THE STRUCTURE AND SYNTHESIS OF OLIGOFLAVANOIDS AND OLIGOSTILBENES FROM *CASSIA ABBREVIATA.*

Dissertation submitted in fulfilment of the requirements for the degree

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by

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M. C. Mthembu

Table of Content

ABSTRACT

Keywords: *Cassia abbreviata,* Leguminosae, flavan-3-ols, bibenzyls, stilbenes, dimeric stilbenes, proguibourtinidins, biomimetic synthesis.

During the present study the acetone extract of the heartwood of *Cassia abbreviata* afforded the known flavan-3-ols, catechin, epicatechin, epiafzelechin, afzelechin and the new (2R,3S) guibourtinidol. This was the first natural occuring 4',7-dihydroxy substituted flavan-3-ol to be isolated. The protocol recently developed in our laboratories using asymmetric dihydroxylation of 1,3-diarylpropenes and subsequent acid-catalyzed cyclization was used to yield the four diastereomers and amongst them the one isolated from *C. abbreviata.*

Proguibourtinidin dimers were identified as the permethyl ether acetates namely the four known dimers only isolated now for the second time, guibourtinidol-(4α→8)-catechin, -epicatechin, -afzelechin and epiafzelechin. (2S,3R,4R)-Guibourtinidol-(4β→8)-afzelechin and (2R,3S,4S)-guibourtinidol-(4α→6)-afzelechin are two new dimers from the heartwood. With extensive NOESY and COSY NMR-experiments it was possible not only to elucidate the structures and the stereochemistry of all the proguibourtinidin dimers but also the preferred conformations of the two rotamers of each compound.

The assignment of the structures and configuration of the two dimeric stilbenes (cyclobutane derivatives) required a special effort due to the similarity and very small differences in their ^{1}H NMR spectra, ^{13}C NMR spectra, mass spectral fragmentation pattern and the inability to grow crystals suitable for X-ray studies.

From the synthesis using photodimerization of the monomeric 3,3',4',5-tetramethoxystilbene and limited structural information from the literature, ^{1}H NMR and MS it was possible to present structures for the two dimeric 3,3',4',5-tetramethoxystilbenes as α-trixillic and β-truxinic configurations.

The monomeric 3,3',4',5-tetrahydroxystilbene, the assumed precursor for the cyclobutane compound was also detected in the acetone extract. Partnering the stilbene were the two bibenzyls namely 3,3',4',5-tetrahydroxy- and 3,4',5-trihydroxydihydrostilbene.

Extensive investigative attempts were conducted to synthesize the trimeric procassinidin, cassiaflavan-(4→8)-epigallacatechin-(4→6)-cassiaflavan isolated from *Cassia petersiana*. From the model reactions executed excellent control regarding regio- and stereoselectivity during C4→C8 formation and good yields were obtained in the synthesis of different cassiaflavan-flavan-3-ol dimers. However, when the same procedure was appplied to accomplish the C4→C6 coupling between the top dimeric unit and the bottom cassiaflavan, it was not successful.

OPSOMMING

Sleutelwoorde: *Cassia abbreviata*; Leguminosae; Flavan-3-ole; bibensiele; stilbene; dimeriese stilbene; proguibourtinidiene; biomimetiese sintese.

Gedurende die huidige ondersoek van die asetoonekstrak van die kernhout van *Cassia abbreviata* is die bekende flavan-3-ole, katesjien, epikatesjien, afzelesjien, epiafzelesjien en die nuwe (2*R*,3*S*)-guibourtinidol geïsoleer. Hierdie was die eerste 4′,7-dihidroksi flavan-3-ol wat uit die natuurlike materiaal kom. Deur gebruik te maak van 'n metodologie wat in hierdie laboratoriums ontwikkel is, deur asimmetriese dihidroksilering van 1,3-diarielpropene, gevolg deur suurgekataliseerde siklisering is die vier diastereomere berei en die een is soortgelyk aan die verbinding wat vanuit *C. abbreviata* geïsoleer is.

Proguibourtinidien dimere is geïdentifiseer as metieleterasetaat derivate waarvan vier bekendes slegs vir die tweede keer geïsoleer is nl. guibourtinidol-(4α→8)-katesjien, -epikatesjien, -afzelesjien en –epiafzelesjien.

(2*S*,3*R*,4*R*)-Guibourtinidol-(4β→8)-afzelesjien en (2*R*,3*S*,4*S*)-guibourtinidol-(4α→6)-afzelesjien is twee nuwe dimere wat vanuit die kernhout gehaal is.

Met behulp van omvattende NOESY en COSY KMR-eksperimente was dit moontlik om die strukture sowel as die stereochemie op te klaar maar hiermee tesame is die konformasies van die twee rotamere van elke dimeer ook vasgestel.

Die toekenning van die strukture en die konfigurasie van die twee dimeriese stilbene (siklobutaan derivate) het 'n grootse poging geverg omrede die klein verskille wat daar is in die ^{1}H KMR, ^{13}C KMR, massaspektrometriese fragmentasie patroon en die feit dat geen bruikbare kristalle vir X-straal analise verkry kon word nie. Fotodimerisasie van die monomeriese 3,3′,4′,5′-tetrametoksi-stilbeen en beperkte informasie vanuit die literatuur, ^{1}H KMR en MS was dit moontlik om die strukture van die twee dimeriese 3,3′,4′,5-tetrametoksistilbene as α-trixillies en β-truxinies voor te stel.

Die monomeriese 3,3′,4′,5-tetrahidroksistilbeen wat aanvaar word as die voorloper van die twee siklobutaan verbindings is ook in die asetoonekstrak opgespoor. Tesame met die stilbeen is die twee bibensiliese verbindings nl. 3,3′,4′,5-tetrahidroksidihidrostilbeen en 3,4′,5-trihidroksidihidrostilbeen gevind.

Omvattende ondersoekende pogings is uitgevoer om die trimeriese procassinidien, cassiaflavaan-(4→8)-epigallokatesjien-(4→6)-casssiaflavaan wat vanuit *Cassia petersiana* geïsoleer is te sintetiseer. Verskeie model reaksies is uitgevoer wat baie goeie regio- en stereoselektiwiteit vir die $C_4 \rightarrow C_8$ binding vorming gelewer het. Verskeie cassiaflavaan-flavan-3-ol dimere is as modelle gebruik. Die finale koppeling van die $C_4 \rightarrow C_6$ binding tussen die dimeriese top eenheid en die onderste cassiaflavaan was na verskeie pogings nie suksesvol nie.

LITERATURE SURVERY

CHAPTER 1

MONOMERIC STILBENES

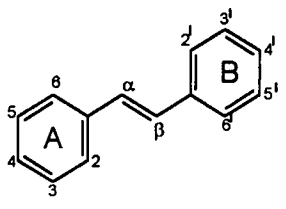
Stilbenes and their derivatives are a small group of plant phenolics but constitute an important group of natural products which often co-occur with flavanoids found in wood, bark and leaves of gymnosperms and angiosperms[1].

Hydroxylated stilbene and some of its derivatives are found to exhibit a variety of biological and pharmaceutical activities, including protein kinase and protein kinase C inhibitory effects[2]. These compounds are also reported to contribute to the insect resistance of wood and are found to oxidize easily to coloured products that contribute to the colour of the wood.

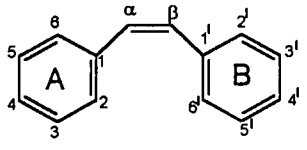
The majority of stilbenes isolated from natural sources, are *trans* (E) in relative stereochemistry while the *cis* (Z) isomers have been reported to be present in small amounts[1].

1.1 NOMENCLATURE

The structures of naturally occurring stilbenes range from the unsubstituted to polysubstituted *trans*- [1] and *cis*- [2] parent hydrocarbons.



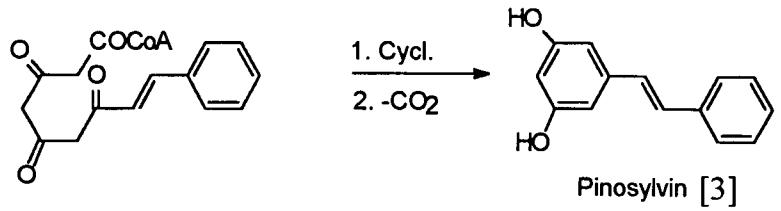
[1]



[2]

The stilbene (1,2-diphenylethylene) with the carbon framework of C_6-C_2-C_6 which originates from the C_6-C_3-C_6 precursor (figure 1.1) by cyclization and decarboxylation

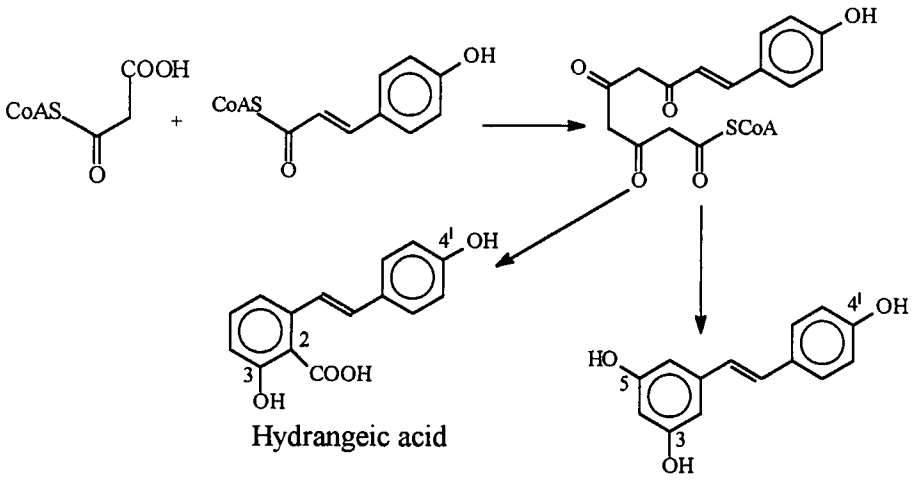
resulting in the 1,2-disubstituted olefin as a product[3]. This process also occurs in a broad spectrum of plants utilizing the acetate and shikimic biosynthetic pathways[4,5]. The 3,5-dioxy substituted compounds are the most common oxygenation pattern of these natural occurring stilbenes[1,6].



Pinosylvin [3]

Figure 1.1 Stilbene biogenesis

The shikimic acid pathway derived compounds are substituted with hydroxy and/or methoxy group(s), located on either the 3,4 and 4' or 3,4' and 5 positions [Scheme1.1][6].



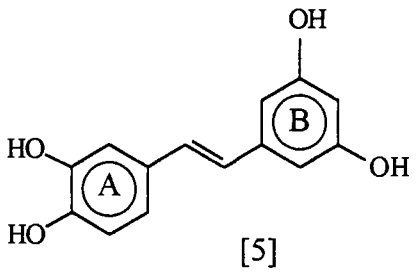
Hydrangeic acid

Resveratrol [4]

SCHEME 1.1

The location of OH groups is found to be of greater importance than their number, as the formation of enzyme-phenol complexes are stereospecific. The 3-hydroxystilbene with either the unsubstituted or substituted 5-position can be antifungal if degraded by extracellular oxidative enzymes of certain species of fungus. The hydroxylation pattern plays a role in the activity of the stilbenes, as in the case of 3,3',4,5'-tetrahydroxystilbene

[5] which is reported to form coloured compounds upon exposure to light mainly due to the *m*-substitution on the B-ring.



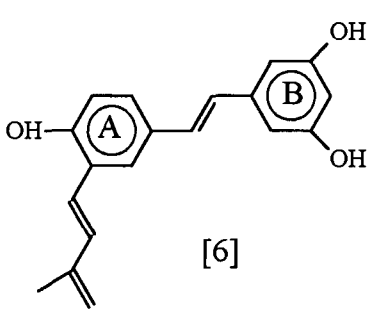
[5]

1.2 STRUCTURE AND NATURAL OCCURRENCE

The structures of a variety of mono- and pentasubstituted (-OH and $-OCH_3$) natural occurring stilbenes and their distribution in woody plants are represented in [Table 1]

Trans-stilbenes undergo photochemical isomerization to yield the corresponding *cis*-isomer. Both *cis*- and *trans*-resveratrol were isolated from diffusates from *Arachis hypogaea* in a darkened laboratory, which indicated that the *cis*-isomer was not the artefact of exposure of the *trans*-isomer to daylight of short wavelength[10]. Isolation of *cis* and *trans*-3,5-dimethoxystilbene confirmed the natural occurrence of the stereoisomers in the bark of *Pinus banksiana*[16].

Cooksey and co-workers[17] characterized another derivative of stilbene from the kernels of *Arachis hypogaea* with an 3-isoprenyl substituent [6], this compound showed antifungal properties.

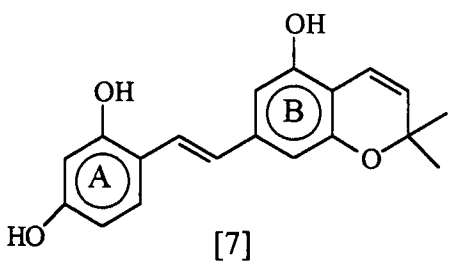


[6]

Table1. Summary of some of natural occurring stilbenes[1]

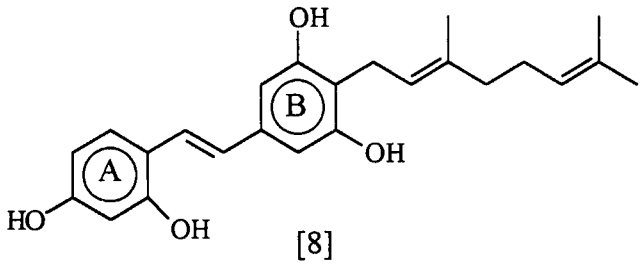
SUBSTITUTION PATTERN		NAME	SOURCE
A-RING	B-RING		
4-OH		4-Hydroxystilbene	*Pinus griffithi*[7]
4-OCH_3		4-Methoxystilbene	*P. griffithi*[7]
3,5-OH		Pinosylvin	*Pinus sylvestris*[8]
3-OH, 5-OCH_3		Pinosylvin monomethyl ether	*P. sylvestris*[8]
3,5-OCH_3		*cis*-or *trans*-Pinosylvin	*Pinus spp.*[9]
3,5-OH	4'-OH	Resveratrol	*Arachis hypogenea*[10]
3,5-OCH_3	4'-OH	Pterostilbene	*Vitis vinifera*[11]
3,4-OH	3',5'-OH	Piceatannol	*Vouacapoua macropetala*[12]
3-OCH_3, 4-OH	3',5'-OH	3-methoxy-4,3',5'-trihydroxystilbene	*Picea spp.*[13]
3,4,5-OH	3',5'-OH	3,4,5,3',5'-Pentahydroxystilbene	*Vouacapoua spp*[12].
2,4-OH	3',5'-OH	2,3',4,5'-Tetrahydroxystilbene	*V. grandiflorum*[14]
2,3-OH, 4-OCH_3	5'-OCH_3	2,3-dihydroxy-4,5'-dimethoxystilbene	*Combretum. caffrum*[15]

Rare stilbenes with a dimethylchromene ring [7], have been isolated from *Artocarpus incisus* and have shown a potential protein kinase inhibitors[2].



[7]

The 2,3',4,5'-tetrahydroxy-4'-geranylstilbene [8] with its potential biogenetic precursors were isolated from the heartwood of *Chlorophora excelsa.* The wood is extensively used for the building and manufacturing of heavy duty furniture[18].



[8]

1.3 STRUCTURE ELUCIDATION

Structural assignment[19] on the basis of UV spectra reveals *cis*-stilbene at the lower wavelength than the *trans* isomer as a result of the short wavelength conjugated system and the decrease in coplanarity. ^{1}H NMR spectra of the *cis*-compound revealed that the olefinic protons were strongly shielded (6.5ppm) as compared to the *trans*-compound (6.75-7.2). The typical values of the coupling constants found to characterize the *cis*-and *trans*-stilbene olefinic protons were J =12.0-12.5 Hz and J =16.0-16.4 Hz respectively.
The X-ray analysis of compounds with the (E)-stilbene skeleton revealed an unusual short ethylene bond which was due to the static orientation disorder and dynamic disorder that originates from the torsion vibration of the C-Ph bond[20].

1.4 SYNTHESIS OF STILBENES

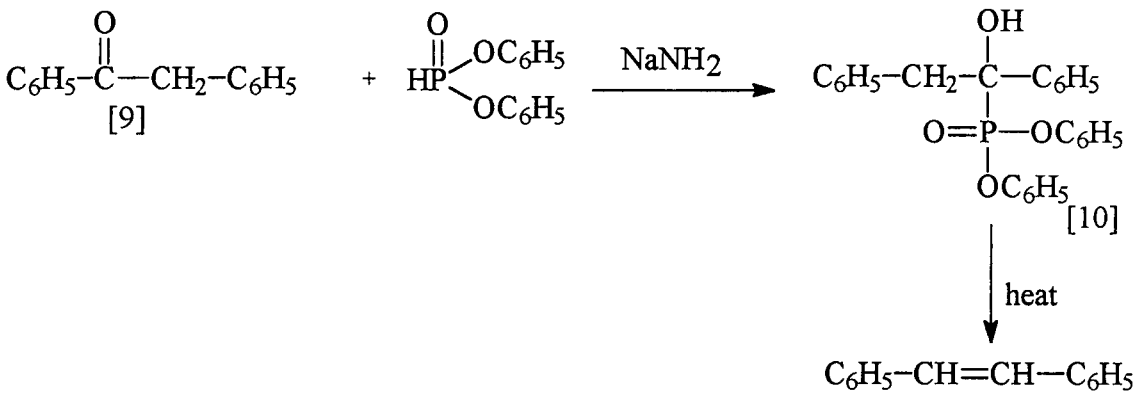
There are no comprehensive review articles dealing specifically with stilbene synthesis but short and useful collections of older methods of synthesis may be found in *Rodd' s Chemistry of Carbon Compounds*[21]. Stilbenes can be synthesized by oxidation, reduction, or elimination reactions from other diaryl compounds as well as from oxidative or eliminative dimerization.

1.4.1 REDUCTION

Reductions were performed on a variety of compounds, for example diarylacetylene[22], benzil, benzoin and deoxybenzoin[23]. Although diarylacetylenes can be hydrogenated to pure (Z)-stilbene, this method was not utilized due to the nonavailablity of diarylacetylenes[24].

The condensation of deoxybenzoin [9] with diethyl phosphite in the presence of catalytic amounts of sodium amide, produce hydroxyethane phosphonate [10][25]. The phosphanate later rearranges on warming to eliminate diethyl phosphate and gives stilbene in a 81% overall yield [Scheme 1.2].

Stilbene oxides, dihalides, diols, episulfides, and halohydrins produce either pure (E)-and (Z)-stilbenes or a racemic mixture because of the combination of stereospecific (oxidative) addition and stereospecific (reductive) elimination which reverses the double bond geometry of the stilbene[43].

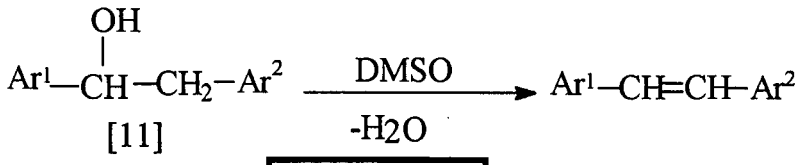


SCHEME 1.2

1.4.2 ELIMINATION OF WATER FROM 1,2-DIARYLETHANOL

The 1,2-diarylethanol [11] water elimination can be accomplished by various reagents such as protic acids[26], iodine[27], or by dissolving in dimethyl sulfoxide[28] to produce (E)-stilbene in a very high yield. [Scheme 1.3].

Syn-elimination was noted on heating acetate esters of 1,2-diarylethanol [11] at 400°C to yield the stilbene. Treatment of the same esters or 2,4,6-trimethylbenzoate with potassium amide or potassium t-butoxide found to yield stilbene with high anti-stereospecificity[29,30]. Sulphonate esters of 1,2-diphenylethanol undergo substitution rather than elimination with base[31].



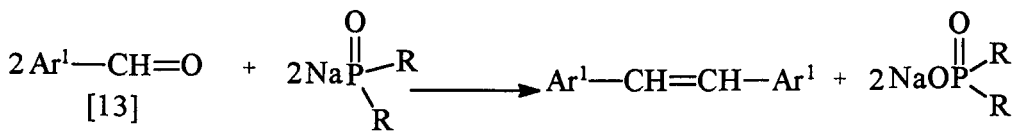
SCHEME 1.3

1.4.3 SYMMETRIC STILBENES BY DIMERIZATION REACTIONS

Stilbenes can be prepaped by oxidative or eliminative and reductive dimerization of methylarenes and their derivatives. Methylarenes [12] were dimerized to give a high yield of different stilbenes when treated with azobenzene and with varying the *para*-heterocyclic substituents[32] [Table 2].

(E)-stilbenes have been synthesized with high selectivity from benzyl hydrosulfide[33], dibenzyl sulfide[34] and phenyldiazomethane[35].

Aryl aldehydes [13] were reductively dimerized to stilbenes on heating with the sodium salt of diphosphite [14][36] or the sodium salt of diphenylphosphine oxide[15][37,38]. These reagents eliminate the intermediate epoxide by deoxygenation [Scheme 1.4].

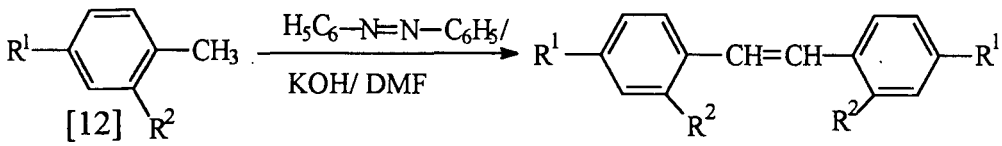


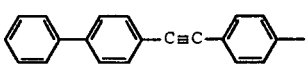
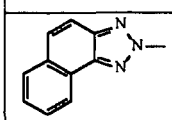
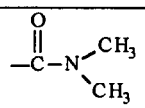
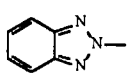
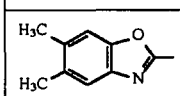
[14] R = OC_2H_5

[15] R = C_6H_5

SCHEME 1.4

Table 2. Stilbenes by Oxidative dimerization of methylarenes with azobenzene

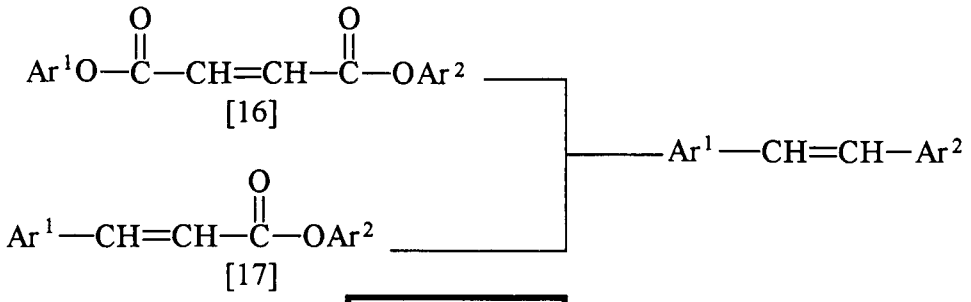


R^1	R^2	OXIDANT	YIELD (%)
	H	$H_5C_6–N=N–C_6H_5$	55
	H	$H_5C_6–N=N–C_6H_5$	60
		$H_5C_6–N=N–C_6H_5$	73
	Cl	$H_5C_6–N=N–C_6H_5$	89
	Cl	$H_5C_6–N=N–C_6H_5$	97

1.4.4 COUPLING OF AROMATIC COMPOUNDS WITH STYRENES AND OTHER VINYLARENES

Asymmetric stilbenes [Scheme 1.5] were obtained from pyrolysis of arylcinnamate [17] while (E)-stilbenes and symmetric alkyl-, halo- and alkoxy-substituted stilbenes were the products formed during the distillation of arylfumarates [16][39,40].

Styrene [18] can be treated with arenediazonium salts[41] (Meerwein arylation reaction) in the presence of copper salts or by arylpalladium compounds[42] in order to synthesize stilbenes [Scheme 1.6]. Coupling of β-halostyrenes with arylmetal compounds were hampered by the fact that the β-substituted styrenes are difficult to prepare as pure geometrical isomers[43].



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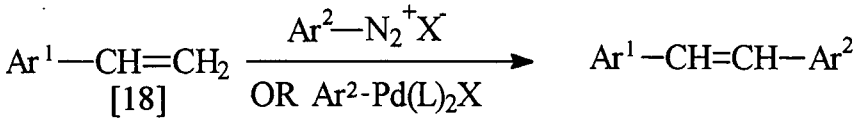
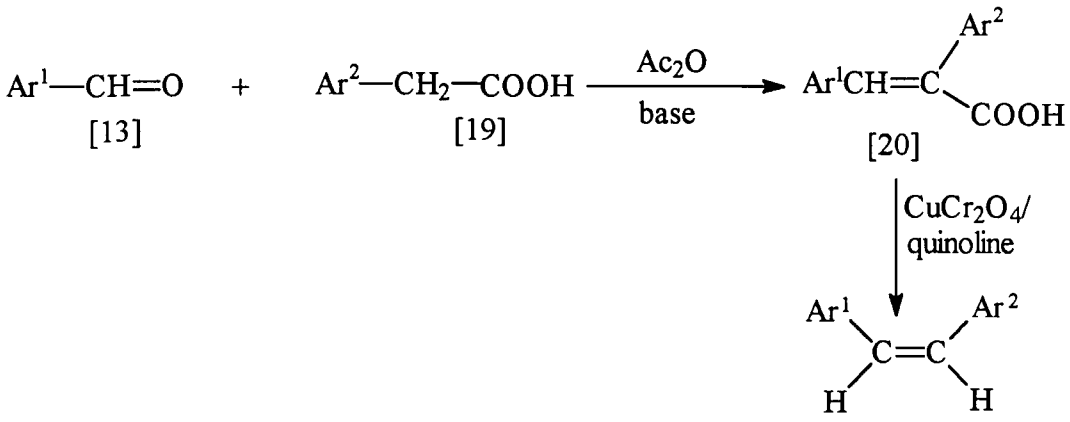


SCHEME 1.6

1.4.5 CONDENSATION OF A NUCLEOPHILIC WITH AN ELECTROPHILIC ARYL COMPOUND

Simple Knoevenagel[44] and Perkin condensations[45] were performed to synthesize stilbenes from arylacetic acids [20] and aryl aldehydes [13]. While asymmetric and a number of symmetrical stilbenes were obtained when a Perkin condensation was used followed by decarboxylation[46] [Scheme 1.7]

The mechanism involves induction of a partial negative charge at the benzyl carbon atom by suitable substituents of the nucleophilic benzyl compounds, then the condensation of aryl aldehyde or benzyl halide to produce stilbene. The method that has gained popularity due to its broad scope, the readily availability of starting materials and mild conditions is the Wittig reaction which produce both (Z)- and (E)-stilbenes. The reaction involves treating of arylmethylhalides with triphenylphosphine, resulting in the arylmethyltriphenylphosphonium halide [21]. Deprotonation of the halide with base produces the phosphonium ylid [22] that reacts with an aryl aldehyde followed by the elimination of phosphine oxide to yield stilbene [scheme 1.8].



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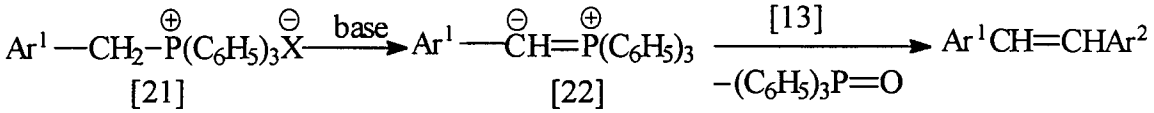
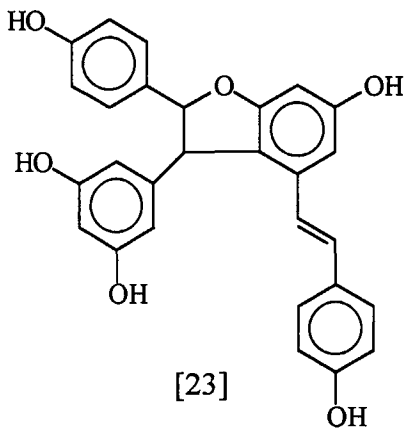


SCHEME 1.8

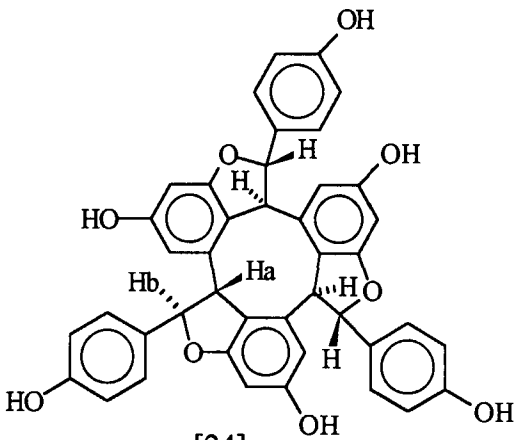
CHAPTER 2

OLIGOMERIC STILBENES

Oligomers of resveratrol, that is dimers [23], trimers [24] and higher oligomers, are found as significant constituents of some woody plants[1,47]. These oligomers are formed via oxidative coupling reactions that are enzymatically controlled to yield optically active products. These oligomers are characterized as antifungal phytoalexins, which are formed in infected grapevine leaves[1].

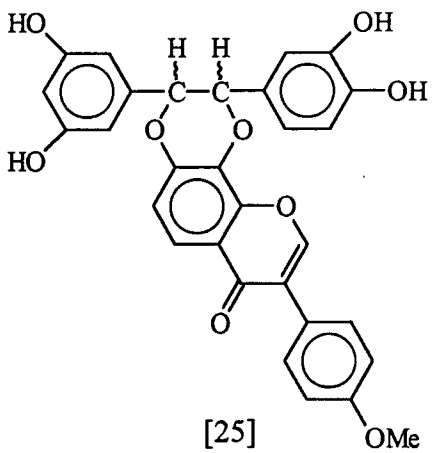


[23]



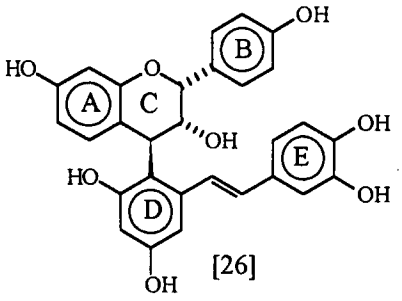
[24]

Occurrence of the dimer maackiasin [25] comprising a stilbene with an isoflavone moeity was reported from the heartwood of *Maackia amurensis*[48].

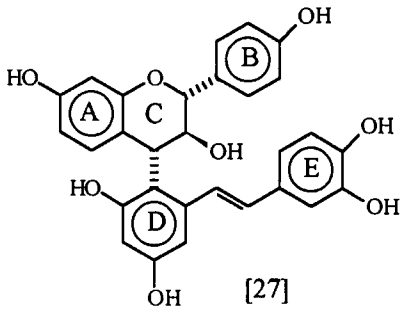


[25]

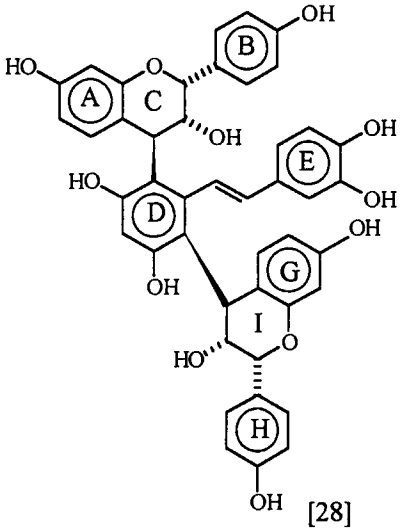
Roux and co-workers, investigated the bark of *Guibourtia coleosperma* and separated the *trans*-3,3',4',5-tetrahydroxystilbene linked to flavanoid units to form dimers [26], [27] and trimers [28], [29][49].



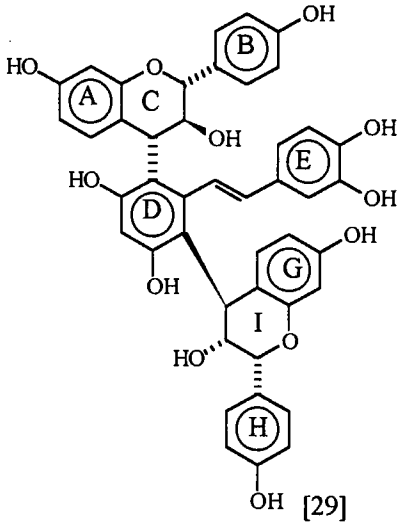
[26]



[27]

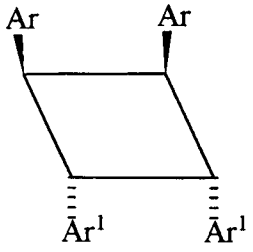


[28]

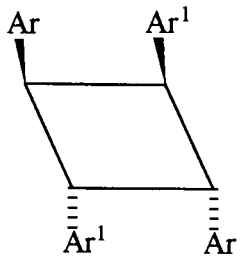


[29]

Dimeric stilbenes with a cyclobutane ring were first isolated from *Crotalaria madurensis* as three isomers for example madurensis A [32], B [31], and C [30]. These isomeric tetra-arylcyclobutanes were characterized by the different configurations of the aryl groups. These cyclobutanes co-occur with the monomeric stilbene, *trans*-3,4,3',5'-tetramethoxystilbene which was assumed to retain their *trans*-orientation during dimerization[50].



[30]



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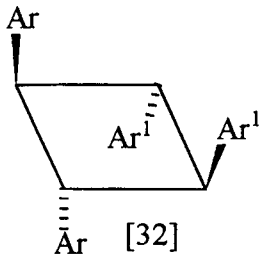
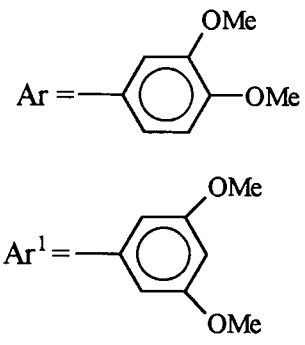
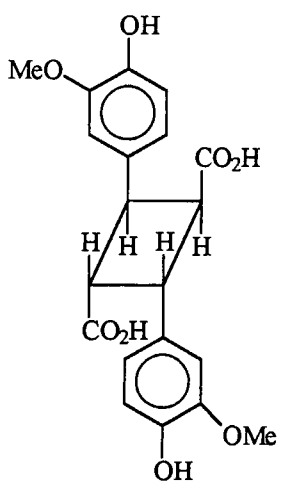


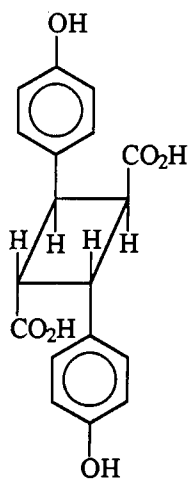
[32]



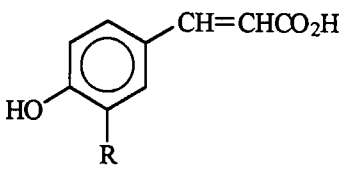
Analogous cyclobutanes [33,34] presumed to be formed photochemically from *p*-coumaric acid [35] and ferulic acid [36] were isolated from *Phyllostachys edulis*[51] and *Sasa kurilensins*[52].



[33]



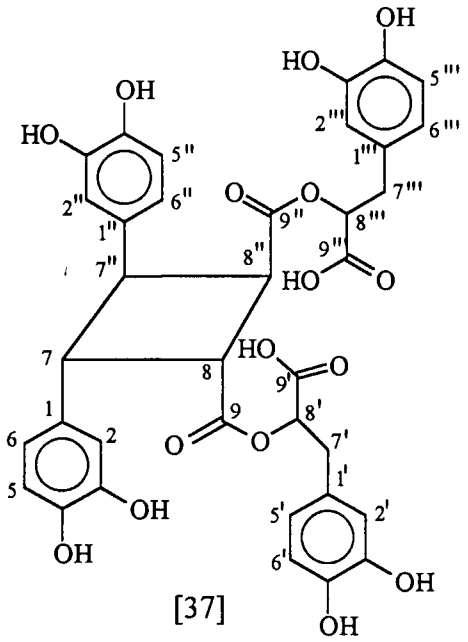
[34]



[35] R = OMe

[36] R = H

Sagerinic acid, a novel cyclobutane [37] was isolated from *Salvia officinalis*[53]. The chemical structure was elucidated by extensive NMR experiments and the cyclobutane protons appeared in the δ 3.60-4.08 ppm region.



[37]

2.1 STRUCTURE ELUCIDATION

2.1.1 SPECTROSCOPIC METHODS

The IR spectra of photodimers [30,31,32] with cyclobutane rings demonstrated the absence of the infrared band at 952-971 cm^{-1}which corresponds to the out-of-plane deformation of the olefinic CH bond in stilbenes[54].

^1H NMR spectra showed symmetricical signals obtained for the four-ring cyclobutane protons corresponding to AA'BB' system of these photodimers. The *cis*-cyclobutane ring proton signals [30,31] are characterized by the presence of the broad singlet at 4.29 ppm corresponding to four protons of tetra-arylcyclobutanes. The chemical shifts of the cyclobutane protons of the *cis*-compound (30,31 δ4.29 ppm) were found to be at the lower field than in the *trans*-compound (30, δ3.52 ppm). It was suggested that the steric

interaction of the *trans* aryl groups have shielding effect on the cyclobutane ring protons[50,54] of compound [32].

The molecular formula was determined by mass spectrometry and the absolute stereochemistry was obtained from X-ray crystallography[50,54].

2.1.2 SYNTHESIS OF OLIGOSTILBENES

Although photochemistry of stilbenes is long known, their dimerization discovered by Ciamician and Silber[55] is one of the least understood reactions. The isolation of two photodimers from the radiation of *trans*-stilbene for a period of two months by Schechter and co-workers[56] enhanced the research on the synthesis of these compounds.

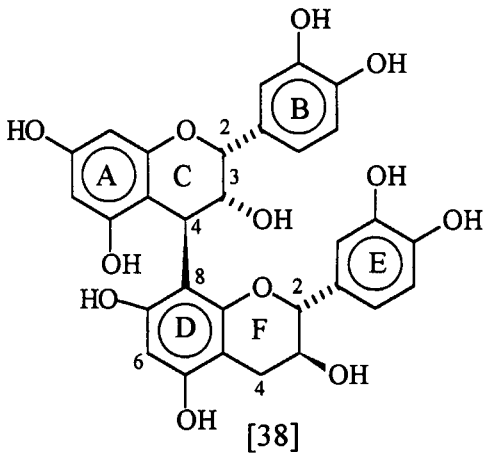
The whole concept involved addition of a photoexcited alkene to a different alkene to form a cyclobutane ring and this phenomenon is known as the [2+2]-photocycloaddition reaction. These irradiation reactions were found to be influenced by steric effects and it was more pronounced for 2,2',4,4'-substituted compared to the 2,2'-or 4,4'-substituted stilbenes[54,57]. The electron withdrawing groups (OCH_3, $NHCOCH_3$ and $NHCOOCH_3$) on either of the aromatic moeities was found to enhance dimerization of stilbenes. The solvents with high polarity lower the extent of dimerization for example, irradiation of 3,5,4'-substituted stilbene in DMF for four hours, using a 450-W source gave 68% while in tetrahydrofuran, under similar conditions, 91.3% dimerization was obtained[54].

The concentration of the reaction mixture also played a role as the high dilution lowered the extent of dimerization due to the competition with isomerization and cyclization.

CHAPTER 3

FLAVAN-3-OLS

Flavan-3-ols are the most abundant monomeric flavans[58] and are widely distributed in leaves, woody parts and fruits of plants[59]. Studies on the biogenetic process in plants suggested that the flavan-3-ols are biosynthesized from flavan-3,4-diols[60] by reductase enzymes. These compounds are also important intermediates in the biogenesis of many proanthocyanidins since they serve as nucleophiles terminating the polymerization process[61] [38].



[38]

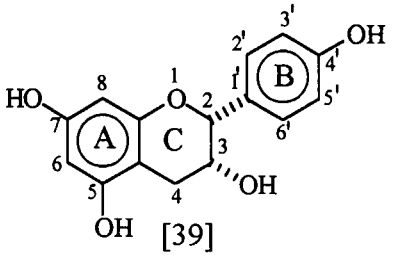
Flavan-3-ol-constituent proanthocyanidins are biologically essential when complexed with other biopolymers for example proteins, carbohydrates or metal ions. These compounds protect plants from insects, diseases and herbivores. Commercially, the above proanthocyanidin are utilized in leather and chipboard manufacturing[62,63].

3.1 NOMENCLATURE

A system of nomenclature for flavans and proanthocyanidins was introduced by Hemmingway and extended by Porter. In this system the monomeric flavanoids are defined in terms of the already established trivial names of flavan-3-ols. Flavan-3-ols

with 2R,3S-configuration and with particular A and B ring hydroxylation patterns are listed in (table 3.1)[60].

The R and S prefix define the absolute stereochemistry of the heterecyclic ring in these compounds to avoid the confusion that had occurred with the informal nomenclature used in the past. Those units with the 2R,3R-configuration are prefixed 'epi' for example epiafzelechin [39] whereas those with 2S-configuration are distinguished by the enantio (*ent-*) prefix[61].

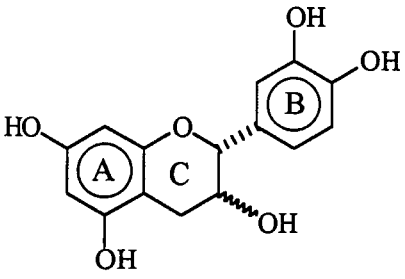


[39]

The flavanoid skeleton [39] is drawn and numbered as described by the IUPAC rules.

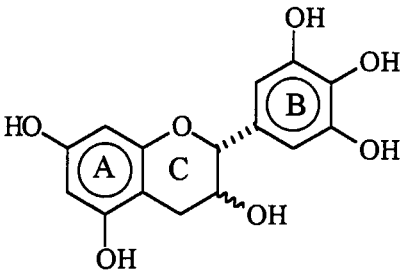
3.2 STRUCTURE AND NATURAL OCCURENCE

From the known natural occurring flavan-3-ols, catechin [40] and epicatechin [41] are distributed throughout the gymnosperms and angiosperms[61].



[40] = ▲

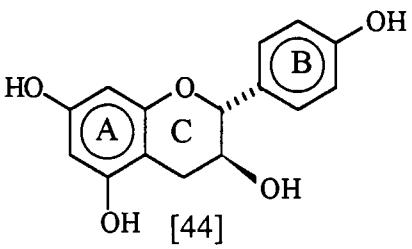
[41] = ⋮



[42] = ▲

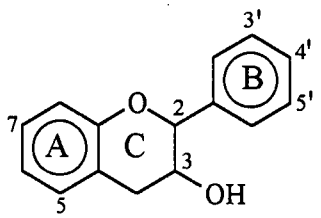
[43] = ⋮

Analogues carrying a pyrogallol B-ring, gallocatechin [42] and epigallocatechin [43] also have wide distribution while afzelechin [44] and epiafzelechin [39], with a monosubstituted B-ring are relatively rare[58].



[44]

Table 3.1 Natural Flavan-3-ols and their sources



Monomer	Hydroxylation Pattern 5	7	8	3'	4'	5'	Stereochemistry 2	3	Sources
Robinetinidol[b]	H	OH	H	OH	OH	OH	R	S	*Acacia spp*[64,65]
Fisetinidol[e]	H	OH	H	OH	OH	H	R	S	*Acacia spp*[66]
Afzelechin[d]	OH	OH	H	H	OH	H	R	S	*Eucalyptus calophylla*[67]
Epiafzelechin[c]	OH	OH	H	H	OH	H	R	R	*Cassia sieberana*[68]
Ent-epiafzelechin[f]	OH	OH	H	H	OH	H	S	S	*Palmae spp*[69]
Catechin[a]	OH	OH	H	OH	OH	H	R	S	Widespread[70,71]
Ent-catechin[c]	OH	OH	H	OH	OH	H	S	R	*Polygonum multiflorum*[72]
Epicatechin[a]	OH	OH	H	OH	OH	H	R	R	Widespread[70,71]
Ent-epicatechin[f]	OH	OH	H	OH	OH	H	S	S	*Palmae spp*[69,72]
Gallocatechin[a]	OH	OH	H	OH	OH	OH	R	S	Widespread[73,74]
Epigallocatechin[a]	OH	OH	H	OH	OH	OH	R	R	Widespread[73,74]
Mesquitol[e]	H	OH	OH	OH	OH	H	R	S	*Prosopin. grandulosa*[75]

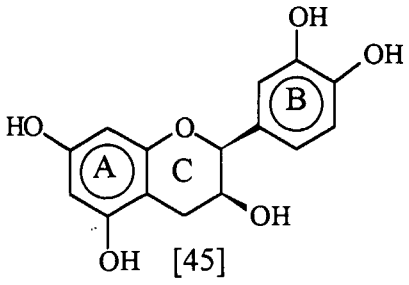
[a] = angiosperm [d] = wood

[b] = bark [e] = heartwood

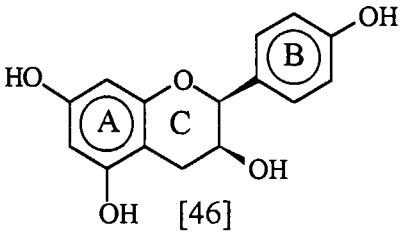
[c] = roots [f] = fruits and leaves

Although a number of flavan-3o-ls with 2S configuration are known, their distribution is quite restricted. *Ent*-epicatechin [45] and *ent*-epiafzelechin [46] are widely distributed in

Palmae species[69]. *Ent*-epicatechin was also isolated from *Polygonum multiflorum*[72] together with its isomer *ent*-catechin (Table 3.1).



[45]



[46]

Eleven O-methylated flavan-3ols are known and most of these compounds are derivatives of catechin and epicatechin.

3.3 STRUCTURE ELUCIDATION

Flavan-3-ols were structural characterized by chemical and spectroscopic methods.

3.3.1 NUCLEAR MAGNETIC RESONANCE METHODS

Nuclear magnetic resonance spectroscopy is a powerful aid in the structure elucidation of flavanoid compounds and determining the stereochemical features of the reduced heterocyclic ring[76]

Structural elucidation of flavan-3-ols is based on the characteristic methylene protons resonating between δ 2,5-3.2 and it was noted that the 4-H_β occurs at the lower field than the 4-Hα in the *cis* and at the higher field than the 4-Hα in the *trans* compound[76].

The relative stereochemistry of the 2,3-protons was defined in terms of the coupling constant (ie. $J_{2,3}$) and the splitting pattern of 2-H. A large coupling constant ($J_{2,3}$ range from 7.5 to 10 Hz) is associated with protons which are *trans* to each other while a small coupling constant ($J_{2,3}$<1 Hz) represents protons *cis* to each other. Splitting pattern of 2-H into an *ortho*-coupled doublet is the characteristic of 2,3-*trans* compound e.g. catechin

and a singlet (due to overlapping of signals) was observed for the *cis*-compound e.g. epicatechin.

The aromatic protons of a phloroglucinol A-ring are the most common in flavan-3-ols (table 3.1) and found to exhibit an AX system demonstrated by two *meta*-coupled doublets at approximately δ 6.09 and δ 6.18 assigned to 6-H(A) and 8-H(A) respectively. An ABX pattern was assigned to a resorcinol A-ring e.g. in fisetinidol (Table 3.1).
B-ring coupling patterns include AA'BB', ABX and AX systems (Table 3.1).

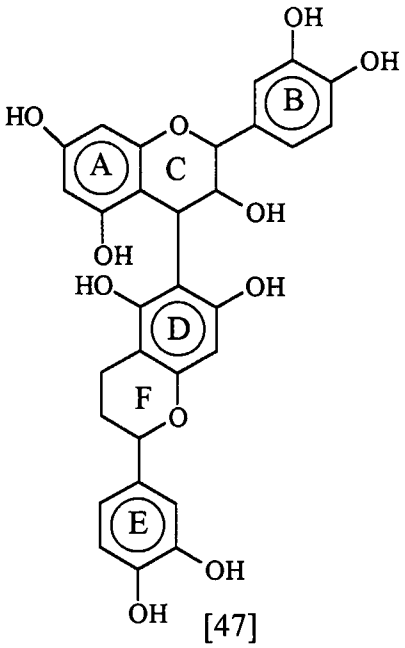
3.3.2 CHIRO-OPTICAL METHODS

The chiroptical methods (CD)is a powerful tool in establishing the absolute configuration at C-4 of flavanoids[77]. It has been used systemically in the studies of flavanones[78], flavan-3-ols[79], 4-arylflavan-3-ols[80,81] and dimeric proanthocyanidins[82,83]. The heterocyclic ring conformation is the prerequisite for unequivocal assessment of absolute stereochemistry at C-4 as it influences the sign of Cotton effect in the low-wavelength region (200-240 nm) in the CD spectra of 4-arylflavan-3-ol[80], biflavonoids[81] and triflavanoids, respectively. The orientation of the C-4 substituent accounts for the contribution towards the sign of the Cotton effect hence the absolute configuration at this stereocentre is positive for 4R- and negative for 4S-configurations, in agreement with the aromatic quadrant rule[84,85]. Flavan-3-ols absolute configuration was obtained from a contribution of the two aromatic chromophores to the L_b transition (280 nm). The 2R absolute configuration with the equatorial aryl substituent results in a negative CD band for the chroman chromatophore.

3.4 FLAVAN-3-OLS AS NUCLEOPHILES

Flava-3-ols constitute important "building blocks" in the condensed tannin molecular framework present in plants. They act as potent nucleophiles during the biosynthesis of oligomers and the *meta*-substituted A-ring is capable of forming C4→C8 [38] and C4→C6 [47] interflavanyl linkages with flavan-3,4-diols[86]. Flavan-3-ols with a

phloroglucinol A-ring such as catechin are stronger nucleophiles than the analogues with a resorcinol A-ring e.g. fisetinidol (Table 3.1)[86].

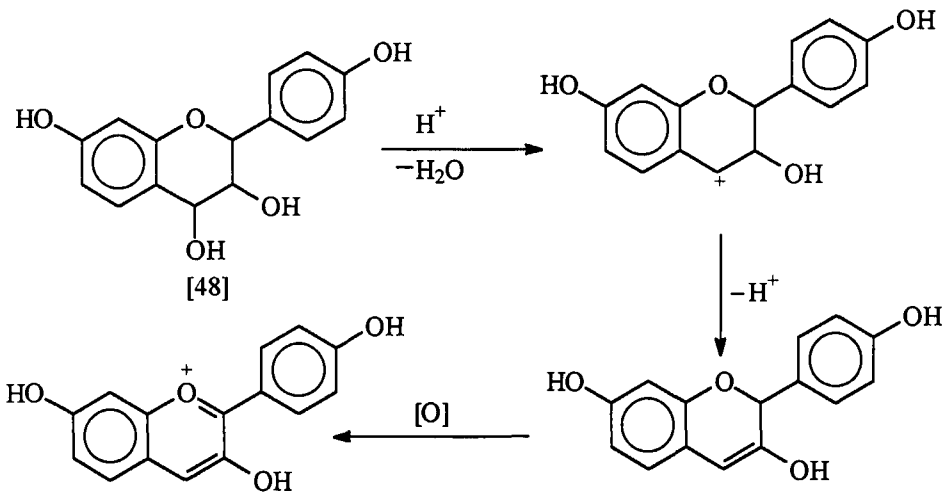


[47]

CHAPTER 4

LEUCOANTHOCYANIDINS

The name leucoanthocyanidins was given to a group of compounds defined as monomeric flavanoids that produce coloured compounds named as anthocyanidins [49] caused by the cleavage of the C4→OH bond on heating with mineral acid[58]. Anthocyanidins are compounds responsible for red to blue colouration of flowers, fruits, and leaves[59] (scheme 4.1).

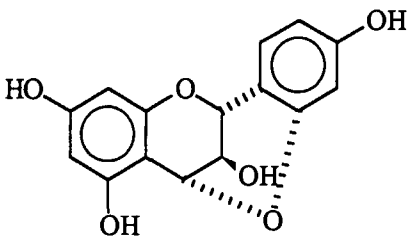


[48]

[49]

SCHEME 4.1

Leucoanthocyanidins are responsible for the broad range of reactions, (precipitation of gelatin and alkaloids, astringent taste and the formation of amorphous polymeric phlobaphens with acids) that are generally attributed to tannins in plants[59].



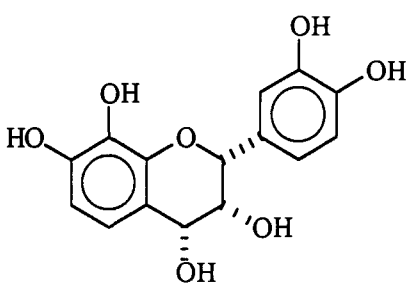
[50]

These flavanoids include flavan-3,4-diols [48], flavan-4-ols and the unusual compound cyanomaclurin [50][58].

4.1 STRUCTURE AND NATURAL OCCURRENCE

4.1.1 FLAVAN-3,4-DIOLS

The vast majority of these compounds are found in the wood or bark of the *Acacia* species and of the Leguminosae. Melacacidin [51] was the first natural flavan-3, 4-diol to be isolated from *Acacia melanoxylon* by King and Bottomley[87], who proved its structure.



[51]

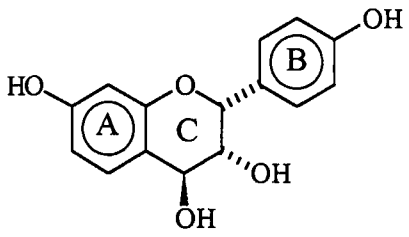
All the known flavan-3, 4-diols can be grouped as follows:

Table 4.1 Classification of flavan-3,4-diols

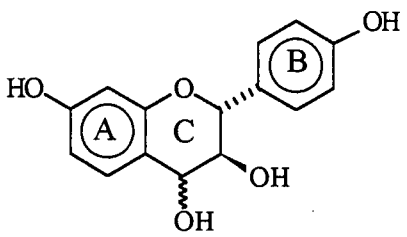
CLASS	HYDROXYLATION
guibourtacacidins	4', 7
mollisacacidins	3', 4', 7
robinetinidins	3', 4', 5,' 7
teracacidins	4', 7, 8
melacacidins	3', 4', 7, 8

Leucoguibourtinidins represent a relatively rare group of compounds which, although occurring as minor components in the heartwoods of *Acacia* spp[88]., predominate in the Southern African species *Guibourtia coleosperma* (large false mopane)[89,90], *Julbenardia*

globiflora (munondo)[91] and *Acacia luederitzii* (bastard umbrella thorn)[92,93]. Steynberg and co-workers reported the occurrence of three diastereomeric leucoguibourtinidins: 2,3-*cis*-3,4-*trans* [52], 2,3-*trans*-3,4-*trans* [54] and 2,3-*trans*-3,4-*cis*-4',7-dihydroxy-3,4-diols



[52]

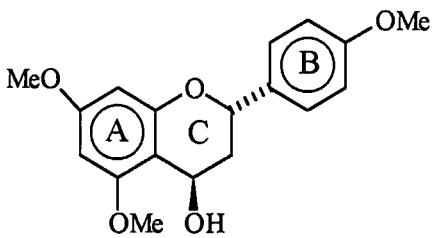


[53] [54]

[53] in proportions of 5:1:1 in *Guibourtia coleosperma*[49]. These compounds were found together with oligomeric proguibourtinidins in the same plant.

