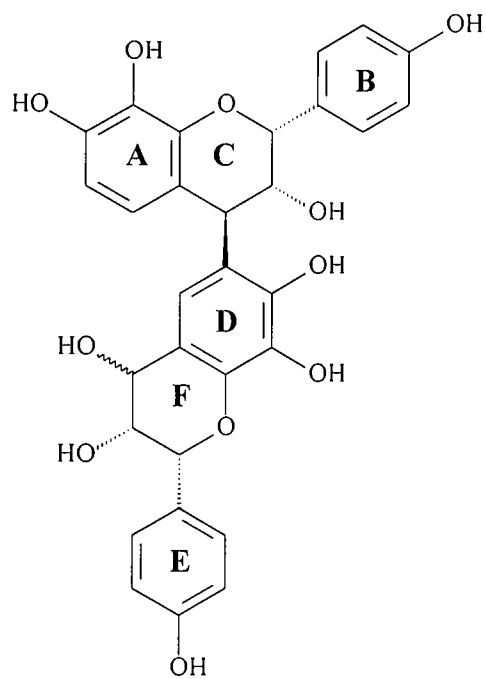
²⁴ E. Malan, and D. G. Roux, *Phytochemistry*, 1975, 14, 1835.

²⁵ E. Malan, *Phytochemistry*, 1995, 40, 1519.

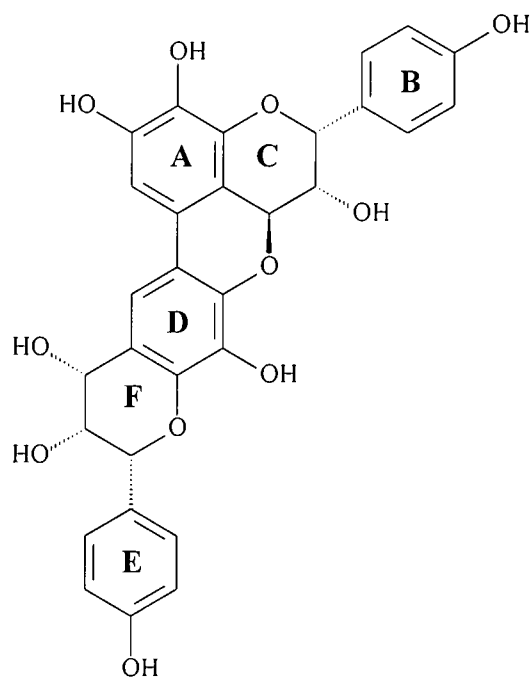
²⁶ E. Malan, A. Sireeparsad, J. F. W. Burger and D. Ferreira, *Tetrahedron Lett.*, 1994, 35, 7415.



(16)



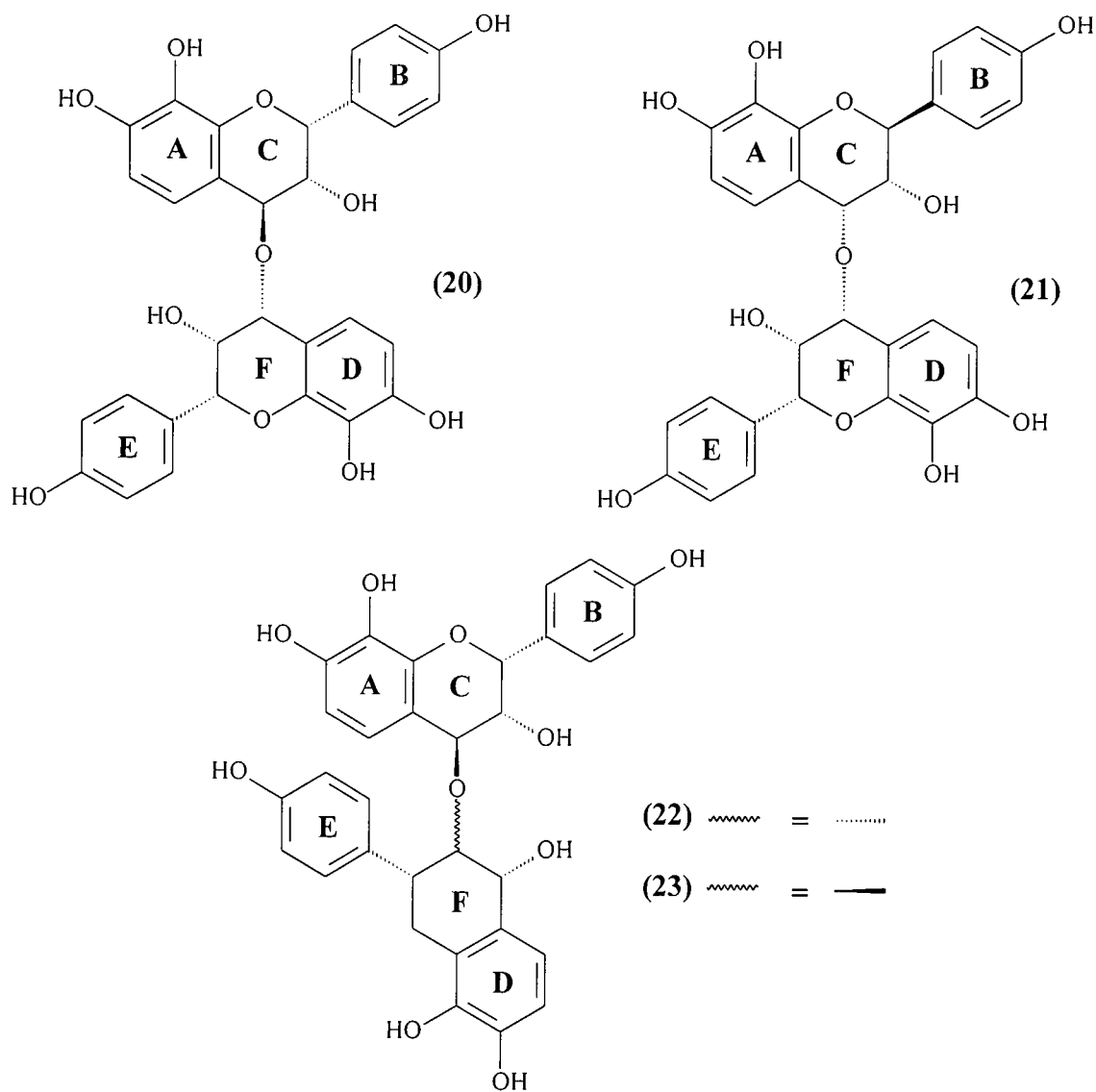
(17) ~~~~~ =
 (18) ~~~~~ = —



(19)

Except for the A-type proanthocyanidins, which contain an ether linkage between the C- and D-rings, ether-linked compounds were limited to the double ether-linked dioxane-

type dimers which were found in *Acacia mearnsii*^{27,28}. Proanthocyanidins possessing a single ether-type interflavanyl linkage are extremely rare. Foo²⁹ was the first to isolate two ether-linked dimers from *A. melanoxylon*. Ten years later Coetzee^{30,31} isolated two (C₄-O-C₄)-linked compounds (20) and (21), as well as the first two (C₄-O-C₃)-linked compounds (22) and (23) from *A. galpinii*.



²⁷ S. E. Drewes and A. H. Ilsley, *J. Chem. Soc. (C)*, 1969, 897.

²⁸ D. A. Young, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2031.

²⁹ L. Y. Foo, *J. Chem. Soc., Chem Commun.*, 1989, 1505.

³⁰ J. Coetzee, E. Malan and D. Ferreira, *J. Chem. Res.*, 1998, (S) 526, (M) 2287.

³¹ J. Coetzee, E. Malan and D. Ferreira, *Tetrahedron*, 1998, 54, 9153.

The co-occurrence of the ether-linked proteracacinidins and some carbon-carbon bonded analogues in the heartwood of *A. galpinii* is a further manifestation of the much reduced nucleophilicity of the pyrogallol A-ring which permits other centers to participate in the formation of interflavanyl bonds.

3.3 Promelacacinidins:

The flavan-3,4-diols, melacacidin (**1**) and teracacidin (**14**) are present in a large number of *Acacia species*^{3,32} but their corresponding proanthocyanidin oligomers are less common, as mentioned in the previous chapter.

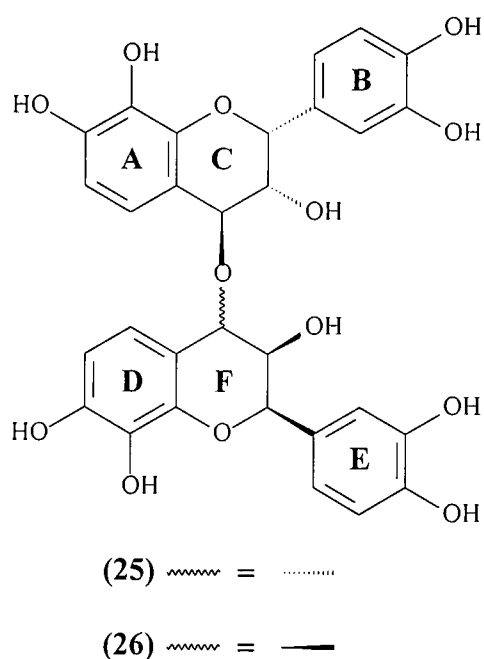
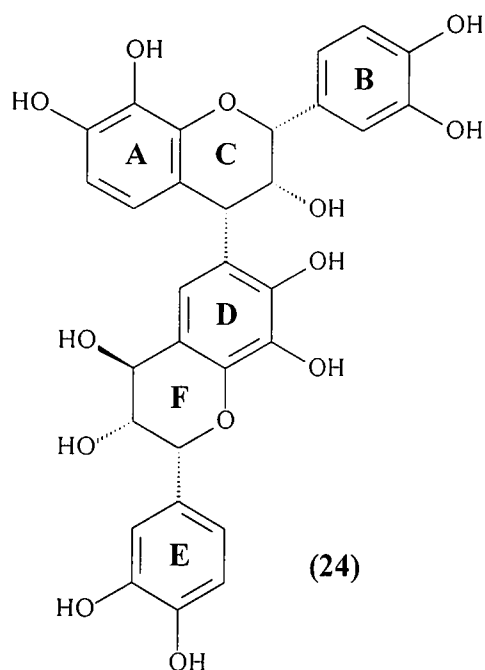
This apparent scarcity was attributed² to the presence of a C-8 hydroxyl functionality, which inhibited electron donation from the C-7 hydroxyl group. Because of this, these flavan-3,4-diols do not easily form C-4 carbocations or A-ring quinone methides, which are required to initiate condensation. Later studies^{33,34,35} showed that flavan-3,4-diols indeed undergo condensation in an acidic medium with phenolic nuclei such as resorcinol and phloroglucinol to give 4-arylflavan-3-ols and therefore suggested the possible formation of proanthocyanidins with a 7,8-dihydroxylated flavanoid pattern. These results were confirmed by the isolation of dimer(**24**) from *A. melanoxylon* by Foo¹¹ and as mentioned the unique ether-linked dimers (**25**) and (**26**) from the same source²⁹.

³² J. W. Clark-Lewis and L. J. Porter, *Aust. J. Chem.*, 1972, 25, 1943.

³³ J. J. Botha, D. Ferreira and D. G. Roux, *J. Chem. Soc., Chem Commun.*, 1978, 698.

³⁴ J. J. Botha, D. A. Young, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1235.

³⁵ L. Y. Foo, *J. Chem. Soc., Chem Commun.*, 1985, 1273.

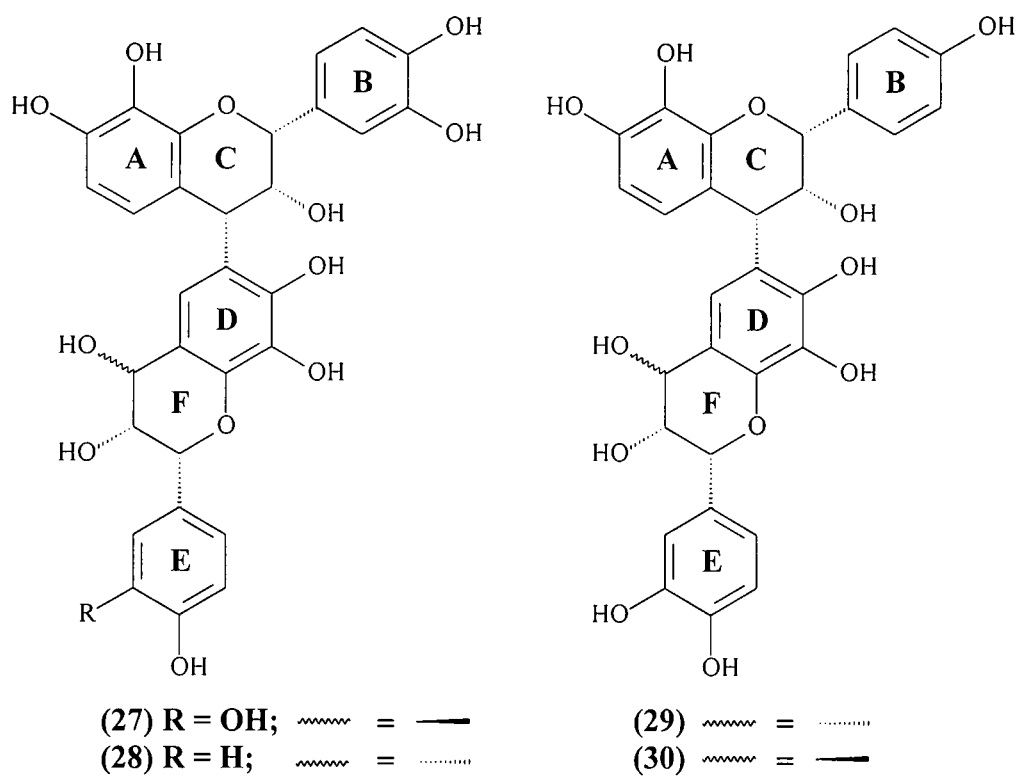


The natural occurrence of these promelacacinidins clearly demonstrated that the pyrogallol A-ring functionality is sufficiently reactive for nucleophilic condensation and that the pyrogallol A-ring functionality can facilitate C-4 carbocation formation from an associated flavan-3,4-diol.

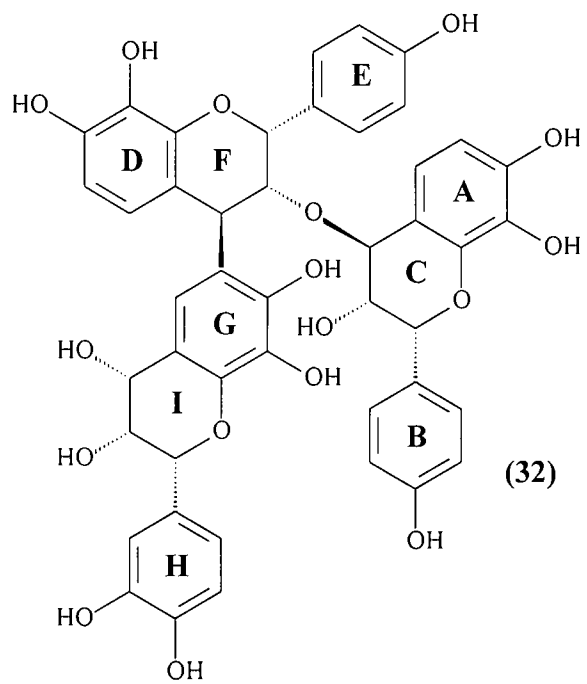
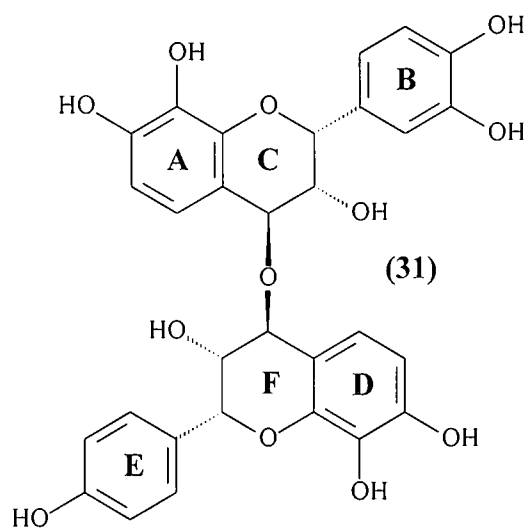
Bennie further confirmed the above facts by isolating the following dimers from *A. caffra*^{36,37}. The C-4(C-ring) → C-6(D-ring) linked promelacacinidin, epimesquitol-(4β → 6)-epimesquitol-4β-ol(**27**) and proanthocyanidins consisting of differently substituted units, epimesquitol-(4β → 6)-epioritin-4α-ol(**28**), epioritin-(4β → 6)-epimesquitol-4α-ol(**29**), epioritin-(4β → 6)-epimesquitol-4β-ol(**30**).

³⁶ L. Bennie, E. Malan, J. Coetzee and D. Ferreira, *Phytochemistry*, 2000, 53, 785.

³⁷ L. Bennie, E. Malan, J. Coetzee and D. Ferreira, *Phytochemistry*, 2001, 57, 1023



The following [C₄-O-C₄] ether-linked dimer consisting of a mesquitol and oritin unit, epimesquitol-(4β → 4)-epioritin-4β-ol(**31**) as well as the trimeric proanthocyanidin, comprising of C-C and C-O-C-bonds, epioritin-(4β → 3)-epioritin-(4β → 6)-epimesquitol-4α-ol(**32**) was also isolated by Bennie³⁷.

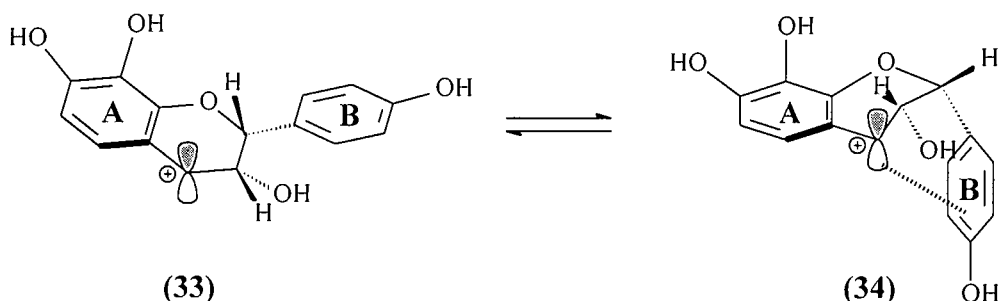


CHAPTER 4: SYNTHETIC METHODS

4.1 Flavan-3,4-diols and flavan-4-thioethers as potential electrophiles:

Flavan-3,4-diols serve as a source of chain extender units in the semi-synthetic approach to oligomers, via their C-4 carbocations, e.g. (33)

The stability of C-4 carbocations is dependent on the degree of delocalization of the positive charge over the A-ring³⁹. It can be predicted by common chemical concepts that this delocalization will be the most effective for C-4 carbocations derived from flavan-3,4-diols with a phloroglucinol-type A-ring, less effective for resorcinol-type of compounds and the least effective for pyrogallol-type melacacidins(1) and teracacidins(14) .



The ability of the B-ring to contribute towards the stabilization of the C-4 carbocation, was first suggested by Brown⁴⁰ and later observed by Ferreira and co-workers^{41,42,43,44,45} . The B-ring stabilizes the C-4 carbocation, e.g. (33), via an A-conformation (34). The A-conformation

³⁹ D. Ferreira, J.P. Steynberg, D.G. Roux and E.V. Brandt, *Tetrahedron*, 1992, 48, 1743.

⁴⁰ B.R. Brown and M.R. Shaw, *J. Chem. Soc., Perkin Trans. 1*, 1974, 2036.

⁴¹ 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; *J. Chem. Soc., Perkin Trans. 1*, 1988, 3323, 3331.

⁴² J.A. Steenkamp, J.C.S. Malan and D. Ferreira, *J. Chem. Soc., Perkin Trans. 1*, 1988, 2179.

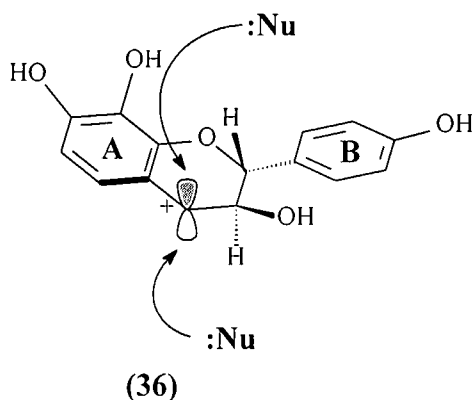
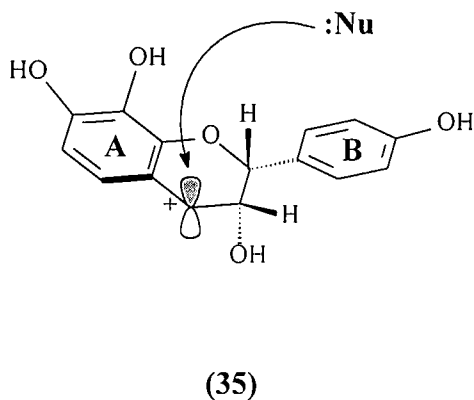
⁴³ J.P. Steynberg, J.F.W. Burger, D.A. Young, E.V. Brandt and D. Ferreira, *Heterocycles*, 1989, 28, 923.

⁴⁴ J.P. Steynberg, E.V. Brandt and D. Ferreira, *J. Chem. Soc., Perkin Trans. 2*, 1991, 1569.

⁴⁵ J.P. Steynberg, E.V. Brandt, D. Ferreira, C.A. Helfer, W.L. Mattice, D. Gornik and R.W. Hemingway, *Magn. Reson. Chem.*, 1995, 33, 611.

represents a half-chair/sofa conformation for the pyran ring, where the 2-aryl group occupies an axial position(34) , in contrast with the conventional equatorial orientation as in structure(33).

Assuming that the carbocation intermediate possesses a sofa conformation, nucleophilic attack on the ion with a (2R,3R)-2,3-*cis*-configuration(35) proceeds from the less hindered "upper" side, presumably with neighbouring group participation of the 3-axial hydroxyl in an E-conformation and by the 2-axial B-ring in an A-conformation. Reaction with a 2,3-*trans* carbocation(36) is directed preferentially from the less hindered "lower" side, i.e. the reaction proceeds with a moderate degree of stereoselectivity³⁹.



The stereochemistry at C-3 and C-4 also influences the reactivity of flavan-3,4-diols as incipient electrophiles. 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 reactions⁴⁶. Such differences are explicable 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^{46,47,48}. Axial C-3 hydroxyl groups may further stabilize C-4 carbocations by formation of a protonated epoxide intermediate⁴⁶ (structure (40) scheme 4.2).

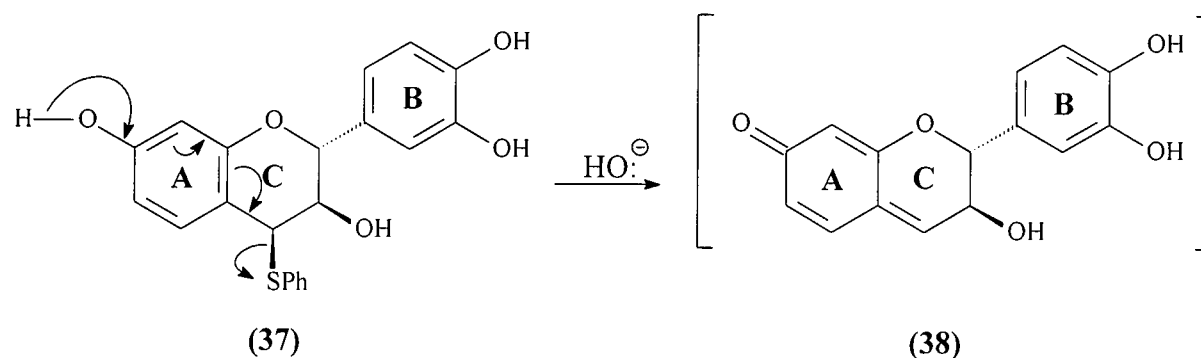
The inductive effect of the 4-hydroxyl function of flavan-3,4-diols or of the C-4 carbocation resulting from its protonation, reduces the nucleophilicity of the aromatic A-ring and thus reduces

⁴⁶ J. W. Clark-Lewis and P. I. Mortimer, *J. Chem. Soc.*, 1960, 4106.

⁴⁷ E. Haslam, *The Flavonoids - Advances in Research*, ed. J.B. Harborne and T.J. Mabry, Chapman and Hall, London, 1982, p. 417.

⁴⁸ L. Y. Foo and H. Wong, *Phytochemistry*, 1986, 25, 1961.

the tendency for self-condensation. Hemingway and Foo^{49,50} overcame this problem, by first synthesizing the flavan-4-thioether e.g. (37) and then adding the flavan-3-ol as a nucleophile. The thio-ether (scheme 4.1) presumably serves as a precursor to an A-ring quinone-methide(38), which is then trapped via interaction with the phenolic A-ring of the added flavan-3-ol.



Scheme 4.1

4.2 Biomimetic synthesis:

4.2.1 Introduction:

Biomimetic syntheses are used to confirm the structures of novel oligomers, by synthesizing the oligomer from the corresponding biogenetic monomeric precursors. Ferreira and co-workers^{7,39,51} extensively reviewed acid-catalyzed reactions, which produce flavan-4-carbocations or A-ring quinone-methides, from flavan-3,4 diols.

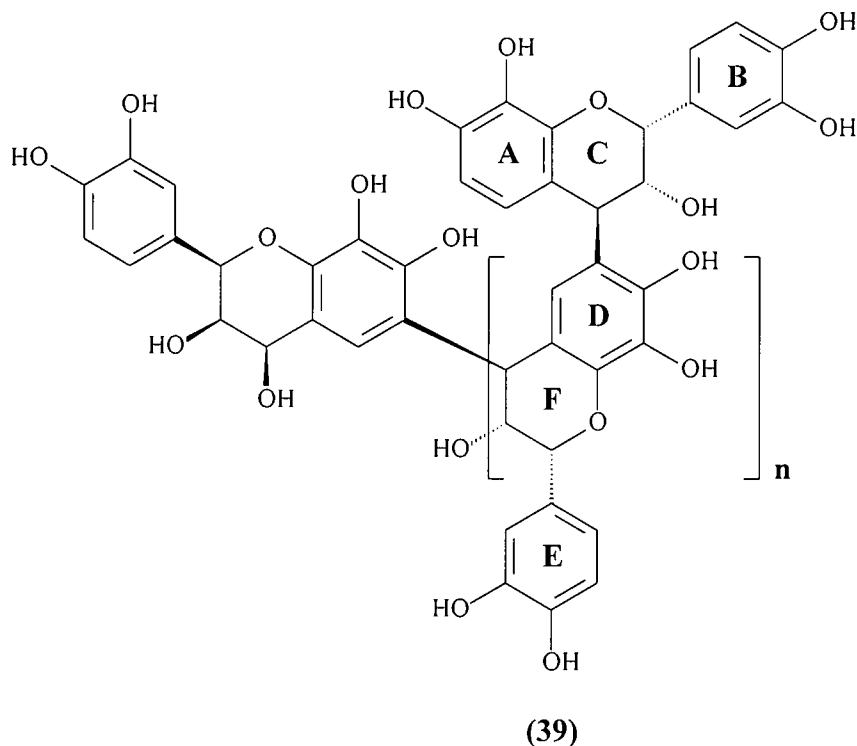
⁴⁹ R. W. Hemingway and L. Y. Foo, *J. Chem. Soc., Chem. Commun.*, 1983, 1035.

⁵⁰ R. W. Hemingway and L. Y. Foo, *J. Chem. Soc., Chem. Commun.*, 1984, 85.

⁵¹ 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.

4.2.2 Acid catalyzed condensation reactions:

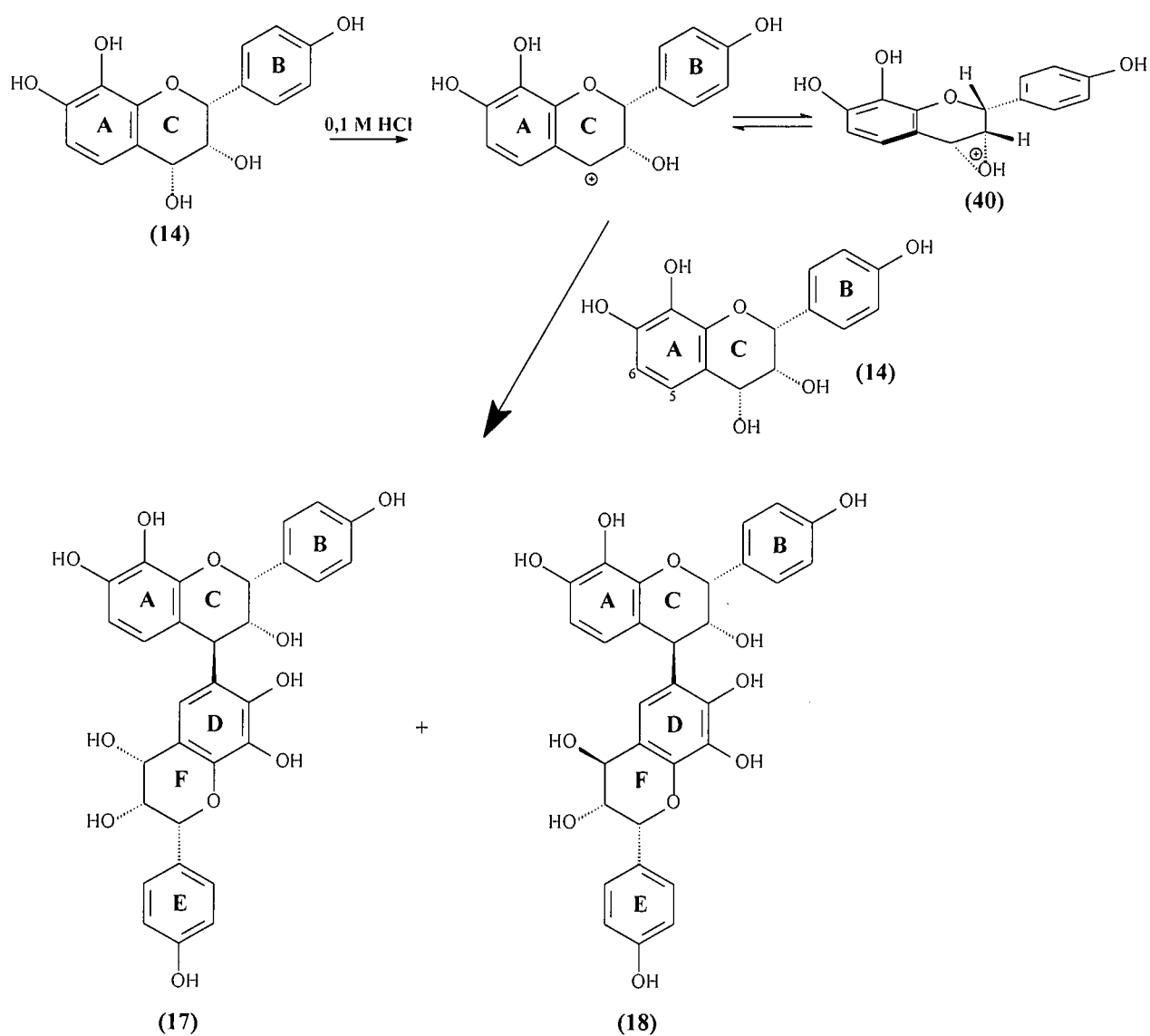
Foo⁵² demonstrated the reactivity of the pyrogallol A-ring with the successful acid catalyzed self-condensation^{52,53} reaction of melacacidin to synthesize compound(39). Acid catalyzed self condensation of the flavan-3,4-diol, epioritin-4 α -ol(14), confirmed the structures of the teracacinidin dimers (17) and (18) which occur in the heartwood of *A. galpinii*⁴.



The flavan-3,4-diols are converted to an intermediate C-4 carbocation under mild acidic conditions (Scheme 4.2). The unprotonated epioritin-4 α -ol then acts as the nucleophile and couples via the C-6 position to the carbocation to stereoselectively form the 4 β -dimers (17) and (18).

⁵² J. J. Botha, D. Ferreira and D. G. Roux, J. Chem. Soc., Chem. Commun., 1978, 700.

⁵³ J. J. Botha, D. Ferreira and D. G. Roux, J. Chem. Soc., Chem. Commun., 1979, 510.



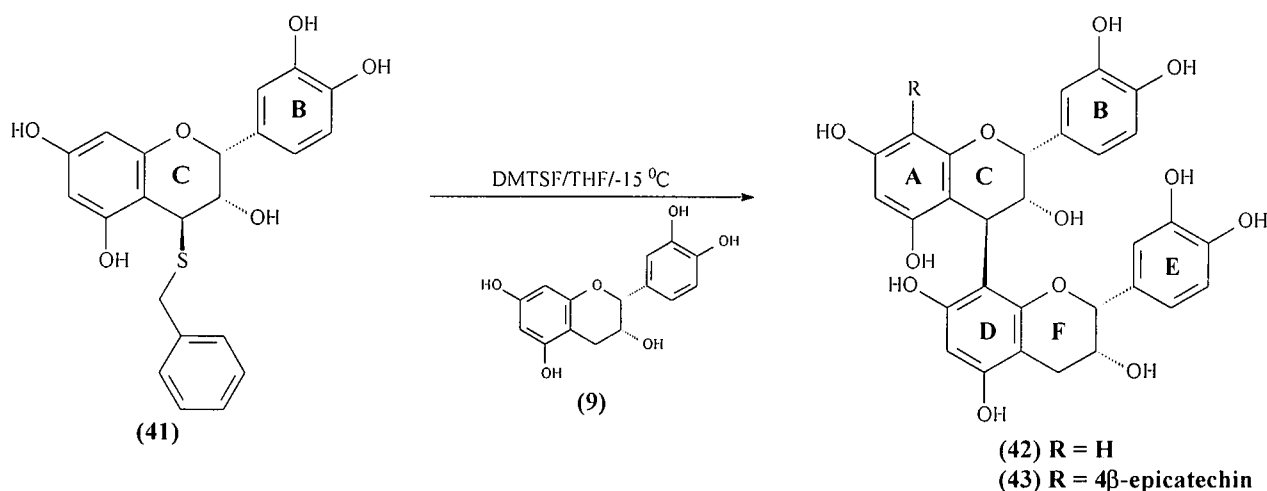
Scheme 4.2

4.2.3 C-4 Thiobenzylethers as electrophiles:

Hemingway and Foo^{49,50} used a different semi-synthetic approach towards the synthesis of oligomeric proanthocyanidins by utilizing 4-thiobenzylethers as incipient electrophiles under mild basic conditions (pH 9). Proanthocyanidin synthesis via the quinone-methide route has yielded

dimers and related derivatives, more cleanly and efficiently than the acid-catalyzed condensation reactions⁴⁹. In this approach an A-ring quinone-methide, formed from the 4-thiobenzylethers (**scheme 4.1**), is trapped by the appropriate nucleophile.

The existing semi-synthetic methods involve coupling of the electrophilic C-4 substituted flavan-3-ols under either acidic or basic conditions^{33,49,50,54}. Under these conditions, the interflavanyl bonds are labile, which invariably leads to equilibrium between substrates and products⁵⁵. The effectiveness of the thiophilic Lewis acids, dimethyl (methylthio) – sulfonium tetrafluoroborate (DMTSF) and silver tetrafluoroborate (AgBF₄), to activate the C₄-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^{39,51}.



Scheme 4.3

A mixture consisting of epicatechin-4β-thiobenzylether(**41**), a 10 molar excess catechin (**9**) as nucleophile and DMTSF (1.1 equivalents) in THF, gave procyanidin B1 (**42**), as well as the analogous trimeric procyanidin(**43**) (**scheme 4.3**). This protocol compares favourably with the classical acid-catalyzed condensation of catechin-4α-ol and catechin, which gave a mixture of procyanidins and trimeric compounds^{33,49,54}.

⁵⁴ J. A. Delcour, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1711.

⁵⁵ P. J. Steynberg, R. J. J. Nel, H. van Rensburg, B. C. B. Bezuidenhout and D. Ferreira, *Tetrahedron*, 1998, 54, 8153.

CHAPTER 5:

SYNTHESIS AND REACTIONS OF FLAV-3-EN-3-OLS AS KEY INTERMEDIATES

5.1 Introduction:

The absence of compelling evidence regarding the biosynthetic pathways to flavan-3-ols, their proanthocyanidins and the key intermediate that could unequivocally explain the formation of these compounds, has evoked much speculation^{56,57,58,59,60,61,62,63}. A logical reductive sequence from the (+)-2,3-*trans*-dihydroflavonol to the flavan-3,4-diol and flavan-3-ol level for the 2,3-*trans* series of both the procyanidins (3,5,7,3',4'-pentahydroxylation) and prodelphinidins (3,5,7,3',4',5'-hexahydroxylation) has been demonstrated in vitro and in vivo^{64,65,66,67}. The latter enzymological studies have shifted the focus from the α -hydroxychalcone^{57,58} and flav-3-en-3-ol^{60-62,68} hypotheses to the stereospecific C-3 hydroxylation of flavanones and hence the intermediacy of 2,3-*trans*- and 2,3-*cis*-dihydroflavonols. This may explain why the synthesis^{63,69} and synthetic potential⁶⁷ of the flav-3-en-3-ols were not extensively explored.

⁵⁶ A. J. Birch, *Chemical Plant Taxonomy*, T. Swain, Ed., Academic Press, London, 1963, 148.

⁵⁷ J. W. Clark-Lewis, D. C. Skingle, *Aust. J. Chem.*, 1967, 20, 2169.

⁵⁸ D. G. Roux and D. Ferreira, *Phytochemistry*, 1974, 13, 2039.

⁵⁹ D. Jacques and E. Haslam, *J. Chem. Soc., Chem. Commun.*, 1974, 231.

⁶⁰ D. Jacques, C. T. Opie, L. J. Porter, E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, 1977, 1637.

⁶¹ E. Haslam, *Phytochemistry*, 1977, 16, 1625.

⁶² R. V. Platt, C. T. Opie, E. Haslam, *Phytochemistry*, 1984, 23, 2211.

⁶³ A. Zanarotti, *Tetrahedron Lett.*, 1982, 23, 3963.

⁶⁴ H. A. Stafford, H. H. Lester, *Plant Physiol.*, 1981, 68, 1035.

⁶⁵ H. A. Stafford, *Phytochemistry*, 1983, 22, 2643.

⁶⁶ H. A. Stafford, H. H. Lester, *Plant Physiol.*, 1985, 78, 791.

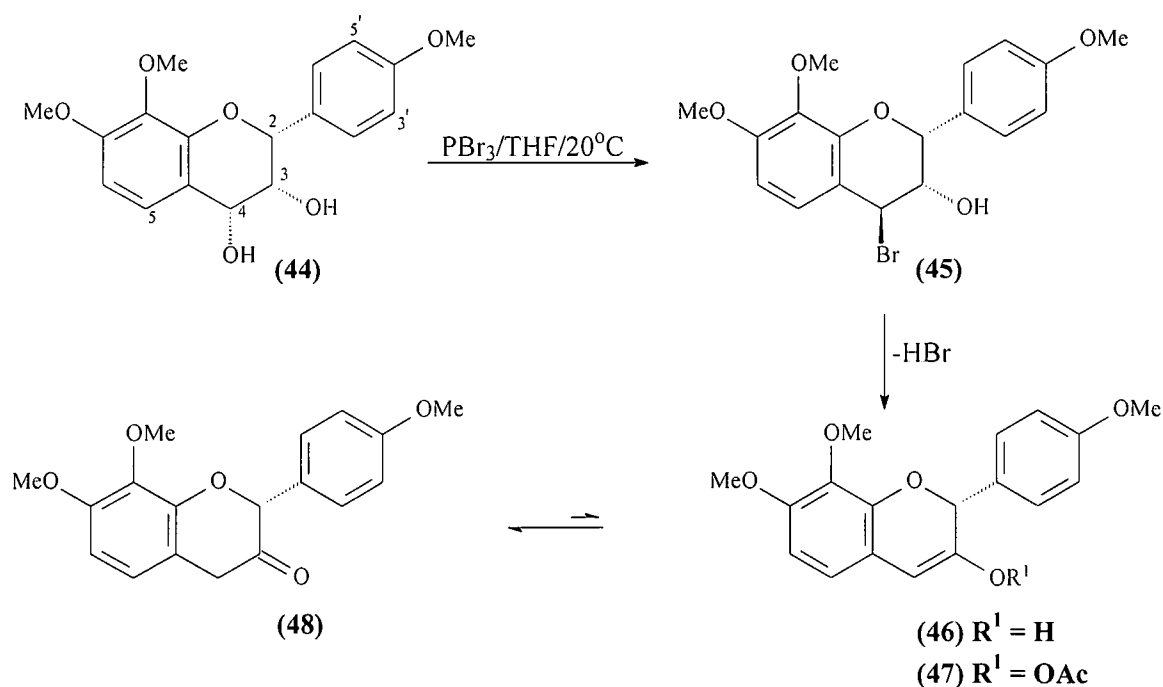
⁶⁷ H. A. Stafford, H. H. Lester, R. M. Weider, *Plant Sci.*, 1987, 52, 99.

⁶⁸ R. W. Hemingway, P. E. Laks, *J. Chem. Soc., Chem. Commun.*, 1985, 746.

⁶⁹ J. W. Clark-Lewis, L. R. Williams, *Aust. J. Chem.*, 1967, 20, 2151.

5.2 Synthesis of flav-3-en-3-ols:

Coetzee⁷⁰ converted (Scheme 5.1) 4',7,8-tri-O-methylepioritin-4 α -ol(**44**) into 4 β -bromoflavan-3-ol(**45**) for use as an intermediate in the synthesis of ether-linked proteracacinidin dimers^{30,31}. The flavan-3,4-diol is first converted into the 4 β -bromoflavan-3-ol(**45**). Since 2,3-*cis*-3,4-*cis*-flavan-3,4-diols are conspicuously resistant to S_N2 processes at C-4^{69,71}, the inversion of configuration at this stereocenter probably results from neighbouring group participation by the axial C-3 hydroxyl function^{30,31} (structure (**40**) scheme 4.2). Spontaneous dehydrobromination leads to the formation of the flav-3-en-3-ol(**46**). The latter compound exists in solution as the keto tautomer(**48**), acetylation with acetic anhydride in pyridine leads to regioselective formation of the flav-3-en-3-acetate(**47**). Similar reactions on fisetinidol-4 α -ol, afforded the same results.



Scheme 5.1

⁷⁰ J. Coetzee, E. Malan and D. Ferreira, *Tetrahedron*, 2000, 56, 1819.

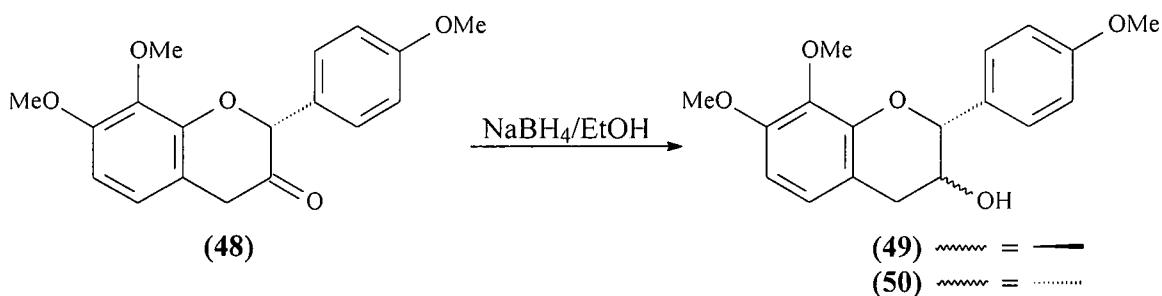
⁷¹ J. Coetzee, E. Malan and D. Ferreira, *Tetrahedron*, 1999, 55, 999.

DISCUSSION

5.3 Reactions of Flav-3-en-3-ols:

The assessment of the possible role of flav-3-en-3-ols in the biosynthesis of flavonoids and especially its role as the electrophilic source of the chain extender units in proanthocyanidin formation prompted us to extend the studies, previously done by Coetzee⁷⁰.

Until now there has been a limited access to pyrogallol A-ring flavan-3-ols from natural sources. The flavan-3-one(**48**) was reduced with sodium borohydride in ethanol (**Scheme 5.2**) to an epimeric mixture of the 2,3-*trans*-flavan-3-ol [4',7,8-tri-O-methyloritin(**49**)] (NMR data **plate 18**) and the 2,3-*cis*-flavan-3-ol [4',7,8-tri-O-methylepioritin(**50**)] (NMR data **plate 17**). This represents the first synthetic access to the hitherto naturally unknown oritin class of flavan-3-ols and the first protocol to manipulate the C-3 stereochemistry of flavan-3-ols, hence complementing the synthetic utility of their facile C-2 epimerization under alkaline conditions⁷².

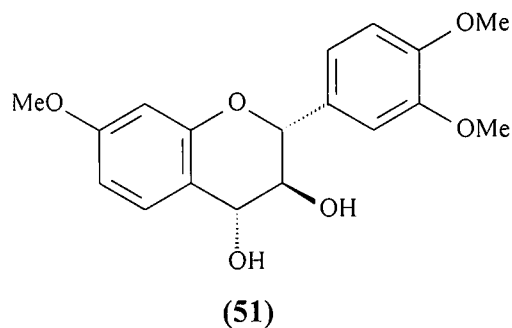


Scheme 5.2

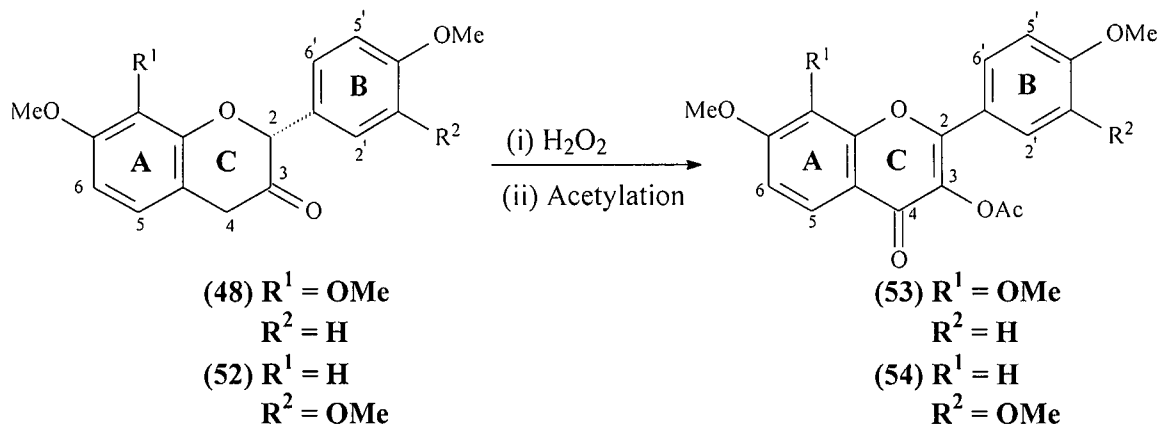
The conversion of the flavan-3,4-diol derivative (**44**) into the flav-3-en-3-ol(**46**), together with the reduction of its keto tautomer(**48**) to give the 2,3-*trans*- and *cis*-flavan-3-ols (**49**) and (**50**), are in agreement with the hypotheses regarding the role of flav-3-en-3-ols^{59-62,68} and A-ring quinone methides^{49,73} in flavan-3-ol and proanthocyanidin biosynthesis. Accordingly, the conversions provide direct in vitro evidence supporting the suggested in vivo role of keto-enol tautomers of types (**48**) and (**46**).

⁷² L. Y. Foo, L. J. Porter, *J. Chem. Soc., Perkin Trans. 1*, 1983, 1535.

⁷³ M. R. Attwood, B. R. Brown, S. G. Lisseter, C. L. Torrero, P. M. Weaver, *J. Chem. Soc., Chem. Commun.*, 1984, 177.



The flavan-3-ones **(48)** and **(52)** prepared from epioritin-4 α -ol tri-O-methylether(**44**) and fisetinidol-4 α -ol tri-O-methylether(**51**) were oxidized with hydrogen peroxide and the corresponding flavonols **(53)** and **(54)** (scheme 5.3) were isolated after acetylation with acetic anhydride in pyridine.



Scheme 5.3

A database compiled by Steynberg^{74,75}, to establish the absolute configuration of (+)-mollisacacidin(**51**) (2R,3S,4R) and the already established absolute configuration of (-)-

⁷⁴ P. J. Steynberg, *Manipulation of the interflavanyl bond in proanthocyanidins*, Ph.D.thesis, U. O. F. S., 1996.

⁷⁵ P. J. Steynberg, J. P. Steynberg, E. V. Brandt, D. Ferreira and R. W. Hemingway, *J. Chem. Soc., Perkin Trans. 1*, 1997, 1943.

teracacidin(**44**) (2R,3R,4R)⁷⁶ was used. Diagnostic from the ¹H NMR spectrum (CDCl₃, 296K, **plate 13**) of (**48**) was the presence of the C-4 methylene doublets (δ 3.73 and 3.63, d, J = 19.0 Hz), which showed prominent benzylic coupling as well as n.O.e association with 5-H(A) (δ 6.80). ⁴J_{HH} coupling between 2-H(C) (δ 5.35) and 2',6'-H(B) (δ 7.33) was also observed and compound (**48**) was identified as (2R)-4',7,8-trimethoxyflav-3-one.