4.1.2 FLAVAN-4-OLS

Most flavan-4-ols were found to co-exist with the corresponding (2S) flavanones, and probably possess the (2S, 4R) absolute configuration. According to Lam and Wang[94] 4', 5,7-triOMe-2,4-*trans*-flavan-4-ol [55] was the first to be isolated from *Dahlia tenuicaulis* leaf[94].

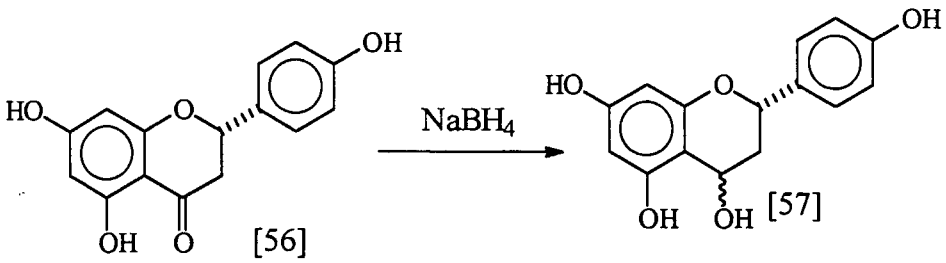


[55]

The structure of these compounds was elucidated by means of UV spectroscopy, ^{1}H NMR spectroscopy, mass spectrometry and chemically, the latter by the mild oxidation of flavan-4-ols to the corresponding flavanones.

Derivatives of flavan-4-ols such as glycosides substituted at position 5 and 6 were isolated from ferns by Tanaka[95,96]. These compounds were found to have (2R,4S) stereochemistry in contrast to the above flavan-4-ols.

Although flavan-4-ols represent a fairly simple substituted molecule they are very difficult to synthesize and can be obtained by the reduction of flavanones (scheme 4.2).



SCHEME 4.2

4.2 STRUCTURE ELUCIDATION

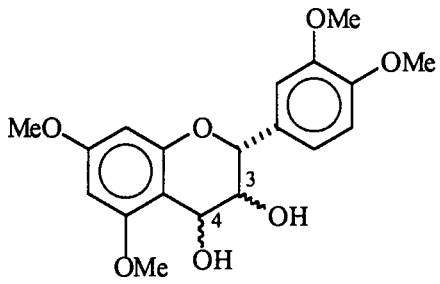
^{1}H NMR and ^{13}C NMR spectroscopy are the primary techniques utilized for the structural elucidation of leucoanthocyanidins. Flavan-3,4-diols are characterized by the coupling constants of the AMX heterocyclic ring protons (table 4.2)[97].

During early research the absolute configuration of these compounds at C-2 and C-3 was confirmed by the conversion of their methyl ethers via hydrogenolysis to corresponding analogous flavan-3-ols. Stereochemistry at C-4 of the flavan-3,4-diols were assessed by the CD method, after stereoselective[98] derivatization (i.e. the 2,3-configuration was retained). The absolute stereochemistry can now be determined by correlation with accumulated CD data.

4.3 FLAVAN-3,4-DIOLS FLAVAN-4-THIOETHERS AS INCIPIENT ELECTROPHILES

Flavan-3,4-diols were found to act as chain extender units in the synthesis of oligoflavanoids due to the ease of formation of the C-4 carbocation. The stability of C-4 electrophiles were influenced by the hydroxylation pattern of the A-ring for the delocalization of charge[61].

Table 4.2 Coupling constants for the heterocyclic protons of 3',4'5,7-tetramethoxy-3,4-diol diastereomers and their acetates



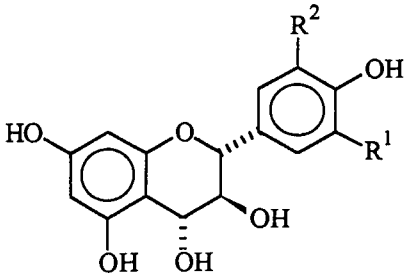
[58] 3= 4=≡ [60] 3= ▮ 4=≡

[59] 3=≡ 4= ▮ [61] 3= 4= ▮

RELATIVE CONFIGURATION	$J_{2,3}$	$J_{3,4}$
2,3-*cis*-3,4-*cis* [58]	1.0	4.8
diacetate	1.6	5.4
2,3-*cis*-3,4-*trans* [59]	0.9	2.5
diacetate	1.4	2.6
2,3-*trans*-3,4-*trans* [60]	10.1	7.3
diacetate	7.4	7.2
2,3-*trans*-3,4-*cis* [61]	10.1	4.1
diacetate	11.1	3.5

It was established that this phenomenon was most effective for flavan-3,4-diols with phloroglucinol A-rings [62-64] and intermediate efficiency for resorcinol A-ring leuco-compounds [65-67].

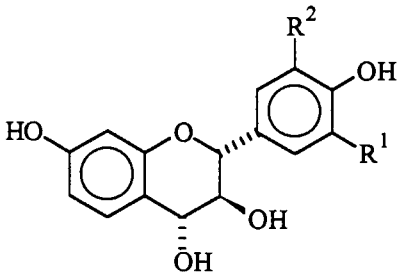
The B-ring was found to enhance the stabilization of C-4 carbocations of substituted diols [69] via an A-conformation [72] representing a half-chair/sofa conformation for the pyran ring (C) in which the 2-aryl group occupies an axial as opposed to the 'customary' equatorial orientation in the E-conformer.



[62] $R^1 = H$, $R^2 = OH$

[63] $R^1 = R^2 = OH$

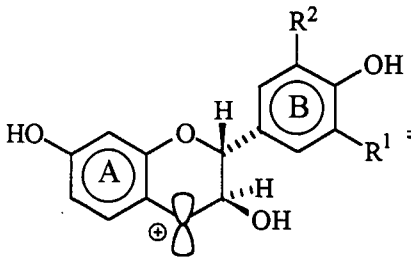
[64] $R^1 = R^2 = H$



[65] $R^1 = H$, $R^2 = OH$

[66] $R^1 = R^2 = OH$

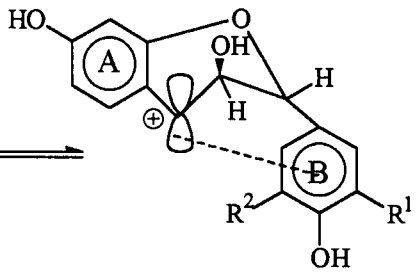
[67] $R^1 = R^2 = H$



[68] $R^1 = H$, $R^2 = OH$

[69] $R^1 = R^2 = OH$

[70] $R^1 = R^2 = H$



[71] $R^1 = H$, $R^2 = O$

[72] $R^1 = R^2 = OH$

[73] $R^1 = R^2 = H$

The effect of the B-ring to additionally stabilize C-4 carbocations via an A-conformation was demonstrated by different rates of condensation observed for leucorobinetinidin [63][99], mollisacacidin [62][99] and guibourtacacidin [64][100].

The more electron-rich pyrogallol function in the leucorobinetinidin carbocations [69]↔[72] is more effective than the pyrocatechol functionality in mollisacacidin analogues [68]↔[71] and mono-oxygenated moiety in the guibourtacacidin ions [70]↔[73] hence the leading condensation rates in decreasing order are [63]>[62]>[64] respectively.

CHAPTER 5

PROANTHOCYANIDIN OLIGOMERS

The oligomeric or polymeric proanthocyanidins represent a large group of phenolic constituents in woody and some herbaceous plants[101]. Together with the biflavanoids they represent the two main classes of complex C_6-C_3-C_6 secondary metabolites[102].

These compounds were reported to be potential substitutes for petroleum-derived phenolic polymers used as industrial adhesives, dispersants and ion exchange materials[103,104,105]. Besides their significance in the economy, a variety of condensed tannins exhibit important physiological activity as antioxidants and their cytotoxicity against human tumor cells.

5.1 NOMENCLATURE

Proanthocyanidins or condensed tannins are substances isolated from plants, which are defined as compounds that produce anthocyanidins by cleavage of the C4→Csp^2 interflavanoid linkage[58].

Hemmingway and co-workers introduced a system of nomenclature for naming proanthocyanidin oligomers in an analogous manner to that of oligo- and polysaccharides. The fundamental flavan structural units of proanthocyanidin oligomers are defined in terms of the familiar monomeric flavan-3-ols (Chapt. 3 table 3.1). The interflavanoid linkage is indicated in the same way as polysaccharides, the bond and its direction contained in brackets (4→). The configuration of the interflavanoid bond at C-4 is indicated by the α(''''') and the β (—) nomenclature (IUPAC rule)[58].

Table 5.1 Names of oligomeric proanthocyanidins with the corresponding monomeric units.

OLIGOMERIC PROANTHOCYANIDINS	MONOMERIC UNITS
Propelargonidin	Afzelechin
Procyanidin	Catechin
Prodelphinidin	Gallocatechin
Proguibourtinidin	Guibourtinidol
Prorobitinedin	Robitinedol
Profisetinidin	Fisetinidol
Proteracacinidin	Oritin
Promelacacinidin	Prosopin
Prodistenidin	Distenin

5.2 STRUCTURE AND NATURAL OCCURRENCE

Proanthocyanidins are divided into two groups, A-type proanthocyanidins which are doubly linked and B-type proanthocyanidins that represent singly linked oligomeric proanthocyanidins[58]. The structure of isolated proanthocyanidins was elucidated and confirmed by synthesis of these compounds in sufficient quantities[61].

5.2.1 B-TYPE PROANTHOCYANIDINS

Proanthocyanidins of the B-type are characterized by singly linked flavanyl units, usually between C-4 of flavan-3,4-diol chain extender unit and C-6 or C-8 of the chain terminating moiety. They are classified according to the hydroxylation pattern of their chain extender units. The classes include proguibourtinidins, profisetinidins, prorobinetinidins, procyanidins, proteracacinidins and promelacacinidins respectively (table 5.1).

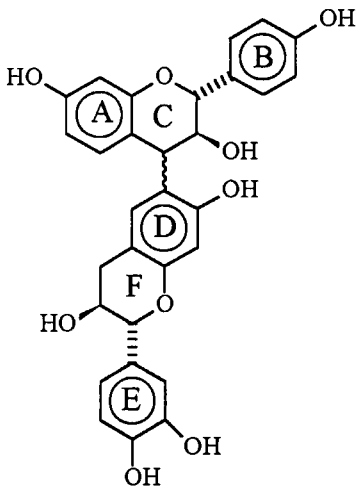
5.2.1.1 PROGUIBOURTINIDINS

Proguibourtinidins with the 4',7-dihydroxy phenolic functionality represent a relatively rare group of condensed tannins. They have been isolated from the Southern African species *Guibourtia coleosperma*[89,90], *Julbernardia globiflora*[91], *Acacia luederitzii*[92,93] and Australian *Acacia* species[88].

A guibourtinidol-epiafzelechin dimer was recently isolated from *Cassia fistula* sapwood for which a (4α→8) interflavanyl linkage was assumed but not confirmed.

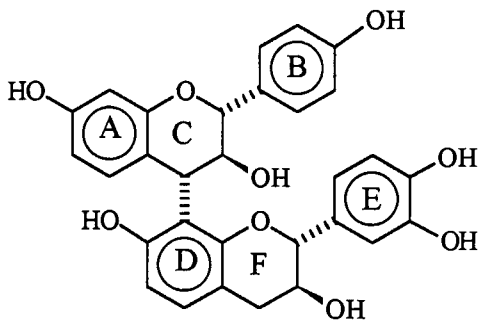
Notable amongst these compounds from *G. coleosperma* were those analogues that carry a 3,3',4,5'-tetrahydroxystilbene terminal unit which was synthesized by substituting the nucleophilic flavan-3-ols with the tetrahydroxystilbene in acid-mediated coupling with the appropriate guibourtinidins[49].

Fisetinidol linked to proguibourtinidins [74], [75], [76], [77] was accompanied in the

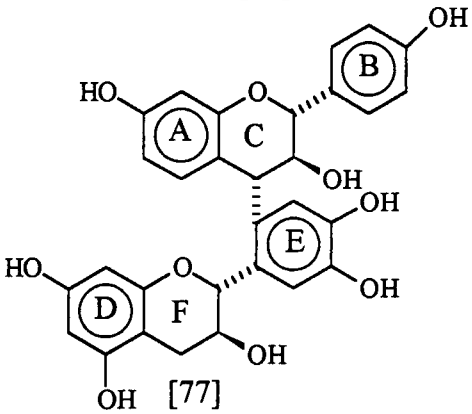


[74] =

[75] =

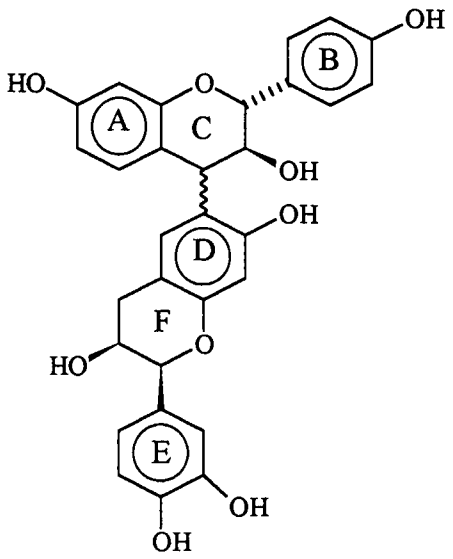


[76]



[77]

heartwood of *Colophospermum mopane* by the guibourtinidol-(4α→6)- [78] and (4β→6)-epifisetinidols [79][100].



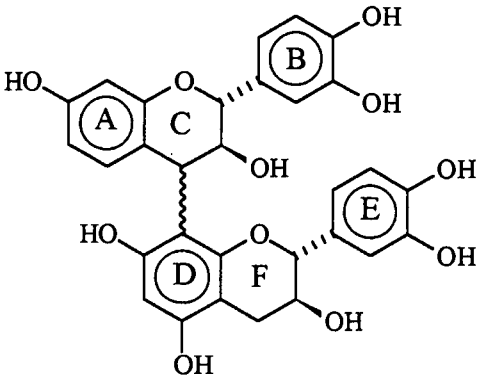
[78] = [79] =

5.2.1.2 PROFISETINIDINS

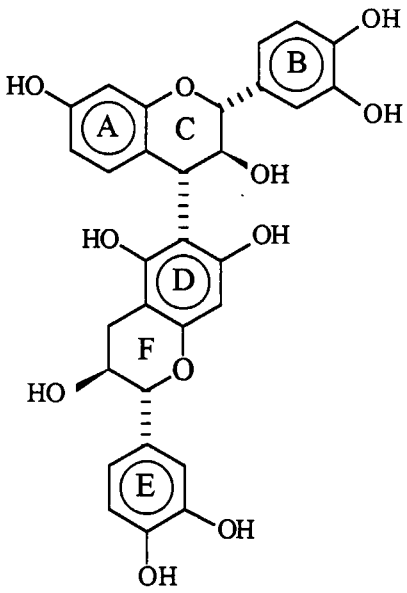
The profisetinidins had been intensively studied at the University of the Orange Free State, Bloemfontein, South Africa. These compounds are the most important polyflavanoids of commercial value, making up the major constituents of the wattle and quebracho tannins[102].

A variety of profisetinidin biflavanoids with a chain-terminating unit catechin were identified, for example, the fisetinidol-(4→8)- and -(4→6)-catechin profisetinidins [80], [81] and [82][102]. These compounds form the $C(sp^3)$-$C(sp^2)$ stable interflavanyl bond which has an effect in both the structural investigation of the polymeric proanthocyanidins in the black wattle bark and of those from other commercial sources, as well as the establishment of the absolute configuration of chain-terminating flavan-3-ol moieties in the oligoflavanoids[102].

The self-condensation of leucofisetinidin results in a variety of 4→6 linked biflavanoids, triflavanoids and higher condensates.



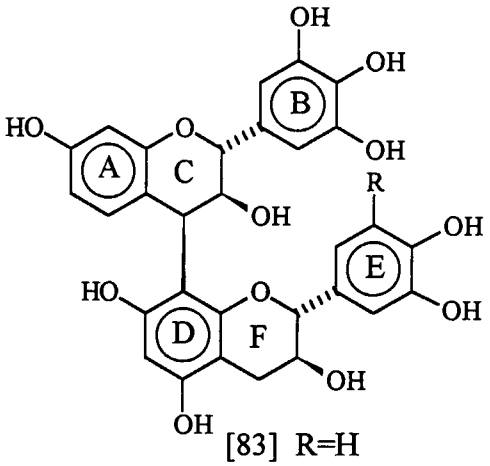
[80] = [81] =



[82]

5.2.1.3 PROROBINETINIDINS

The prorobinetinidin-type oligoflavanoids predominately occurs in the wattle bark extract and these metabolites are based on either catechin or gallocatechin chain-terminating units[58]. Two dimers robinetinidol-(4β→8)-catechin [83], and robinetinidol-(4β→8)-gallocatechin [84] were recently identified from the extract[102].



[83] R=H

[84] R=OH

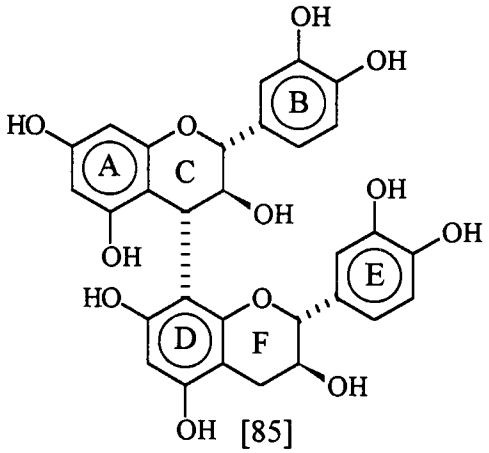
5.2.1.4 PROTERACACIDINS AND PROMELACACIDINS

Although the flavan-3,4-diols e.g. melacacidin, its C-4 epimer isomelacacidin and teracacidin are present in a small number of the *Acacia*[88,89] species, their corresponding proanthocyanidin oligomers are sparsely represented.

The oligomeric proanthocyanidins possessing the pyrogallol A-ring were restricted to two examples of the promelacacinidin class in the heartwood of *Prosopis glandulosa*[106,107] and *Acacia melanoxylon*[108] and until recently the isolation of proteracacinidin dimers was restricted to the heartwood of *Acacia galpinii*[106] and *Acacia caffra*[107,108]. The occurrence of these compounds in nature demonstrates that the pyrogallol A-ring is sufficiently reactive for nucleophilic condensation and also facilitates C-4 carbocation formation from the associated flavan-3,4-diols.

5.2.1.5 PROCYANIDINS

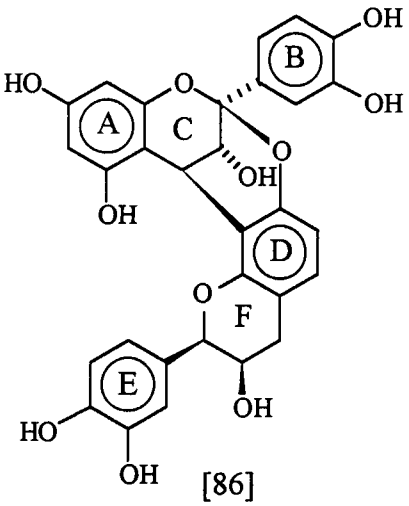
Procyanidins are the most dominant and ubiquitous group of natural proanthocyanidins and they represent condensation products of highly reactive leucocyanidins [58] that react with nucleophilic substrates such as the catechin or gallocatechin respectively[102]. The 4β interflavanoid bond is attributed to all 2,3-*cis* procyanidins [38] whereas 4α-bond represent most of natural occurring 2,3-*trans* procyanidins[85][61].



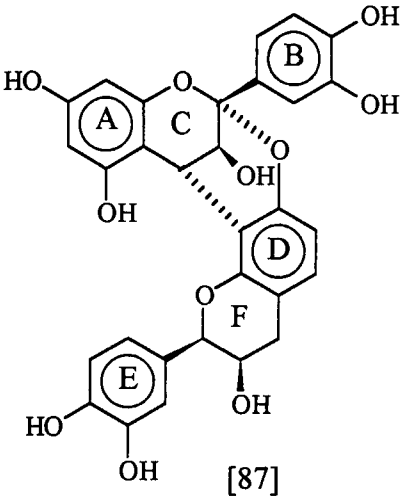
[85]

5.2.2 A-TYPE PROANTHOCYANIDINS

Apart from the well-known $sp^3 \rightarrow sp^2$ bond these compounds also possess a second ether linkage from C-2 of the top unit and found to exhibit either a (2β,4β) [86] or a (2α,4α)-configuration [87] for the doubly-linked units[102].



[86]



[87]

The structure of [87] was deduced by Haslam and his collaborators from the spectroscopic and chemical evidence and has recently been unequivocally proved by X-

ray crystallography[103]. Constituents other than catechin and epicatechin were encountered eg. flavonols, flavans, epigallocatechin and the afzelechins.

5.3 STRUCTURE ELUCIDATION

5.3.1 SPECTROSCOPIC METHODS

5.3.1.1 NUCLEAR MAGNETIC RESONANCE SPECTROMETRY

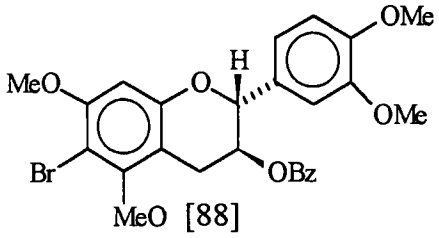
The structure of oligomeric proanthocyanidins is mainly about the position and absolute stereochemistry of the interflavanoid bond.

^{13}C NMR spectroscopy has proved to be a very useful technique for the study of proanthocyanidins, especially as the phenols provide good quality spectra, whereas their ^{1}H NMR spectra have the tendency to give broadened peaks due to proton exchange processes. The ^{13}C NMR spectra of oligomers provide information regarding the A- and B-ring substitution pattern, the relative stereochemistry of the C-ring and, in favourable cases, the position of the interflavanyl bond[58].

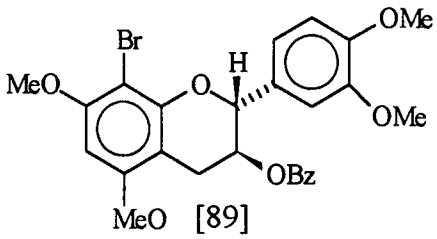
^{1}H NMR spectra of the free phenolic proanthocyanidins reveal a complicated spin pattern at ambient temperature, due to the dynamic rotational isomerism about the C4→C6/C8 bond. The above problem was solved by the derivatization of proanthocyanidins as phenolic permethyl 3-O-acetates which enabled the conducting of the experiments at higher temperature to minimize the effect of rotational isomerism. The complex pattern of heterocyclic and aromatic protons in the oligomeric proanthocyanidins was resolved by the use of high-resolution ^{1}H NMR (at 300 or 500MHz) spectroscopy.

In differentiating between the alternatives of C4→C6 and C4→C8 interflavanoid linkages Hunt and Roux[112] synthesized both 6- [88] and 8-bromo derivatives [89] of brominated catechin and used X-ray crystallography during their studies. These compounds were converted via lithio intermediates into analogues bearing 6- and 8-substituents which possess both electron withdrawing (COOH) and donating (OH, CH_2OMe) properties[112]. Study of absolute values of the chemical shifts of these

compounds indicated that H-8 consistently resonates at significantly lower field than the H-6 without overlap and also that the differential values mainly devolve upon the axial H-2[112].



[88]



[89]

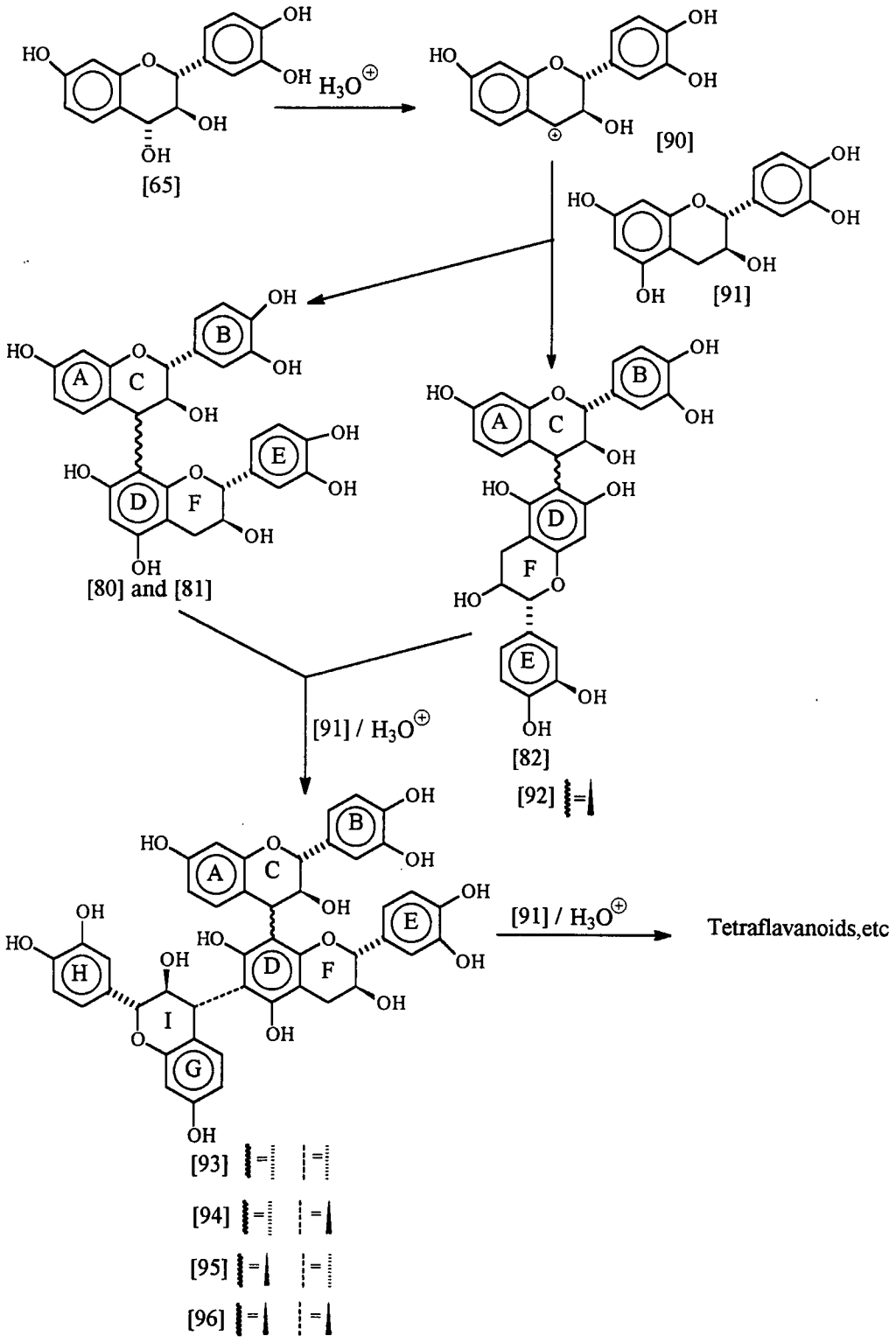
5.3.1.2 CIRCULAR DICHROIC SPECTROSCOPY

The CD method supplemented the indirect method based on the ^{1}H and ^{13}C NMR spectra chemical shift differences[113]. Thus, the absolute configuration of the interflavanoid bond correlated with the position and sign of the CD band. See chapter 3.

5.3.2 BIOMIMETIC SYNTHESIS OF PROANTHOCYANIDINS

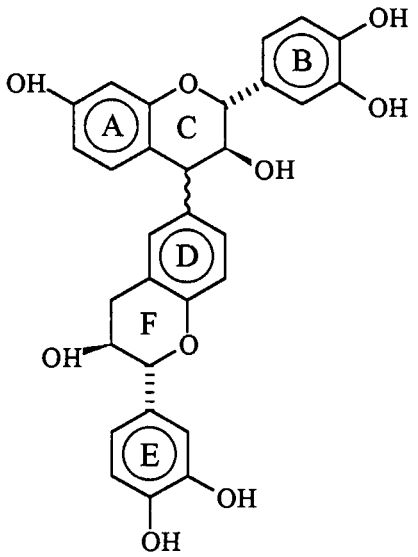
Acid catalyzed reactions to produce flavan-4-carbocations or A-ring quinone-methides either from flavan-3,4-diols or from interflavanoid bond cleavage of oligomeric/polymeric proanthocyanidins, that react with the A-ring of flavan-3-ols to produce oligomeric proanthocyanidins [scheme 5.1] have been employed and named biomimetic synthesis. From these the following generalizations are possible.

The condensation of leucofisetinidin [61] with an excess of catechin as the bifunctional nucleophile gave four products regio- and stereoselectively. The C-8 on the catechin unit is sterically less hindered than the C-6 under conditions of attack by bulky nucleophile. The 3,4-*trans* attachment of the flavan-3-ol to the carbanion found to exhibit the degree of preference over the 3,4-*cis* attachment at C-4 [scheme 5.1][114].


[65]
$H_3O^{\oplus}$
[90]
[91]
[80] and [81]
[82]
[92]
[91] / $H_3O^{\oplus}$
[91] / $H_3O^{\oplus}$
Tetraflavanoids,etc
[93]
[94]
[95]
[96]

SCHEME 5.1

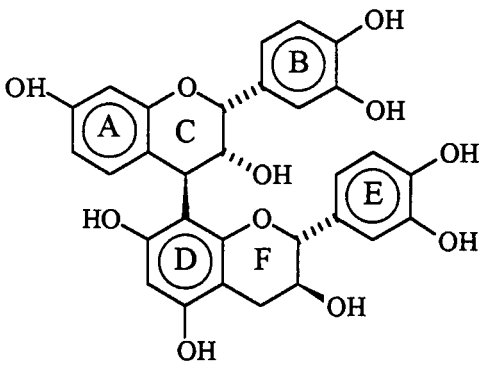
It was also suggested that leucofisetinidins undergo the regiospecific condensation if the substrate is a resorcinol-type flavan-3-ol, e.g. fisetinidol which results in the production of 4→6-linked dimers [97] and [98][114].



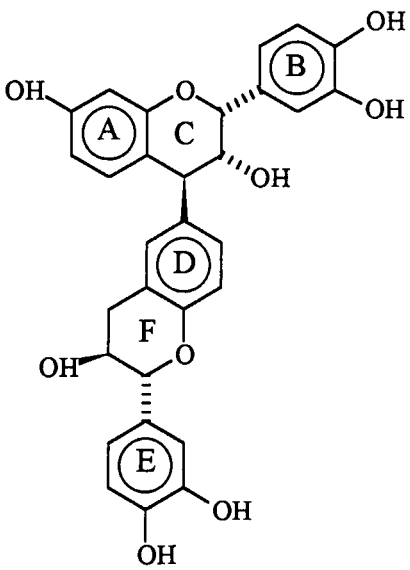
[97] 

[98] 

Changing the stereochemistry to all *cis* e.g. epifisetinidin, the intermediate flavan-4-carbocation controls the course of the condensation reactions to yield only biflavanoids [99] and [100] that possess only the 4β interflavanyl linkage[114].



[99]



[100]

DISCUSSION

CHAPTER 6

METABOLITES FROM CASSIA ABBREVIATA

Cassia abbreviata is a small (6 m) umbrella-shaped deciduous tree with a very distinctive cylindrical pod fruit. The tree belongs to the Caesalpiniaceae family and is occurs on sandy soil in the Kruger National Park[115,116]. The bark has a brownish-grey colour while the dark brown heartwood is heavy (900 kgm^{-3}) and hard.

The tree features in South African medicine and infusions of the bark were used to treat blackwater fever, abdominal pain and toothache while seeds are sucked as a tonic[115,116].

Investigation of the acetone extract of *Cassia abbreviata* heartwood revealed the co-occurrence of flavan-3-ols, stilbenes, biphenyls, dimeric stilbenes and proguibourtinidins. All these compounds were isolated as permethyl ether acetate derivatives and structures were elucidated by NMR experiments.

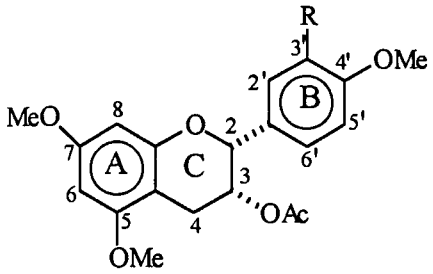
6.1 FLAVAN-3-OLS

Four known flavan-3-ols namely catechin, epicatechin, afzelechin, epiafzelechin were isolated. The ratio of catechin to epicatechin was 1:5, whereas the ratio of epiafzelechin to afzelechin was 1:18, the latter being the major flavan-3-ol in the heartwood.

Epiafzelechin **[101]**, epicatechin **[102]**, afzelechin **[103]** and catechin **[104]** were obtained after methylation and subsequent acetylation of fractions C_7 and C_9 from a Sephadex LH 20 column/separation.

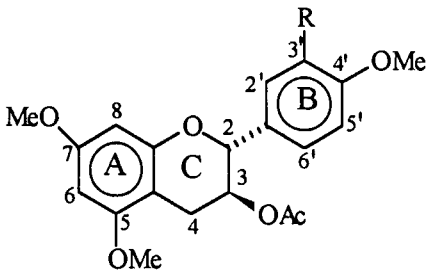
^1H NMR data (table 1, plates 1, 2, 3 and 4) of derivatives **101-104** showed the characteristic ABMX system in the heterocyclic region with an AX system (2x d, J = 2.5 Hz) for the aromatic A-rings. AA'BB' (2x d, J = 9.0 Hz) patterns were evident for the B-

rings of derivatives **101** and **103** while the derivatives **102** and **104** showed a B-ring with



[101] R = H

[102] R = OMe



[103] R = H

[104] R = OMe

an ABX (d, J = 2.0; dd, J = 2.0, 8.5 and d, J = 8.5) spin system.

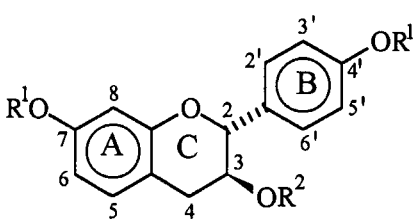
Table 1. ^{1}H NMR (300 MHz, $CDCl_3$ at 296K) data of flavan-3-ols **101, 102, 103**, and **104.** Splitting patterns and Coupling constants (Hz) are given in parentheses.

Position	**101 (Plate 1)**	**102 (Plate 2)**	**103 (Plate 3)**	**104 (Plate 4)**
2	5.05(s)	5.04(s)	5.06(d,6.5)	5.04(d,6.5)
3	5.44(m)	5.46(m)	5.35(m)	5.36(m)
4_{ax}	2.91(dd,3.0,18.0)	2.90(dd,3.0,18.0)	2.69(dd,7.0,16.5)	2.68(dd,7.0,16.5)
4_{eq}	2.99(dd,5.0,18.0)	3.00(dd,5.0,18.0)	2.89(dd,5.5,16.5)	2.93(dd,5.5,16.5)
6	6.21(d,2.5)	6.23(d,2.5)	6.19(d,2.5)	6.19(d,2.5)
8	6.13(d,2.5)	6.14(d,2.5)	6.11(d,2.5)	6.11(d,2.5)
2'/6'	7.39(d,9.0)		7.30(d,9.0)	
3'/5'	6.92(d,9.0)		6.90(d,9.0)	
2'		7.06(d,2.0)		6.91(d,2.0)
5'		6.88(d,8.5)		6.89(d,8.5)
6'		6.99(dd,2.0,8.5)		6.93(dd,2.0,8.5)
OMe	3.80,3.81,3.84 (each s)	3.80,3.81,3.91, 3.93 (each s)	3.79(2xs),3.81(s)	3.78,3.79,3.87, 3.88 (each s)
OAc	1.93 (s)	1.94 (s)	1.97(s)	1.97(s)

The 2,3-*cis* configuration was confirmed by the appearance of 2-H(C) as broad singlets at δ 5.05 and δ 5.04 for derivatives **101** and **102** respectively. The $^3J_{2,3}$-value of 6.5 Hz, observed for both **103** and **104,** indicated the 2,3-*trans* relative configuration of their C-rings. Two doublets of doublets in the region of δ 2.68-2.99 were assigned to 4-H_{ax}(C) and 4-H_{eq}(C) respectively. The absolute configuration of compounds **101-104** were confirmed from the CD data of flavan-3-ol derivatives[79,117].

6.1.1 GUIBOURTINIDOL

The novel free phenolic guibourtinidol **[105]** was purified and isolated as a yellowish-brown amorphous solid. The 4',7-di-O-dimethyl-3-O-acetyl derivative **[106]** from the C_6 fraction of the acetone extract of heartwood of *Cassia abbreviata* was prepared to facilitate the ^{1}H NMR experiments.



[105] $R^1 = R^2 = H$

[106] $R^1 = Me$, $R^2 = Ac$

[107] $R^1 = R^2 = Ac$

^{1}H NMR data (table 2, plate 5) showed an ABMX spin system in the heterocyclic region together with a ABX and AA'BB' spin system for the aromatic A- and C-rings. The presence of an O-acetyl (δ 1.98) and two O-methyl (δ 3.80, 3.82) resonances featured the typical substituents of a 4',7-di-O-methyl-3-O-acetylflavan-3-ol[117].

A COSY experiment permitted the assignment of the 2-H(C), 3-H(C) and 4-H(C) protons of the heterocyclic C-ring at δ 5.12, (J = 6.5 Hz), δ 5.30, (m) δ 2.82, (J = 7.0, 16.0 Hz) and δ 2.99, (J =5.0, 16.0 Hz). The 2,3-*trans* relative configuration was evident from the $^3J_{2,3}$ of 6.5 Hz, such a relatively smaller coupling constant presumably reflecting

significant contributions of A-conformers to the ensemble of conformers related to the C-ring[118].

The COSY data also showed the *meta*-coupling (J = 2.5 Hz) for 8-H(A) at δ 6.54 and correlated with the 6-H(A) at δ 6.53 (dd, J = 2.5, 9.0 Hz) as well as the 5-H(A) at δ 6.95 (J = 9.0 Hz) which represents the A-ring aromatic proton signals. Benzylic coupling between 5-H(A) and the 4-H_{eq}(C), observed in the NOESY experiment, confirmed the ABX pattern to the A-ring and the A/C ring system conjunction.

Table 2 ^{1}H (300 MHz) and ^{13}C (75.46 MHz) NMR data for guibourtinidol **105** and derivative **106** at 296K. Splitting patterns and Coupling constants (Hz) are given in parentheses.

Position	**106 ($CDCl_3$) (Plate 5a)**	**105 [$(CD_3)_2CO$] (Plate 5b)**	**105 [$(CD_3)_2CO$] (Plate 5b)**
2	5.12(d,6.5)	4.66(d,8.0)	82.4
3	5.30(m)	4.02(m)	67.7
4_{ax}	2.82(dd,7.0,16.0)	2.74(dd5.0,16.0)	33.3
4_{eq}	2.99(dd,5.0,16.0)	2.93(dd,9.0,16.0)	33.3
5	6.95(d,9.0)	6.88(d,9.0)	130.5
6	6.53(dd,2.5,9.0)	6.39(dd,2.5,9.0)	108.4
7			157.2
8	6.54(d,2.5)	6.30(d,2.5)	102.8
9			155.6
10			111.9
1'			130.7
2'/6'	7.30(d,9.0)	7.33(d,9.0)	129.0
3'/5'	6.90(d,9.0)	6.82(d,9.0)	115.2
4'			157.6
OMe	3.80,3.82 (each s)		
OAc	1.98		

NOE correlation was observed between the resonances of 2-H(C) at δ 5.12 and 2',6'-H(B) at δ 7.30 which linked the AA'BB' pattern to the B-ring.

A high amplitude negative Cotton effect (-3864) at 283.9 nm in the CD spectrum of derivative **106** (plate 5a) was in accordance with chiroptical data[79,117] of flavan-3-ol derivatives with (2R,3S) absolute configuration. The FAB mass spectrum showed a molecular ion at m/z 327 $[M-H]^+$, thus confirming the $C_{19}H_{20}O_5$ molecular formula for compound **106**.

Guibourtinidol **105** was also isolated and purified as peracetate **107,** which afforded the free phenolic form via alkaline hydrolysis. Assignment of the ^{13}C NMR spectra (table 2, plate 5b) of derivative **105** were based on HMBC and HMQC experiments.

The identification of guibourtinidol as the first natural occurring flavan-3-ol with the 4',7-dihydroxy phenolic substitution presented the opportunity to synthesize the four diastereomers by adapting a developed protocol[119] towards flavan-3-ol derivatives via asymmetric dihydroxylation of 1,3-diarylpropenes and subsequent acid-catalyzed cyclization to give the first synthesis of free phenolic flavan-3-ols[120]. This synthesis of free phenolic diastereomers led to confirmation of the structure of the free phenolic natural product by comparing the ^1H and ^{13}C NMR spectra and data[120].

CHAPTER 7

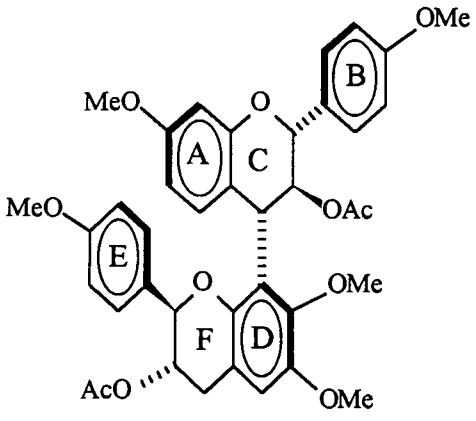
PROGUIBOURTINIDINS

Natural proanthocyanidin dimers, guibourtinidol-(4α→8)-catechin, -(4α→8)-epicatechin, -(4α→8)-afzelechin and -(4α→6)-catechin were isolated from the heartwood of *Acacia luedertzii*[97]. Guibourtinidin dimers containing -(4α→8)-catechin, epiafzelechin and –(4β→8) epicatechin, epiafzelechin were isolated from the bark of *C. abbreviata* and synthesized[121].

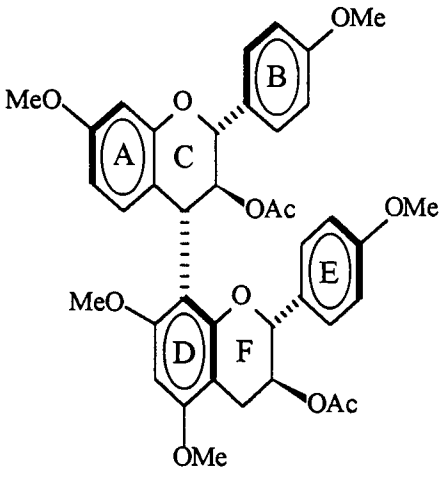
The ^{1}H NMR spectra of methyl ether acetate derivatives of dimeric proguibourtinidins displayed two overlapped spectra due to the presence of restricted rotation about the interflavanyl bond[111,113].

The presence of methoxyl and acetoxyl groups in the derivatives found to enhance the restricted rotation on the NMR time-scale of the rotational isomers[122]. The rotational isomers are designated either (+) or (-) on the basis of the sign of A(10)-C(4)-D(6 or 8)-D(7) dihedral angle to be consistent with earlier molecular modeling work by Mattice and co-workers[123,124]. Therefore, the (4→8) linked compounds in which the E-ring of the lower unit extends out and away from the A- and C-ring plane are designated (+) whereas (-) assigned for rotamer with the E-ring that is behind the A- and C-ring plane. The conformation in which the A(10)-C(4)-D(6)-D(7) dihedral angle is (-) in (4→6) linked compounds corresponds to the rotamer with the pyran oxygen of the F-ring is out away from, and (+) behind the plane of the interflavanoid bond (figure 7.1)[122].

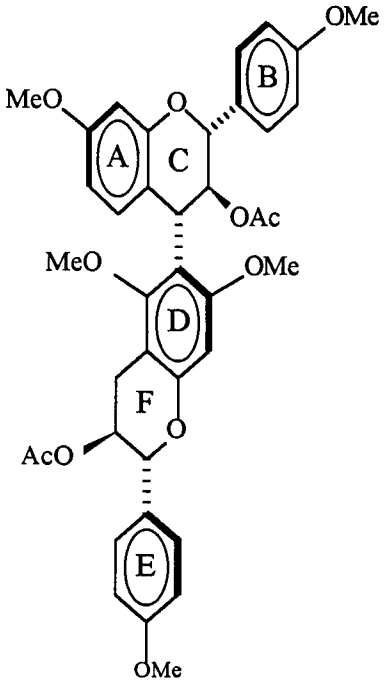
The assignment of the major and the minor rotamer conformations was confirmed after a series of NOE experiments and the allocation of signals for the top and bottom units of the specific dimer.



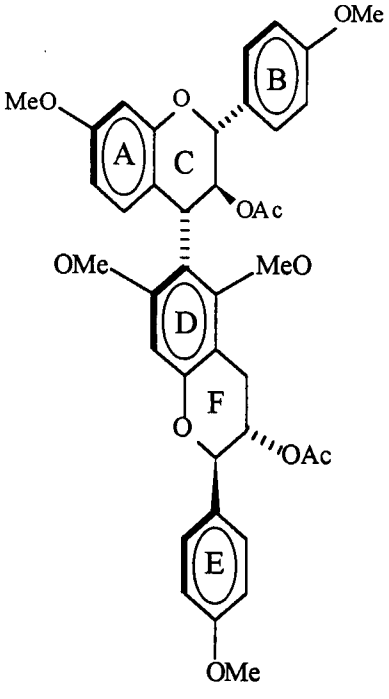
A(10)-C(4)-D(8)-D(7) = (-)



A(10)-C(4)-D(8)-D(7) = (+)



A(10)-C(4)-D(6)-D(7) = (-)



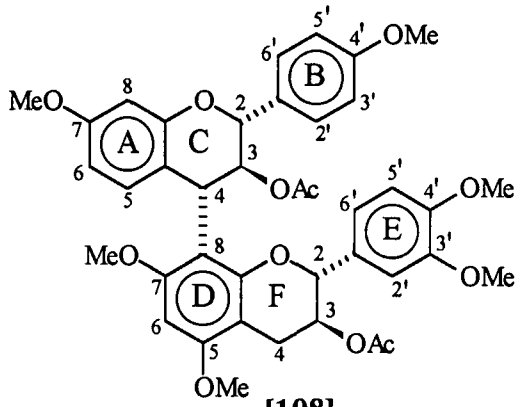
A(10)-C(4)-D(6)-D(7) = (+)

Figure 7.1 Conformations of rotamers of (4→8) and (4→6) linked dimers based on dihedral angles.

7.1 GUIBOURTINIDIN-CATECHIN DIMERS

7.1.1 GUIBOURTINIDOL-(4α→8)-CATECHIN

The proguibourtinidin derivative, **108** was isolated after methylation and acetylation of the C_{10} fraction from the acetone extract of the heartwood of *Cassia abbreviata*.



[108]

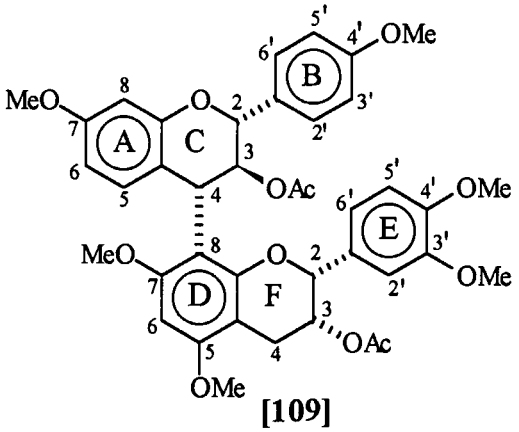
The 300 MHz ^{1}H NMR data (table 3, plate 6) of the methyl ether acetate derivative of guibourtinidol-(4α→8)-catechin **108** in $CDCl_3$ showed an AMX spin system with resonances at δ 4.82, (2-H, J = 10 Hz), δ 6.00, (3-H, J = 10 Hz) and δ 4.84, (4-H, J = 10 Hz) and an ABMX resonances at δ 4.83, (2-H, J = 9.0 Hz), δ 5.00, (3-H, m), δ 2.61, (4-H_{ax}, J = 6.0, 17.0 Hz) andδ 3.08, (4-H $_{eq}$, J = 9.0, 17.0 Hz) systems in the heterocyclic regions which were attributed to the C- and F-rings respectively. The aromatic protons for the substituted ABC top unit were attributed to an ABX pattern with resonances at δ 6.30, (8-H, J = 2.5 Hz), δ 6.45, (6-H, J = 2.5, 8.5 Hz) and δ 6.70, (5-H, J = 8.5 Hz) and an AA'BB' system with resonances at δ 6.52 (2',6'-H, J = 9.0 Hz) and δ 7.04, (3',5'-H, J = 9.0 Hz). A proton singlet 6-H atδ 6.18 in the aromatic region of the D-ring and an ABX pattern with resonances at δ 6.58, (2'-H, J = 2.0 Hz), δ 6.48, (6'-H, J = 2.0, 8.5 Hz) and δ 6.73, (5'-H, J = 8.5 Hz) was assigned to the E-ring of the DEF bottom unit. When taken in conjunction with the AMX- and ABMX-systems for the heterocyclic region with the presence of two O-acetyls group proton resonances (δ 1.61, 1.90) and six O- methyls

group proton resonances (δ 3.74, 3.77, 3.78, 3.85, 3.89 ppm) the substitution of the dimer could be confirmed.

The coupling constants of $J_{2,3} = J_{3,4} = 10.0$ Hz characterized the 2,3-*trans*-3,4-*trans* relative configuration for C-ring. The chemical shift and coupling constants as per (table 3) are reminiscent of the catechin[125] and the 2,3-*trans* relative configuration of the F-ring was defined. The high amplitude negative Cotton effect $[\theta]_{234}$ -1.44×10^{-5} in the CD spectrum of **108** (plate 6) determined a 4α (C) linkage and thus 4S absolute configuration. When taken in conjunction with 2,3-*trans*-3,4-*trans* relative configuration as defined by ^{1}H NMR coupling constants of the heterocyclic AMX system of absolute configuration of the top unit was confirmed to be 2R,3S,4S.

7.1.2 GUIBOURTINIDOL-(4α→8)-EPICATECHIN

The methyl ether acetate derivative **109** was obtained after methylation and acetylation of the C_{12} fraction from the acetone extract of the heartwood of *Cassia abbreviata*.



[109]

The ^{1}H NMR spectrum (plate 7) of the methyl ether acetate derivative of guibourtinidol-(4α→8)-epicatechin **109** showed the presence of two rotamers that occurred in a relative proportion of 1:1.1 as defined by the signal intensities. Efforts were concentrated on defining the conformation of the major rotamer.

Inspection of the ^{1}H NMR data (table 3, plate 7) of both major and the minor rotamer indicated the presence of an AMX and ABMX systems for the heterocyclic C- and F-

rings. The aromatic protons of the top unit were defined by an ABX and AA'BB' spin system. A singlet in the aromatic region and an ABX system for the E-ring was evident for DEF bottom unit.

The COSY spectrum permitted the assignment of 2-H(C), 3-H(C) and 4-H(C) heterocyclic C-ring protons with resonances occurring at δ 4.94, (J = 10.0 Hz), δ 6.06, (J = 10.0 Hz) and δ4.84, (J = 10.0 Hz). The 2,3-*trans*-3,4-*trans* relative configuration of the heterocyclic C-ring was evident from the coupling constant of $J_{2,3} = J_{3,4}$ = 10.0 Hz and a strong NOE association between 2-H(C) and 4-H(C) which suggested the 2,4-*cis* configuration of the heterocyclic ring. The ABMX system of the F-ring was comprised of a broad singlet at δ 5.00 for 2-H(F) and a multiplet at δ 5.30 for 3-H(F) (table 3) which indicated the 2,3-*cis* relative configuration typical for epicatechin[125].

To assign the methoxy group proton signals and hence the rotational conformation of the major rotamer, it was first necessary to define the aromatic proton signals of the A- and D-rings. An ABX pattern of resonances at δ 6.48, (J = 2.5 Hz), δ 6.46, (J = 2.5, 8.5 Hz) and δ 6.80, (J = 8.5 Hz) for the A-ring was confirmed by the COSY spectrum and supported by a strong NOE associations between 8-H(A) and 7-OMe(A) group proton resonances at δ 3.78.The benzylic coupling observed between resonances of 5-H(A) and 4-H(C) confirmed the A/C ring junction.

The proton singlet at δ 6.20 was assigned to 6-H(D) due to the strong NOE association with resonances of 7-OMe(D) at δ 3.90 and 5-OMe(D) at δ 3.86 respectively, thereby establishing a (4→8) interflavanoid bond.

$^4J_{HH}$ long distance coupling between 2-H(C) and 2',6'-H(B) with resonances at δ 6.75 (J = 9.0 Hz) and 7.08, (J = 9.0 Hz) confirmed an AA'BB' system of the top unit and the assignment of 4'-OMe(B) at δ 3.76 was confirmed by NOE association to the 2',6'-H(B) doublet at δ 7.08. An ABX spin system with resonances at δ 6.58, (5'-H, J = 8.5 Hz), δ 6.38, (6'-H, J = 2.0, 8.5 Hz) and δ 6.70, (2'-H, J = 2.0 Hz) was assigned to the E-ring. The 4'-OMe(E) group protons resonance at δ 3.77 was confirmed by NOE experiment with the *ortho*-doublet 5'-H(E) at δ 6.58 and the NOE between this methoxyl and 2'-H(E) and

the methoxy group protons at δ 3.77 confirmed both the 3'-OMe and the ABX substituted pattern.

Table 3 ^{1}H NMR (300MHz, $CDCl_3$) of derivative **108 and 109** at 296K. Splitting patterns and Coupling constants (Hz) in parentheses.