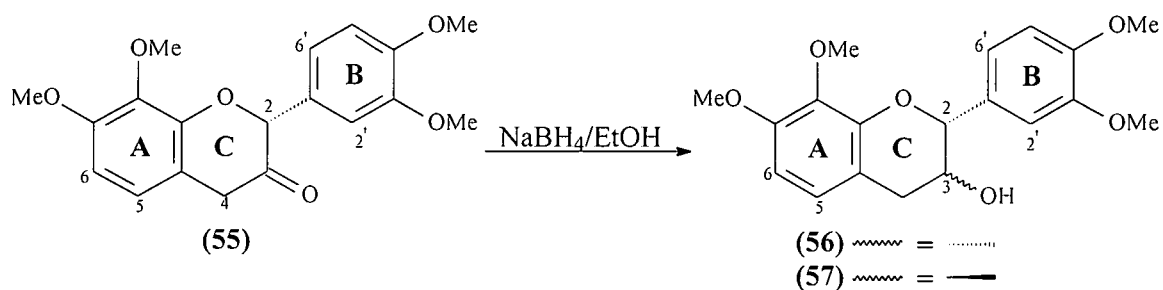
Characteristic of compound (**53**) is the absence of any heterocyclic resonances in the ¹H NMR spectrum (**plate 19**) and the presence of the two deshielded doublets, 5-H(A) (δ 7.99, d, J = 9.0 Hz) and 2',6'-H(B) (δ 7.93, d, J = 9.0 Hz) because of the excessive deshielding exercised by the carbonyl functionality and the 3-OAc(C) substituent. Compound (**53**) was therefore identified as 3-acetoxy-4',7,8-trimethoxyflavonol.

The characteristic C-4 methylene doublets (δ 3.72 and 3.62, d, J = 19.5 Hz) were also evident in the ¹H NMR spectrum (**plate 15**) of the flavan-3-one(**52**). The two aromatic ABX-systems in conjunction with a broad heterocyclic singlet (2-H(C)) and three methoxyl signals identified compound (**52**) as (2-R)-3',4',7-trimethoxyflavan-3-one.

The presence of the deshielded doublet, 5-H(A) (δ 8.17, d, J = 8.5 Hz) and doublet of doublets 6-H(A) (δ 7.52, dd, J = 8.5 Hz) caused by the deshielding exercised by the carbonyl functionality, the presence of two aromatic ABX-systems and the absence of any heterocyclic resonances in the ¹H NMR spectrum (CDCl₃, 296K, **plate 20**) characterized and identified compound (**54**) as 3-acetoxy-3',4',7-trimethoxyflavonol.

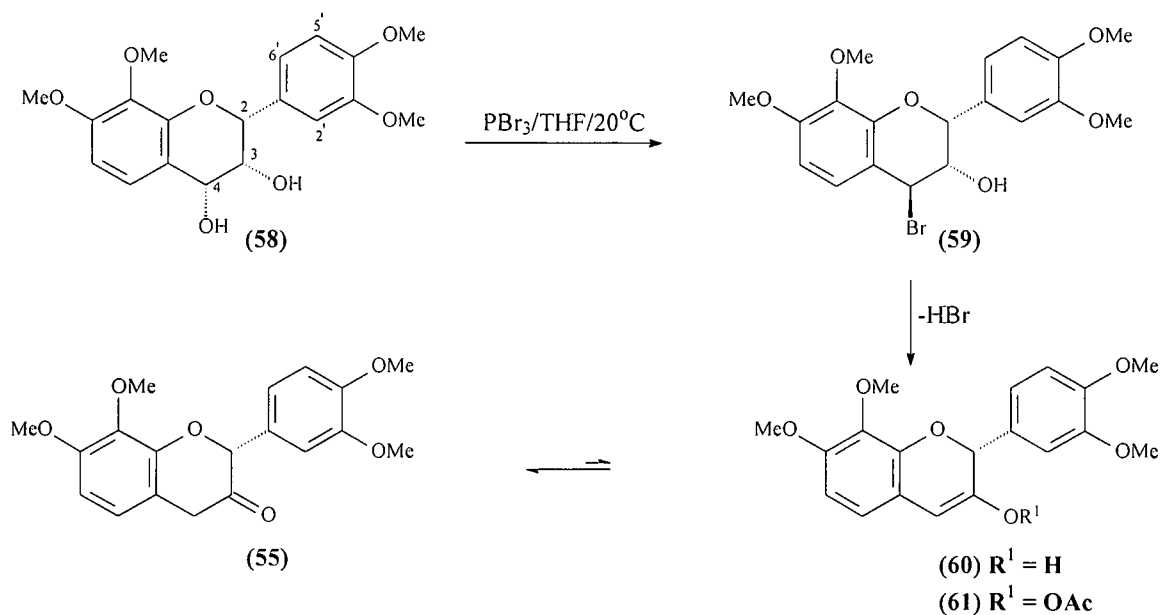
Due to the scarcity and the absence of 3',4',7,8-tetrahydroxyflavan-3-ol (mesquitol)(**8**) in *Acacia nigrescens* the next step was to synthesize mesquitol. As previously discussed an epimeric mixture of the flavan-3-ols (**56**) and (**57**) (**scheme 5.4**) can be synthesized by reduction of the flavan-3-one(**55**) with sodium borohydride in ethanol.

⁷⁶ J. W. Clark-Lewis and D. G. Roux, *J. Chem. Soc.*, 1959, 1402; J. W. Clark-Lewis and D. G. Roux, *J. Chem. Soc.*, 1962, 2502.



Scheme 5.4

Mesquitol-4 α -ol tetra-O-methylether(**58**) (scheme 5.5) was thus dissolved in dry THF, treated with 0.35 equivalents of PBr_3 under N_2 and stirred for two hours at room temperature. After several attempts it became clear that the 3',4',7,8-tetramethoxyflavan-3-one(**55**) could not be easily synthesized, because the required product was not obtained.



Scheme 5.5

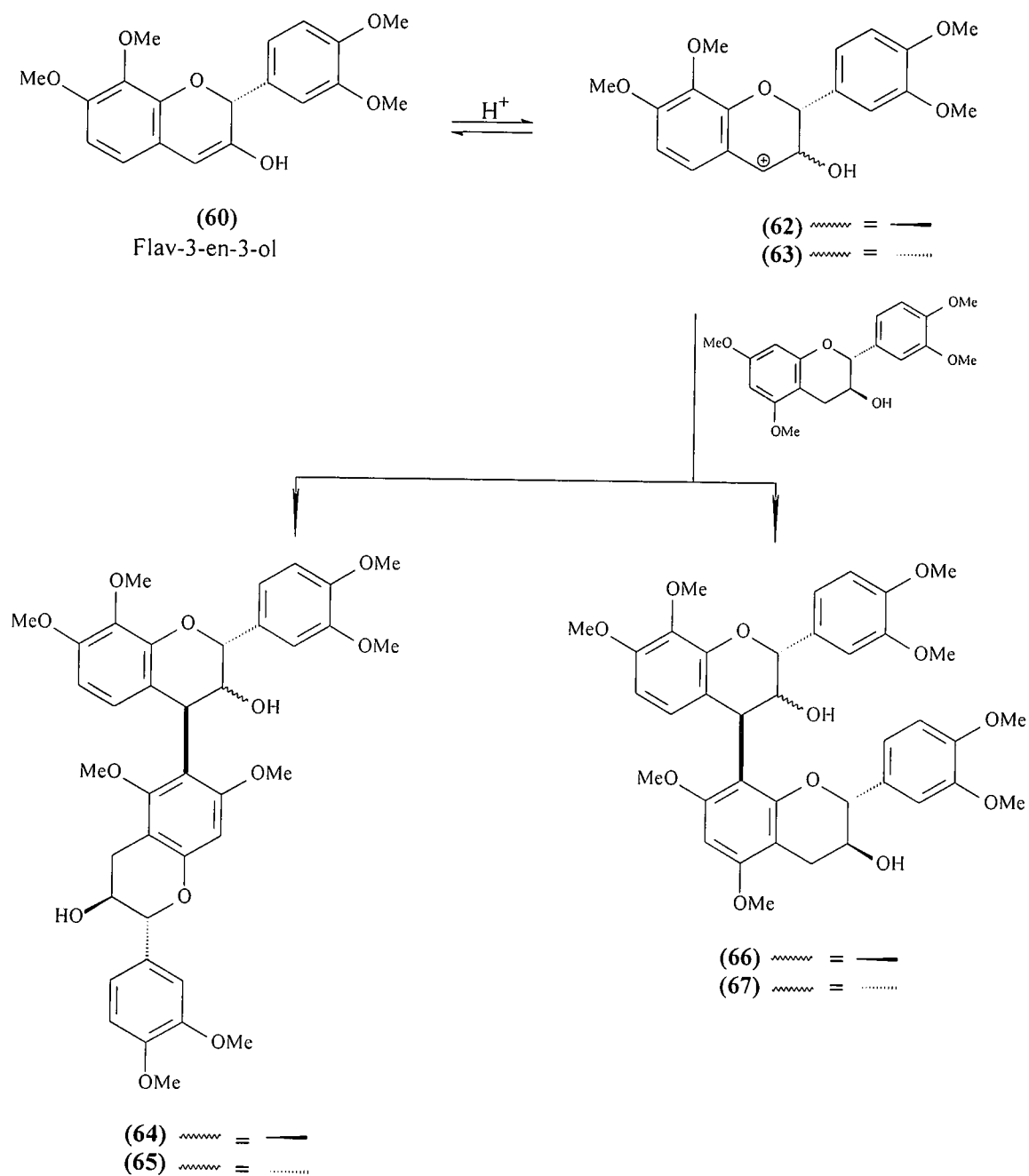
The reaction was repeated, but before PLC separation, the reaction mixture was acetylated with acetic anhydride in pyridine. PLC separation followed with the subsequent isolation of 3',4',7,8-tetramethoxyflavan-3-en-3-acetate(**61**) in a very low yield (6.0 %).

The ¹H NMR spectrum (CDCl₃, 296K, **plate 16**) (**table 5.1**) showed a deshielded singlet 4-H(C) (δ 6.54) and a heterocyclic singlet 2-H(C) (δ 5.85) as well as an ABX-system and an AB-system. The 4-H(C) singlet showed benzylic coupling with 5-H(A) (δ 6.76) and resonated to lower field (δ 6.54) mainly because of the deshielding exercised by 3-OAc(C) substituent and the 3,4-double bond together with the aromatic A-ring. Compound (**61**) was characterized as (2R)-3-acetoxy- 3',4',7,8-tetramethoxyflavan-3-ene. Due to time restrictions this very interesting synthesis could not be further pursued.

Table 1: ¹H NMR peaks (δ_C) for compound **61** at 300 MHz (296K). Splitting patterns and J values (Hz) are given in parentheses.

Ring	Carbon	¹ H-CDCl ₃
A	5	6.82(d,8.0)
	6	6.48(d,8.0)
B	2'	7.03(d,2.0)
	5'	6.76(d,8.0)
	6'	7.01(dd,2.0,8.0)
C	2	5.85(br.s,1.5)
	3	-
	4	6.33(br.s,1.5)
OMe		3.88,3.85,3.83,3.65 (all s)
OAc		2.08 (s)

The flav-3-en-3-ol(**60**) may in principle serve as an electrophile in the biosynthetic and enzymatic sequence leading to oligoflavanoids. Protonation (**scheme 5.6**) of the flav-3-en-3-ol(**60**) could give the 2,3-*cis* and 3,4-*trans* carbocations (**62**) and (**63**) respectively, which may then be trapped by a potent nucleophilic flavan-3-ol to form the (4,6)- and (4,8)-linked biflavanoids as the first step in oligomer formation.



Scheme 5.6

We venture the prediction that the orientation of the C(2)-phenyl group will determine the stereochemical course of the protonation step, hence leading to the simultaneous formation of oligomers belonging to the series of naturally occurring analogues with 2,3-*trans*- and 2,3-*cis*-chain extender units.

CHAPTER 6:

LEUCOANTHOCYANIDINS FROM *ACACIA*

NIGRESCENS:

6.1 Introduction:

Acacia nigrescens represents the first South African species, which contains flavanoid analogues with a 3',4', 7,8-tetrahydroxy phenolic substitution pattern^{2,77}.

The monomer epimesquitol-4 α -ol(**1**) dominates in the heartwood extract and the diastereomer, epimesquitol-4 β -ol(**2**), are also present.

Fractions A1 up to A17 obtained from a Sephadex LH20 column separation of the MeOH-extract of the heartwood of *A. nigrescens* followed by derivatization yielded the two diols as well as the flavanol(**5**), dihydroflavonol(**4**) and the flavanone(**6**).

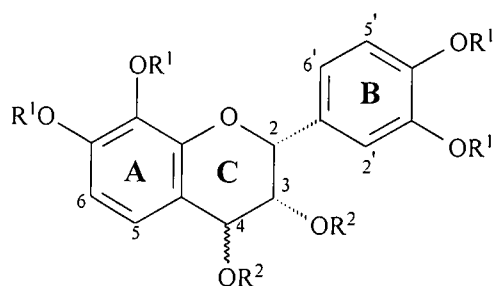
6.2 Epimesquitol-4 α -ol(**1**):

¹H NMR[CDCl₃, 296K] data (plate 4) (table 6.1) of the tetramethylether diacetate derivative(**51**) of epimesquitol-4 α -ol(**1**), previously isolated from *A. galpinii*¹⁸ and *A. caffra*^{78,79}, was compared and the absolute configuration confirmed to be (2R,3R,4R)-2,3-*cis*-3,4-*cis*-flavan-3,4, 3',4',7,8, -hexaol (epimesquitol-4 α -ol).

⁷⁷ E. Malan, *Phytochemistry*, 1993, 33, 733.

⁷⁸ A. Sireeparsad, *The Structure and Synthesis of Natural Products isolated from Acacia galpinii and Acacia caffra*, M.Sc.-thesis, University of Durban-Westville, 1996.

⁷⁹ L. Bennie, *The Structure and Chemical Elucidation of the Heterogeneous Interflavanyl Bonds in Oligomeric Proteracacinidins from Acacia caffra*, Ph.D.-thesis, University of the Orange Free State, 1999.



- | | | | | |
|------|--|---|--|--|
| (1) | | = | | R ¹ = H |
| (51) | | = | | R ¹ = Me, R ² = Ac |
| (2) | | = | | R ¹ = H |
| (52) | | = | | R ¹ = Me, R ² = Ac |

6.3 Epimesquitol-4 β -ol(2):

The 300MHz ¹H NMR[CDCl₃, 296K] data (**plate 5**) (**table 6.1**) of the tetramethylether diacetate derivative(**52**) indicated an ABX- and an AB-system in the aromatic region together with a heterocyclic AMX-system. These systems, together with the four methoxy and two acetoxy signals, confirmed the flavan-3,4-diol nature of the compound.

A 2D COSY experiment revealed the benzylic coupling to be between 5-H(A, δ 7.20,d,8.5 Hz) and 4-H(C, δ 5.90,d,3.0 Hz), this in conjunction with the coupling constant of 8.5 Hz for 5-H(A) and 6-H(A) confirming the AB-system of the A-ring. The long range coupling (⁴J_{HH}) of 2'-H(B, δ 7.06) and 6'-H(B, δ 7.05) with 2-H(C, δ 5.33) defined the ABX-system of the B-ring.

The small J_{2,3} value (1.5 Hz) in the ¹H NMR spectra of (**52**) indicated a 2,3-*cis* relative configuration, while the J_{3,4} value of 3.0 Hz is reminiscent of a 3,4-*trans* relative configuration^{4,76,80}. Comparison of the CD data of (**52**) with similar derivatives (epioritin-4 β -ol), confirmed (**52**) to be (2R,3R,4S)-2,3-*cis*-3,4-*trans*-flavan-3,4,7,8,3',4'-hexaol (epimesquitol-4 β -ol).

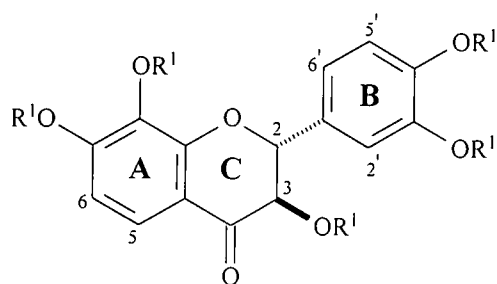
⁸⁰ S. E. Drewes and D. G. Roux, *Biochem. J.*, 1964, 90, 343; *ibid.*, 1965, 94, 482; *ibid.*, 1965, 96, 681; *ibid.*, 1966, 98, 493.

Table 6.1: ^1H NMR peaks (δ_{C}) for compounds **51** and **52** at 300 MHz (296K). Splitting patterns and J values (Hz) are given in parentheses.

Ring	Carbon	51 -CDCl ₃	52 -CDCl ₃
A	5	6.93(d,9.0)	7.20(d,8.5)
	6	6.63(d,9.0)	6.63(d,8.5)
B	2'	7.05(d,2.0)	7.06(d,2.0)
	5'	6.89(d,8.5)	6.90(d,8.5)
	6'	7.04(dd,2.0,8.5)	6.05(dd,2.0,8.5)
C	2	5.35(br.s,1.0)	5.33(br.s,1.5)
	3	5.67(dd,1.0,4.0)	5.27(dd,1.5,3.0)
	4	6.33(d,4.0)	5.90(d,3.0)
OMe		3.93,3.91,3.91,3.90 (all s)	3.94,3.91,3.91,3.90 (all s)
OAc		2.13,1.94 (all s)	2.16,1.90 (all s)

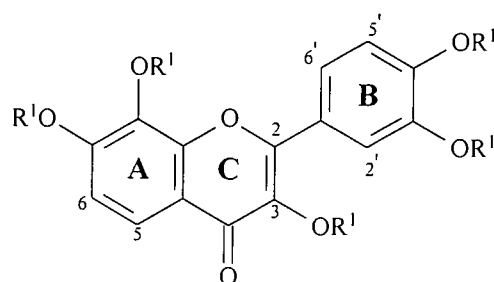
6.4 Dihydroflavonol(4):

Compound (**4**) was isolated as the penta-acetate derivative(**53**). An ABX- and an AB-system were present in the aromatic region of the ^1H NMR spectrum (**plate 1**) (**table 6.2**) and two doublets, 2-H(C, δ 5.48, d, 12.0 Hz) and 3-H(C, δ 5.72, d, 12.0 Hz) in the heterocyclic region. 2-H(C) showed benzylic coupling of 1.0 Hz with 2'-H(B) and 6'-H(B), thus confirming it as one of the two protons in the heterocyclic area. The large $J_{2,3}$ value (12.0 Hz) in the ^1H NMR spectra of (**53**) indicated a 2,3-*trans* relative configuration. The presence of a deshielded doublet, 5-H(A) (δ 7.85, d, $J = 9.0$ Hz) and the doublet of doublets, 6'-H(B δ 7.40, dd, $J = 2.0$ Hz & 8.5Hz) are because of the electron withdrawing effect exercised by the carbonyl functionality at C-4. The presence of the 3-OAc(C) substituent, along with the above information indicated and identified compound (**53**) as a (2R)-3,3',4',7,8-penta-acetoxydihydroflavonol.



(4) $R^1 = H$

(53) $R^1 = Ac$



(5) $R^1 = H$

(54) $R^1 = Me$

6.5 Melanoxetin [flavonol](5):

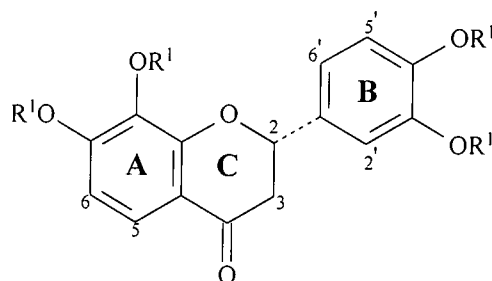
Characteristic of compound (54) is the absence of any heterocyclic resonances in the 1H NMR spectrum (plate 3) (table 6.2) and the presence of a deshielded doublet, 5-H(A) (δ 8.00, d, $J = 9.0$ Hz) and doublet of doublets, 6'-H(B) (δ 7.88, dd, $J = 2.0$ Hz & 8.5 Hz) due to the deshielding exercised by the carbonyl functionality at C-4 and the 3-OAc(C) substituent. An ABX- and an AB-system further confirmed compound (54) as 3,3',4',7,8-pentamethoxyflavonol.

Table 6.2: 1H NMR peaks (δ_C) for compounds 53 and 54 at 300 MHz (296K). Splitting patterns and J values (Hz) are given in parentheses.

Ring	Carbon	53- $CDCl_3$	54- $CDCl_3$
A	5	7.85(d,9.0)	8.00(d,9.0)
	6	6.99(d,9.0)	7.06(d,9.0)
B	2'	7.30(d,2.0)	7.85(d,2.0)
	5'	7.27(d,8.5)	7.04(d,8.5)
	6'	7.40(dd,2.0,8.5)	7.88(dd,2.0,8.5)
C	2	5.48(d,12.0)	
	3	5.72(d,12.0)	
OMe			4.04,4.02,4.00,4.00,3.91 (all s)
OAc		2.35,2.34,2.33,2.28,2.19 (all s)	

6.6 Flavanone(6):

Compound(6) was isolated as the tetra-acetate derivative(55) and very characteristic was the two methylene protons, which resonated at δ 3.20. An ABX- and an AB-system were also evident from the ^1H NMR $[(\text{CD}_3)_2\text{CO}]$ data (plate 2) (table 6.3) and in the heterocyclic region there was only one additional proton 2-H(C, δ 5.75, dd, 3.0 Hz & 13.0 Hz), which coupled with both the methylene protons 3eq-H(C, δ 3.20, dd, 13.0 Hz & 13.0 Hz) and 3ax-H(C, δ 3.00, dd, 3.0 Hz & 13.0 Hz). The low field appearance 5-H(A) (δ 7.79, d, $J = 8.5$ Hz) was because of the deshielding exercised by the carbonyl functionality at C-4. Compound(55) was identified as 3',4',7,8-tetra-acetoxyflavanone.



(6) $\text{R}^1 = \text{H}$

(55) $\text{R}^1 = \text{Ac}$

Table 6.3: ^1H NMR peaks (δ_{C}) for compound 55 at 300 MHz (296K). Splitting patterns and J values (Hz) are given in parentheses.

Ring	Carbon	55- $(\text{CD}_3)_2\text{CO}$
A	5	7.79(d,8.5)
	6	7.02(d,8.5)
B	2'	7.46(d,2.0)
	5'	7.34(d,8.0)
	6'	7.50(dd,2.0,8.0)
C	2	5.75(dd,3.0,13.0)
	3-eq	3.20(dd,13.0,13.0)
	3-ax	3.00(dd,3.0,13.0)
OAc		2.33,2.30,2.30,2.27 (all s)

CHAPTER 7:
C-C LINKED PROANTHOCYANIDIN
DIMERS
FROM *ACACIA NIGRESCENS*

7.1 Introduction:

The very recent occurrence of condensed tannins with a pyrogallol A-ring was limited to the heartwoods of *Acacia galpini*^{4,30,31} and *Acacia caffra*^{26,36} for proteracacinidins and to *Prosopis glandulosa*²³ and *Acacia melanoxylon*^{11,29} for the presence of promelacacinidin dimers.

The notable variety of the types and positions of interflavanyl bonds e.g. carbon-carbon, ether-linked, from the C-3, C-4, C-5 and C-6 positions, present in these flavonoid compounds isolated to date is a manifestation of the relatively reduced nucleophilicity of the pyrogallol A-ring.

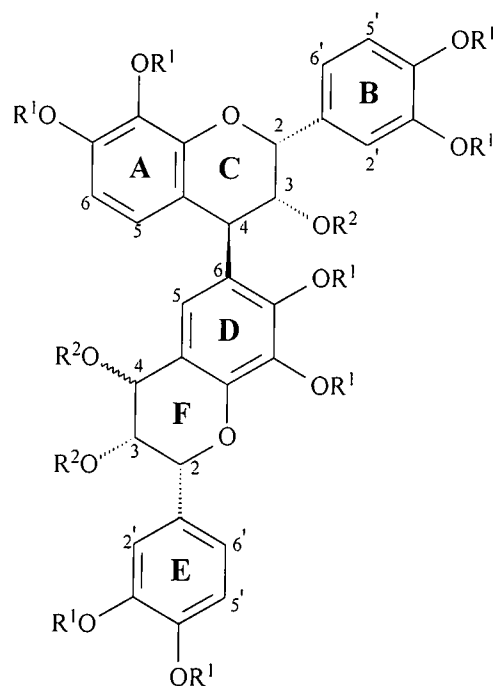
The present investigation of the MeOH extract of *Acacia nigrescens* heartwood revealed the occurrence of several novel promelacacinidins and two known promelacacinidins. All the compounds were identified as their permethylaryl ether acetate derivatives.

7.2 C-4(C-RING) → C-6(D-RING) LINKED PROMELACACINIDINS:

The 300 MHz ¹H NMR data of the octamethylether triacetates of compound (57) (C₆D₆) (plate 6) and (58) (CDCl₃) (plate 7) in table 7.1, exhibited in each case two heterocyclic AMX-systems and two ABX-systems, an AB-system and a broad singlet in the aromatic region. This together with eight *O*-methyl and three *O*-acetyl resonances suggested the compounds to be dimers comprising two mesquitol moieties both with a flavan-3,4-diol terminal unit.

7.2.1 Epimesquitol-(4 β →6)-epimesquitol-4 α -ol(**56**) and epimesquitol-(4 β →6)-epimesquitol-4 β -ol(**59**):

With the assistance of NOESY and COSY experiments it was possible to identify and assign the AMX-systems of the top and bottom moieties for compounds (**57**) and (**58**) respectively.



- (**56**) $\text{---} = \text{---} \quad \text{R}^1 = \text{R}^2 = \text{H}$
 (**57**) $\text{---} = \text{---} \quad \text{R}^1 = \text{Me}, \text{R}^2 = \text{Ac}$
 (**58**) $\text{---} = \text{---} \quad \text{R}^1 = \text{Me}, \text{R}^2 = \text{Ac}$
 (**59**) $\text{---} = \text{---} \quad \text{R}^1 = \text{R}^2 = \text{H}$

The lower field 4-H (F, d) protons for both compounds (**57**) and (**58**) at δ 6.49 and δ 5.67 were utilized as references to identify the F-ring heterocyclic systems as well as the 5-H (D, s) protons at δ 6.92 and δ 6.64 respectively from n.O.e. associations. The latter

protons in turn showed an association in both compounds with the 4-H(C, d) protons at δ 4.98 and δ 4.46, which served as reference to establish the C-ring heterocyclic systems (**table 7.1**).

COSY experiments showed coupling between the respective 2-H resonances of the C- and F-rings of both compounds (**57**) and (**58**) with the 2'-H and 6'-H signals of the D- and E-rings which enabled the identification of the four different ABX-systems (**table 7.1**) present in the two dimers. The long range COSY coupling (${}^4J_{HH}$) between the 4-H(C) protons and the 5-H(A) protons confirmed the AB-system of the A-ring. The ${}^4J_{HH}$ coupling of 5-H(D, δ 6.92 & δ 6.64) protons with both 4-H(C, δ 4.98 & δ 4.46) and 4-H(F, δ 6.49 & δ 5.67) support the NOESY results for an (4 \rightarrow 6) interflavanyl bond in both dimers.

The coupling constants of both the AMX-systems for compound (**58**) and the one AMX-system of (**57**) are characteristic of 2,3-*cis*-3,4-*trans* ($J_{2,3} = 1.5$ and $J_{3,4} = 3.0$ Hz) relative stereochemistry, while the other AMX-system of (**57**) showed coupling reminiscent of 2,3-*cis*-3,4-*cis* ($J_{2,3} = 1.0$ and $J_{3,4} = 4.5$ Hz) relative stereochemistry^{4,25}.

The high amplitude positive Cotton effects at $[\theta]_{241.7} 8262$ and $[\theta]_{241.7} 8898$ for compounds (**57**) and (**58**) respectively are indicative of β -orientation at C-4 at both the C-rings and hence 2*R*,3*R*,3*R* absolute configurations^{4,25}. The relatively high concentration of epimesquitol-4 α -ol and epimesquitol-4 β -ol present in the heartwood, in conjunction with the relative stereochemistry of 2,3-*cis*-3,4-*cis* (bottom unit) and 2,3-*cis*-3,4-*trans* (bottom unit) of the dimers (**57**) and (**58**) make them the logical precursors to these compounds with an absolute stereochemistry assigned to the dimers of 2*R*,3*R*,4*R*(C-ring)-2*R*,3*R*,4*R*(F-ring) for (**57**) and 2*R*,3*R*,4*R*(C-ring)-2*R*,3*R*,4*S*(F-ring) for (**58**).

FAB-MS (m/z 832) confirmed the molecular ions required for the molecular formula of $C_{44}H_{48}O_{16}$ for both compounds.

Table 7.1: ^1H NMR peaks (δ_{C}) for compounds (57) and (58) at 300 MHz (296K).

Splitting patterns and J values (Hz) are given in parentheses.

Ring	Carbon	(57)- C_6D_6	(58)- CDCl_3
A	5	6.69(d,8.5)	6.70(d,8.5)
	6	6.44(d,8.5)	6.58(d,8.5)
B	2'	7.30(d,2.0)	7.03(d,2.0)
	5'	6.64(d,8.5)	6.82(d,8.5)
	6'	7.15(dd,2.0,8.5)	6.87(dd,2.0,8.5)
C	2	5.78(br.s,1.5)	5.18(br.s,1.5)
	3	6.13(dd,1.5,3.0)	5.40(dd,1.5,3.0)
	4	4.98(d,3.0)	4.45(d,3.0)
D	5	6.92(s)	6.64(s)
	6	-	-
E	2'	7.08(d,2.0)	7.03(d,2.0)
	5'	7.27(d,8.5)	6.91(d,8.5)
	6'	6.95(dd,2.0,8.5)	7.03(dd,2.0,8.5)
F	2	4.87(br.s,1.0)	5.30(br.s,1.5)
	3	5.93(dd,1.0,4.5)	5.25(dd,1.5,3.0)
	4	6.49(d,4.5)	5.67(d,3.0)
OMe		4.22,4.17,4.00,3.54, 3.52,3.47,3.46,3.44, (all s)	4.03,4.00,4.00,3.92, 3.91,3.90,3.88, 3.87(s)
OAc		1.91,1.65,1.60, (all s)	2.11,1.91,1.90, (all s)

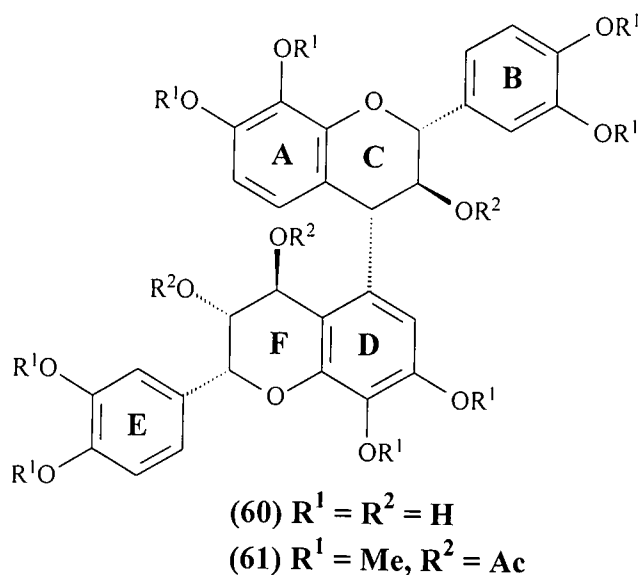
7.3 C-4(C-RING) \rightarrow C-5(D-RING) LINKED PROMELACACINIDINS:

7.3.1 Mesquitol-(4 α \rightarrow 5)-epimesquitol-4 β -ol(60):

The 300 MHz ^1H NMR[(CD_3) $_2\text{CO}$] data (plate 8) of the octamethylether triacetate derivative(61) of compound(60) in table 7.2, exhibited two heterocyclic AMX-systems and two ABX-systems, an AB-system and a broad singlet in the aromatic region. This together with eight *O*-methyl and three *O*-acetyl resonances suggested the compound to be a dimer comprising two mesquitol moieties.

By utilizing the shielded proton 4-H(F, d, δ 6.0) as reference^{23,25} it was possible to identify the two AMX heterocyclic systems belonging to C- and F-rings respectively (**table 7.2**). The aromatic singlet at δ 6.70 showed no $^4J_{HH}$ coupling to 4-H(F) but very clearly to 4-H(C) and the OMe at δ 3.74 (C-7, D-ring), which justified the identification as 6-H(D). From the ($^4J_{HH}$) benzylic coupling between 4-H(C, δ 4.50, d, 10.0 Hz) and 5-H(A, δ 6.21, d, 8.5 Hz) it was possible to establish the AB-system belonging to the A-ring.

The 2D COSY experiment also showed coupling between the 2- and 2',6'-protons (**table 7.2**) which assisted the identification of the respective ABX-systems of the B- and E-rings. Phase sensitive NOESY experiment confirmed the above observations and showed association between 6-H(D, δ 6.70) with both the 7-OMe(D) and 4-H(C). Together the above information strongly indicated a 4-C (C-ring) to 5-C (D-ring) interflavanyl bond.



The heterocyclic AMX-system of the C-ring showed coupling constants ($J_{2,3} = 9.0$; $J_{3,4} = 10.0$ Hz) typical of a 2,3-*trans*-3,4-*trans* relative stereochemistry, which was confirmed by the n.O.e. association between 2-H(C) and 4-H(C) suggesting the protons to be on the same side of the heterocyclic ring²⁶.

Table 7.2: ^1H NMR peaks (δ_{C}) for compounds (**61**) at 300 MHz (296K). Splitting patterns and J values (Hz) are given in parentheses.

Ring	Carbon	(61)- $(\text{CD}_3)_2\text{CO}$
A	5	6.21(d,8.5)
	6	6.56(d,8.5)
B	2'	7.22(d,2.0)
	5'	6.96(d,8.5)
	6'	7.10(dd,2.0,8.5)
C	2	5.06(d,9.0)
	3	5.94(dd,9.0,10.0)
	4	4.50(d,10.0)
D	5	-
	6	6.70(s)
E	2'	7.20(br.d,2.0)
	5'	6.99(d,8.5)
	6'	7.14(dd,2.0,8.5)
F	2	5.46(br.s,1.5)
	3	5.42(very br.s)
	4	6.10(d,3.5)
OMe		3.85,3.84,3.79, 3.76,3.75(all s), 3.83(x3)
OAc		2.24,1.98,1.66(s)

HMBC, HMQC and ^{13}C analysis confirmed the number of *O*-methyl and *O*-acetyl groups as well as the suggested carbon structure (**table 7.3**). The chemical shift for the 4-C(C) at δ_{C} 43.7 is in accordance with a phenyl substituent at this position³⁵.

The long-range HMBC correlations between H-4(C, δ 4.50) and 5-C (D, 112.1, $^2\text{J}_{\text{CH}}$), H-4(F, δ 6.10) and 5-C (D, $^3\text{J}_{\text{CH}}$), together with couplings of H-6(D, δ 6.70) to 5-C (D, $^2\text{J}_{\text{CH}}$) and to 7-C (D, δ 154.6, $^2\text{J}_{\text{CH}}$), as well as to 8-C (D, δ 136.4, $^3\text{J}_{\text{CH}}$) confirmed the 4-C(A) to 5-C(D) interflavanyl linkage.

Table 7.3: ^{13}C NMR peaks (δc) for compound **(61)**.

Ring	Carbon	(61) - $(\text{CD}_3)_2\text{CO}$
A	5	123.2
	6	106.1
B	2'	111.8
	5'	111.5
	6'	121.0
C	2	80.9
	3	71.9
	4	43.7
D	5	-
	6	105.9
E	2'	111.0
	5'	111.8
	6'	119.6
F	2	73.7
	3	68.3
	4	63.6

MS-FAB showed a molecular mass of 832 units thus supporting the molecular formula of $\text{C}_{44}\text{H}_{48}\text{O}_{16}$ and the structure of **(61)**.

The negative Cotton effect of -15580 near 246 nm is reminiscent of a 4α C-ring substituent⁸¹, which in conjunction with the 2,3-*trans*-3,4-*trans* relative stereochemistry supported a 2*R*,3*S*,4*S* absolute configuration for the top unit of **(61)**. Coupling constants ($J_{2,3} = 1.5$; $J_{3,4} = 3.5$ Hz) of the F-ring are representative of a 2,3-*cis*-3,4-*trans* relative stereochemistry⁴. Epimesquitol-4 β -ol is present in the same extract of the plant and therefore the logical precursor with a 2*R*,3*R*,4*S* absolute configuration was assigned to the F-ring.

Due to the broadening of all the heterocyclic protons in the ^1H NMR (296 K) spectrum with no sharpening of the peaks at elevated temperatures suggested a preferred

⁸¹ J. H. van der Westhuizen, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1220.

conformation^{25,45}. This was in fact confirmed by specific n.O.e. interactions between 4-H(F, δ 6.10) to 4-H(C, δ 4.50) but no associations with 5-H(A) and 3-H(C). From the above information and in conjunction with associations from 6-H(D, δ 6.70) to 3-H(C) but none to 4-H(C) and 5-H(A) (See figure 7.1) it was decided to construct a model of molecule (**61**) with the use of the PC Spartan Pro Mechanics Program (PC/x86) 6.0.6, which coincides with these observations. The minimum energy conformer as shown in figures 7.1 and 7.2 has a calculated energy of 212.464 kcal/mole and the measured distances of 2.22 Å between H-6(D) to H-3(C), 2.45 Å between 4-H(C) to 4-H(F), 3.73 Å between H-6(D) and H-5(A) confirmed the above positive n.O.e. observations. The previously suggested preferred conformation obtained from the use of Dreiding models²⁵ was now confirmed by computer modelling (figure 7.1) where the bottom unit (DEF) is perpendicular to the plane of the top unit (ABC) with the D-ring below the plane and the E- and F-rings on the same level as the plane of the top unit.

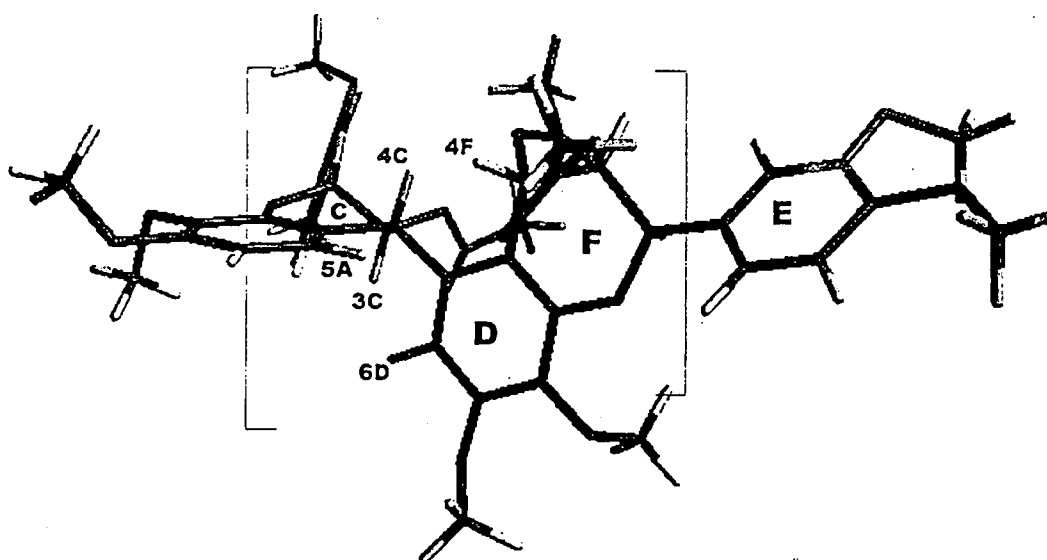


Figure 7.1. The lowest energy conformer of compound (61)

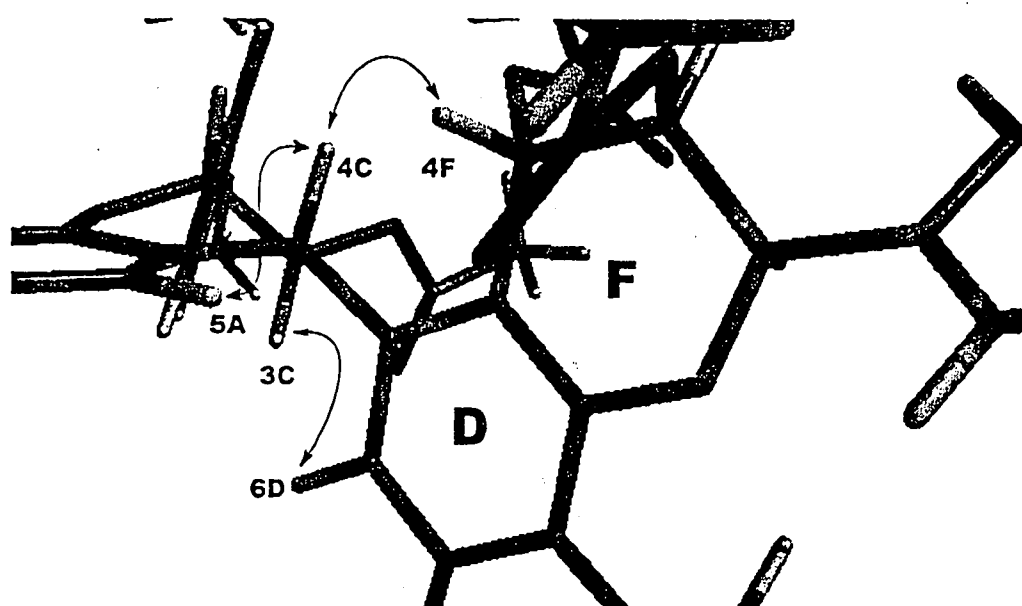


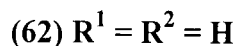
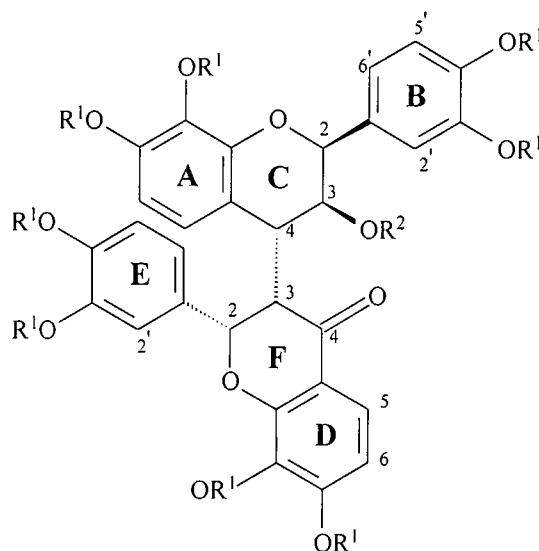
Figure 7.2. Close-up of the important n.o.e. correlations for compound (61)

7.4 C-4(C-ring) → C-3(F-ring) Linked promelacacinidins:

7.4.1 Ent-epimesquitol-(4 α →3)-3',4',7,8-tetrahydroxy flavanone(62):

The *ent*-epimesquitol-(4 α →3)-3',4',7,8-tetrahydroxy flavanone (**62**) was isolated as the octa-methyl ether acetate derivative (**63**). By using the 4-H(C, δ 3.43) as reference, the ¹H NMR (Me₂CO) data (**plate 9**) (**table 7.4**) showed (⁴J_{HH}) benzylic coupling between 4-H(C, δ 3.43, dd, 2.5Hz, 10.0 Hz) and 5-H(A, δ 7.15, d, 8.5 Hz), this made it possible to establish the AB-system belonging to the A-ring.

The 2D COSY experiment showed coupling between the 2- and 2',6'-protons (**table 7.4**), which assisted the identification of the ABX-system of the B-ring of compound (**63**).



The ABX- and AB-units of the D- and E-rings of the bottom unit were also determined by a similar method (**table 7.4**) using 2-H(F, δ 5.91) as reference. The F-ring of (**63**) proved to be different from the corresponding rings of the previous compounds (**57**), (**58**) and (**61**) with only two protons at δ 5.91 (d, 3.5 Hz) and δ 3.59 (dd, 3.5 and 10.0 Hz) in the heterocyclic region. These two protons showed coupling with each other (3.5 Hz) and the proton at δ 3.59 also showed coupling with 4-H(C) of 10.0 Hz and hence the above two protons were assigned to the 2-C(F) and 3-C(F) carbons respectively.

FAB-MS with a molecular ion of *m/z* 730 confirmed the dimeric character of the *ent*-epimesquitol-flavanone (**63**) with a molecular formula of C₄₀H₄₂O₁₃. The much

deshielded chemical shift at δ 7.60 (d, 9.0 Hz) of 5-H(D) (**table 7.4**) strongly suggested an adjacent carbonyl group at 4-C(F), which was confirmed by ^{13}C NMR resonance at δ_{C} 191.7 (**table 7.5**)⁸².

Table 7.4: ^1H NMR peaks (δ_{C}) for compound (**63**) at 300 MHz (296K). Splitting patterns and J values (Hz) are given in parentheses.

Ring	Carbon	(63)-(CD ₃) ₂ CO
A	5	7.15(d,8.5)
	6	6.76(d,8.5)
B	2'	7.07(d,2.0)
	5'	6.98(d,8.5)
	6'	7.03(dd,2.0,8.5)
C	2	5.65(d,2.0)
	3	5.42(dd,2.0,2.5)
	4	3.43(dd,2.5,10.0)
D	5	7.60(d,8.5)
	6	6.82(d,8.5)
E	2'	7.08(d,2.0)
	5'	6.83(d,8.5)
	6'	6.89(dd,2.0,8.5)
F	2	5.91(d,3.5)
	3	3.59(dd,3.5,10.0)
	4	-
OMe		3.95,3.91,3.83, 3.74,3.72, (all s) 3.83(x3)
OAc		1.70(s)

The chemical shift of 4-C(C) at δ_{C} 38.7 and 4-H(C) at δ 4.43 is reminiscent of the 4-C coupling position of the top unit. Due to the carbonyl functional group at the 4-C(F) of

⁸² P. K. Agrawal, Carbon-13 of Flavonoids, London: Elsevier, 1989, 96.

the bottom moiety of **(63)**, together with the complete ABX and AB aromatic proton system of the D- and E-rings displayed in the ^1H NMR (**table 7.4**), the only obvious coupling position that remained was at 3-C(F). This position was confirmed by the proton coupling of 4-H(C) to 3-H(F) of 10.0 Hz. The COSY experiment displayed couplings from 4-H(C, δ 3.43) to 3-H(F, δ 3.89, $^3J_{\text{HH}}$) and from 2-H(F, δ 5.91) to 3-H(F, δ 3.59, $^3J_{\text{HH}}$). HMBC showed couplings from 3-H(F, δ 3.59) to 3-C(C, δ_{c} 69.6, $^3J_{\text{CH}}$), with 4-C(C, δ_{c} 38.7, $^2J_{\text{CH}}$) and to 4-C(F, δ_{c} 191.7, $^2J_{\text{CH}}$).

Table 7.5: ^{13}C NMR peaks (δ_{c}) for compound **(63)**.

Ring	Carbon	(63) - $(\text{CD}_3)_2\text{CO}$
A	5	125.7
	6	105.2
B	2'	111.1
	5'	111.8
	6'	119.3
C	2	74.0
	3	69.6
	4	38.7
D	5	123.2
	6	106.6
E	2'	110.8
	5'	111.7
	6'	119.6
F	2	79.4
	3	53.9
	4	191.7

The negative cotton effect of $[\theta]_{245.2} -10240$ for **(63)** is indicative of a 4α C-ring substituent and in conjunction with a relative 2,3-*cis*-3,4-*trans* configuration supports an 2*S*,3*S*,4*S* absolute stereochemistry for the top flavanyl unit.

The data (**table 7.6**) obtained from the computer modelling of the two most likely low energy conformations compare very well as shown in figures 7.3 and 7.5 of compound

(63) *viz.* C-ring-phenyl (equatorial)-F-ring-phenyl (equatorial) and C-ring-phenyl (equatorial)-F-ring-phenyl (axial) respectively. Only slight differences with regard to the n.O.e. association distances between protons and the calculated low energy values of 203.75 kcal/mole and 201.60 kcal/mole were evident.

However from the observed n.O.e. associations (**table 7.6**) the proton to proton distances are slightly in favour of figure 7.3 as well as the parallel stacked position of the two aromatic rings A and E ($4.073 \text{ \AA}^{\circ}$), which could be favourable for π -interaction^{83,44} and which shows the A-ring to be perpendicular to the plane of the E-ring in figure 7.5.

⁸³ C. A. Hunter and J. K. M. Sanders, *J. Amer. Chem. Soc.*, 1990, 112, 5525.

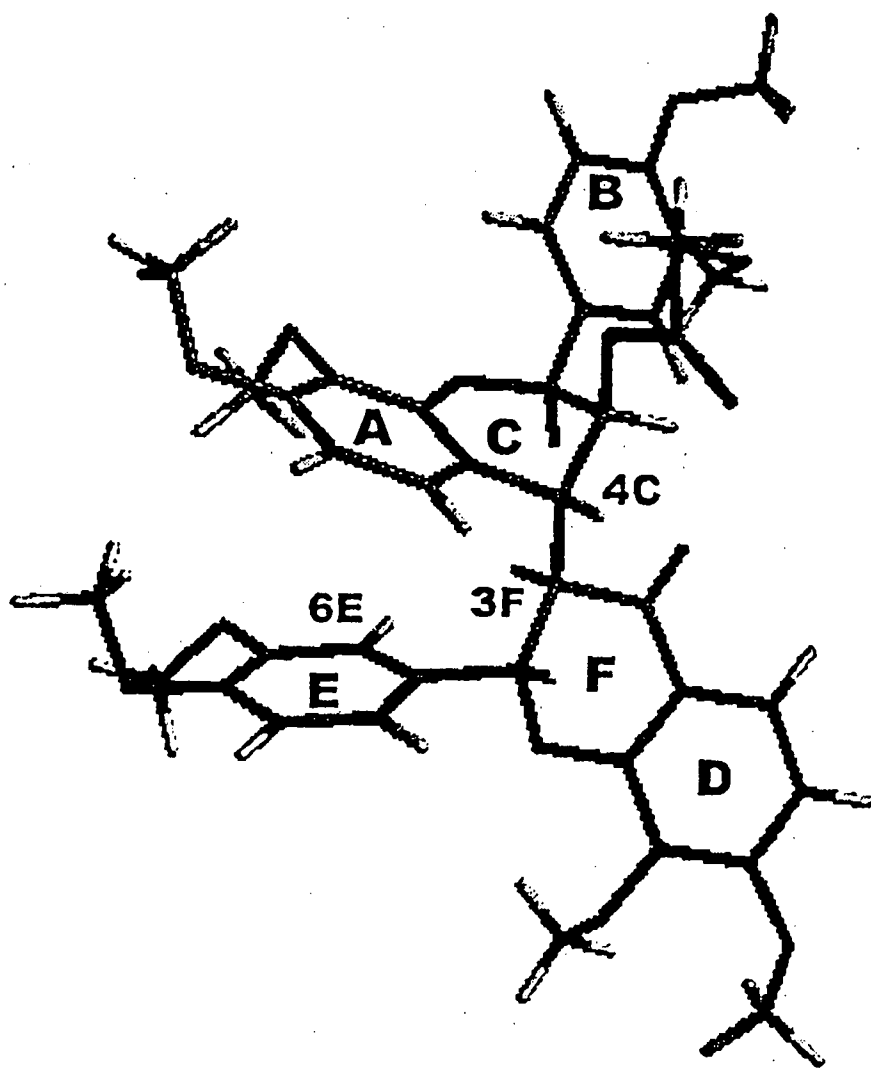


Figure 7.3. Low energy conformation of compound (63) with the E-ring equatorial.

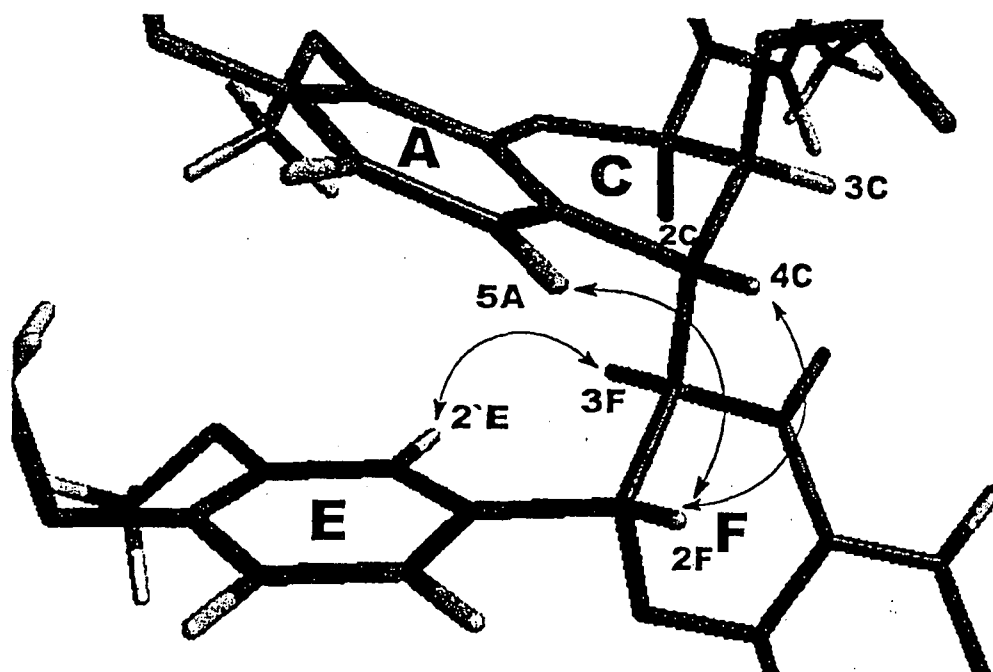


Figure 7.4. Close-up of the low energy structure of Fig. 7.3.

Table 7.6 Comparative data of the two possible low energy conformations in figures 3 and 5 for compound (63).

Protons	Distance between protons (Å^0)		NOESY Associations
	Figure <u>3</u>	Figure <u>5</u>	
2-H(C)→3-H(F)	2.240	2.220	positive
2'-H(E)→3-H(F)	2.156	2.530	positive
4-H(C)→5-H(A)	2.410	2.460	positive
4-H(C)→2-H(F)	2.430	2.890	slight
3-H(F)→3-H(C)	3.330	3.270	slight
2-H(F)→5-H(A)	2.930	2.34	positive
>C=O→H-3(C)	2.246	2.564	
Energy kcal/mole	203.75	201.60	

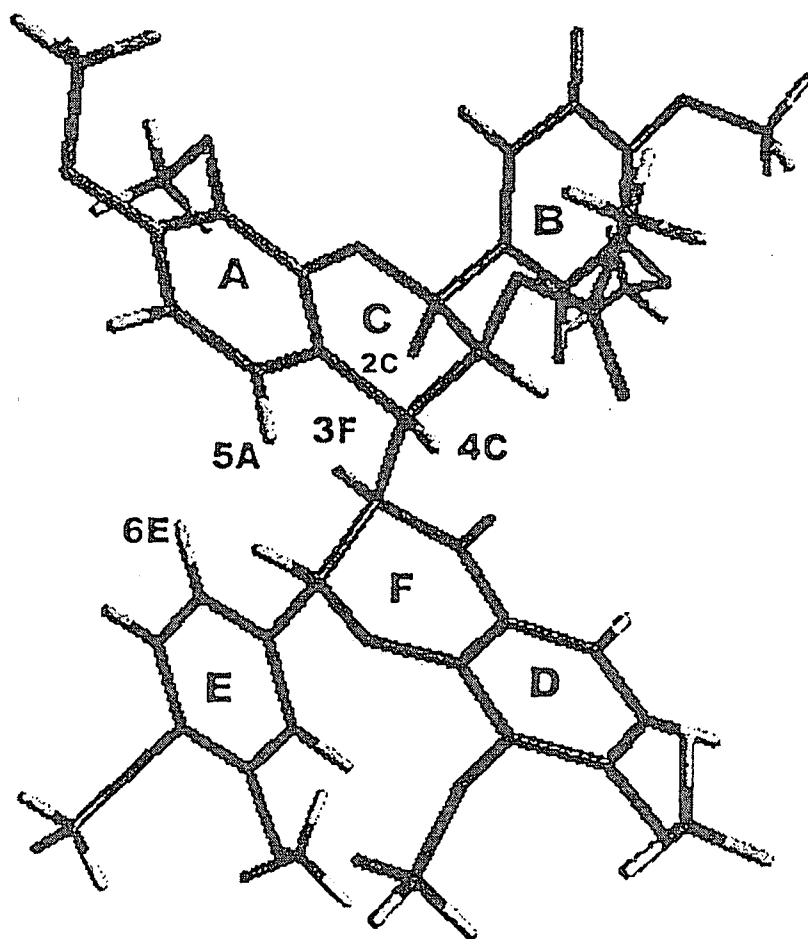


Figure 7.5. Low energy conformation of compound (63) with the E-ring axial.

CHAPTER 8:
NOVEL FLAVANOL – FLAVONOL
PROMELACACINIDIN DIMERS FROM *ACACIA*
NIGRESCENS

8.1 Introduction:

Previous studies^{2,77} on this tree led to the isolation of a variety of 3',4',7,8-tetrahydroxyflavonoids but no dimeric flavonoids were found. As part of our ongoing research^{4,26,36} for the occurrence of oligomeric flavonoids having a pyrogallol A-ring component, two new flavanol-flavonol C₄(C) to C₅(D) linked promelacacinidin dimers were isolated from the heartwood of *Acacia nigrescens*.

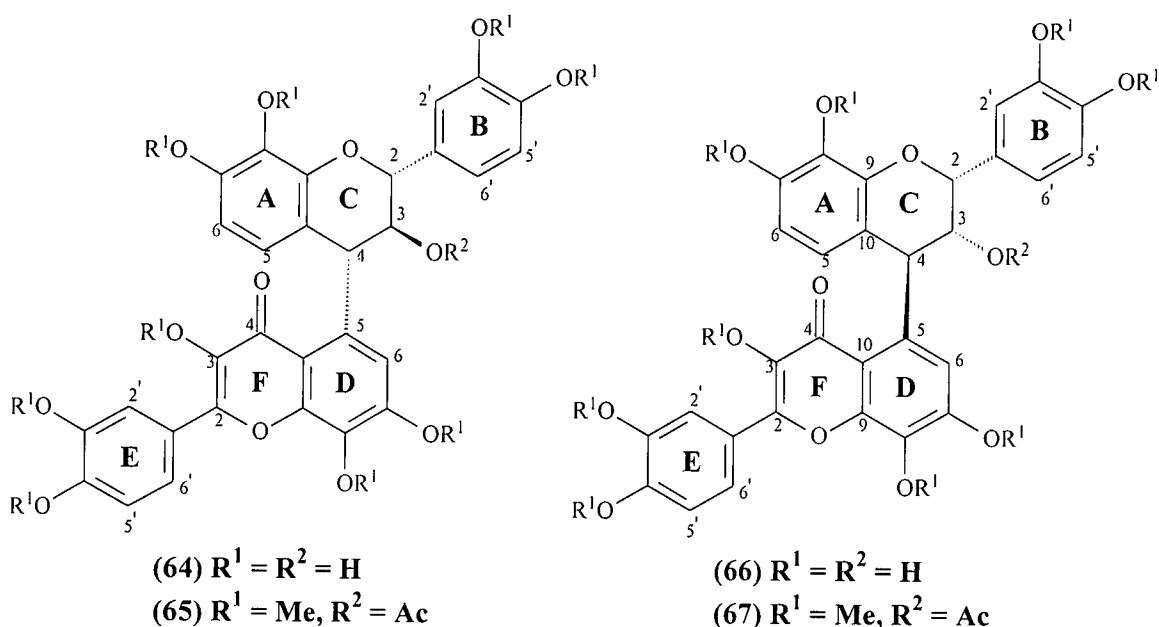
Due to the complexity of the phenolic fraction in which the proanthocyanidins (64) and (66) were found and the presence of tetrameric and higher polymeric compounds, they were purified and identified as the nona-methyl ether acetate derivatives (65) and (67), respectively.

Detailed ¹H, ¹³C and 2D experiments (¹H-¹H COSY, NOESY, HMQC and HMBC) were utilized for the structural elucidation.

8.2 Mesquitol-(4 α →5)-3,3',4',7,8-pentahydroxy flavonone(64) and epimesquitol-(4 β →5)-3,3',4',7,8-pentahydroxy flavonone(66):

¹H-NMR data (table 8.1) of compounds (65) (plate 10) and (67) (plate 11) were used to establish the structures and relative configurations. The presence of nine *O*-methyl and one *O*-acetyl proton signals, together with two AB- and two ABX proton spin systems in the spectra of (65) and (67) suggested the dimeric nature of the two derivatives. The FAB-MS analysis indicated molecular ions of m/z 758 for both the compounds and confirmed their dimeric nature.

Only one AMX-system (C-ring) observed in the $^1\text{H-NMR}$ spectra for both compounds along with a very deshielded pair of E-ring 2',6'-protons at δ 7.88/7.92 and 7.89/7.93 (**table 8.1**) respectively, disclosed the presence of a conjugated carbonyl in the bottom moiety (F-ring) and was indeed supported by the $^{13}\text{C-NMR}$ appearance of δ_{C} signals at 176.7 and 176.0 (Table 8.2) for both (**65**) and (**67**). Combined, this information suggested a flavonol terminal unit. Contrary to what was observed for proanthocyanidin derivatives with C-C interflavanil linkages where the 4-H(C) of the top unit is shielded (1.32-1.82 ppm) relative to the same proton in the permethylaryl ether 3,4-di-*O*-acetyl derivative of the flavan-3,4-diol precursor^{4,75}, the 4-H(C) in both (**65**) and (**67**) was deshielded to δ 6.66 and δ 6.15 respectively, because of the nearby carbonyl at 4-C(F).



Phase sensitive NOESY experiments of (**65**) and (**67**) showed associations between 2-H(C) and 2'-H(B), 6'-H(B); from 4-H(C) to 5-H(A) (**table 8.1**), which facilitated the identification of the systems (A- and B-rings) belonging to the ABC-units. Important is that 2'-H(E) associated with both the 3-OMe(F) and 3'-OMe(E).

HMBC data for compounds (**65**) and (**67**) showed coupling between the 4-H(C) to 4-C(F, $^4\text{J}_{\text{CH}}$), to 5'-C(F, $^2\text{J}_{\text{CH}}$) \rightarrow 5-C(D, $^2\text{J}_{\text{CH}}$) and 6-C(D, $^3\text{J}_{\text{CH}}$), the 6-H(D) coupled to 7- and 8-C(D, $^2\text{J}_{\text{CH}}$, $^3\text{J}_{\text{CH}}$), 4-C(F, $^4\text{J}_{\text{CH}}$) and 10-C(D, $^3\text{J}_{\text{CH}}$); 2'-H(E) to 3-C(F, $^3\text{J}_{\text{CH}}$) \rightarrow 3-C(F,

$^4J_{CH}$). COSY experiments showed coupling between 4-H(C) and 6-H(D, $^4J_{HH}$). This supported the flavanol-flavonol structures as well as the 4-C(C) to 5-C(D) linkage between the units.

Table 8.1 1H NMR peaks (δ_H) of compounds **(65)** and **(67)** at 300 MHz (296K), Splitting patterns and J values (Hz) are given in parentheses.

Ring	Proton	(65)-CDCl ₃	(67)-CDCl ₃
A	5	6.45(d,9.0)	6.77(d,9.0)
	6	6.49(d,9.0)	6.63(d,9.0)
B	2'	6.90(d,2.0)	6.94(d,2.0)
	5'	6.79(d,9.0)	6.78(d,9.0)
	6'	7.00(dd,2.0,9.0)	6.82(dd,2.0,9.0)
C	2	5.29(d,8.0)	5.08(d,1.0)
	3	5.74(dd,8.0,8.0)	5.48(dd,1.0,2.0)
	4	6.66(d,8.0)	6.15(d,2.0)
D	5	-	-
	6	6.59(s)	6.51(s)
E	2'	7.88(d,2.0)	7.89(d,2.0)
	5'	7.05(d,9.0)	7.05(d,9.0)
	6'	7.92(dd,2.0,9.0)	7.93(dd,2.0,9.0)
OMe		3.76,3.81,3.86, 3.87,3.92,3.94, 3.98,4.00(2)	3.75,3.85(2),3.87, 3.93,4.00(2),4.03, 4.04
OAc		1.76	1.96

The ^{13}C analysis confirmed the nine *O*-methyl and one *O*-acetyl groups as well as the suggested carbon structures **(65)** and **(67)** (**table 8.2**). The chemical shifts for the 4-C(C) at δ_C 42.3 (Table 8.2) and 42.6 for **(65)** and **(67)** are in accordance with a phenyl substituent³⁵ at the 4-carbon of the top unit (ABC) of a dimer and confirmed this carbon as a linkage point.

The coupling constants of the heterocyclic proton systems [$J_{2,3}(C) = 8.0$ Hz; $J_{3,4}(C) = 8.0$ Hz] for **(65)** and [$J_{2,3}(C) = 1.5$ Hz; $J_{3,4}(C) = 2.5$ Hz] for **(67)** are reminiscent of a 2,3-*trans*-3,4-*trans* and 2,3-*cis*-3,4-*trans* relative stereochemistry^{4,77} for the respective C-rings. When taken in conjunction with a negative Cotton effect, $[\theta]_{245.2} -8.765 \times 10^3$ for **(65)** and a positive Cotton effect of $[\theta]_{235.9} = 1.528 \times 10^4$ for **(67)**, these are indicative of

4 α - and 4 β -configurations^{83,84} at the 4-C positions respectively. From the above results it was possible to assign a 2*R*,3*S*,4*S*(C-ring) and a 2*S*,3*R*,4*R*(C-ring) absolute configurations⁸⁴ to the top (ABC) units of compounds (65) and (67).

Notwithstanding the identical DEF-units in both derivatives (65) and (67), the different configurations at the stereogenic centers of the top moieties (ABC-unit) resulted in somewhat different preferred conformations (Figures 8.1 and 8.2). The diagnostic NOESY interaction between 3-H(C) and 6-H(D) in the case of (65), prompted the building of a Dreiding model of the dimer, which was complemented by computer modeling and resulted in the structure of the preferred conformation as depicted in Figure 8.1. The distance between 3-H(C) and 6-H(D) was measured at 2.595 Å, between 4-H(C) and the carbonyl at 4-C(F) was measured at 2.165 Å. The energy of this lowest energy conformer was calculated at 240.82 kcal/mole. The bottom unit (DEF) is perpendicular to the plane of the top unit (ABC), but from Figure 1 the bottom moiety is below the plane of the top unit.

⁸³ J. J. Botha, D. A. Young, D. Ferreira and D. G. Roux, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1213.

⁸⁴ M. W. Barrett, W. Klyne, P. M. Scopes, A. C. Fletcher, L. J. Porter, E. Haslam, *J. Chem. Soc., Perkin Trans. 1*, 1979, 2375.

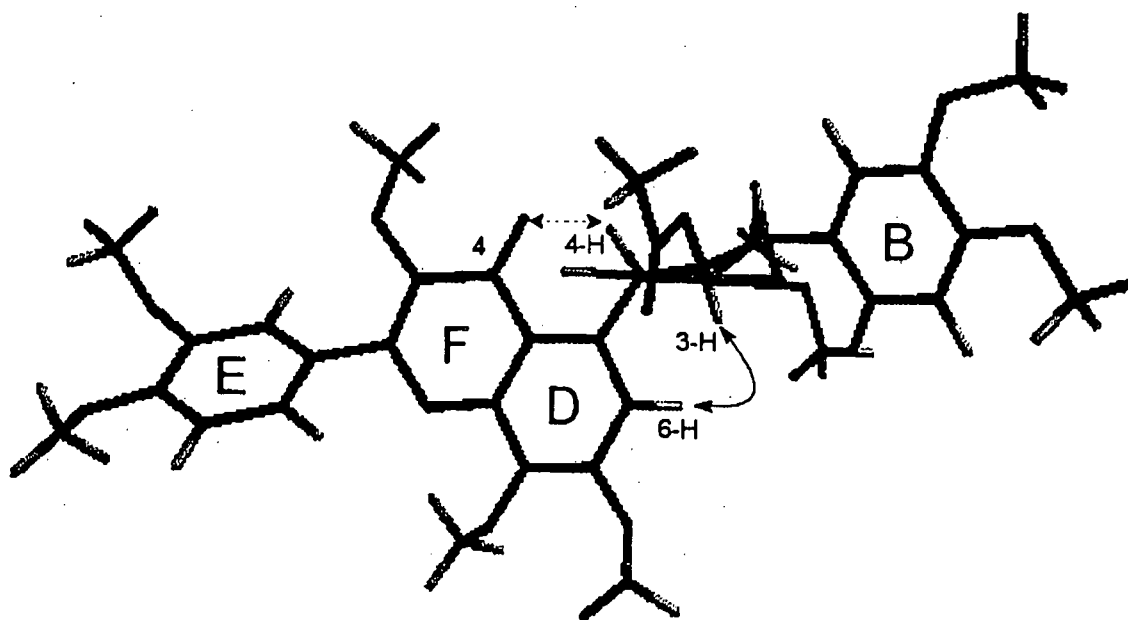


Figure 8.1. Preferred conformation of (**65**) with NOESY interactions (solid arrow) and 4-H(C) to carbonyl distance (dotted arrow).

NOESY interaction between 2-H(C) and 6-H(D) was used as reference for the modelling of compound (**67**) (Figure 8.2), and a distance of 2.261 Å was measured between these protons. The distance between 4-H(C) and the carbonyl functional group was measured at 2.117 Å. The calculated energy of this conformer amounted to 241.52 kcal/mole. The DEF-moiety is perpendicular and at right angles to the plane of the ABC-unit with the D-ring above the plane and the E- and F-rings in the plane of the top unit. The small difference in the calculated relative energy values of the two conformers (Figures 8.1 and 8.2) are insignificant although there is a difference in the absolute stereochemistry of the two dimers (**65**) and (**67**).

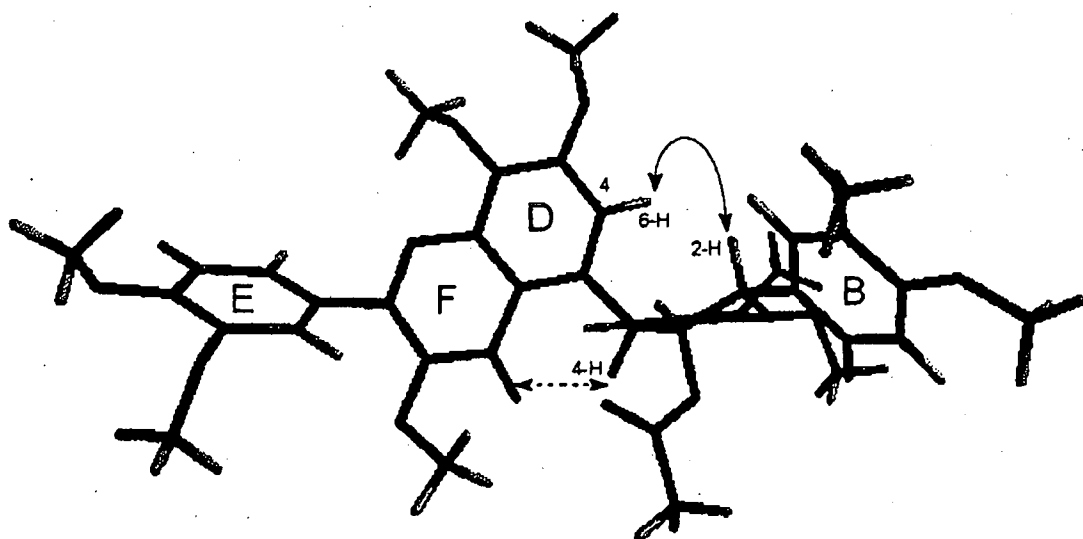


Figure 8.2. Preferred conformation of (67) with NOESY interactions (solid arrow) and 4-H(C) to carbonyl distance (dotted arrow).

Table 8.2: ^{13}C Assignment for compounds (65) and (67). (CDCl_3 , 296K)

Ring	Carbon	(65)	(67)
A/C	2	79.8	73.8
	3	74.2	72.2
	4	42.3	42.6
	5	124.7	125.8
	6	105.9	105.9
	7	152.4	149.1
	8	137.4	137.2
	9	148.6	149.4
	10	118.7	115.3
	B	1'	130.1
2'		110.4	110.2
3'		149.1	152.6
4'		148.8	149.0
5'		111.0	111.0
6'		119.9	119.4
D/F	2	153.3	153.2
	3	141.0	141.4
	4	176.7	176.0
	5	138.9	140.9
	6	111.4	112.7
	7	155.5	155.2
	8	135.9	136.2
	9	150.6	153.2
	10	117.7	116.6
	E	1'	123.8
2'		111.4	111.3
3'		149.2	149.4
4'		151.5	151.4
5'		111.4	111.4
6'		122.4	122.4
OMe		56.2(x2),56.3, 56.4,56.5,56.6, 60.3,61.4,61.8	56.1,56.2,56.3, 56.4,56.5,56.7, 60.3, 61.4,61.8
OAc		21.2	21.4
OAc		169.9	168.9

EXPERIMENTAL

CHAPTER 9:

STANDARD EXPERIMENTAL TECHNIQUES

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

9.1 CHROMATOGRAPHY:

9.1.1 Thin Layer Chromatography:

Qualitative thin layer chromatography (TLC) was conducted on "Merck TLC-aluminium sheets: Silica Gel F₂₅₄" (0.2 mm layer) cut into strips 3 x 5 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 20mm) coated with a layer (1.0 mm) of unactivated Merck Kieselgel 60 PF₂₅₄ (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 PF₂₅₄" 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 120x4 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 (54x6.5 cm) charged with Merck Kieselgel 60 (230-400 mesh) using benzene-Me₂CO as eluent at a flow rate of 60 ml/min.

9.2 DEVELOPMENT OF CHROMATOGRAMS WITH VANILLIN-SULFURIC ACID:

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

9.3 ANHYDROUS SOLVENTS:

THF and diethyl ether was refluxed over sodium-benzophenone under an N₂ –atmosphere until a dark blue colour persisted and was freshly distilled under N₂ prior to use.

DCM and DMF were respectively refluxed over CaH₂ for 12 hours with subsequent fresh distillation under N₂ 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 [200 mg 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 SPECTROMETRICAL METHODS:

9.6.1 Nuclear Magnetic Resonance Spectroscopy (NMR):

¹H NMR spectra were recorded on an AVANCE DPX₃₀₀ Bruker spectrometer with tetramethylsilane as internal standard. The ¹H NMR spectra were recorded at 296K (23 °C).

The solvents used were deuteriochloroform (CDCl_3, δ 7.24), deuteriobenzene ($\text{C}_6\text{D}_6, \delta$ 7.15) and deuterioacetone [$(\text{CD}_3)_2\text{CO}/\text{acetone-d}_6, \delta$ 2.04]. Chemical shifts were expressed in terms of parts per million (ppm) on the δ scale and coupling constants were measured in Hz.

Abbreviations were used as follows:

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

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 $[\theta]$ was:

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

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

CHAPTER 10:

ISOLATION OF PHENOLIC METABOLITES

FROM *ACACIA NIGRESCENS*

10.1 Extraction and enrichment of the heartwood components:

Drillings (11,3 kg) from the heartwood of *A. nigrescens* were first extracted with $(\text{CH}_3)_2\text{CO}$ (3x3.0 l) for 24h periods at room temperature (25°C). Secondly the dried drillings were extracted with MeOH (3x3.0 l) under the same conditions as above. The extract was concentrated by evaporating the MeOH under vacuum (35°C). The concentrate was dissolved in water and then freeze dried to give a dark-brown powder (315 g). An enriched extract was obtained by repeated FCC of 7x6 g quantities of the MeOH extract, using Merck Kieselgel 60 as stationary phase and benzene-Me₂CO (8:2, v/v) as eluent. The following combinations were obtained:

Fraction	Tubes	Yield
A	21 - 25	9.09 g
B	53 - 143	14.82 g
C	144 - 360	6.72 g

10.2 Separation of the phenolic components:

The enriched combination C (6.72 g) was separated on a Sephadex LH-20 column using EtOH as eluent. The first 2000 ml of EtOH was discarded and the fractions were combined as indicated in **table 10.1**.

All the fractions were analyzed by TLC and certain selected fractions were analyzed by PLC. According to the initial analyses, fractions A1 up to A17 (3.582 g) consisted mainly of known monomeric flavonoids². Small quantities of these selected fractions were derivatized to identify the the melacacinidin monomers for later use in the synthetic study.

Table 10.1:

Fraction	Tubes	Yield (mg)
A1	1 - 25	43.3
A2	26 - 66	105.7
A3	67 - 90	23.3
A4	91 - 120	61.6
A5	121 - 151	15.6
A6	152 - 189	109.4
A7	190 - 250	664.6
A8	251 - 278	64.9
A9	279 - 300	41.9
A10	301 - 349	268.4
A11	349 - 363	36.2
A12	364 - 415	395.6
A13	416 - 440	114.7
A14	441 - 500	1034.4
A15	501 - 556	314.2
A16	557 - 597	236.2
A17	597 - 621	66.8
A18	622 - 682	618.0
A19	683 - 714	157.5
A20	715 - 753	325.8
A21	753 - 779	26.5
A22	780 - 802	26.5

Fractions A18 to A22 (named A20) were combined (1.153 g) and after methylation subjected to FCC separation using benzene-Me₂CO (9:1, v/v) as eluent at a flow rate of 60

ml/min. With the use of TLC the following 22 fractions were collected, combined and identified (table 10.2).

Table 10.2:

Fractions	Yield (mg)
A20/1	49.0
A20/2	24.5
A20/3	21.2
A20/4	196.8
A20/5	108.1
A20/6	67.6
A20/7	39.3
A20/8	96.9
A20/9	31.0
A20/10	41.0
A20/11	43.4
A20/12	13.8
A20/13	24.9
A20/14	44.0
A20/15	67.6
A20/16	100.7
A20/17	13.8
A20/18	64.5
A20/19	40.3
A20/20	104.3
A20/21	29.0
A20/22	80.4

Most of the fractions comprised of polymeric material except four fractions, which were acetylated and subsequently purified by TLC as reported with the specific compounds isolated. All R_f values are given in (B:A; 9:1, v/v) , unless otherwise stated.

10.3 Analysis of fraction A7:

Acetylation of a portion (50 mg) of fraction A7 followed by PLC (B:A; 9:1, v/v) gave two main bands A7.1 (R_f 0.54, 7.7 mg) and A7.2 (R_f 0.41, 11.5 mg).

10.3.1 (2R)-3,3',4',7,8-penta-acetoxylidihydroflavonol(53):

Band A7.1 comprised of the title compound(53) as an *amorphous solid*.

^1H NMR data : Plate 1

10.3.2 3',4',7,8-tetra-acetoxylflavanone(55):

Band A7.2 comprised of the title compound(55) as an *amorphous solid*.

^1H NMR data : Plate 2

10.4 Analysis of fraction A10:

10.4.1 3,3',4',7,8-pentamethoxyflavonol(54):

Methylation of a portion (50 mg) of fraction A10 followed by PLC (B:A; 9:1, v/v) gave one main band (R_f 0.61, 34.7 mg), which comprised of the title compound(54) as an *amorphous solid*.

^1H NMR data : Plate 3

10.5 Analysis of fraction A16:

Methylation and acetylation of a portion (50 mg) of fraction A16 followed by PLC (B:A; 9:1 x2, v/v) gave two main bands A16.1 (R_f 0.17, 24 mg) and A16.2 (R_f 0.15, 19 mg).

10.5.1 (2R,3R,4R)-2,3-*cis*-3,4-*cis*-3,4-diacetoxy-3',4',7,8-tetramethoxyflavan (51) [epimesquitol-4 α -ol tri-O-methylether diacetate]

Band A16.1 comprised of the title compound(51) as an *amorphous solid*.

$^1\text{H NMR}$ data : Plate 4

CD data : Plate 1

10.5.2 (2R,3R,4S)-2,3-*cis*-3,4-*trans*-3,4-diacetoxy-3',4',7,8-tetramethoxyflavan (52) [epimesquitol-4 β -ol tri-O-methylether diacetate]

Band A16.2 comprised of the title compound(52) as an *amorphous solid*.

$^1\text{H NMR}$ data : Plate 5

CD data : Plate 2

10.6 Analysis of fraction A20/21:

10.6.1 Epimesquitol-(4 β →6)-epimesquitol-4 α -ol octa-O-methylether triacetate(57):

Acetylation of fraction A20/21 followed by PLC (B:A; 8:2 x3, v/v) purification yielded one main band, the title compound A20/21.1 (R_f 0.57, 35.0 mg), as a *white amorphous solid*.

Found : M^+ , 832.2941. $C_{44}H_{48}O_{16}$ requires M^+ , 832.2942

1H NMR data : Plate 6, Table 7.1

CD data : Plate 3

10.7 Analysis of fraction A20/16:

The acetylated fraction A20/16 was separated on PLC (B:A; 8:2 x2, v/v) and gave five main bands:

A20/16.1 (R_f 0.55, 47.9 mg)
A20/16.2 (R_f 0.48, 12.3 mg)
A20/16.3 (R_f 0.40, 26.1 mg)
A20/16.4 (R_f 0.28, 6.4 mg)
A20.16.5 (R_f 0.16, 2.5 mg)

10.7.1 Epimesquitol-(4 β →6)-epimesquitol-4 β -ol octa-O-methylether triacetate(58):

PLC separation of A20/16.1 (H:B:A:EA; 4:2:1:1.5 x3, v/v) gave three bands, A20/16.1.1 (R_f 0.58, 10.1 mg), A20/16.1.3 (R_f 0.53, 7.1 mg) and the title compound A20/16.1.2 (R_f 0.55, 13.9 mg) as a *white amorphous solid*.

Found : M^+ , 832.2939. $C_{44}H_{48}O_{16}$ requires M^+ , 832.2942

1H NMR data : Plate 7, Table 7.1

CD data : Plate 4

10.7.2 Mesquitol-(4 α →5)-epimesquitol-4 β -ol octa-O-methylether triacetate(61):

PLC separation of A20/16.3 (H:B:A:EA; 4:2:1:1.5 x3, v/v) gave two bands, A20/16.3.1 (R_f 0.46 – 0.37, 3.2 mg) and the title compound A20/16.3.2 (R_f 0.40, 14.1 mg) as a *white amorphous solid*.

Found : M^+ , 832.2940. $C_{44}H_{48}O_{16}$ requires M^+ , 832.2942

1H NMR data : Plate 8, Table 7.2

CD data : Plate 5

Bands A20/16.4 and A20/16.5 were not further investigated.

10.8 Analysis of fraction A20/6:

After acetylation of fraction A20/6 it was separated on PLC (B:A; 9:1 x4, v/v) and yielded four bands:

A20/6.1 (R_f 0.63 – 0.49, 21.2 mg)

A20/6.2 (R_f 0.53, 19.2 mg)

A20/6.3 (R _f 0.49, 4.4 mg)
A20/6.4 (R _f 0.49, 3.2 mg)

10.8.1 Ent-epimesquitol tetra-O-methyltriacetyl-(4 α →3)-3',4',7,8-tetra-O-methylflavanone(63):

PLC separation of A20/6.1 (H:B:A:EA; 4:2:1:1.5 x4, v/v) gave four bands, A20/6.1.1 (R_f 0.60, 2.7 mg), A20/6.1.2 (R_f 0.59 – 0.51, 9.9 mg), A20/6.1.3 (R_f 0.52, 1.8 mg) and the title compound A20/6.1.4 (R_f 0.50, 6.2 mg) as a *white amorphous solid*.

Found : M⁺, 730. C₄₀H₄₂O₁₃ requires M⁺, 730.368

¹H NMR data : Plate 9, Table 7.4

CD data : Plate 6

Fraction A20/6.1.2 was separated by PLC, but only polymeric material was found. No investigation was done on fractions A20/6.1.1 and A20/6.1.3 because of the lack of material.

10.8.2 Mesquitol-(4 α →5)-melanoxetin nona-O-methyl ether acetate(65):

PLC separation of A20/6.2 (H:B:A:EA; 4:2:1:1.5 x4, v/v) gave two bands, A20/6.2.1 (R_f 0.58, 2.5 mg) and the title compound A20/6.2.2 (R_f 0.55, 14.1 mg) as a *yellowish amorphous solid*.

Found : M^+ , 758.2564. $C_{40}H_{42}O_{13}$ requires M^+ , 7582566.
 1H NMR data : Plate 10, Table 8.1
CD data : Plate 7

10.9 Analysis of fraction A20/10:

10.9.1 Epimesquitol-(4 β →5)-melanoxetin nona-O-methyl ether acetate(67):

PLC separation (B:A; 8:2 x2, v/v) of fraction A20/10 gave three bands, A20/10.1 (R_f 0.67, 12.3 mg), A20/10.2 (R_f 0.55, 8.1 mg) and the title compound A20/10.3 (R_f 0.50, 17.0 mg) as a *yellow-brown amorphous solid*.

Found : M^+ , 758.2567. $C_{40}H_{42}O_{13}$ requires M^+ , 758.2566.
 1H NMR data : Plate 11, Table 8.1
CD data : Plate 8

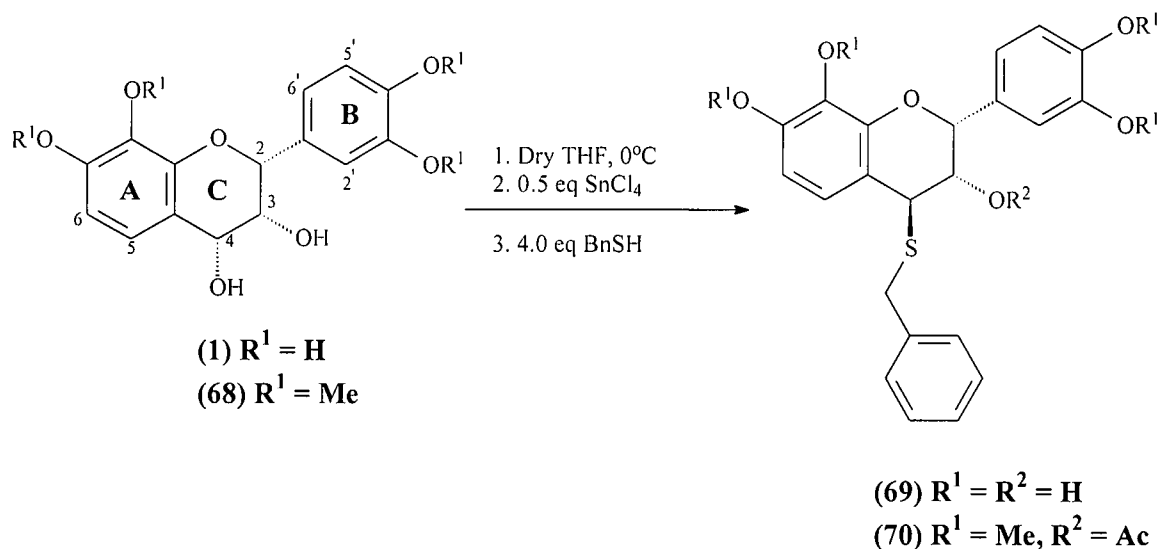
Fractions A20/10.1 and A20/10.2 contained polymeric material.

CHAPTER 11:
SYNTHESIS OF C-C LINKED
PROMELACACINIDINS

11.1 SYNTHESIS OF (2R,3S,4S)-2,3-CIS-3,4-TRANS-3,3',4',7,8-PENTAHYDROXY-4-BENZYLTHIOFLAVAN(69):

[epimesquitol-4 β -benzylthioether]

Epimesquitol-4 α -ol(**1**) (100 mg) was dissolved in dry THF (10 ml) and cooled to 0°C. Under a blanket of N₂ 0.5 equivalents of SnCl₄ (0.13 ml) was added, followed by immediate addition of 4 equivalents of BnSH (0.14 ml). The mixture was stirred for 2 h at 0°C. The solvent was removed under a stream of N₂ and the mixture separated by PLC (B:A; 9:1 x2, v/v).



Two main bands were obtained; B2 (R_f 0.56, 12.1 mg) and the title compound B1 (R_f 0.76, 85.6 mg) as a *yellow amorphous solid*. B1 comprised the unreacted starting material, a portion (10 mg) was derivatized and ¹H NMR identification was done on compound (**70**).

11.2 ATTEMPTED SYNTHESIS OF C-C LINKED PROMELACACINIDINS:

11.2.1 Standard procedure for the synthesis of proanthocyanidin dimers:

The epimesquitol-4 β -thiobenzylether(**69**) and epimesquitol-4 α -ol(**1**) (2 eq) were dissolved in dry THF (10 ml). The mixture was stirred under a N₂-atmosphere for 15 min at 0°C. Solid AgBF₄ (2.5 eq) or DMTSF (1 eq) was added and the reaction was stirred for a further 90 min at 0°C. After the addition of ice (50 g) the mixture was extracted with EtOAc (50 ml x 6). The combined organic layers were dried (Na₂SO₄) and evaporated under vacuum.

11.2.2 Attempted synthesis of epimesquitol-(4 β →6)-epimesquitol-4 α -ol(**68**):

A mixture of epimesquitol-4 β -thiobenzylether(**69**) (45 mg, 8.8206 x 10⁻⁵ mole), epimesquitol-4 α -ol(**1**) (74.6 mg, 2.1254 x 10⁻⁴ mole, 2.4 eq) and AgBF₄ (50 mg, 2 eq)/DMTSF (20 mg, 1 eq) was stirred at 0°C for different reaction times. PLC (B:A:M; 6:3:1, v/v) separation of the reaction mixture yielded nothing but epimesquitol-4 α -ol(**1**). After repeated attempts this methodology was abandoned and due to time constraints no further reactions were attempted.

CHAPTER 12: PROCEDURES CARRIED OUT ON FLAVAN-3-ONES

12.1 Standard procedure for the preparation of flavan-3-ones:

The appropriate flavan-3,4-diol (50 mg) was dissolved in dry THF (10 ml) and treated with 0.35 equiv. of PBr₃ (0.049 ml). The reaction mixture was stirred under an N₂-atmosphere for 2 h at room temperature (22°C), the volume was reduced under vacuum and the products were separated by PLC.

12.2 Epioritin-4 α -ol: (starting compound)

12.2.1 (2R)-4',7,8-Trimethoxyflavan-3-one(48):

Preparation according to the standard procedure followed by PLC (B:A; 8:2, v/v) separation yielded the title compound(48) (R_f 0.80, 37 mg) as an *amorphous solid*.

Found : M⁺, 314.1152. C₁₈H₁₈O₅ requires M⁺, 314.1154.

¹H NMR data : Plate 13

12.2.2 (2R)-3-Acetoxy-4',7,8-trimethoxyflav-3-ene(47):

After acetylation and purification by means of PLC (B:A; 9:1, v/v) the title compound(47) (R_f 0.66, 35.8 mg) was obtained as an *amorphous solid*.

Found : M⁺, 356.1260. C₂₀H₂₀O₆ requires M⁺, 356.1259.

¹H NMR data : Plate 14

12.3 Fisetinidol-4 α -ol: (starting compound)

12.3.1 (2R)-3',4',7-Trimethoxyflavan-3-one(52):

Preparation according to the standard procedure followed by PLC (B:A 8:2, v/v) separation yielded the title compound(52) (R_f 0.78, 38.2 mg) as an *amorphous solid*.

Found : M^+ , 314.1154. $C_{18}H_{18}O_5$ requires M^+ , 314.1154.

1H NMR data : Plate 15

12.4 Mesquitol-4 α -ol: (starting compound)

The (2R)-3',4',7,8-tetramethoxyflavan-3-one(55) could not be isolated.

12.4.1 (2R)-3-Acetoxy-3',4',7,8-tetramethoxyflav-3-ene(61):

The reaction mixture was prepared according to the standard procedure and because compound (55) could not be isolated, the whole reaction mixture was acetylated and then PLC (B:A; 9:1 x2, v/v) separation afforded the title compound(61) (R_f 0.44, 3.1 mg) as a pure *amorphous solid*.

1H NMR data : Plate 16

12.5 Standard procedure for the reduction of flavan-3-ones:

Compound (48) (20.1 mg) was dissolved in EtOH (10 ml), $NaBH_4$ (4 mg) added and the mixture was stirred at room temperature (25°C) for 3 h. The mixture was quenched with 0.3 M HCl (10 ml), filtered and the solvent removed under vacuum. The epimeric

mixture was acetylated and separated by PLC (H:A:EA; 92:4:4 x5, v/v) to give compounds (49) and (50) as pure products.

12.5.1 (2R,3R)-2,3-*cis*-3-Acetoxy-4',7,8-trimethoxyflavan(50):

The title compound(50) (R_f 0.36, 7.6 mg) was isolated as a *white amorphous solid*.

$^1\text{H NMR}$ data : Plate 17

CD data : Plate 9

12.5.2 (2R,3S)-2,3-*trans*-3-Acetoxy-4',7,8-trimethoxyflavan(49):

The title compound(49) (R_f 0.28, 3.9 mg) was isolated as a *white amorphous solid*.

$^1\text{H NMR}$ data : Plate 18

CD data : Plate 10

12.6 Standard procedure for the oxidation of flavan-3-ones:

The appropriate flavan-3-ones (10 mg) were dissolved in THF (10 ml), 5% H_2O_2 (4 ml) added and the mixture was stirred at room temperature (25°C) for 1 h. The mixture was quenched with ice and work-up comprised extraction with Et_2O (3 x 20 ml). The combined extract was evaporated under vacuum.

12.6.1 3-Acetoxy-4',7,8-trimethoxyflavonol(53):

After acetylation, PLC (B:A; 9:1, v/v) separation afforded the title compound(53) (R_f 0.58, 4.2 mg) as a pure *yellow amorphous solid*.

$^1\text{H NMR}$ data : Plate 19

12.6.2 3-Acetoxy-3',4',7-trimethoxyflavonol(54):

After acetylation, PLC (B:A; 9:1, v/v) separation afforded the title compound(54) (R_f 0.54, 2.4 mg) as a pure *yellow amorphous solid*.