RING	PROTONS	[108] (Plate 6)	[109] & Rotamer (*) (Plate 7)
	5	6.70(d,8.5)	6.80,6.65*(d,8.5)
A	6	6.45(dd,2.5,8.5)	6.46(dd,2.5,8.5)
	8	6.30(d,2.5)	6.48,6.48* (d,2.5)
B	2'/6'	7.04(d,9.0)	7.08,7.43*(d,9.0)
	3'/5'	6.52(d,9.0)	6.75,6.94*(d,9.0)
C	2	4.82(d,10.0)	4.94,4.93*(d,10.0)
	3	6.00(t,10.0)	6.06,6.00*(t,10.0)
	4	4.84(d,10.0)	4.92,5.04*(d,10.0)
D	6	6.18(s)	6.20,6.11*(s)
E	2'	6.58(d,2.0)	6.70(d,2.0)
	5'	6.73(d,8.5)	6.58(d,8.5)
	6'	6.48(dd,2.0,8.5)	6.38,6.36*(dd,2.0,8.5)
F	2	4.83(d,9.0)	5.00,5.15*(s)
	3	5.00(m)	5.30,5.55*(m)
	4_{ax}	2.61(dd,6.0,17.0)	2.87(dd,2.0,18.0)
	4_{eq}	3.08(dd,9.0,17.0)	2.98(dd,5.0,18.0)
OMe		3.74,3.77,3.78,3.85, 3.89,3.90 (each s)	3.76-4'B,3.77-4'E, 3.78-7A, 3.86-5A,3.79-3'E,3.90-7D, 3.59*,3.74*,3.82*,3.84*, 3.92*,3.95* (each s)
OAc		1.61,1.90 (each s)	1.75,1,78,1.79* 1.78* (each s)

* Signals of the rotamer.

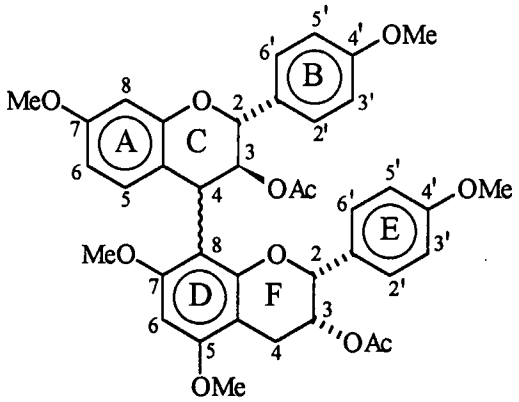
The CD spectrum of derivative **109** (plate 7) exhibited a high amplitude negative Cotton effect of $[\theta]_{245}$ $-1.243x10^5$, indicating an 4α-flavanyl C-ring substituent. When taken in conjugation with the relative stereochemistry of 2,3-*trans*-3,4-*trans*, the CD data supported a 2R,3S,4S absolute configuration for the ABC top unit.

The rotational conformation of the interflavanoid bond was established by the NOE associations observed between 7-OMe(D) with protons 3-H(C), 4-H(C) and 5-H(A). Therefore the major rotamer is (+) which implies that the E-ring of the lower unit extends outwards and away from the A-and C-ring plane.

7.2 GUIBOURTINIDIN-AFZELECHIN DIMERS

7.2.1 GUIBOURTINIDOL-(4α→8)- AND -(4β→8)-EPIAFZELECHIN

Methylation and subsequent acetylation of the phenolic analogue isolated from the acetone extract of *Cassia abbreviata* heartwood afforded the proguibourtinidin derivative **110** and **111** as discussed.



[110] =

[111] =

^{1}H NMR spectra of both methyl ether acetate derivatives of -(4α→8)- **110** (table 4, plate 8) and -(4β→8)- **111** (table 4, plate 9) both displayed rotamer ratios of 1:2 when recorded in $CDCl_3$.

Table 4 ^{1}H NMR (300 MHz, $CDCl_3$) of derivative 110 and 111 at 296K. Splitting patterns and Coupling constants (Hz) in parentheses.

RING	PROTONS	110 & Rotamer (Plate 8)	111 & Rotamer (Plate 9)
A	5	6.82,6.64*(d,8.5)	6.66,6.88*(d,8.5)
	6	6.49,6.38*(dd,2.5,8.5)	6.16,6.44*(dd,2.5,8.5)
	8	6.54,6.47*(d,2.5)	6.62,6.58*(d,2.5)
B	2',6'	7.11,6.79*(d,9.0)	7.36,7.26*(d,9.0)
	3',5'	6.77,6.94*(d,9.0)	6.90,6.85*(d,9.0)
C	2	4.95(d,10.0)	5.50,5.34*(d,7.0)
	3	5.97(t,10.0)	5.64,5.69*(dd,5.5,7.0)
	4	4.93(d,10.0)	4.84,5.01*(d,5.5)
D	6	6.18,6.11*(s)	6.18,6.13*(s)
E	2',6'	6.73,6.94*(d,9.0)	6.96,7.26*(d,9.0)
	3',5'	6.68,6.74*(d,9.0)	6.76,6.72*(d,9.0)
F	2	5.15,5.03*(s)	4.39,5.03*(s)
	3	5.30,5.49*(s)	5.37,5.45*(m)
	4_{ax}	2.86,(dd,2.5,18.0)	2.88,2.90*(dd,2.5,18.0)
	4_{eq}	2.95(dd,5.0,18.0)	2.99,3.02*(dd,5.5,18.0)
OMe		3.76-4'E, 3.80-4'B, 3.81-5A, 3.86-5D, 3.90-7D,3.59*, 3.74*, 3.80*, 3.84*,3.85* (each s)	3.47-7A,3.78-4'E,3.79-7D, 3.82-4'B,3.86-5D,3.42*, 3.76*,3.81*,3.83*,3.84* (each s)
OAc		1.65,1.74,1.78*,1.81* (each s)	1.83,1.92,1.71*,1.92* (each s)

Analysis of the ^{1}H NMR data (table 4, plate8 and plate 9) of both derivatives **110** and **111** showed an AMX and ABMX systems for the heterocyclic C- and F-rings respectively. The ABC-substituted unit was defined by an ABX and AA'BB' spin system. A singlet in the aromatic region and an AA'BB' pattern were attributed to the bottom unit (DEF). The data obtained correlated with the literature ^{1}H NMR data[121].

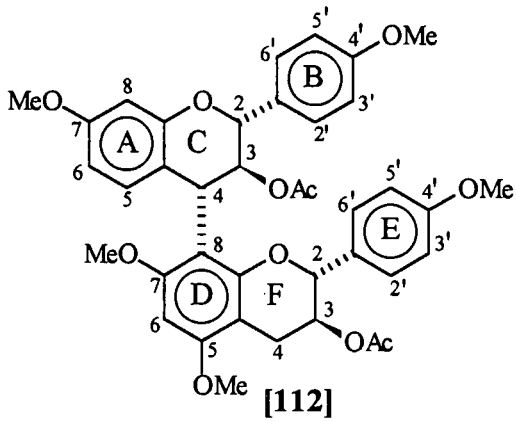
The 2,3-*trans*-3,4-*trans* relative configuration of the heterocyclic C-ring of derivative **110** was ascertained by the coupling constants of $J_{2,3}$ = $J_{3,4}$ = 10.0 Hz and hence the conformation of the upper unit was approximately half chair (E-conformation). Whereas the coupling constant of $J_{2,3}$ = 7.0 Hz and $J_{3,4}$ =5.5 Hz characterized the 2,3-*trans*-3,4-*cis* relative configuration for derivative **111.** The relative 2,3-*cis* configuration of the F-rings for both derivatives **110** and **111** was confirmed by $J_{2,3}$ of *ca* 1.0 Hz.

The absolute configuration of 2R,3S,4S was assigned for derivative **110** (plate 8) from the CD data which displayed a high negative Cotton Effect of $[\theta]_{245}$ $-1.082x10^5$ and high positive Cotton effect at $[\theta]_{244}$ $3.618x10^5$ confirmed the 4β interflavanyl linkage in derivative **111** (plate 9) therefore was assigned the absolute stereochemistry of 2R,3S,4R.

The assignment of the methoxy groups protons of derivative **110,** 4'-OMe(E) at δ 3.76, 4'-OMe(B) at δ 3.80, 5-OMe(D) at δ 3.86 and 7-OMe(D) at δ 3.90 was done by NOE correlation experiments. NOE associations observed between the 7-OMe(D) protons with both 4-H(C) and 2-H(C) together with the association between the 5-OMe(D) protons and the methylene 4-H(F) protons confirmed the (-) major rotamer, in which the E- and F-rings of the DEF unit are folded back under the plane of the AC-ring of the upper unit. The absence of the NOE between the 7-OMe(D) protons and 5-H(A) of the major rotamer of derivative **110** suggested that more time was spent in the crowded conformation and hence there was no free rotation around the interflavanoid bond.

The rotational conformation of the interflavanoid bond for derivative **111** was perfectly assigned by the NOE associations observed between the protons of 7-OMe(D) which revealed at δ 3.80 with 4-H(C), 5-OMe(D) at δ 3.86 with 4-H(F), 7-OMe(A) proton resonance at δ 3.47 with 3',5'-H(E), 2-H(F) with 5-H(A) and 2-H(C) with 2',6'-H(E). The absence of NOE between the 7-OMe(D) proton resonance and 5-H(A) resonance indicated that free rotation was hindered around the interflavanoid bond. Therefore the conformation of derivative **111** clearly showed the (-) or compact rotamer. The preference of the more crowded conformation of these molecules was explained in terms of minimization of the surface area to enhance solute-solvent interaction.

7.2.2 GUIBOURTIDOL-4α→8-AFZELECHIN



[112]

Methylation and subsequent acetylation of the phenolic analogue isolated from the acetone extract of heartwood of *Cassia abbreviata* afforded the proguibourtinidin derivative, **112.**

The ^{1}H NMR spectrum of the pentamethyl ether acetate derivative, **112** (plate 10) displayed two rotamers in the relative proportion of 1:1.5.

Analysis of the ^{1}H NMR data (table 5, plate 10) of the pentamethyl ether acetate derivative, **112** exhibited an AMX system which was attributed to the heterocyclic C-ring substitution pattern with resonances at δ 4.83 (2-H, J = 10.0 Hz), δ 5.95 (3-H, J = 10.0 Hz), δ 4.86 (4-H, J = 10.0 Hz) which defined the 2,3-*trans*-3,4-*trans* relative configuration for the C-ring. The 2,3-*trans* relative configuration was evident for the heterocyclic F-ring from the $J_{2,3}$ of 9.0 Hz.

An ABX pattern with resonances at δ 6.73 (5-H, J = 8.5 Hz), δ 6.48 (6-H, J = 2.5, 8.5 Hz), δ 6.40 (8-H, J = 2.5 Hz) indicated the 7-substituted A-ring. A strong NOE association observed between the 7-OMe(A) group proton resonances at δ 3.78 and 8-H(A) resonance at δ 6.40 and the benzylic coupling between the resonances of 5-H(A) at δ 6.73 and 4-H(C) at δ 4.86 confirmed the ABX spin system for the A-ring and the A/C junction.

An aromatic proton singlet appearing at δ 6.17 was typical of the residual 6-H(D) proton resonance of the (4→8) linked biflavanoid with a phloroglucinol D-ring. The correlation

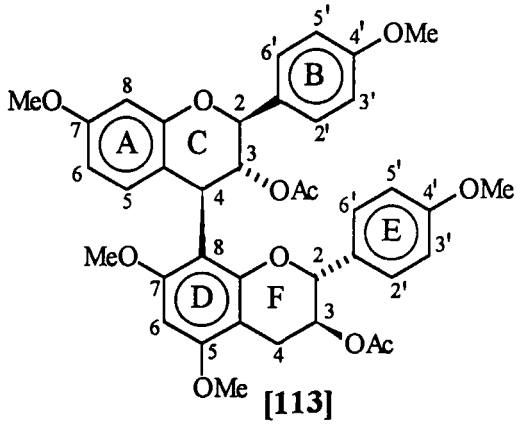
observed for the 6-H(D) resonance with the group proton resonances of 7-OMe(D) at δ 3.89 and 5-OMe(D) at δ 3.84 confirmed the structure.

The $^4J_{HH}$ long range couplings between 2-H(C) resonance at δ 4.83 with 2',6'-H(B) resonance at δ 7.06 and 2-H(F) resonance at δ 4.94 with 2',6'-H(E) enabled the differentiation of the spin systems of the top and bottom units. NOE associations between the two methoxy group proton resonances at δ 3.78 and 3.81 with 3',5'-H(E) and 3',5'-H(B) confirmed 4'-OMe(B) and 4'-OMe(E) respectively.

The high amplitude negative Cotton Effect $[\theta]_{243}$ -208600 in the CD spectrum of **112** (plate 10) suggested an 4α interflavanoid bond. The presence of a NOE association between the resonances of 2-H(C) and 4-H(C) suggested a 2,4-*cis* relative configuration and altogether with the acquired data confirmed a 2R,3S,4S absolute stereochemistry for the top unit.

The rotational conformation of the interflavanoid bond for derivative **111** was perfectly assigned by the NOE associations observed between 7-OMe(D) group proton resonances at δ 3.80 with 4-H(C), 5-OMe(D) protons at δ 3.86 with 4-H(F), 7-OMe(A) at δ 3.47 with 3',5'-H(E), 2-H(F) with 5-H(A) and 2-H(C) with 2',6'-H(E). The absence of any NOE associations between 7-OMe(D) proton resonances and 5-H(A) resonance indicated that the free rotation was hindered around the interflavanoid bond.

7.2.3 GUIBOURTINIDOL-(4β→8)-AFZELECHIN



[113]

The proguibourtinidin derivative, **113** was isolated after methylation and acetylation of the C_8 fraction from the acetone extract of the heartwood of *Cassia abbreviata.*

The ^{1}H NMR spectrum (plate 11) of the pentamethyl ether acetate derivative, **113** showed two rotamers in approximately equal proportions of 1:1,1.
Analysis of the ^{1}H NMR data (table 5, plate 11) of the pentamethyl ether acetate derivative, **113** of the major rotamer showed the presence of an AMX system with resonances at δ 5.28 (2-H, *J*=10 Hz), δ 5.58 (3-H, *J* = 7.0, 10.0 Hz) and δ 4.94 (4-H, *J* = 7.0 Hz) and an ABMX system with resonances at δ 4.14 (2-H, *J* = 9.0), δ 5.20 (3-H, m *ca* 1.0 Hz), δ 2.60 (4-H_{ax}, *J* = 9.0, 17.0 Hz) and δ 3.14 (4-H_{eq}, *J* = 6.0, 17.0 Hz) for the heterocyclic C- and F-rings.

A 2,3-*trans*-3,4-*trans* relative configuration for the heterocyclic C-ring was defined by the coupling constants of $J_{2,3}$ = 10.0 Hz, $J_{3,4}$ = 7.0 Hz, which is different from the 2R,3S *trans-trans* compound **112**. The appearance of the 2-H(F) resonance as clear doublet at δ 4.14 is a very the characteristic signal for the 4β *trans-trans* compound and suggested that the top unit could very likely be an *ent-* moiety. The NOE associations between the resonances of 2-H(C) at δ 5.28 and 4-H(C) at δ 4.94 strongly suggested the 2,4-*cis* configuration of the heterocyclic C-ring. The $J_{2,3}$ of 9.0 Hz indicated 2,3-*trans* relative stereochemistry for the heterocyclic F-ring.

The aromatic proton singlet of 6-H(D) resonance at δ 6.19 showed strong NOE associations with the group proton resonances of 5-OMe(D) at δ 3.86 and 7-OMe(D) at δ 3.83 which is indicative for the (4→8) interflavanyl bond. The 7-OMe(A) protons resonance at δ 3.53 was assigned by NOE observed to the 8-H(A) resonance at δ 5.81 and 6-H(A) resonance at δ 6.26. The benzylic coupling observed between the resonances of 5-H(A) at δ 6.69 and 4-H(C) at δ4.86 confirmed an ABX substituted A-ring and the A/C ring junction.
The $^4J_{HH}$ long distance couplings between 2-H(C) and 2',6'-H(B) and 2-H(F) with 2',6'-H(E) differentiated the spin systems of the top (ABC) and bottom (DEF) units.

Table 5 ^{1}H NMR (300 MHz, $CDCl_3$) of derivative 112, 113 and 114 at 296K. Splitting patterns and Coupling constants (Hz) in parentheses.

RING	H	[112] & Rotamer (Plate 10)	[113] & Rotamer (Plate 11)	[114] & Rotamer (Plate 12)
A	5	6.73,6.64*(d,8.5)	6.69,6.80*(d,8.5)	6.65,6.74*(d,8.5)
	6	6.48,6.39*(dd,2.5,8.5)	6.26,6.43*(dd,2.5,8.5)	6.426.45*(dd,2.5,8.5)
	8	6.40,6.48*(d,2.5)	5.81,6.55*(d,2.5)	6.50,6.55*(2.5)
B	2',6'	7.06,7.44*(d,9.0)	7.28,7.31*(d,9.0)	7.45,7.46*(d,9.0)
	3',5'	6.77,6.92*(d,9.0)	6.87,6.88*(d,9.0)	6.93,6.92*(d,9.0)
C	2	4.83,(d,10)	5.28,5.33*(d,10)	4.95,4.89*(d,10.0)
	3	5.95,6.12*(t,10.0)	5.58,5.57*(dd,7.0,10.0)	6.08,5.59*(t,10.0)
	4	4.86,(d,10.0)	4.94,5.01*(d,7.0)	4.89,4.91*(d,10.0)
D	6	6.17,6.10*(s)	6.19,6.11*(s)	
	8			6.30,6.33*(s)
E	2',6'	7.06,7.27*(d,9.0)	7.02,7.24*(d,9.0)	7.34,7.33*(d,9.0)
	3',5'	6.73,6.87*(d,9.0)	6.78,6.79*(d,9.0)	6.93,6.91*(d,9.0)
F	2	4.94,(d,9.0)	4.14,(d,9.0)	5.00,4.87*(7.5)
	3	4.90,5.21*(m)	5.20,5.39*(m)	5.35,5.25*(m)
	4_{ax}	2.61,280*(dd,9.0,16.0)	2.60(dd,9.0,17.0)	2.80,2.69*(dd,9.0,16.0)
	4_{eq}	3.03,2.80*(dd,6.0,16.0)	3.14(dd,6.0,17.0)	3.18.3.19*(dd,6.0,16.0)
OMe			3.53-7A,3.79-4'E,3.81-4'B,3.83-7D,3.87-5D 3.36*,3.79*,3.80*, 3.81*,3.83* (each s)	3.57-7D,3.76-7A,3.78-5D, 3.83-4'E,3.84-4'B 3.75*,3.81*,3.83*, 3.84*,3.85* (each s)
OAc		1.61,1.90	1.73,1.83,1.82*,1.98* (each s)	1.64,1.94,1.66*,1.87*

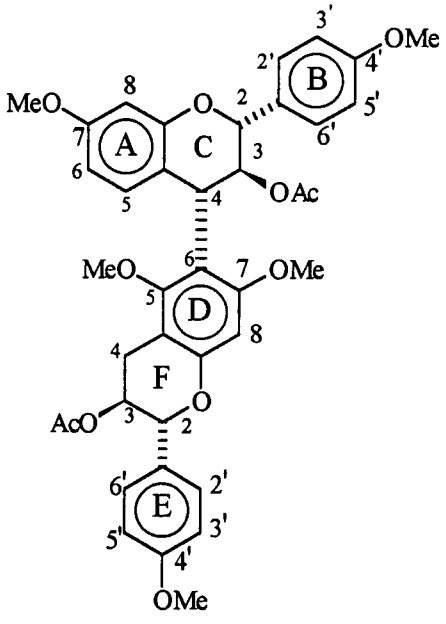
The high-amplitude positive Cotton Effect ($[\theta]_{244}$ $3.2x10^5$) in the CD spectrum of derivative,**113** (plate 11) pointed towards an 4β linkage and thus a 4R absolute stereochemistry. When taken in conjugation with the relative stereochemistry of 2,3-*trans*-3,4-*trans*, the CD data supported the 2S,3R,4R absolute configuration of the ABC top unit.

The conformation of the major rotamer is (-), confirming that the E- and F-rings of the lower unit are folded back under the AC plane of the top unit as shown by the NOE associations resonances of 3',5'-H(E) to 7-OMe(A), 7-OMe(D) to 4-H(C) and 4'-OMe(E) to 8-H(A). The absence of NOE associations between the resonances of 7-OMe(D) protons to the 5-H(A) and 3-H(C) proved that the free rotation was hindered around the interflavanoid bond.

7.2.4 GUIBOURTINIDOL-(4α→6)-AFZELECHIN

The proguibourtinidin derivative **114** was isolated after methylation and acetylation of the C_{13} fraction obtained from the acetone extract of the heartwood of *Cassia abbreviata.*

The ^1H NMR spectrum (plate 11) of the methyl ether acetate derivative **114** in $CDCl_3$ showed 2-rotamers that occurred in a relative proportion of 1:2.4.



[114]

Inspection of the ^1H NMR data (table 5, plate 12) of derivative **114** showed an AMX and ABMX spin systems for the heterocyclic C- and F-rings respectively. The ABC substituted top unit was defined by an ABX and an AA'BB' spin patterns for the aromatic ring protons.

A singlet in the aromatic region and an AA'BB' spin system was evident for the bottom (DEF) unit (table 5). When taken in conjuction with the presence of two O-acetyl and five O-methyl resonances the data collectively indicated a dimeric structure.

The heterocyclic C-ring predominately showed the E-conformation on the basis of $^3J_{HH}$ coupling ($J_{2,3}$ = $J_{3,4}$ = 10.0 Hz) of 2,3-*trans*-3,4-*trans* for the top unit. The heterocyclic ring protons of the terminal F-ring were assigned from the COSY spectrum as resonances δ 5.00 (2-H, J = 7.5 Hz), δ 5.35 (3-H, m), δ 2.80 (4-H, J = 9.0, 16.0 Hz) and δ 3.18 (4-H, J = 6.0, 16.0 Hz). The $^3J_{HH}$ coupling (J = 7.5 Hz) defined the 2,3-*trans* relative configuration and suggested a small proportion of the A-conformer present according to the approach by Steynberg *et al.*[126] to an arithmetic time-averaging molar ratio of the A- and E-conformers which are responsible for the observed coupling constants.

Assignment of the methoxy group proton signals especially of the A- and D-rings were crucial in determining the conformation of the major rotamer. An aromatic proton singlet 8-H(D) resonance at δ 6.30 showed strong NOE association with 7-OMe(D) group proton resonance at δ 3.57 which characterized the (4→6) interflavanoid bond. NOE association observed between the methoxy group proton resonance at δ 3.78 and 5-H(A) resonance at δ 6.65 confirmed the 5-OMe(D). Strong NOE associations were observed between the 7-OMe(A) group protons resonance and 8-H(A) at δ 6.50 (J=2.5 Hz) and 6-H(A) at δ 6.43 (J = 2.5, 8.5 Hz) resonances. The benyzlic coupling observed between 5-H(A) at δ 6.65 and 4-H(C) at δ 4.89 confirmed the ABX system of the A-ring and the A/C junction.

COSY experiments indicated the coupling of 2-H(C) at δ 4.95 with 2',6'-H(B) at δ 7.45 and 2-H(F) at δ 5.00 with 2',6'-H(E) at δ 7.34 resonances, which confirmed and differentiated the two AA'BB' spin patterns for the top and the bottom unit. The 4'-OMe(E) and that of 4'-OMe(B) group protons resonance at δ 3.83and 3.84 respectively may interchange due to the overlapping of the signals of the B- and E-rings.

The presence of NOE association between 2-H(C) and 4-H(C) indicate the 2,4-*cis* relative configuration of the C-ring. The CD spectrum of **114** (plate 11) exhibited a high amplitude negative effect of $[\theta]_{244}$ -61220 indicating a 4α-flavanyl C-ring substituent of

which together with the 2,3-*trans*-3,4-*trans* relative configuration, indicated a 2R,3S,4S absolute stereochemistry for the heterocyclic C-ring.

NOE experiments indicated associations of 7-OMe(D) group protons resonance with 3-H(C) resonance and 5-OMe(D) group protons resonance with 4-H(C) resonance which evident for (+) A(10)-C(4)-D(6)-D(7) dihedral angle corresponding to the pyran oxygen behind the plane of the C4→C6 linkage and defined the compact rotamer conformation. The absence of NOE associations beween 7-OMe(D) and 5-H(A), 5-OMe(D) and 8-H(D) resonances confirmed that the free rotation was hindered around the interflavanoid bond. It was obvious from the above data that the derivative **114** spent more time in the compact conformation.

CHAPTER 8

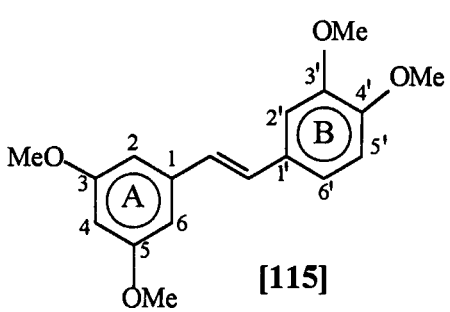
STILBENE DERIVATIVES

8.1 TETRAHYDROXYSTILBENES

In this study only one tetrahydroxystilbene was isolated as an O-methyl derivative from the acetone extract of the heartwood of *Cassia abbreviata*.This was found to be the monomeric precursor of the cyclodimers and to co-occur with its corresponding bibenzyl **127** in a very small quantity.

8.1.1 3,3',4',5-TETRAMETHOXYSTILBENE

The tetramethyl ether stilbene derivative **115** was obtained after methylation of the C_{10} fraction from the acetone extract of heartwood of *Cassia abbreviata.*



[115]

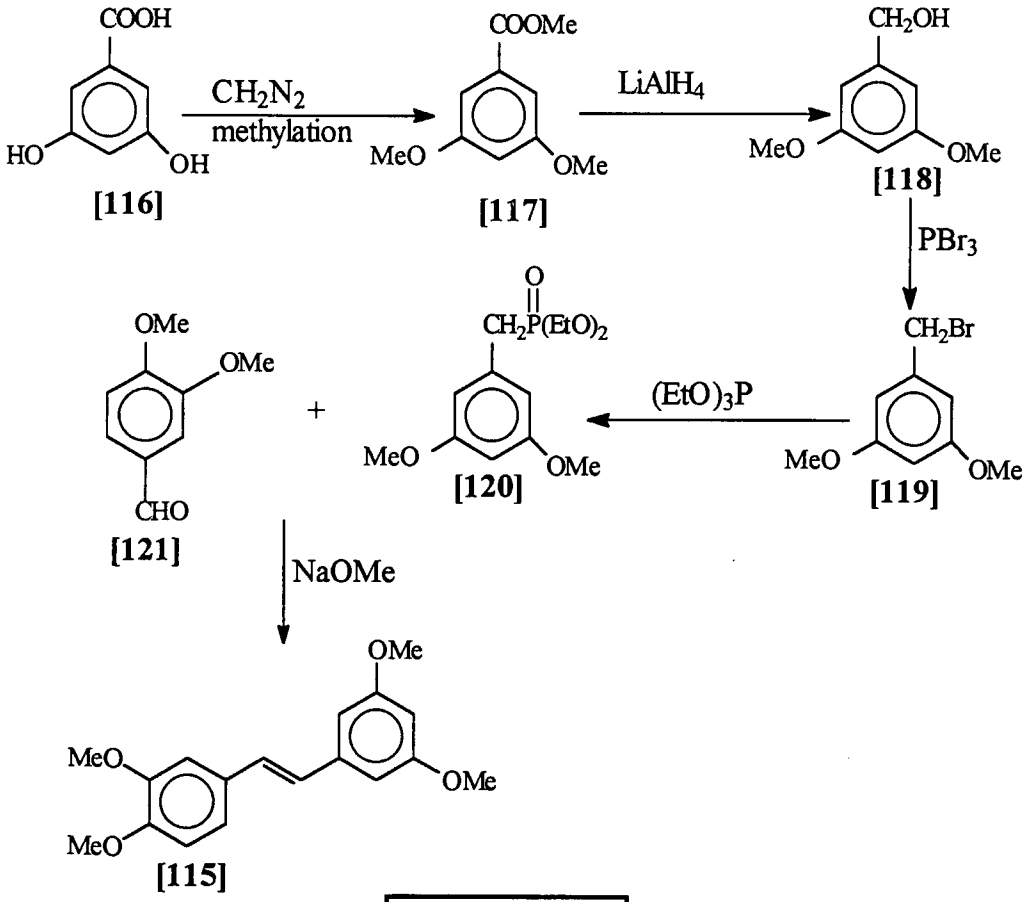
^{1}H NMR spectrum data (table 6, plate 13) of tetramethoxystilbene **115** showed *trans*-olefinic proton signals at δ 6.92 and 7.06 (both with J = 16.0) and the presence of an AX_2 and ABX spin systems (each with three protons) typical of a stilbene moiety.

COSY and NOESY experiments were used to assign the aromatic proton resonances of 2-H(A), 4-H(A) and 6-H(A) at δ 6.68(J = 2.0 Hz), δ 6.40(J = 2.0 Hz) and δ 6.68 (J = 2.0 Hz) respectively to the AX_2 spin system and confirmed the ABX system with resonances at δ 7.10 (2-H, J = 2.0 Hz), δ 6.88 (5-H, J = 8.0 Hz) and δ 7.07 (6-H, J = 2.0, 8.0 Hz).

The oxygenation pattern was assigned from the NOE associations of 2-H(A) and 4-H(A) with the methoxy group protons resonance at δ 3.85 which confirmed the resonances of 3-OMe(A) and 5-OMe(A) groups of the A-ring. The protons resonances of 3'-OMe(B) at δ 3.97 and 4'-OMe(B) at δ 3.92 was confirmed by NOE association observed between 2'-H(A) and 5'-H(A) proton resonances.

8.1.2 SYNTHESIS OF 3,3',4',5-TETRAMETHOXYSTILBENES

Due to the small quantity of monomeric stilbene isolated from the heartwood a multistep synthesis was used as outlined in (scheme 8.1) to prepare more of the required stilbene **115**.

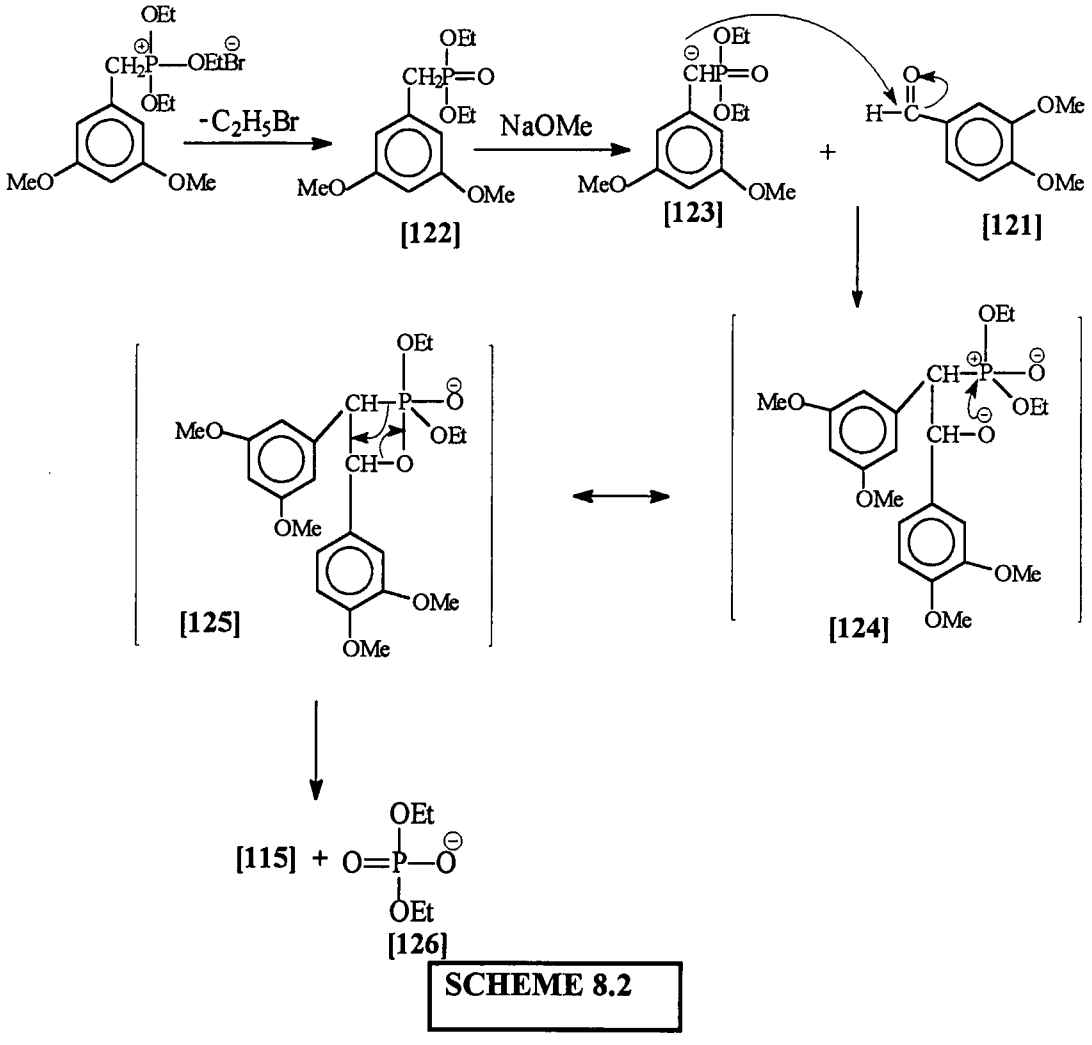


SCHEME 8.1

The Wittig reaction[35] was used in the synthesis of 3,3',4',5-tetramethoxystilbene. The reaction is about coupling of diethyl benzylphosphonate with the appropriate aryl

aldehyde in basic conditions to produce *trans*-stilbene exclusively in situ. The diethyl benzylphosphonate was synthesized by starting with 3,5-dihydroxybenzoic acid **[116].** After methylation with diazomethane to 3,5-dimethoxybenzoate **[117]** and then selectively reduced to 3,5-dimethoxybenzyl alcohol **[118]** which was treated with PBr_3 and resulted in the alkyl halide, 3,5-dimethoxybenzyl bromide **[119]** which was converted to the phosphonate **[120]** on heating with excess triethylphosphite.

The mechanism involved the deprotonation of diethyl benzylphosphonate **122** to the phosphonate anion **123,** which then reacted with the carbonyl group of 3,4-dimethoxybenzaldehyde **[121]** to form the intermediate **124.**



SCHEME 8.2

Wadsworth and Emmons[127,128] suggested that it could be in equilibrium with the four-membered ring **125**. The oxophosphotane **126** eliminated the phosphine oxide to yield the stilbene **115** (scheme 8.2).

Table 6 ^{1}H NMR (300 MHz) data for stilbene derivatives **115, 127** and **128** at 296K. Splitting patterns and Coupling constants (Hz) are given in parentheses

RING	PROTON	115 (Plate 13)	127 (Plate 14)	128 (Plate 15)
A	2	6.68(d,2.0)	6.36(d,2.1)	6.82(d,2.0)
	4	6.40(t,2.0)	6.33(t,2.1)	6.78(t,2.0)
	5			
	6	6.68(d,2.0)	6.36(d,2.1	
-CH_2-	4xH		2.87	2.92(s)
-CH-	α	7.06(d,16.0)		
	β	6.92(d,16.0)		
B	2'	7.10(d,2.0)	6.69(d,2.0)	
	5'	6.88(d,8.0)	6.82(d,8.0)	
	6'	7.07(dd,2.0,8.0)	6.75(dd,2.0,8.0)	
	2',6'			7.18(d,8.5)
	3',5'			7.01(d,8.5)
OMe		3.85-3A,3.92-4'B, 3.97-3'B,3.85-5A (each s)	3.79(x2),3.87, 3.88 (each s)	
OAc				2.29,2.30,2.31 (each s)

8.2 DIHYROSTILBENES

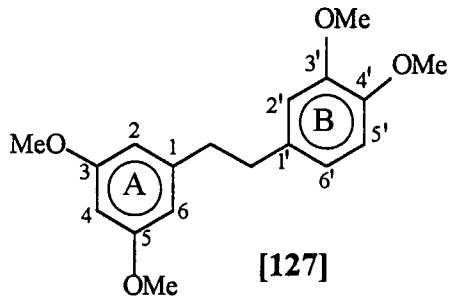
Dihyrostilbenes were found in *Pinus spp.*[129] (only the Haploxylon subgroup) and in *Morus spp.*[130] They are, together with the stilbenes, useful taxonomic markers. It was

noticeable that some of dihyrostilbenes (eg. 2,3'-dihydroxy-5-methoxydihydrostilbene) were identified as plant-growth inhibitors[131]. A natural occurring benzyl derivative, which appear to be biogenetically related to stilbenes, was isolated from Leguminosae species, *Zollernia paraensis*[132], and it contained to 2,4,2'-trihydroxy-4'-methoxybenzyl groups.

Two bibenzyls **127** and **128** were isolated as free phenolic (3,4',5- and 3,3'4',5-) during this study as there were no natural O-methyl groups present after acetylation and separations of the C_7 fraction. The 3,4',5-trihydroxybibenzyl[133] was isolated as 3,4'-dihydroxy-5-methoxybibenzyl and was also synthesized by catalytic hydrogenation of resveratrol with Pd charcoal catalyst[134].

8.2.1 3,3',4'5-TETRAMETHOXYBIBENZYL

Methylation of the C_7 fraction from the acetone extract of the *Cassia abbreviata* heartwood afforded derivative **127.**



[127]

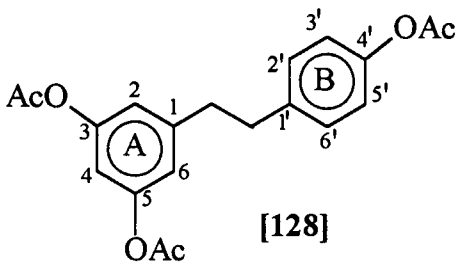
^{1}H NMR spectrum data (table 6, plate 14) revealed two benzylic methylene protons at δ 2.87 and six aromatic protons on the two benzene rings, which represented the characteristic pattern of the bibenzyl[129].

The AX_2 system represented by proton resonances at δ 3.36 (J = 2.1 Hz), δ 6.33 (J = 2.1 Hz) and δ 6.36 (J = 2.1 Hz) was assigned to the A-ring. The B-ring proton resonances were assigned at δ 6.69 (J = 2.0), δ 6.82 (J = 8.0 Hz) and δ 6.75 (J = 2.0, 8.0 Hz).

Methoxy group protons resonance at δ 3.79 were identified as 3- and 5-OMe(A) from their associations with 2-H(A) and 4-H(A) proton resonances during NOE experiments.

8.2.2 3,4',5-TRIACETOXYBIBENZYL

Acetylation of the C_6 fraction from the acetone extract of *Cassia abbreviata* heartwood afforded derivative **128.**



[128]

^{1}H NMR spectrum data (table 6, plate 15) showed two benzylic methylene protons together with an AA'BB' and AX_2 systems for the two aromatic rings.

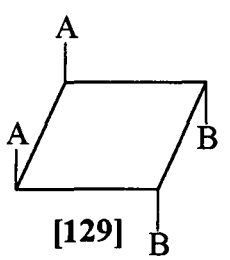
NOE associations of two pairs of methylene protons at δ 2.92 with 2',6'-H(B) δ 7.18 and 2-H(A) δ 6.82 confirmed the bibenzyl structure of this compound. The AA'BB' system was represented by two pairs of proton resonances at δ 7.18 and δ 7.01 (both J = 8.5 Hz) and was confirmed from the coupling tof the proton pair at δ 7.18 to the O-acetyl at δ 2.31.

The two O-acetyls at δ 2.30 showed association with 2-H(A) δ 6.82 and 4-H(A) δ 6.78 which confirmed the 3,5-substitution of the A-ring.

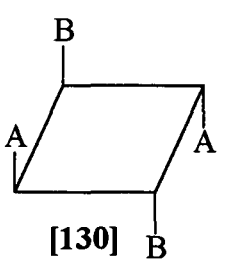
CHAPTER 9

STILBENE CYCLODIMERS

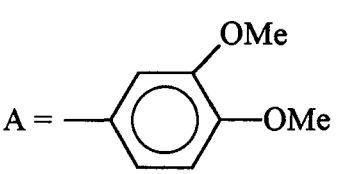
Two compounds, **[129]** and **[130]** were isolated from the acetone extract of *Cassia abbreviata*. The initial ^1H NMR (table 7, plate 16 and 17) reminded very much of a bibenzyl spectrum except that the chemical shifts of the $-CH_2-$ protons were different e.g. δ 2.80-2.85 ($CDCl_3$) for bibenzyls and δ 4.31 ($CDCl_3$) for the unknown compounds respectively. According to the chemical shifts in (table 7) the two compounds were different although closely related and the most obvious difference was the singlet at δ 4.31 for compound **129** as oppose to a multiplet at δ 4.31 for compound **130**.

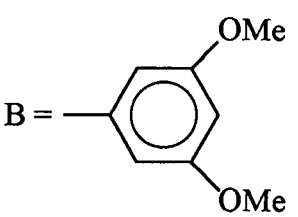


[129]



[130]

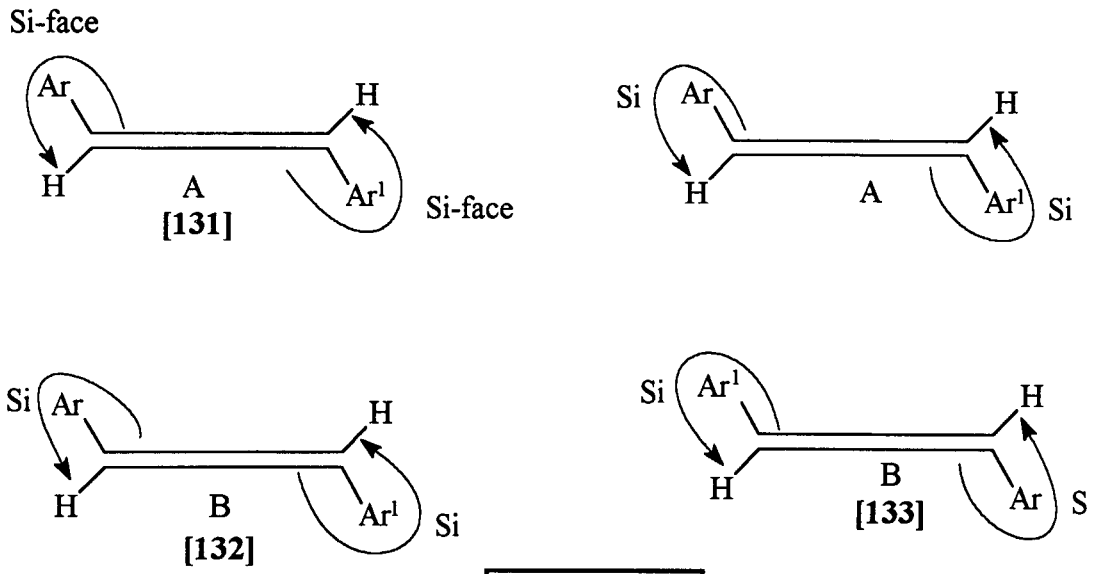




Mass spectral analysis showed accurate masses of m/z 600.27246 and m/z 600.27197 for compounds **129** and **130** respectively, suggesting two dimeric structures. Apart from this information and the difference in R_f values of 0.65 and 0.61 for compound **129** and **130** respectively. There was no other information to facilitate the elucidation of the structures. An attempt to grow crystals of both compounds resulted in tiny crystals not good enough to be used for X-ray diffraction studies.

The monomeric stilbene **[115]** found in the same extract was accepted to be the logic precursor to the dimers **129** and **130**. Photodimerization using the synthesized monomeric stilbene yielded two major compounds with identical ^1H NMR (table 7, plate 16 and 17) as the two dimers, which in fact confirmed their molecular formula of $C_{36}H_{40}O_8$.

According to the midpoint analysis of the AA'BB' cyclobutane protons of compounds **129** and **130** it can be expected to have the same chemical shift at δ4.45-4.49 ppm. The photodimers of the same monomeric stilbene suggested that they occur as indicated in (scheme 9.1).



SCHEME 9.1

The top Si-Si face of A was attacked by the Re-Re face of the B **[131]** resulting in a β-truxinic configuration. The Si-Si face of A was attacked by the Re-Re face of B **[132]**, but in a different orientation by attacking monomer to result in a α-truxillic configuration.

The cyclobutane derivatives (dimeric stilbenes) isolated from *Crotalaria madurensis*[135] were assigned configurations **129** and **130** as the result of the X-ray studies and the ^1H NMR of these compounds showed multiplets at δ 4.29 (NMR solvent not reported) for the cyclobutane protons.

Theoretical calculations made by Ulrich and co-workers[136] in order to determine the fine structure of the cyclobutane proton multiplets, have shown the multiplets to appear in the region of δ 4.40, with a splitting pattern similarly to that of the structure **130**. This finding was confirmed by Ben-Efriam and co-workers[137] by using their mid-point prediction method as well as NMR data obtained from experimental analysis of tetra-arylcyclobutanes.

The cyclobutane proton signal pattern of configuration **129** (table 7, plate 16) was a poorly resolved multiplet (a broad singlet) while the cyclobutane protons of compound **130** (table 7, plate 17) showed fairly well resolved multiplet, both at δ 4.43.

Table 7 ^{1}H NMR (300 MHz, $CDCl_3$) of tetra-arylcyclobutanes [129] and [130]. Splitting patterns and Coupling constants (Hz) in parentheses.

RING	**PROTONS**	**[129]** **[Plate 16]**	**[130]** **[Plate 17]**
A	2	6.51(d,2.0)	6.55(d,2.0)
	5	6.69(d,8.0)	6.71(d,8.0)
	6	6.74(dd,2.0,8.0)	6.77(dd,2.0,8.0)
B	2'	6.32(d,2.0)	6.29(d,2.0)
	4'	6.23(t,2.0)	6.21(t,2.0)
	6'	6.32(d,2.0)	6.29(d,2.0)
CYCLOBUTANE	4XH	4.43(s)	4.43(ddd,5.0,7.0,14.0)
OMe		3.67-3A,3.82-4A, 3.66-3'B,3.66-5'B (each s)	3.68-3A,3.82-4A, 3.66-3'B,3.66-5'B (each s)

Electron-impact MS-analysis was not very convincingly and the only difference was a fragment at m/z 285 (25%) for dimer **129**, which could be ascribed to the higher abundance of the fragment after loss of CH_3 from the A-A cleaved product from the *para* methoxyl groups. From the above information it was possible to assign the structures for compounds **129** and **130** respectively.

9.1 SYNTHESIS OF TETRA-ARYLCYCLOBUTANES FROM THEMONOMERIC STILBENES

Photodimerization is the direct irradiation of monoolefins giving rise to high energy singlets which undergo intersystem crossing[137]. This process is known as [2+2] cycloaddition reaction where the triplet excited olefin react with the ground state olefin[137]. Although photodimers only reported to be synthesized by irradiation of monoolefins either in solution or solid state. It was stated that photodimerization of trans-substituted stilbenes in solid state occurs if the crystal structure parallel double bond spacing is 3.7- 4.2Å[138]. Therefore stilbenes were generally irradiated in solution.

3,3',4',5-Tetramethoxystilbene **115** was irradiated with mercury lamps at 325nm wavelength in different dry solvents in the presence of different sensitizers (Table 9.1) to produce the two required dimers **129** and **130**.

Table 9.1 The results obtained from irradiation of trans-stilbene.

SOLVENT	SENSITIZER	% YIELD
THF		2
THF	0.05M Me_2CO	35
THF	0.05 BENZOPHENONE	25
Me_2CO	0.05 BENZOPHENONE	26
Me_2CO		48

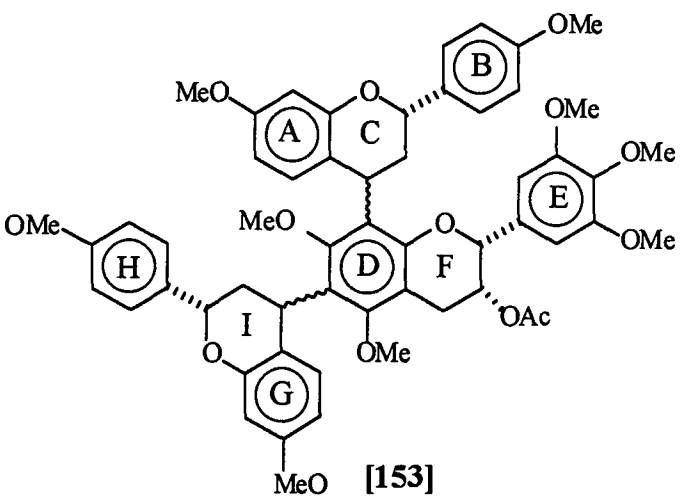
The above results were explained in terms of the excitation of the olefin as the dimers are the products of S_1 state and the T_1 state give rise to the *cis* isomer. The stilbene in THF absorbed the light excited into the S_1 and then to T_1 state that resulted in a very low yield of dimers and higher yield of *cis*-isomer. Whereas the addition of sensitizers dramatically increased the yields as these compounds act as donor by absorbing the light into T_1 state and donating it to the stilbene to and give the dimeric products. High yields also depend on the highest triplet energy of the sensitizer and it must have the total triplet energy of approximately 3 kcal/mol or greater than that of the acceptor. Acetone (78 kcal/mol) has the higher triplet energy than benzophenone (69 kcal/mol) which explained the good yield obtained with acetone (table 9.1)[137,139]. The fact that aliphatic ketones have the higher triplet energies than aromatic ketones therefore they were used to increase the yields of dimers.

CHAPTER 10

INTERFLAVANYL BOND FORMATION IN PROCASSINIDINS

Dimeric proanthocyanidins with flavan chain extender units are relatively rare. The diastereomers of cassiaflavan-epiafzelechins were the first flavan dimers to be isolated from the leaves of *Cassia fistula*[141] and they were revealed in the introduction of the new class procassinidins. Recent additions to this class of oligomeric flavanoids include flavan-flavan-3-ols dimers from *Cassia nomame*[142], a (2S)-4',7,8-trihydroxyflavan-(4β→6)-epioritin-4α-ol from *Acacia caffra*[143] and probutinidins and procassinidins from *Cassia petersiana*[144]. The physiological activity such as lipase- and insect growth inhibition[142] was reported for some of these flavan compounds justify the synthesis of these compounds.

The novel trimer cassiaflavan-epigallocatechin-cassiaflavan was isolated from the acetone extract of the bark of *Cassia petersiana*. With the extensive use of ^{1}H NMR, COSY and NOESY experiments it was possible to put forward a structure **[153]** (table 12) for the trimer but the stereochemistry posed a problem.



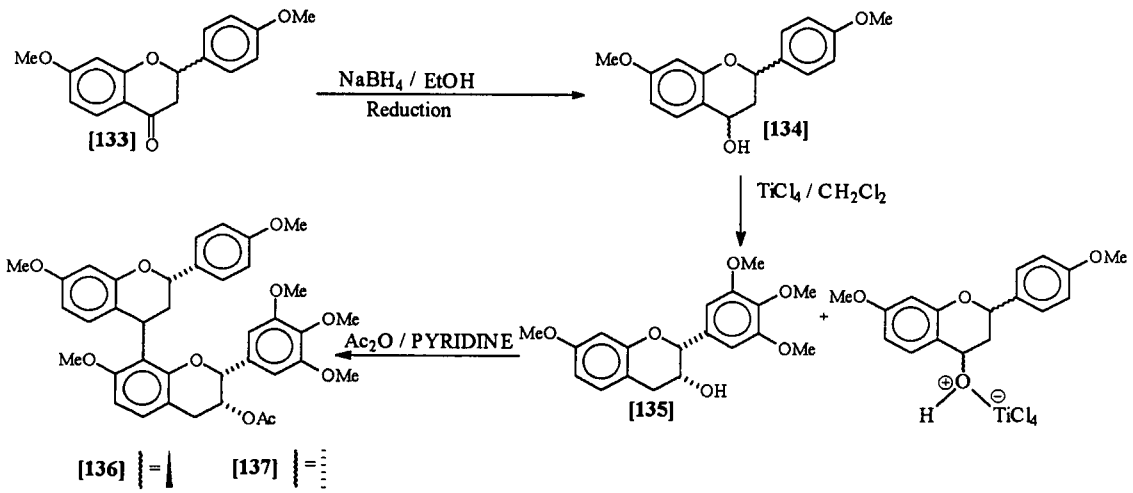
[153]

In the attempt to synthesize compound **[153]** basic compounds were used for the synthesis in conjuntion with and flavanoid monomers (scheme 10.1, 10.2, 10.3). A number of model reactions were conducted (scheme 10.3) to optimize conditions and develop the methodology for the final synthesis.

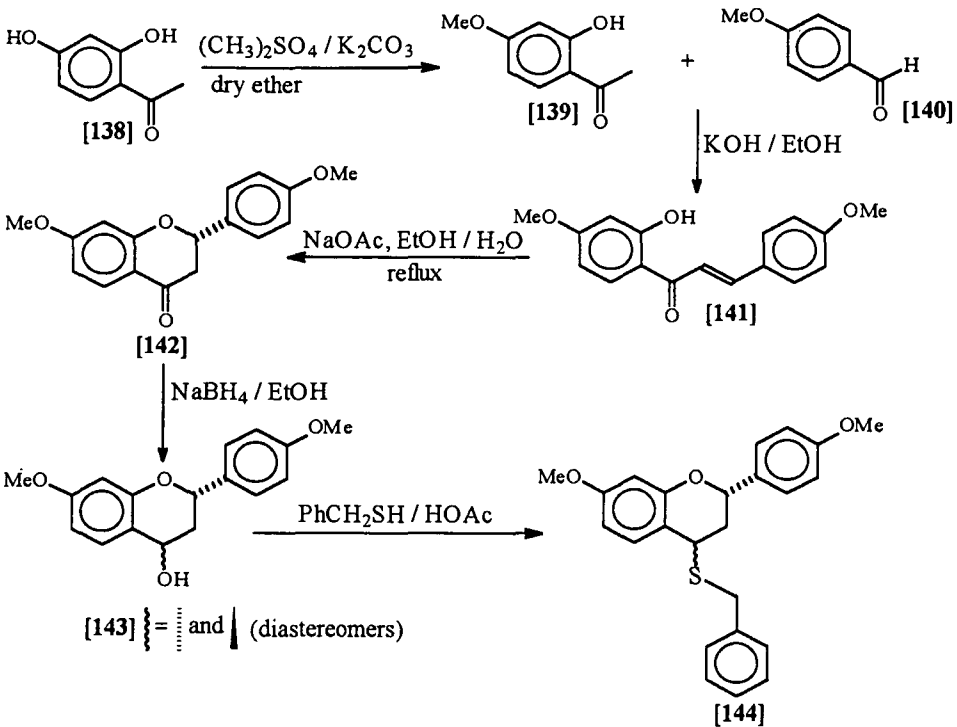
Flavan dimers were synthesized by reduction of the racemic flavanone **[133]** to the diastereomeric flavan-4-ols **[134]** and condensation with epigallocatechin **[135]** using titanium tetrachloride as a Lewis acid to give four diastereomers in a very low yields of which only two of them were successfully purified **[136]** and **[137]** [scheme 10.1][144].

Owing to the fact that these compounds may be of biological interest, optimization of their synthetic yields was justified. The synthesis of flavan dimers where the flavan-4-ol C4-OH group was replaced with benzylmercaptan and condensed with the nucleophile in the presence of $AgBF_4$/THF as Lewis acid was exploited.

The method was reported to be effective regarding regio- and stereoselectivity during polymerization and good yields were obtained for most of the free phenolic procyanidins[145].



SCHEME 10.1

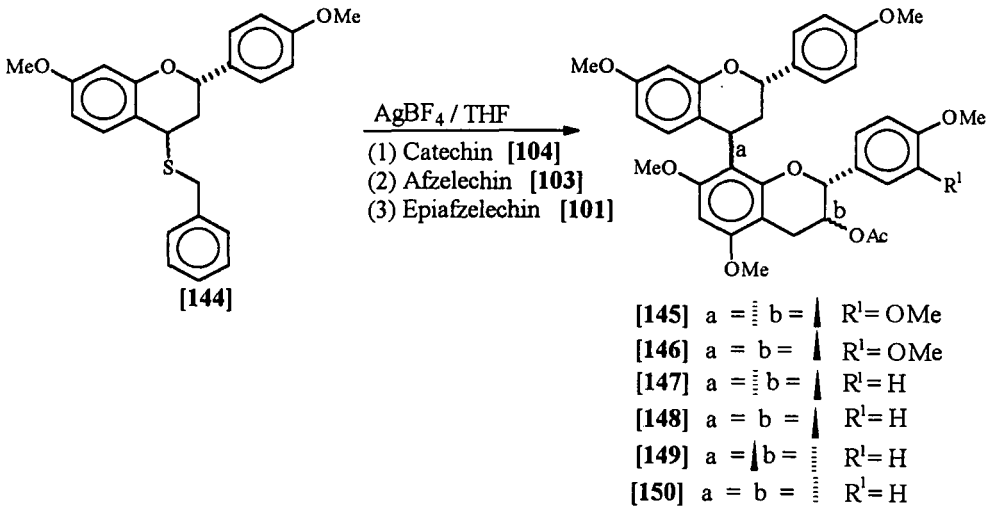


SCHEME 10.2

The monomeric 4',7-dimethoxyflavan-4-ol was synthesized as shown in [scheme 10.2]. 2-hydroxy-4-methoxyacetophenone **[139]** was coupled to 4-methoxybenzaldehyde **[140]** *via* aldol condensation to chalcone **[141]**. The chalcone was cyclized on treatment with sodium acetate in ethanol water to flavanone **[142],** which was later reduced to diastereomeric flavan-4-ol **[143]**.

The monomeric species catechin, afzelechin and epiafzelechin used as nucleophiles in the model reactions were isolated as methyl ethers from the acetone extract of the heartwood of *Cassia abbreviata*. Free phenolic epigallocatechin was obtained from green tea (*Camellia sinensis*).

The proposed synthesis involved the activation of the C4-S bond of the 4',7-dimethoxyflavan-4-thiol towards carbon nucleophiles by the effective thiophilic Lewis acid, $AgBF_4$ to generate the $C(sp)^2$-$C(sp)^3$ bond.



SCHEME 10.3

The flexibility of the method using procassinidins was investigated by carrying out model reactions between the 4-thiobenzyl ether of flavan-4-ol and different nucleophiles with phloroglucinol A-rings such as catechin, afzelechin and epiafzelechin (scheme 10.3). Treatment of a solution 4',7-dimethoxyflavan-4-thiol with each of the above nucleophiles in THF with $AgBF_4$ (2.5eq.) at 65°C overnight, afforded 4β→8 and 4α→8 linked procassinidin dimers. The above procedure was adopted to synthesize the assumed dimeric precursors **136** and **137** for the trimer. From a maximum of four possible diastereomers only two of them were obtained in sufficient quantities for use of NMR spectroscopy to enable the structure elucidation.

Table 8 ^{1}H NMR (300 MHz, C_6D_6) of derivatives **145** and **146** at 343K. Splitting patterns and Coupling constants (Hz) in parentheses.

RING	PROTON	[145] (Plate 21)	[146] (Plate 22)
A	5	7.07(d,8.5)	7.04(d,8.5)
	6	6.60(dd,2.5,8.5)	6.58(dd,2.5,8.5)
	8	6.81(d,2.5)	6.67(d,2.5)
B	2',6'	7.43(d,9.0)	7.41(d,9.0)
	3',5'	6.84(d,9.0)	6.84(d,9.0)
C	2	5.67(dd,3.5,7.0)	5.67(dd,3.5,7.0)
	3	2.54(m)	2.55(m)
	3	2.88(ddd,6.5,7.0,13.5)	2.82(ddd,6.5,7.0,13.5)
	4	5.03(t,7.0)	5.02(t,7.0)
D	6	6.11(s)	6.12(s)
E	2'	6.87(d,2.0)	6.97(d,2.0)
	5'	6.70(d,8.5)	6.71(d,8.5)
	6'	6.80(dd,2.0,8.5)	6.91(dd,2.0,8.5)
F	2	5.06(d,8.0)	4.82(d,8.0)
	3	5.51(m)	5.61(m)
	4	3.02(dd,9.0,17.0)	3.00(dd,9.0,17.0)
	4	3.35(dd,6.0,17.0)	3.38(dd,6.0,17.0)
OMe		3.41,3.45,3.52,3.54, 3.57,3.60 (each s)	3.44,3.46,3.52,3.53, 3.55,3.62 (each s)
OAc		1.66(s)	1.65(s)

The interflavanyl linkage, absolute and relative configurations of the procassinidin derivatives were determined by ^{1}H NMR experiments (tables 8-11). Due to the rotational isomerism about the (4→8) interflavanyl bond the spectra had to be recorded at 70-75°C to ensure the first order characteristics.

The ^{1}H NMR spectra of derivatives **136, 137** and **145-150**, indicated similarly substituted ABC-units comprising AA'BB'- and ABX-systems for the aromatic protons and an AMNX-coupled pattern for the heterocyclic protons of the C-4 substituted flavan moiety[144].

A one-proton aromatic singlet for the D-ring, with an AMXY system [(2-H, J = 8.0 Hz), 3-H, (m *ca* 1.0 Hz) 4-H, (J = 8.0, 17.0 Hz) and (J = 5.5, 17.0 Hz)] heterocyclic system for the F-ring defined the two rings of the bottom unit for derivatives **145-148.** An aromatic ABX [2'-H, (J = 2.0 Hz), 5'-H, (J = 8.5 Hz), 6'-H, (J = 2.0, 8.5 Hz)] spin system was attributed to the E-rings of derivatives **145** and **146.** The E-rings of derivatives **147** and **148** assigned an AA'BB' pattern from the observed two pairs of *ortho*-coupled doublets in their respective spectra as per (table 9).

The lack of NOE associations between 2-H(C) at δ 5.67 and δ5.66 and 4-H(C) at δ 5.02 and δ 5.01 resonances confirmed the 2,4-*trans* configuration of derivative **145** and **148.** The phase sensitive NOESY experiment of derivative **146** and **147** showed association between 2-H(C) at δ 5.67 and 4-H(C) at δ 5.03 and δ 5.02 indicating a 2,4-*cis* configuration of the C-ring.

The singlet in the aromatic region for all the derivatives **145-148** was assigned to 6-H(D) as the result of the observed correlation with the two adjacent methoxy group proton resonances hence the (4→8) interflavanyl linkage was established.

The CD spectra of derivative **146** (plate 22) and **147** (plate 23) exhibited high-negative Cotton effects at $[\theta]_{250}$ −102900 and $[\theta]_{235}$ −174600 which confirmed the 4α configuration at C-4(C). When taken in conjuction with the relative stereochemistry of 2,4-*cis*, the CD data supported the 2S,4S absolute stereochemistry[84,146] of the cassiaflavan ABC-unit.

The large positive Cotton effects at $[\theta]_{250}$ 79800 and $[\theta]_{245}$ 95950 for **145** (plate 21) and **148** (plate 24), respectively, indicated a 4β configuration at C-4(C) for both and together

with the allocated 2,4-*trans* relative stereochemistry confirmed the 2S,4R absolute configuration of the top units of the two compounds.

Table 9 ^{1}H NMR (300 MHz, C_6D_6) of derivative **147** and **148** at 343K. Splitting patterns and Coupling constants (Hz) in parentheses.

RING	PROTON	[147] (Plate 23)	[148] (Plate 24)
A	5	7.06(d,8.5)	7.01(d,8.5)
	6	6.61(dd,2.5,8.5)	6.61(dd,2.5,8.5)
	8	6.81(d,2.5)	6.72(d,2.5)
B	2',6'	7.42(d,9.0)	7.41(d,9.0)
	3',5'	6.83(d,9.0)	6.83(d,9.0)
C	2	5.67(dd,3.5,7.0)	5.66(dd,3.5,7.0)
	3	2.90(m)	2.82(ddd,6.5,7.0,13.5)
	3	(ddd,6.5,7.0,13.5)	(ddd,6.5,7.0,13.5)
	4	5.02(7.0)	5.01(t,7.0)
D	6	6.10(s)	6.12(s)
E	2',6'	7.10(d,9.0)	6.84(d,9.0)
	3',5'	6.82(d,9.0)	6.82(d,9.0)
F	2	5.04(d,8.0)	4.85(d,8.0)
	3	5.45(ddd,6.5.7.0,13.0)	5.58(ddd,6.5,7.0,13.0)
	4	2.99(dd,8.0,18.0)	2.98(dd,8.0,18.0)
	4	3.32(dd,5.5,18.0)	3.34(dd,5.5,18.0)
OMe		3.41,3.45,3.47,3.51,3.55 (each s)	3.42,3.43,3.46,3.52,3.53 (each s)
OAc		1.67(s)	1.67(s)

A proton singlet in the aromatic region was evident for the substituted phloroglucinol in ring the bottom unit of flavan-3-ols and an AMXY spin system at-H, (s),3-H, (m), 4-H, (J = 5.0, 18.0 Hz) and (J = 2.5, 18.0 Hz) confirmed the 2,3-*cis* relative configuration of the heterocyclic F-rings of derivatives **149**, **150**, **136** and **137.** An AA'BB' spin system observed for protons 2',6'-H(E) and 3',5'-H(E) was assigned to the E-ring of the

derivatives **149** and **150.** The E-ring of derivatives **136** and **137** with 2'-H(E) and 6'-H(E) protons assigned at δ 6.63 and δ 6.67 was evident for an A_2 aromatic spin system.

Table 10 ^{1}H NMR (300 MHz, C_6D_6) of derivative **149** and **150** at 343K. Splitting patterns and Coupling constants (Hz) in parentheses.

RING	PROTON	[149] (Plate 25)	[150] (Plate 26)
A	5	7.06(d,8.5)	7.09(d,8.5)
	6	6.57(dd,2.5,8.5)	6.64(dd,2.5,8.5)
	8	6.81(d,2.5)	6.95(d,2.5)
B	2',6'	7.46(d,9.0)	7.42(d,9.0)
	3',5'	6.89(d,9.0)	6.83(d,9.0)
C	2	5.75(dd,3.5,7.0)	5.63(t,7.0)
	3	2.57(m)	2.43(m)
	3	3.00(ddd,6.5,7.0,13.5)	3.03(ddd,6.5,7.0,13.5)
	4	5.07(t,7.0)	5.04(dd,7.0,10.0)
D	6	6.11(s)	6.11(s)
E	2',6'	6.91(d,9.0)	6.91(d,9.0)
	3',5'	6.83(d,9.0)	6.85(d,9.0)
F	2	4.71(s)	4.89(s)
	3	5.64(m)	5.56(m)
	4	3.05(dd,5.0,18.0)	3.03(dd,5.0,18.0)
	4	3.34(dd,2.5,18.0)	3.31(dd,2.5,18.0)
OMe		3.39,3.46,3.50,3.51,3.53 (each s)	3.39,3.46,3.52,3.53,3.59 (each s)
OAc		1.62 (s)	1.73 (s)

Aromatic proton singlets at δ 6.13 and 6.11 for derivatives **136** and **137 149** and **150,** were assigned to 6-H(D) because of NOE associations with the two methoxy group proton resonances of 7-OMe(D) and 5-OMe(D) respectively, and thereby establishing the (4→8) interflavanoid bond.

The phase sensitive NOESY experiment of derivatives **150** and **137** showed associations between 2-H(C) at (δ 5.63, 5.60) and 4-H(C) at (δ 5.04, 5.07), indicating a 2,4-*cis* relative configuration of the C-rings. The conspicuous absence of the NOE associations between 2-H(C) at (δ 5.75, 5.77) and 4-H(C) at (δ 5.07, 5.04) in compounds **149** and **136** strongly suggested a 2,4-*trans* relative configuration for the C-rings.

The CD spectra of derivatives **150** (plate 26) and **137** (plate 28), exhibited a high negative amplitude Cotton effects at $[\theta]_{245}$ −138800 and $[\theta]_{238}$ −213900 which confirmed the 4α configuration at C-4(C). When taken in conjugation with the relative stereochemistry of 2,4-*cis*, the CD data supported the 2S,4S absolute stereochemistry.
The large positive Cotton effects at $[\theta]_{246}$ 73360 and $[\theta]_{244}$ 91870 for compounds **149** (plate 25) and **136** (plate 27) respectively, indicated a 4β configuration at C-4(C) for both and together with the allocated 2,4-*trans* relative stereochemistry confirmed the 2S,4R absolute stereochemistry of the top units for the two compounds.
The preference of 4β products was due to the contribution of 4'-methoxy group in replacement of the 4-thiol group by enhancing the π-bonding from the B-ring and stability of carbocation intermediates therefore the incoming group had to approach *trans* to the B-ring. The (4→8) linkages are also a result of the stable intermediates that allow for the regioselective attack of the nucleophile via C-8 where the HOMO displays maximum amplitude[147].

The excellent control regarding regio- and stereoselectivity during the C4→C8 bond formation and the good yields obtained in the synthesis of procassinidin dimers, prompted an investigation into the expansion of this method towards the synthesis of higher oligomers. In an attempted synthesis of trimer [21] the procassinidin derivatives **136** and **137** were coupled with 4-benzylsulfanylflavan epimers using $AgBF_4$ in THF, but resulted only in the starting materials, therefore the method was unsuccessful. The reason could be that the methyl ether derivatives are poor nucleophiles due to the bulkiness of the O-methyls. During a second synthesis the procassinidin derivatives with the bottom unit in the free phenolic state were successfully obtained from benzylsulfanylflavan and

the free phenolic gallocatechin in THF and $AgBF_4$. The reaction was carried out at 0°C for 1 hour to give the derivatives **151** and **152**.

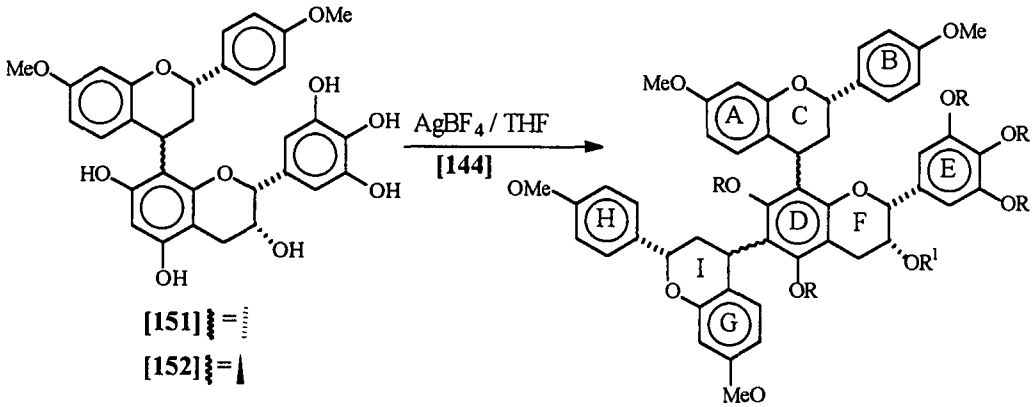
Table 11 ^1H NMR (300 MHz, C_6D_6) of derivatives **136** and **137** at 348K. Splitting patterns and Coupling constants (Hz) in parentheses.

RING	PROTON	[136] (Plate 27]	[137] (Plate 28)
A	5	7.02(d,8.5)	6.98(d,8.5)
	6	6.52(dd,2.5,8.5)	6.52(dd,2.5,8.5)
	8	6.68(d,2.5)	6.87(d,2.5)
B	2',6'	7.45(d,9.0)	7.40(d,9.0)
	3',5'	6.86(d,9.0)	6.86(d,9.0)
C	2	5.77(dd,3.5,7.0)	5.60(dd,4.0,11.5)
	3	2.54(ddd,6.5,7.0,13.5)	2.54(m)
	3	2.93(ddd,6.5,7.0,13.5)	3.00(ddd,6.5,7.0,13.5)
	4	5.04(7.0)	5.07(7.0)
D	6	6.13(s)	6.13(s)
E	2'	6.67(s)	6.63(s)
	6'	6.67(s)	6.63(s)
F	2	4.60(br s)	4.94(br s)
	3	5.58(m)	5.60(m)
	4	3.11(dd,5.0,18.0)	3.08(dd,5.0,18.0)
	4	3.31(dd,2.5,18.0)	3.32(dd,2.5,18.0)
OMe		3.44,3.48,3.50,3.55, 3.69,3.70,3.93 (each s)	3.38,3.48,3.54,3.56, 3.61,3.62,3.91 (each s)
OAc		1.65(s)	1.57(s)

Methylation and acetylation of compounds **151** and **152** gave the derivatives **136** and **137**, which were obtained in higher yields compared with the previous coupling (100% more). The experiment to couple the procassinidin dimers **151** and **152** with 4-

benzylsulfanylflavan using $AgBF_4$ in THF starting from 0°C and raising the temperature to 65°C was unsuccessful to produce **153**.

An alternative attempt to synthesize the trimer from coupling the procassinidin dimers **151** and **152** with 4',7-dimethoxyflavan-4-ol using 0.1M HCl, was also unsuccessful. This method was used quite successfully by Roux and co-workers.



[151] ⋮ =

[152] ⋮ =

[153] R = Me, R^1 = Ac

[154] R = R^1 = H

SCHEME 10.4

During the acid catalysed synthesis the acid was first used with methanol as the solvent but tended to methylate the derivatives **151** and **152** thereby hindering the coupling. reaction. Another attempt where acid in dichloromethane as solvent was used was also unsuccessful.

After all the attempted procedures failed we then concluded that the (4→8)-coupled analogues were the most preferred dimers as compared with the (4→6) procassinidins, as manifested from the many unsuccessful experiments conducted.

Table 12 ^{1}H NMR (300 MHz, C_6D_6) of cassiaflavan-epigallocatechin-cassiaflavan at 353K. Splitting patterns and Coupling constants (Hz) in parentheses.

RING	PROTON	[153] (Plate 29)
A	5	6.92(d,8.5)
	6	6.49(dd,2.5,8.5)
	8	6.61(d,2.5)
B	2',6'	7.41(d,7.0)
	3',5'	6.87(d,7.0)
C	2	5.76(dd,3.5,6.0)
	3	2.42(m)
	3	3.03(ddd,8.5,8.5,13.5)
	4	4.69(dd,5.5,11.0)
E	2	6.64(d,1.0)
	6	6.64(d,1.0)
F	2	4.53(s)
	3	5.57(m)
	4	3.12(d,5.0)
G	5	6.96(d,8.5)
	6	6.54(dd,2.5,8.5)
	8	6.93(d,2.5)
H	2',6'	7.38(d,7.0)
	3',5'	6.83(d,7.0)
I	2	5.71(dd,3.5,6.0)
	3	2.49(m)
	3	3.13(ddd,7.5,10.0,13.5)
	4	4.72(dd,5.5,7.0)
OMe		3.27,3.29,3,45,3.46,3.47,3.57, 3.77,3.78,3.94 (each s)
OAc		1.67 (s)

EXPERIMENTAL

CHAPTER 11

STANDARD EXPERIMENTAL PROCEDURE

The following standard experimental techniques as detailed below will be briefly referred to in subsequent chapters.

11.1 CHROMATOGRAPHIC METHODS

11.1.1 COLUMN CHROMATOGRAPHY

Three sizes of glass columns were available, with dimensions of 33 x 900 50 x 300 and 50 x 1200 mm. These will be referred to small (L), medium (M) and large (L) columns respectively.

11.1.1.1 SILICA GEL AS ABSORBENT

A column was packed by adding a slurry of Merck Kieselgel Art 7734 (170-230 mesh) to the column. The slurry was prepared by using the same solvent as used for the eluent. The column was vibrated with its tap open to ensure dense, efficient packing free of the air bubbles. The ratio of material to be separated to silica gel was 1:20. The material was first absorbed onto a small quantity of silica gel and then transferred to the top of the column. An eluent rate of 30 cm^3 per 30 minutes was used, and fractions were collected in test tubes.

11.1.1.2 LH-20 AS ABSORBENT

An ethanol slurry of LH-20 was allowed to stand for 24 hours. The slurry was subsequently poured into the column with the tap left open to ensure compact packing. The ratio of material to be separated to LH-20 resin was 1:25. The material was dissolved

in minimum ethanol and loaded in the column. Fractions were collected in test tubes at the elution rate of 30 cm^3 per 30 minutes.

11.1.2 THIN LAYER CHROMATOGRAPHY (TLC)

Preparative thin layer chromatography (PLC) plates were prepared by uniformly spreading (2 mm) slurry of 200 g of Merck Kieselgel (Art 7747) silica gel in 475 cm^3 of water over 200 x 200 mm glass plates. The plates were dried and the heated at 80°C for 24 hours before being used. The loading of material to be separated was 10 to 25 mg per plate. A separating tank was set up with the appropriate solvent and the chromatogram developed therein. After development the plates were dried and examined under the ultraviolet light (λ 254 and 360 nm). The relevant bands of compounds were marked and scraped off. Acetone was used to extract the compounds from the scraped silica gel. The acetone was removed from under reduced pressure and the residue dried in a vacuum oven.

Micro-separations were carried out on commercially available aluminium backed silica gel plates (Merck Art 5554) with a loading of 3-5 mg per plate.

11.1.2.1 FORMALDEHYDE-SULPHURIC ACID SPRAY[88]

All TLC plates were sprayed lightly with a 2% (v/v) solution of formaldehyde (40%) in concentrated sulphuric acid and subsequently heated to ensure optimum colour development.

11.2 PECTROSCOPIC METHODS

11.2.1 NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY

^1H NMR, NOESY, COSY, HOMODEC and ^{13}C NMR experiments were recorded on a 300 MHz Bruker DRX 300 MHz spectrometer at 296K. Solvents used were $CDCl_3$, acetone-d_6 and benzene-d_6.

Chemical shifts are given in parts per million (ppm) on the delta (δ) scale and coupling constants (*J*) are accurate to 0,1 Hz.

The abbreviations used:

s	=	singlet
d	=	doublet
dd	=	doublet of doublet
t	=	triplet
q	=	quartet
m	=	multiplet
b	=	broad

11.2.2 MASS SPECTROSCOPY

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

11.2.3 CIRCULAR DICHROISM (CD)

The spectropolarimeter used was a Jasco J-710 and the CD-chromatograms were run in methanol.

11.3 CHEMICAL METHODS

11.3.1 ACETYLATION WITH ACETIC ANHYDRIDE[148]

The completely dried material was dissolved in the minimum amount of pyridine and an excess of acetic anhydride was added. The solution was heated at about 60°C for 8 hours and then poured over crushed ice to precipitate the acetate derivative. The precipitate was

filtered, washed free of excess pyridine and acetic anhydride with ice water, dried in a vacuum oven.

11.3.2 METHYLATION WITH DIAZOMETHANE[149]

Dried phenolic material (150-200 mg) was dissolved in methanol (50 cm^3) and the solution cooled to -10°C in an ice-salt bath. Diazomethane generated by the reaction of KOH (8 g) in ethanol (48 cm^3) and water (2 cm^3) with N-methyl-N-nitroso-p-toluene sulfonamide (diazald, 10 g) in ether under mild refluxing, was transferred directly (by distillation) into the pre-prepared phenolic solution. The reaction mixture was left in a deep freeze at -10°C for 48 hours. The excess diazomethane was evaporated in a fume cupboard at room temperature.

11.4 ABBREVIATIONS

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

A/Me_2CO	=	acetone
Ac	=	acetyl
B	=	benzene
DCE	=	dichloroethane
EtOAc	=	ethyl acetate
EtOH	=	ethanol
MeOH	=	methanol
Me	=	methyl
H	=	hexane
OH	=	hydroxyl
OMe	=	methoxyl

CHAPTER 12

ISOLATION OF METABOLITES FROM CASSIA ABBREVIATA

12.1 EXTRACTION OF THE HEARTWOOD

Heartwood drillings (2.7 kg) were repeatedly extracted with acetone (3 x 2.5 ℓ) for 24h periods at room temperature (25°C). The extract was concentrated by evaporation under vacuum at 35°C. The concentrate was dissolved in water and then freeze dried to give a pale-brown powder (340.0 g).

12.2 SEPARATION

Two portions (25 g) of the acetone were separated on Sephadex LH-20/EtOH columns (5 x 160cm) with flow rate of 1 ml/min, 32minutes fractions.

The fractions from column A and B were combined as follows: C_1 (tubes 17-28, 594.9 mg), C_2 (29-40, 126 mg), C_3 (48-57, 232 mg), C_4 (69-77, 144 mg), C_5 (78-86, 132 mg), C_6 (91-103, 400 mg), C_7 (109-133, 2.27 g), C_8 (134-144, 1.34 g), C_9 (145-154, 3.67 g), C_{10} (155-189, 7.34 g), C_{11} (190-207, 1.69 g), C_{12} (208-237, 3.34 g), C_{13} (238-288, 2.33 g), C_{14} (289-341, 2.75 g), C_{15} (342-376, 508 mg), C_{16} (377-404, 432 mg), C_{17} (405-442, 615 mg), C_{18} (443-471, 235 mg), C_{19} (472-940, 614 mg) and C_{20} (942-1500, 802 mg). Only fraction C_6-C_{12} will be discussed here.

12.2.1 Methylation of a PLC purified portion (200mg) [R_f 0.64, B:A:MeOH (6:3:1) of fraction C_6 followed by PLC separation in B:A (9:1 x1) afforded four bands [R_f 0.91 (4.7 mg), 0.71 (7.0 mg) 0.54 (10.4 mg), 0.42 (13 mg).

12.2.1.1 *Guibourtinidol*

Acetylation of R_f 0.42 (13 mg) band and purification in pure benzene yielded the 4',7-di-O-methyl-3-acetyl derivative **106**, R_f 0.22 (12.3 mg). The remaining portion of fraction C_6 was acetylated and purified by PLC in B:A (96:4, x2) to afford a main band at R_f 0.54 (5.8 mg) which gave the full acetate of guibourtinidol **107**. The acetylated compound was hydrolyzed in 1% KOH and MeOH for 5 min. at reflux temperature to give free phenolic compound **105**, R_f 0.46 (5.7 mg).

^{1}H NMR data: plate 5a,b, table 2

^{13}C NMR data: plate 5b, table 2

CD data: plate 5a

12.2.1.2 *3,4',5-triacetoxybibenzyl*

Acetylation of the remaining fraction C_6 and purification in DCE:A (96:4 x2) yielded derivative **128,** R_f 0.55 (1.1 mg)

^{1}H NMR data: plate 15, table 6

12.2.2 Methylation of a PLC purified portion (200 mg) [R_f 0.59, B:A:MeOH (6:3:1)] of fraction C_7 followed by PLC separation in B:A (96:4 x1) afforded three bands [R_f 0.43 (4.3 mg), 0.22 (48 mg) and 0.13 (4.7 mg).

12.2.2.1 3,3',4',5-*tetramethoxystilbene*

Methylation of C_{10} form the acetone extract of heartwood of *Cassia abbreviata* afforded the tetramethyl ether stilbene derivative **127,** R_f 0.43 (4.3 mg).

^{1}H NMR data: plate 14, table 6

12.2.2.2 *Afzelechin*

Acetylation of R_f 0.22 (48 mg) band and purification in pure benzene yielded the 4',5,7-tri-O-methyl-3-O-acetyl derivative **103**, R_f 0.64 (35,8 mg).

^{1}H NMR data: plate 3, table 1

CD data: plate 3

12.2.2.3 *Epiafzelechin*

Acetylation of R_f 0.22 (48 mg) band and purification in pure benzene yielded the 4',5,7-tri-O-methyl-3-O-acetyl derivative **101**, R_f 0.49 (1.9 mg).

^{1}H NMR data: plate 1, table 1

CD data: plate 1

12.2.3. Methylation of a PLC purified portion (200 mg) [R_f B:A:MeOH (6:3:1)] of fraction C_8 followed by purification by PLC separation in B:A (9:1 x2) afforded seven bands [R_f 0.9 (14.9 mg), 0.58 (2.7 mg), 0.43 (3.9 mg), 0.41 (4.3 mg), 0.29 (21 mg), 0.2 (8.8 mg), 0.11 (6.5 mg).

12.2.3.1 *Ent-Guibourtinidol-(4β→8)-afzelechin*

Acetylation of R_f 0.29 (21 mg) band and purification in $CHCl_3$:ether (98:2 x2) yielded the acetate derivative **113**, R_f 0.96 (4.5 mg).

^{1}H NMR data: plate 11, table 5

CD data: plate 11

12.2.3.2 *Guibourtinidol-(4β→8)-epiafzelechin*

Acetylation of R_f 0.29 (21 mg) band and purification in $CHCl_3$:ether (98:2 x2) yielded the acetate derivative **111**, R_f 0.93 (7.5 mg).

^{1}H NMR data: plate 9, table 4

CD data: plate 9

The remaining bands still comprised of mixtures and were not further investigated.

12.2.4. Methylation of a PLC purified portion (200 mg) [R_f 0.40 B:A:MeOH (6:3:1)] of fraction C_9 followed by purification by PLC separation in B:A (9:1 x1) afforded eight bands [R_f 0.89 (5.5 mg), 0.63 (6.8 mg), 0.57 (6.6 mg), 0.42 (10.4 mg), 0.5 (25 mg). 0.38 (23.6 mg), 0.21 (59.9 mg), 0.11 (9.2 mg).

12.2.4.1 *Catechin*

Acetylation of R_f 0.5 (25 mg) band and purification in toluene-methylketone (9:1 x2) yielded the permethylaryl ether acetate **104**, R_f 0.62 (20.8 mg).

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

CD data: plate 4

12.2.4.2 *Epicatechin*

Acetylation of R_f 0.5 (25 mg) band and purification in toluene-methylketone (9:1 x2) yielded the permethylaryl ether acetate **102**, R_f 0.55 (4.0 mg).

^{1}H NMR data: plate 2, table 1

CD data: plate 2

12.2.4.3 *Guibourtinidol-(4α→8)-afzelechin*

Acetylation of R_f 0.21 (59.9 mg) and purification in B:A (96:4 x3) afforded R_f 0.57 (7.6 mg) band which was further purified in H:A:EOAc (65:22:13 x2) to yield acetate derivative **112**, R_f 0.63(5.6 mg).

^{1}H NMR data: plate 10, table 5

CD data: plate 10

12.2.4.1 *Guibourtinidol –(4α→8)-epiafzelechin*

Acetylation of R_f 0.21 (59.9 mg) and purification in B:A (96:4 x3) afforded R_f 0.43 (36.2mg) band which was further purified in H:A:EOAc (65:22:13 x2) to yield acetate derivative **110**, R_f 0.58 (16.3 mg).

^{1}H NMR data: plate 8, table 4
CD data: plate 8

The remaining bands were containing small quantities and mixtures of proanthocyanidins.

12.2.5 Methylation of a PLC purified portion (200 mg) [R_f 0.65 B:A:MeOH (6:3:1)] of fraction C_{10} followed by purification by PLC separation in B:A (9:1 x1) afforded six bands [R_f 0.79 (74.7 mg), 0.53 (6.7 mg), 0.43 (11.0 mg), 0.34 (11.4 mg), 0.2 (21.1 mg), 0.14 (20.9 mg).

12.2.5.1 *3,3',4',5-tetramethoxystilbene*

Acetylation of R_f 0.79 (74.7 mg) and purification in B:A (96:4 x1) yielded derivative **115**, R_f 0.75 (39.6 mg).

^{1}H NMR data: plate 14, table 6

12.2.5.2. *cis-Tetra-arylcyclobutane*

Acetylation of R_f 0.43 (11.0 mg) and purification in B:A (9:1 x2) yielded derivative **130**, R_f 0.65 (3.2 mg).

^{1}H NMR data: plate 17, table 7
^{13}C NMR data: plate 17

12.2.5.3 *trans-Tetra-arylcyclobutane*

Acetylation of R_f 0.43 (11.0 mg) and purification in B:A (9:1 x2) yielded derivative**129**, R_f 0.61 (2.9 mg).

^{1}H NMR data: plate 16, table 7
^{13}C NMR data: plate 17

12.2.5.4 *Guibourtinidol-(4α→8)-catechin*

Acetylation of R_f 0.2 (22.1mg) band and purification in B:A (96:4 x1) afforded R_f 0.53 (1.3 mg) band which was further purified in H:A:EOAc (65:22:13 x2) to yield the acetate derivative **108**, R_f 0.67 (1.1 mg).

^{1}H NMR data: plate 6, table 3
CD data: plate 6

12.2.6 Methylation of a PLC purified portion (200 mg) [R_f 0.41 B:A:MeOH (6:3:1)] of fraction C_{12} followed by purification by PLC separation in B:A (9:1 x2) afforded three bands [R_f 0.43 (11.3 mg), 0.37 (9.4 mg), 0.15 (51.4 mg)].

12.2.6.1 *Guibourtinidol-(4α→8)-epicatechin*

Acetylation of R_f 0.15 (51.4 mg) band and purification in dichloroethane-acetone (96:4 x3) yielded the acetate derivative **109**, R_f 0.32 (3.3 mg).

^{1}H NMR data: plate 7, table 3
CD data: plate 7

12.2.7. Methylation of a PLC purified portion (200 mg) [R_f B:A:MeOH (6:3:1)] of fraction C_{13} followed by purification by PLC separation in B:A (9:1 x2) afforded six bands [R_f 0.64 (4.7 mg), 0.57 (2 mg), 0.34 (7.9 mg). 0.29 (10.2 mg), 0.2 (10.0 mg), 0.11 (34 mg)].

12.2.7.1 *Guibourtinidol-(4α→6)-afzelechin*

Acetylation of R_f 0.11 (34 mg) band and purification in B:A (9:1 x1) yielded acetate derivative **114**, R_f 0.5 (1.2 mg).

^{1}H NMR data: plate 12, table 5

CD data: plate 12

CHAPTER 13

SYNTHESIS OF MONOMERIC AND OLIGOMERIC STILBENES

The isolated tetrahydroxystilbene and two dimeric stilbenes were synthesized in order to elucidate the structure of these compounds. Different methods were reported for the synthesis of monomeric stilbenes of which Witting reaction found to be the most convenient method due to the availability of the starting material. The presence of monomeric stilbene in large quantities enabled the photosynthesis of the stilbene dimers.

13.1 FORMATION OF 3,5-DIMETHOXYBENZYL PHOSPHONATE SALT

13.1.1 *Methyl-3,5-Dimethoxybenzoate* [117]

A methanol solution containing 3,5-dihydroxybenzoic acid (15 g) was methylated by diazomethane (see Chpt. 11 section 11.3.2 for procedure) for an hour.

^{1}H NMR data: δ 7.18 (2-H, d, J = 2.5 Hz), δ 6.64 (4-H, t, J = 2.5 Hz), δ 3.82 and 3.91 2x OCH_3

13.1.2 *3,5-Dimethoxybenzyl alcohol* [118]

An ether solution (25 ml) of 3,5-dimethoxybenzoate (13 g, 66.3 mmol) was added dropwise to (2.7 g) lithium aluminium hydride in (75 ml) dry ether and the reaction mixture was stirred at room temperature for an hour. Water was added slowly to decompose the excess lithium aluminium hydride. Removal of organic phase and extraction of acidified aqueous layer with ether (3x 30 ml) then drying with Na_2SO_4 and removal of the solvent gave the crude compound as the yellow oil (12.2 g, 94%) of 3,5-dimethoxybenzyl alcohol M.p. 44-47°.

^{1}H NMR data: δ 6.50 (2-H, d, J = 2.5 Hz), δ 6.37 (4-H, t, J = 2.5 Hz), δ 6.50 (6-H, d, J = 2.5 Hz), δ 4.59, (-CH_2-, s, *ca* 1.0 Hz), δ 3.77 2 x OCH_3.

13.1.3 *3,5-Dimethoxybenzyl bromide* [119]

To a solution of 3,5-dimethoxybenzyl alcohol (12.2 g) in dry THF (20 ml), was added 1.2 eq. of PBr_3 and the mixture was stirred at room temperature for an hour. Addition of water and extraction with ether (3 x 30 ml) gave the crude products after drying (Na_2SO_4) and evaporation of the solvent.

^{1}H NMR data: δ 6.57 (2-H, d, J = 2.5 Hz), δ 6.42 (4-H, t, J = 2.5 Hz), δ 6.57 (6-H, d, J = 2.5 Hz), δ 4.44, (-CH_2-, s), δ3 .81 2 x OCH_3

13.1.4 *3,5-Dimethoxy-diethyl-benzylphosphonate* [120]

To a solution of 3,5-dimethoxybenzyl bromide (10.64 g, 46.7 mmol) in dry acetonitrile triethyl phoshite (1.2 *eq.*) was added and the mixture was refluxed for an hour at the boiling temperature of the solvent. The cooled mixture was filtered and the precipitate dried.

A solution of 3,5-dimethoxybenzyl bromide (10.64 g, 46.7 mmol) in excess triethyl phosphite (1.2 *eq.*) was heated to 130°C until the evolution of ethyl bromide had ceased. Excess triethyl phosphite was removed by vacuum distillation.

13.2 SYNTHESIS OF MONOMERIC STILBENE

Scheme 8.1

13.2.1 *3,3',4',5-Tetramethoxystilbene*

A mixture of 3,5-dimethoxy-triethyl-benzylphosphonate (8 g, 20,3 mmol) and dimethylformamide (20 ml) containing sodium methoxide (2 g) was cooled to 0°C for 30 minutes. This was followed by addition of 3,4-dimethoxybenzaldehyde (4.01 g) to the mixture and refluxing for an hour at room temperature. The reaction mixture was then heated on the steam bath (50°C) for 2 hours and allowed to stand overnight at room

temperature. A quantity of water (20 ml) was added and the product extracted with ether (3 x 30 ml). Drying of the extract (Na_2SO_4) followed by evaporation and flash column chromatography, gave the pure stilbene (6.28 g).

^{1}H NMR data: same as that of derivative **115.**

13.3 SYNTHESIS OF CYCLOBUTANE STILBENE DERIVATIVES

13.3.1 *Tetra-arylcyclobutanes*

A concentrated solution of 3,3',4',5-tetramethoxystilbene (500 mg) in dry acetone was irradiated overnight in a 250ml water-cooled quartz flask using mercury lamps of 325 nm wavelength. After disappearance of trans-stilbene the mixture was purified by PLC (B:A, 9:1 x2, v/v) into two pure dimers,.

^{1}H NMR data: same as those of derivative **129** and **130** respectively.

CHAPTER 14

FORMATION OF C-C INTERFLAVANYL BOND IN PROCASSINIDINS

14.1 SYNTHESIS OF FLAVAN-4-OL

14.1.1 *2-hydroxy-4-methoxyacetophenone*

A solution of 2,4-dihydroxyacetophenone (10 g) and anhydrous potassium carbonate (10 g) in dry acetone (150 ml) was stirred. Dimethylsulphate was added dropwise and the mixture was refluxed for 4 hours at 60°C. The excess K_2CO_3 was removed by filtration, rinsed with dry acetone and the filtrate dried. Evaporation of the solvent under reduced pressure followed by flash column chromatography gave an oily product, which was crystallized from ethanol to give pure 2-hydroxy-4-methoxyacetophenone (10.4567 g).

^{1}H NMR data: δ 2.57 (-CH_3-, s), δ 6.43 (3-H, d, J = 2.5 Hz), δ 6.46 (5-H, dd, J = 2.5, 9.0 Hz), δ 3.85 (1 x OCH_3, s).

14.1.2 *4,4'-dimethoxy-2-hydroxychalcone*

To a solution of 2-hydroxy-4-methoxyacetophenone (10.4567 g) in EtOH (50 ml) a 50% (m/v) *aq* KOH (0.4 ml/mmol) was added and the mixture was stirred at room temperature for 30-40 minutes. The excess 4-methoxybenzaldehyde (1.2 *eq* in EtOH) was added dropwise and the reaction and followed by TLC. After the disappearance of acetophenone (24 hours), H_2O (40 ml) was added and the products extracted with ether (4 x 40 ml). Drying of extracts (Na_2SO_4) followed by evaporation and the flash column chromatography, gave the crude product, which was crystallized from EtOH to yellow crystals of pure chalcone and a 70% yield.

^{1}H NMR data: δ 7.68 (2,6-H, d, J = 9.0 Hz), δ 6.97 (3,5-H, d, J = 9.0 Hz), δ 6.49 (3'-H, d, J = 2.5 Hz), δ 6.52 (5'-H, dd, J = 2.5, 8.5 Hz), δ 7.85 (6'-H, d, J = 8.5 Hz), δ 7.48 (Hα, d, J = 16.0 Hz), δ 7.87 (Hβ, d, J = 16.0 Hz), δ3 .89 (2 x OCH_3, s).

14.1.3 *4',7-dimethoxyflavanone*

To an ethanol:H_2O (10:1) solution of 4,4'-dimethoxy-2-hydroxychalcone (7.3456 g) was added NaOAc and the mixture was refluxed for 12 hours at temperature. After cooling the excess NaOAc was precipitated by acetone and filtered. The filtrate was evaporated under reduced pressure followed by flash column chromatography to yield a product of 4.278 g.

^{1}H NMR data: δ 7.88 (5-H, d, J = 8.5 Hz), δ 6.50 (8-H, d, J = 2.5 Hz), δ 6.63 (6-H, J = 2.5, 8.5 Hz), δ 7.42 (2',6'-H, d, J = 8.5 Hz), δ 5.43(2-H, dd, J = 3.0, 13.0 Hz), δ 2.81(3-H, dd, J = 3.0, 12.0 Hz), δ 3.08 (3-H, dd, J = 12.0, 13.0 Hz), δ 3.54 and δ 3.85 (2 x OMe, s).

14.1.4 *4',7-dimethoxyflavan-4-ol*

A methanol solution of $NaBH_4$ (1.4 g) was added dropwise to the solution of 4',7-dimethoxyflavanone (4.278 g) in ethanol (20 ml) and the reaction mixture was stirred at room temperature for 12 hours. Addition of water (80 ml), acidification by *aq* HCl followed by removal of organic phase and extraction of the aqueous layer with ethyl acetate (3 x 80 ml) gave the crude product after drying (Na_2SO_4) and evaporation of the solvent. Flash chromatography yielded the pure product.

^{1}H NMR data: δ 5.12 (2-H, dd, J = 3.0, 13.0 Hz), δ 2.15 (3-H, ddd, J = 11.5, 11.5, 13.0 Hz), δ 2.48 (3-H, ddd, J = 3.0, 6.5, 13.0 Hz), δ 5.05 (4-H, br m), δ 7.42 (5-H, d, J = 8.5 Hz), δ 6.59 (6-H, dd, J = 2.5, 8.5 Hz), δ 6.49 (8-H, d, J = 2.5 Hz), δ 7.38 (2',6'-H, d, J = 8.5 Hz), δ 6.95 (3',5'-H, d, J = 8.5 Hz), δ 3.78 and δ 3.84 (2 x OMe, s).

14.1.5 *4-benzylsulfanylflavan*

A solution of flavan-4-ol (200 mg) in EtOH (10 ml) was purged with N_2 for 1 hour. Phenylmethanethiol (0.4 ml) was added and the mixture was cooled to 0°C under inert atmosphere. And 50% HOAc (10 ml) was added to the cooled solution followed by refluxing for 12 hours at 70°C.

After addition of water (70 ml), the excess phenylmercaptan was washed out with hexane (2x15 ml), the aqueous layer was extracted with EtOAc (3x50 ml), the organic layers

combined, dried (Na_2SO_4), evaporated to dryness and separated by PLC in benzene to give 4-benzylsulfanylflavan (100 mg, 50% yield).

14.2 GENERAL PROCEDURE FOR THE SYNTHESIS OF PROCASSINIDINS

A solution of 4-benzylsulfanylflavan (60 mg) and methyl ether flavan-3-ol (3 *eq*) in THF was treated with solid $AgBF_4$ (2.5 *eq*) and refluxed at 65°C for 12 hours. Water (30 ml) was added followed by extraction with EtOAc (4x30 ml), drying (Na_2SO_4) and evaporating under reduced pressure gave mixture of products.

14.2.1 CONDENSATION OF 4',7-DIMETHOXYFLAVAN-4-THIOL AND PERMETHYL ETHER OF CATECHIN.

The product mixture was resolved by preparative TLC in H:A:EA (65:20:15) gave two bands at R_f 0.57 (37.8 mg) and 0.39 (34.7mg). The band of R_f 0.57 (37.8 mg) was the starting material.

14.2.1.1 *Cassiaflavan-(4β-8)-catechin*

Acetylation of R_f 0.39 (34.7 mg) band and purification in H:A:EA (65:20:15 x 4) afforded cassiaflavan-(4β→8)-catechin **146** R_f 0.74 (8.6 mg).

^{1}H NMR data: plate 22, table 8
CD data: plate 22

14. 2.1.2 *Cassiaflavan-(4α-8)-catechin*

Acetylation of R_f 0.39 (34.7 mg) band and purification in H:A:EA (65:20:15 x 4) afforded cassiaflavan-(4β→8)-catechin **145**,R_f 0.71 (12.3 mg).

^{1}H NMR data: plate 21, table 8

CD data: plate 21

14.2.2 CONDENSATION OF 4',7-DIMETHOXYFLAVAN-4-THIOL AND PERMETHYL ETHER OF AFZELECHIN.

The product mixture was separated into two bands by preparative TLC in H:A:EA (65:20:15) eg. R_f 0.54 (61.3 mg) and 0.35 (41.5 mg). The first band (61.3 mg) was afzelechin and the band of R_f 0.35 (41.5 mg) was acetylated and purified in H:A:EA (65:20:15) to yield two bands R_f 0.67 (0.5 mg) and 0.56 (23.1 mg).

14.2.2.1 *Cassiaflavan-(4β-8)-afzelechin*

Further purification of R_f 0.65 (23.1 mg) in H:B:A (60:30:10 x3) afforded derivative **148**, R_f 0.55 (5.7 mg).

^{1}H NMR data: plate 24, table 9

CD data: plate 24

14.2.2.2 *Cassiaflavan-(4α-8)-afzelechin*

Further purification of R_f 0.65 (23.1 mg) in H:B:A (60:30:10 x3) afforded derivative **147**, R_f 0.54 (6.1 mg).

^{1}H NMR data: plate 23, table 9

CD data: plate 23

14.2.3 CONDENSATION OF 4',7-DIMETHOXYFLAVAN-4-THIOL AND PERMETHYL ETHER OF EPIAFZELECHIN.

The product mixture was resolved by preparative TLC in H:A:EA (60:30:10) and gave two bands at R_f 0.53 (46.4 mg) and 0.38 (39.5 mg). The first band 0.53 (46.4 mg) was

epiafzelechin and the band of R_f 0.38 (39.5 mg) was acetylated and purified in B:A (96:4 x2) to yield two bands R_f 0.48 (18.7 mg) and 0.39 (10.3 mg).

14.2.3.1 *Cassiaflavan-(4β-8)-epiafzelechin*

Further purification of R_f 0.48 (18.7mg) band in $CHCl_3$:B:A (50:45:5 x2) afforded derivative **149**, R_f 0.75 (13.0mg).

^{1}H NMR data: plate 25, table 10
CD data: plate 25

14.2.3.2 *Cassiaflavan-(4α-8)-epiafzelechin*

Further purification of R_f 0.39 (10.3mg) band in $CHCl_3$:B:A (50:45:5 x2) afforded derivative **150**, R_f 0.59 (7.9mg).

^{1}H NMR data: plate 26, table 10
CD data: plate 26

14.2.4 CONDENSATION OF 4',7-DIMETHOXYFLAVAN-4-THIOL AND PERMETHYL ETHER OF EPIGALLOCATECHIN.

The product mixture was resolved by preparative TLC in H:A:EA (65:20:15) yielding two bands at R_f 0.50 (66 mg) and 0.35 (50.7 mg). The band of R_f 0.50 (66 mg) was the starting material epigallocatechin.

Alternative method: To a solution of 4-benzylsulfanylflavan (100 mg) in THF was added solid $AgBF_4$ (2.5 *eq*) and stirred at 0°C for 15 minutes. Free phenolic epigallocatechin (3 *eq*) was added to the mixture and stirred at 0°C for 1 hour. Water (50 ml) was added followed by extraction with EtOAc (4x50 ml), drying (Na_2SO_4) and evaporating under reduced pressure gave a mixture of products. Separation by PLC in B:A:M (7:2:1) gave two bands of dimers R_f 0.57 (150.8 mg) and 0.45 (81.5 mg) which were later methylated and acetylated.

14.2.4.1 *Cassiaflavan-(4β-8)-epigallocatechin*

Acetylation of R_f 0.350 (50.7 mg) band and purification in H:A:EA (65:20:15 x 1) afforded derivative **136** R_f 0.5 (31.9 mg).

^{1}H NMR data: plate 27, table 11

CD data: plate 27

14.2.4.2 *Cassiaflavan-(4α-8)-epigallocatechin*

Acetylation of R_f 0.350 (50.7 mg) band and purification in H:A:EA (65:20:15 x 1) afforded derivative **137** R_f 0.49 (14.9 mg).

^{1}H NMR data: plate 28, table 11

CD data: plate 28

ATTEMPTED PROCEDURES FOR THE SYNHESIS OF TRIMER

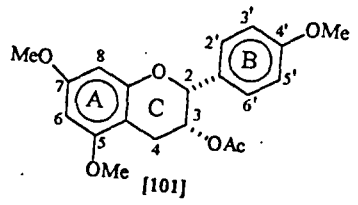
A solution of 4-benzylsulfanylflavan (60 mg) and methyl ether of casssiaflavan-epigallocatechin (3 *eq*) in THF was treated with solid $AgBF_4$ (2.5 *eq*) and refluxed at 65°C for 12hours. Water (30 ml) was added followed by extraction with EtOAc (4x30 ml), drying (Na_2SO_4) and evaporating under reduced pressure gave a mixture of products, but none of the required compound.

As an alternative to the previous reaction; To a solution of 4-benzylsulfanylflavan (100 mg) in THF was added solid $AgBF_4$ (2.5 *eq*) and stirred at 0°C for 15 minutes. Procassinidin with free phenolic epigallocatechin bottom unit (3 *eq*) was added to the mixture and stirred at (0-40)°C for 1-24 hours. Water (50 ml) was added followed by extraction with EtOAc (4x50 ml), drying (Na_2SO_4) and evaporating under reduced pressure gave the mixture of products, but the required compound could not found.

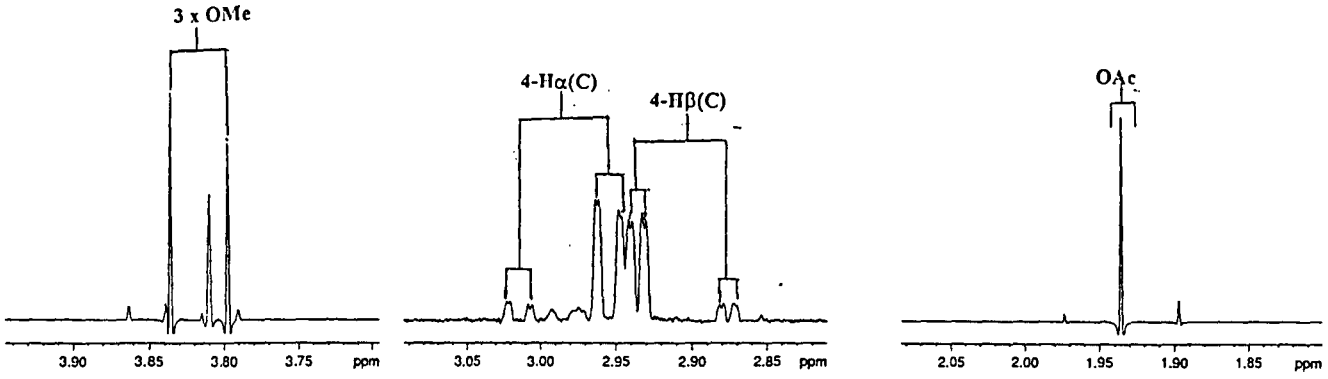
The last attempted method: The solution of nucleophile (procassinidin dimer 1mmole) in methylene chloride was added to 0.1M HCl under nitrogen atmosphere. The electrophile (flavan-4-ol 0.5 mmole) was added dropwise to this solution over a period of an hour. The reaction was stirred at room temperature for 3 days monitored by TLC. After the disappearance of starting materials water was added and the product extracted with ethyl acetate (4x50 ml). The extract was dried (Na_2SO_4) and the solvent evaporated under reduced pressure to give a light brown residue, chromatographed on a column which gave unwanted material.