^1H NMR data : Plate 20

APPENDIX A: NMR SPECTRA

(2R)-3,3',4',7,8-penta-acetoxylflavonol(53)

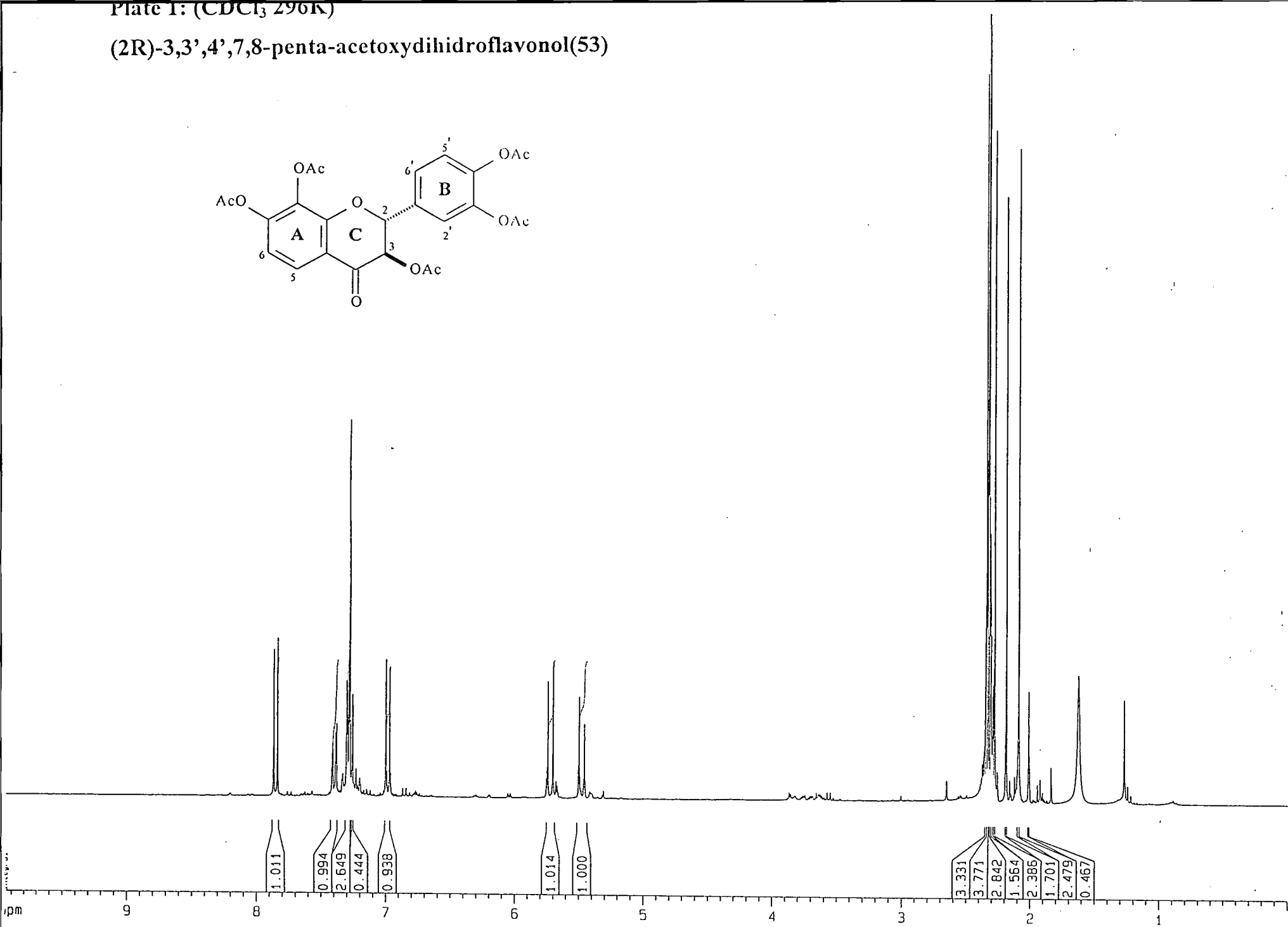
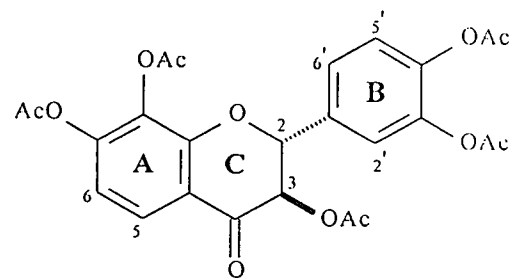


FIGURE 1. (CDCl₃ 25°C)

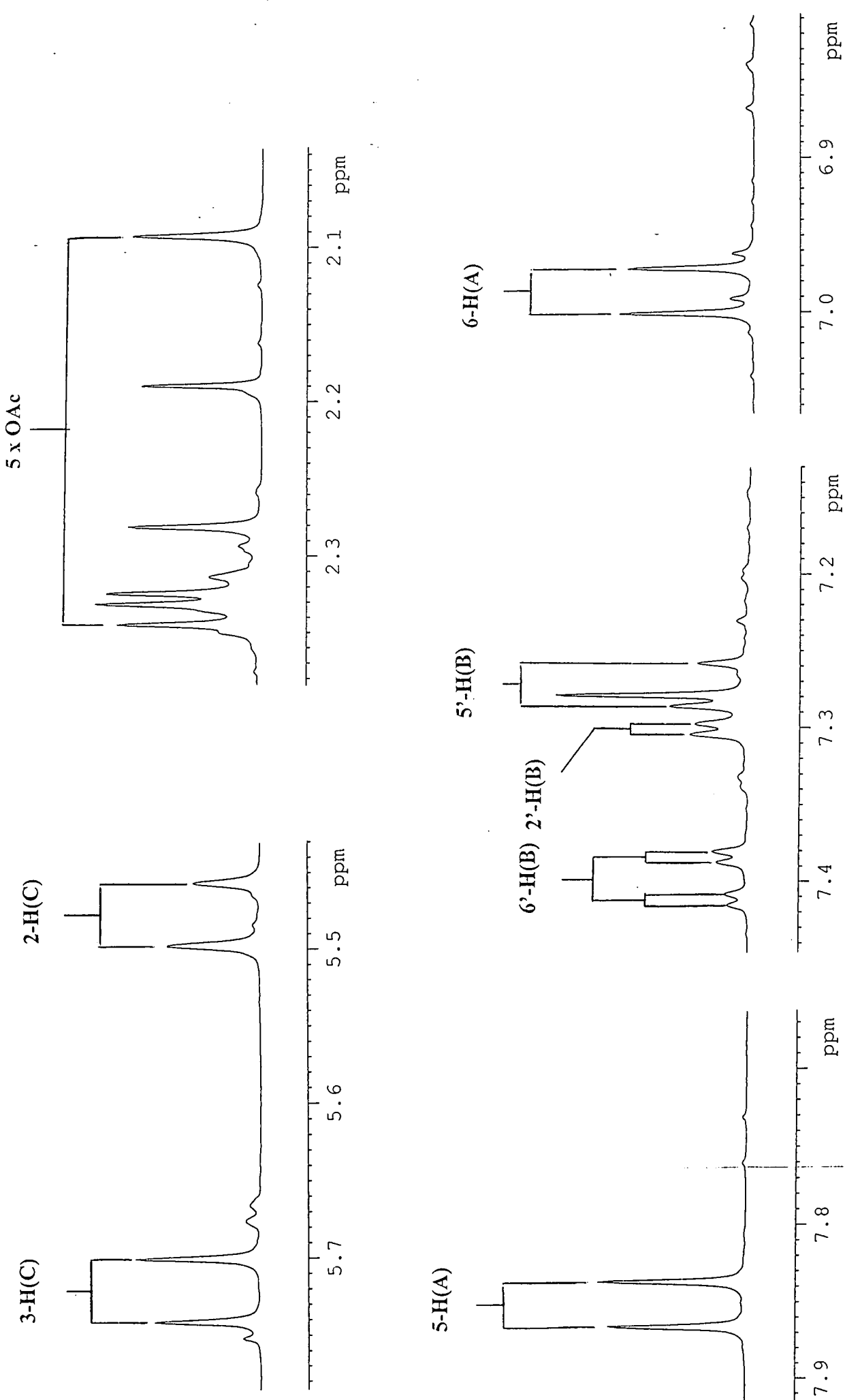
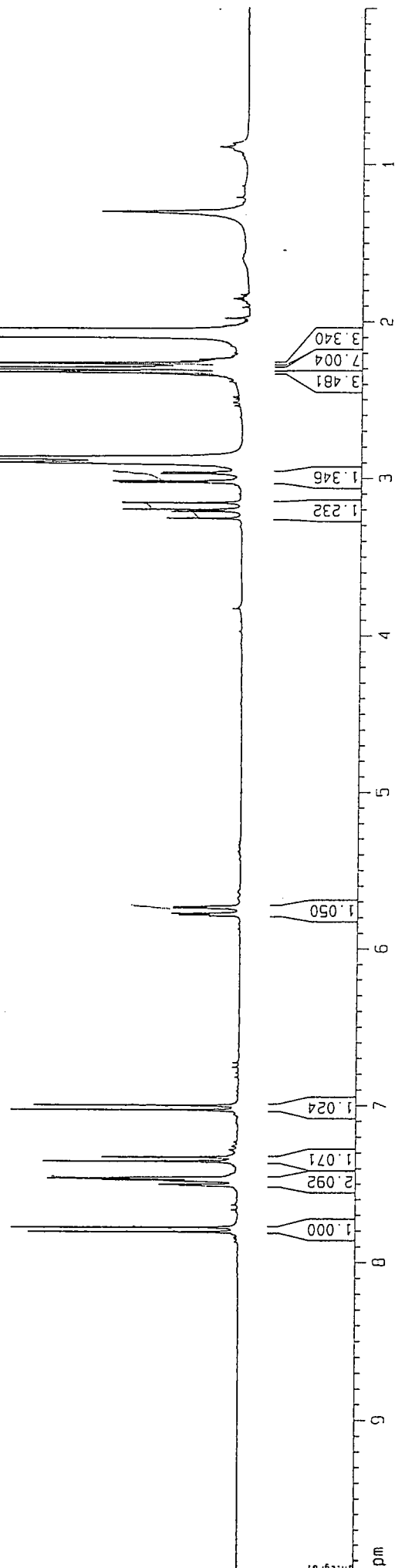
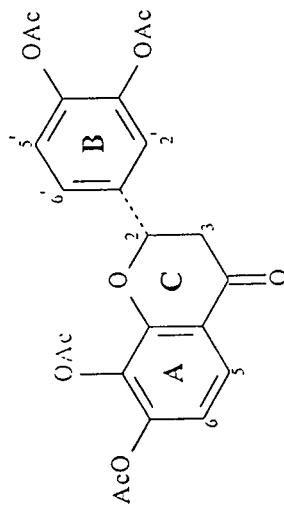
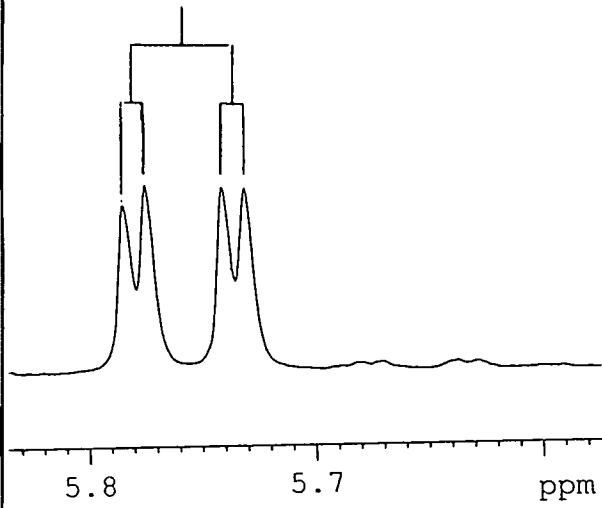


Plate 2: [(CD)₃CO, 296K]

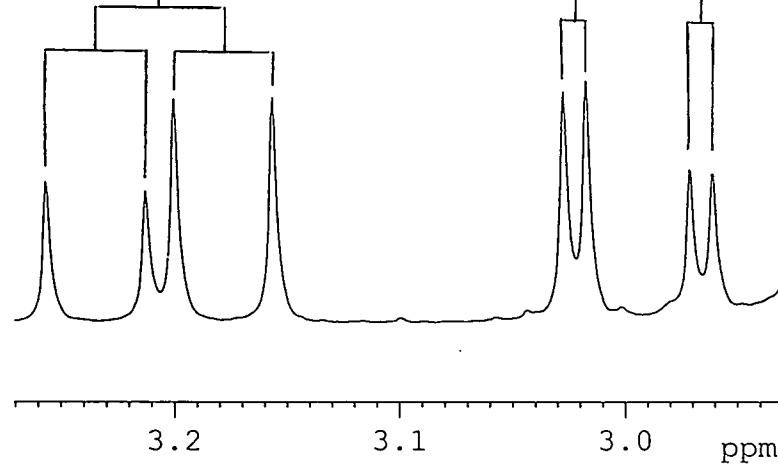
3',4',7,8-tetra-acetoxyflavanone(55)



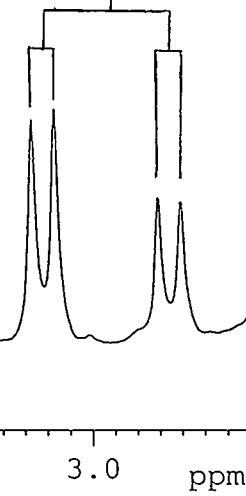
2-H(C)



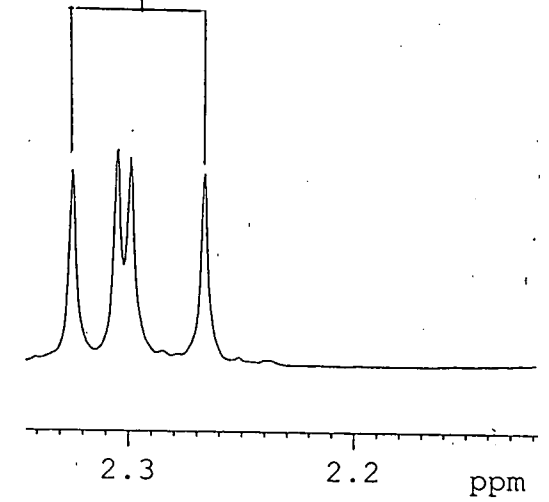
3eq



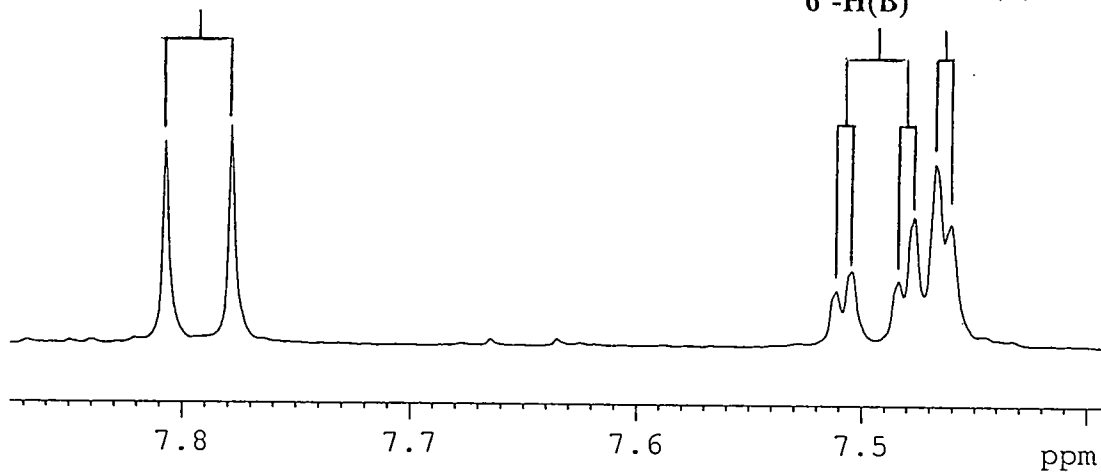
3ax



4 x OAc

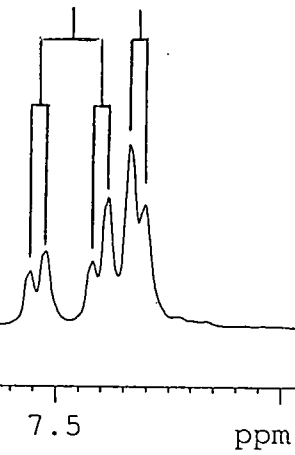


5-H(A)

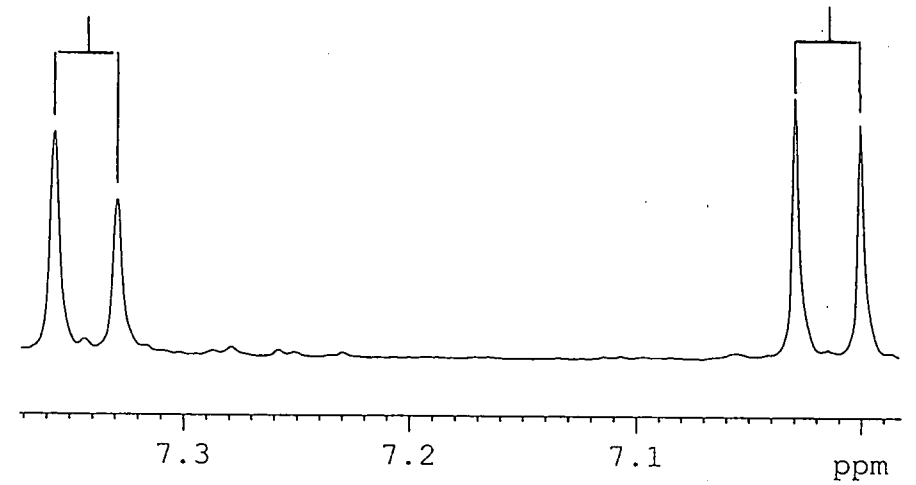


6'-H(B)

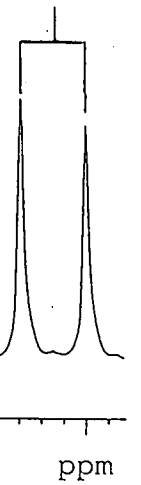
2'-H(B)



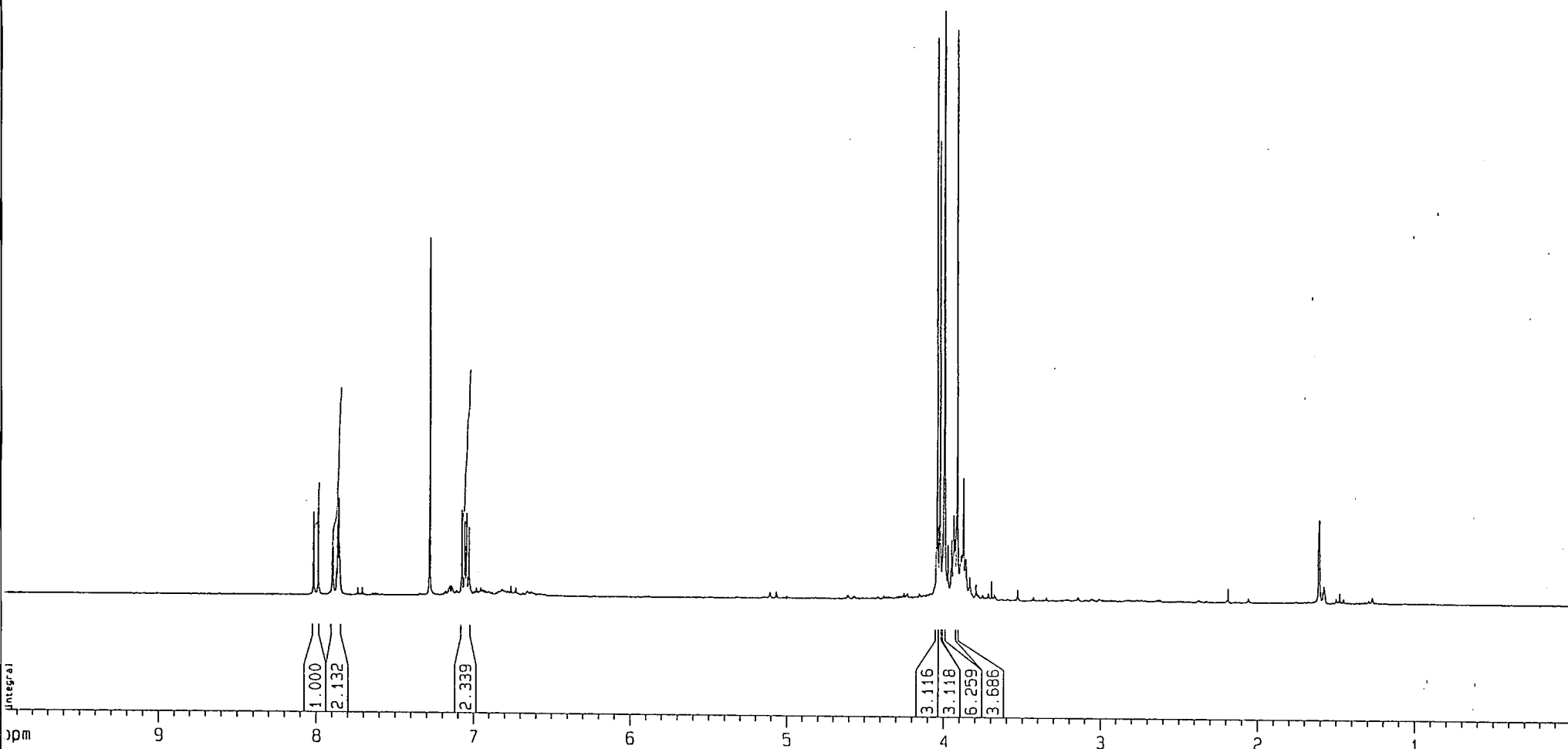
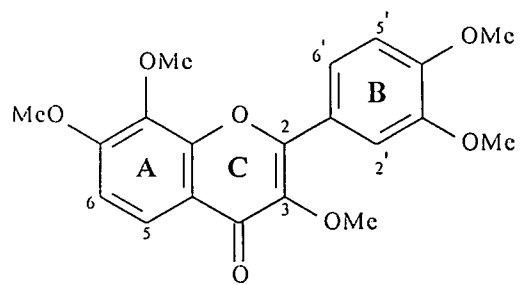
5'-H(B)



6-H(A)



3,3',4',7,8-pentamethoxyflavonol(54)



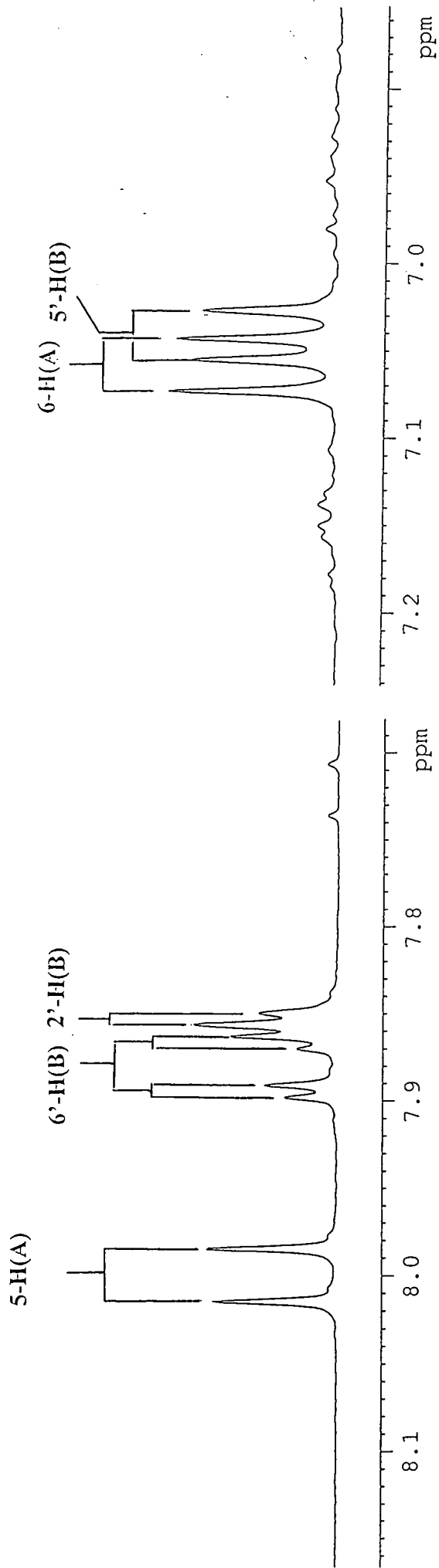
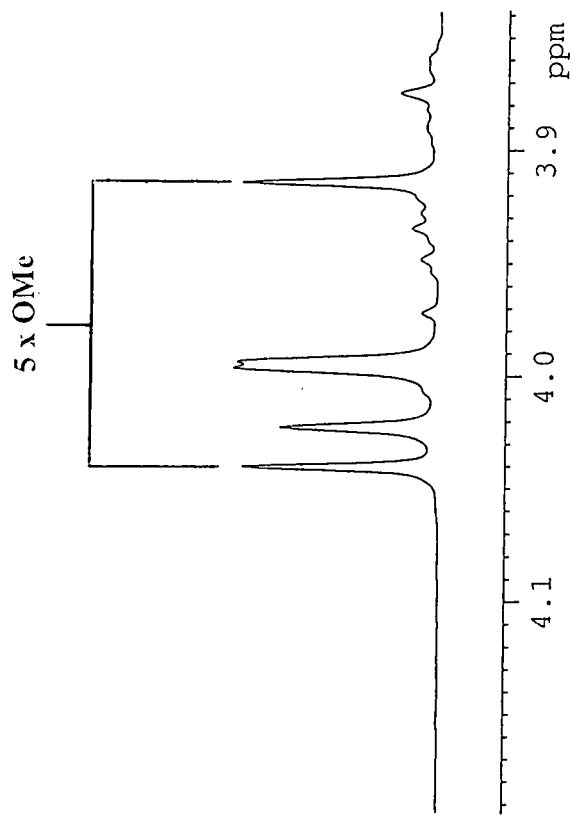
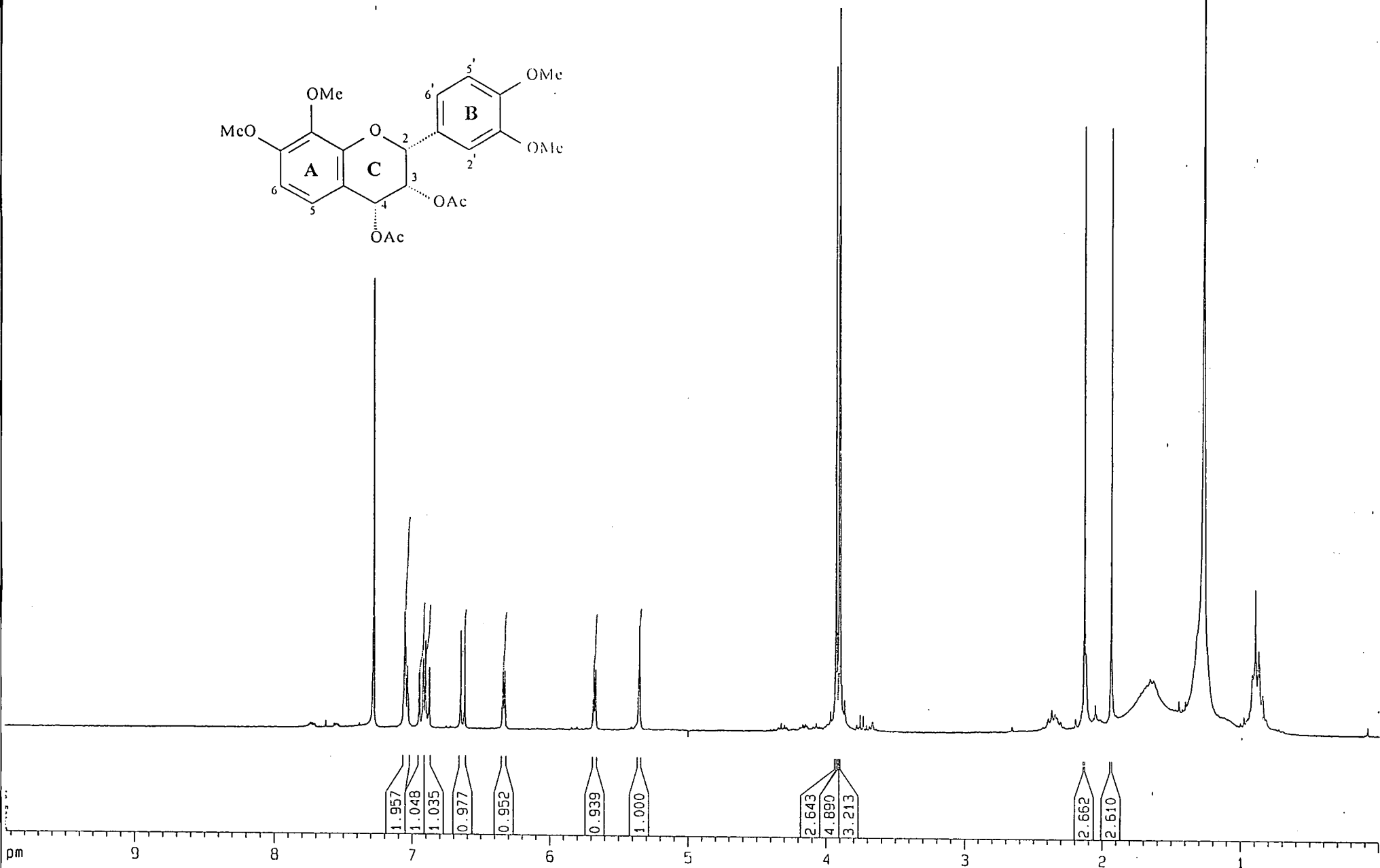
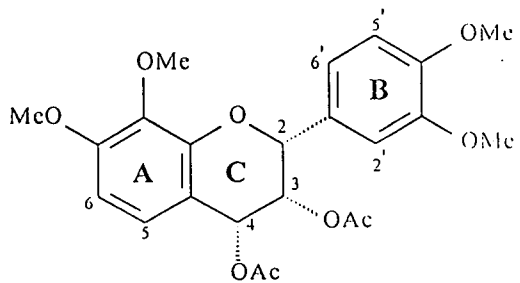
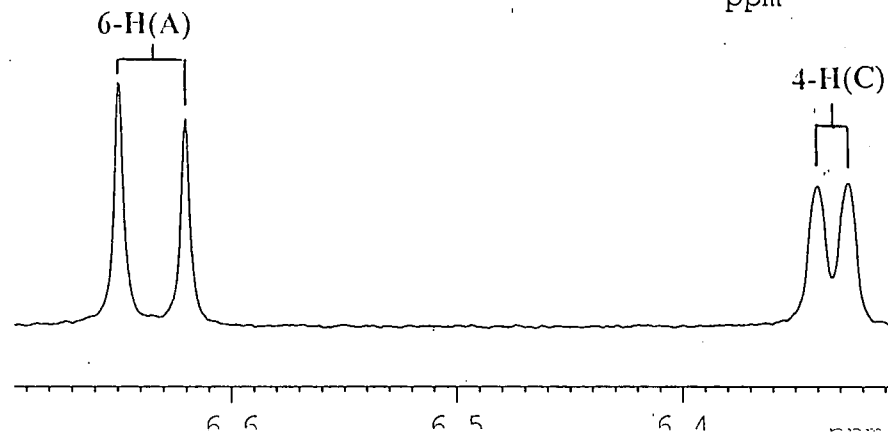
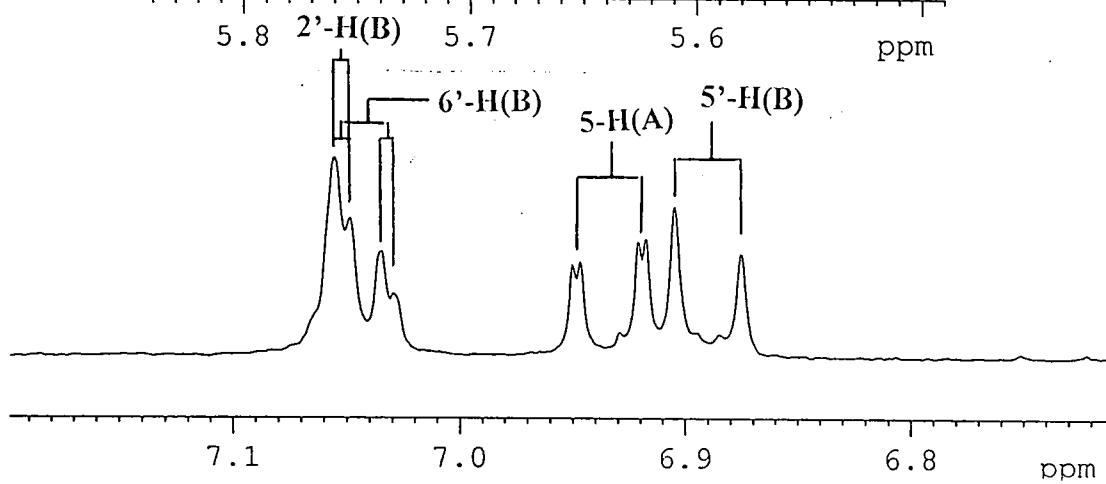
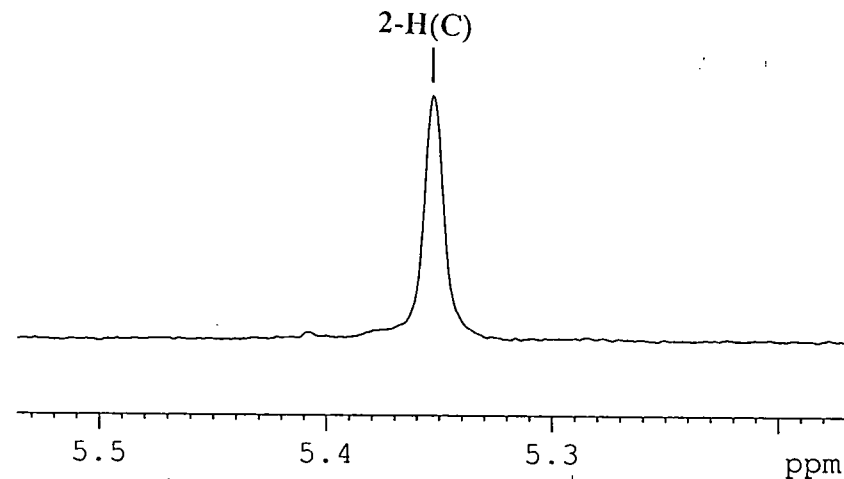
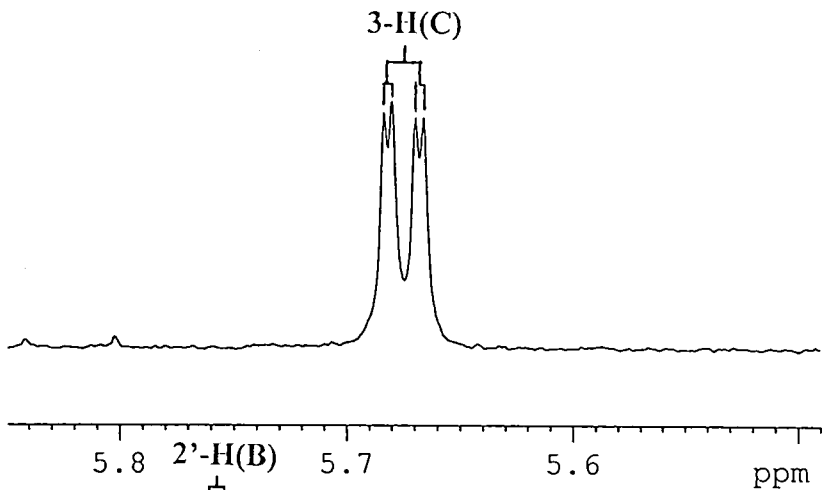
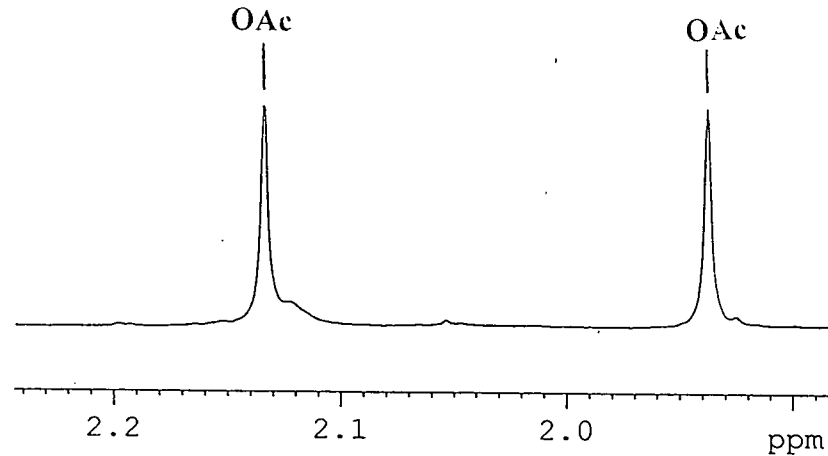
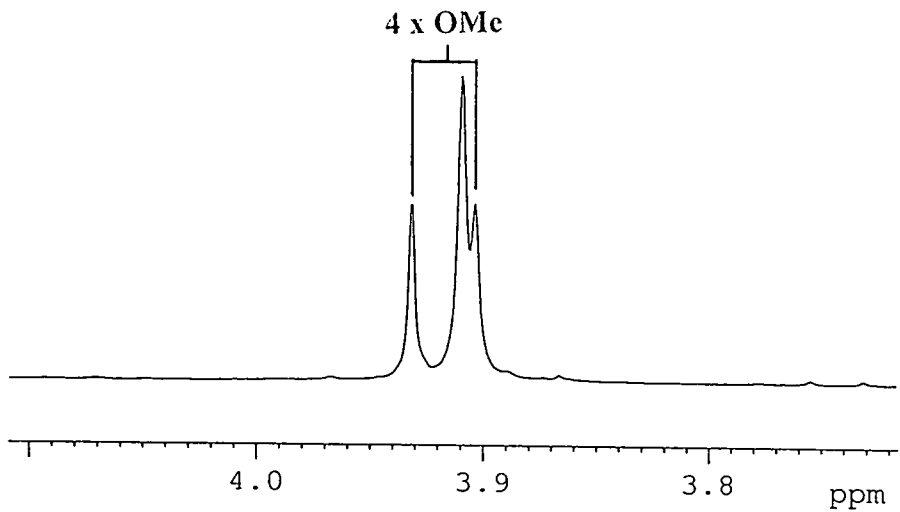


Plate 4: (CDCl₃, 296K)

(2R,3R,4R)-2,3-cis-3,4-cis-3,4-diacetoxy-3',4',7,8-tetramethoxyflavan

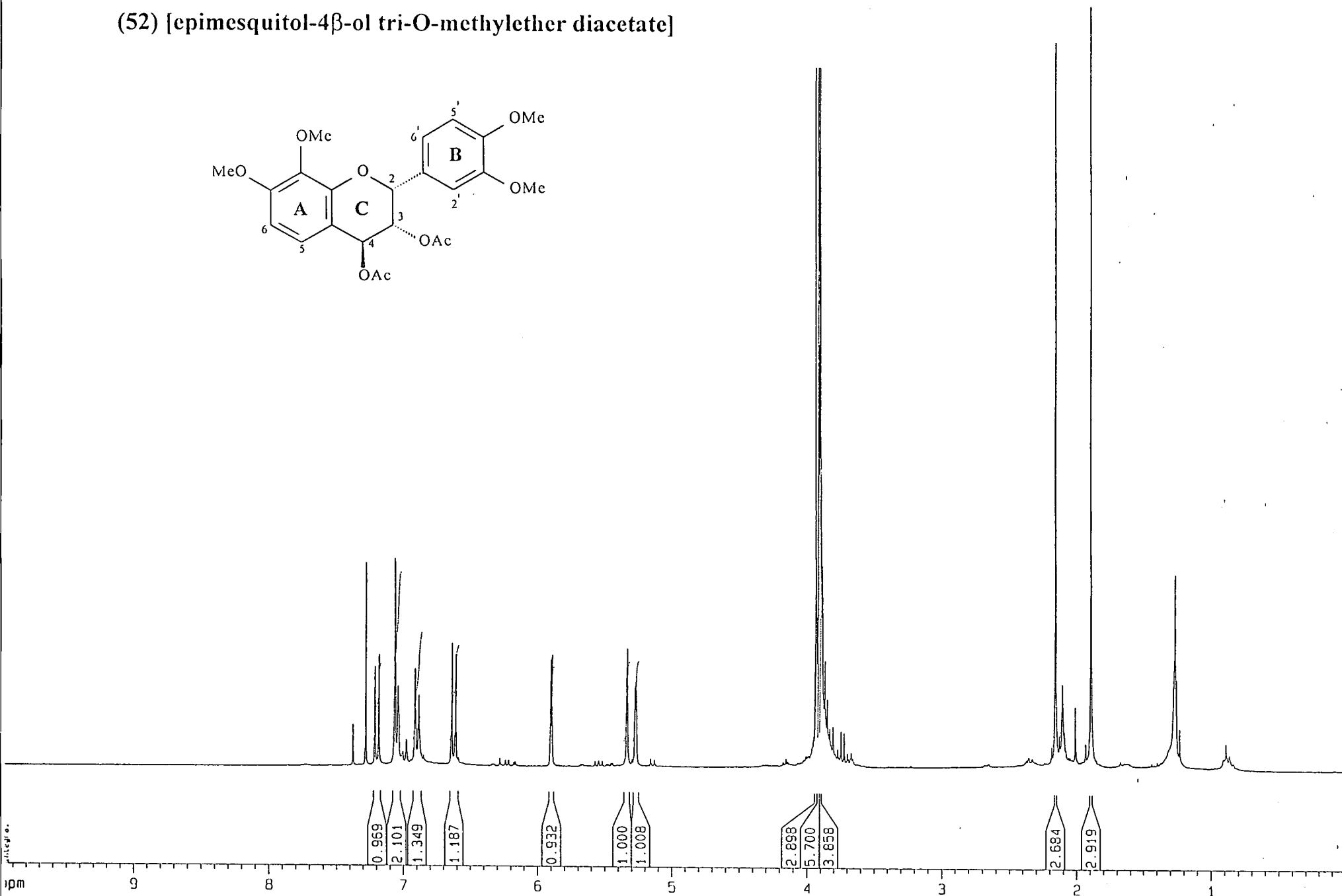
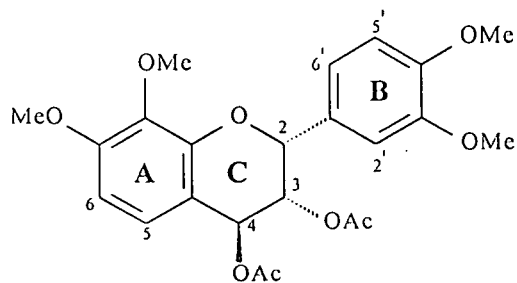
(51) [epimesquitol-4 α -ol tri-O-methylether diacetate]

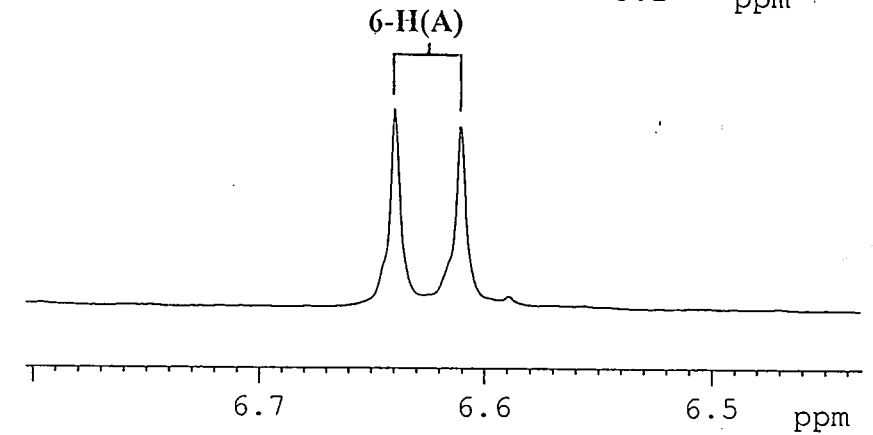
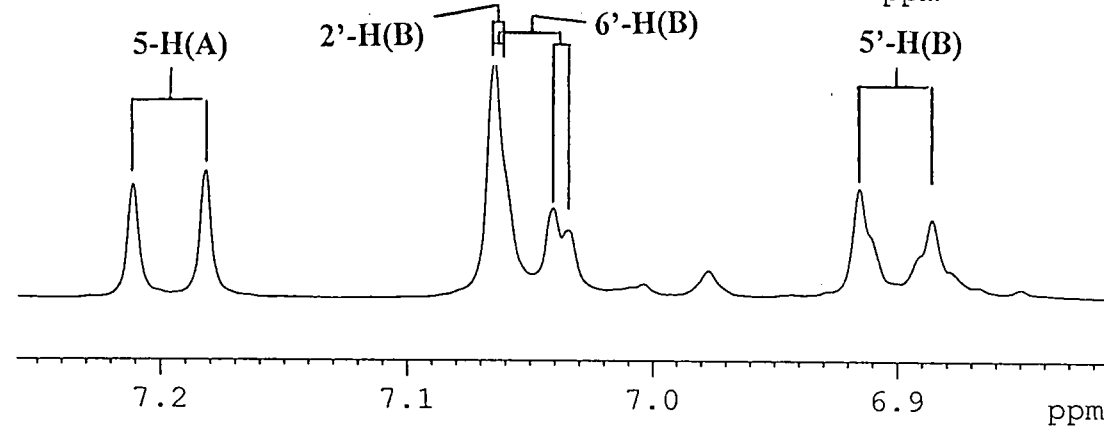
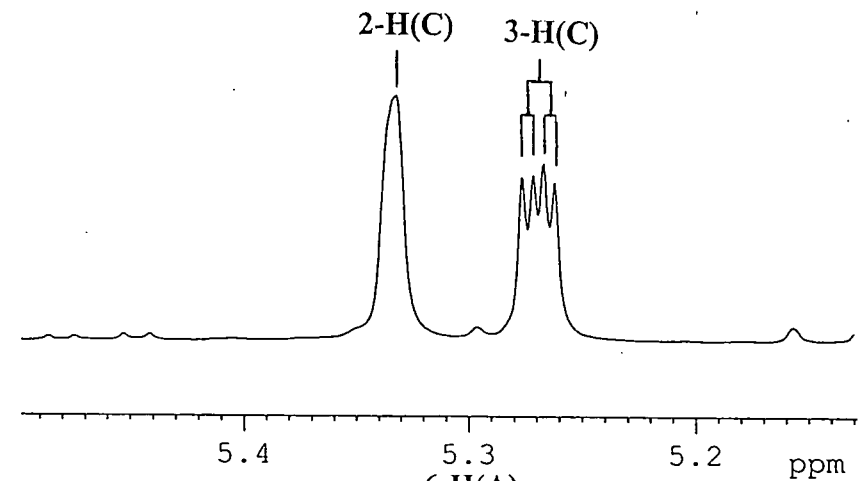
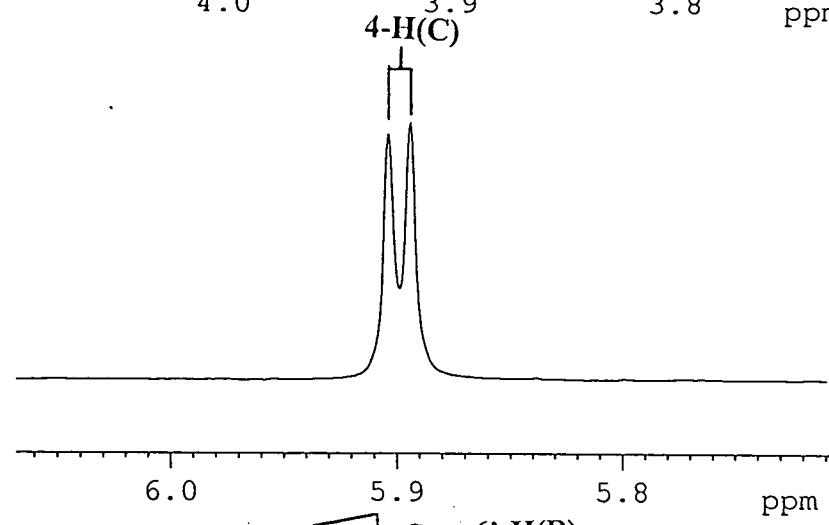
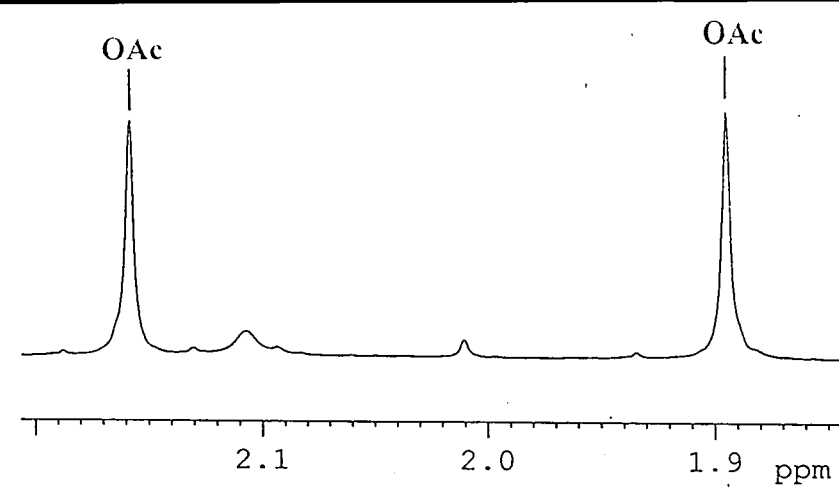
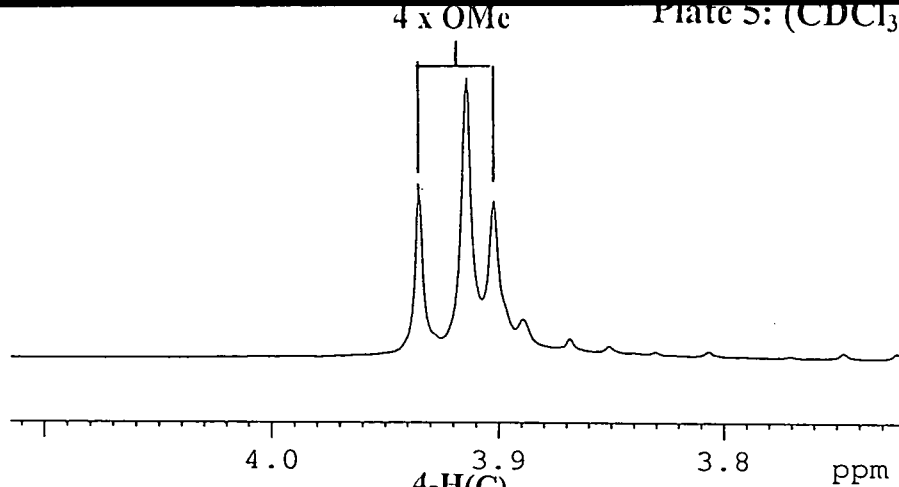




(2R,3R,4S)-2,3-cis-3,4-trans-3,4-diacetoxy-3',4',7,8-tetramethoxyflavan

(52) [epimesquitol-4 β -ol tri-O-methylether diacetate]





Epimesquitol-(4 β →6)-epimesquitol-4 α -ol octa-O-methylether
 triacetate(57)

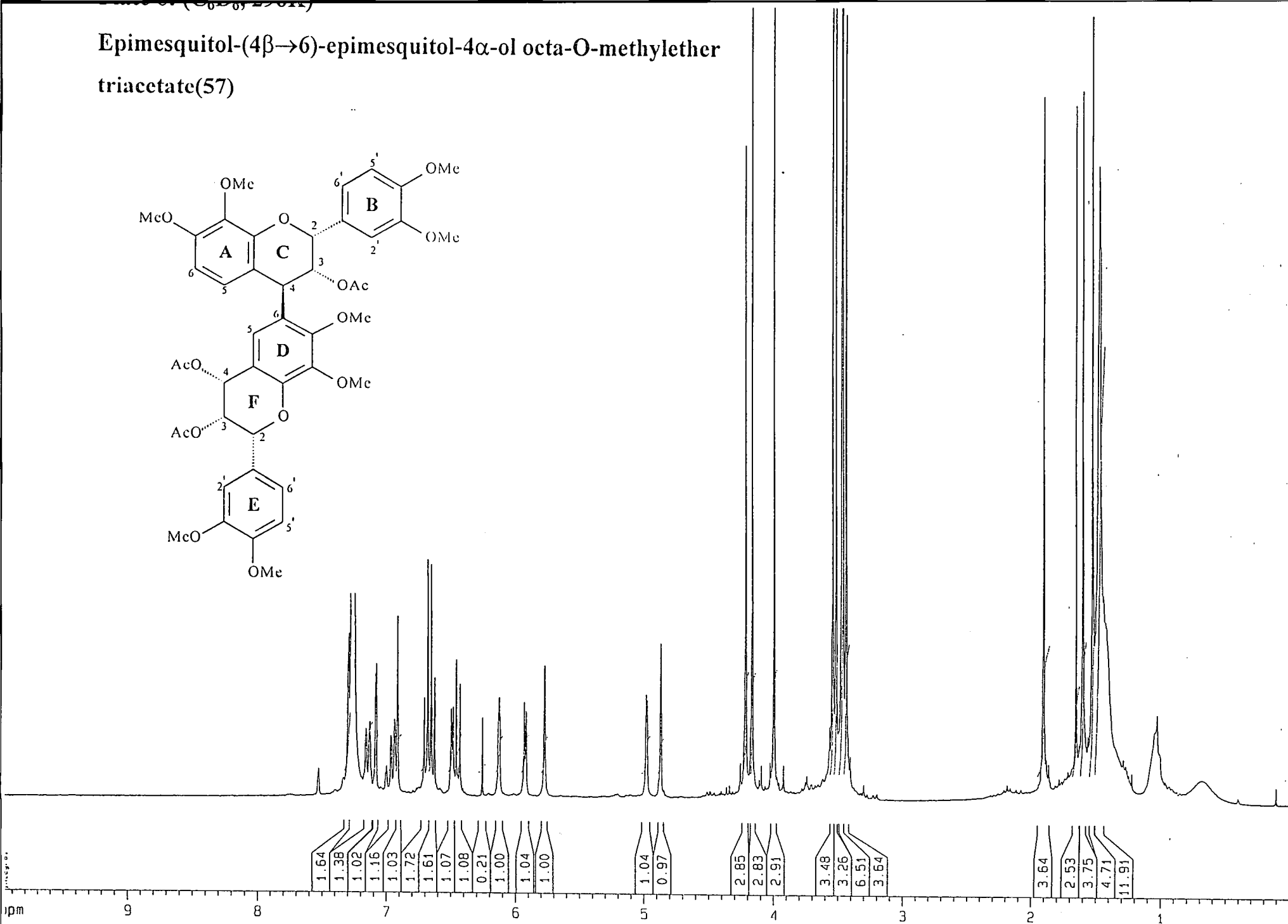
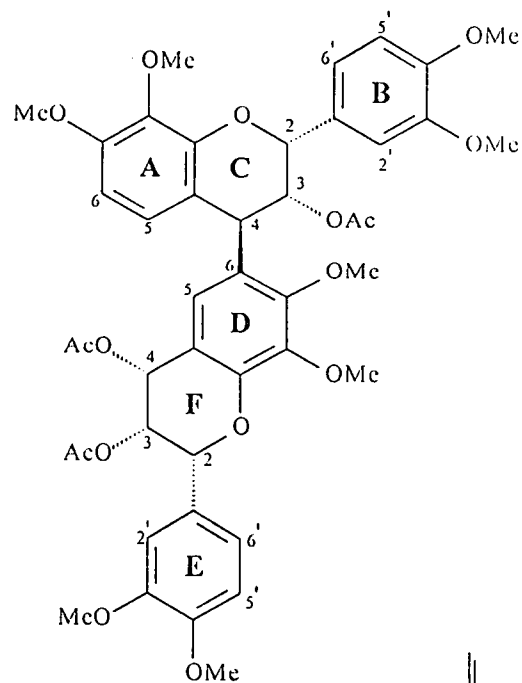


Plate 6: (C₆D₆, 296K)

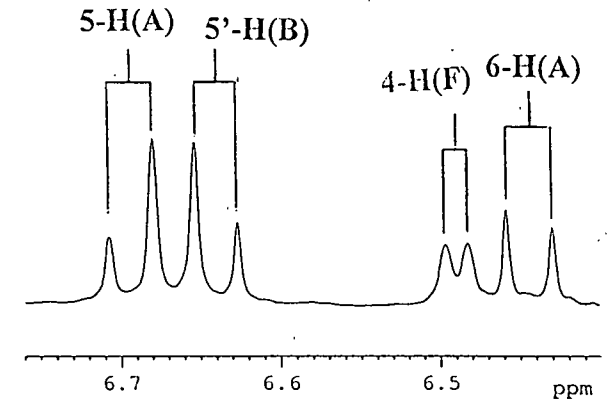
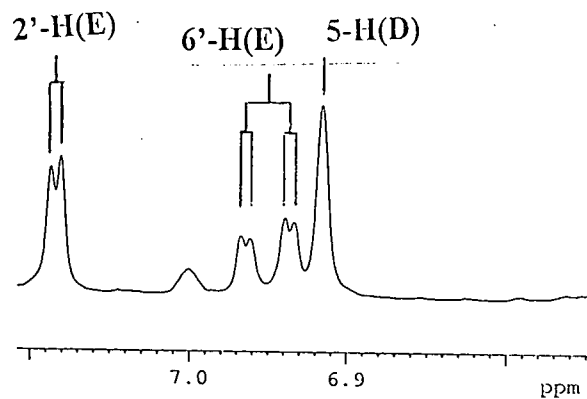
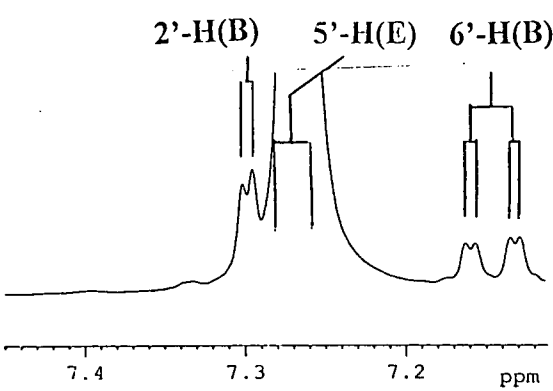
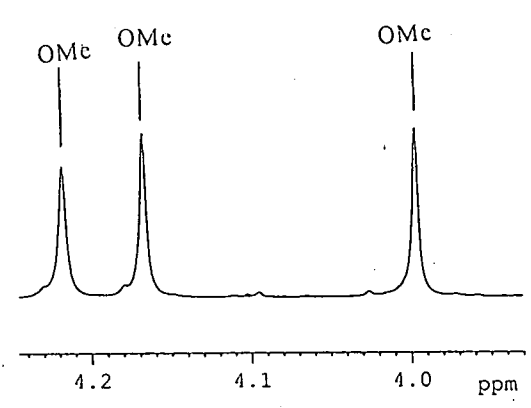
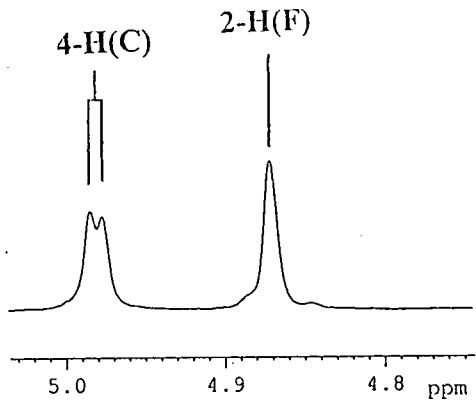
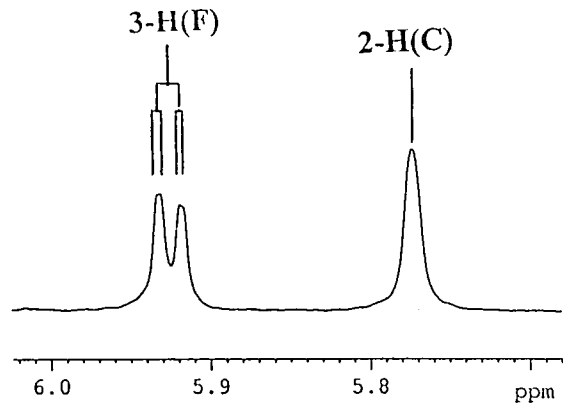
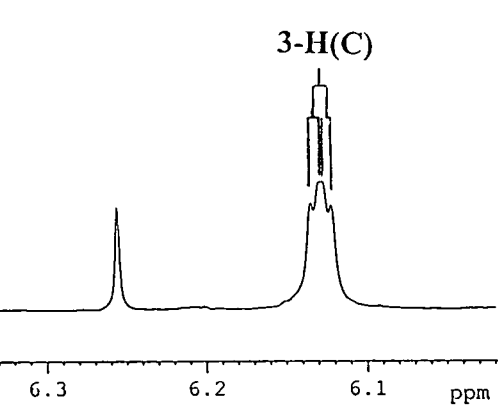
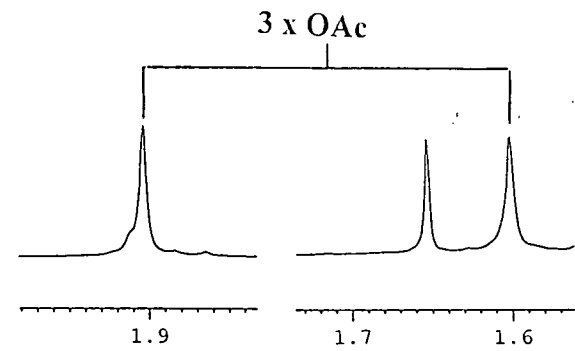
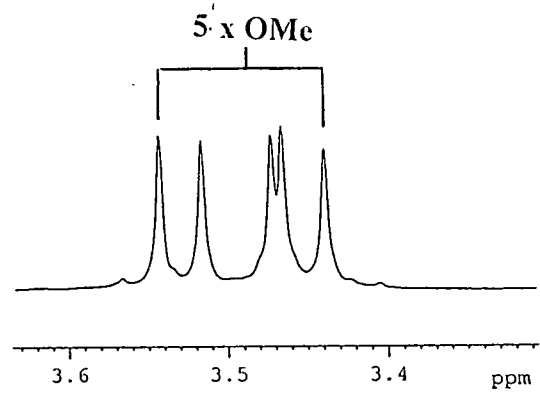


Plate 7: (CDCl₃ 296K)

Epimesquitol-(4 β →6)-epimesquitol-4 β -ol octa-O-methylether
triacetate(58)

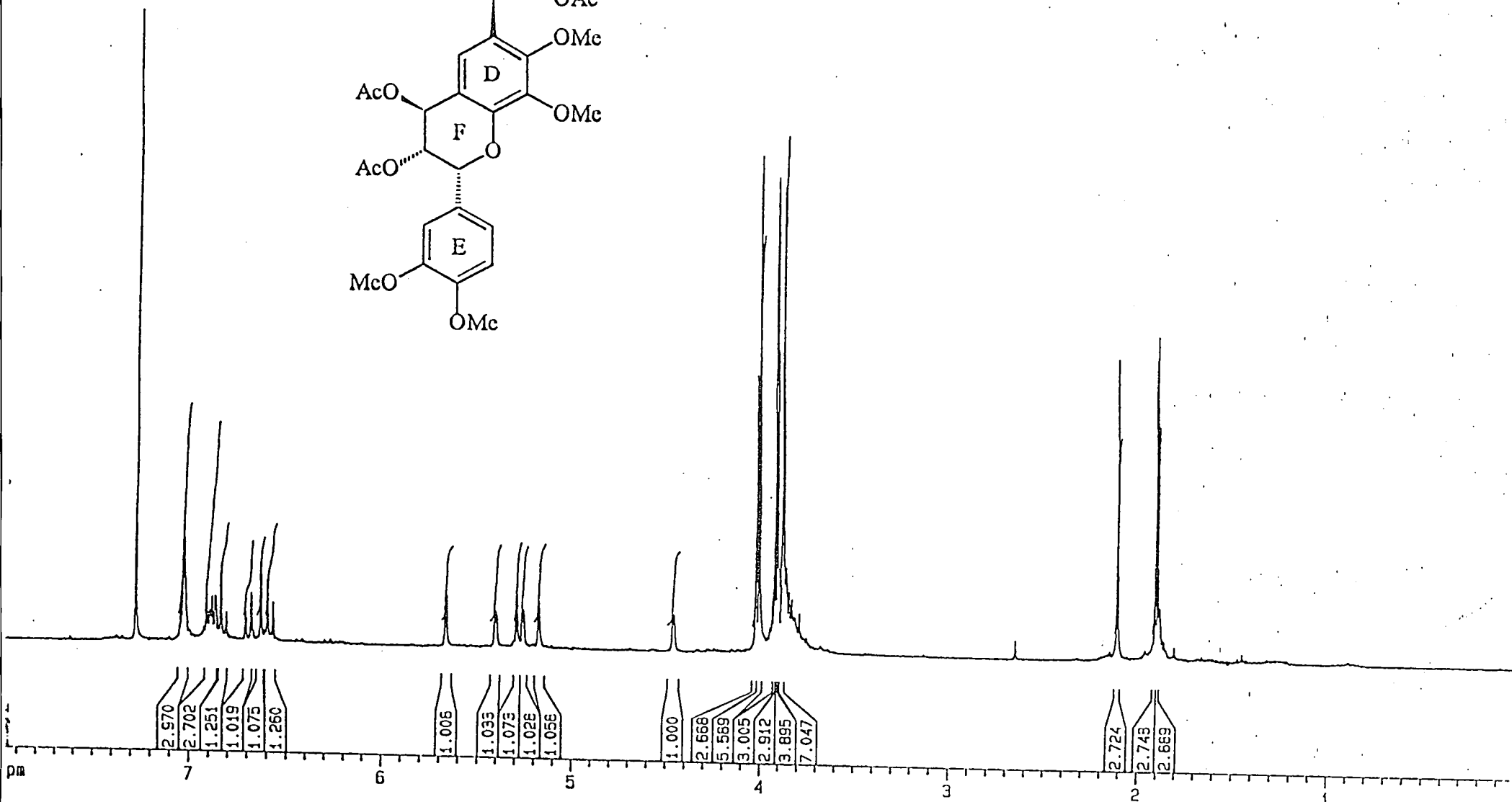
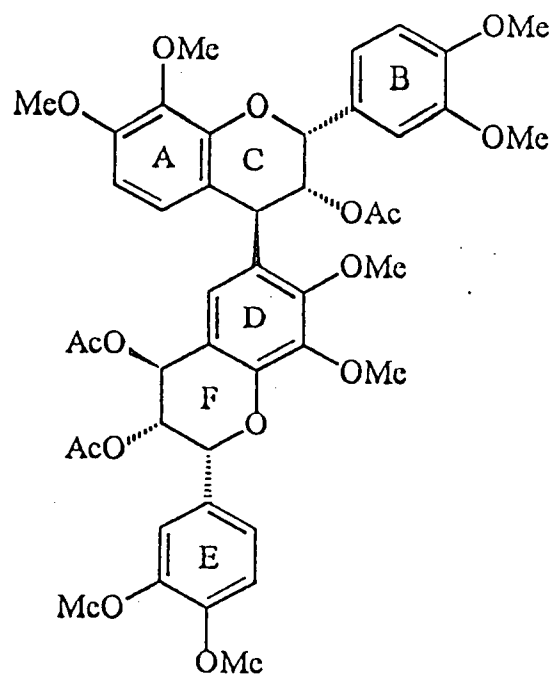
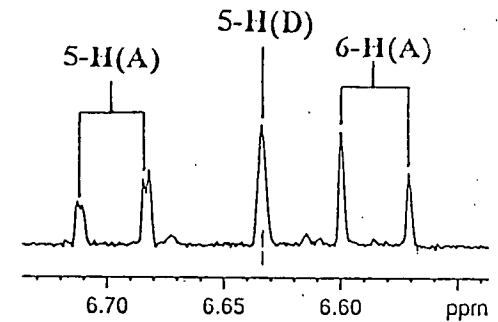
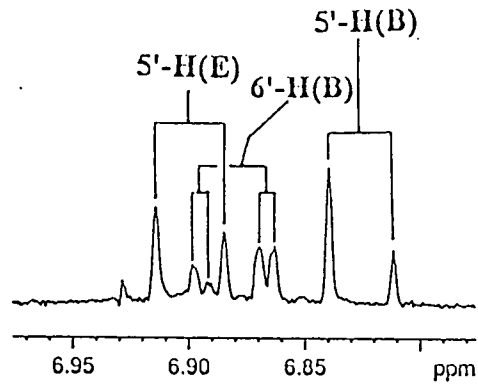
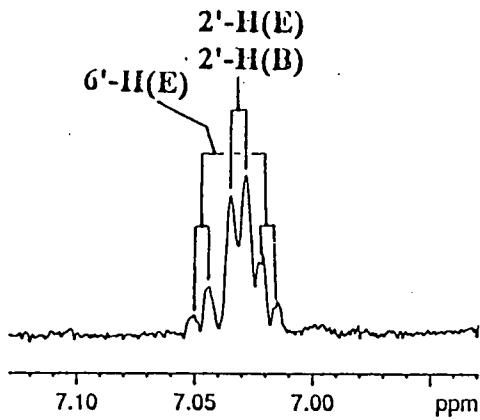
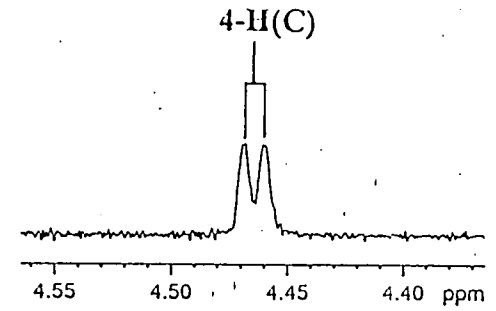
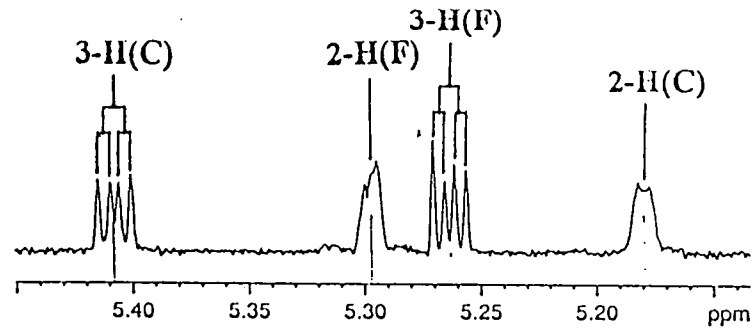
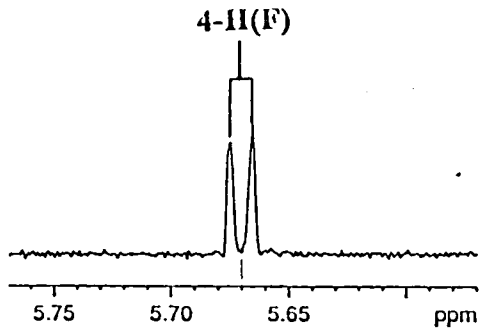
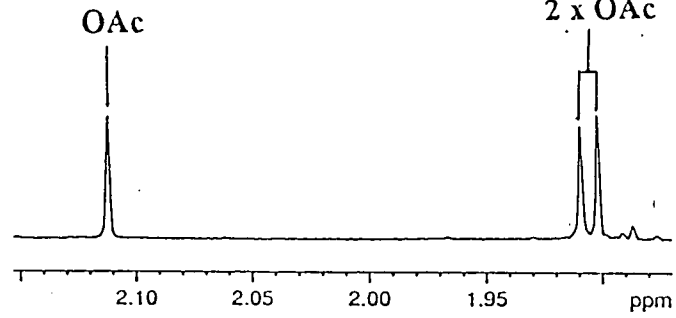
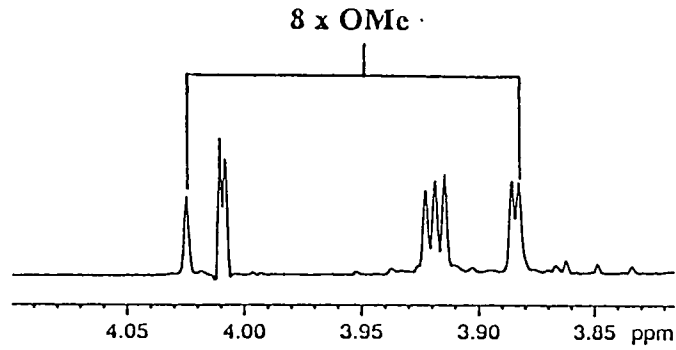


Plate 7: (CDCl₃ 296K)



Mesquitol-(4 α →5)-epimesquitol-4 β -ol octa-O-methylether triacetate(61)

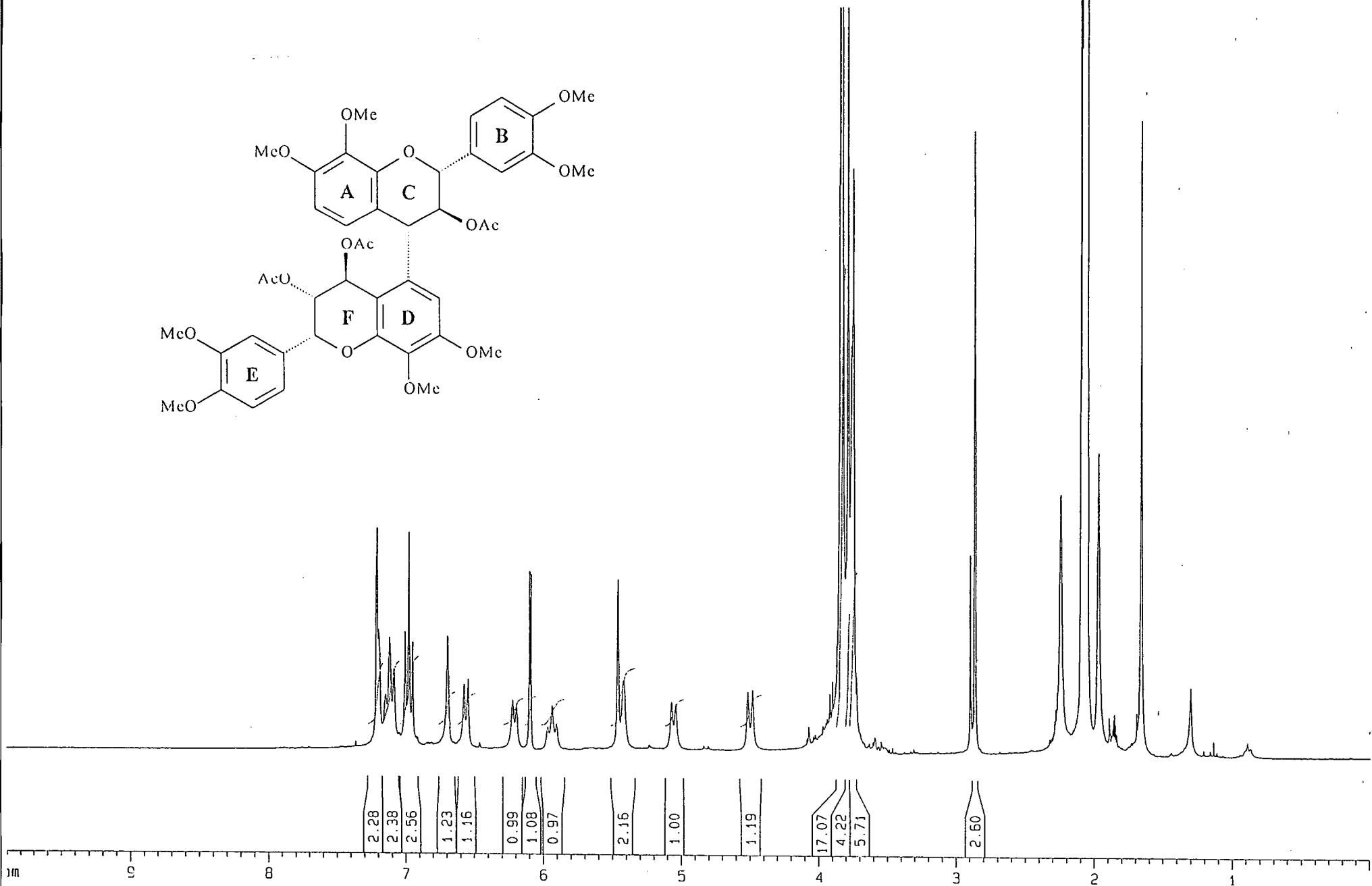
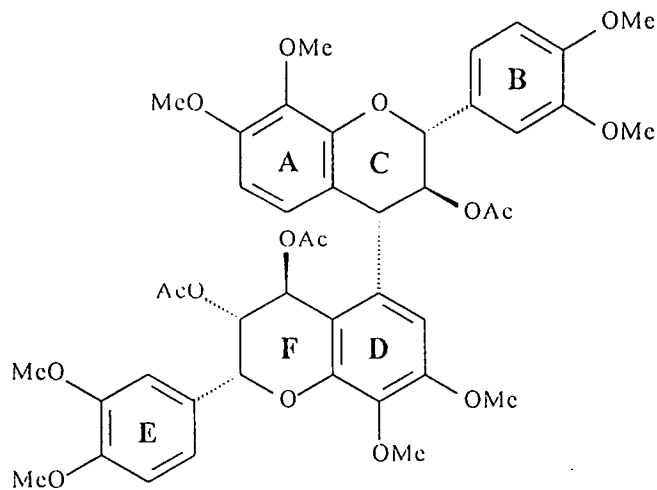
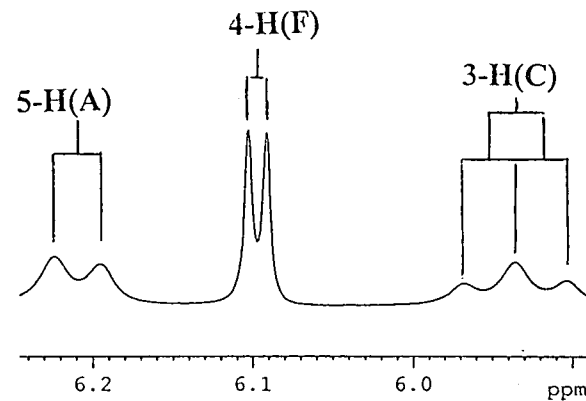
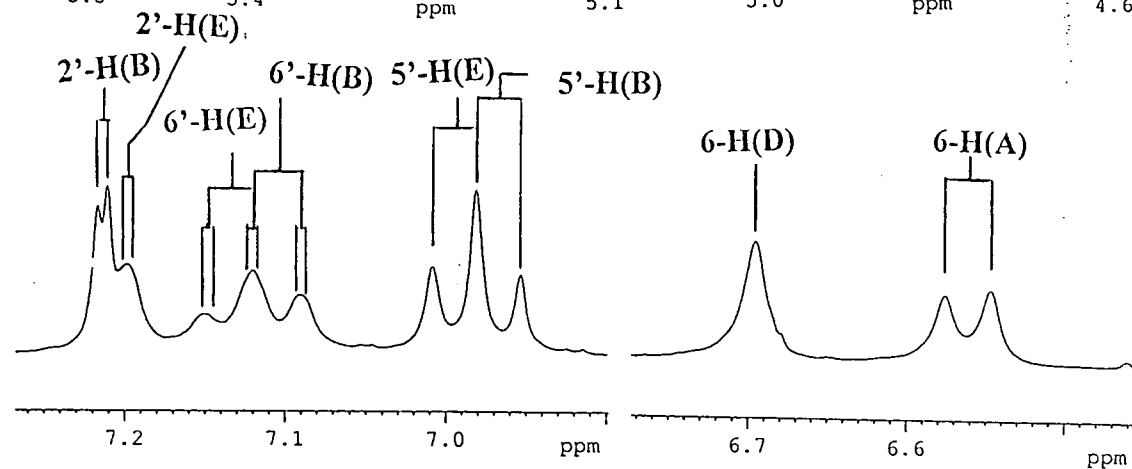
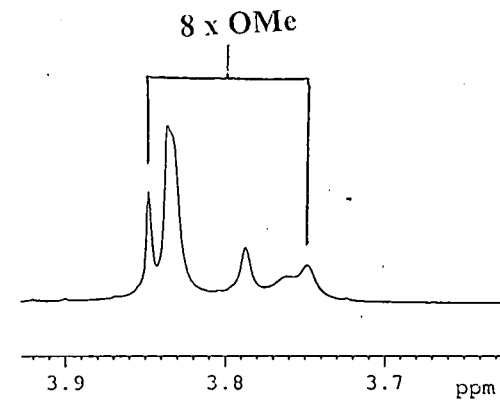
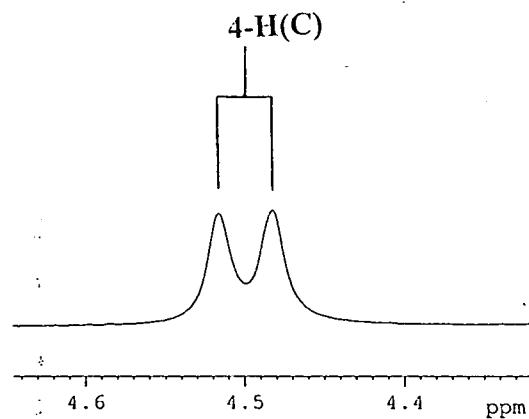
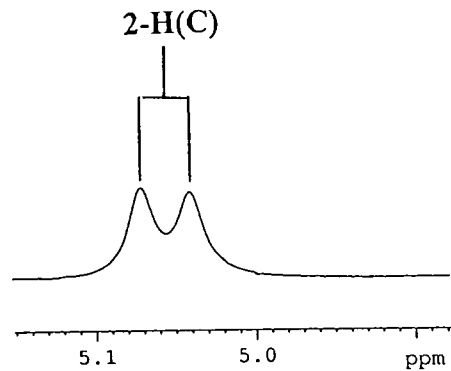
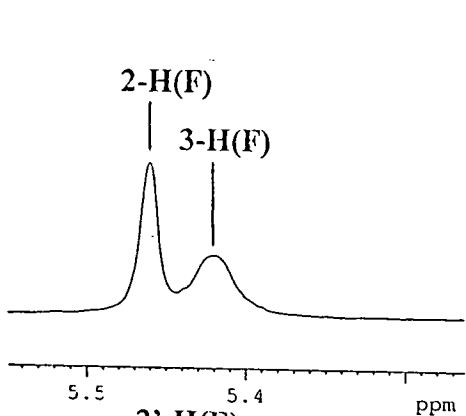
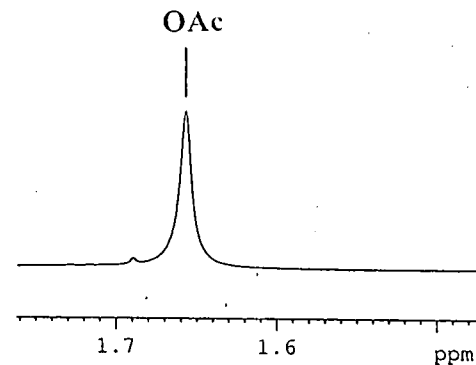
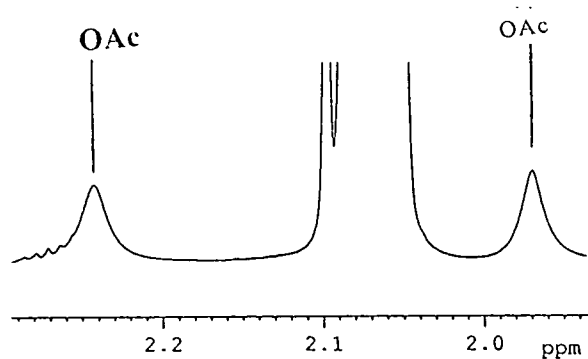


Plate 8: $[(CD)_3CO, 296K]$



Ent-epimesquitol tetra-O-methyltriacetyl-(4 α - \rightarrow 3)-3',4',7,8-tetra-O-methylflavanone(63)

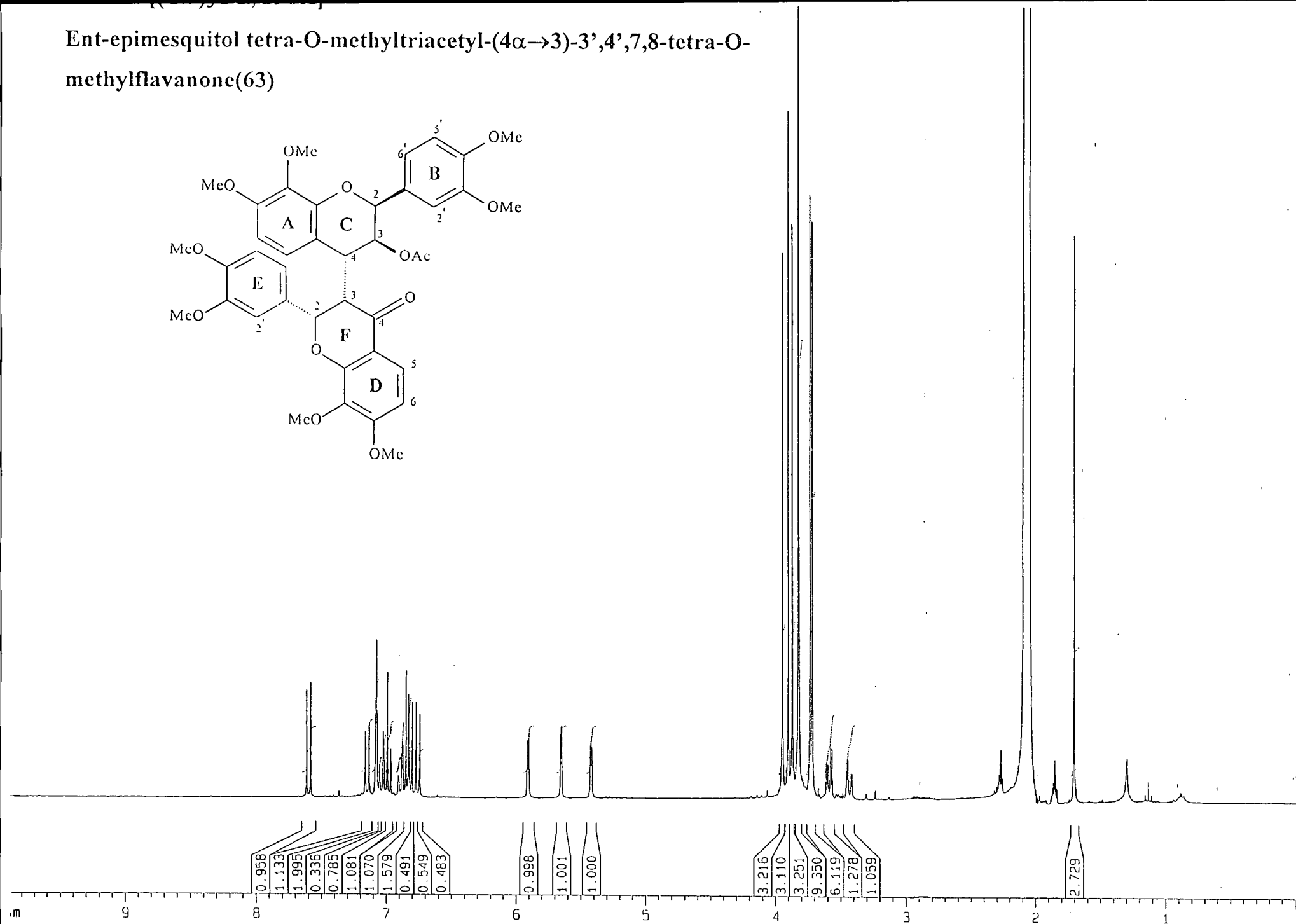
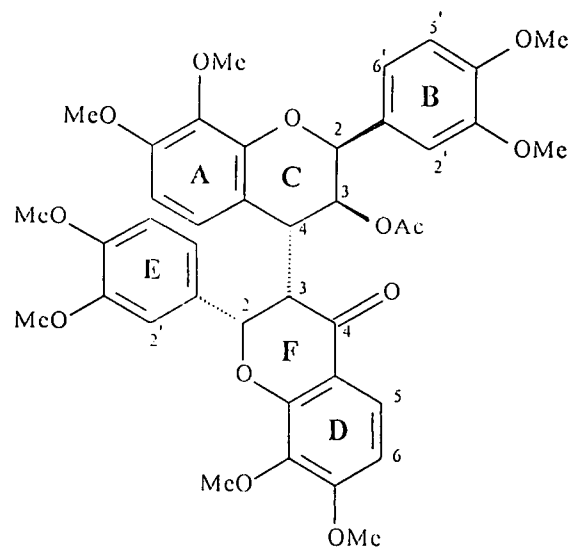
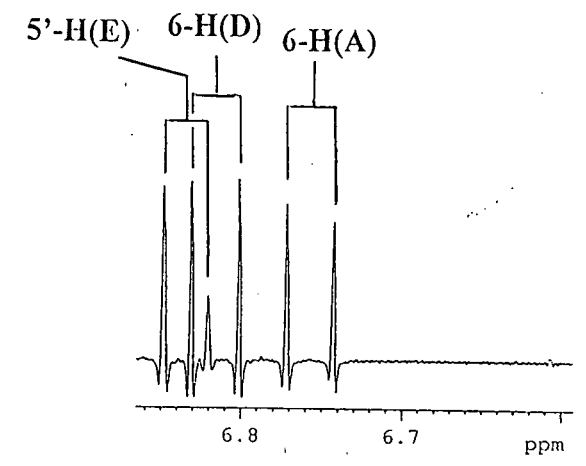
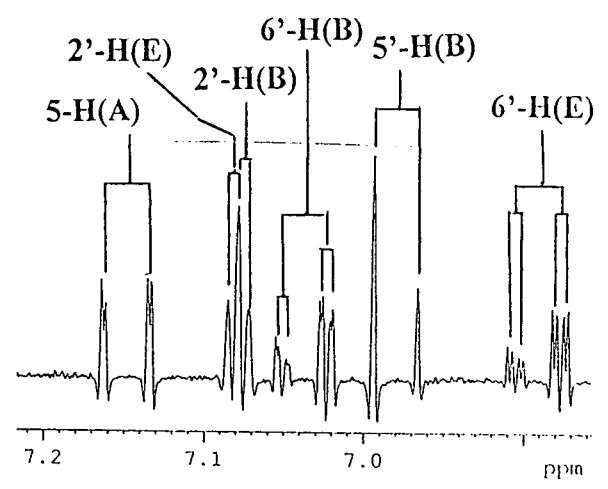
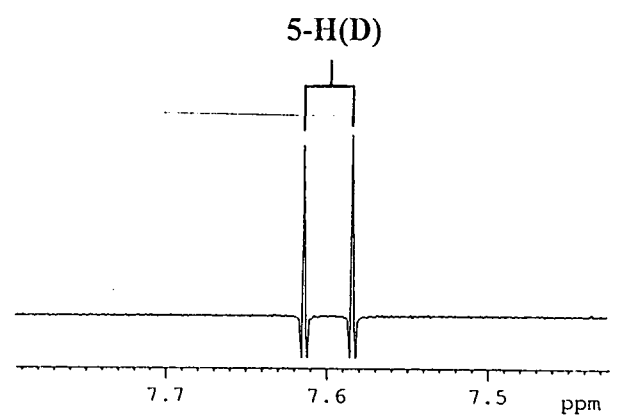
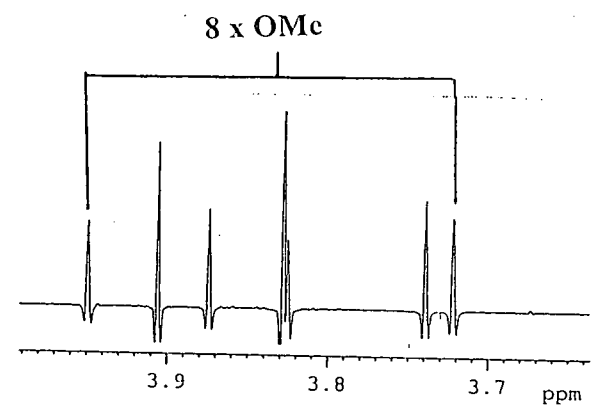
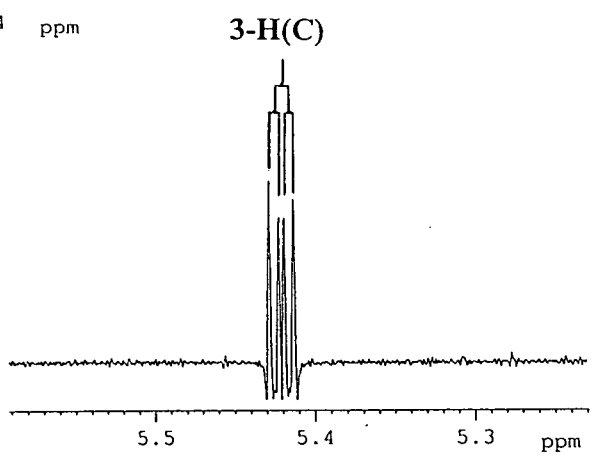
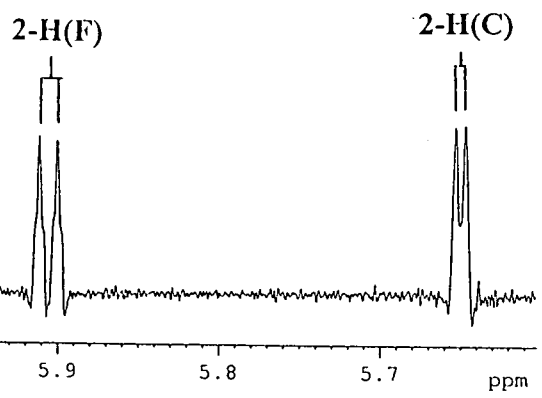
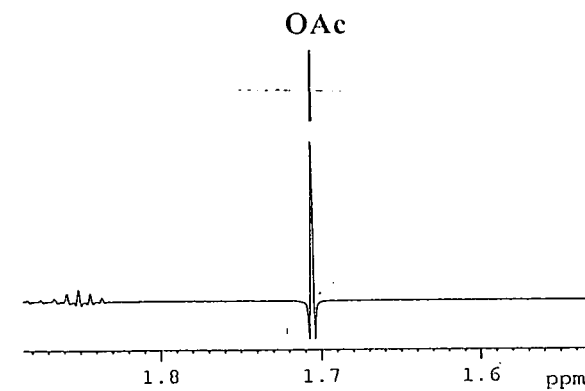
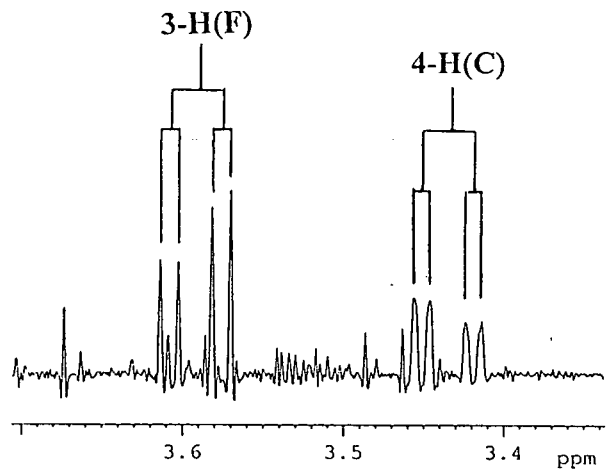


Plate 9: $[(CD)_3CO, 296K]$



Mesquitol-(4 α →5)-melanoxetin nona-O-methyl ether acetate(65)

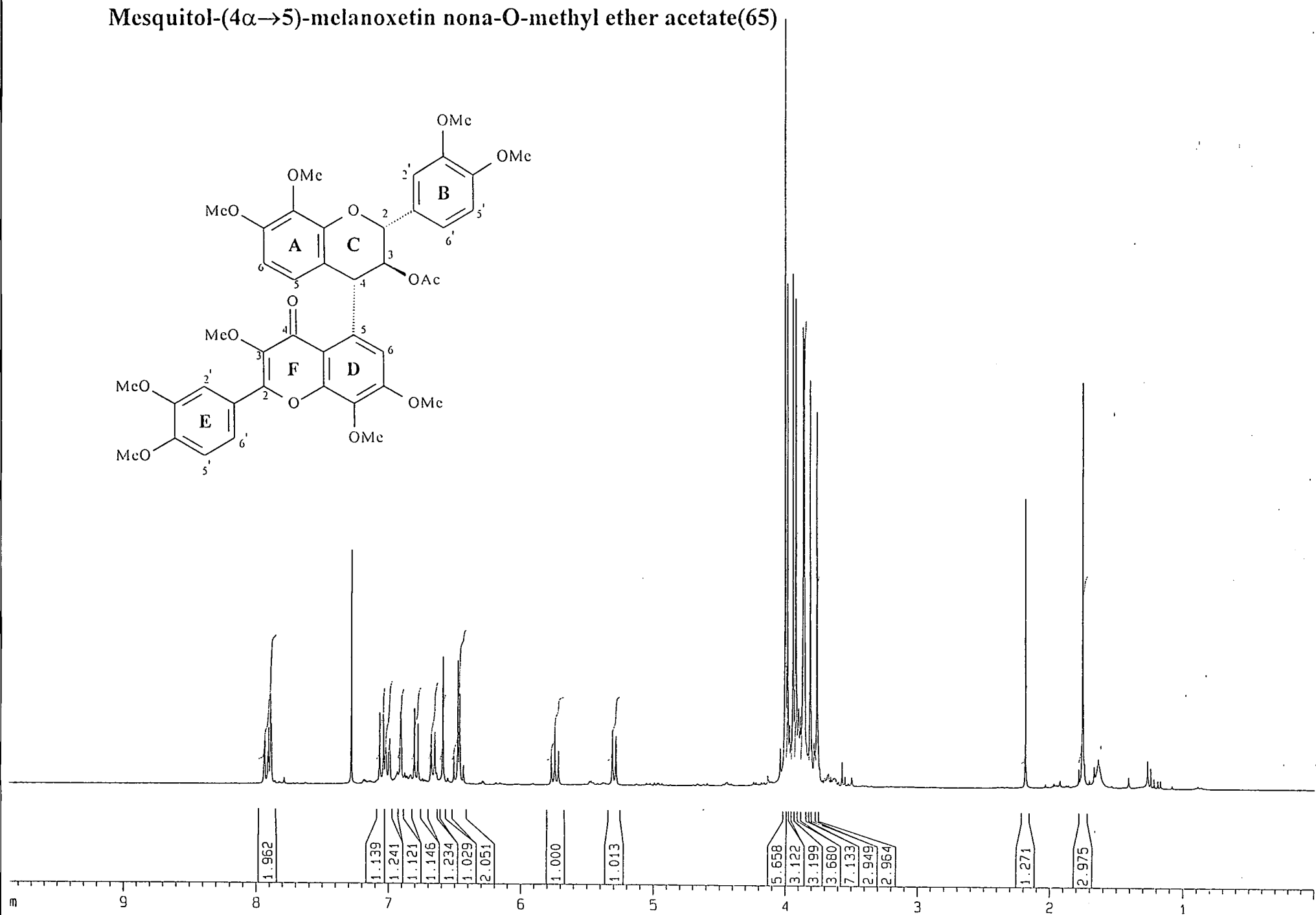
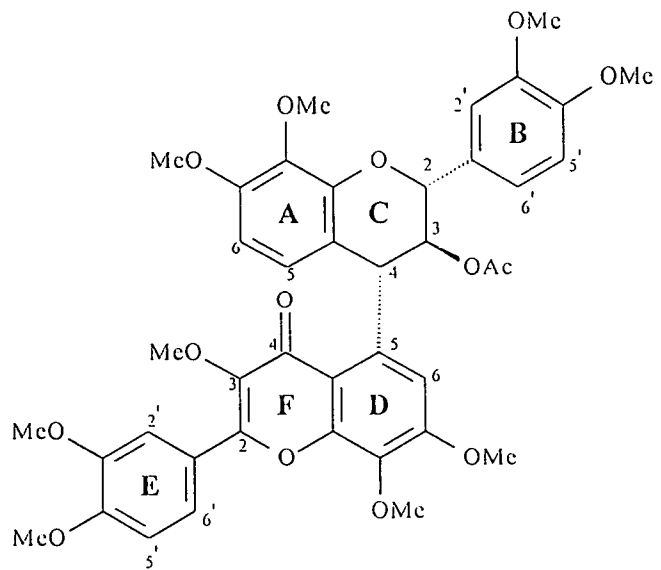
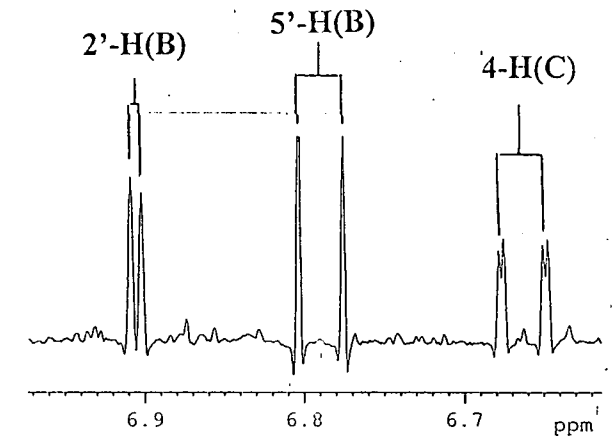
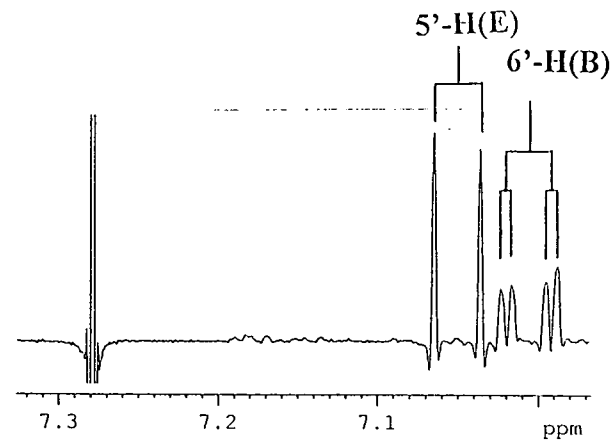
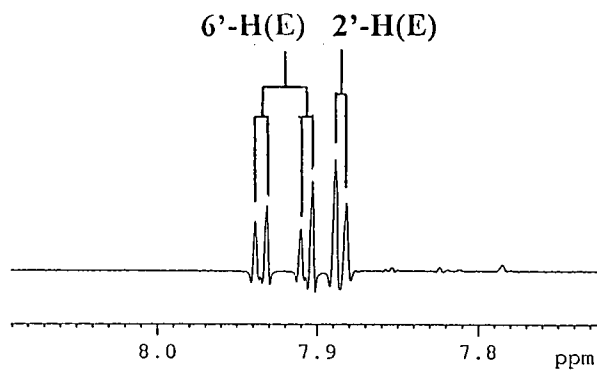
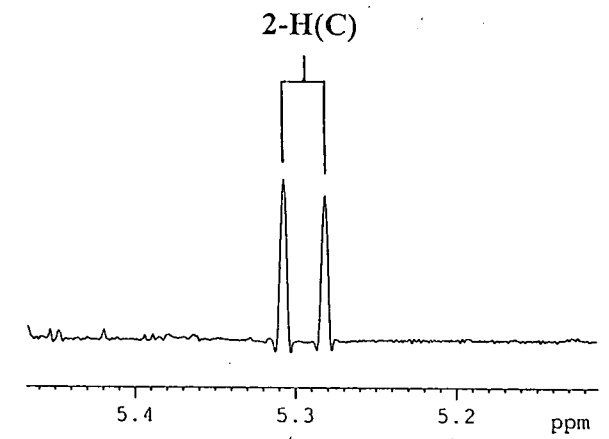
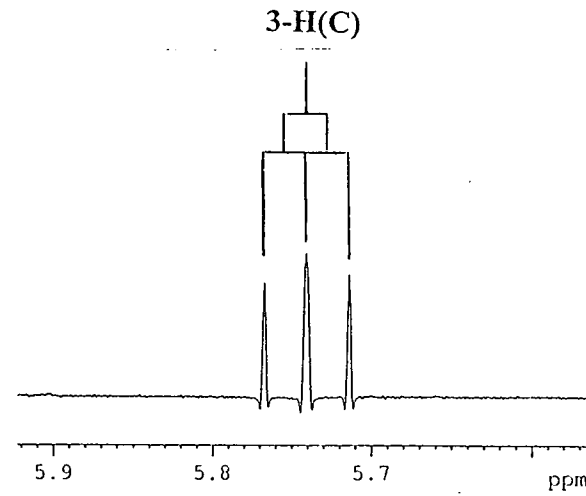
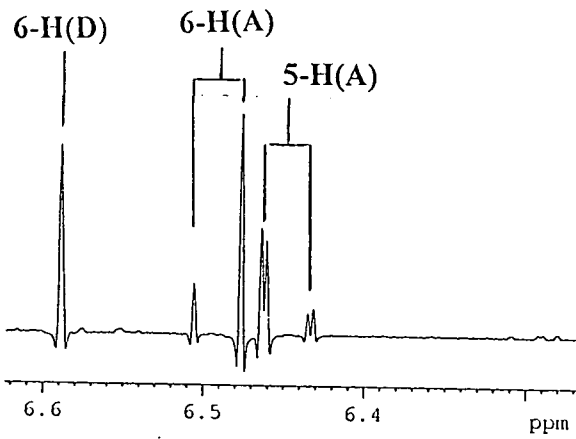
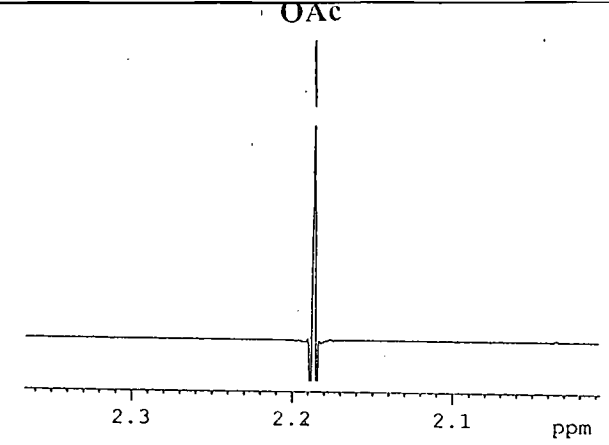
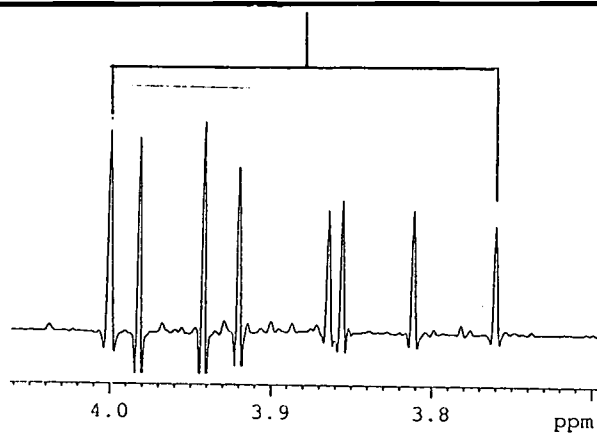