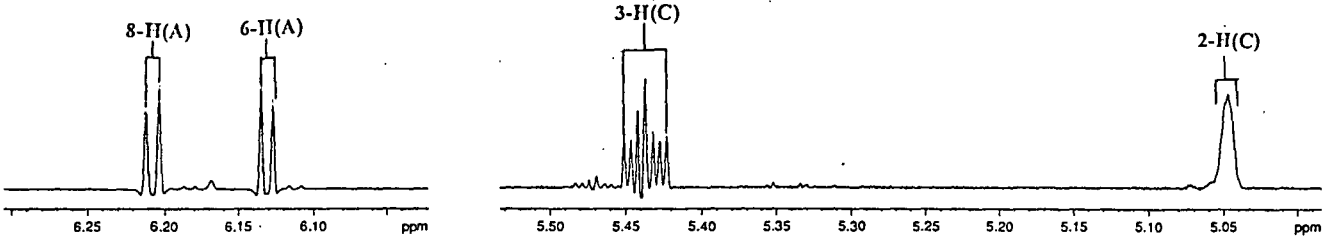
APPENDIX

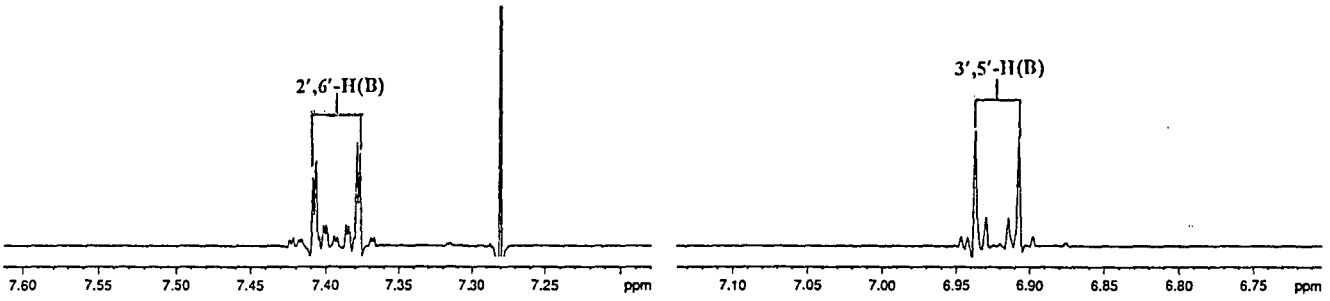
Plate 1 ($CDCl_3$ 296K)

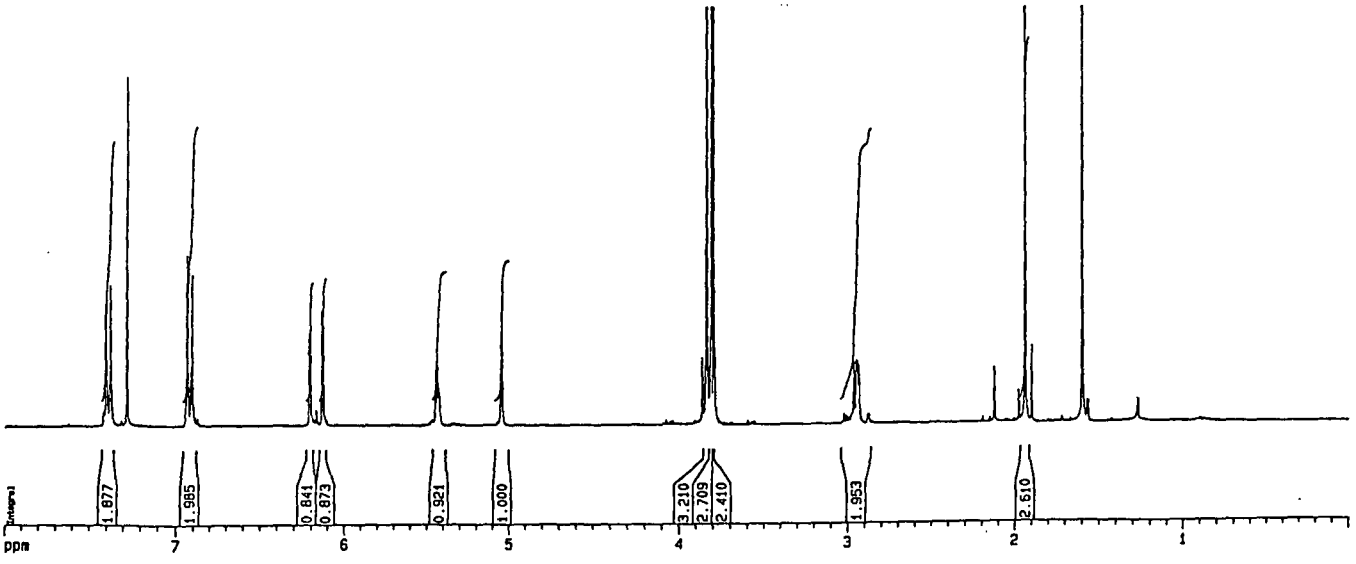


[101]









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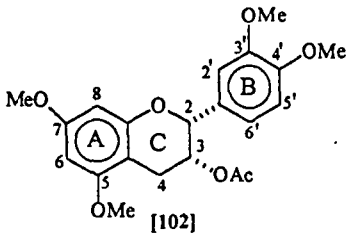

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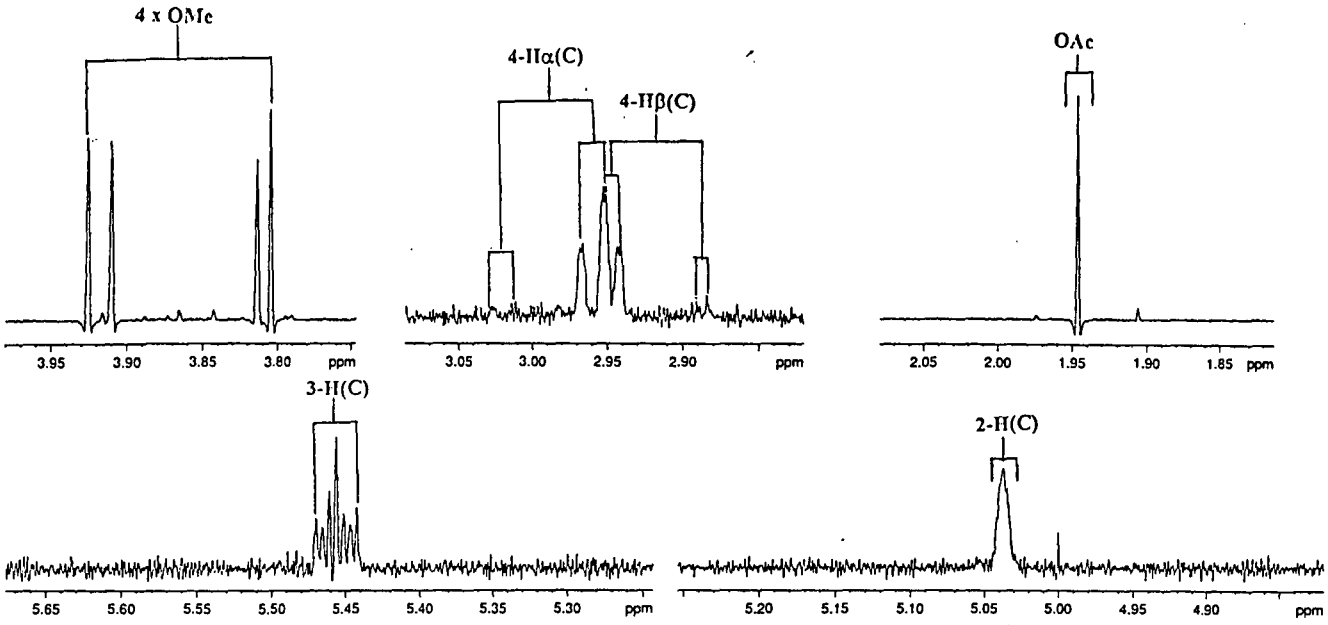
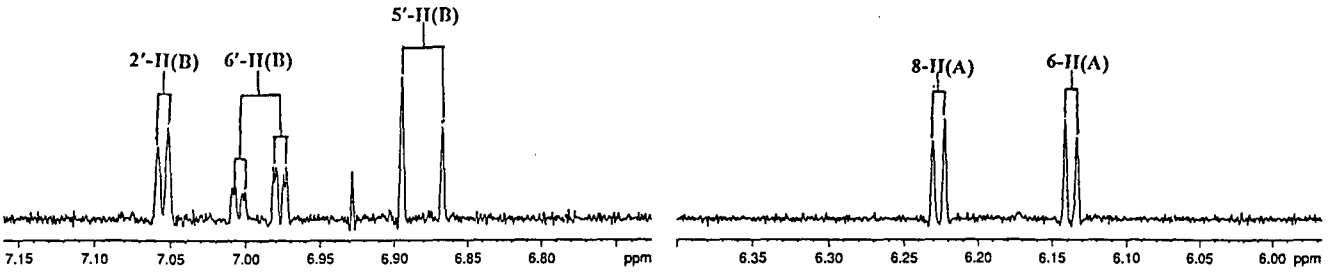




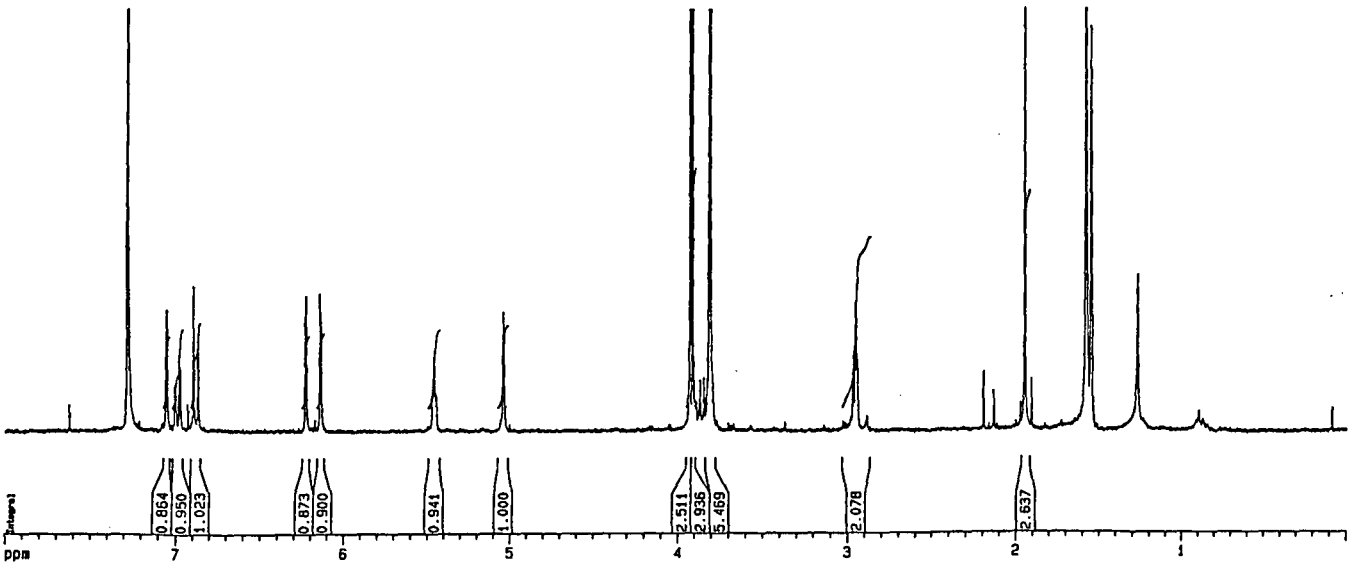
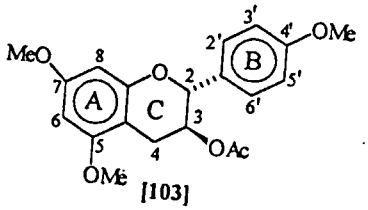


Plate 3 ($CDCl_3$ 296K)



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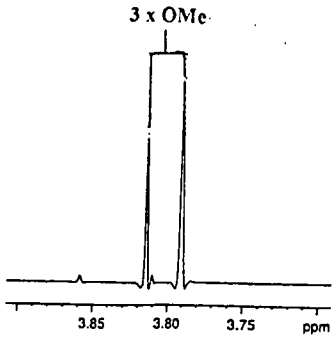
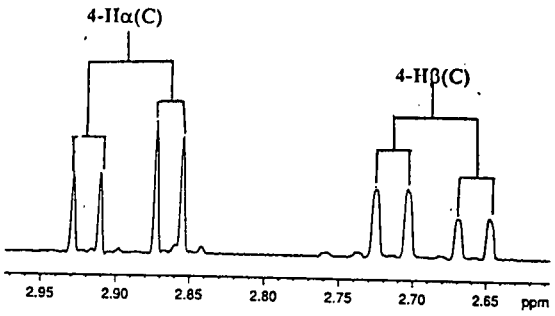
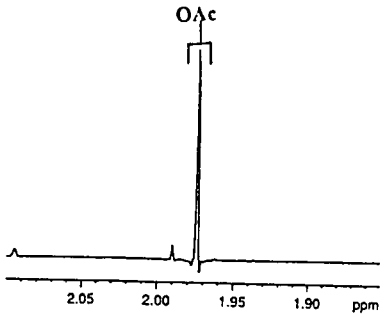
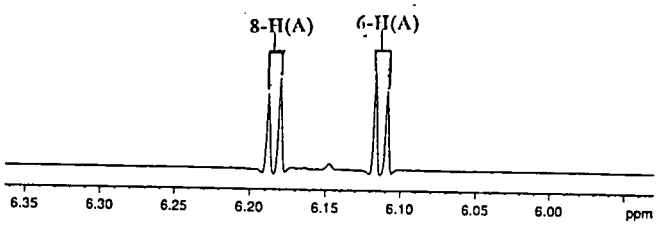
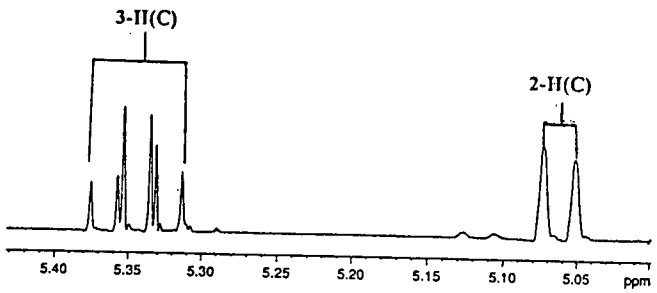
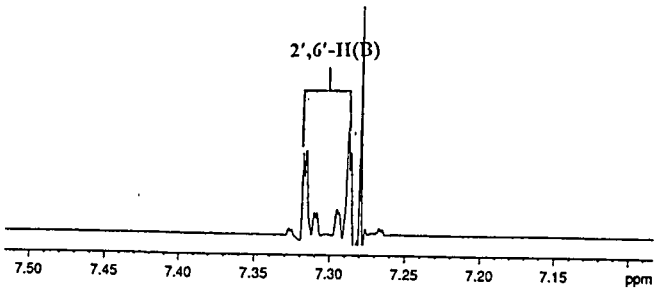


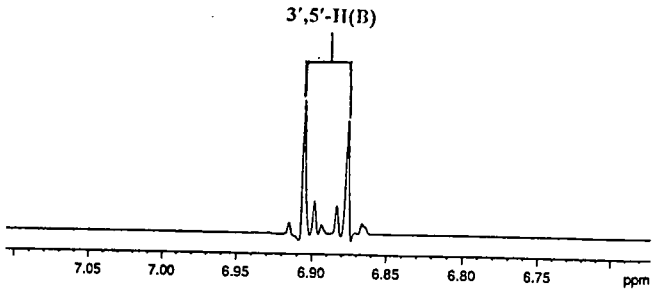












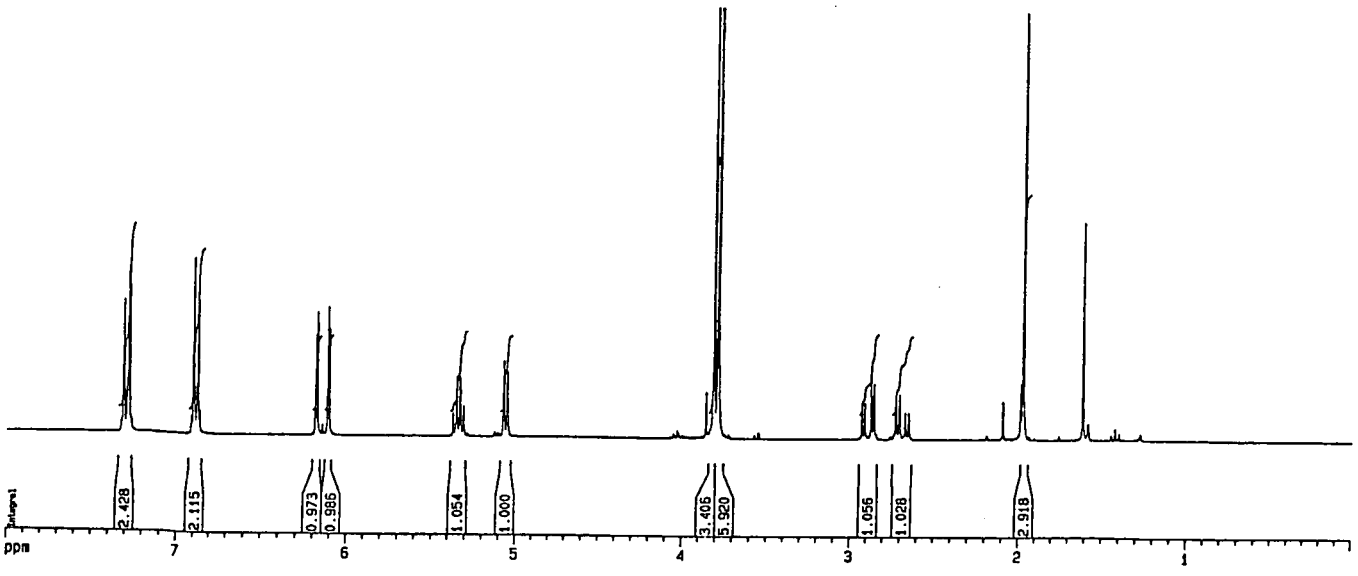
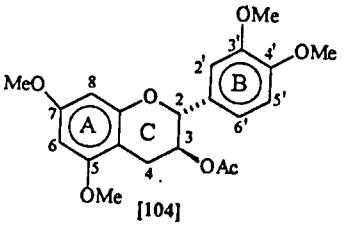


Plate 4($CDCl_3$ 296K)



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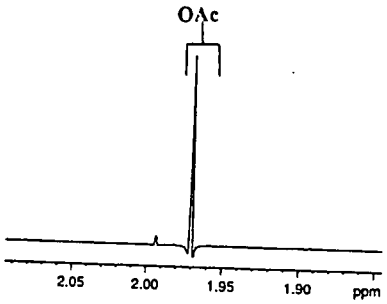
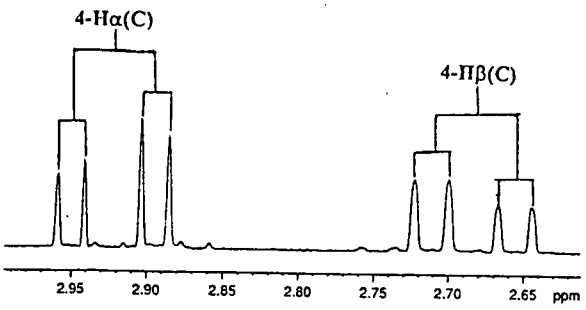
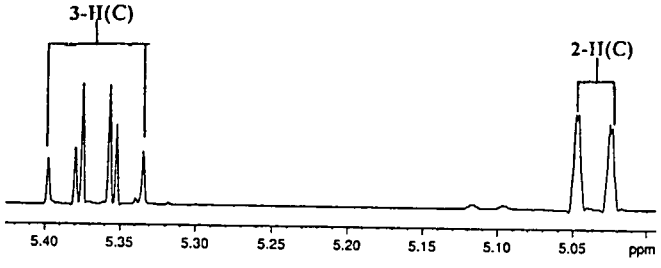
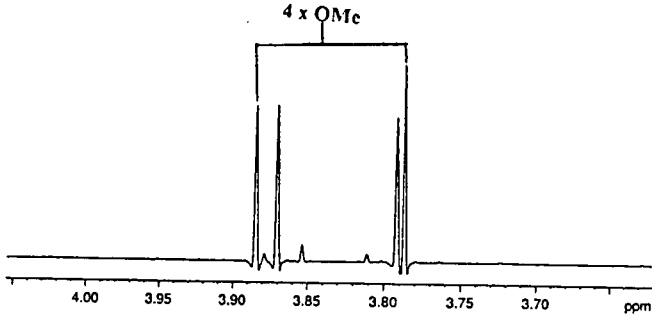
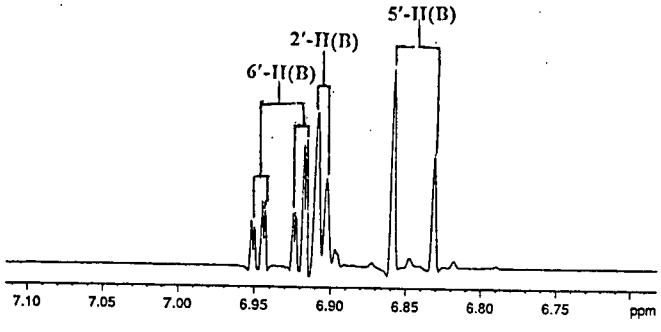
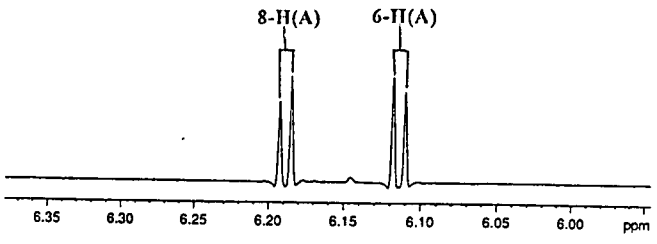












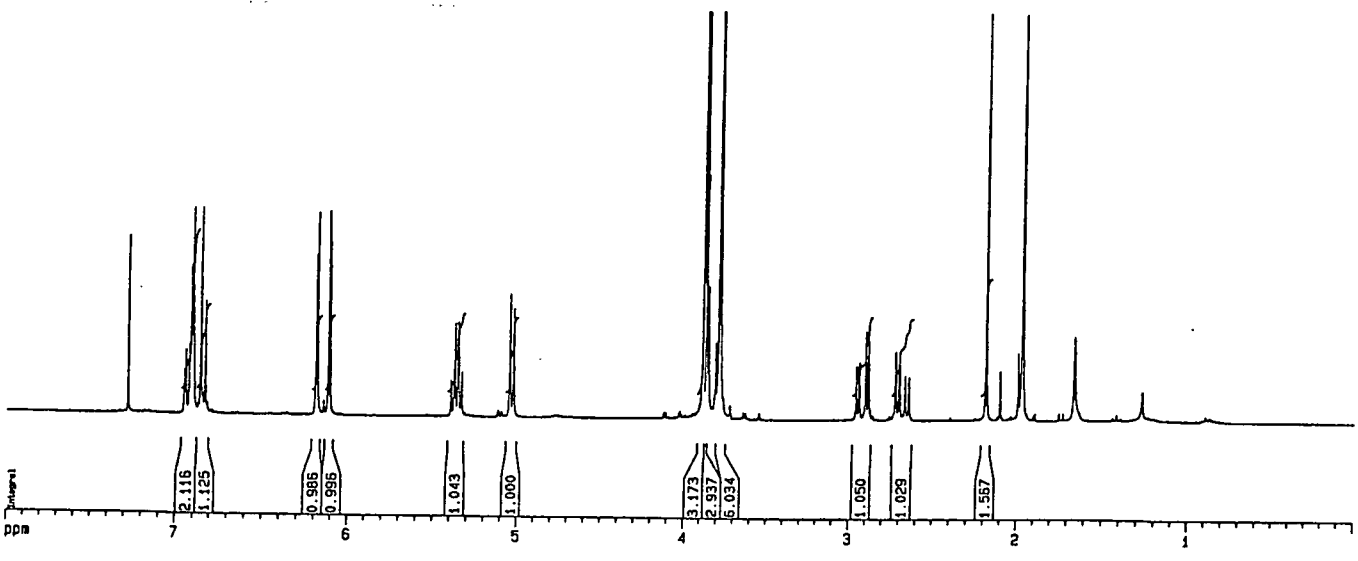
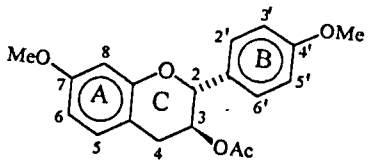


Plate 5a ($CDCl_3$ 296K)



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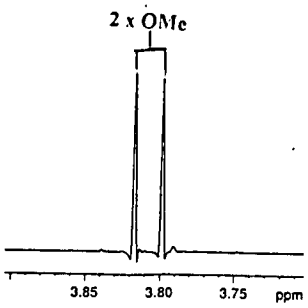
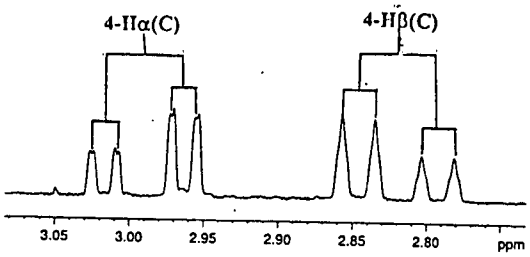
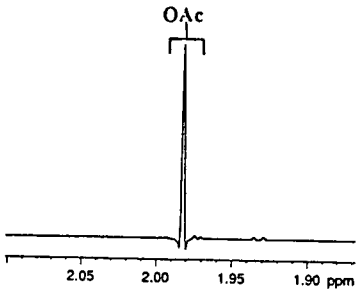
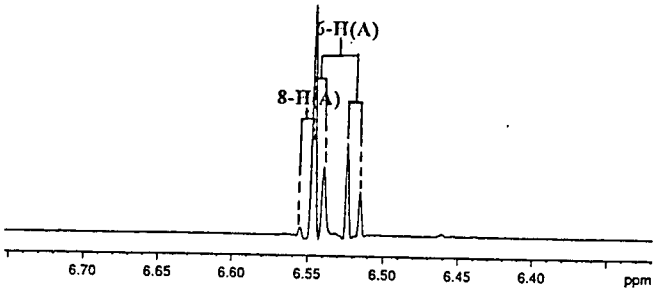
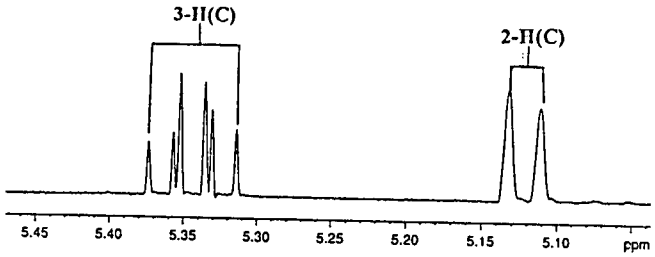
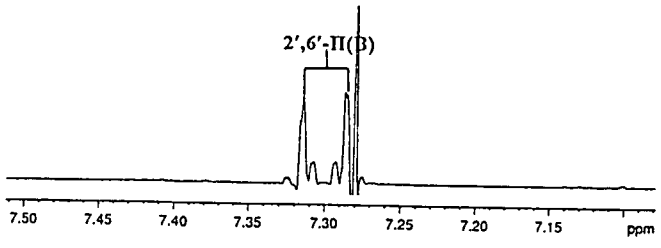


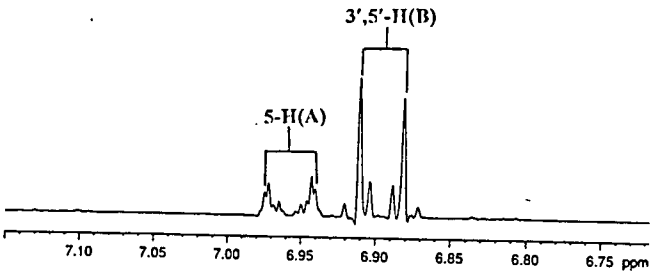












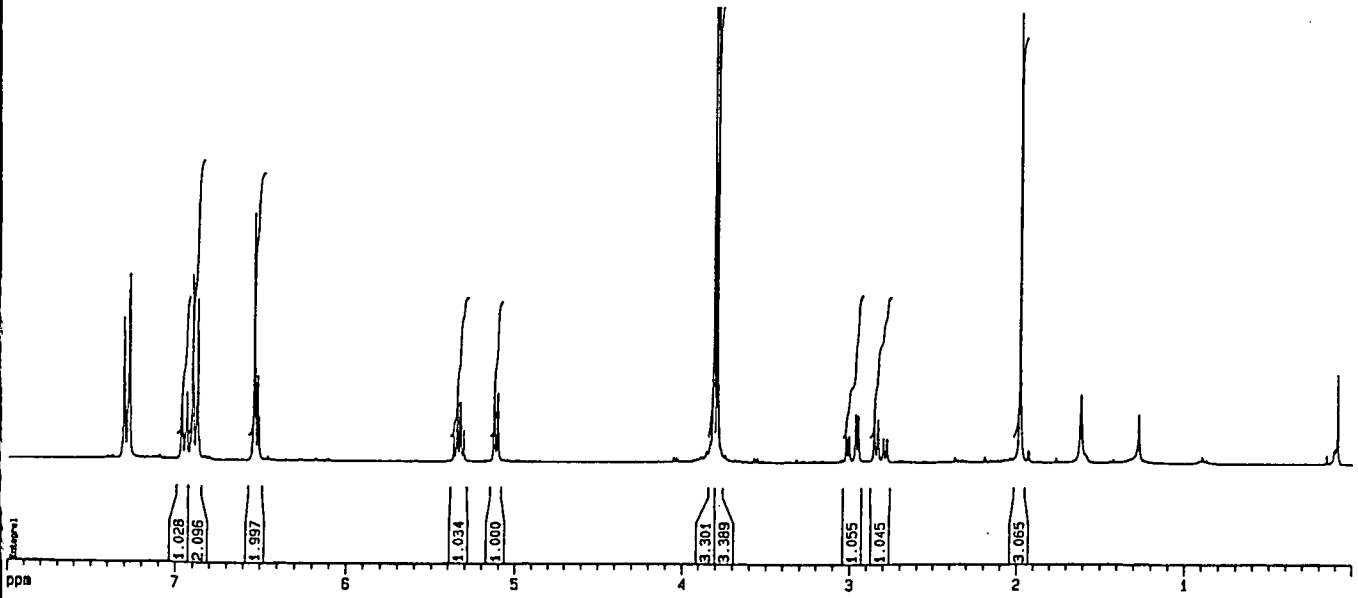
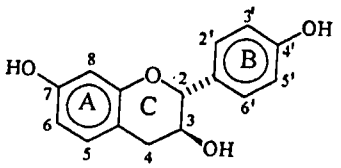


Plate 5b $(CD_3)_2CO$ 296K



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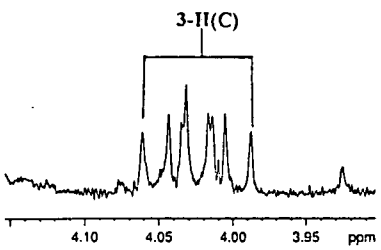
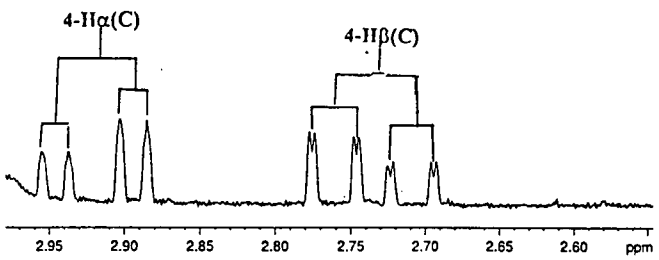
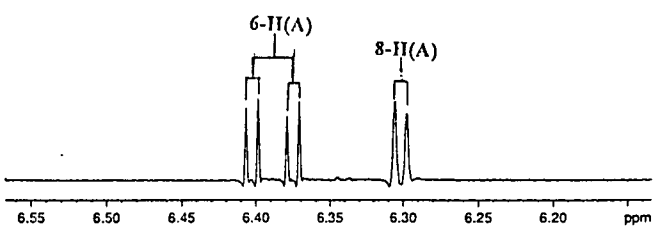
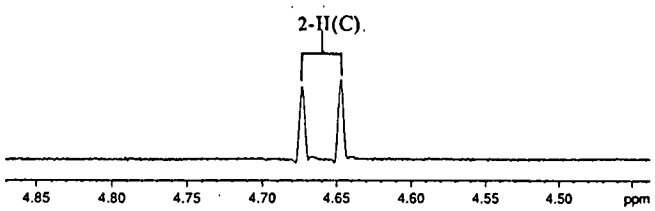
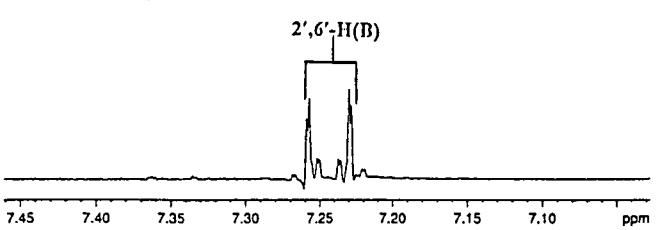
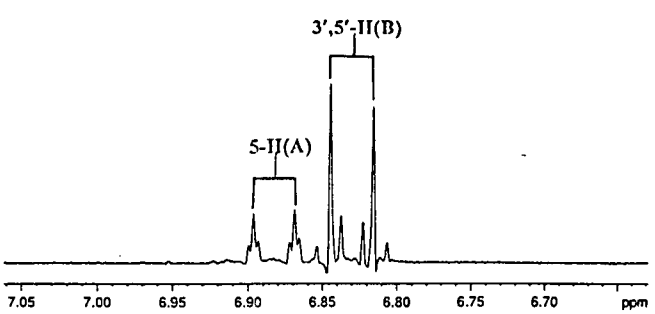












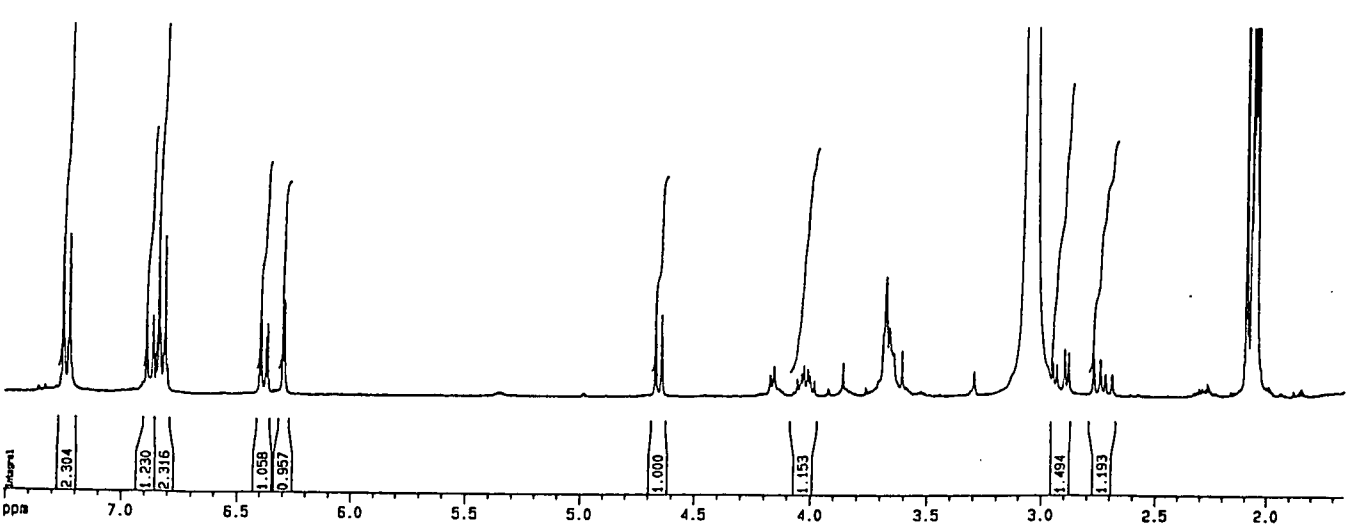
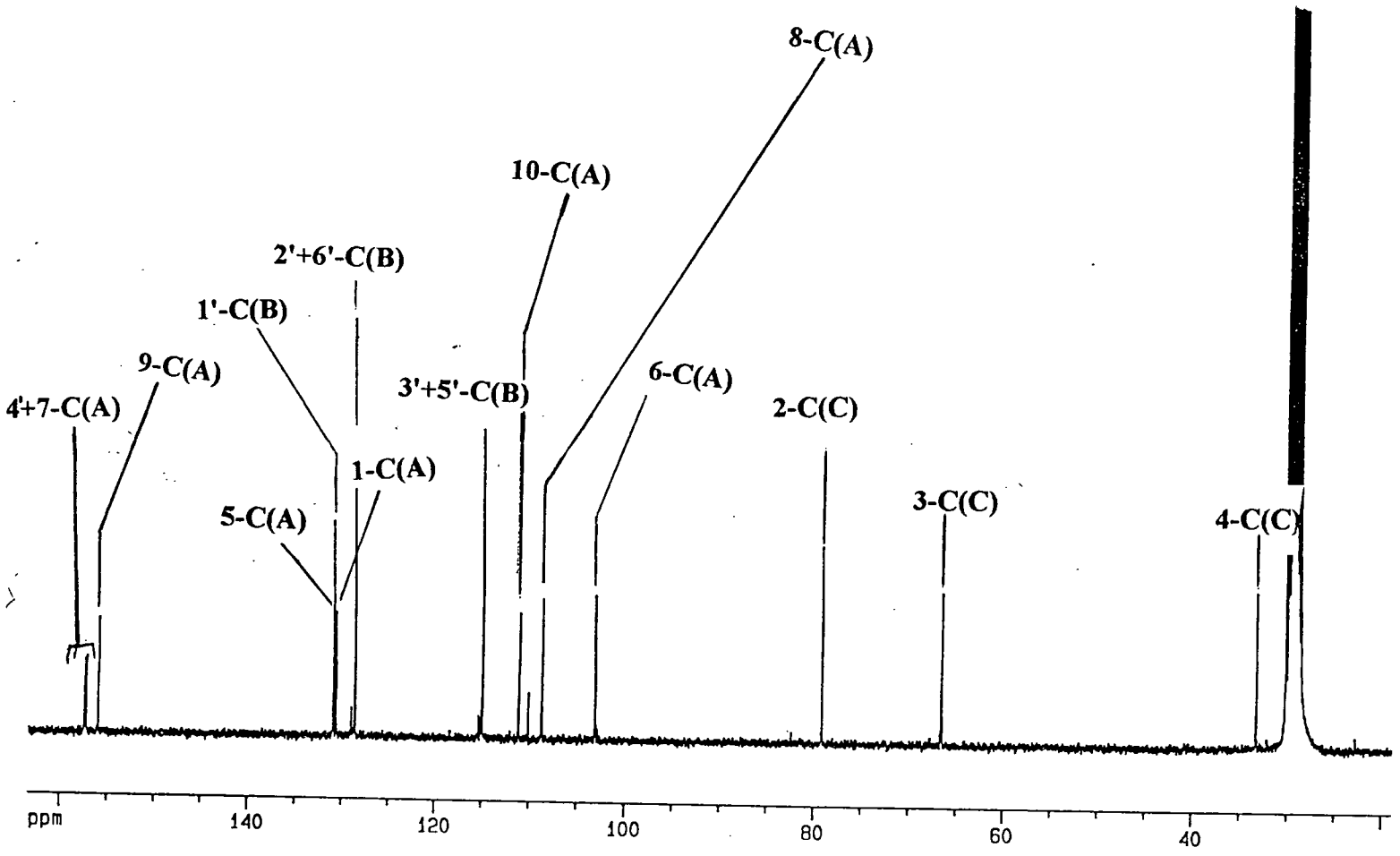
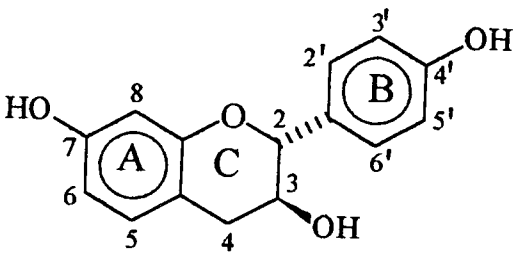


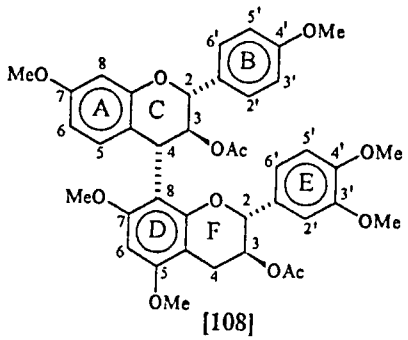
Plate 5b $(CD_3)_2CO$ 296K



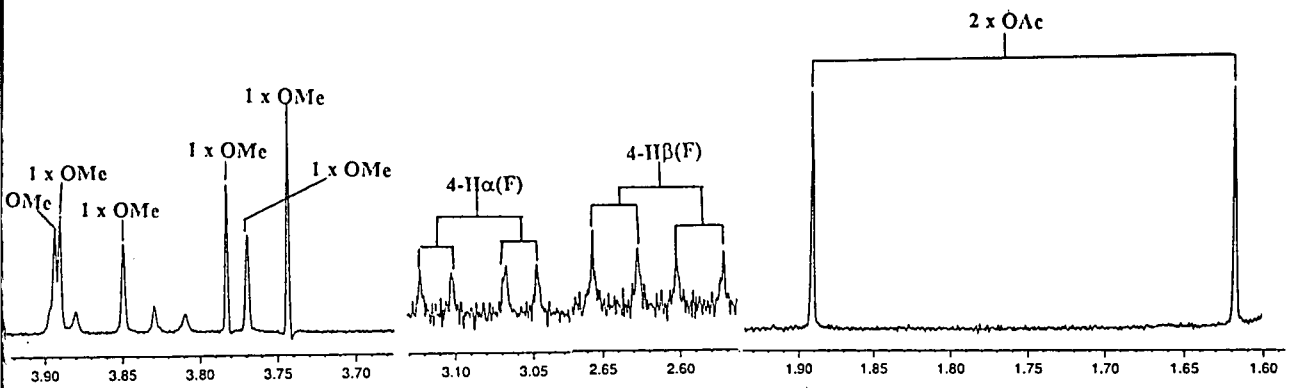


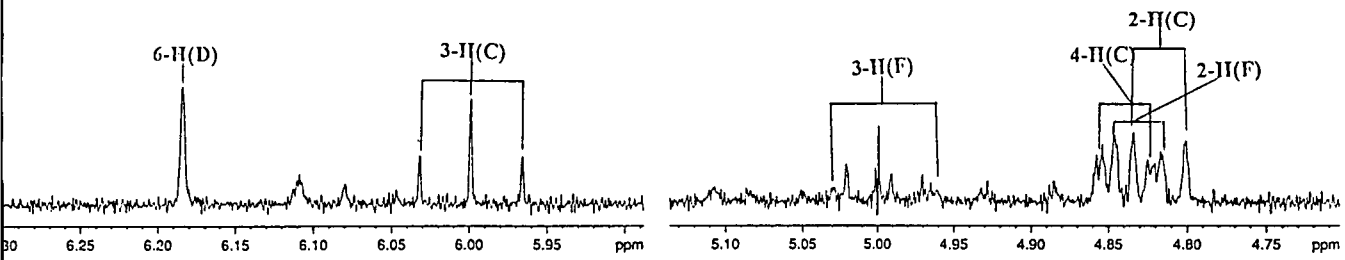
[105]

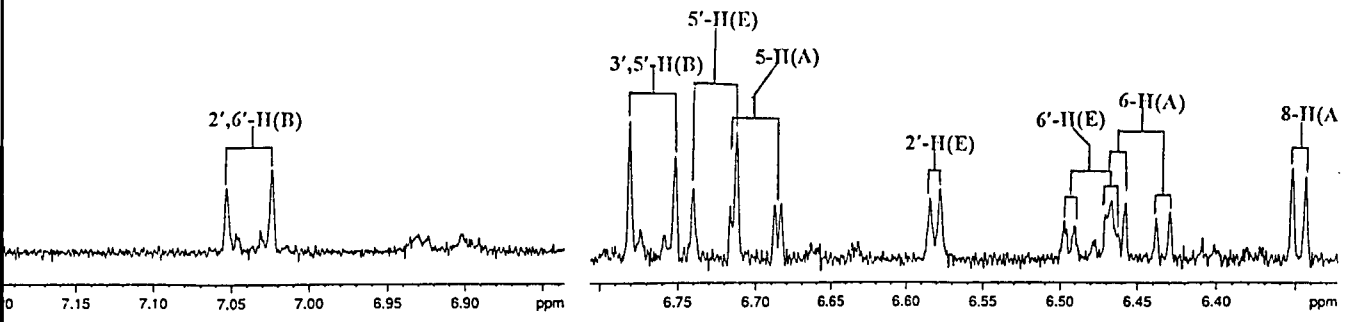
Plate 6 ($CDCl_3$ 296K)



[108]







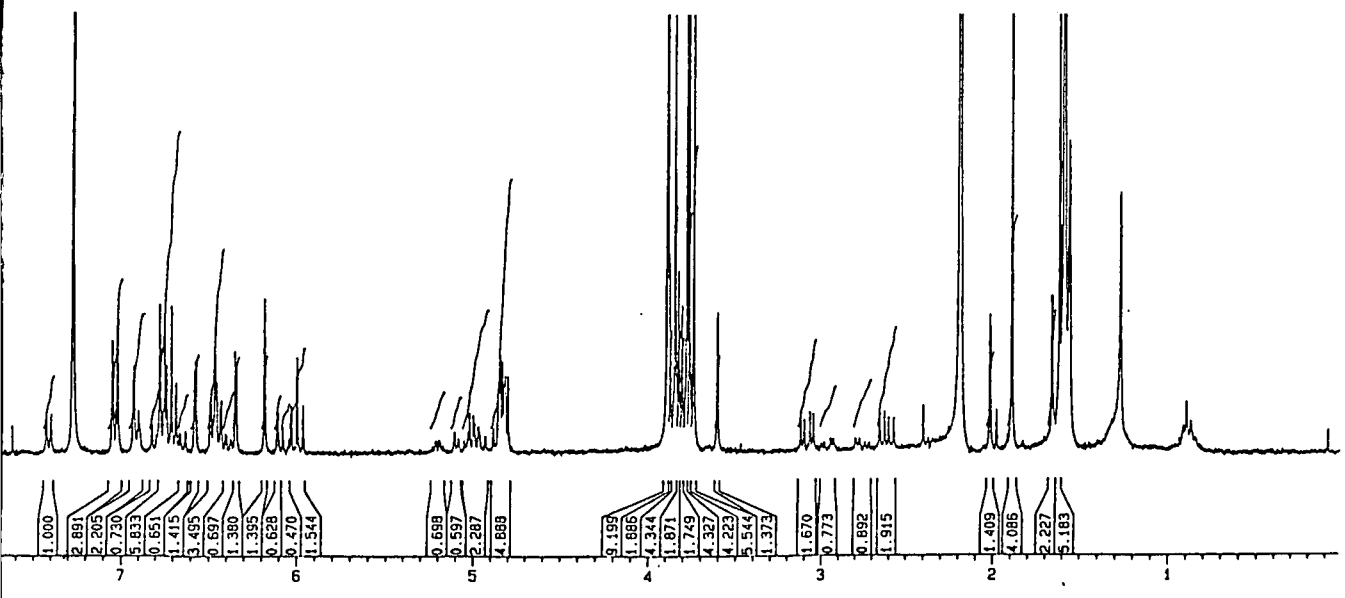
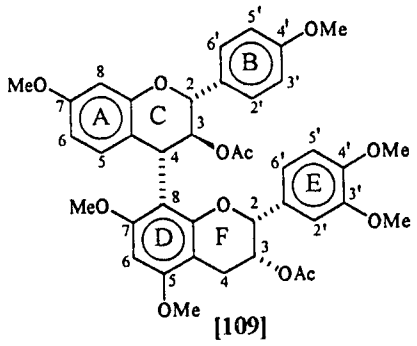
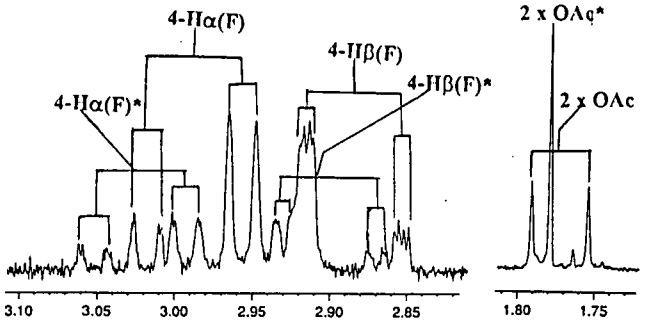
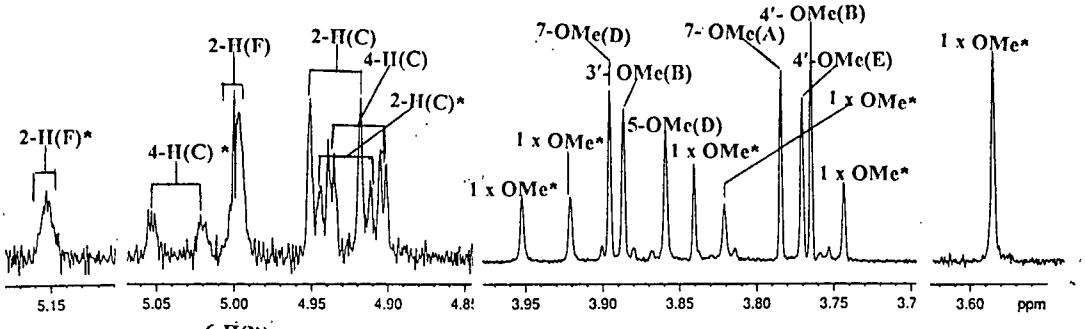
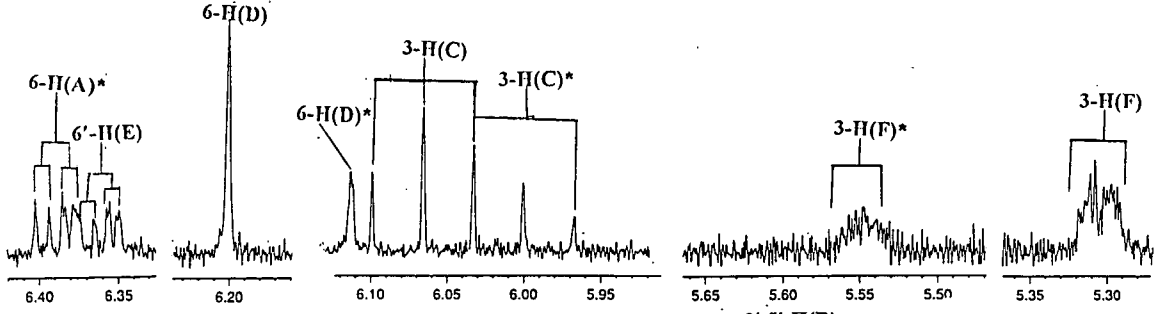


Plate 7 ($CDCl_3$ 296K)









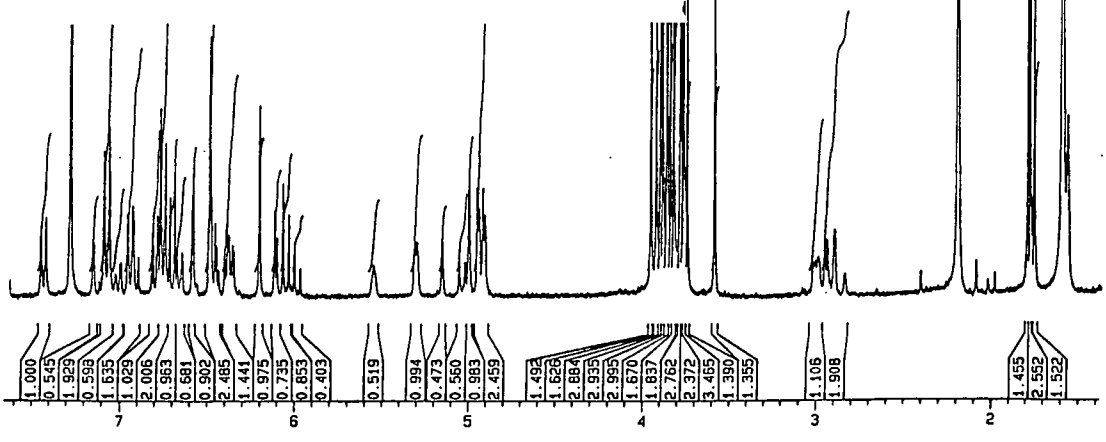
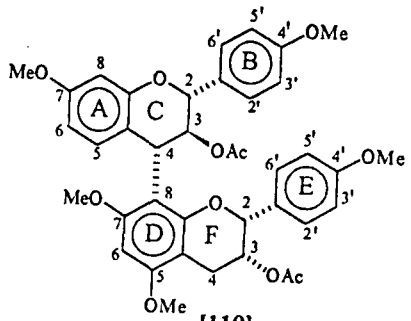


Plate 8 ($CDCl_3$ 296K)



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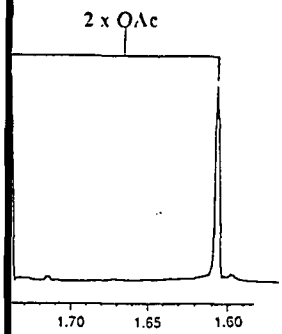
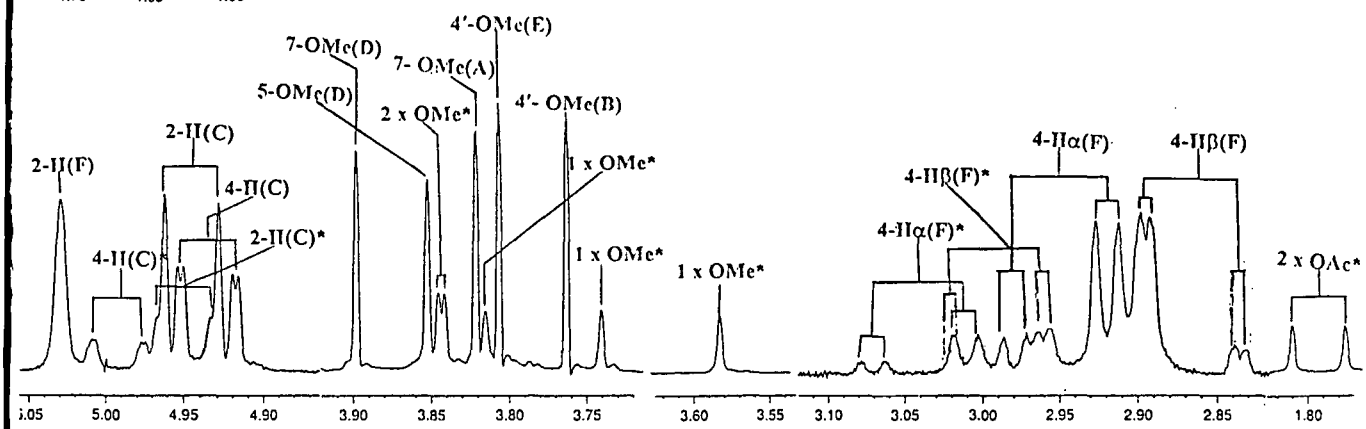
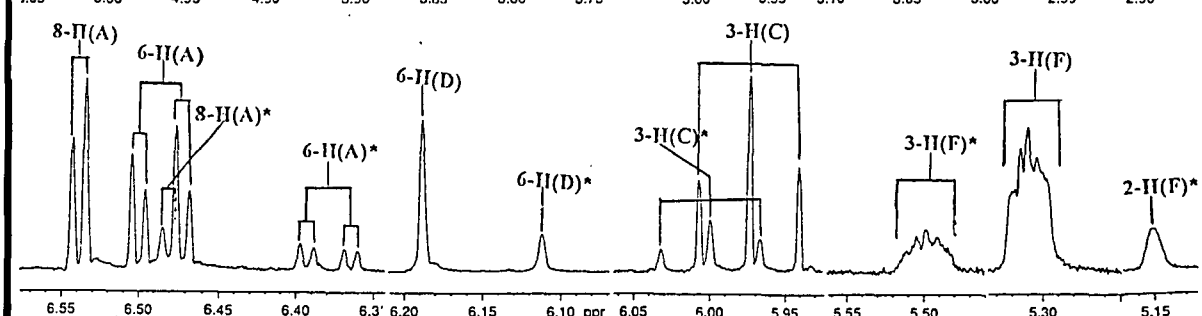
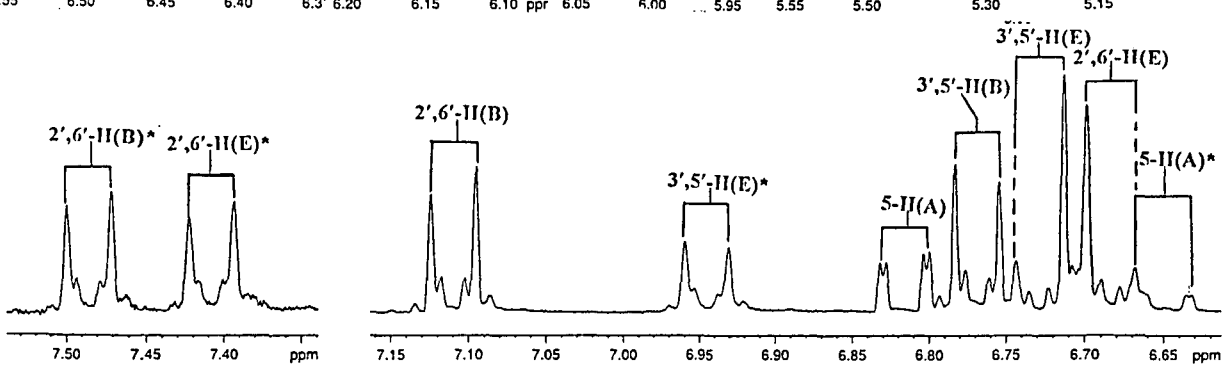