Plate 10: (CDCl₃ 296K)



Epimesquitol-(4 β →5)-melanoxetin nona-O-methyl ether acetate(67)

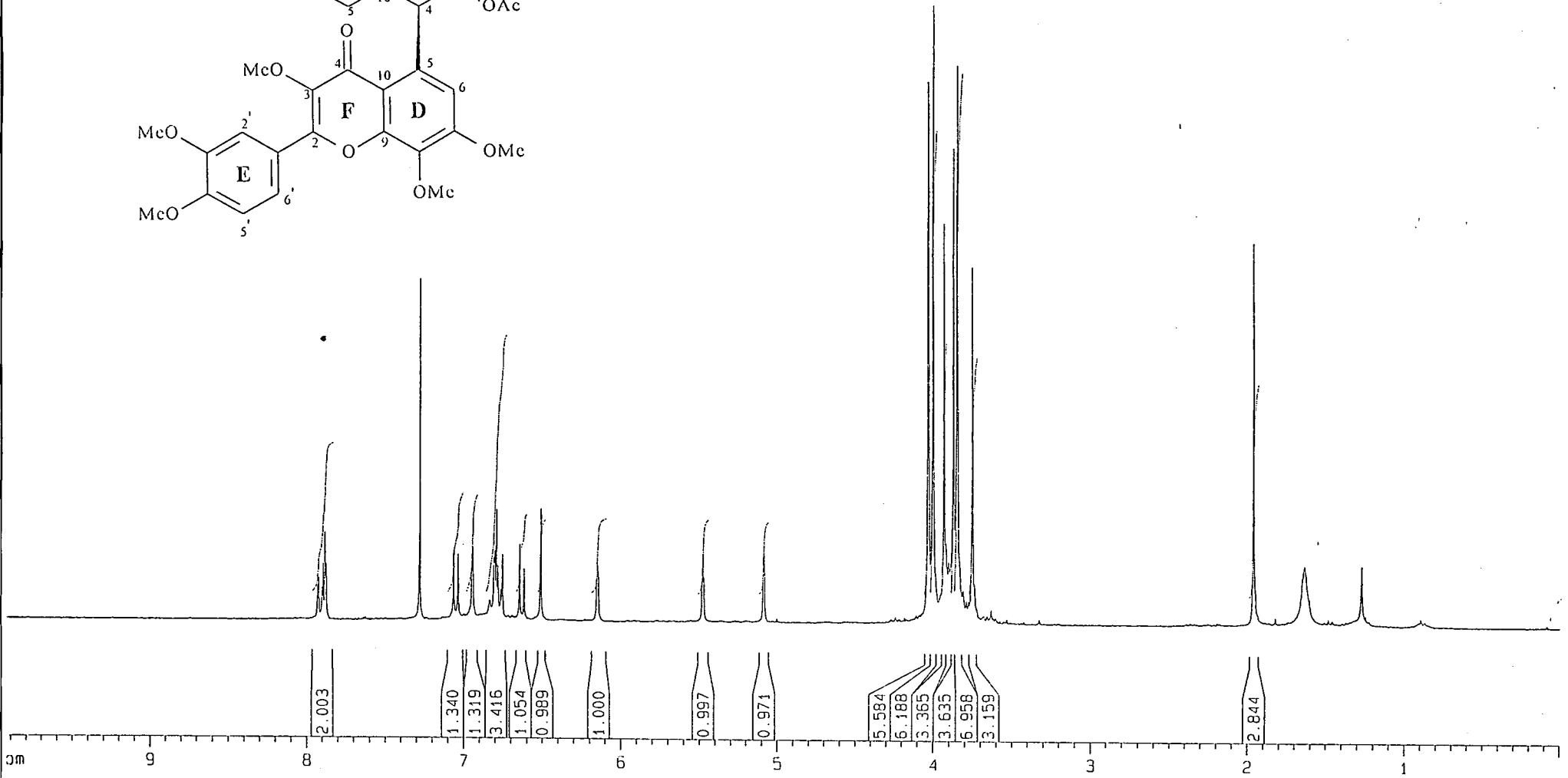
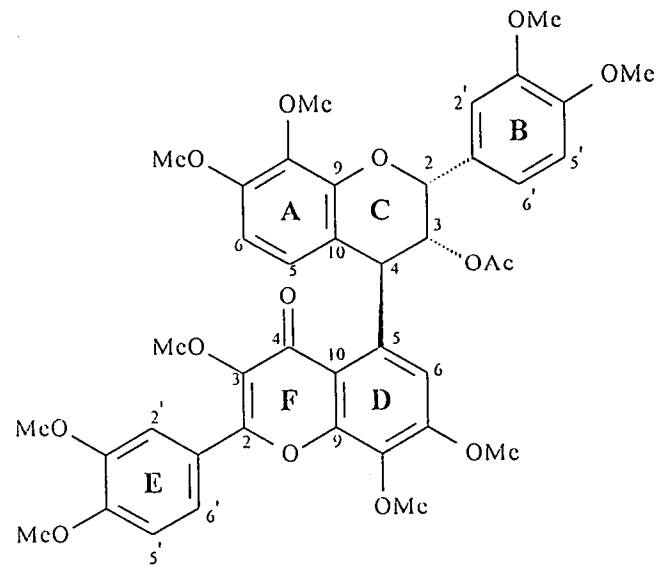
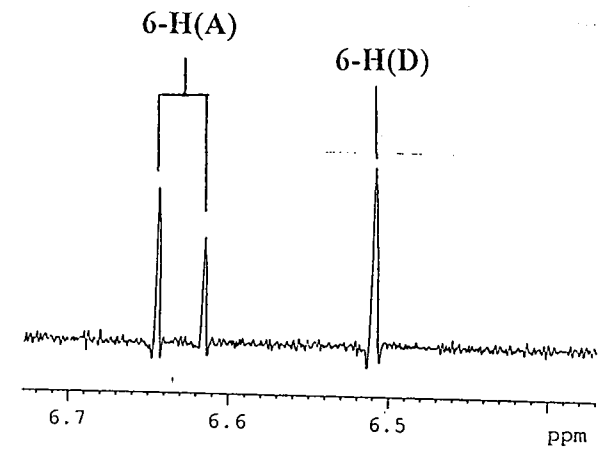
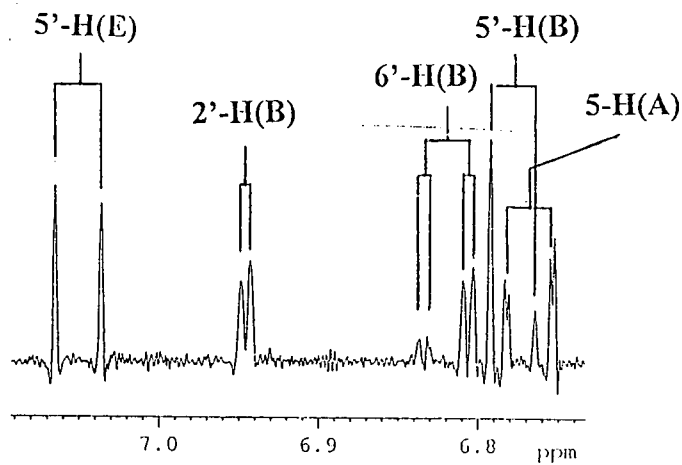
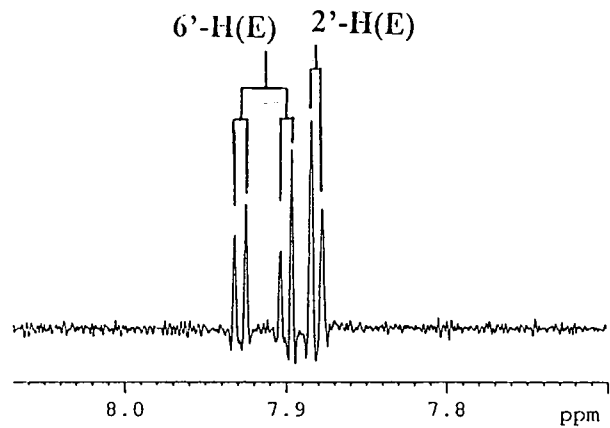
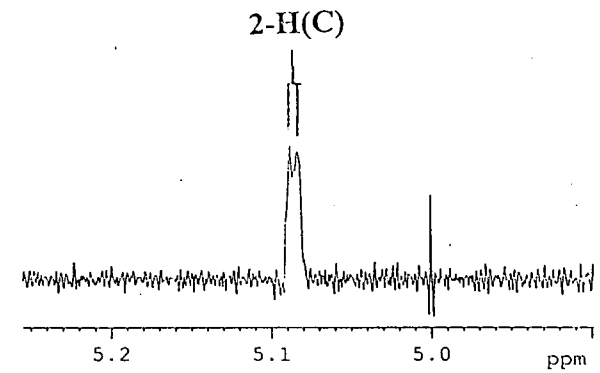
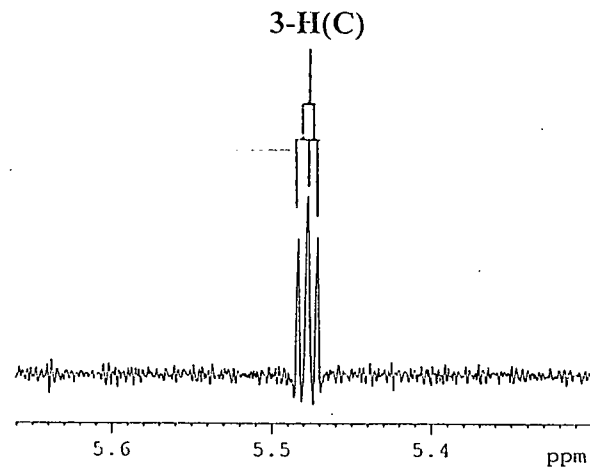
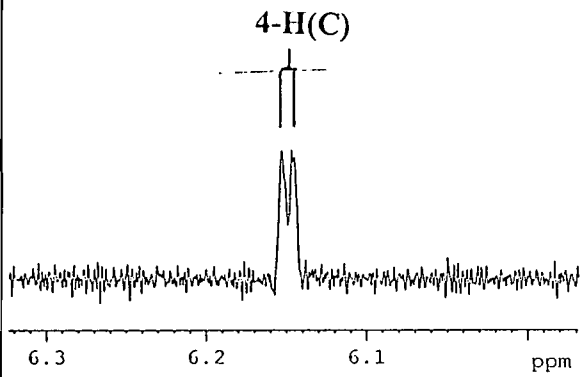
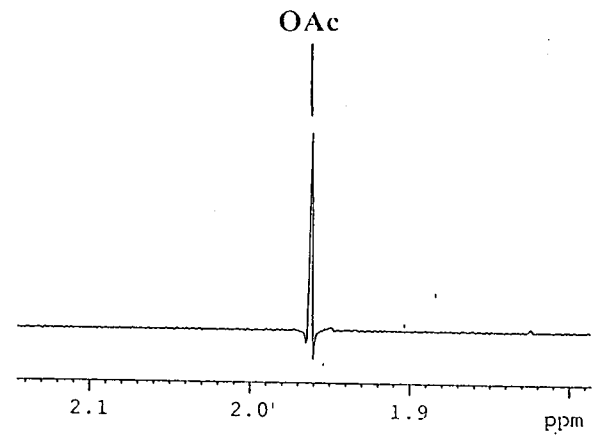
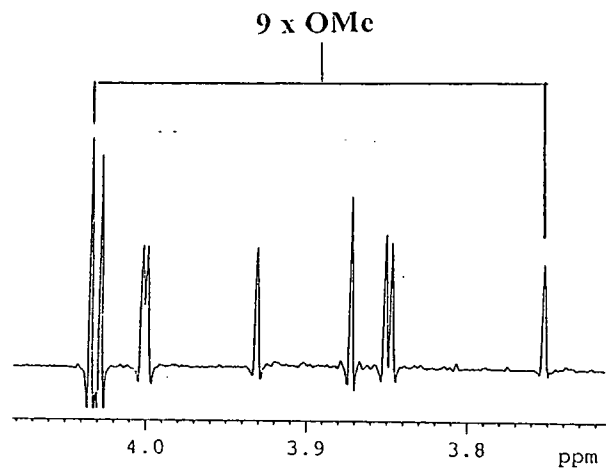


Plate 11: (CDCl₃ 296K)



(2R,3S,4S)-2,3-cis-3,4-trans-3-acetoxy-3',4',7,8-tetramethoxy-4-benzylthioflavan: [epimesquitol-4 β -benzylthioether]

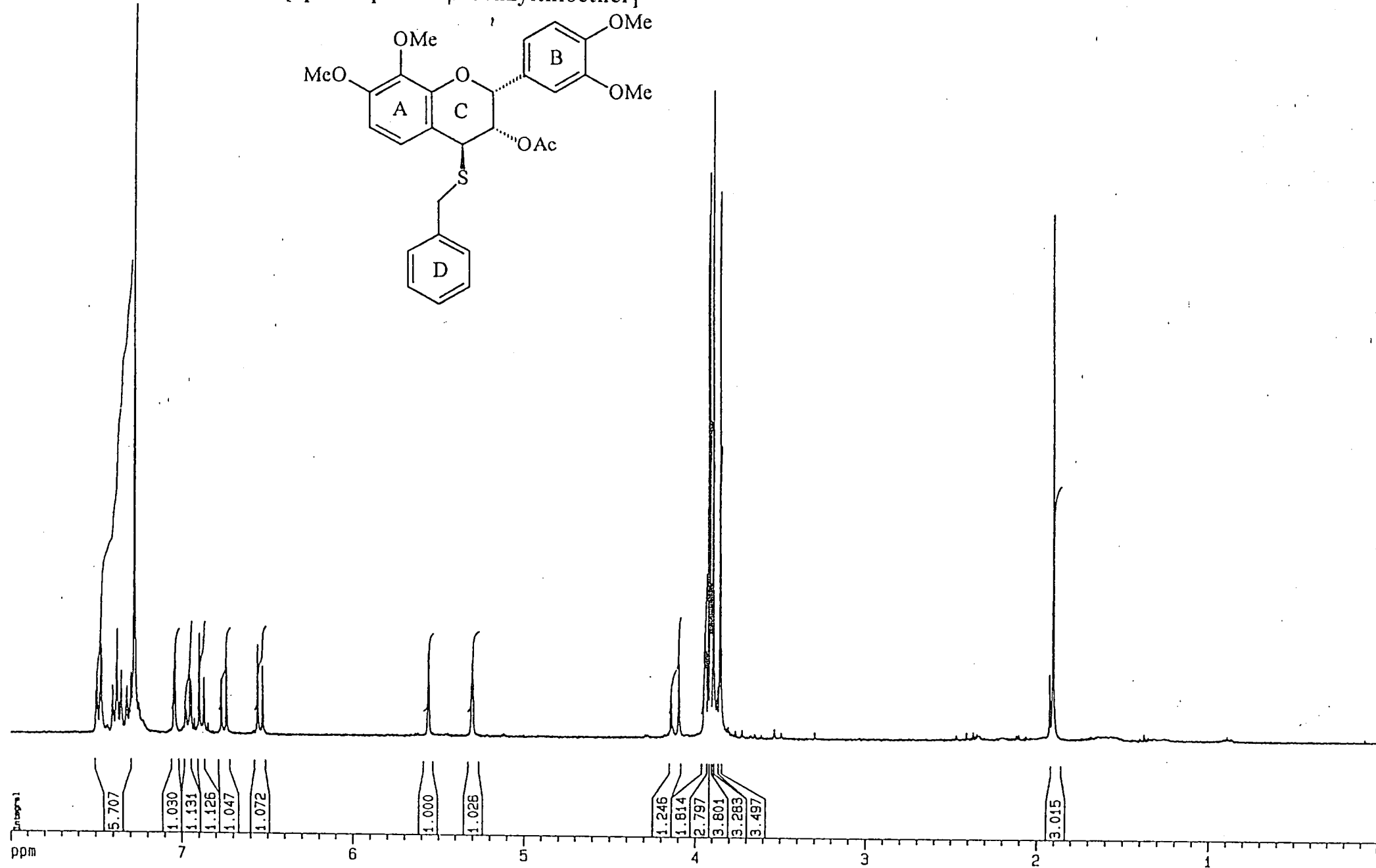
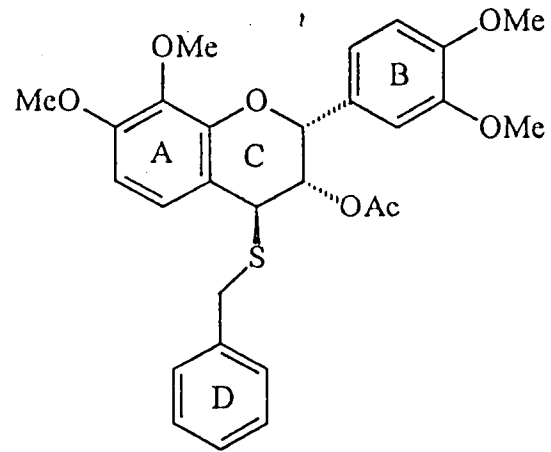
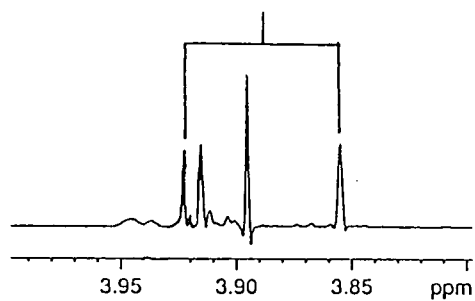
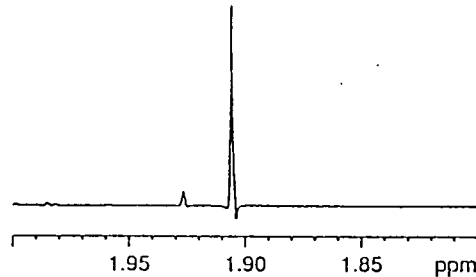


Plate 12: (CDCl₃, 296K)

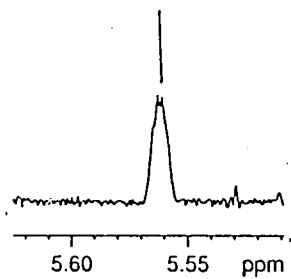
4 x OMe



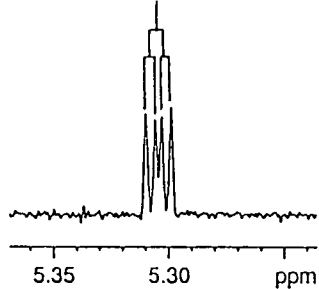
OAc



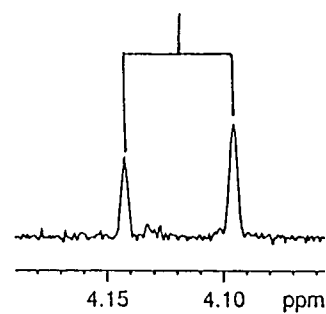
2-H(C)



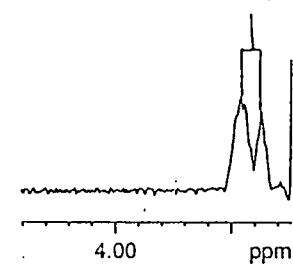
3-H(C)



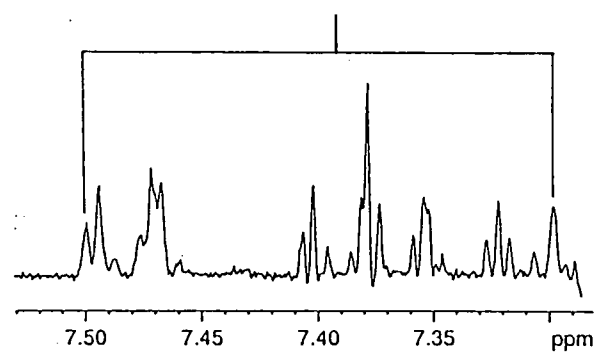
CH₂



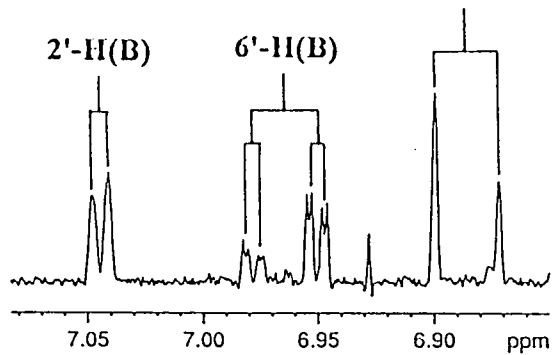
4-H(C)



D-ring



5'-H(B)



2'-H(B)

6'-H(B)

5-H(A)

6-H(A)

2'-H(B)

6'-H(B)

5-H(A)

6-H(A)

2'-H(B)

6'-H(B)

5-H(A)

6-H(A)

2'-H(B)

6'-H(B)

5-H(A)

6-H(A)

2'-H(B)

6'-H(B)

5-H(A)

6-H(A)

2'-H(B)

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2'-H(B)

6'-H(B)

5-H(A)

6-H(A)

2'-H(B)

6'-H(B)

5-H(A)

6-H(A)

2'-H(B)

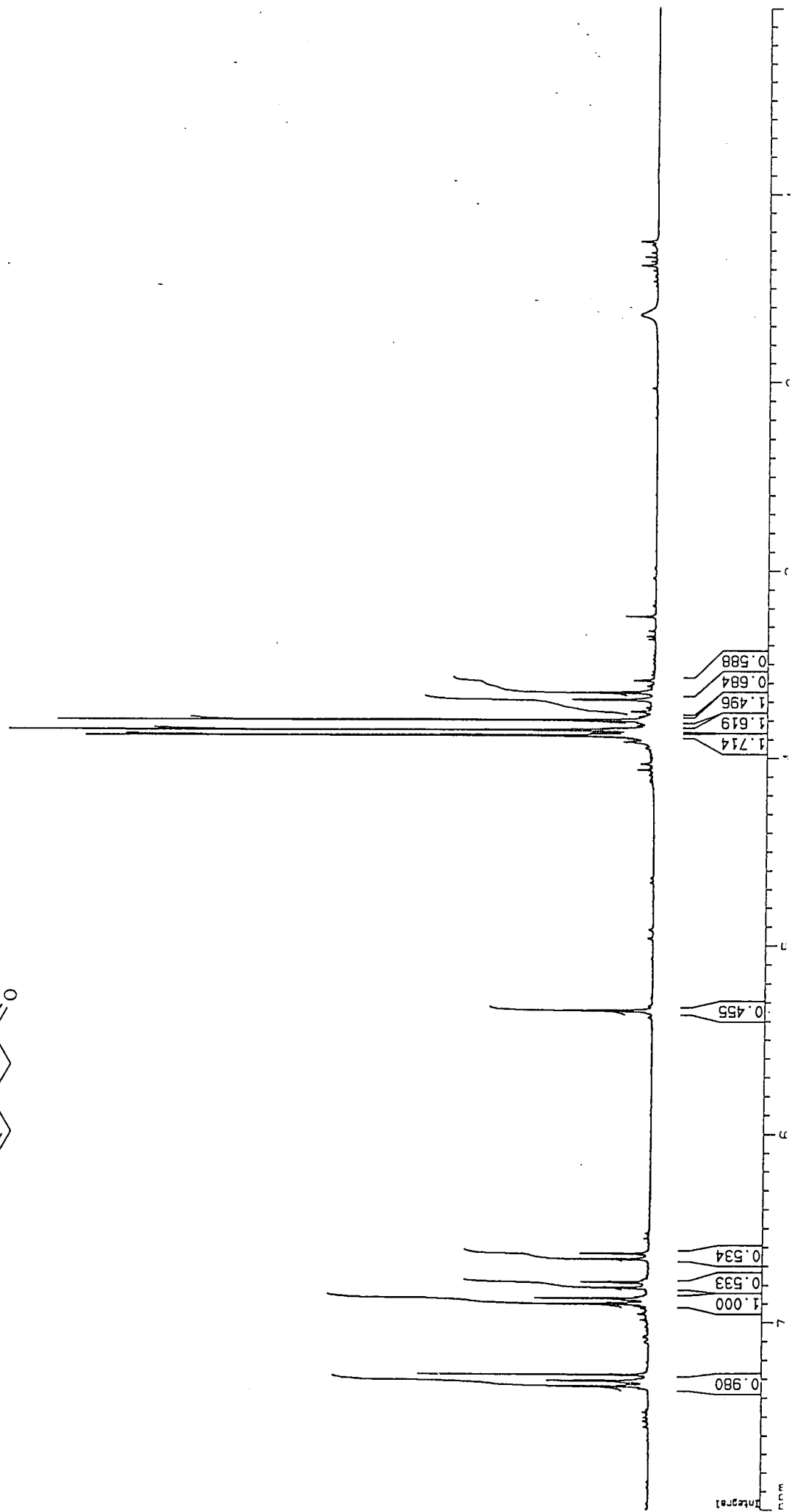
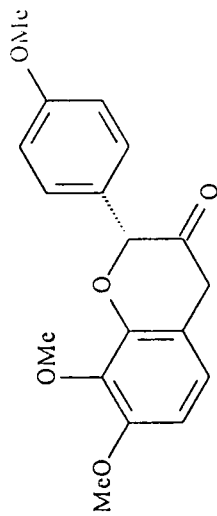
6'-H(B)

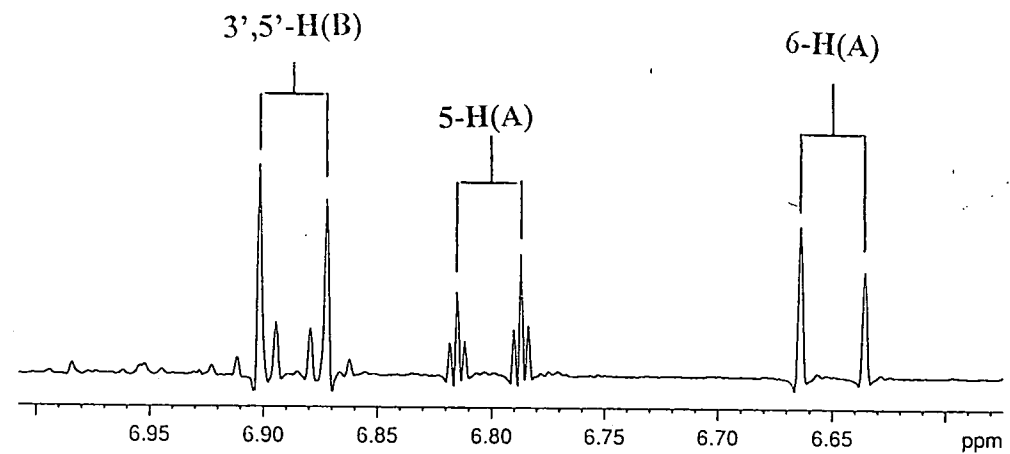
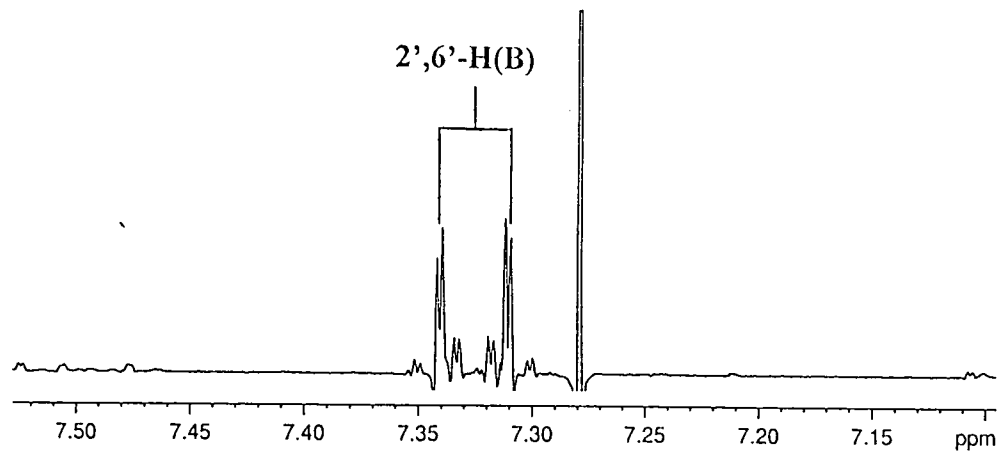
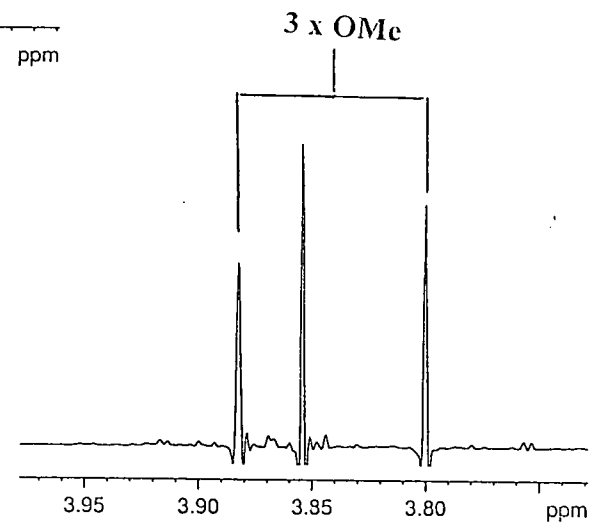
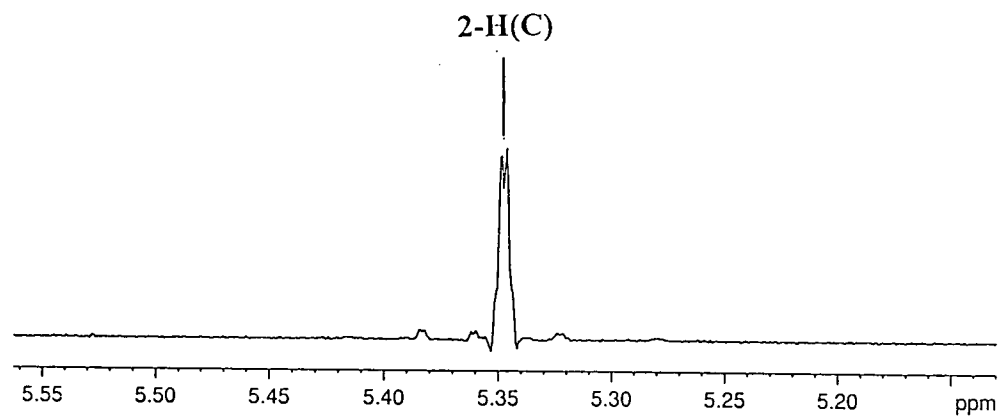
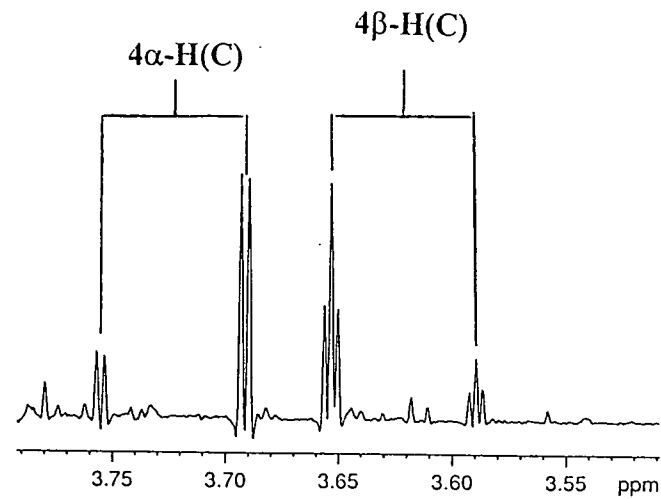
5-H(A)

6-H(A)

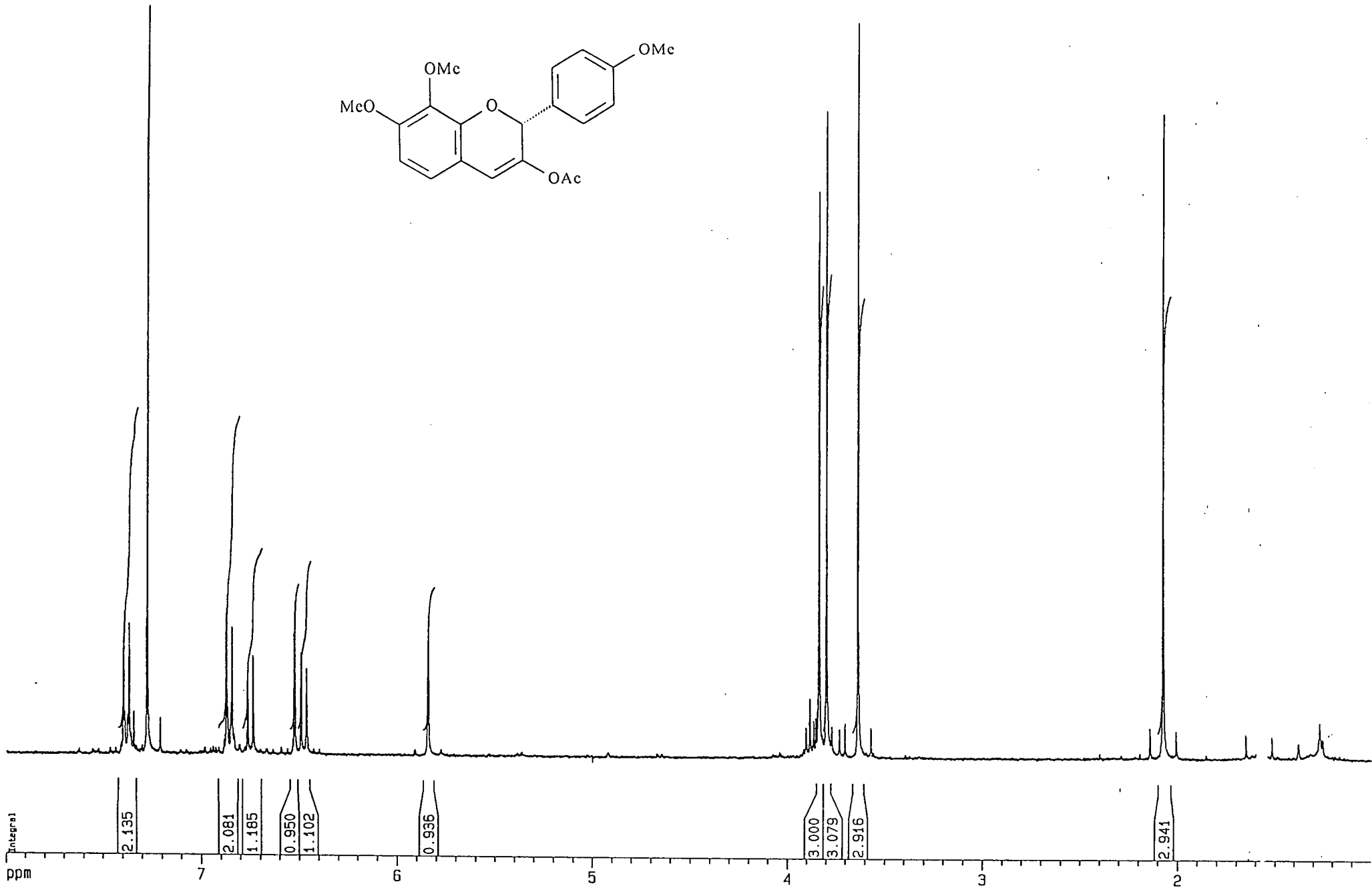
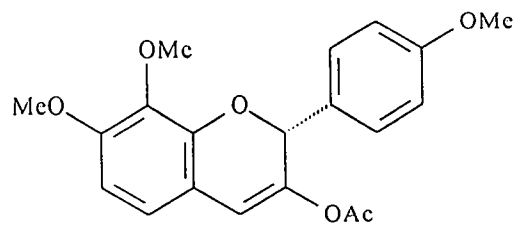
Plate 13: (CDCl₃ 296K)

(2R)-4',7,8-Trimethoxyflavan-3-one(48)





(2R)-3-Acetoxy-4',7,8-trimethoxyflav-3-ene(47)



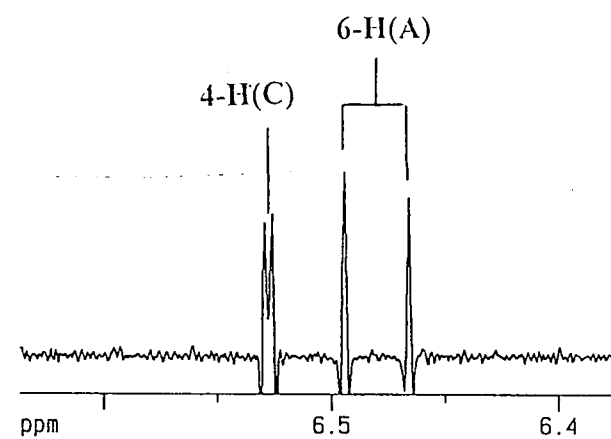
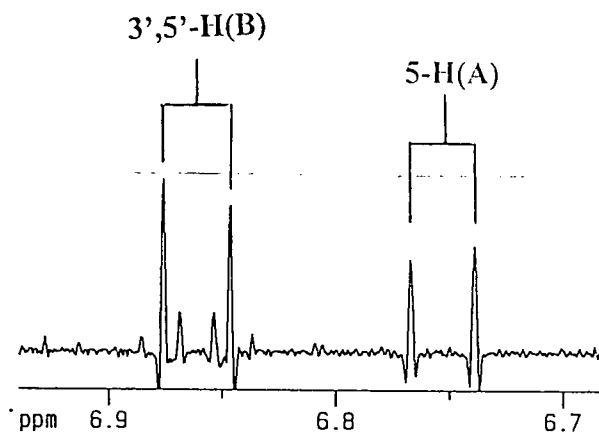
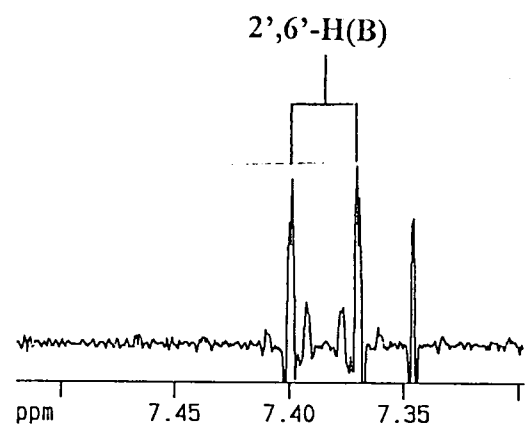
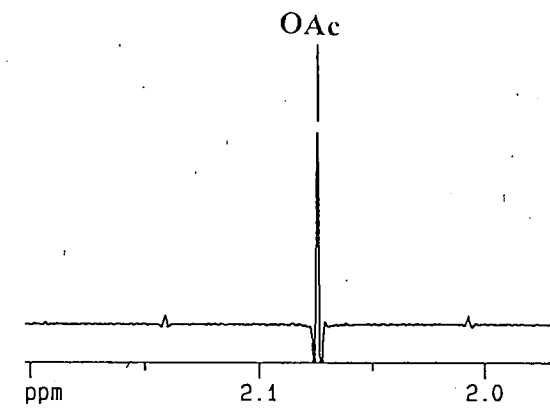
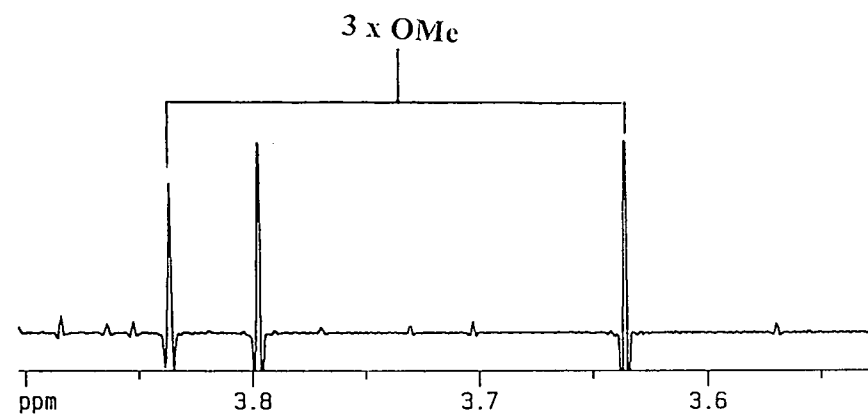
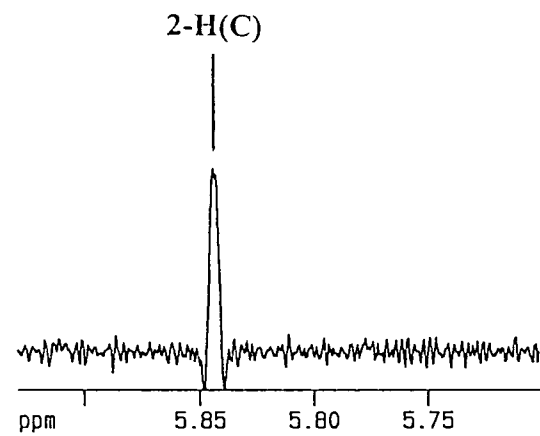
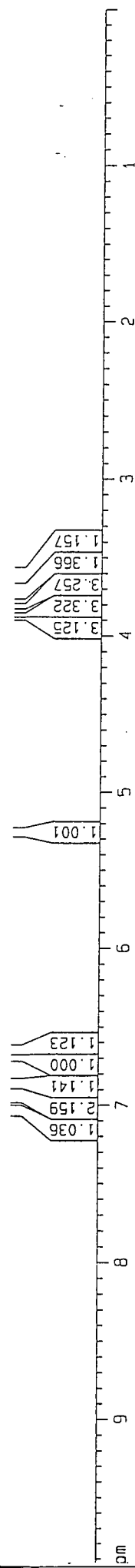
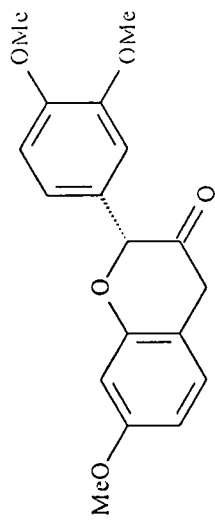
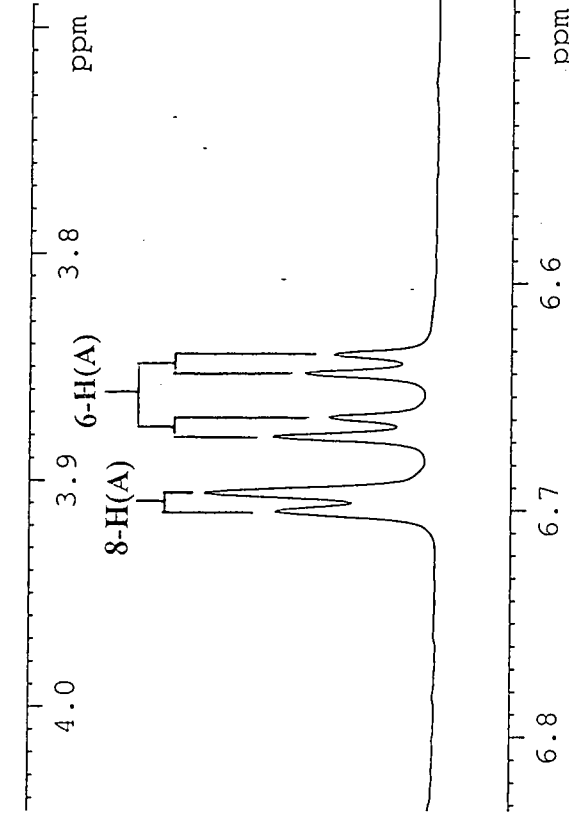
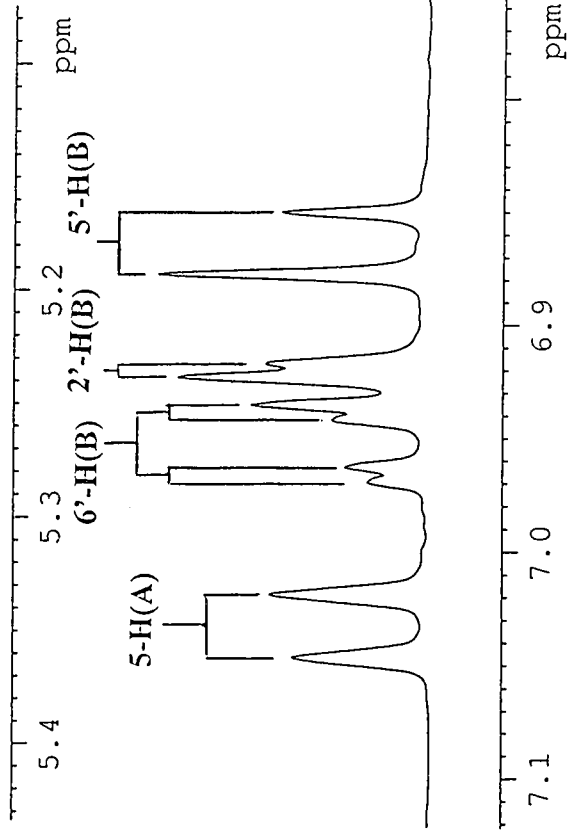
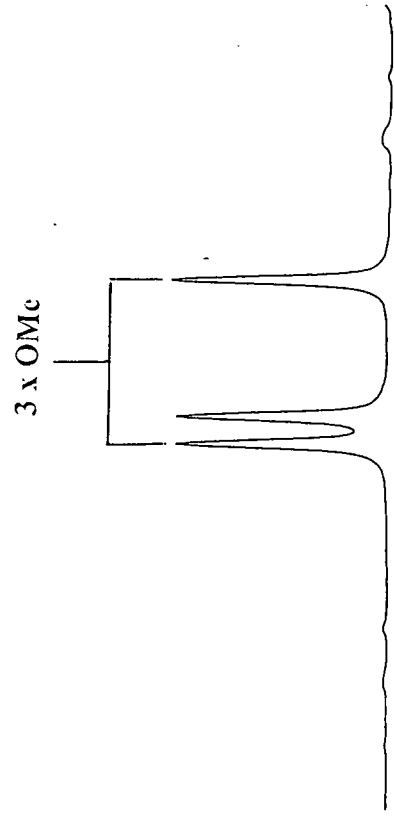
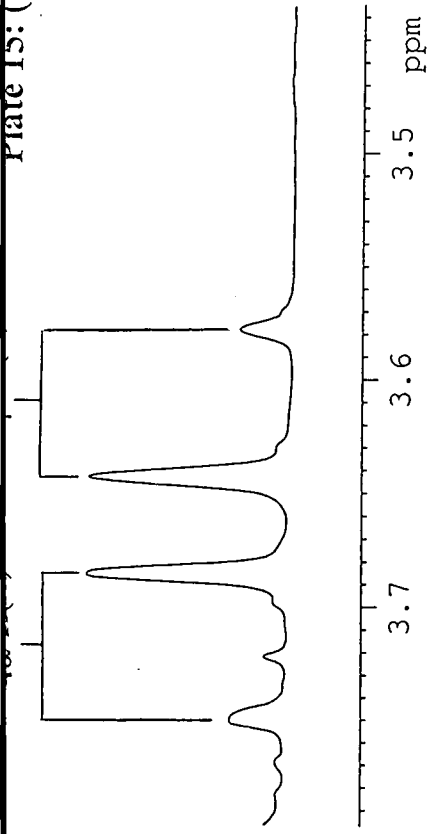