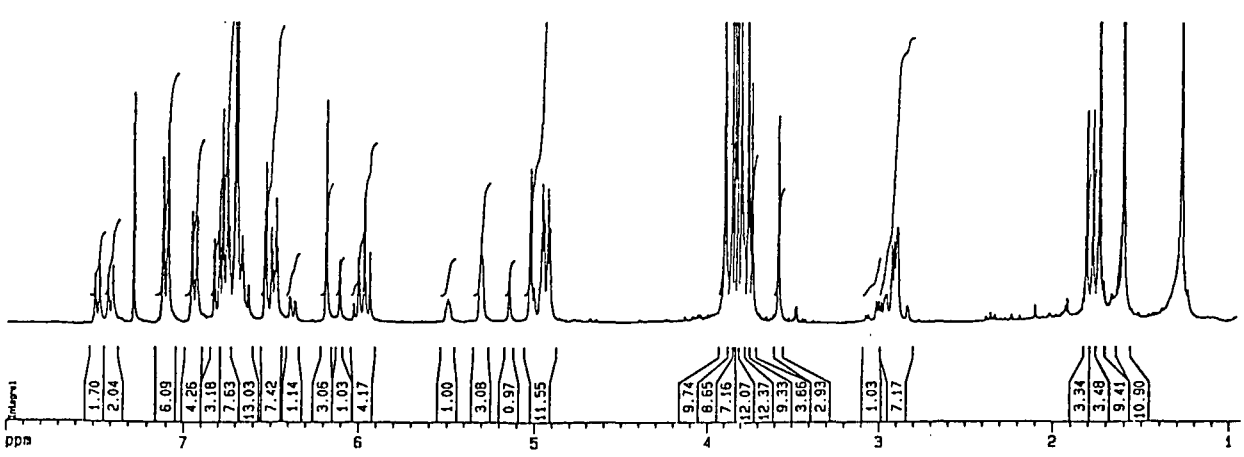
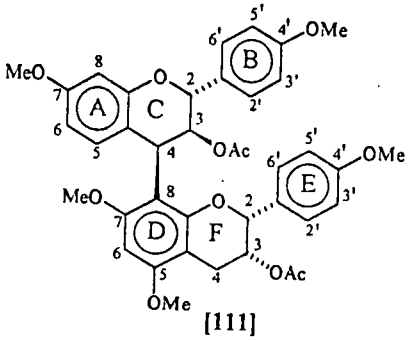


Plate 9 ($CDCl_3$ 296K)



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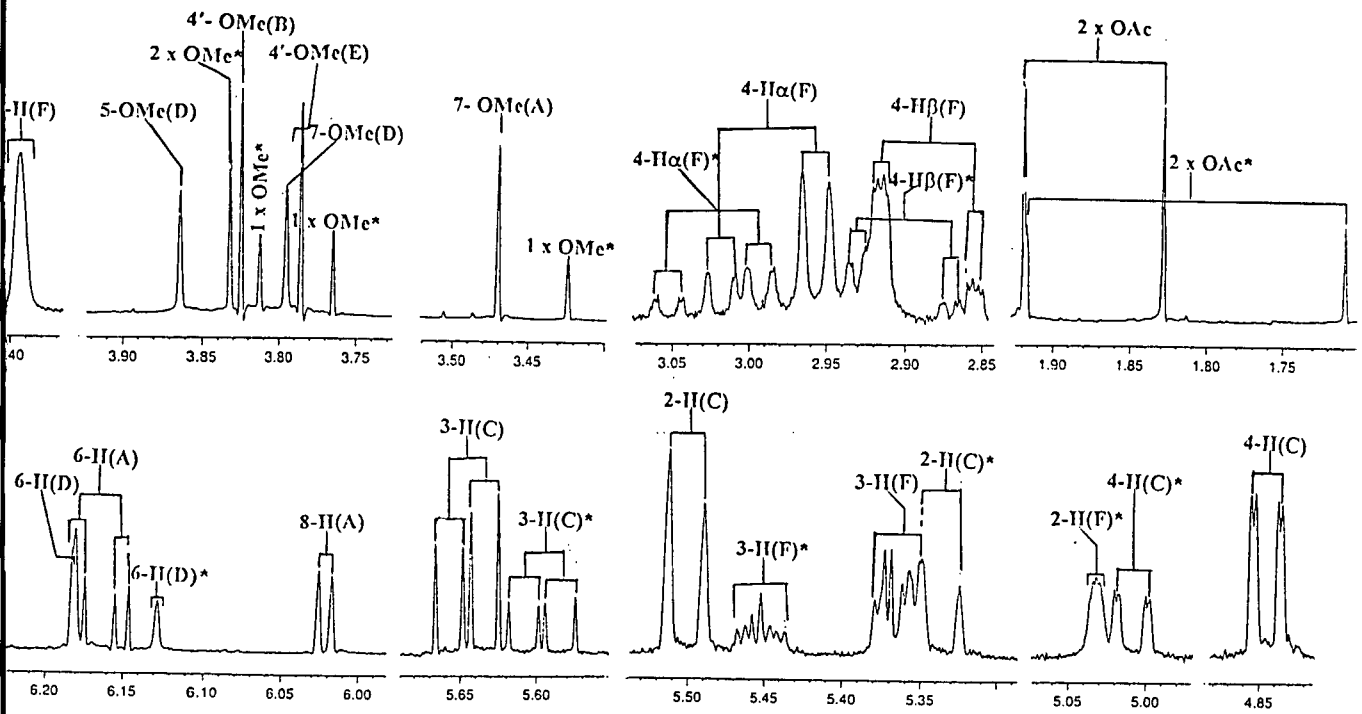


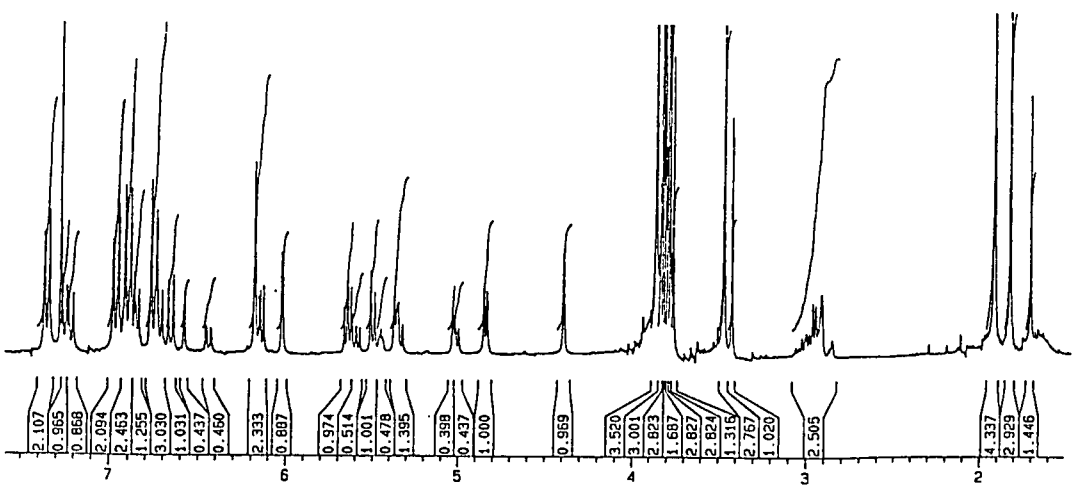
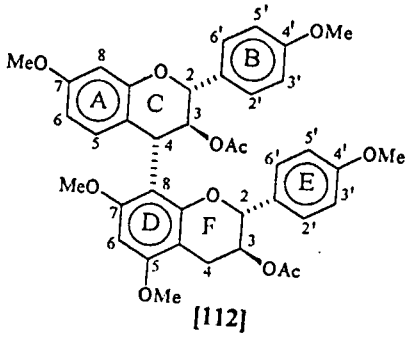


Plate 10 ($CDCl_3$ 296K)



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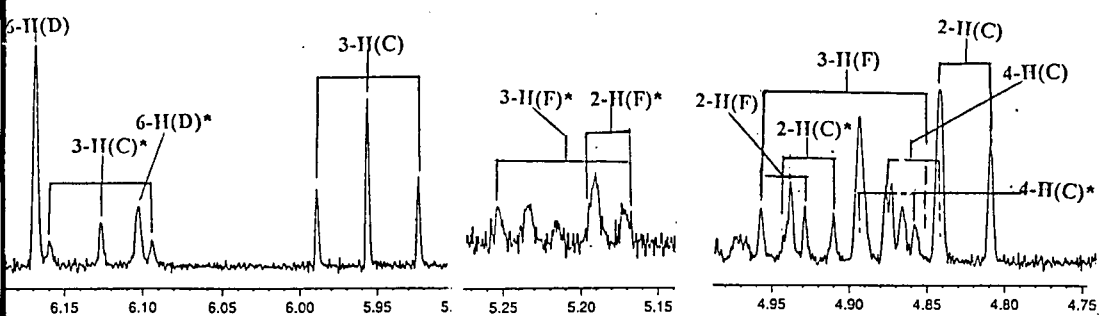
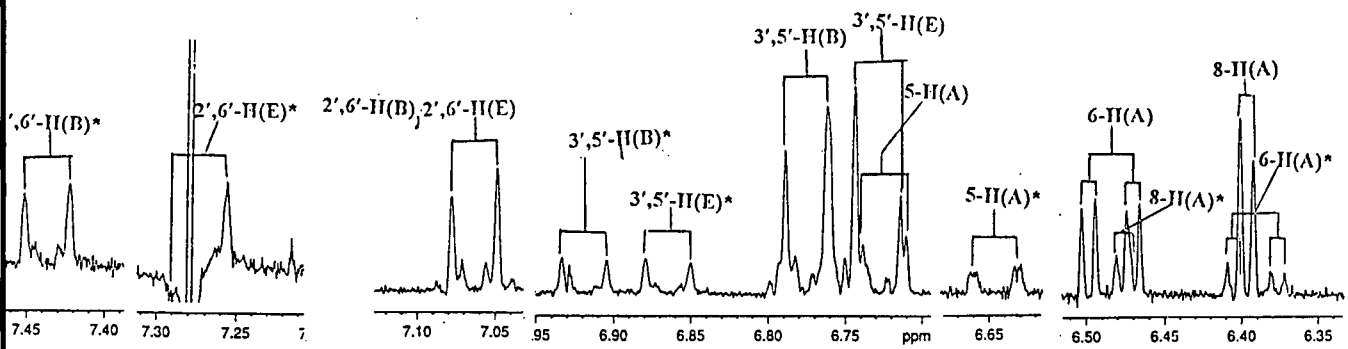




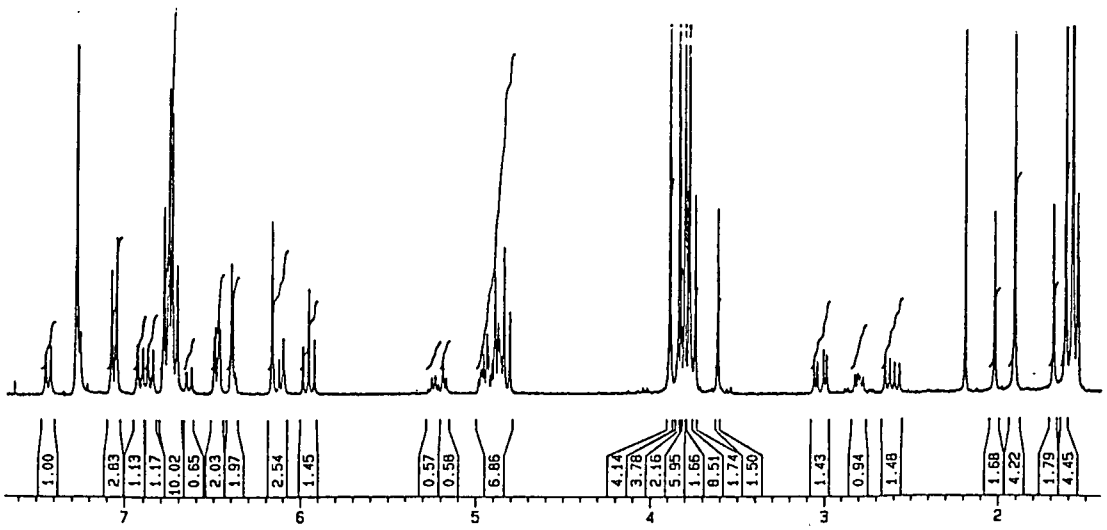


Plate 11 ($CDCl_3$ 296K)

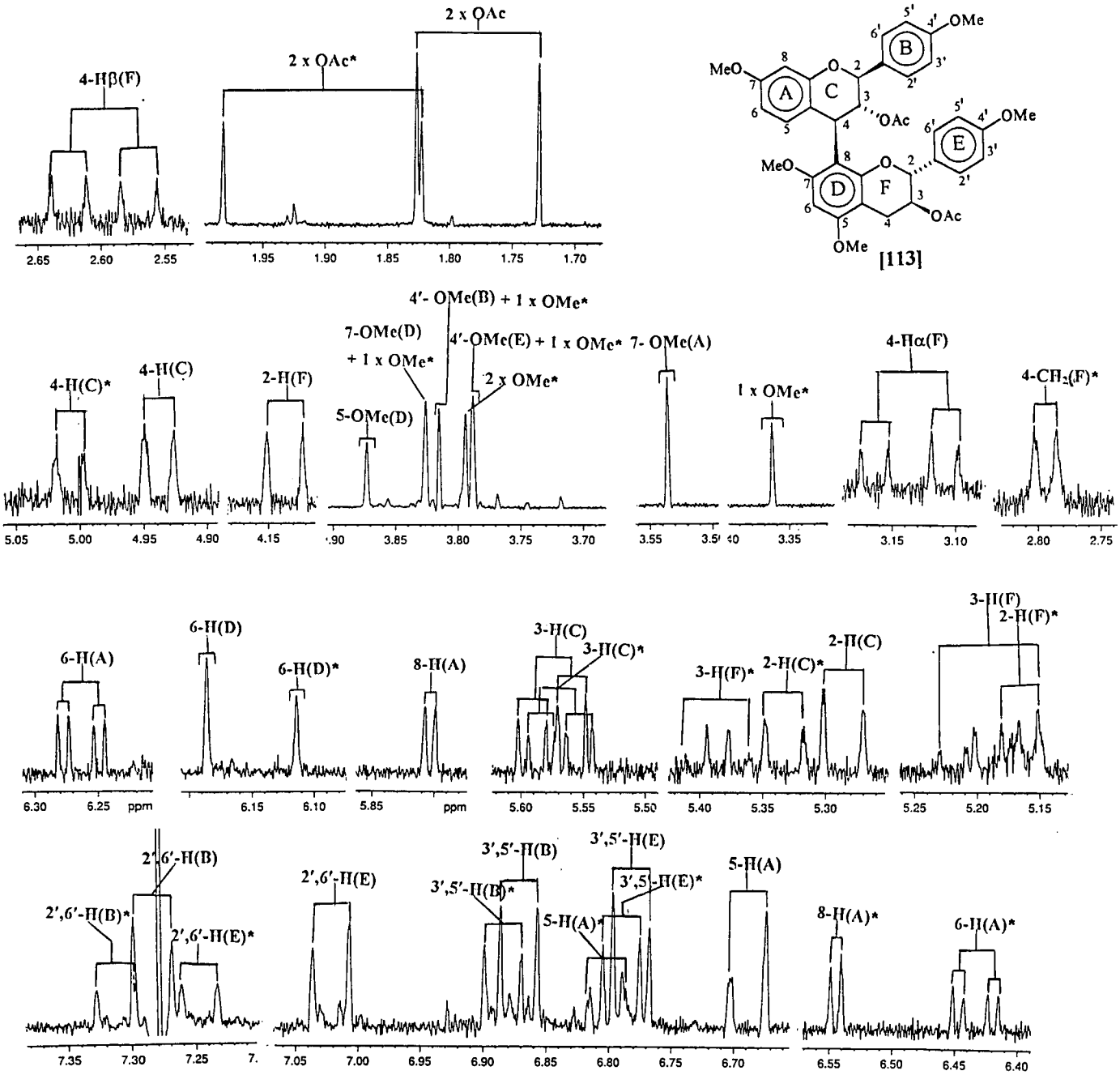
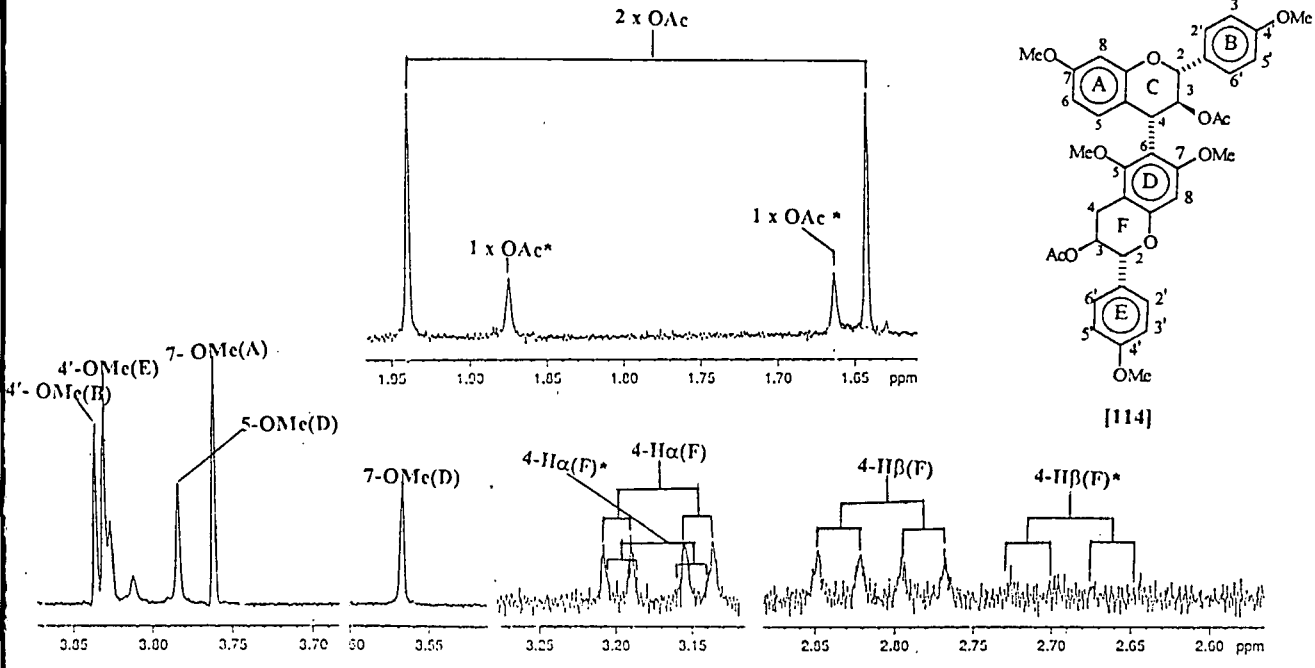


Plate 12 ($CDCl_3$ 296K)



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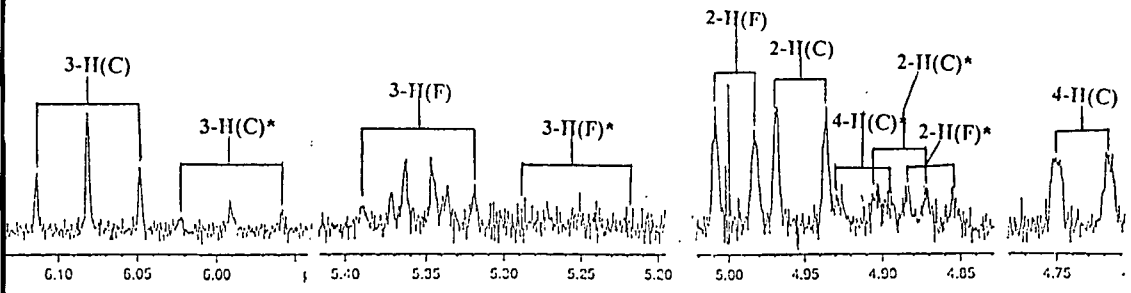
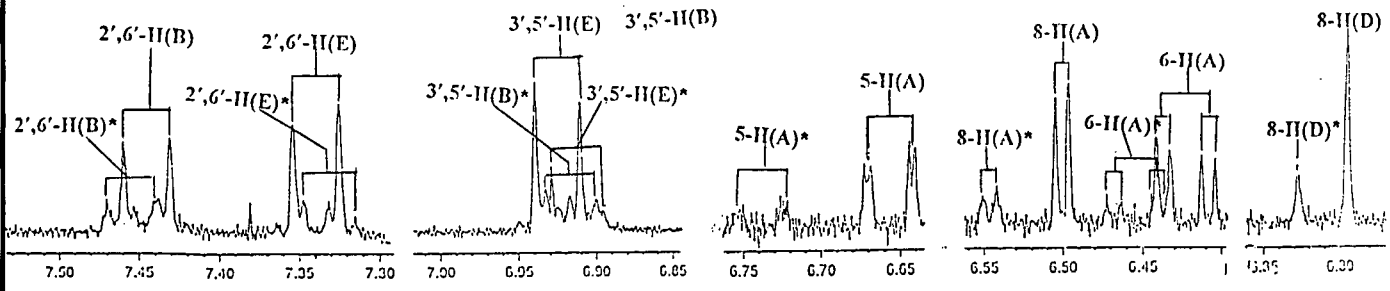




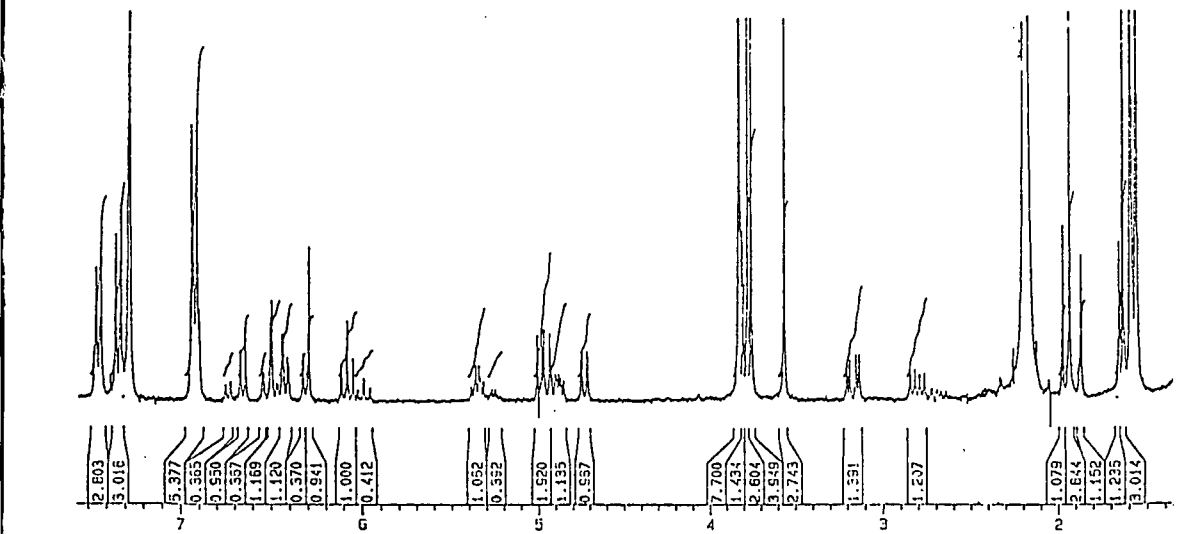
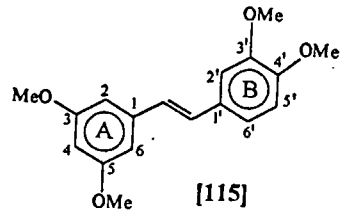


Plate 13 ($CDCl_3$ 296K)



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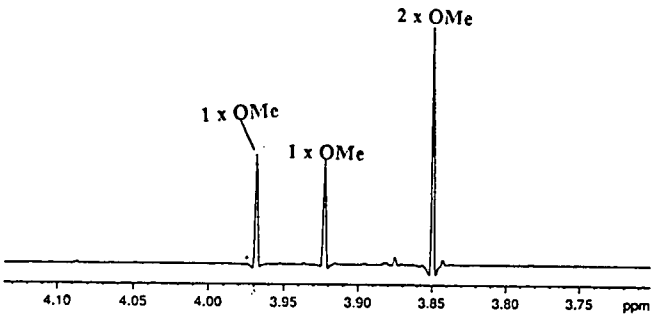
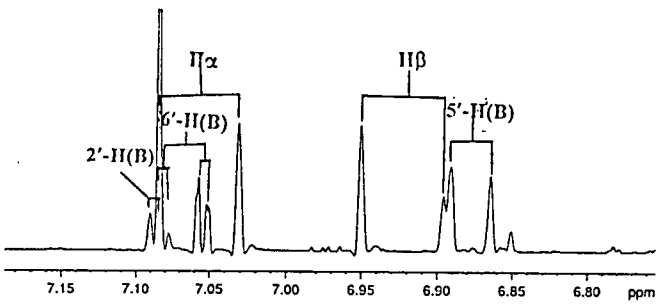
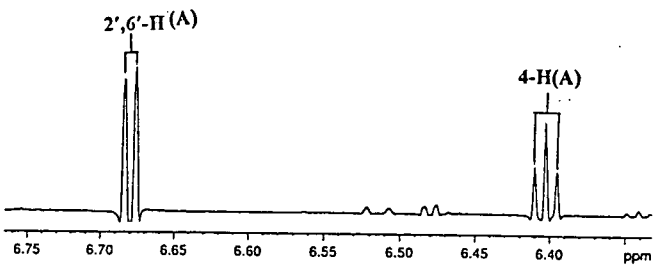






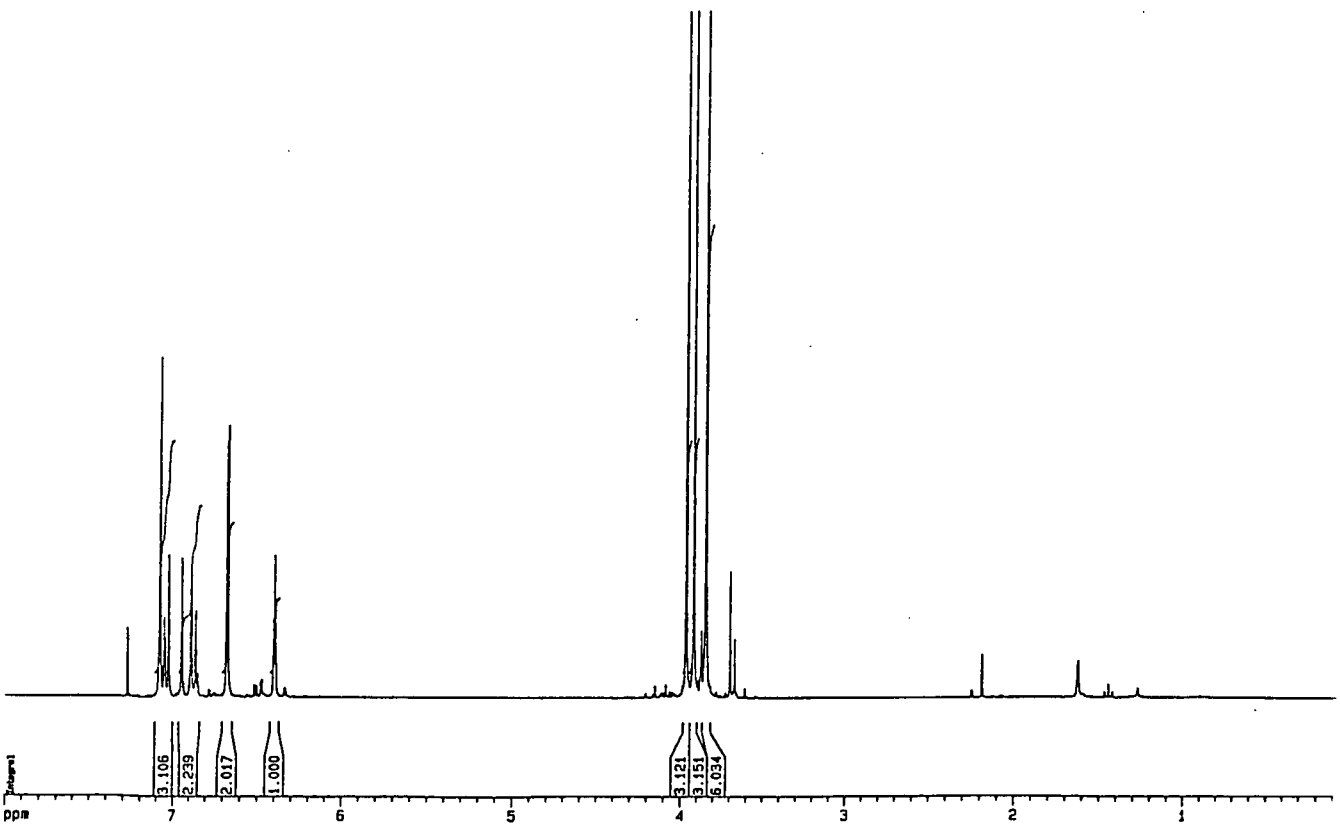
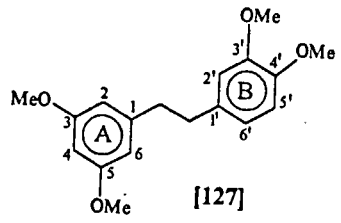


Plate 14 ($CDCl_3$ 296K)



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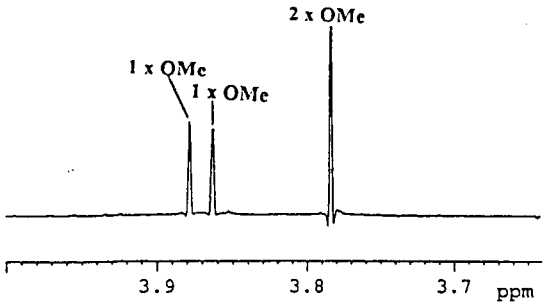
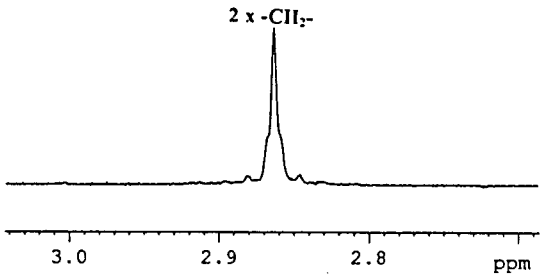
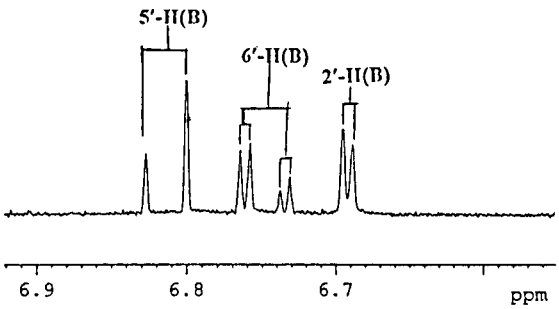
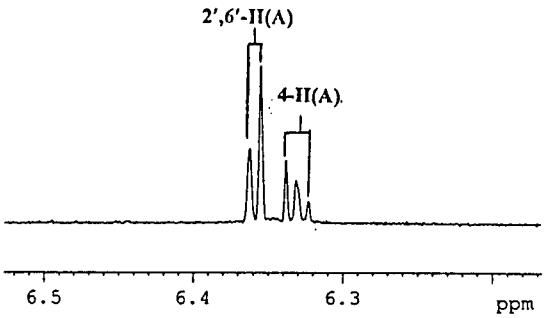








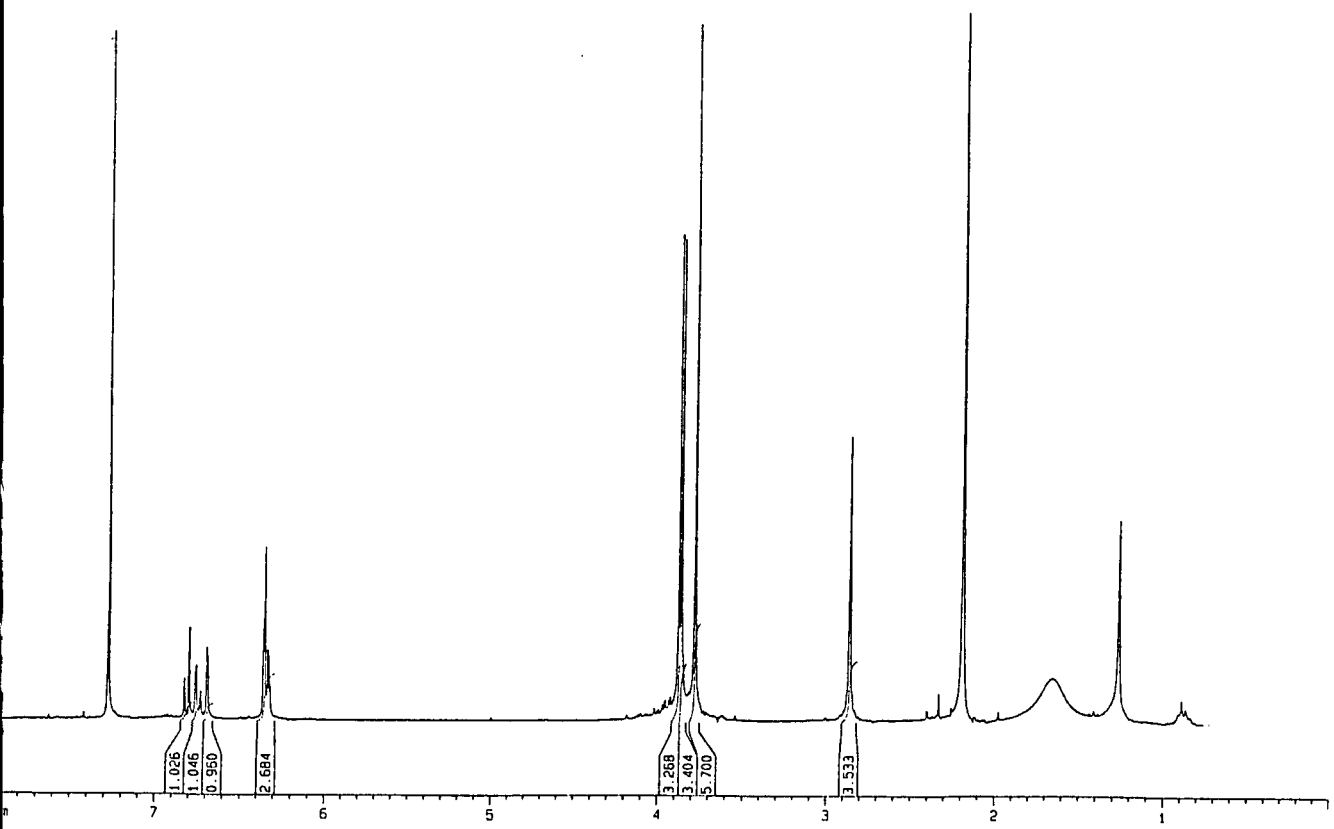
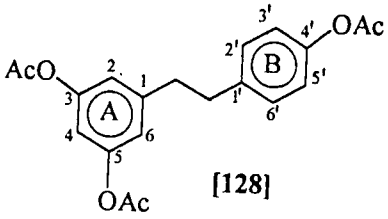


Plate 15 ($CDCl_3$ 296K)



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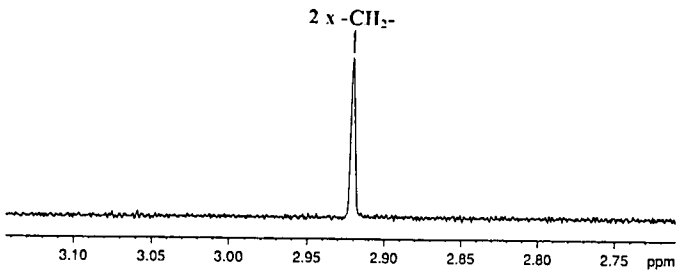
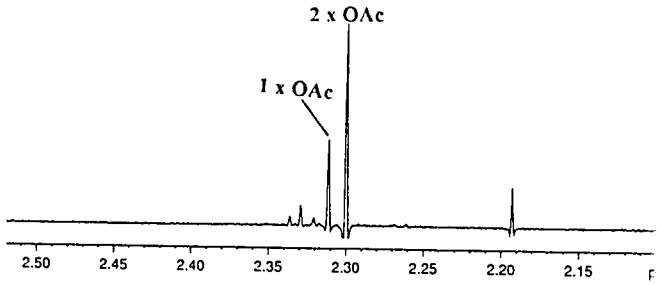
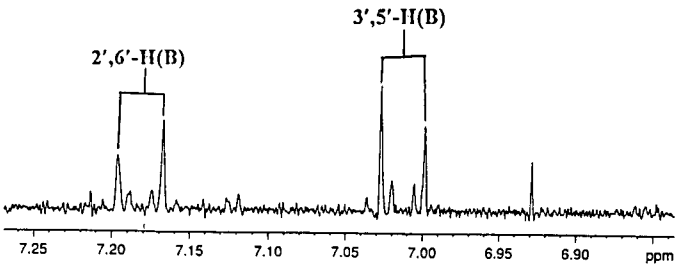
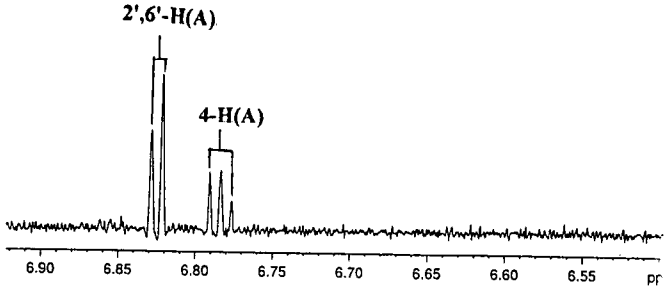








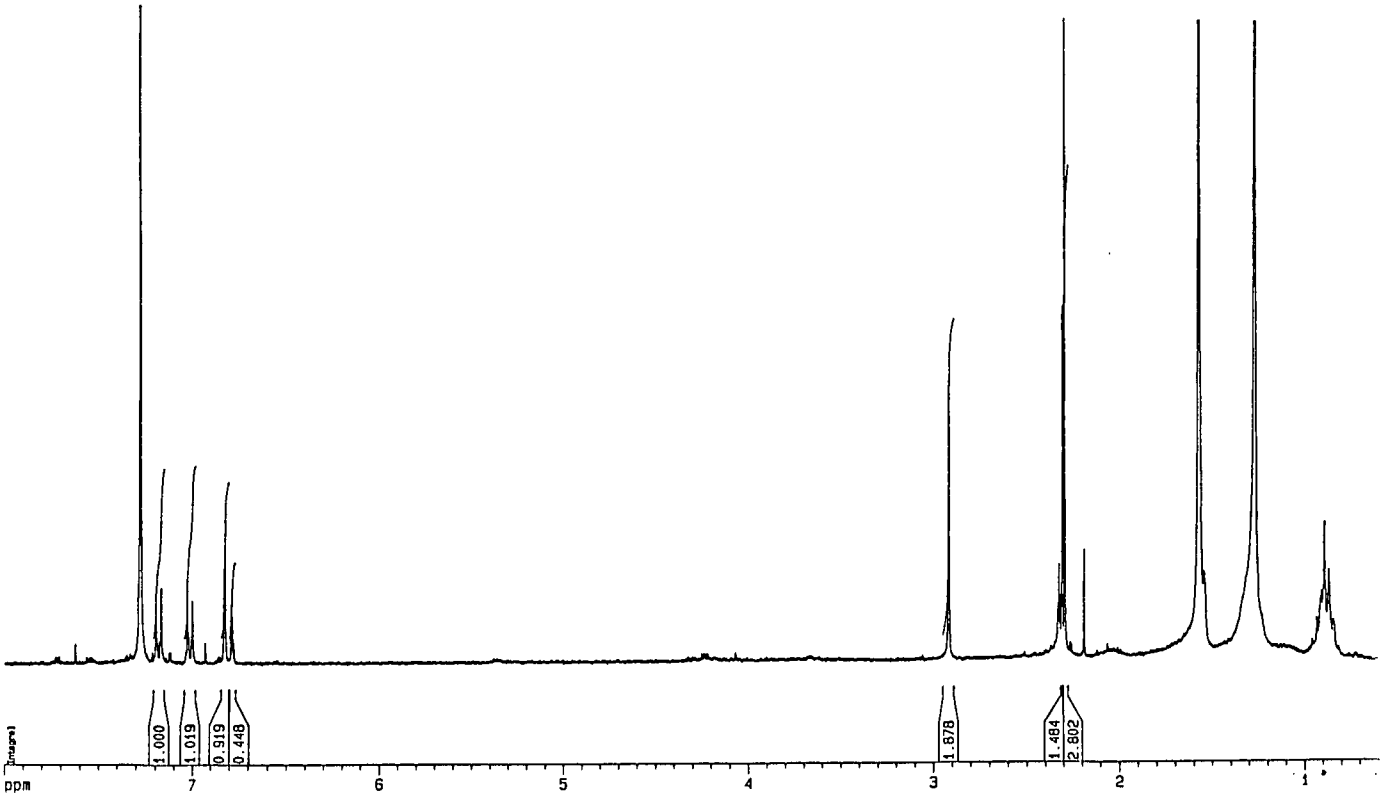
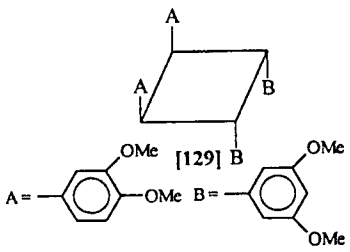
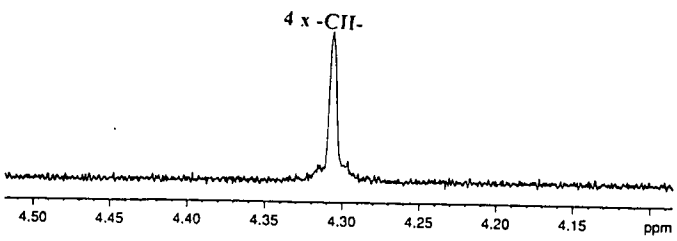
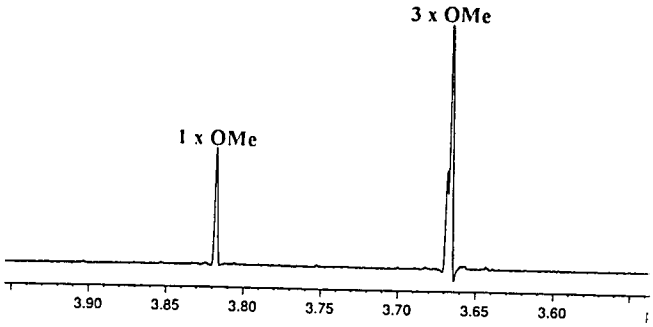
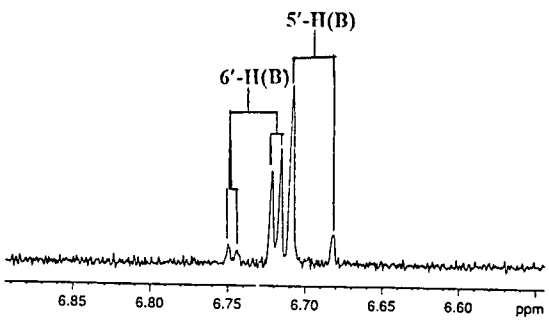


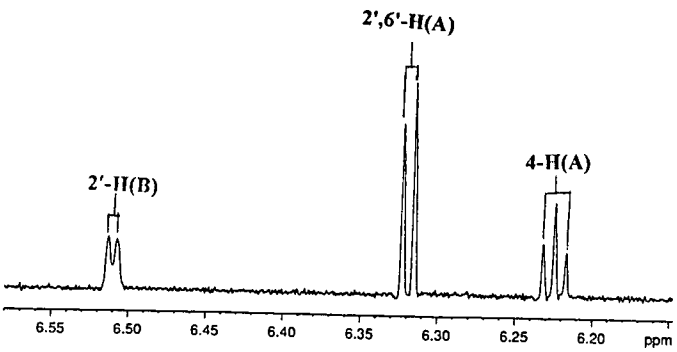
Plate 16 ($CDCl_3$ 296K)











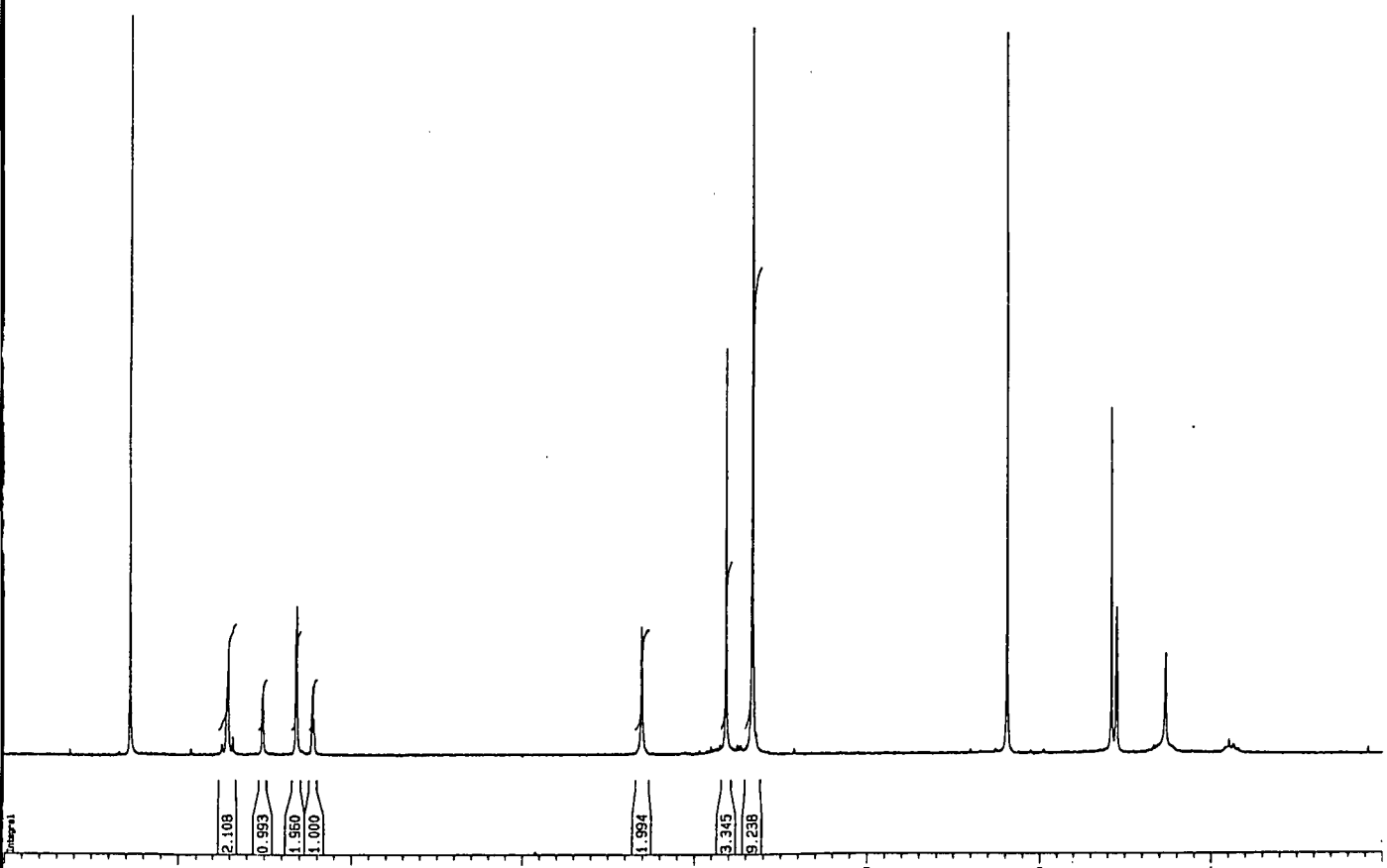
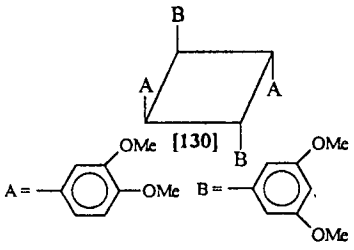
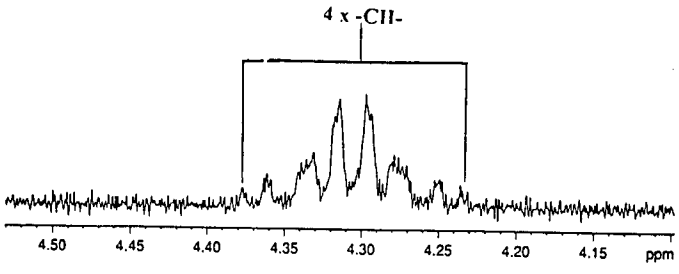
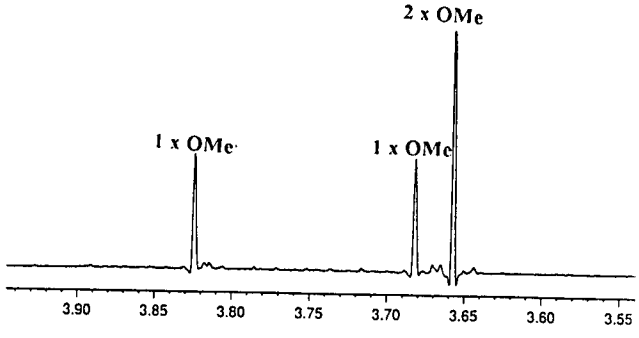
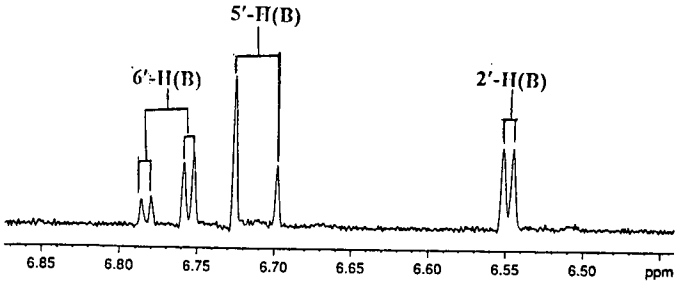


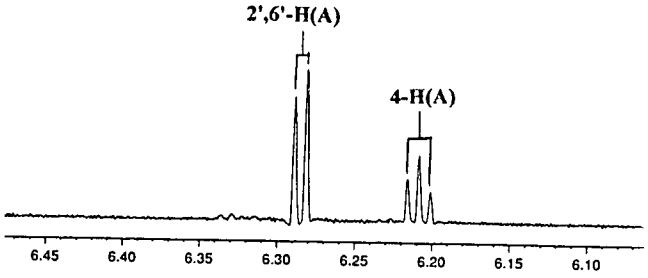
Plate 17 ($CDCl_3$ 296K)











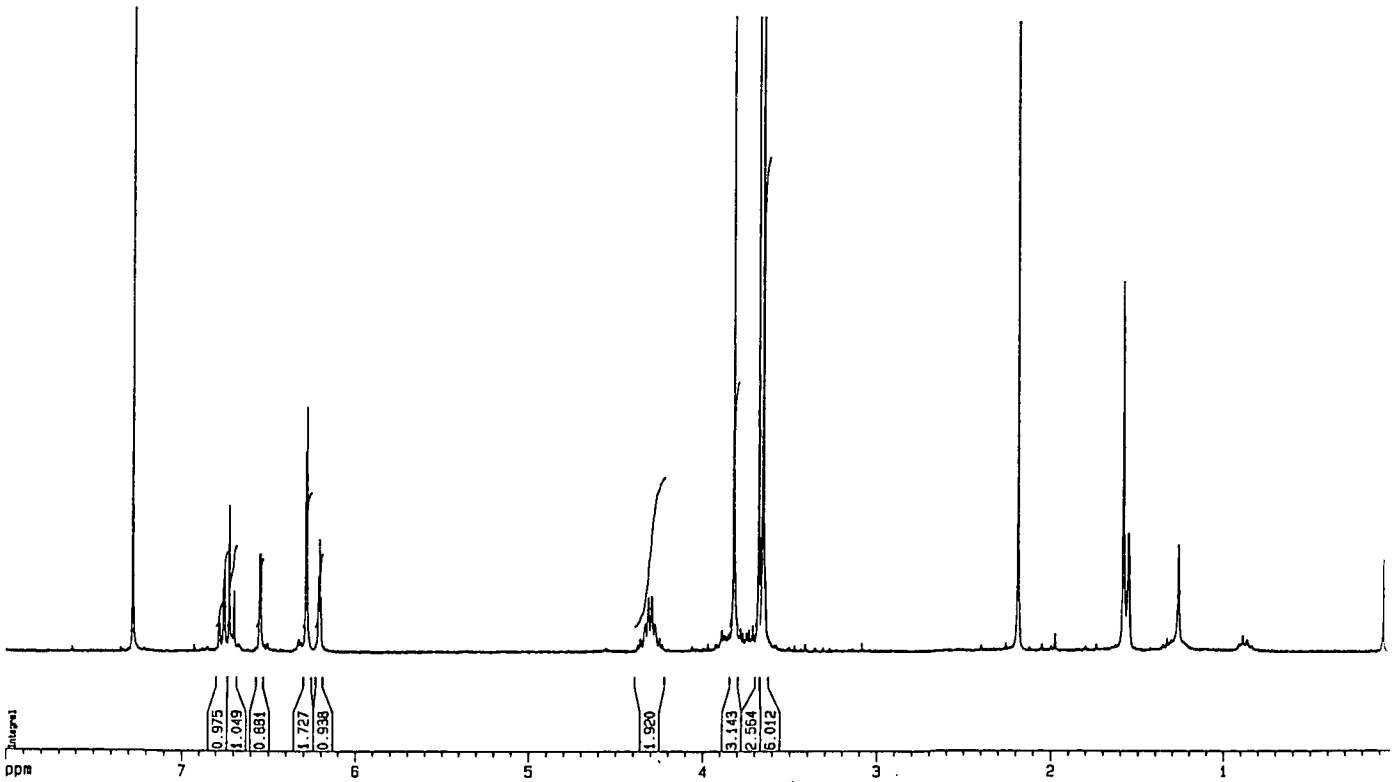
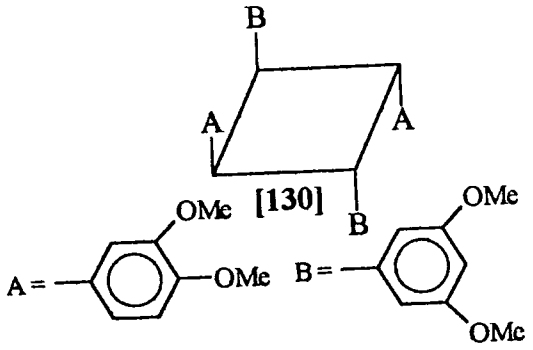
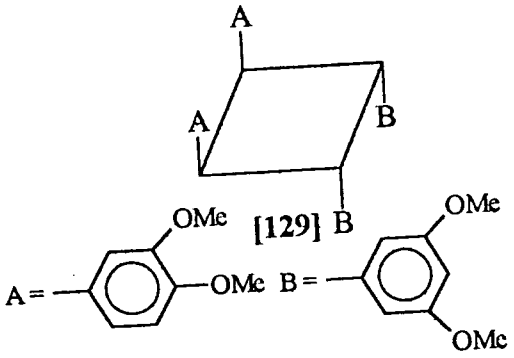


Plate 17 ($CDCl_3$ 296K)



[130]



[129]

CARBONS	CHEMICAL SHIFTS
1'	133.79
2'+6'	106.91
3'+5'	160.83
4'	98.17
1	143.72
2	112.12
3	148.70
4	147.65
5	111.07
6	120.36

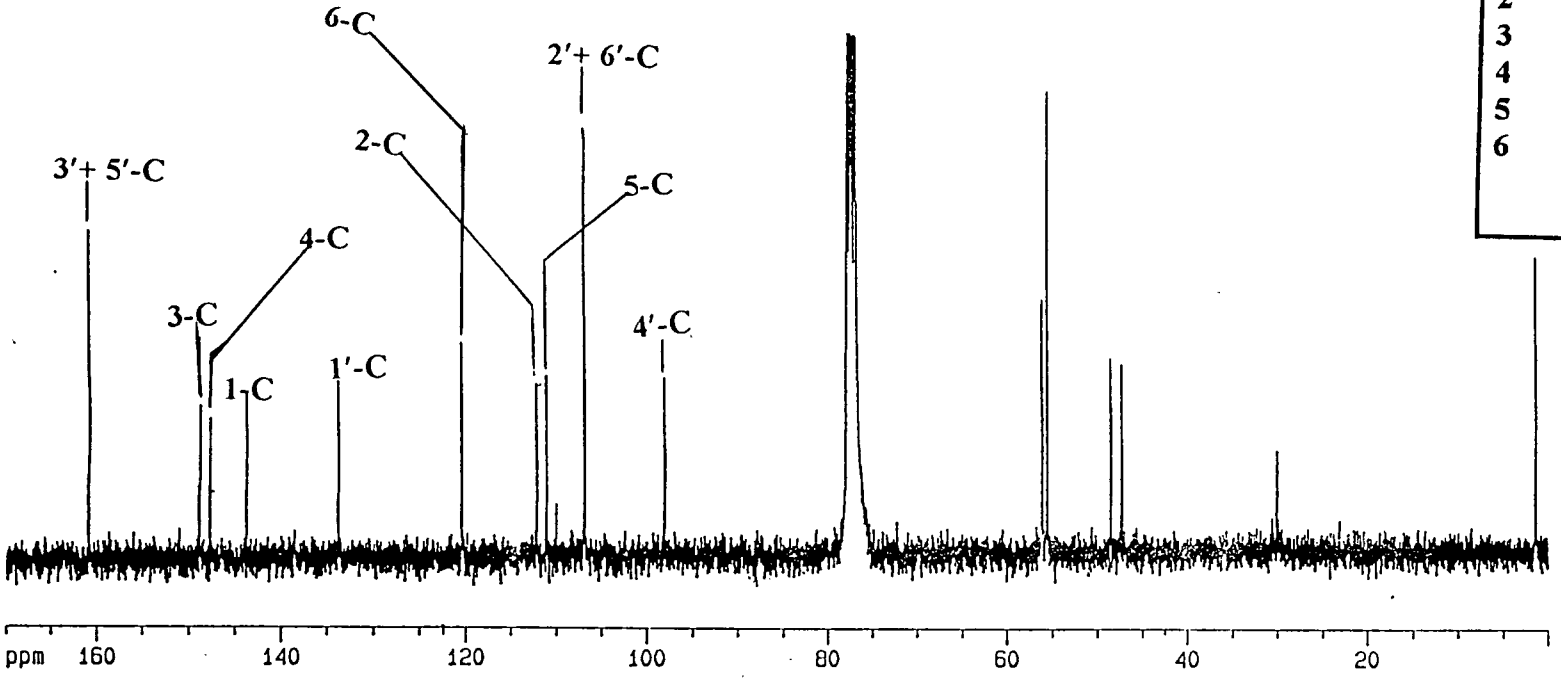
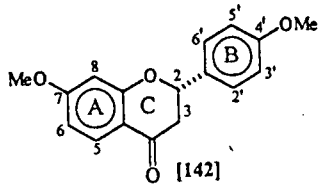
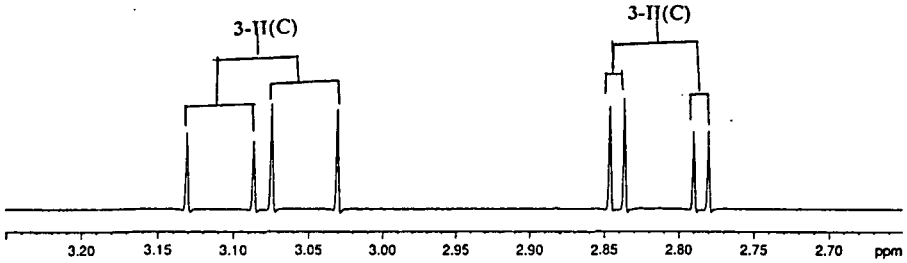
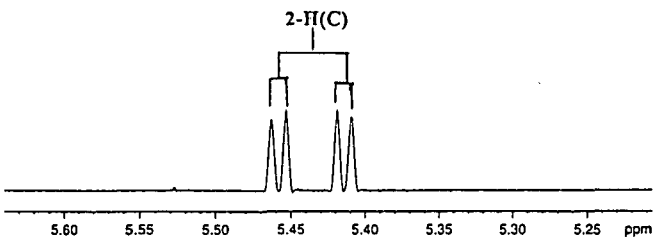
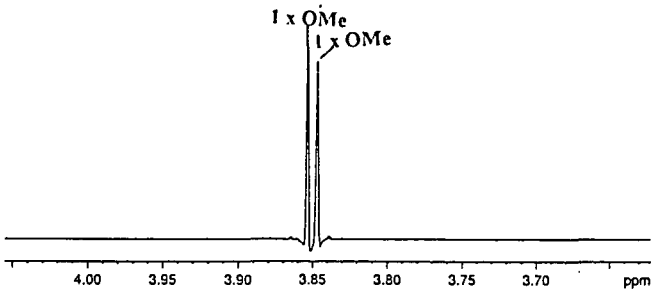


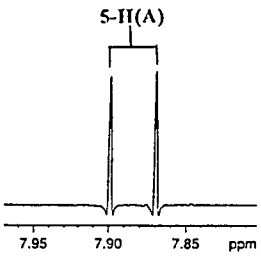
Plate 18 ($CDCl_3$ 296K)

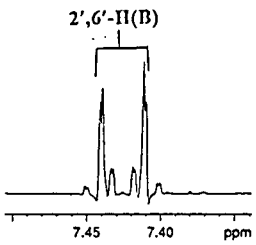


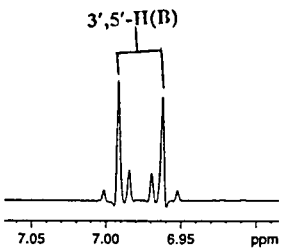


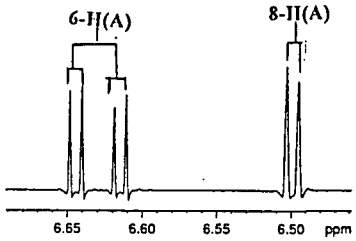












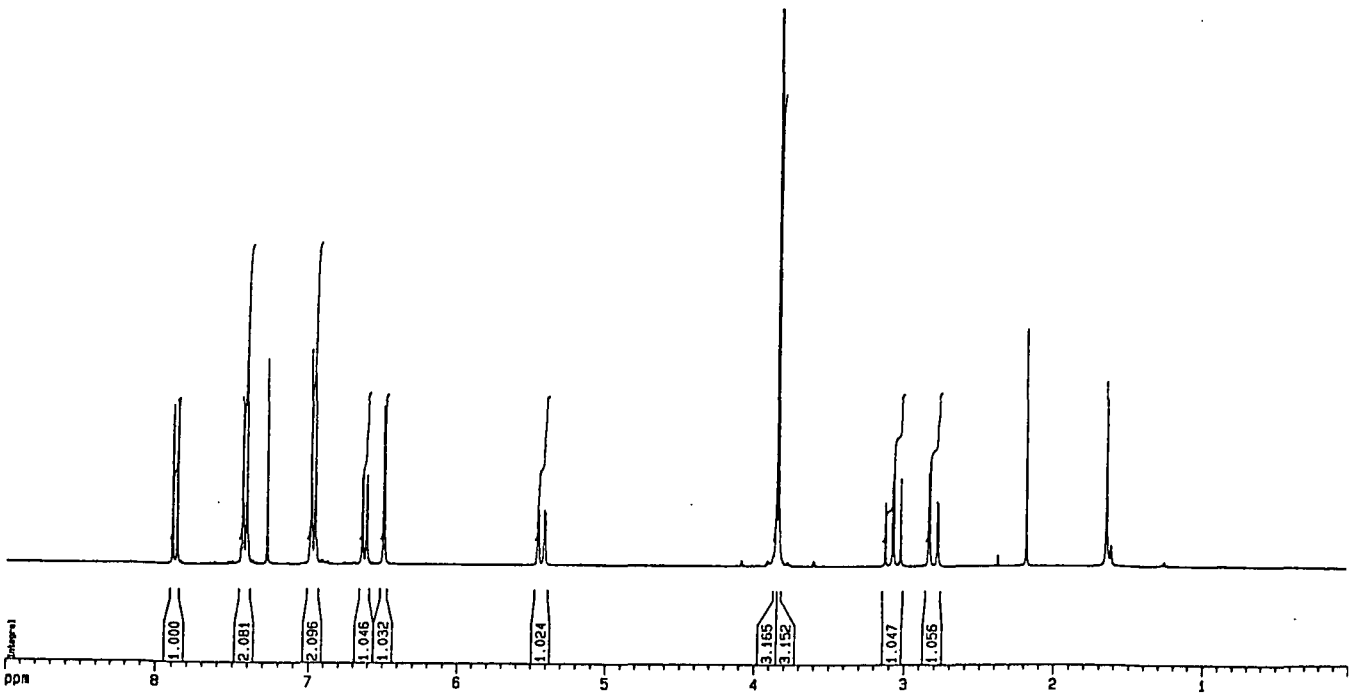
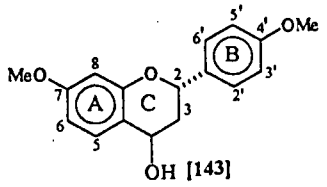
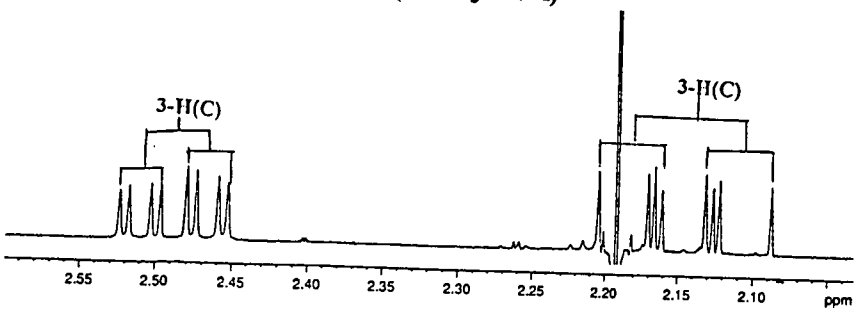
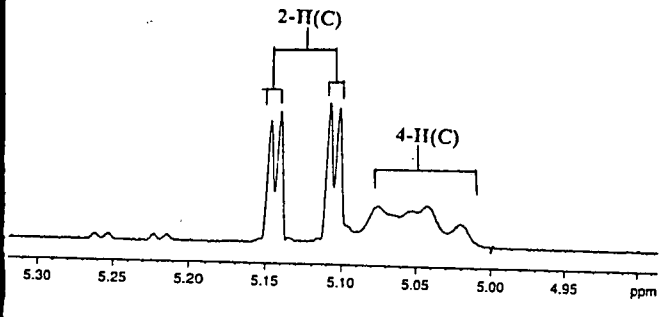
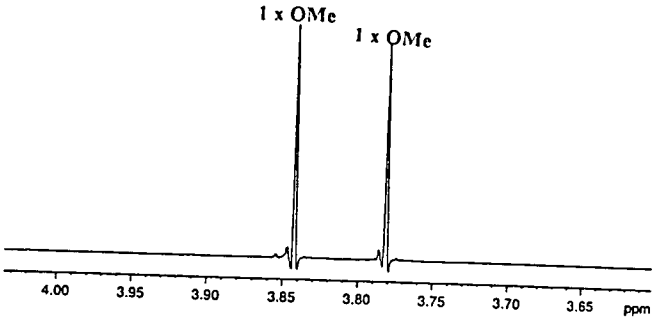


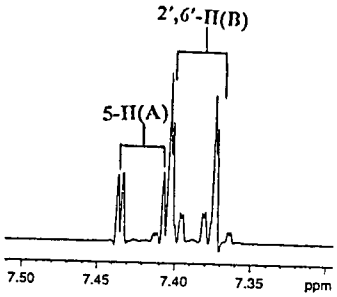
Plate 19 ($CDCl_3$ 296K)

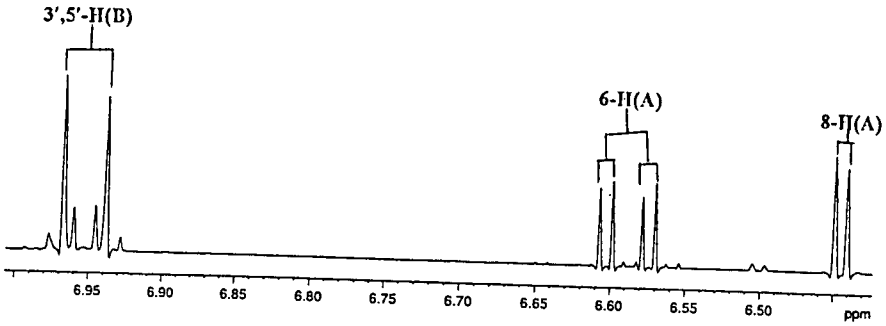












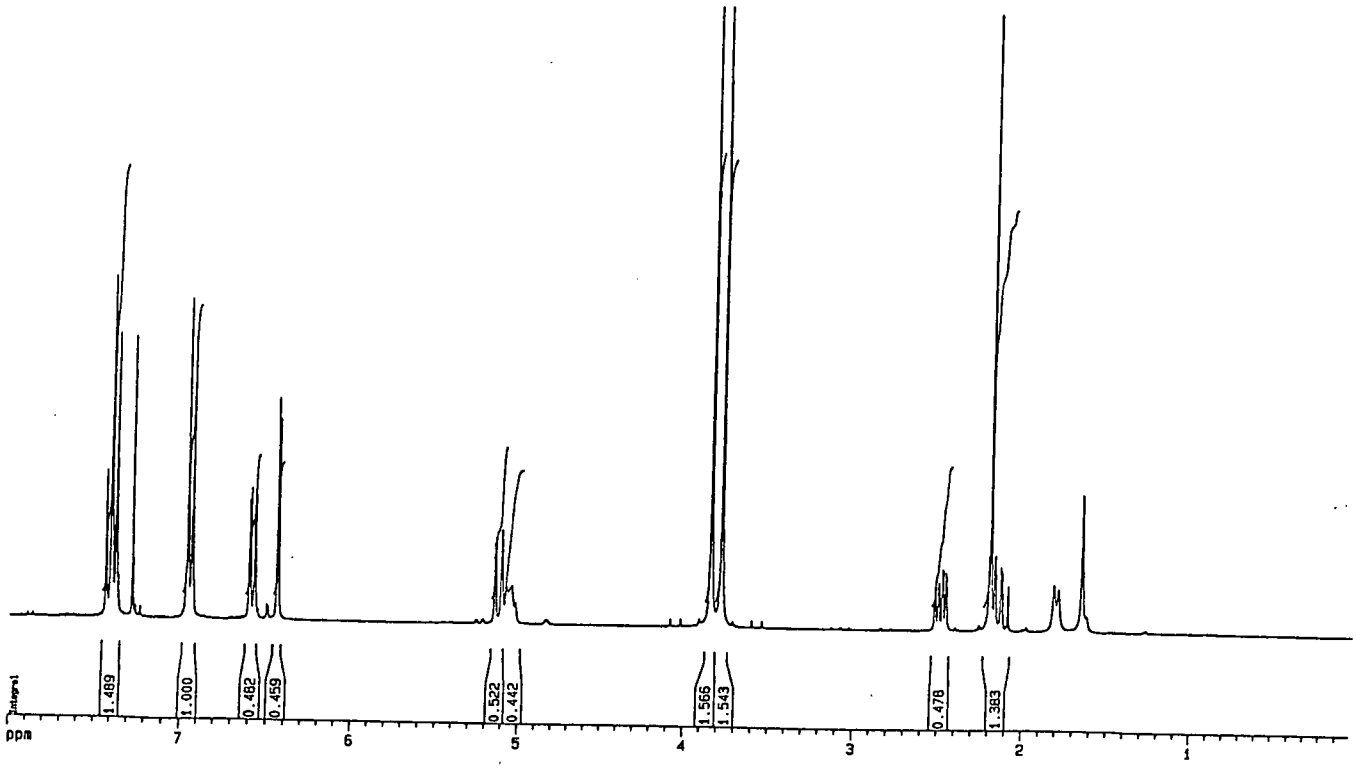
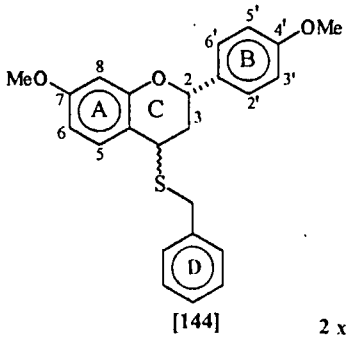
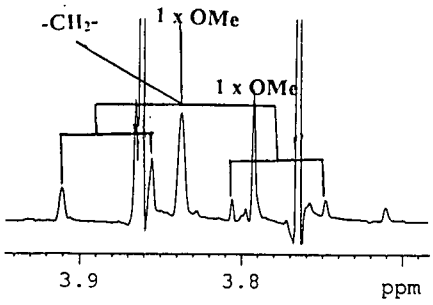
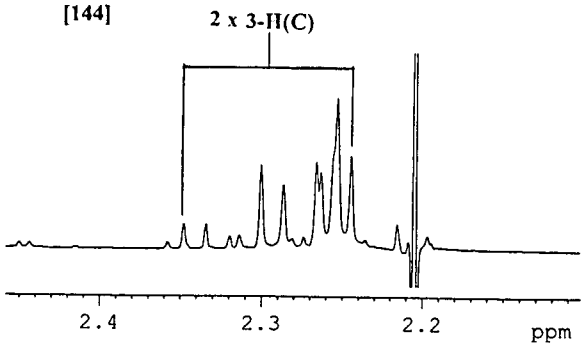


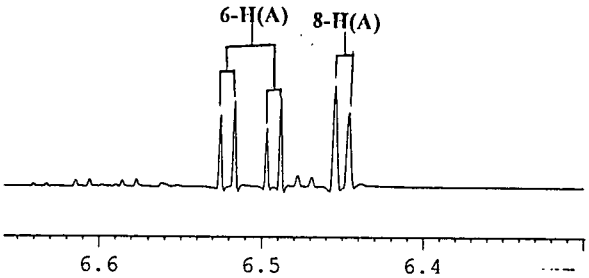
Plate 20 ($CDCl_3$ 296K)

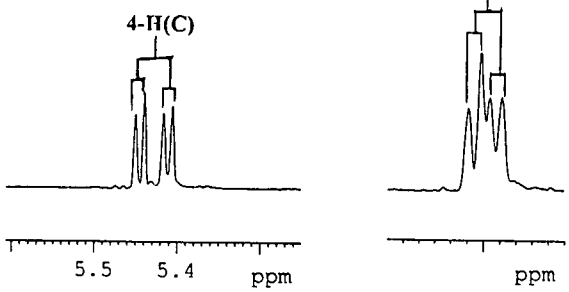


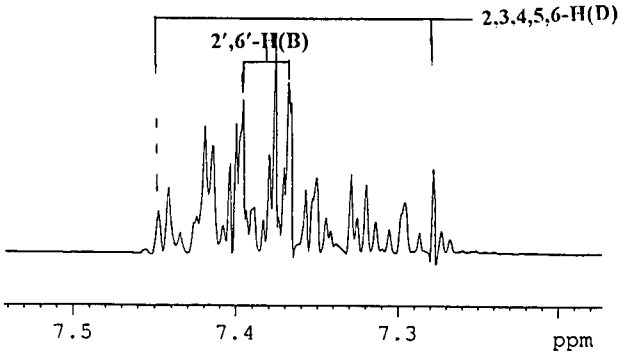
[144]

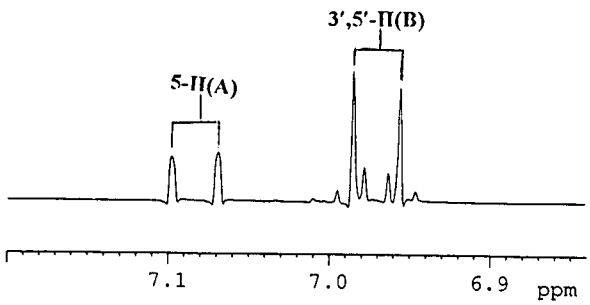












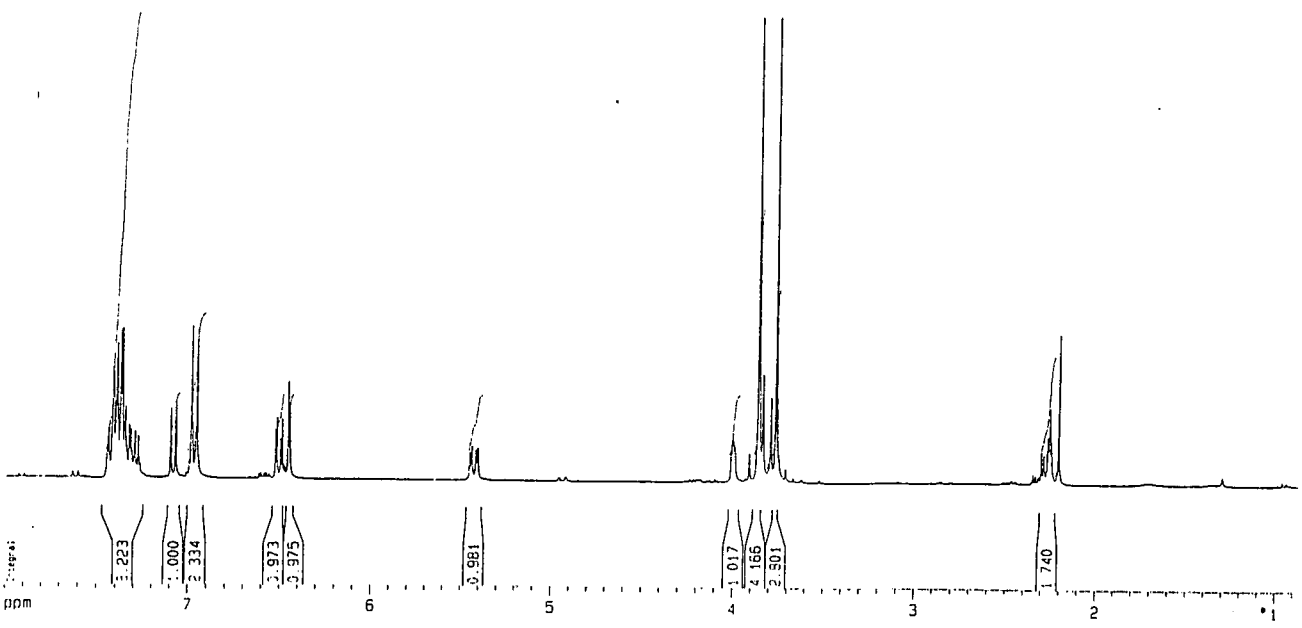


Plate 21 (C_6D_6 343K)

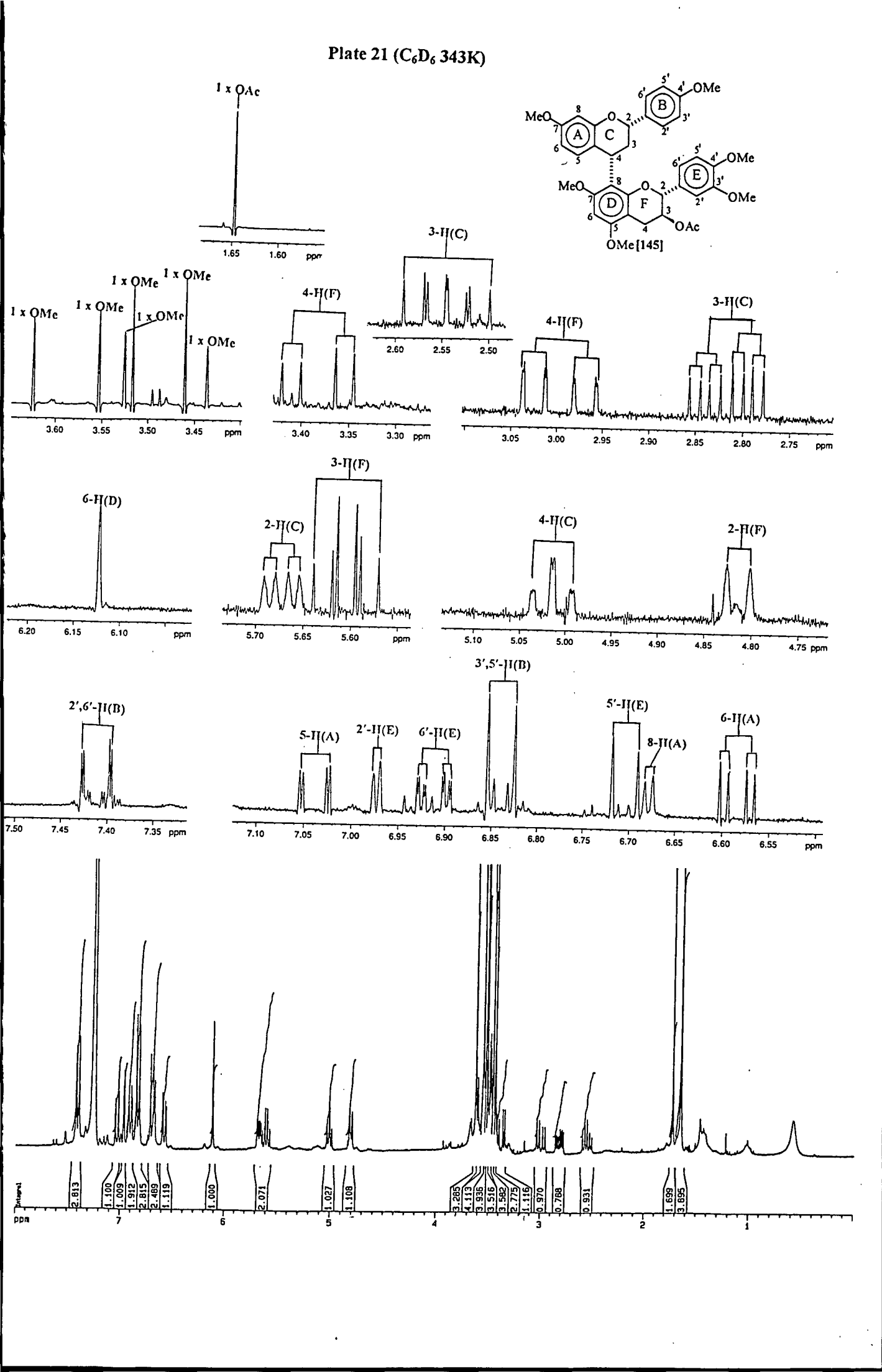
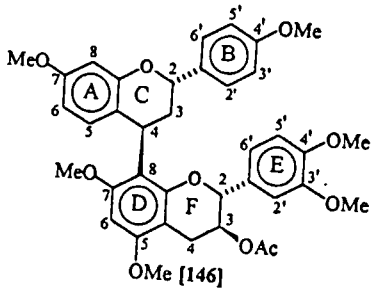
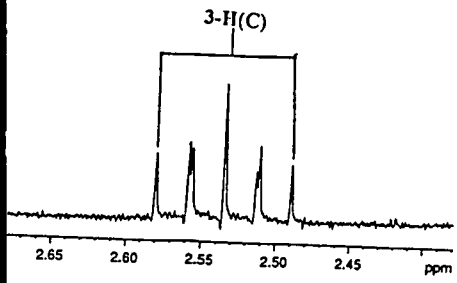
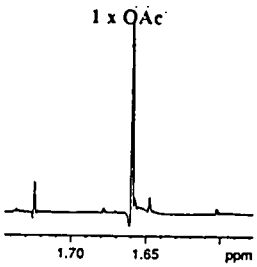
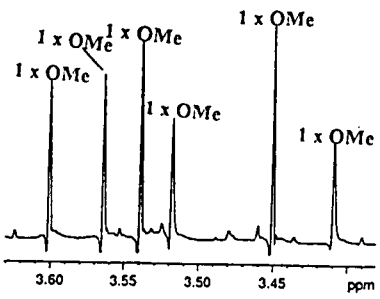


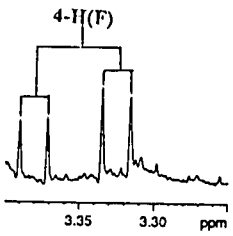
Plate 22 (C_6D_6 343K)

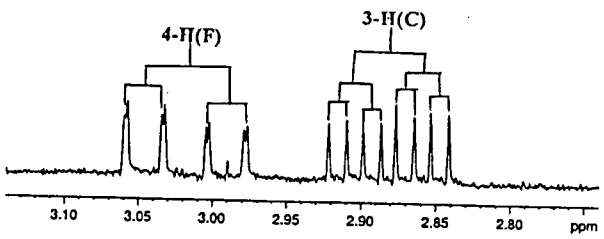


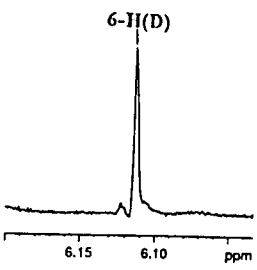


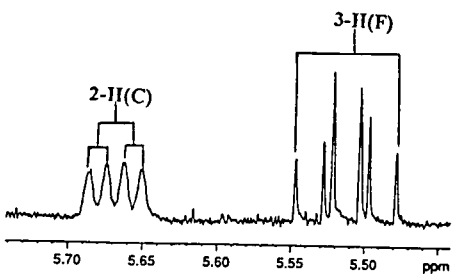


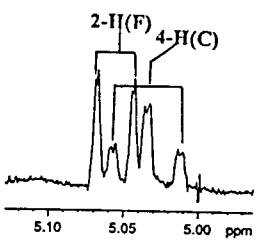


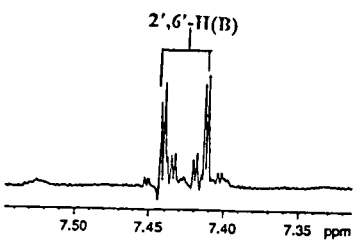


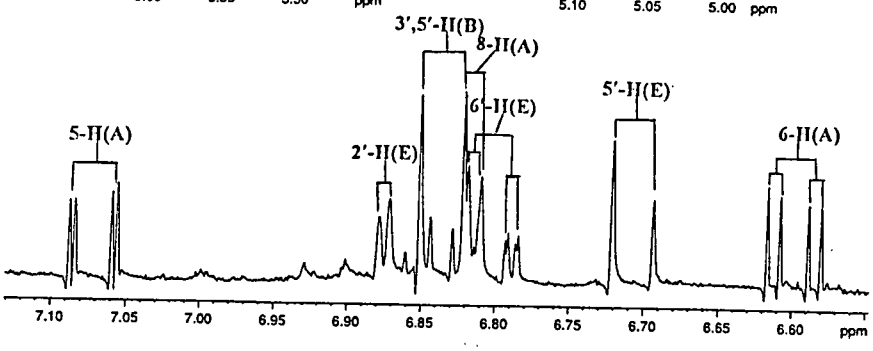












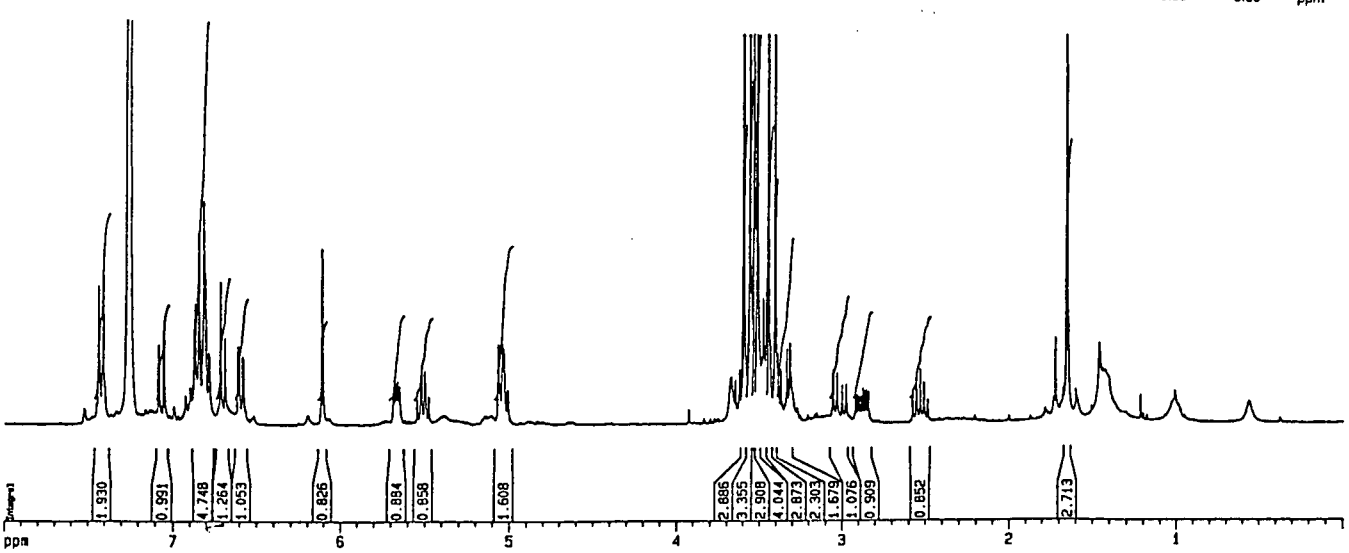
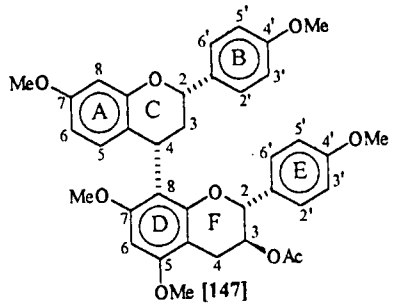
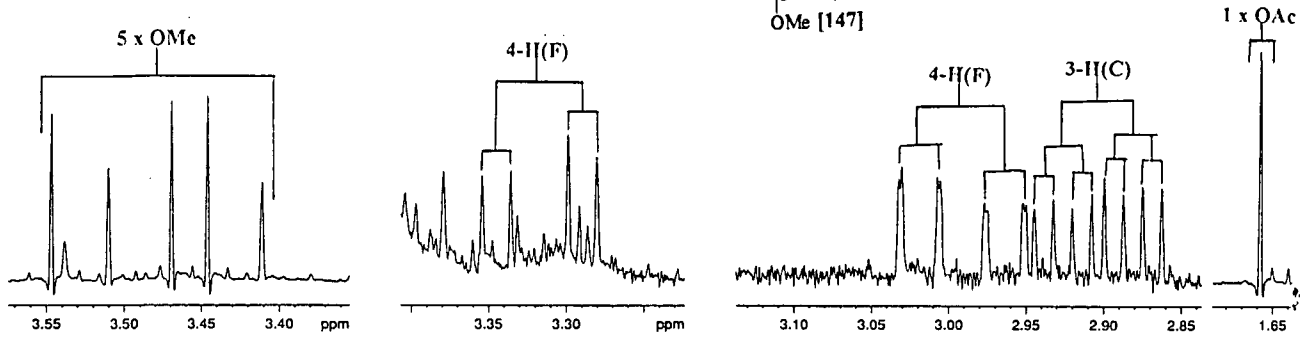
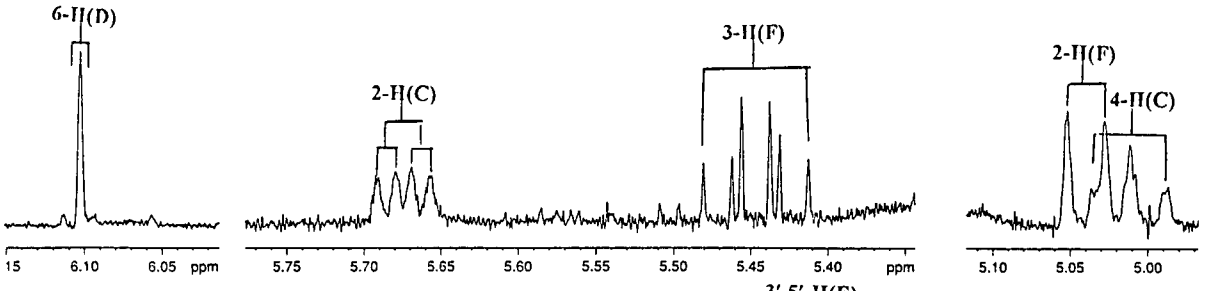
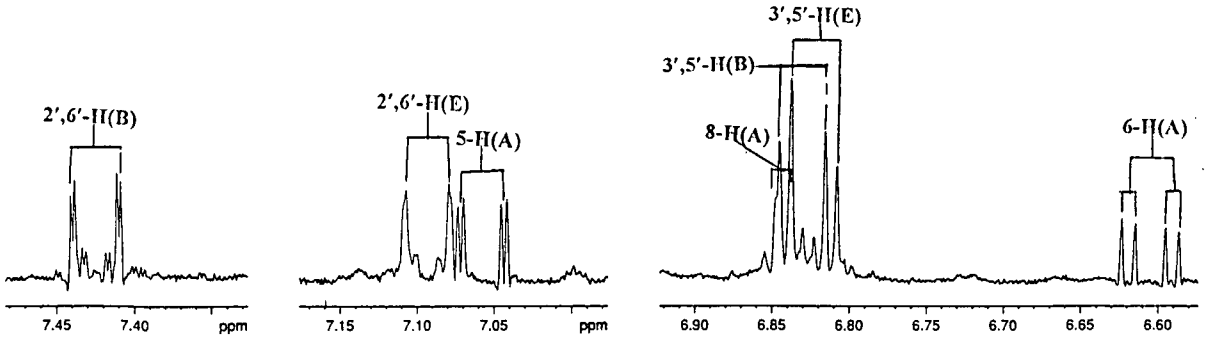


Plate 23 (C_6D_6 343K)









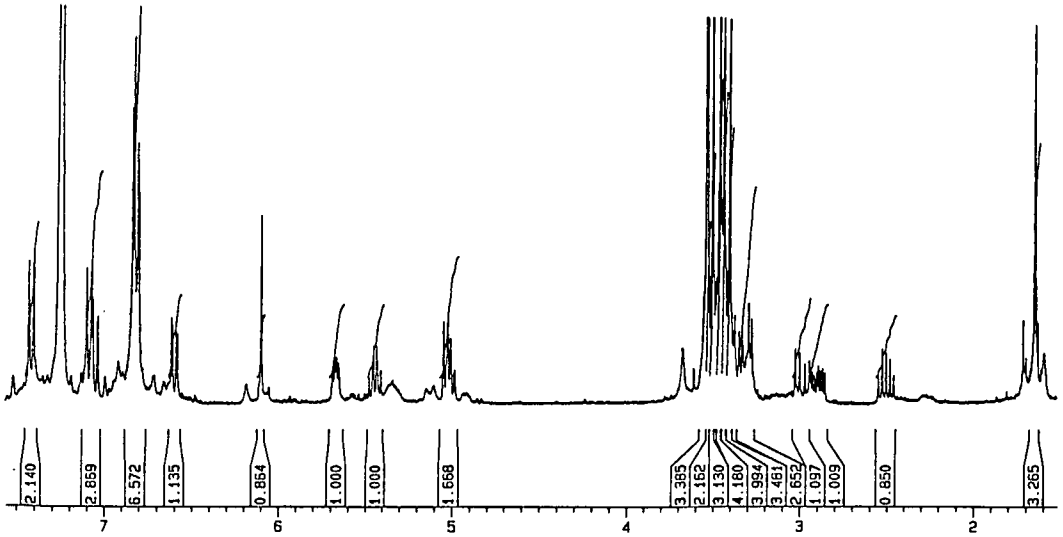
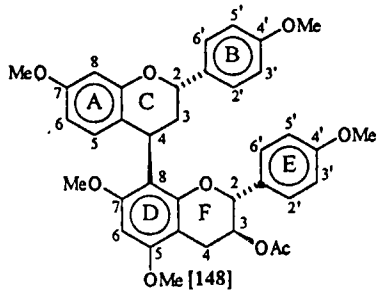
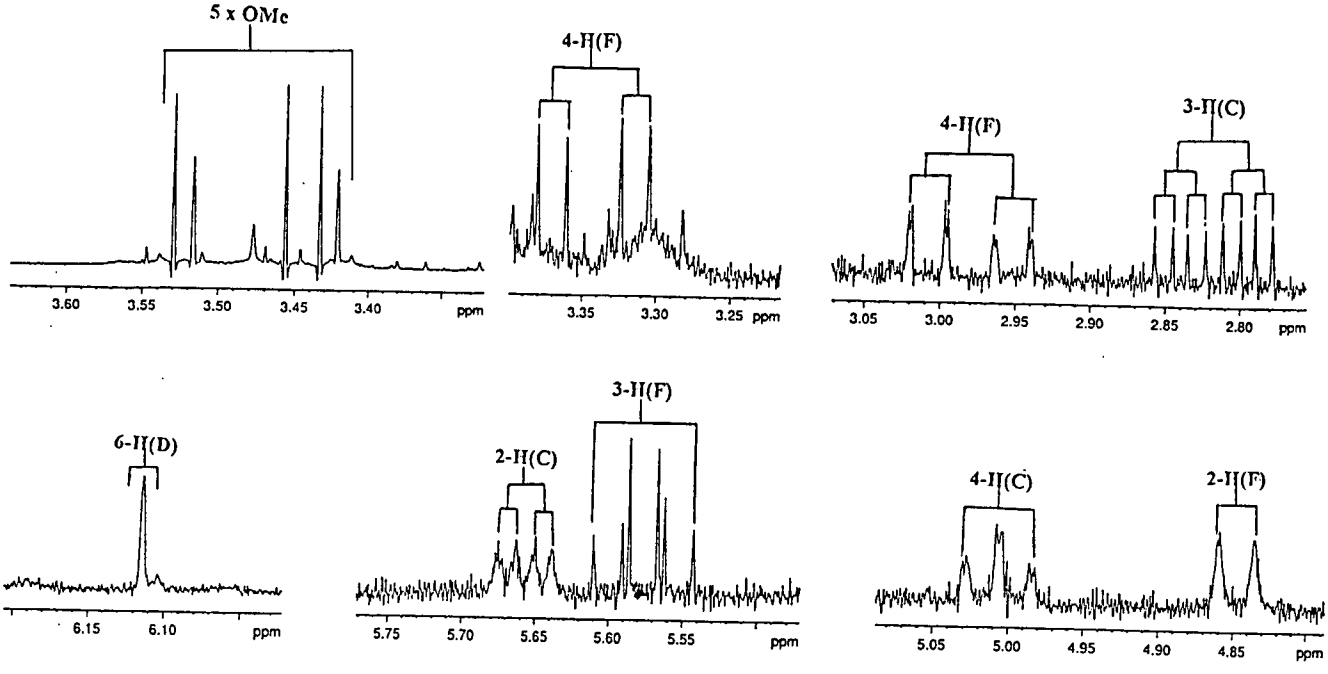


Plate 24 (C_6D_6 343K)





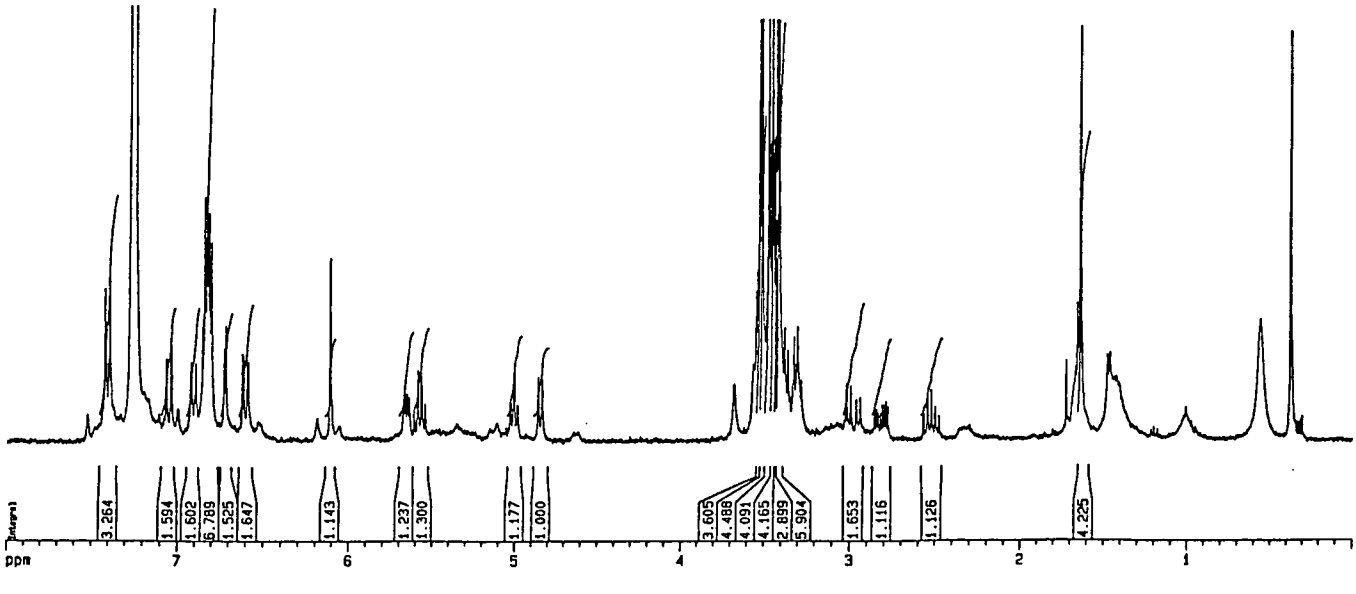
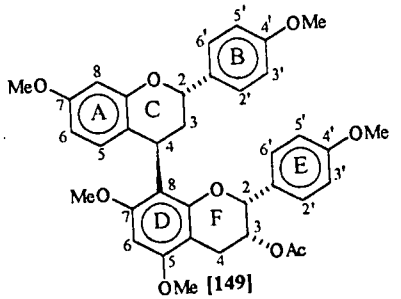
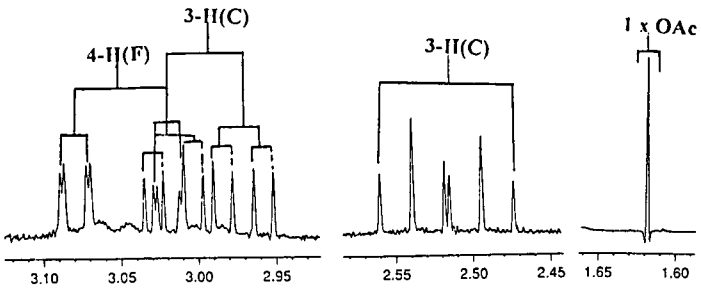
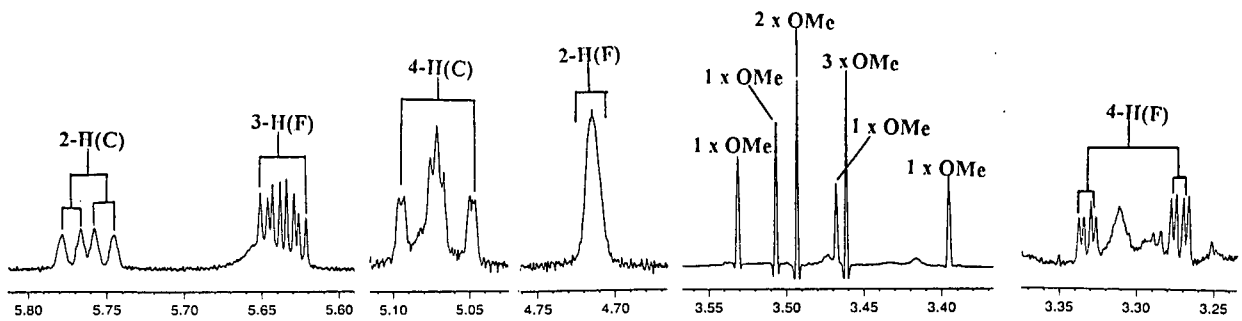
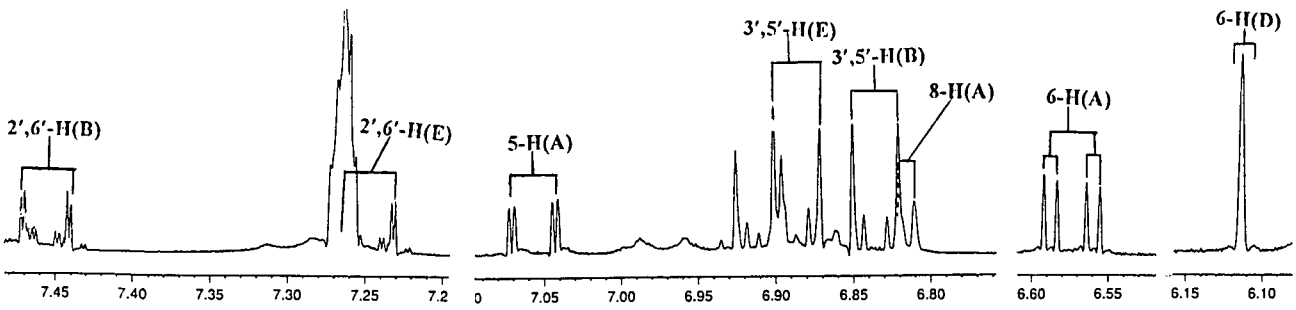


Plate 25 (C_6D_6 343K)









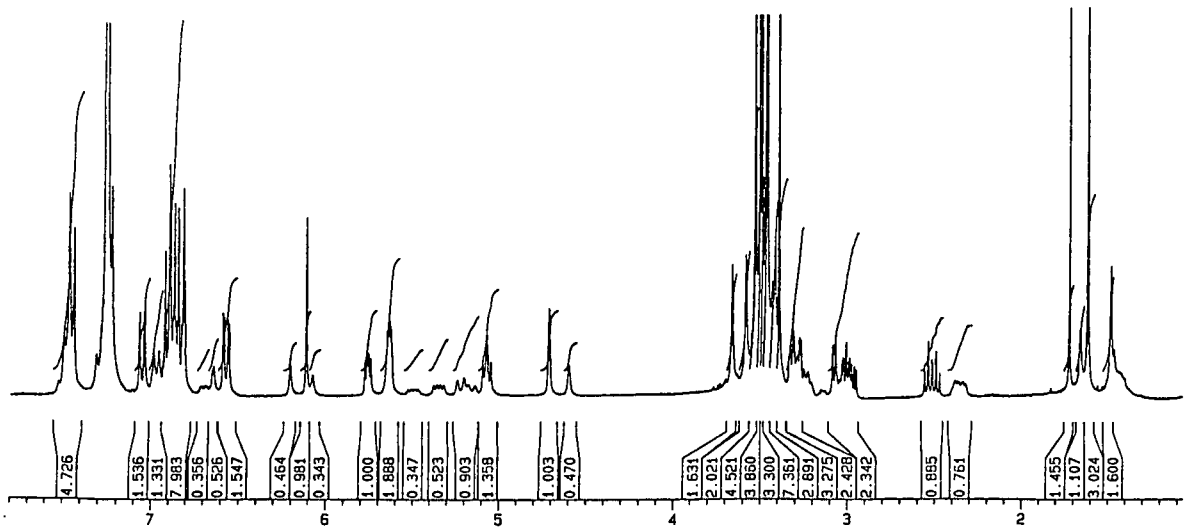
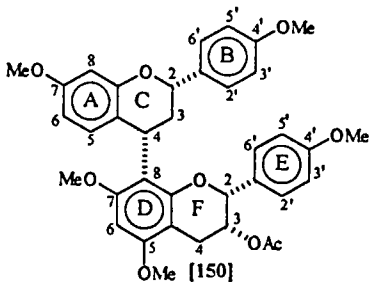