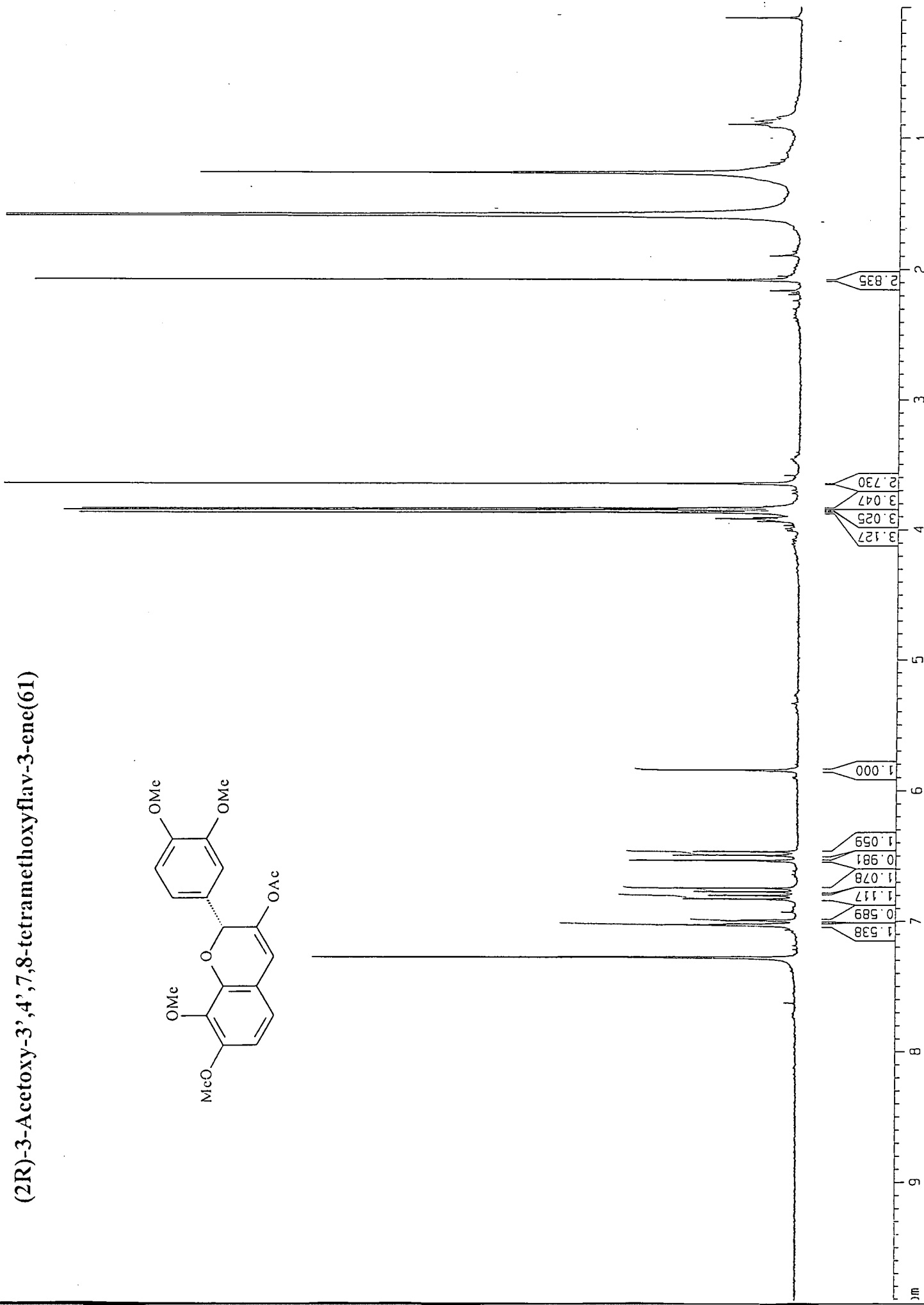
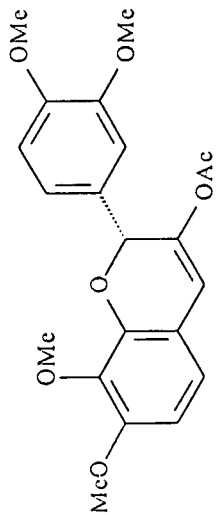
Plate 15: (CDCl₃, 296K)

(2R)-3',4',7-Trimethoxyflavan-3-one(52)





(2R)-3-Acetoxy-3',4',7,8-tetramethoxyflav-3-ene(61)



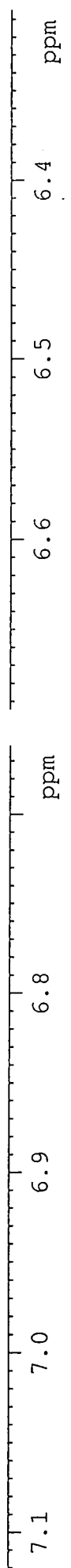
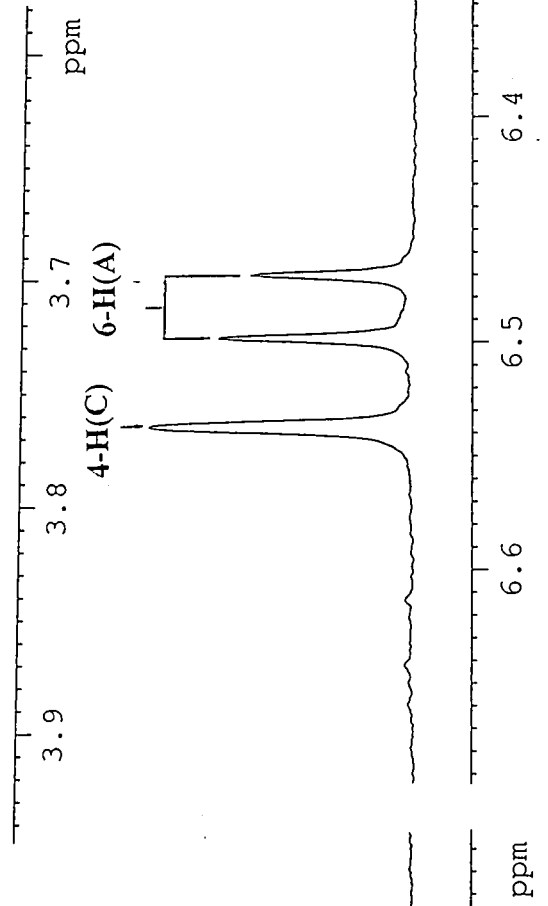
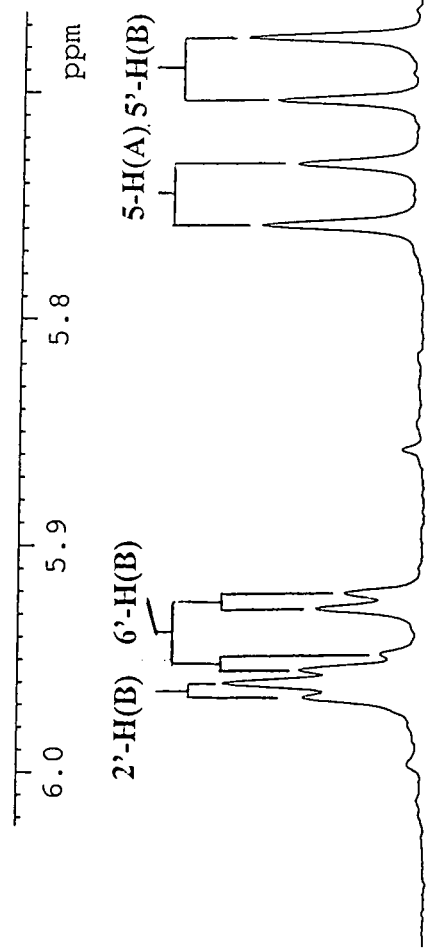
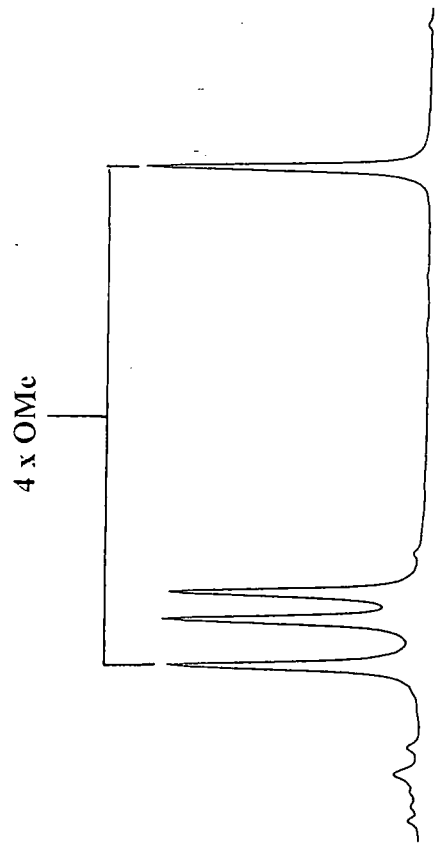
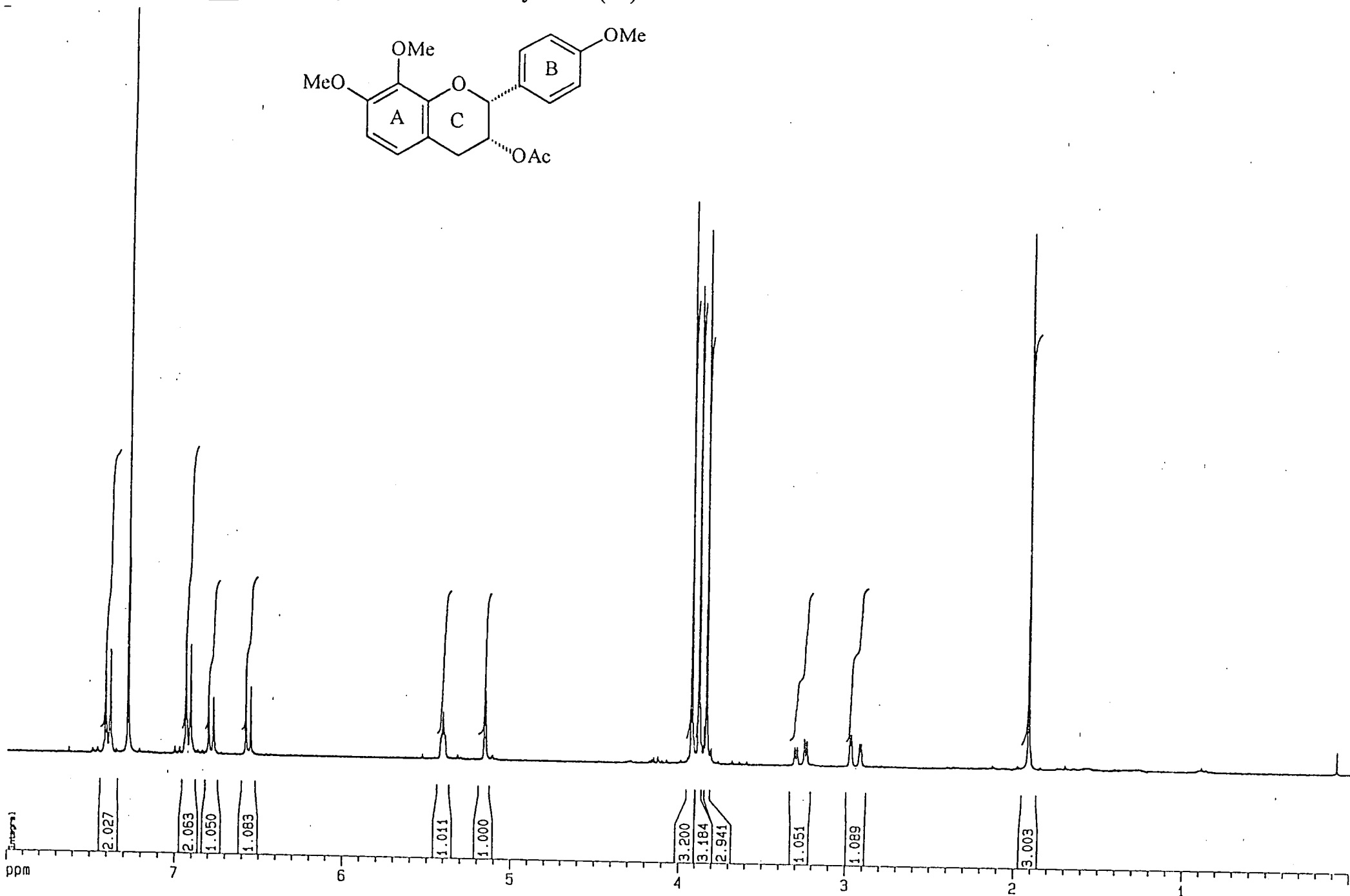
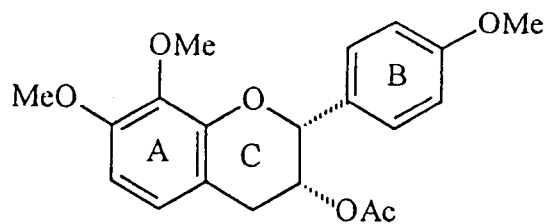
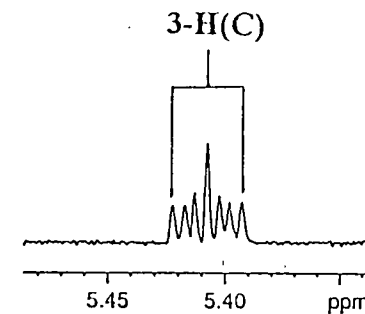
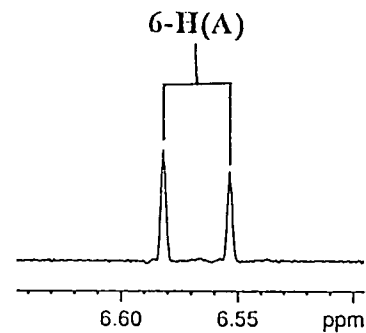
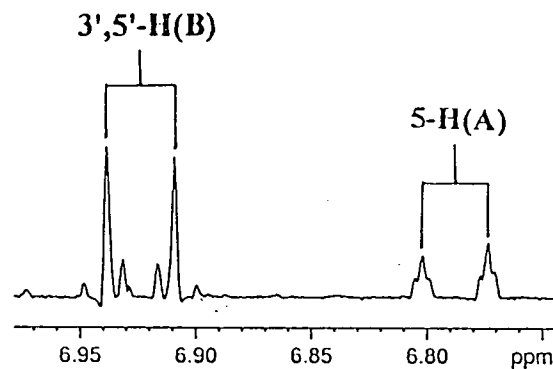
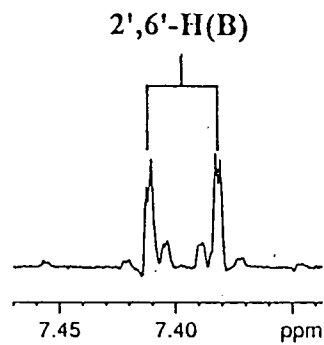
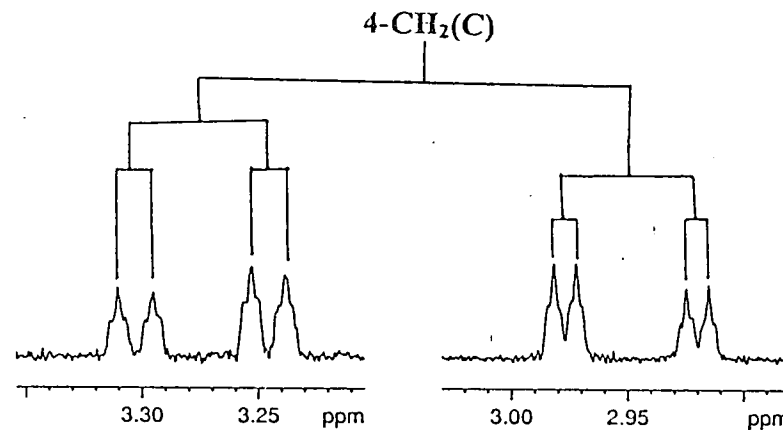
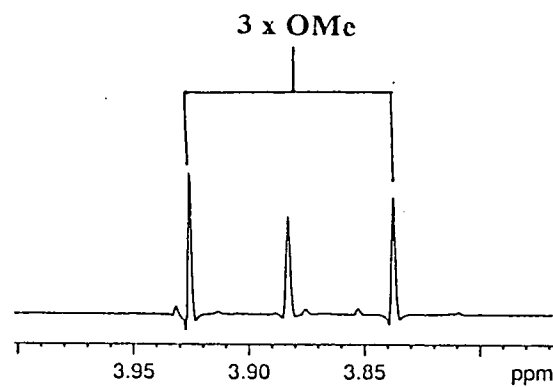
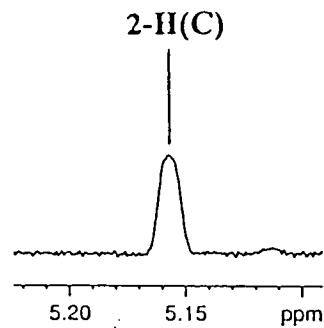
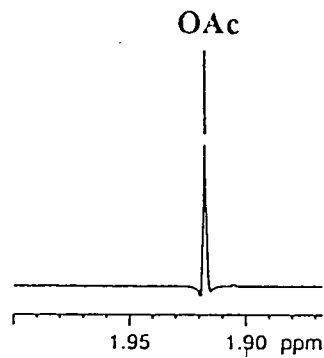


Plate 17: (CDCl₃ 296K)

(2R,3R)-2,3-cis-3-Acetoxy-4',7,8-trimethoxyflavan(50)





(2R,3S)-2,3-trans-4',7,8-trimethoxyflavan(49)

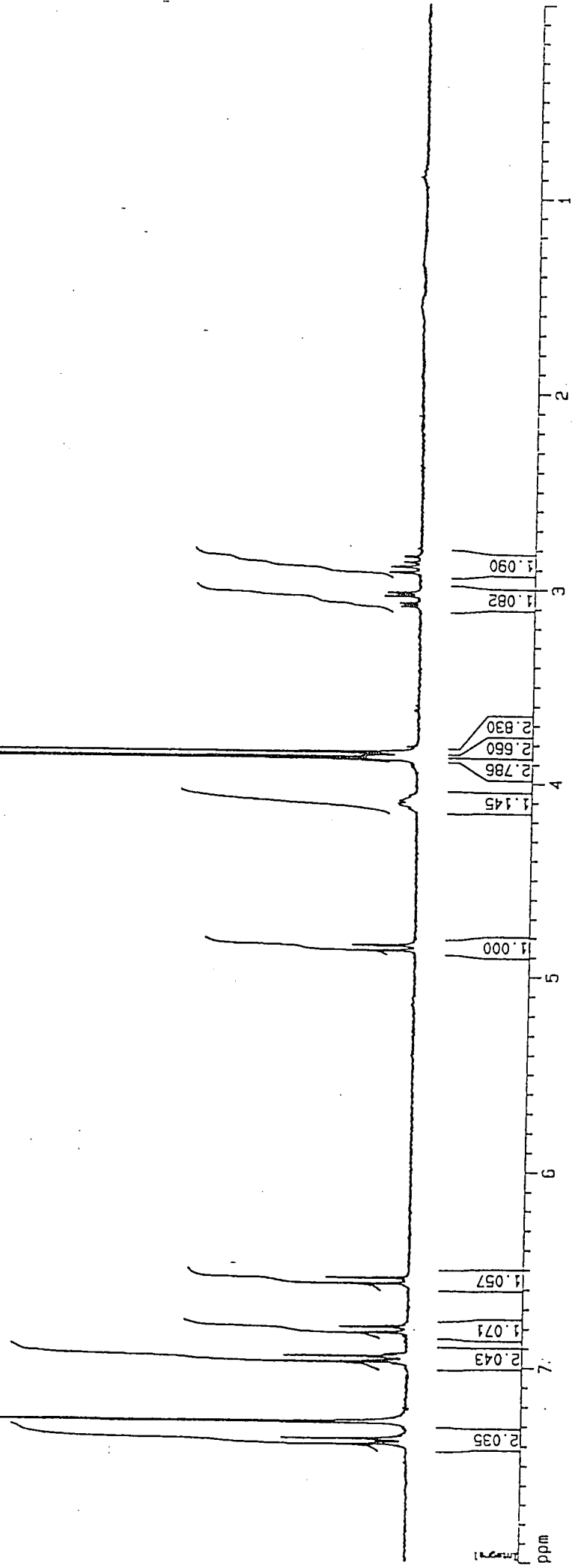
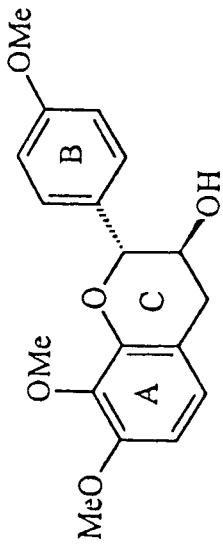
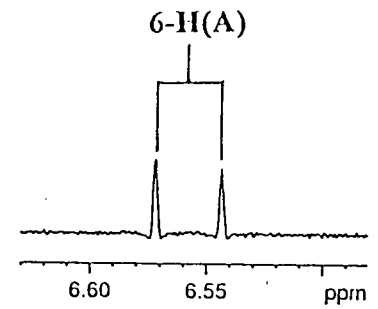
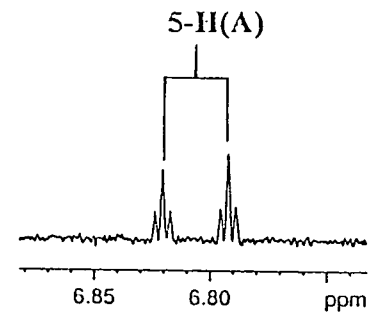
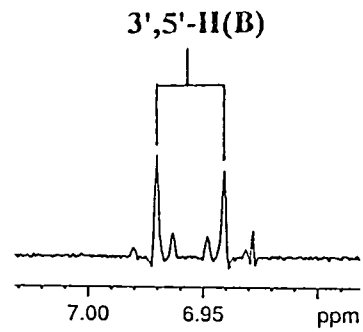
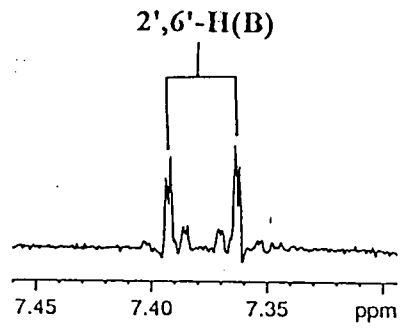
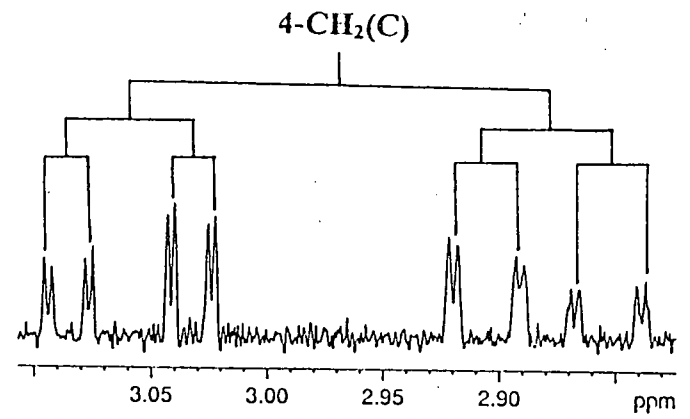
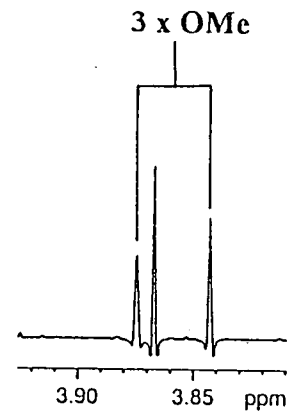
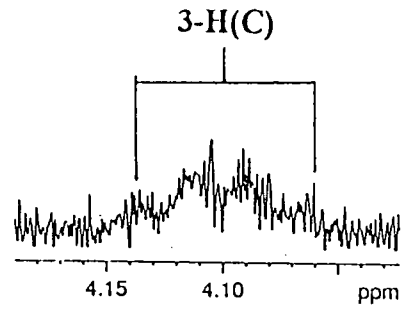
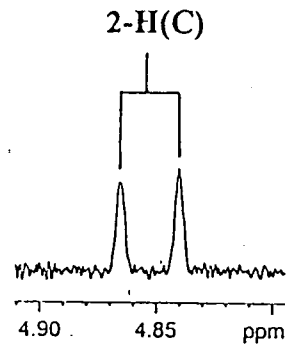
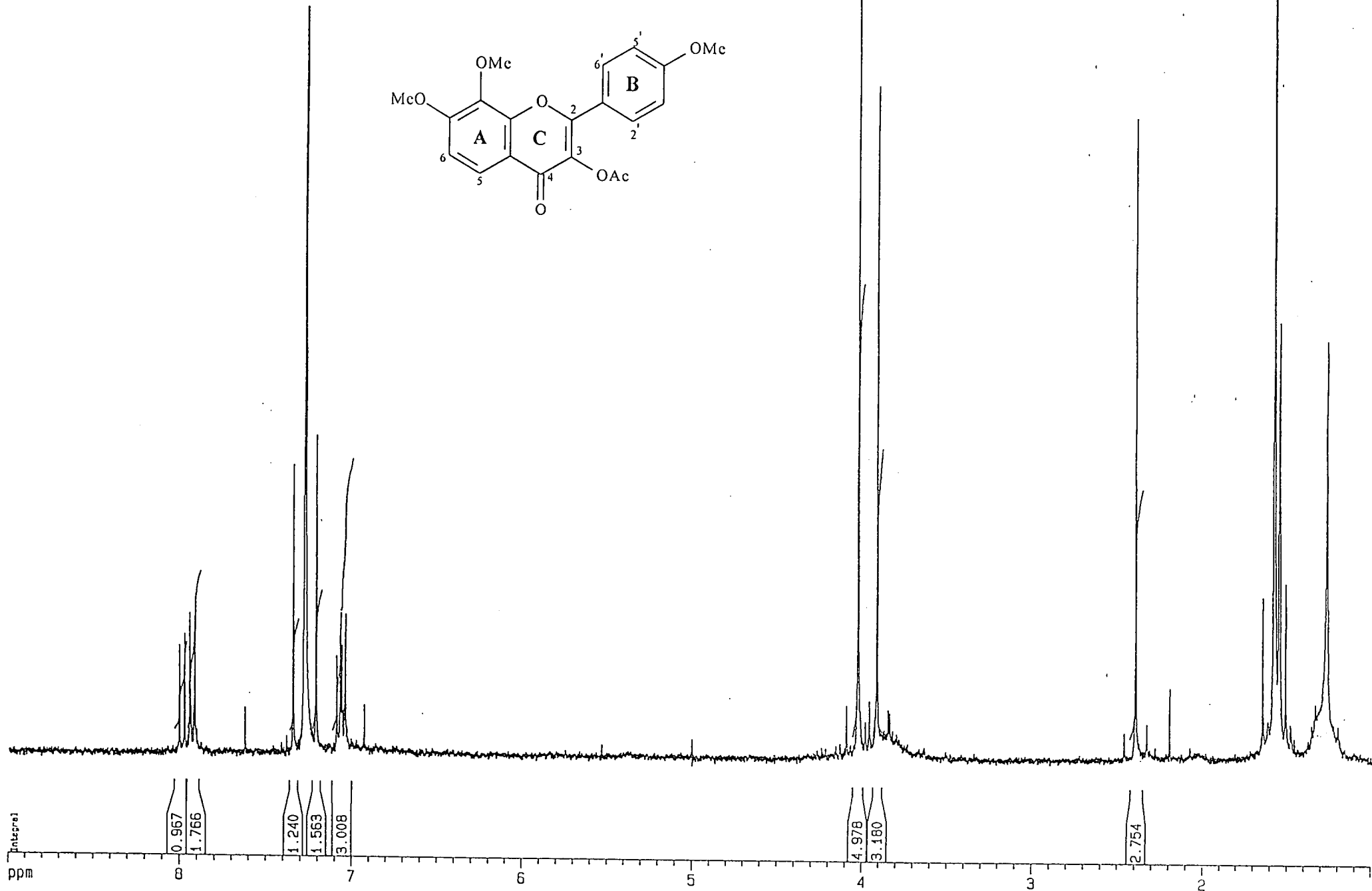
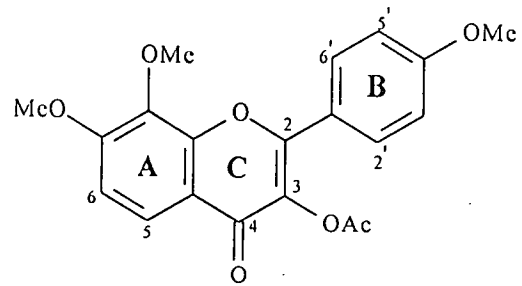


Plate 18: (CDCl₃ 296K)



3-Acetoxy-4',7,8-trimethoxyflavonol(53)



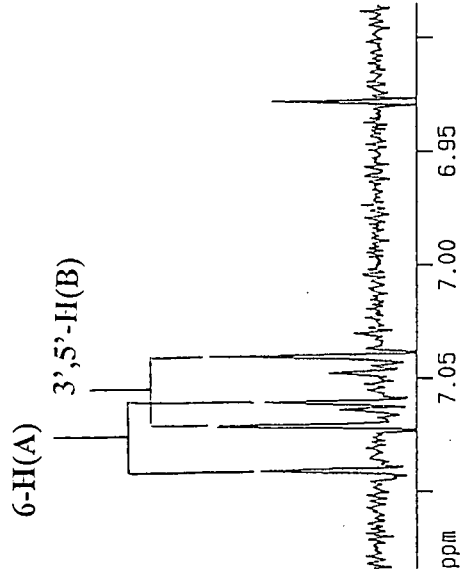
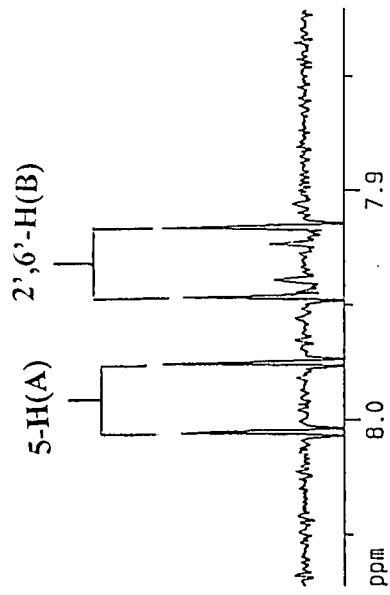
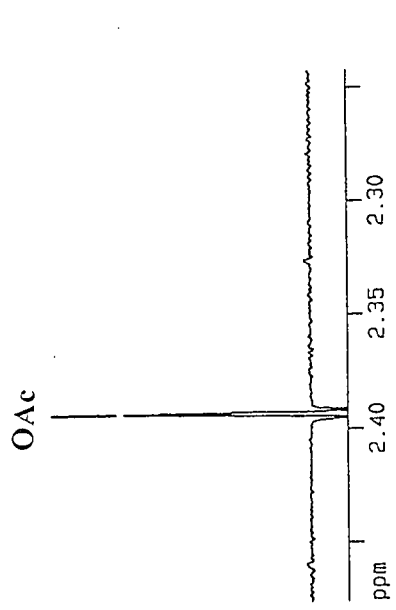
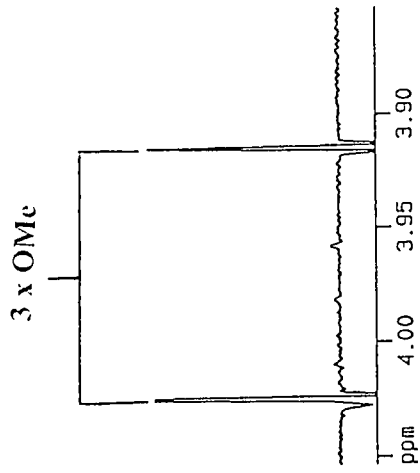


Plate 20: (CDCl₃ 296K)

3-Acetoxy-3',4',7-trimethoxyflavonol(54)

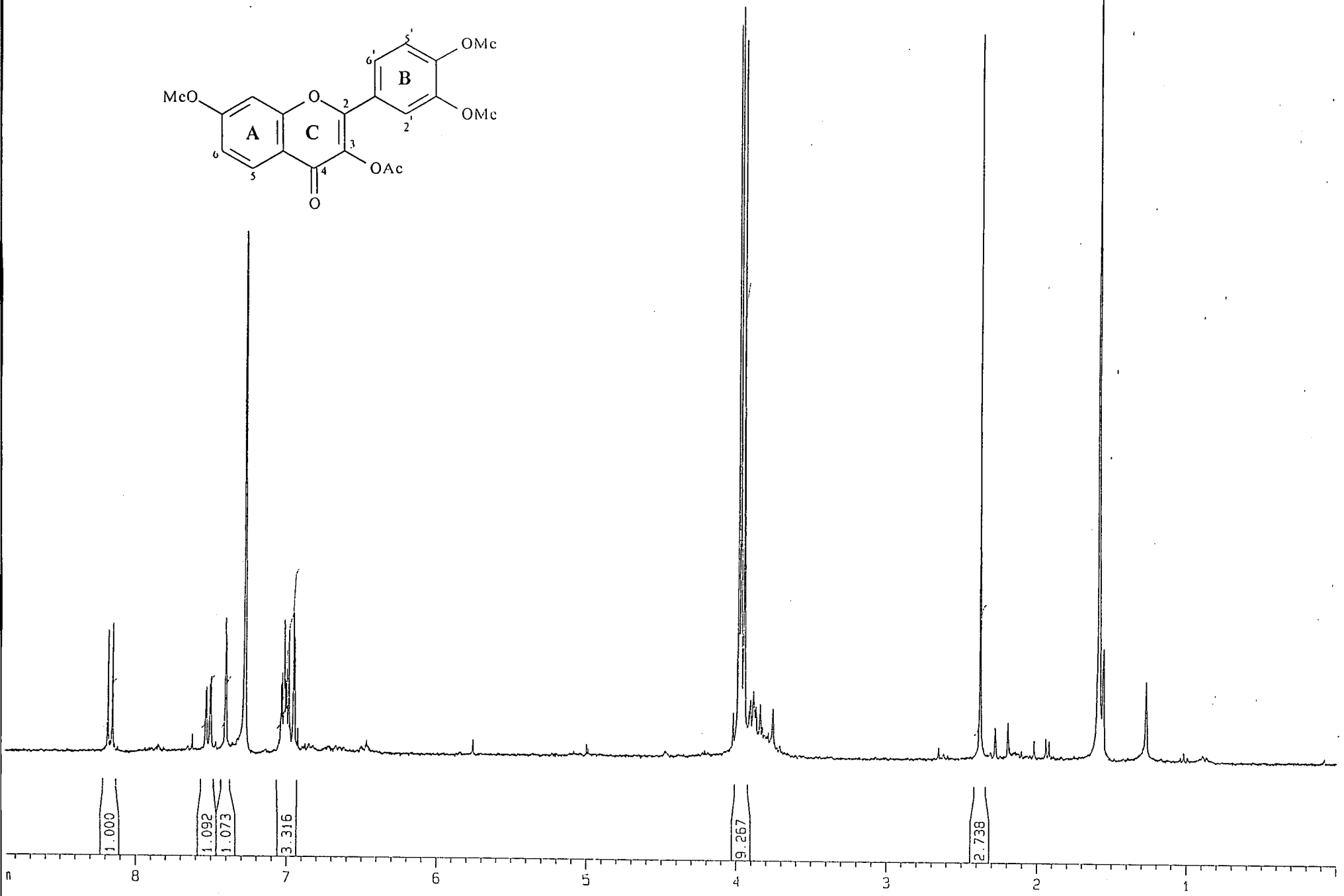
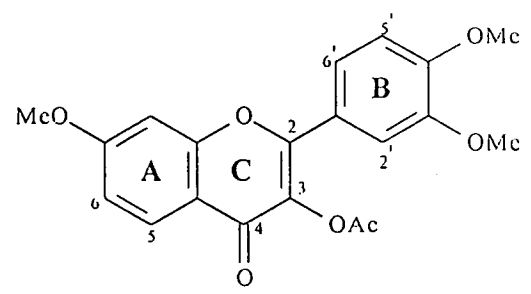
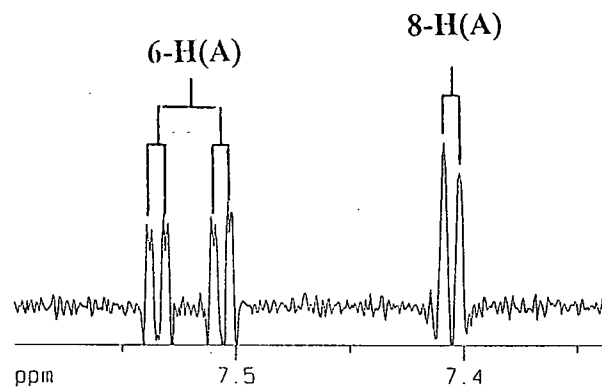
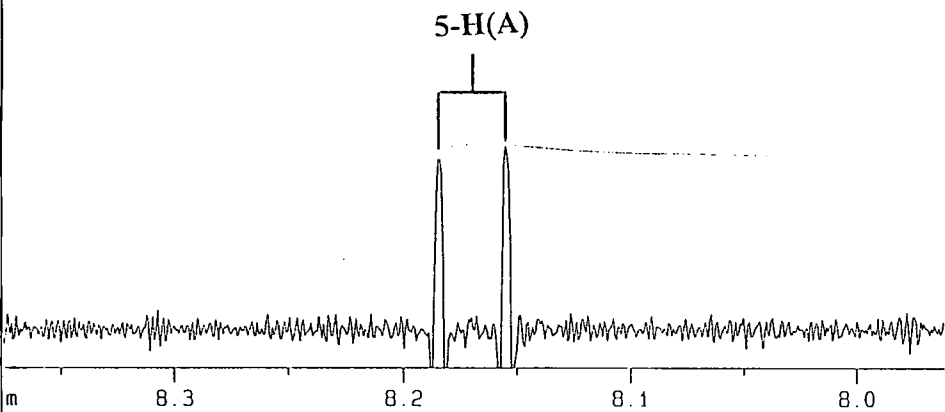
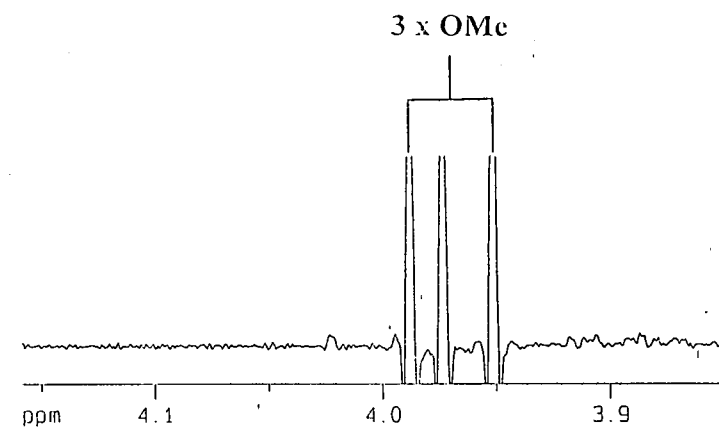
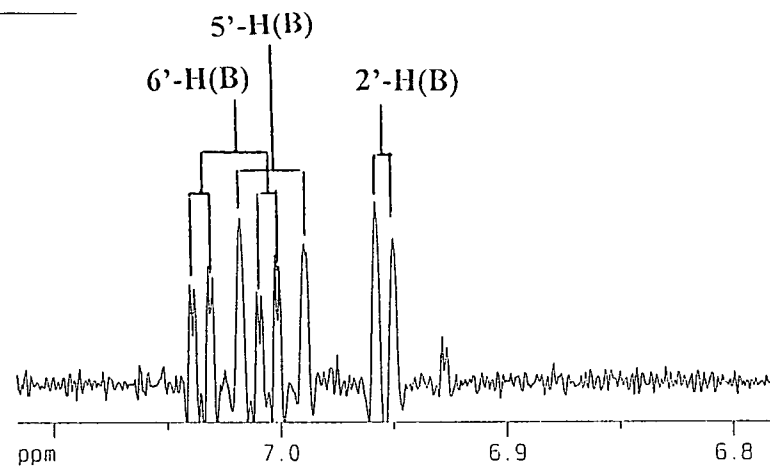
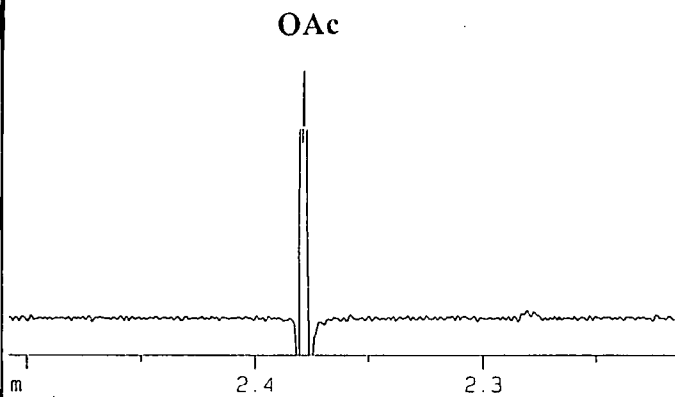
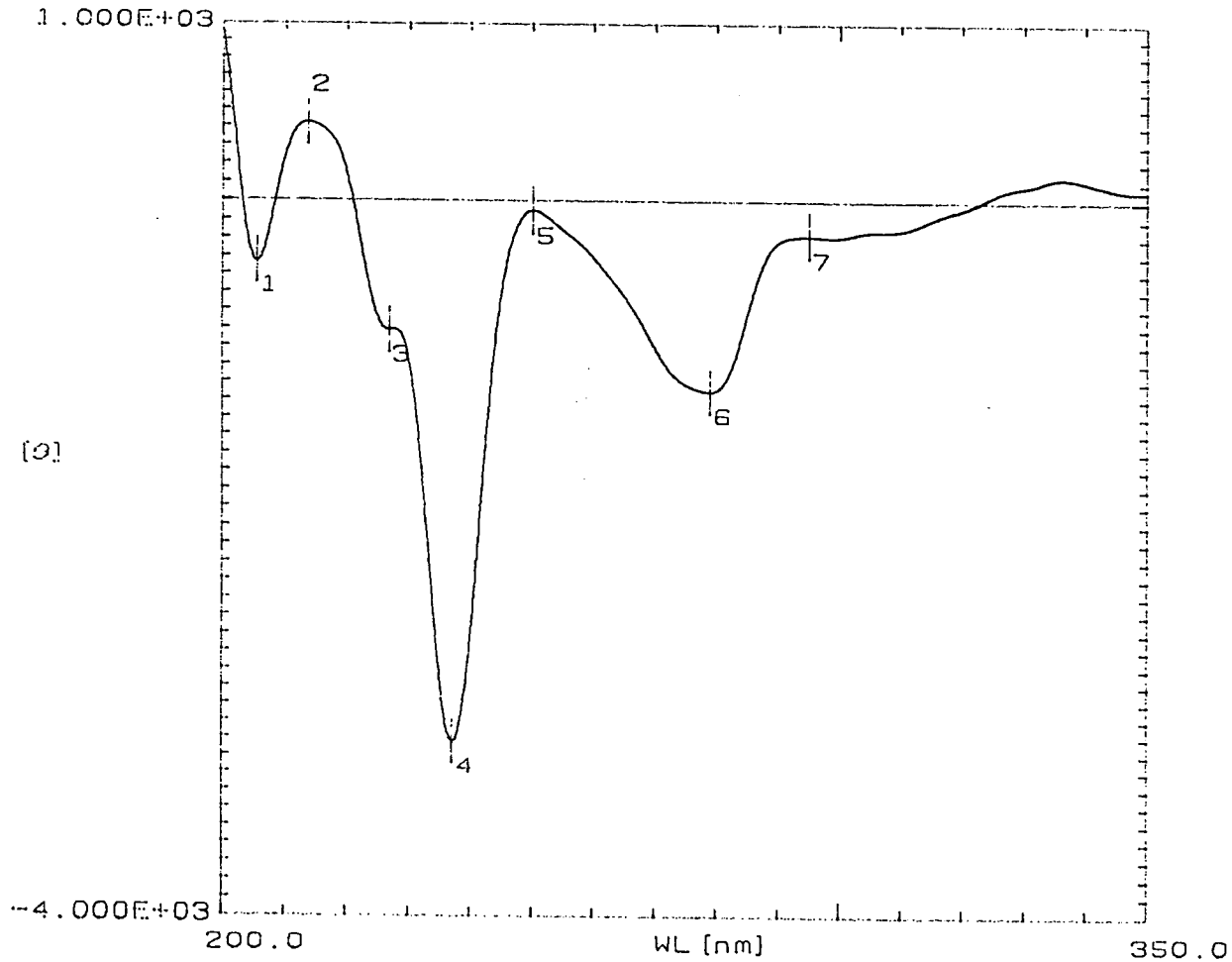


Plate 20: (CDCl₃ 296K)



APPENDIX B: CD SPECTRA

Plate 1:



No.	Wavelength	Value
1	205.60 nm	-3.307E+02
2	213.70 nm	4.226E+02
3	226.90 nm	-7.084E+02
4	237.20 nm	-3.024E+03
5	250.20 nm	-4.938E+01
6	279.00 nm	-1.052E+03
7	295.10 nm	-1.810E+02

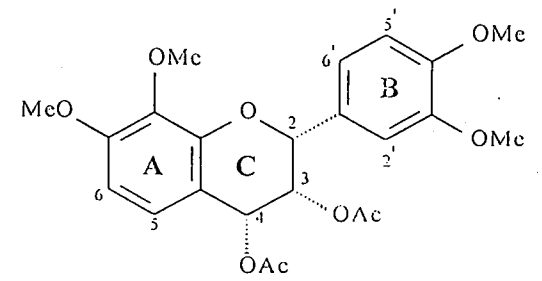
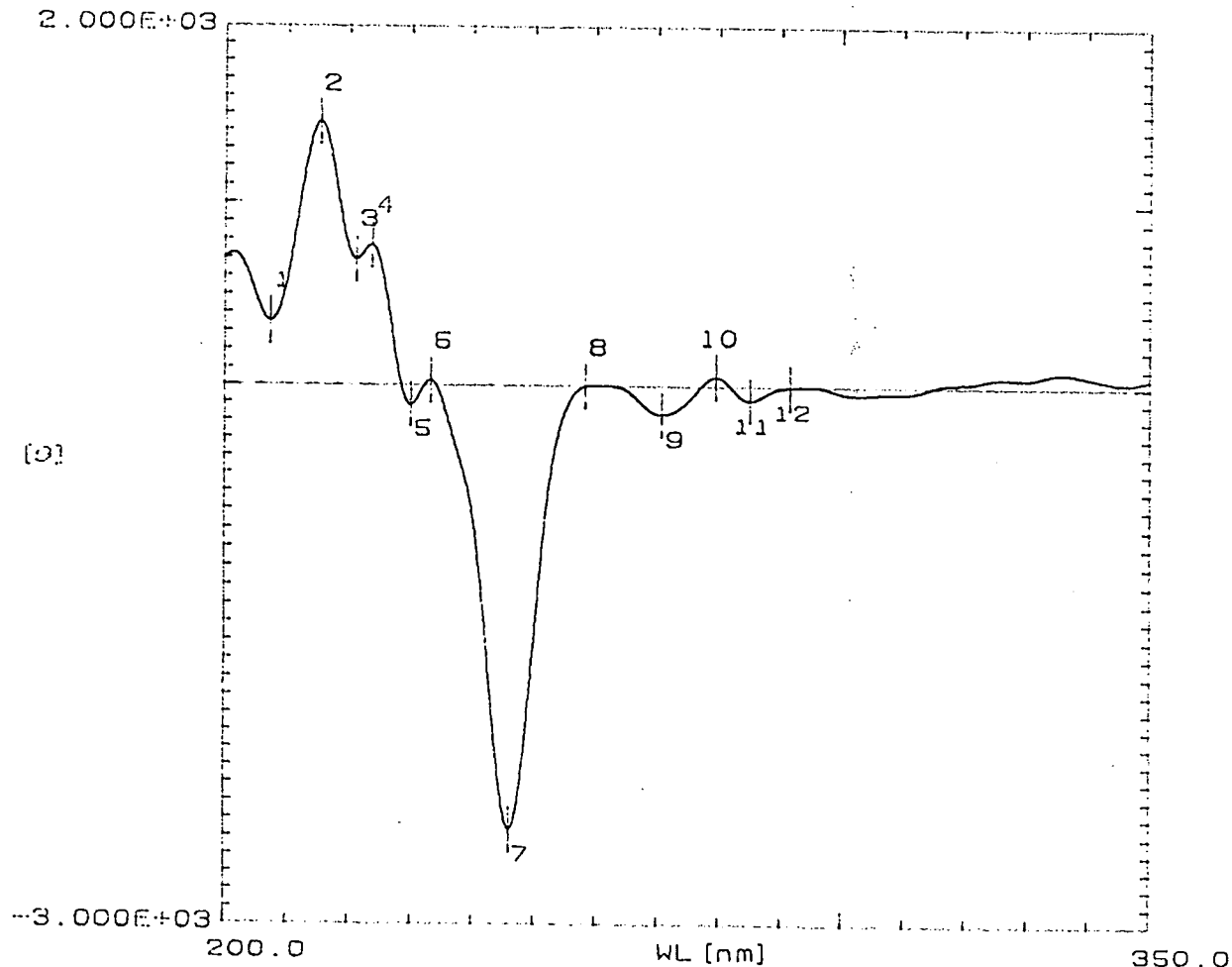
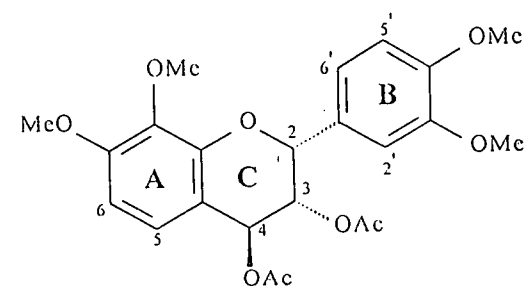


Plate 2:

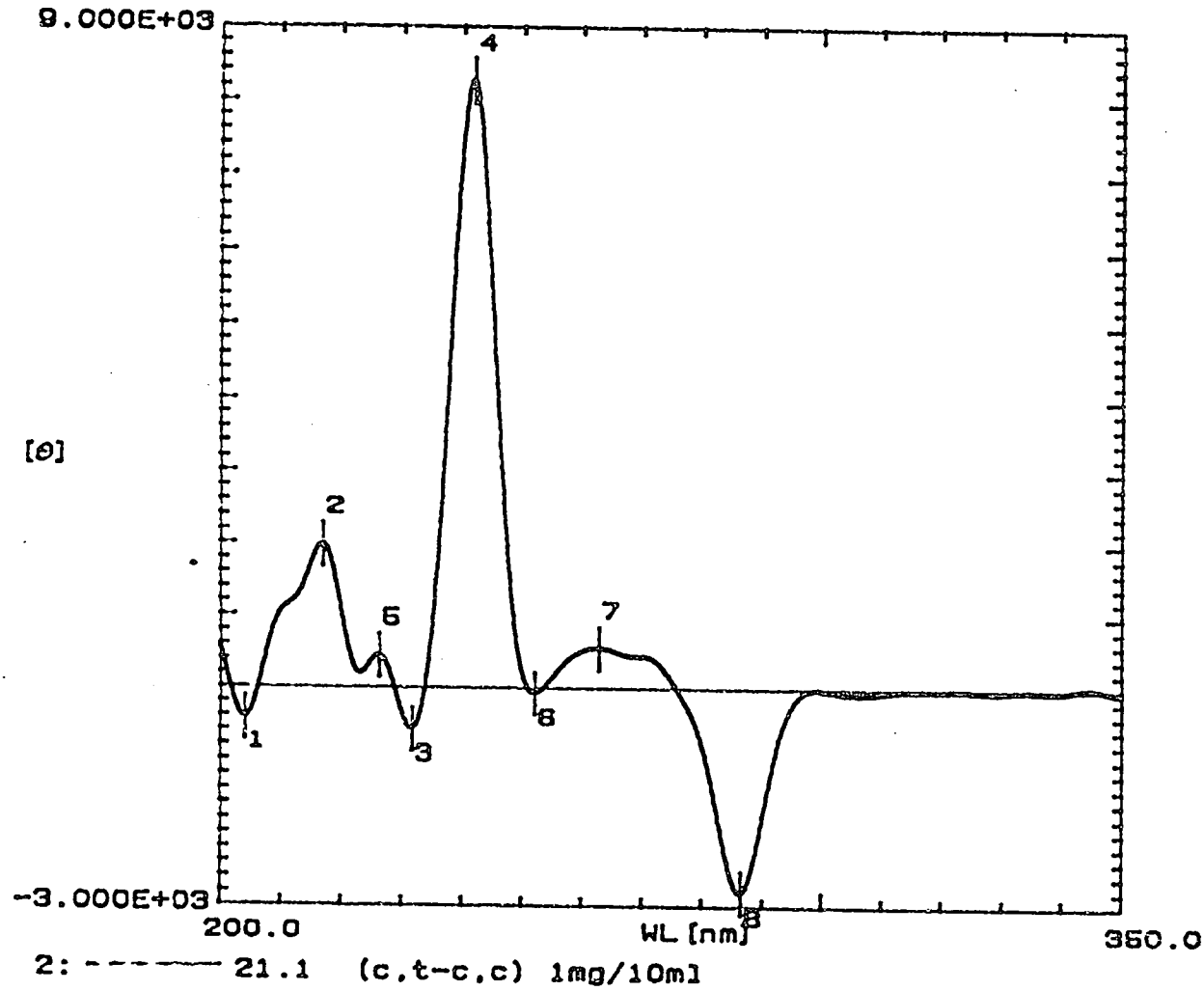


No.	Wavelength	Value
1	207.40 nm	3.526E+02
2	215.30 nm	1.447E+03
3	221.20 nm	6.931E+02
4	223.60 nm	7.705E+02
5	230.00 nm	-1.084E+02
6	233.20 nm	2.913E+01
7	246.10 nm	-2.459E+03
8	258.40 nm	5.174E-01
9	270.80 nm	-1.599E+02
10	279.50 nm	5.581E+01
11	285.00 nm	-7.711E+01
12	291.50 nm	-1.013E+00



2: 6 cis-trans mela 1mg/10ml

Plate 3:



No.	Wavelength	Value
1	204.00 nm	-4.267E+02
2	218.80 nm	1.986E+03
3	231.60 nm	-5.798E+02
4	241.70 nm	8.282E+03
5	228.10 nm	4.506E+02
6	262.00 nm	-7.526E+01
7	282.70 nm	5.593E+02
8	288.40 nm	-2.802E+03

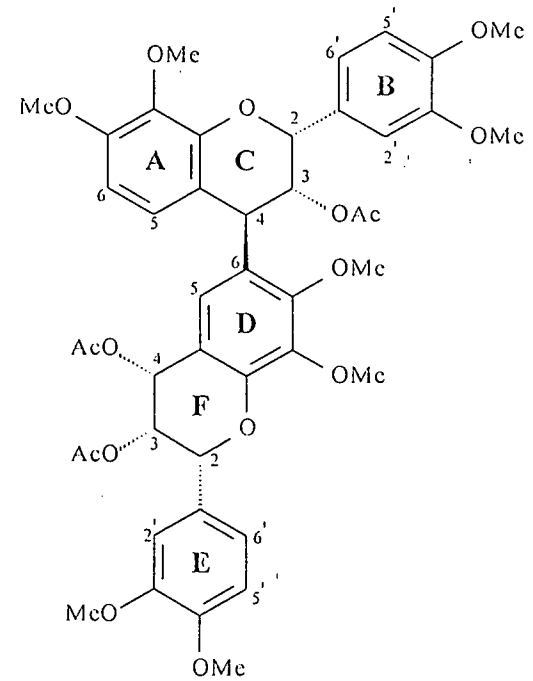
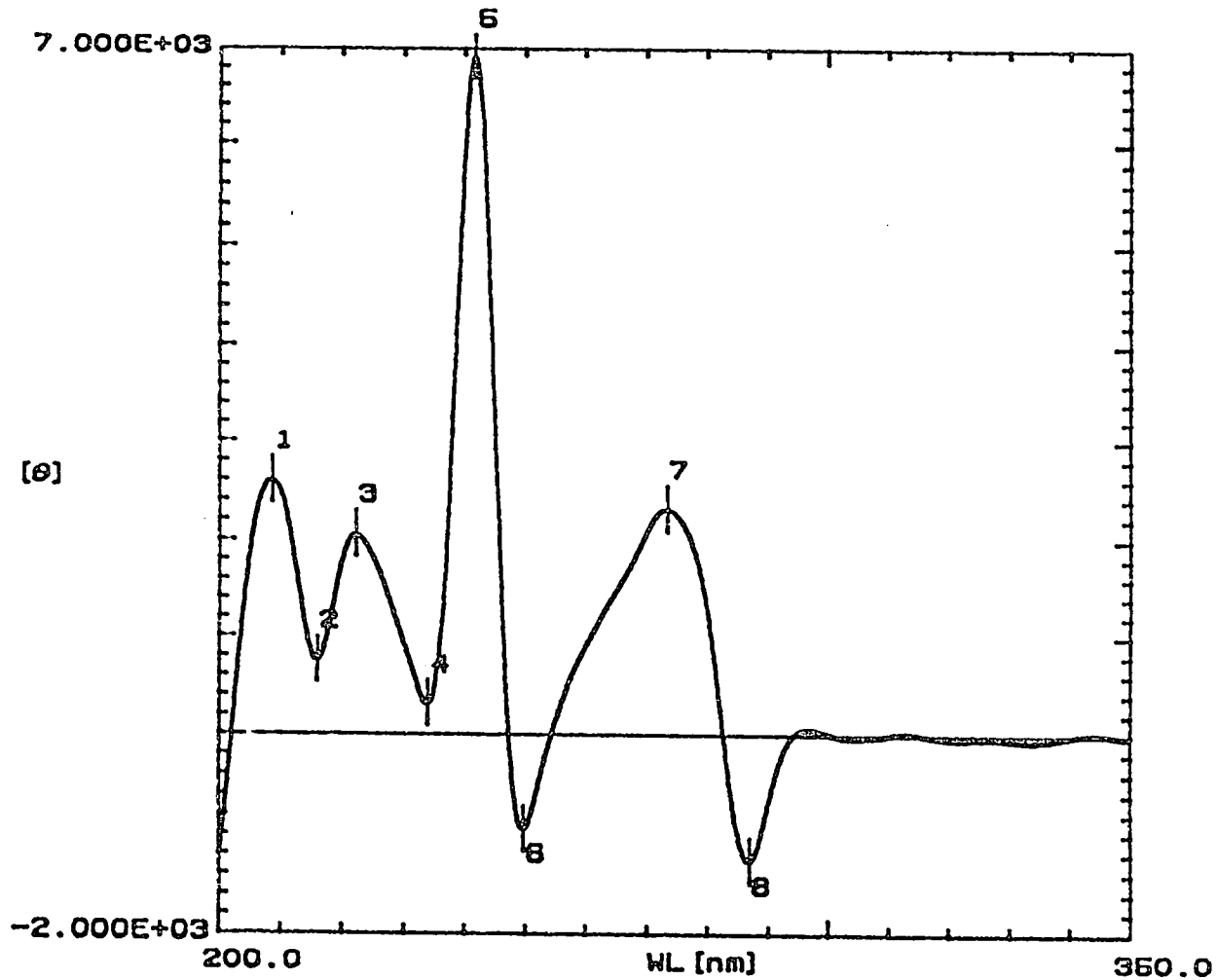


Plate 4:



2: - - - - - 20.18.1.2 (c.t-c.t) 1mg/10ml

No.	Wavelength	Value
1	208.70 nm	2.691E+03
2	216.00 nm	7.489E+02
3	222.30 nm	2.043E+03
4	234.10 nm	3.048E+02
5	241.70 nm	6.898E+03
6	249.80 nm	-9.686E+02
7	273.40 nm	2.308E+03
8	286.80 nm	-1.274E+03

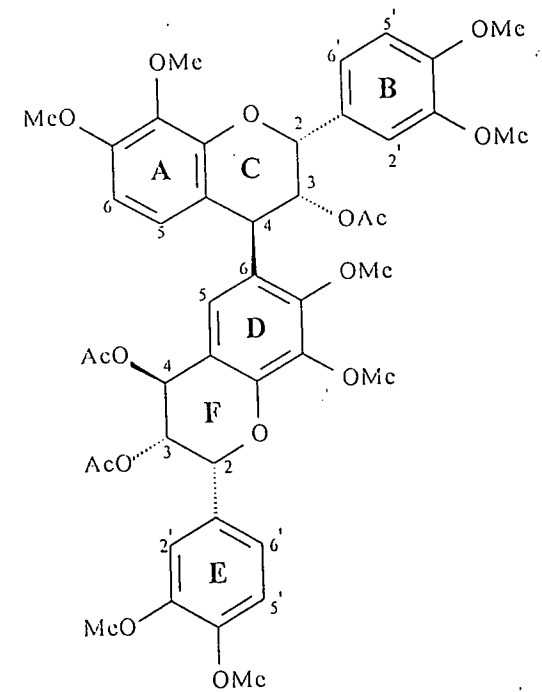
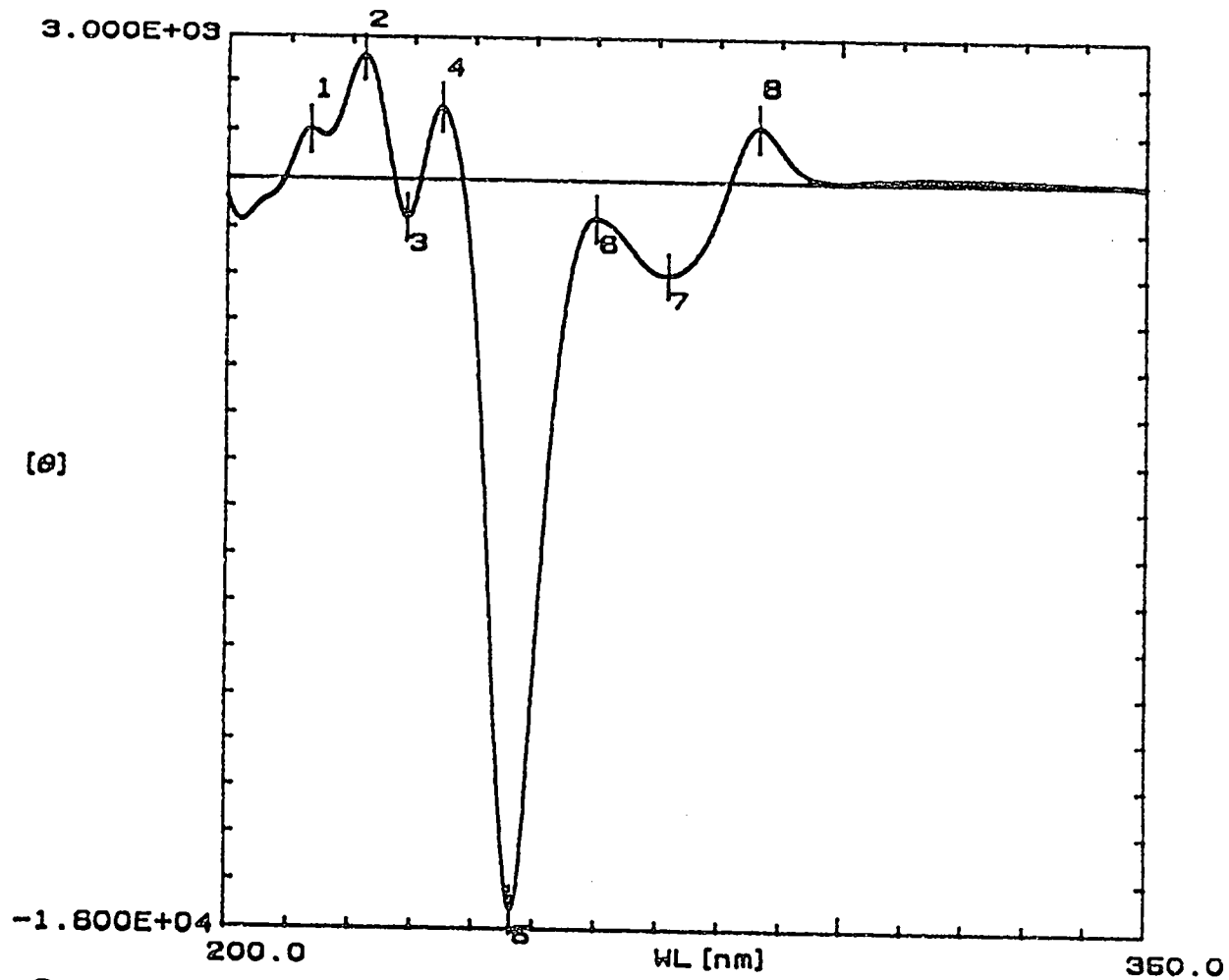


Plate 5:



No.	Wavelength	Value
1	213.50 nm	1.041E+03
2	222.10 nm	2.553E+03
3	229.00 nm	-7.819E+02
4	234.70 nm	1.507E+03
6	248.40 nm	-1.558E+04
8	260.00 nm	-7.699E+02
7	271.70 nm	-1.989E+03
8	286.50 nm	1.143E+03

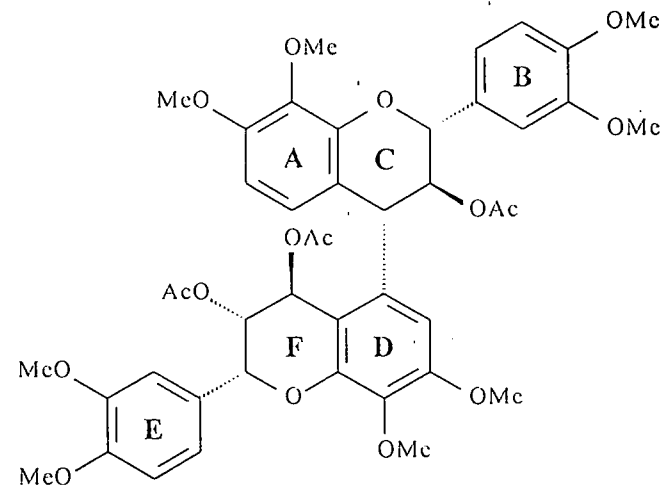
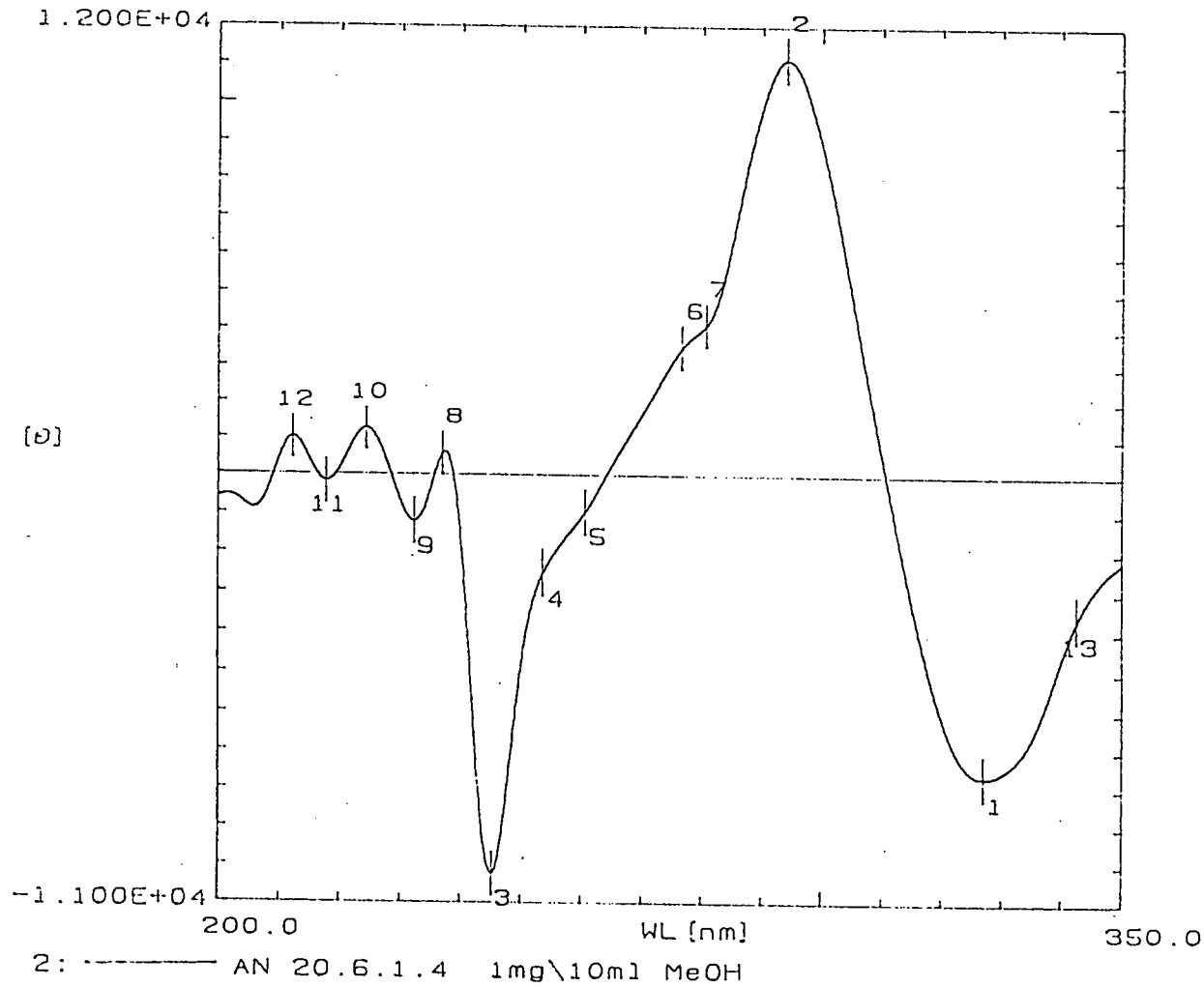


Plate 6:



No.	Wavelength	Value
1	327.10 nm	-7.676E+03
2	294.10 nm	1.114E+04
3	245.20 nm	-1.024E+04
4	253.60 nm	-2.504E+03
5	260.60 nm	-9.530E+02
6	276.60 nm	3.532E+03
7	280.60 nm	4.133E+03
8	236.90 nm	5.799E+02
9	232.20 nm	-1.212E+03
10	224.30 nm	1.253E+03
11	217.70 nm	-1.772E+02
12	212.10 nm	1.019E+03
13	342.60 nm	-3.587E+03

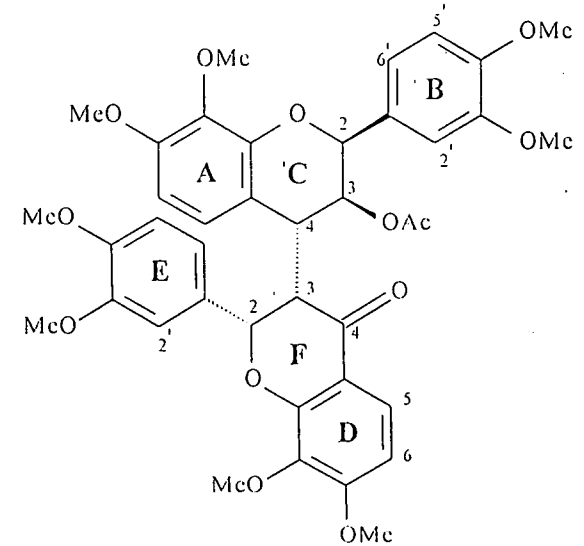
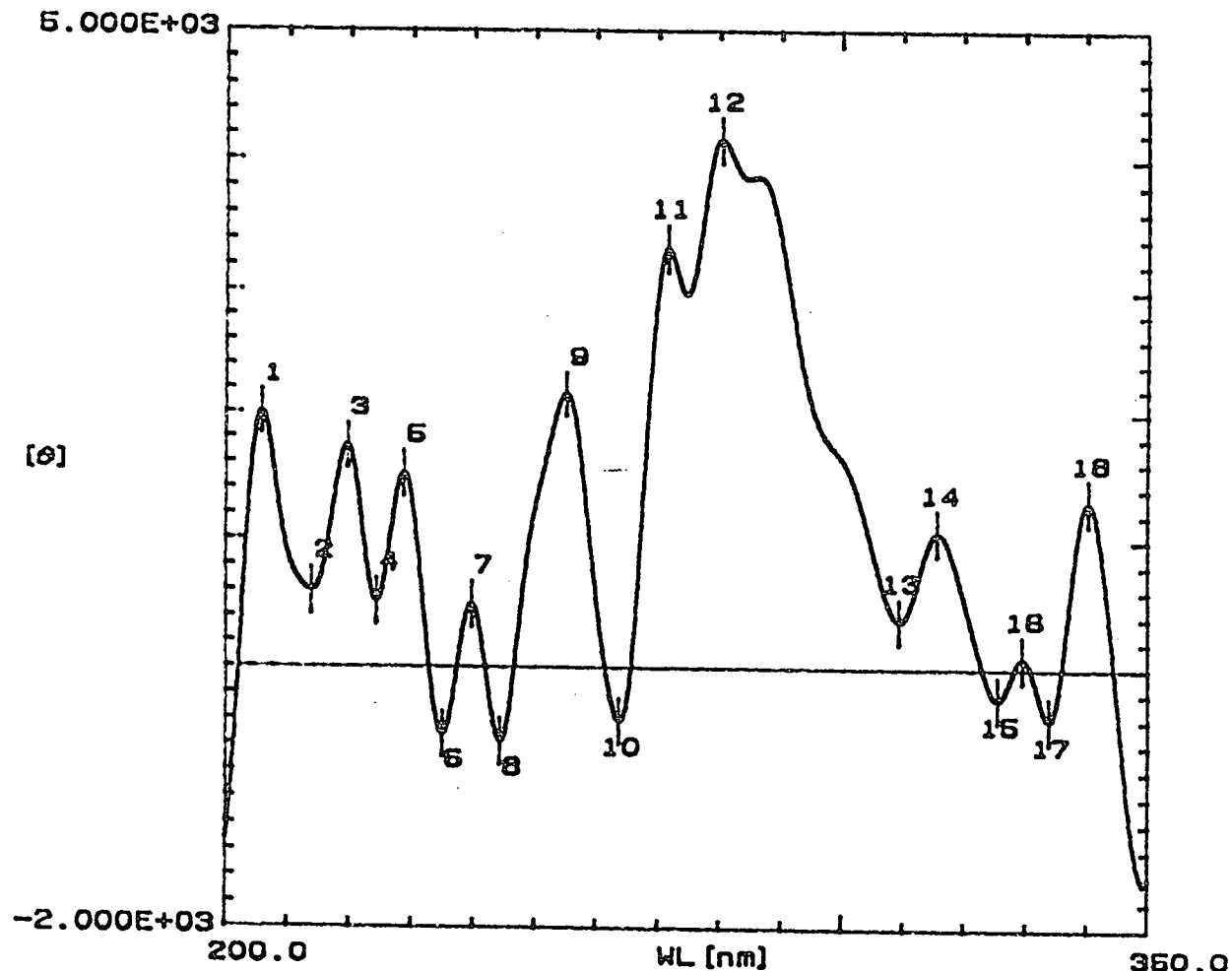


Plate 7:



2: - - - - - 20.8.2.2 (t,t-flavonol) 1mg/10ml

No.	Wavelength	Value
1	205.70 nm	2.011E+03
2	213.80 nm	6.888E+02
3	219.80 nm	1.738E+03
4	224.30 nm	6.173E+02
5	228.70 nm	1.619E+03
6	234.80 nm	-6.146E+02
7	239.70 nm	4.868E+02
8	244.30 nm	-6.722E+02
9	255.10 nm	2.162E+03
10	263.70 nm	-4.130E+02
11	271.60 nm	3.930E+03
12	280.30 nm	4.168E+03
13	309.40 nm	3.764E+02
14	316.70 nm	1.088E+03
15	325.60 nm	-2.926E+02
16	329.80 nm	8.701E+01
17	333.80 nm	-3.898E+02
18	340.30 nm	1.919E+03

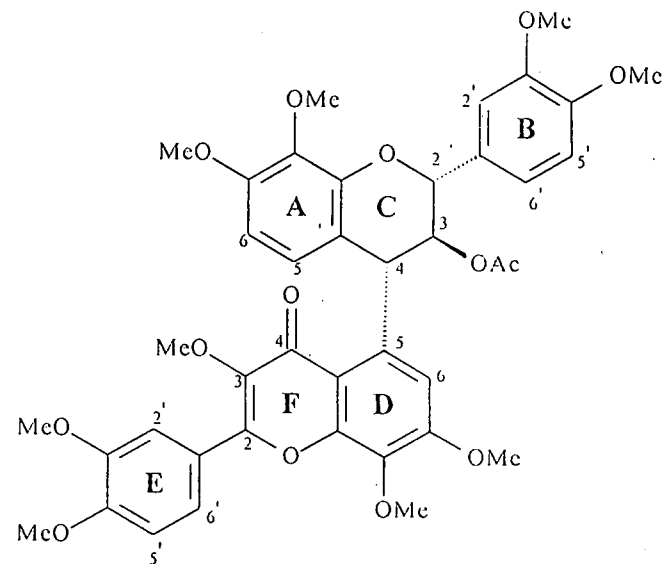
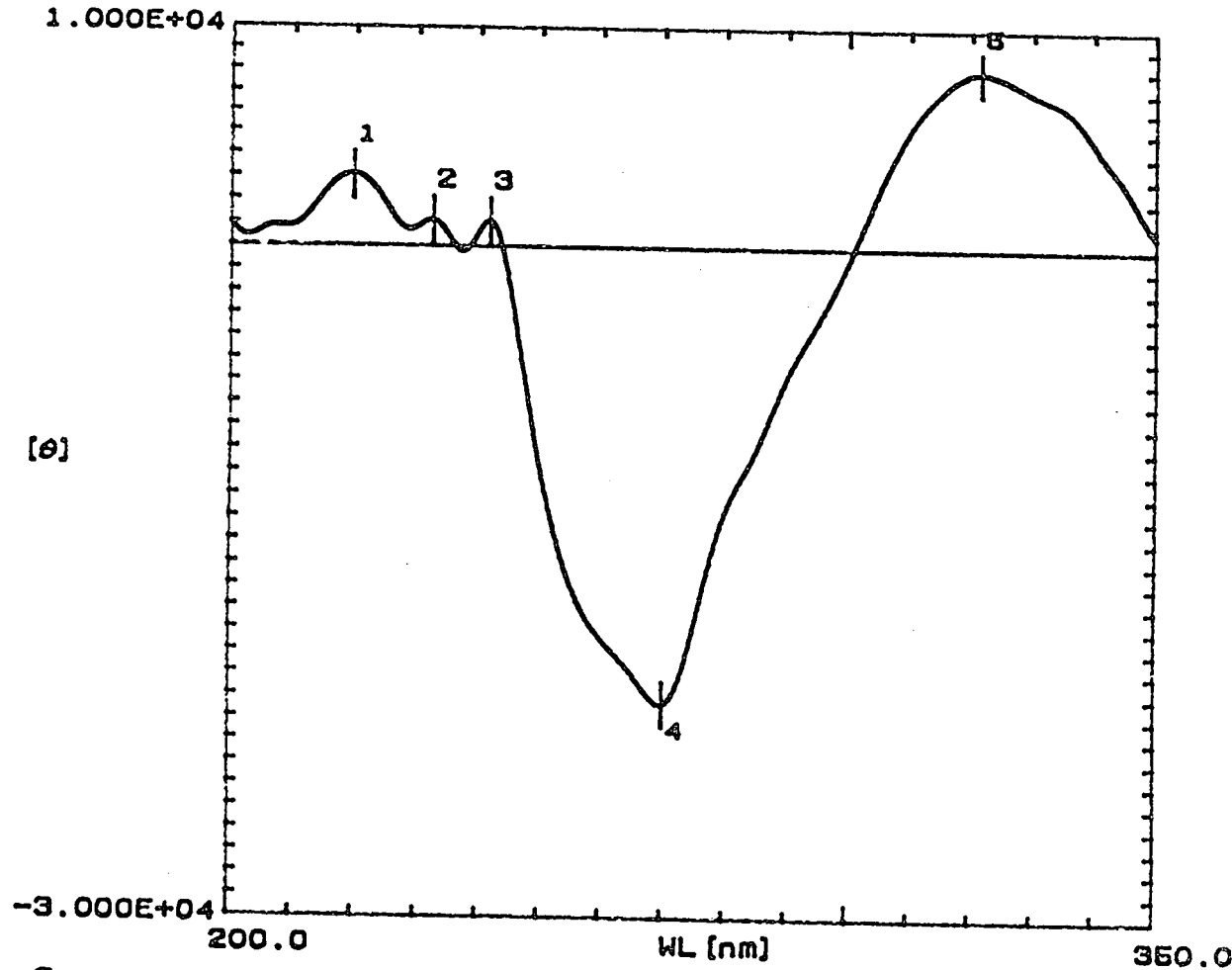


Plate 8:



No.	Wavelength	Value
1	219.60 nm	9.090E+03
2	232.40 nm	1.146E+03
3	241.60 nm	1.095E+03
4	289.80 nm	-2.034E+04
5	321.30 nm	7.986E+03

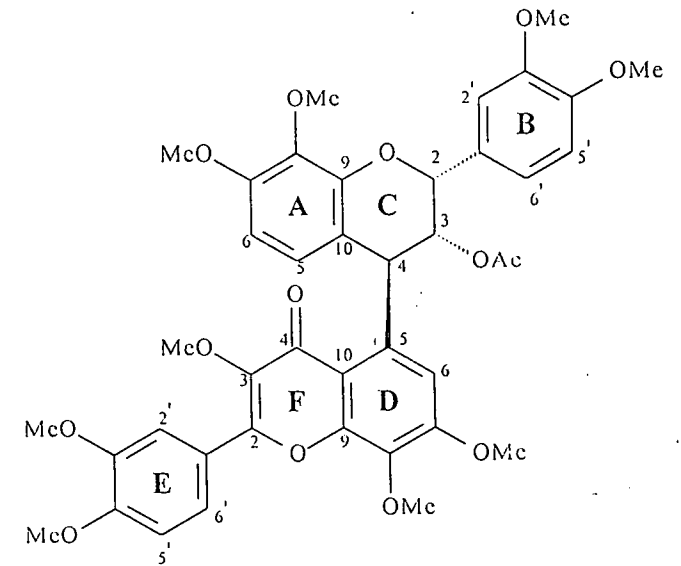
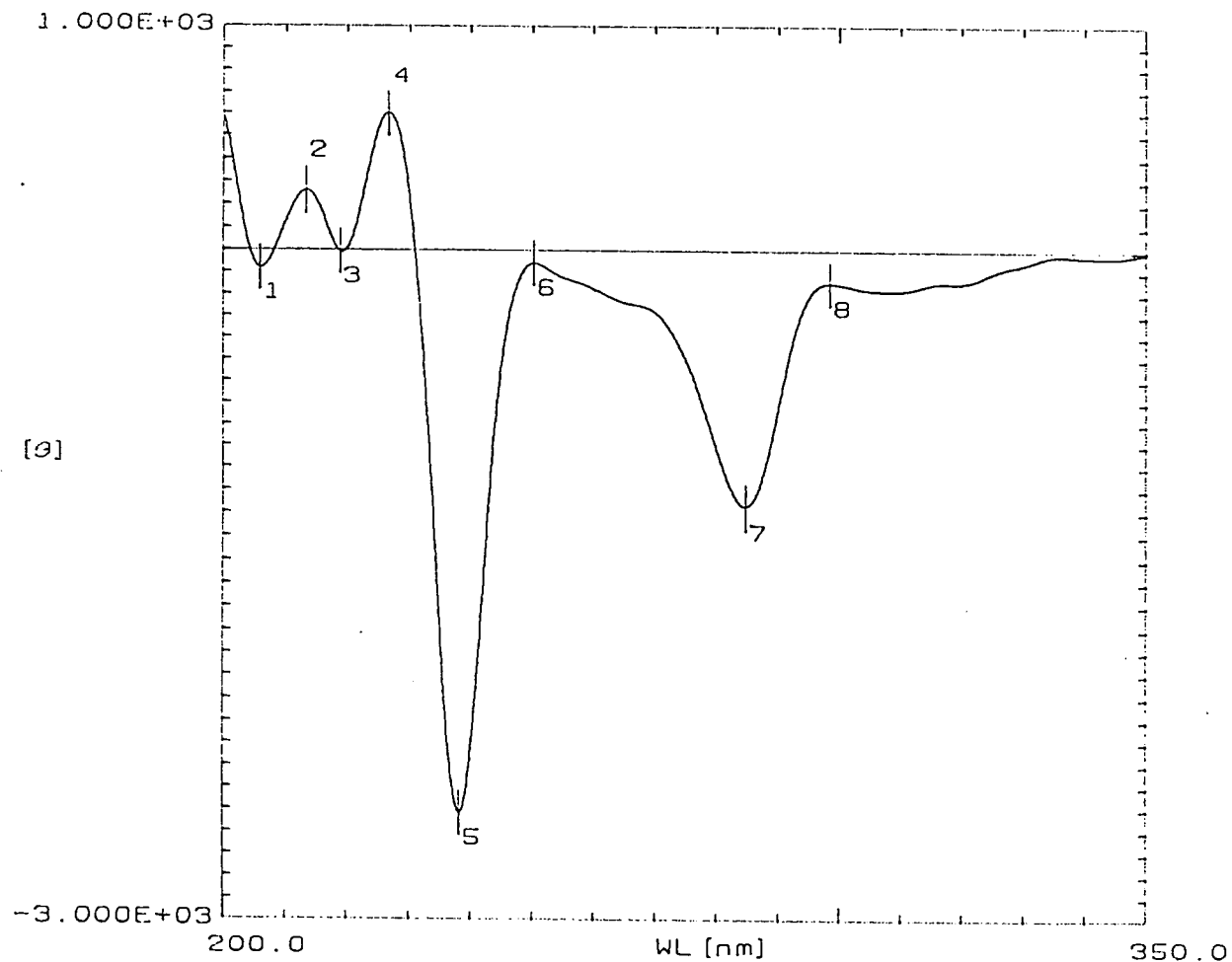


Plate 9:



2: ----- 1 2,3-cis 1mg/10ml

No.	Wavelength	Value
1	206.00 nm	-8.089E+01
2	213.30 nm	2.607E+02
3	218.80 nm	-6.383E+00
4	226.60 nm	6.013E+02
5	238.20 nm	-2.516E+03
6	250.30 nm	-5.740E+01
7	284.80 nm	-1.170E+03
8	298.50 nm	-1.468E+02

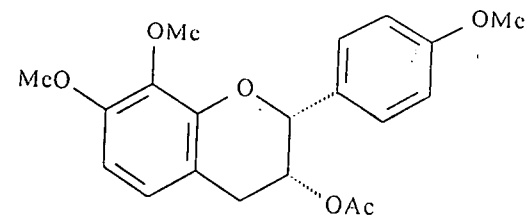
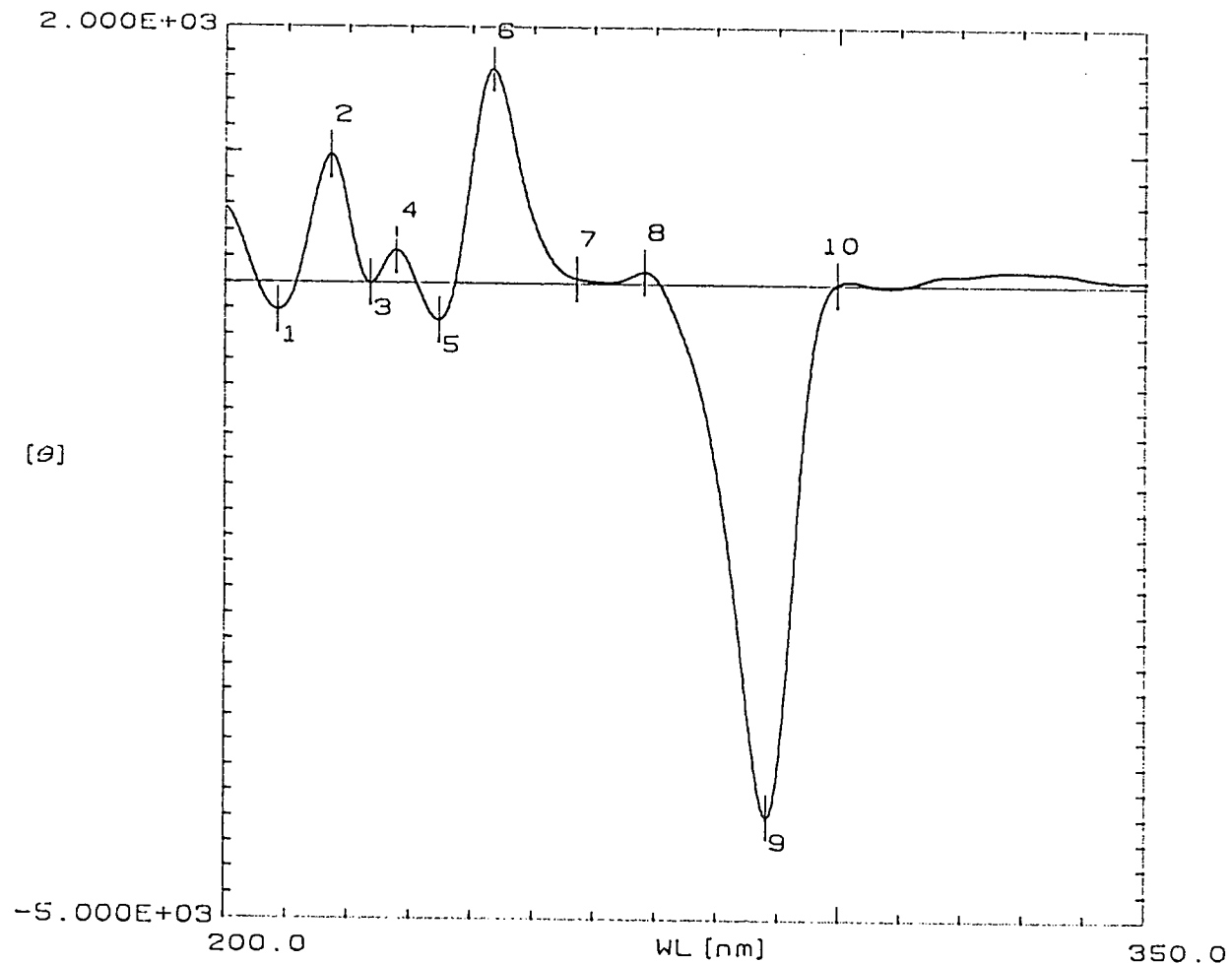
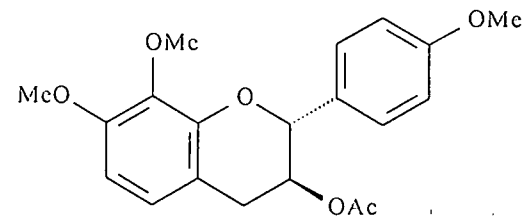


Plate 10:



No.	Wavelength	Value
1	208.30 nm	-2.119E+02
2	216.80 nm	9.797E+02
3	223.30 nm	-8.594E-01
4	227.50 nm	2.528E+02
5	234.40 nm	-2.850E+02
6	243.10 nm	1.662E+03
7	256.90 nm	3.990E+01
8	267.90 nm	9.381E+01
9	287.90 nm	-4.192E+03
10	299.50 nm	9.951E+00



2: - - - - - 2 2,3-trans 1mg/10ml

SUMMARY

The previous investigation, by Fourie in the 1970's, revealed the presence of phenolic compounds in the heartwood of Knobwood (*Acacia nigrescens*), comprising a series of leucomelacacinidins. Early suggestions that oligomers containing the pyrogallol-type A-ring moieties are unlikely to exist, prompted a detailed reinvestigation for possible higher oligomers in the heartwood of this tree. A number of promelacacinidin dimers were discovered.

Enrichment of the heartwood extract followed by a number of gel separations resulted in complex phenolic mixtures of dimeric- and polymeric material. The purification of which was facilitated by derivatization of the phenolic compounds to methylether acetates. Structural elucidation of the compounds was carried out with the utilization of mass spectrometry and detailed ^1H NMR spectroscopy (300 MHz), ^{13}C , COSY, NOESY, HMQC and HMBC experiments on the methylether acetate derivatives.

Apart from the presence of epimesquitol-4 α -ol, epimesquitol-4 β -ol, dihydroflavonol, flavonol (melanoxetin) and the flavanone in the heartwood of *A. nigrescens* it also afforded a variety of C-C linked dimeric promelacacinidins. Two C-4 (C-ring) \rightarrow C-6 (D-ring) linked dimers, epimesquitol-(4 β \rightarrow 6)-epimesquitol-4 α -ol and epimesquitol-(4 β \rightarrow 6)-epimesquitol-4 β -ol were isolated from this tree.

The unique class of proanthocyanidins with a C-4 (C-ring) \rightarrow C-5 (D-ring) linkage was extended by the present isolation of mesquitol-(4 α \rightarrow 5)-epimesquitol-4 β -ol and the two novel promelacacinidin dimers, mesquitol-(4 α \rightarrow 5)-3,3',4',7,8-pentahydroxy flavonone and epimesquitol-(4 β \rightarrow 5)-3,3',4',7,8-pentahydroxy flavonone.

The novel promelacacinidin dimer, with a unique C-4 (C-ring) \rightarrow C-3 (F-ring) interflavanyl linkage e.g. ent-epimesquitol-(4 α \rightarrow 3)-3',4',7,8-tetrahydroxyflavanone was also isolated and the structure elucidated.

Unlike previous studies, the synthetic approach where epimesquitol-4 β -thiobenzylether was used with epimesquitol-4 α -ol as nucleophile and AgBF₄ as thiophylic Lewis acid, no dimeric promelacacinidins were isolated. This prompted the alternative use of DMTSF as initiator for nucleophilic substitution and indeed a C-O-C-linked promelacacinidin was isolated, but not enough material was available for structural elucidation.

There is a great deal of uncertainty concerning the general pathway in flavanoid biosyntheses. It was suggested that flav-3-en-3-ols could be key intermediates in this pathway. During previous studies, flav-3-en-3-ols was synthesized in high yield. This encouraged a further investigation as to the reactions of the keto tautomer of the flav-3-en-3-ol, namely the flavan-3-one. A series of successfully oxidations and reductions were carried out on the flavan-3-one, in order to synthesize the flavonol and mesquitol (flavan-3-ol) for future use in synthesis.

OPSOMMING

In die 1970's het Fourie verskillende leucomelacacinidiese uit die Knoppiesdoring (*Acacia nigrescens*) geïsoleer. Daar is egter getwyfel oor die bestaan van oligomere, met 'n pirogallol-tipe A-ring en dit het verdere navorsing op die kernhout tot gevolg gehad. Tydens hierdie ondersoek is 'n verskeidenheid dimeriese promelacacinidiese geïsoleer.

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 ^1H KMR-spektrometrie (300 MHz), ^{13}C , COSY, NOESY, HMQC and HMBC eksperimente gebruik te maak.

Afgesien van epimesquitol- 4α -ol, epimesquitol- 4β -ol, dehidroflavonol, flavonol (melanoxetin) en die flavanone het die kernhout van *A. nigrescens* ook 'n verskeidenheid C-C gebonde promelacacinidiese gelewer. Twee bekende, C-4 (C-ring) \rightarrow C-6 (D-ring) gebonde dimere, epimesquitol-($4\beta\rightarrow 6$)-epimesquitol- 4α -ol en epimesquitol-($4\beta\rightarrow 6$)-epimesquitol- 4β -ol is geïsoleer.

Die unieke groep proanthosianidiese met 'n C-4 (C-ring) \rightarrow C-5 (D-ring) interflavaniel bindings is uitgebrei deur die isolasie van mesquitol-($4\alpha\rightarrow 5$)-epimesquitol- 4β -ol en die twee baie seldsame verbindings, mesquitol-($4\alpha\rightarrow 5$)- $3,3',4',7,8$ -pentahidroksie flavanone and epimesquitol-($4\beta\rightarrow 5$)- $3,3',4',7,8$ -pentahidroksie flavanone.

Die eerste promelacacinidiese met 'n unieke C-4 (C-ring) \rightarrow C-3 (F-ring) interflavaniel binding naamlik, ent-epimesquitol-($4\alpha\rightarrow 3$)- $3',4',7,8$ -tetrahidroksieflavanone is ook geïsoleer en gekarakteriseer.

Die sintetiese benadering waartydens epimesquitol-4 β -bensieltioeter saam met epimesquitol-4 α -ol as nukleofiel en AgBF₄ as tiofiliese Lewissuur gebruik is, het afgesien van vorige suksesvolle navorsing, geen positiewe resultate opgelewer nie. Die nukleofiele substitusie van epimesquitol-4 β -bensieltioeter, met DMTSF as aktiveerder is ook ondersoek en 'n C-O-C gebonde promelacacinidien is geïsoleer, maar nie genoeg material vir die opklaring van die struktuur nie.

Daar is steeds groot onsekerheid insake die tussenprodukte gedurende die biosintese van flavanoïede. Flav-3-en-3-ole is voorgestel as sleutel tussenprodukte in sommige gepostuleerde biosintetiese roetes. Tydens vorige sintetiese studies is flav-3-en-3-ole in hoë opbrengste gesintetiseer. Dit het navorsing op die keto tautomeer van die flav-3-en-3-ol, naamlik die flavan-3-oon aangemoedig. Om mesquitol (flavaan-3-ol) en die ooreenkomstige flavonol te sintetiseer is verskeie reduksies en oksidasies op die flavan-3-oon met sukses uitgevoer.

U.O.V.S. BIBLIOTEK