Plate 26 (C_6D_6 343K)



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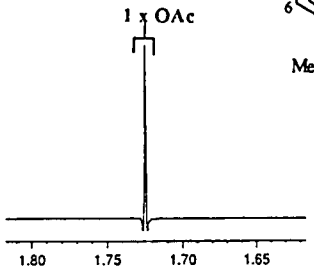
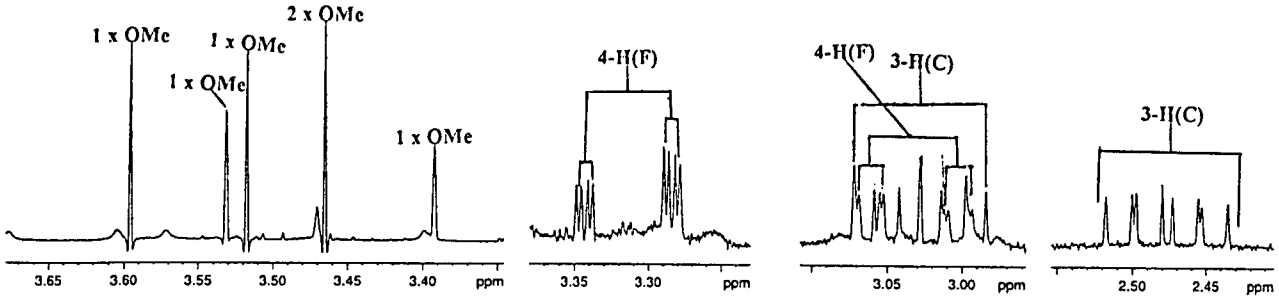
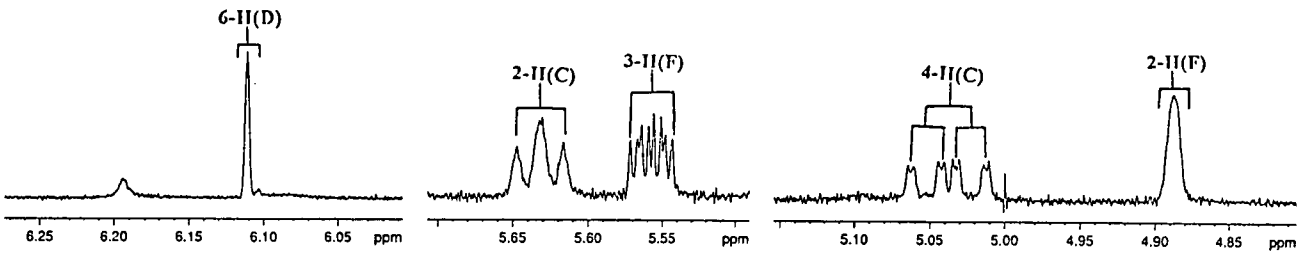
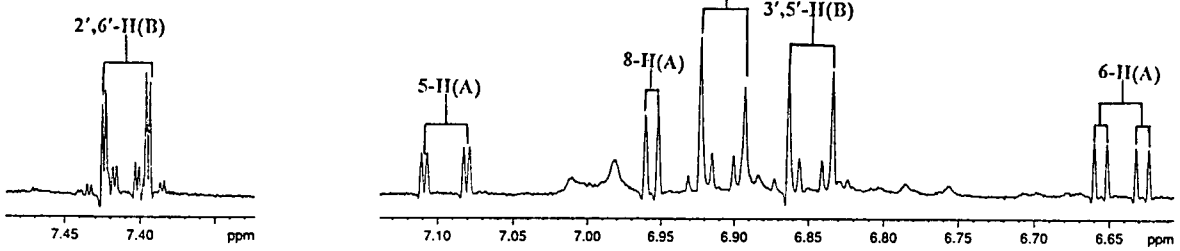








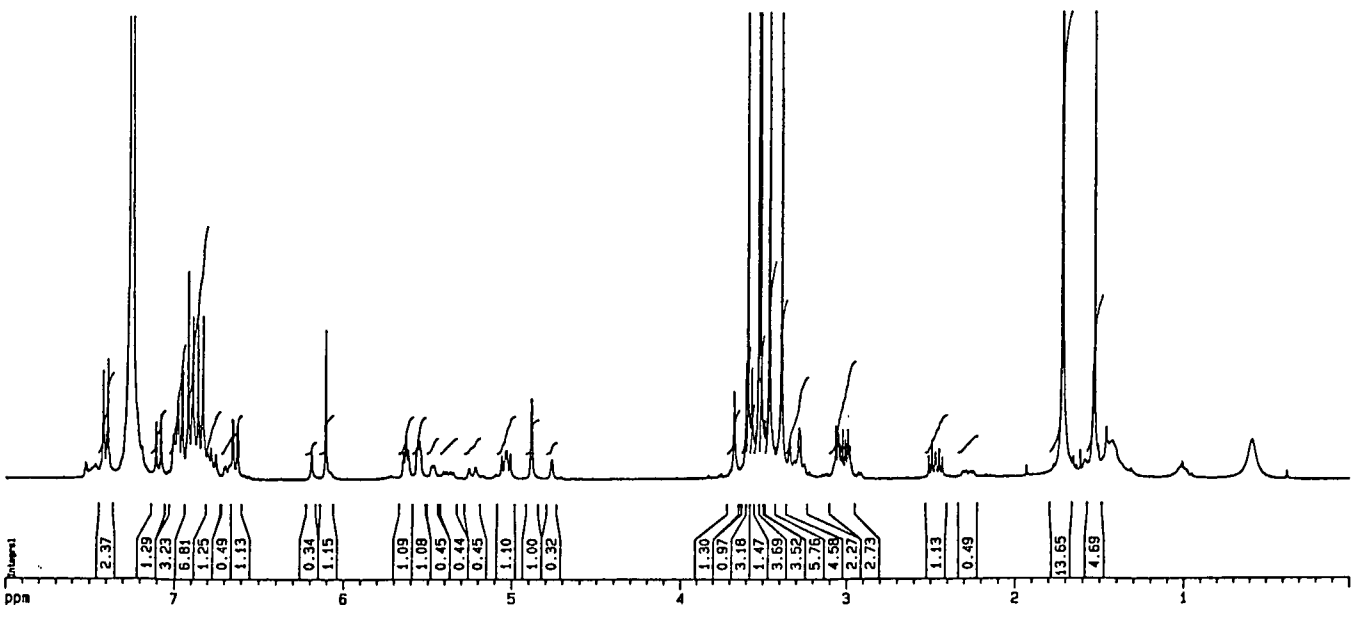
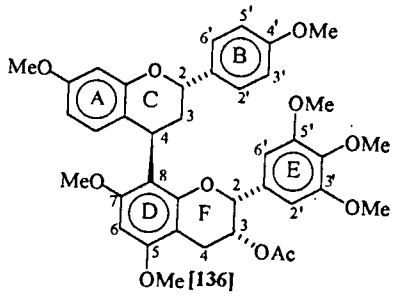
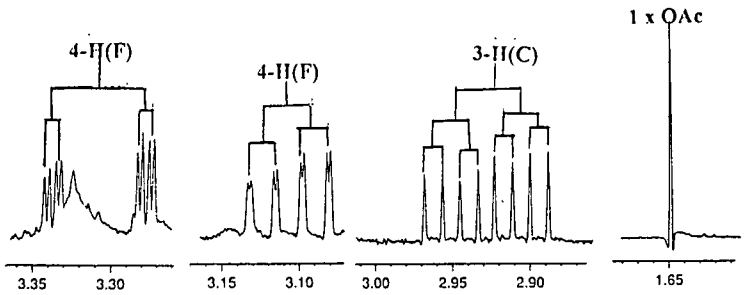
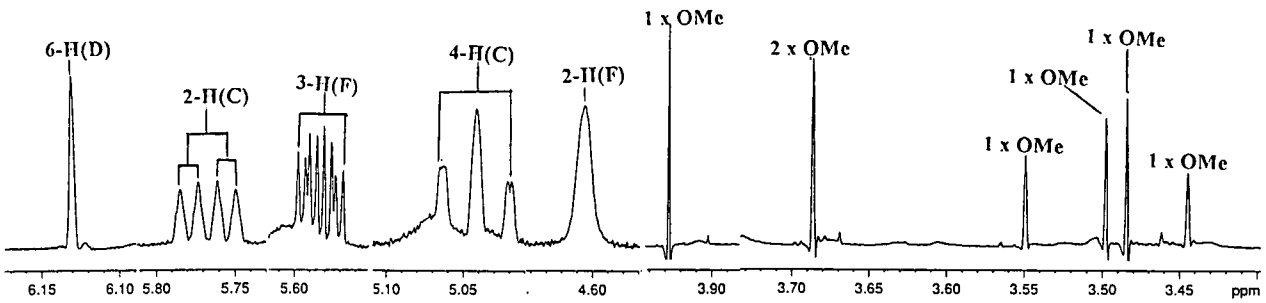
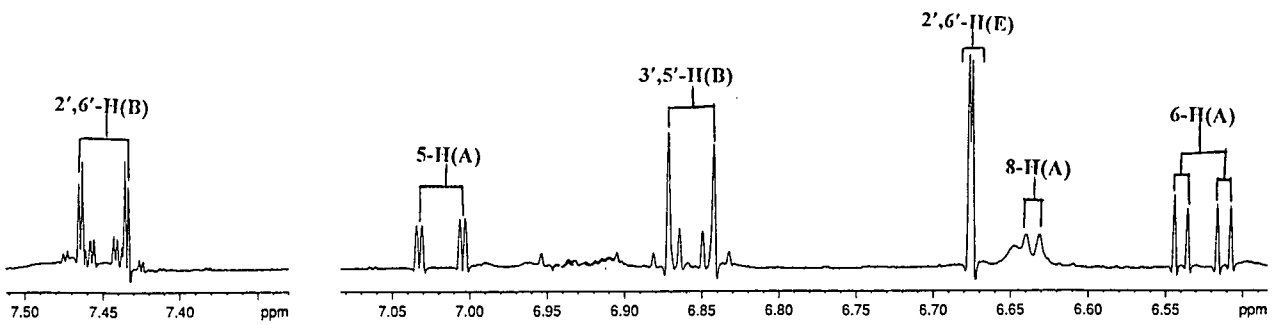


Plate 27 (C_6D_6 348K)









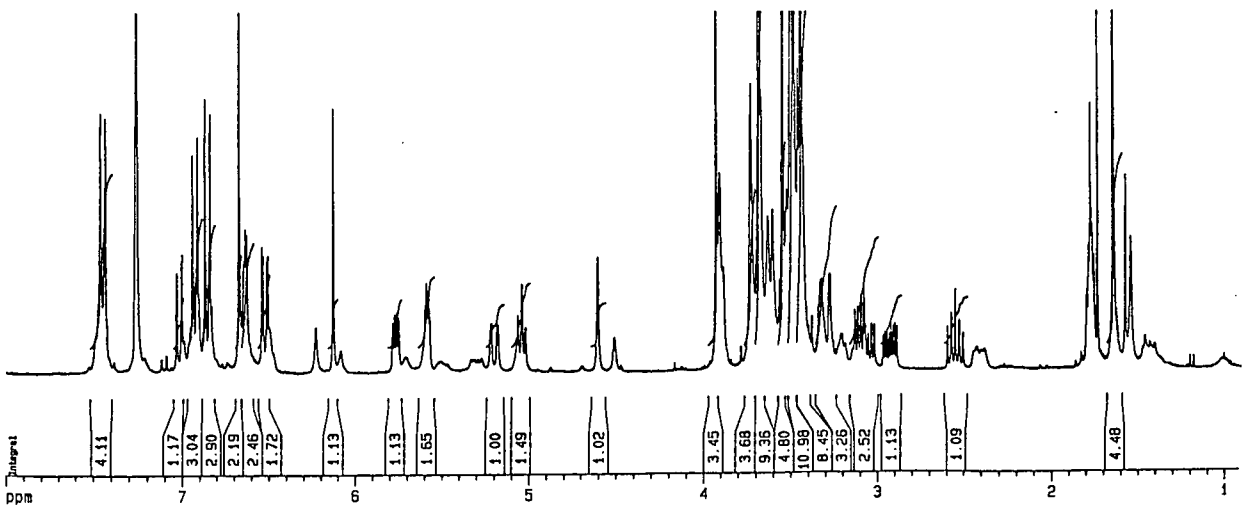
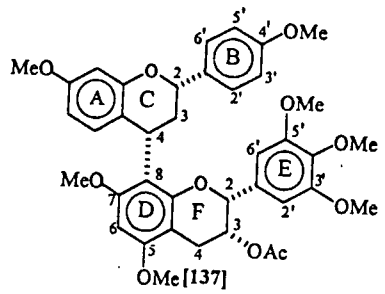
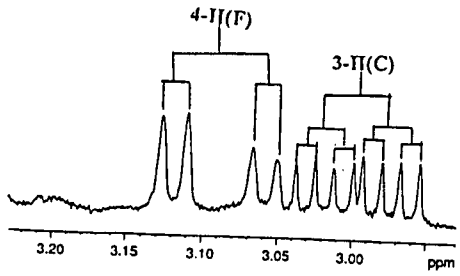
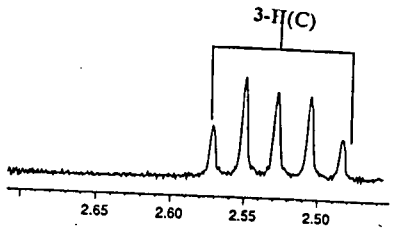
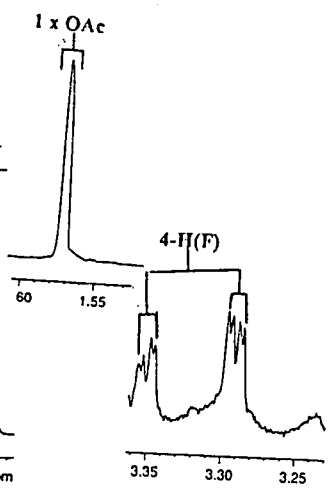


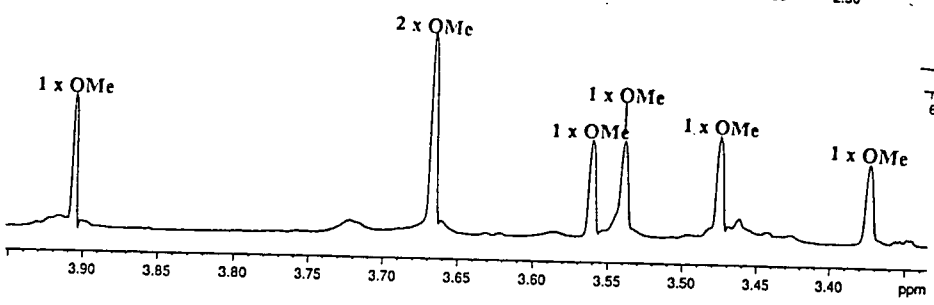
Plate 28 (C_6D_6 348K)

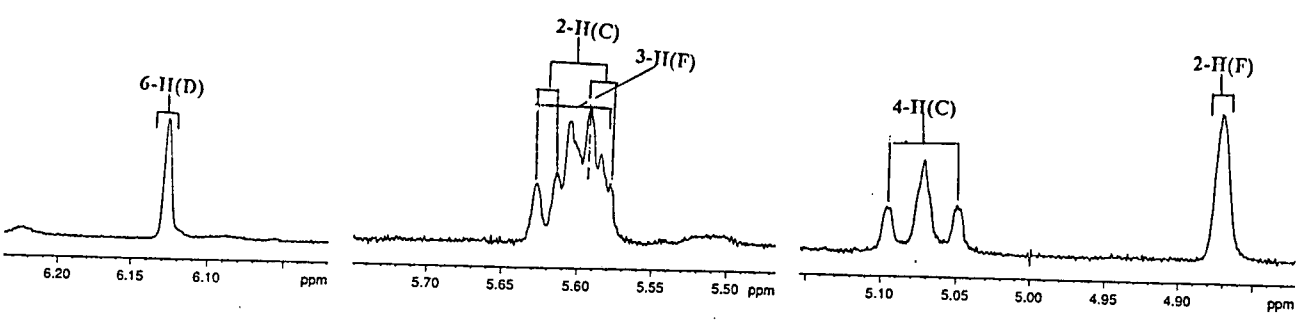


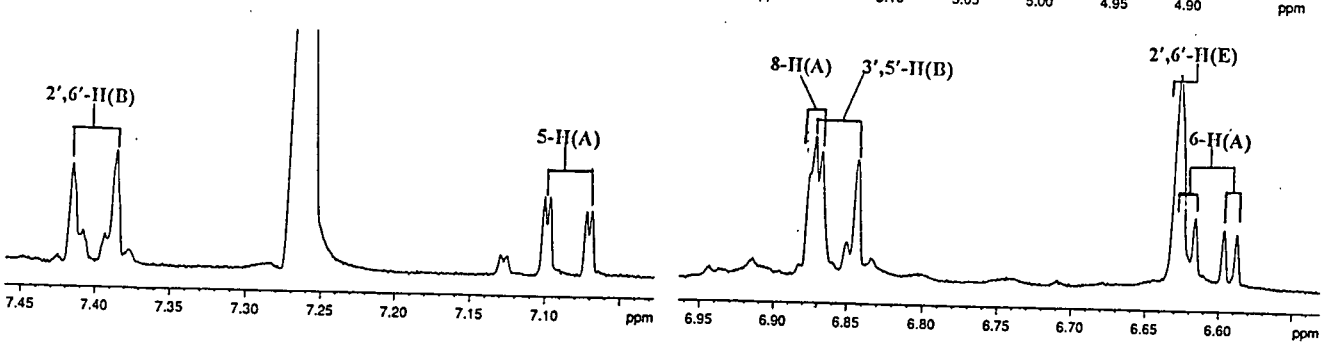












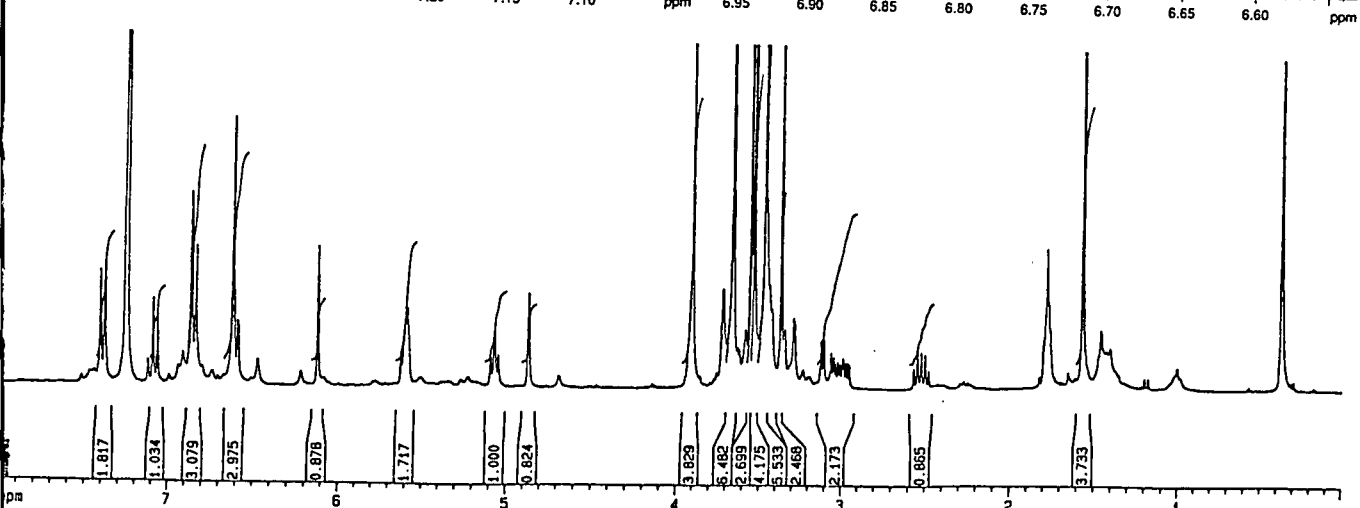
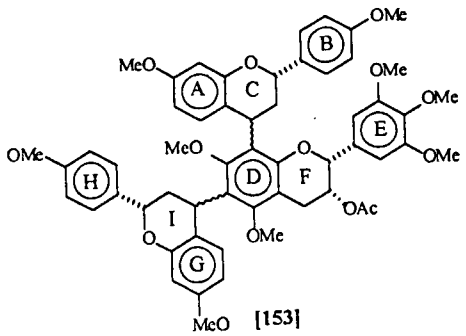
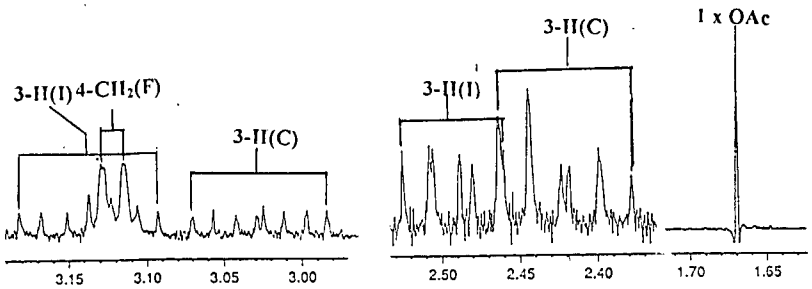
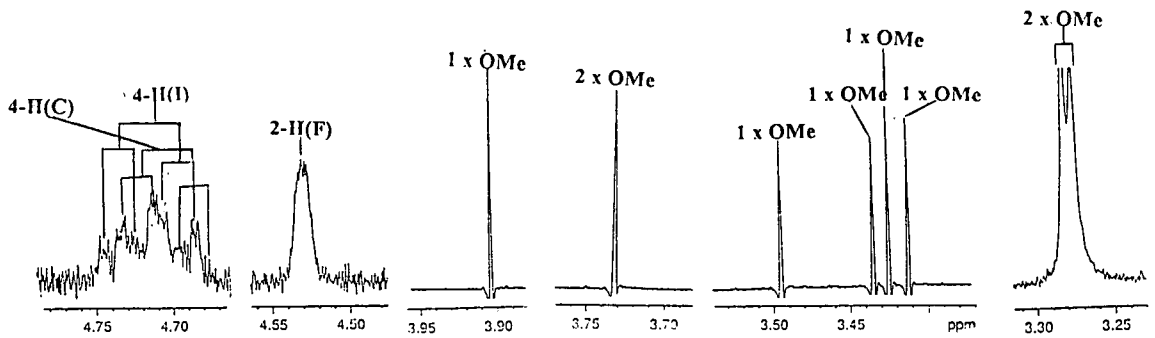


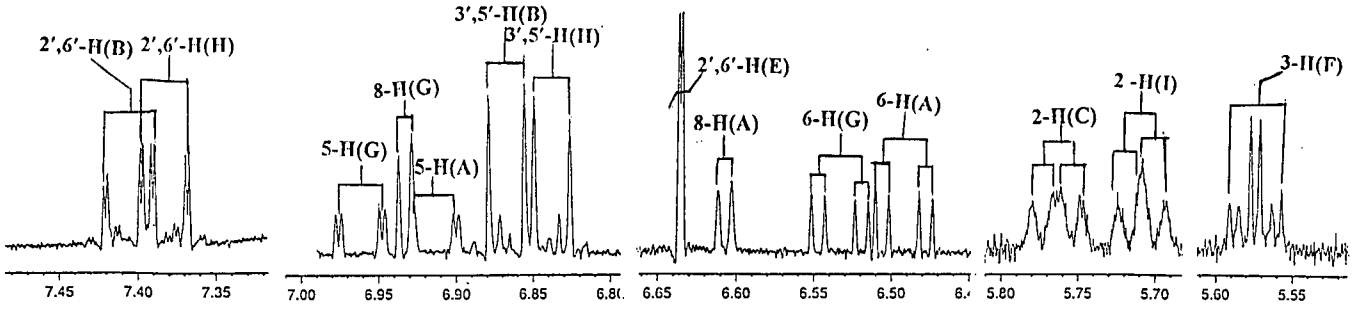
Plate 29 (C_6D_6 353K)

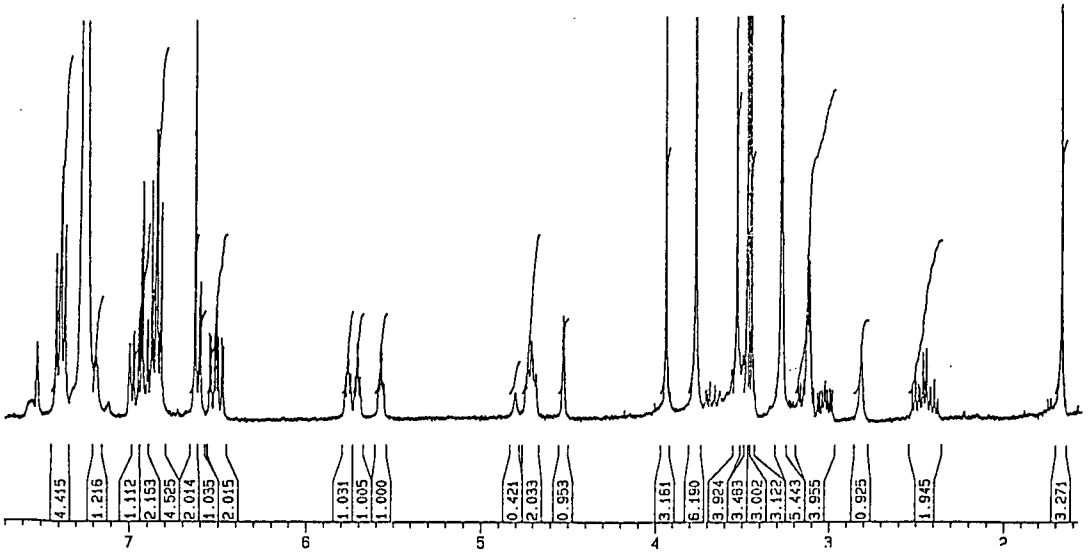


[153]

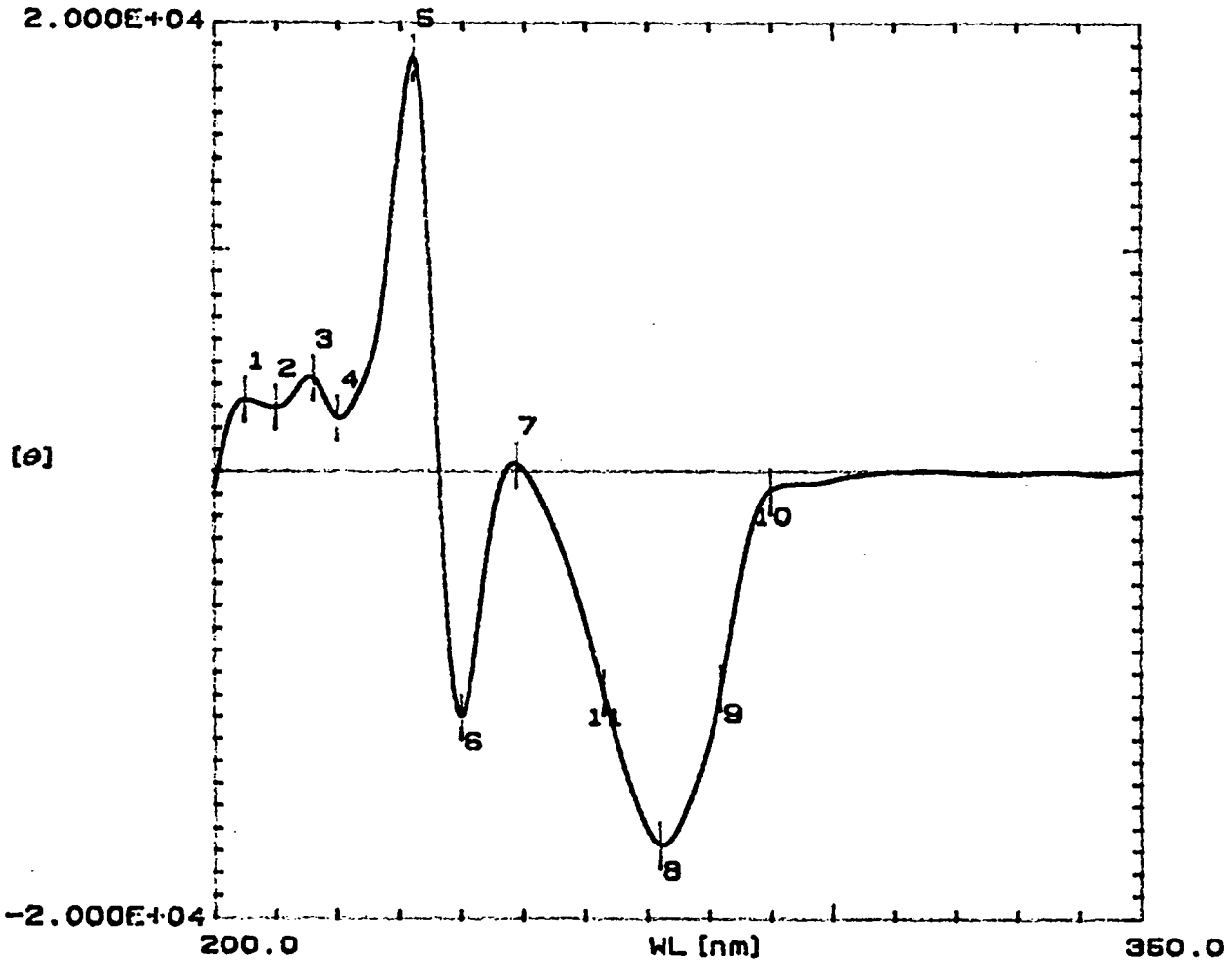




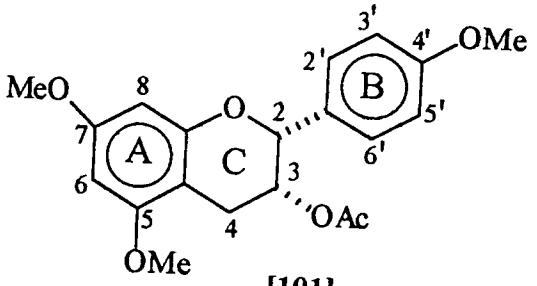




CD SPECTRAS

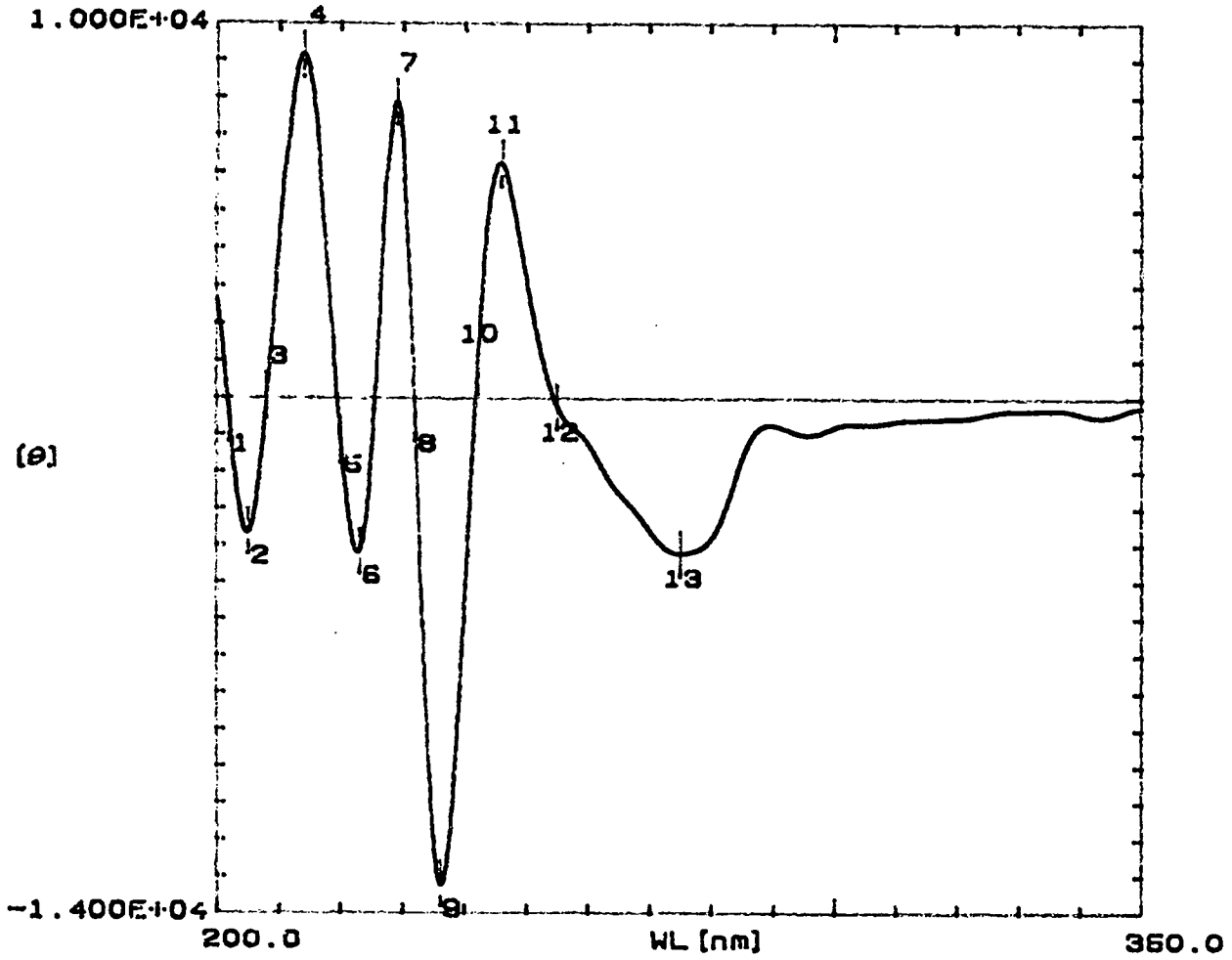


No.	Wavelength	Value
1	205.00 nm	3.296E+03
2	210.00 nm	2.941E+03
3	216.00 nm	4.282E+03
4	220.00 nm	2.482E+03
5	232.00 nm	1.844E+04
6	240.00 nm	-1.098E+04
7	249.00 nm	3.538E+02
8	272.00 nm	-1.671E+04
9	282.00 nm	-8.658E+03
10	290.00 nm	-8.396E+02
11	283.00 nm	-8.854E+03

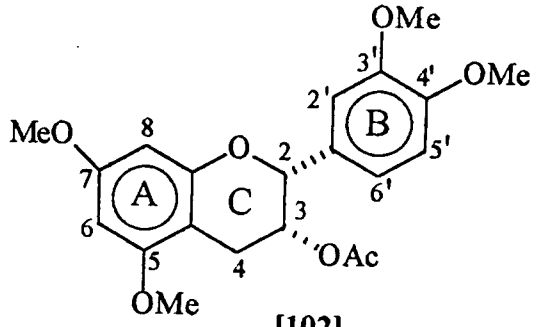


[101]

Plate1

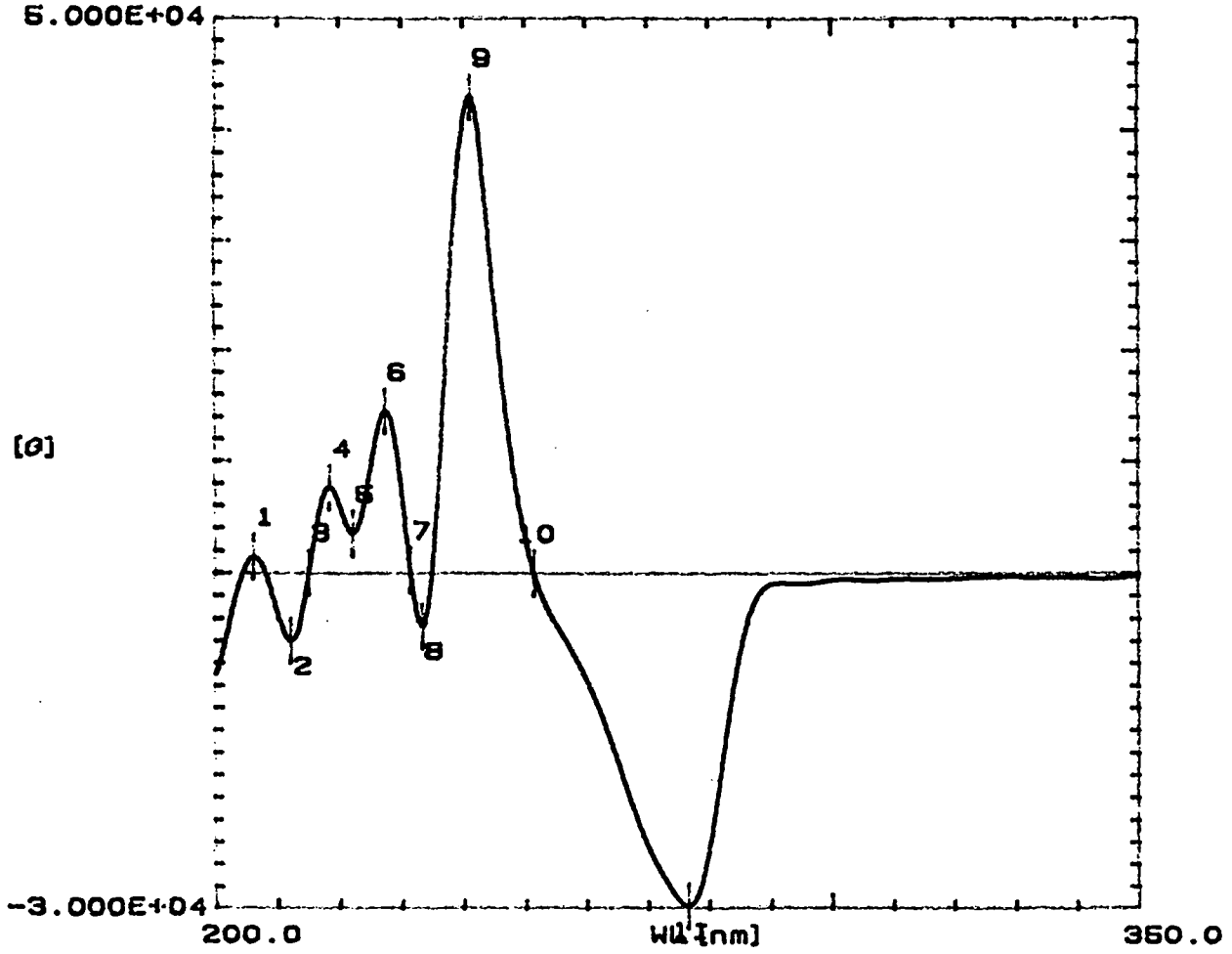


No.	Wavelength	Value
1	202.00 nm	-5.957E+02
2	205.00 nm	-3.627E+03
3	208.00 nm	6.680E+01
4	214.00 nm	8.162E+03
5	220.00 nm	-1.142E+03
6	223.00 nm	-4.146E+03
7	229.00 nm	7.887E+03
8	232.00 nm	-5.636E+02
9	236.00 nm	-1.319E+04
10	242.00 nm	6.928E+02
11	246.00 nm	8.249E+03
12	255.00 nm	-2.506E+02
13	275.00 nm	-4.204E+03

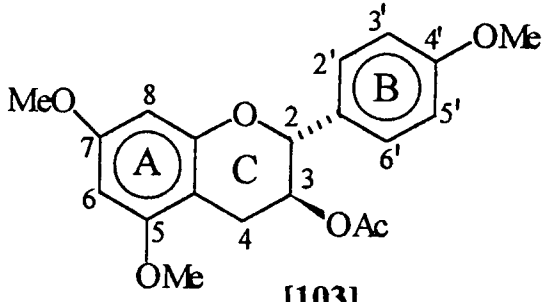


[102]

Plate 2

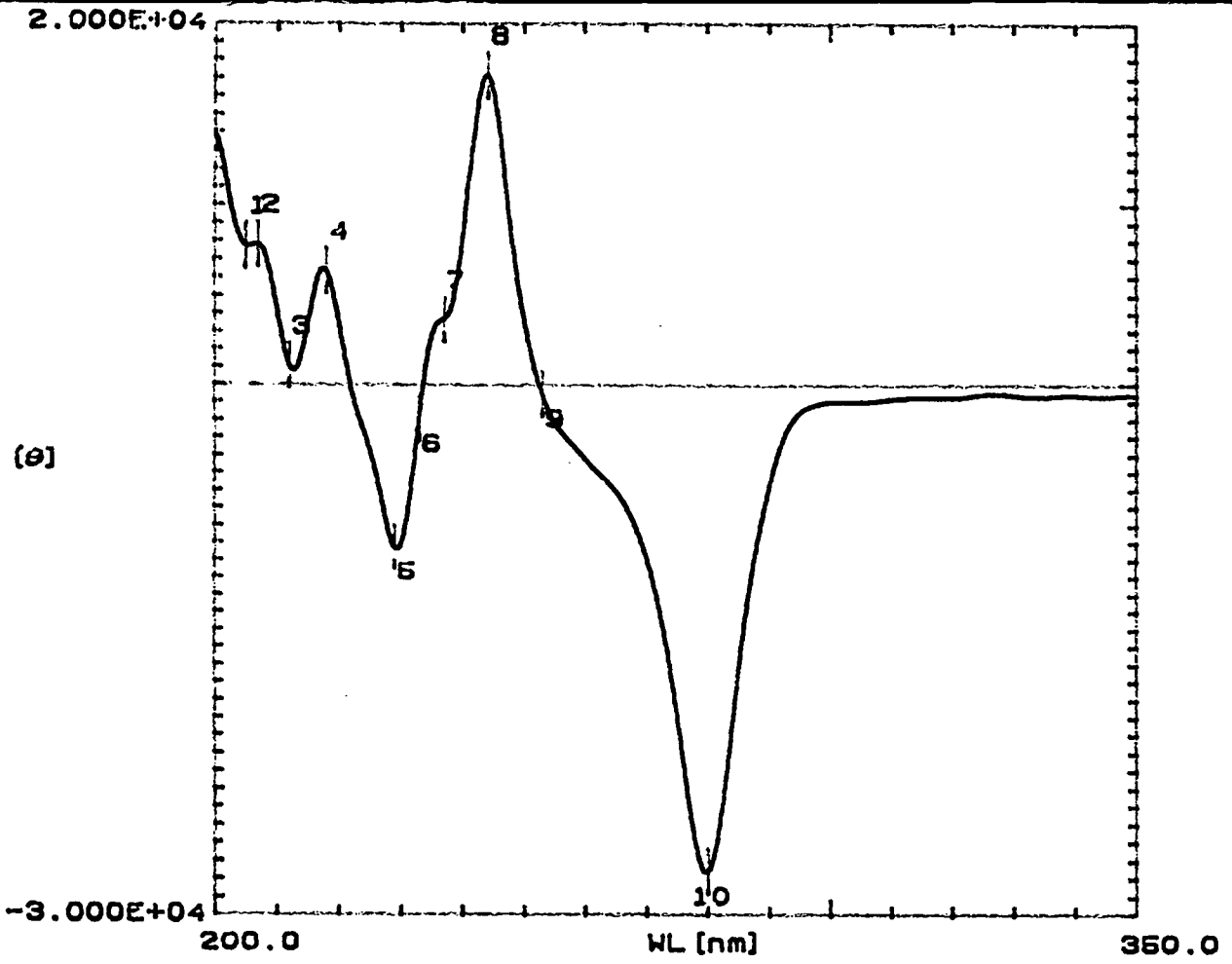


No.	Wavelength	Value
1	206.20 nm	1.426E+03
2	212.10 nm	-5.997E+03
3	215.10 nm	7.381E+01
4	218.40 nm	7.634E+03
5	222.20 nm	3.517E+03
6	227.60 nm	1.463E+04
7	231.50 nm	3.089E+02
8	233.30 nm	-4.636E+03
9	241.10 nm	4.303E+04
10	251.60 nm	7.478E-01
11	276.40 nm	-2.976E+04

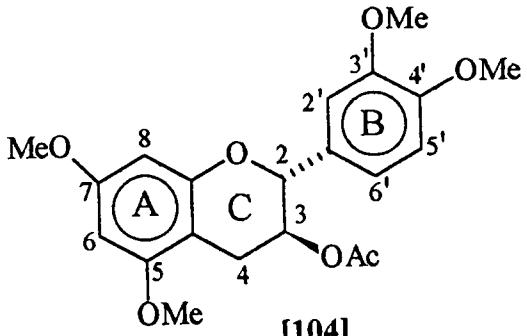


[103]

Plate 3

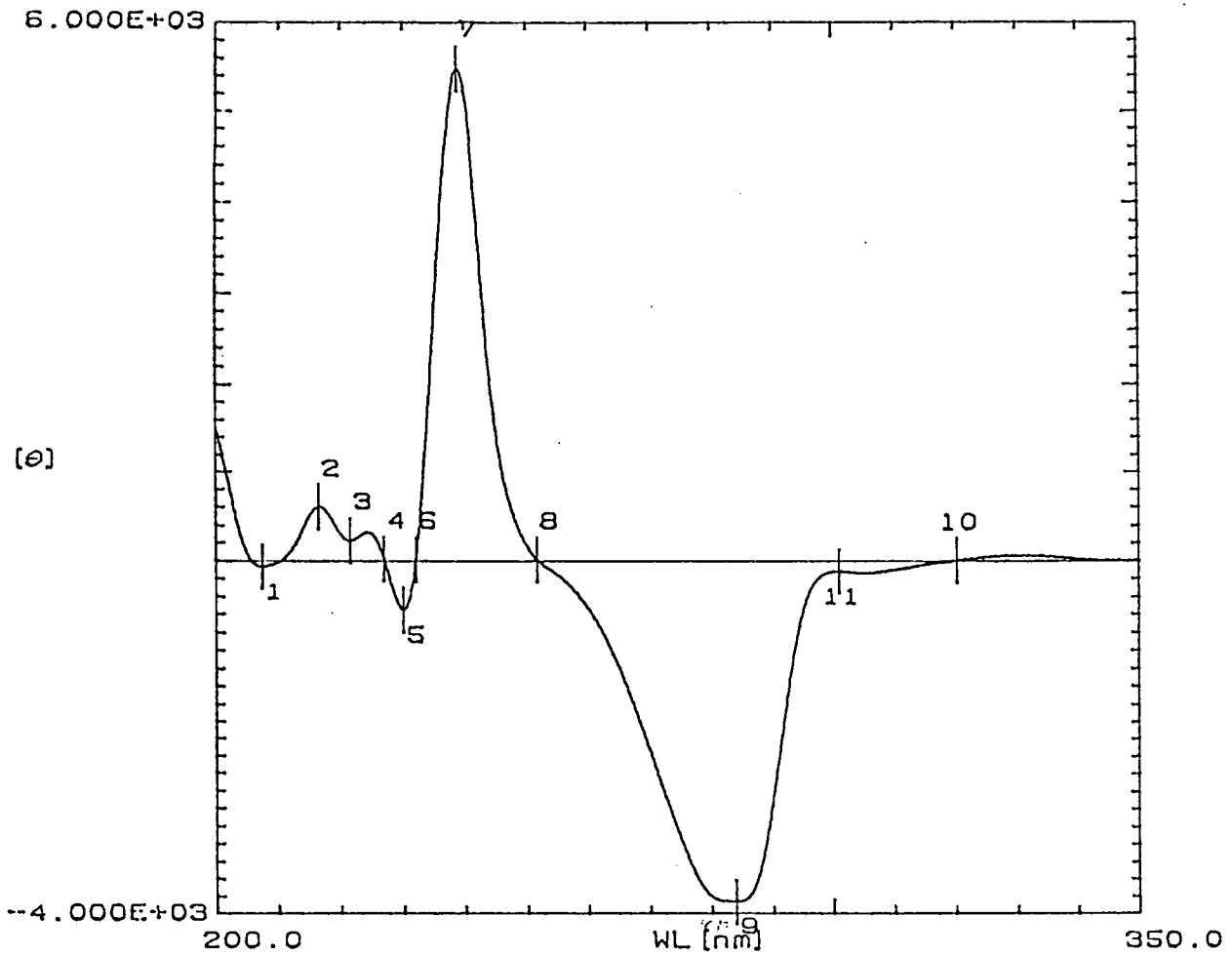


No.	Wavelength	Value
1	205.00 nm	7.758E+03
2	207.00 nm	7.837E+03
3	212.00 nm	1.087E+03
4	218.00 nm	6.388E+03
5	229.00 nm	-9.247E+03
6	233.00 nm	-1.931E+03
7	237.00 nm	3.685E+03
8	244.00 nm	1.717E+04
9	253.00 nm	-4.912E+02
10	280.00 nm	-2.752E+04

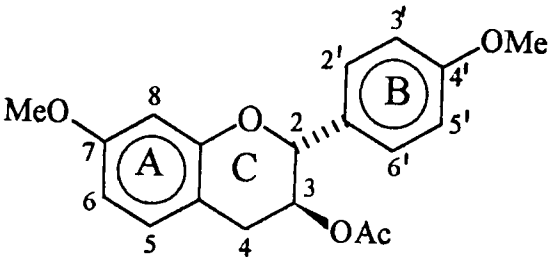


[104]

Plate 4

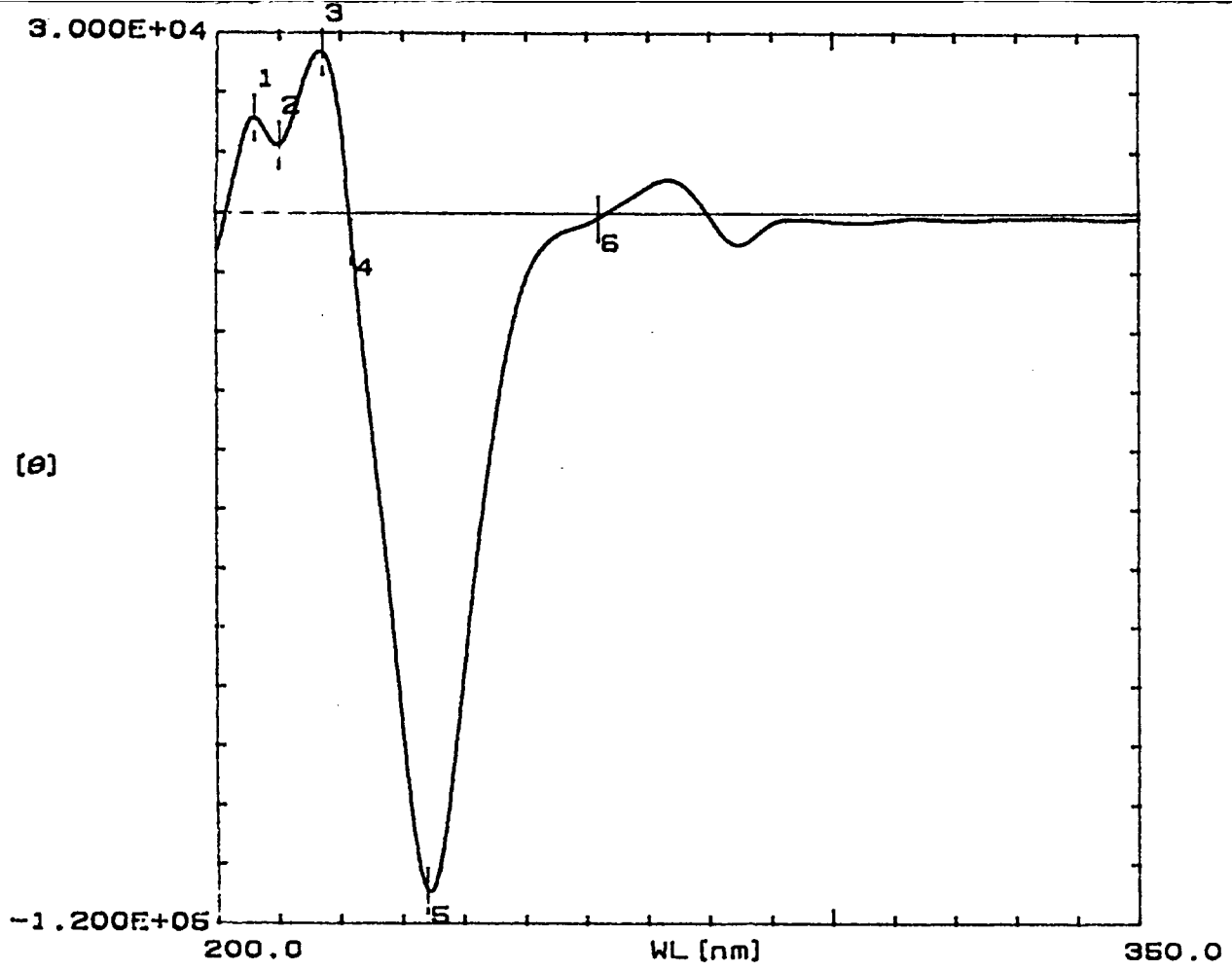


No.	Wavelength	Value
1	207.40 nm	-6.987E+01
2	216.60 nm	6.033E+02
3	221.60 nm	2.230E+02
4	227.00 nm	2.178E+01
5	230.10 nm	-5.510E+02
6	232.10 nm	9.656E+00
7	238.80 nm	5.481E+03
8	251.80 nm	6.346E+00
9	283.90 nm	-3.864E+03
10	320.20 nm	6.508E-01
11	301.00 nm	-1.210E+02

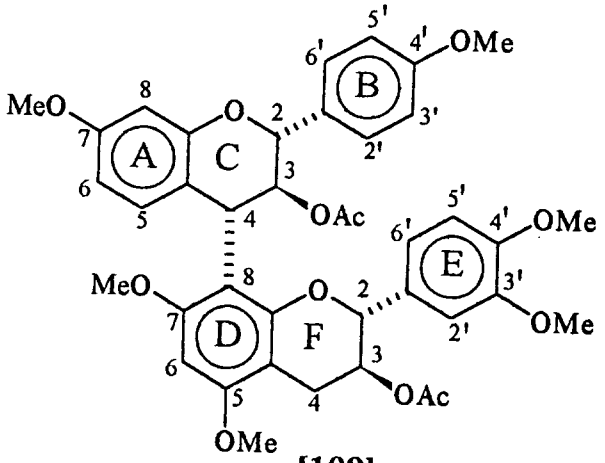


[106]

Plate 5a

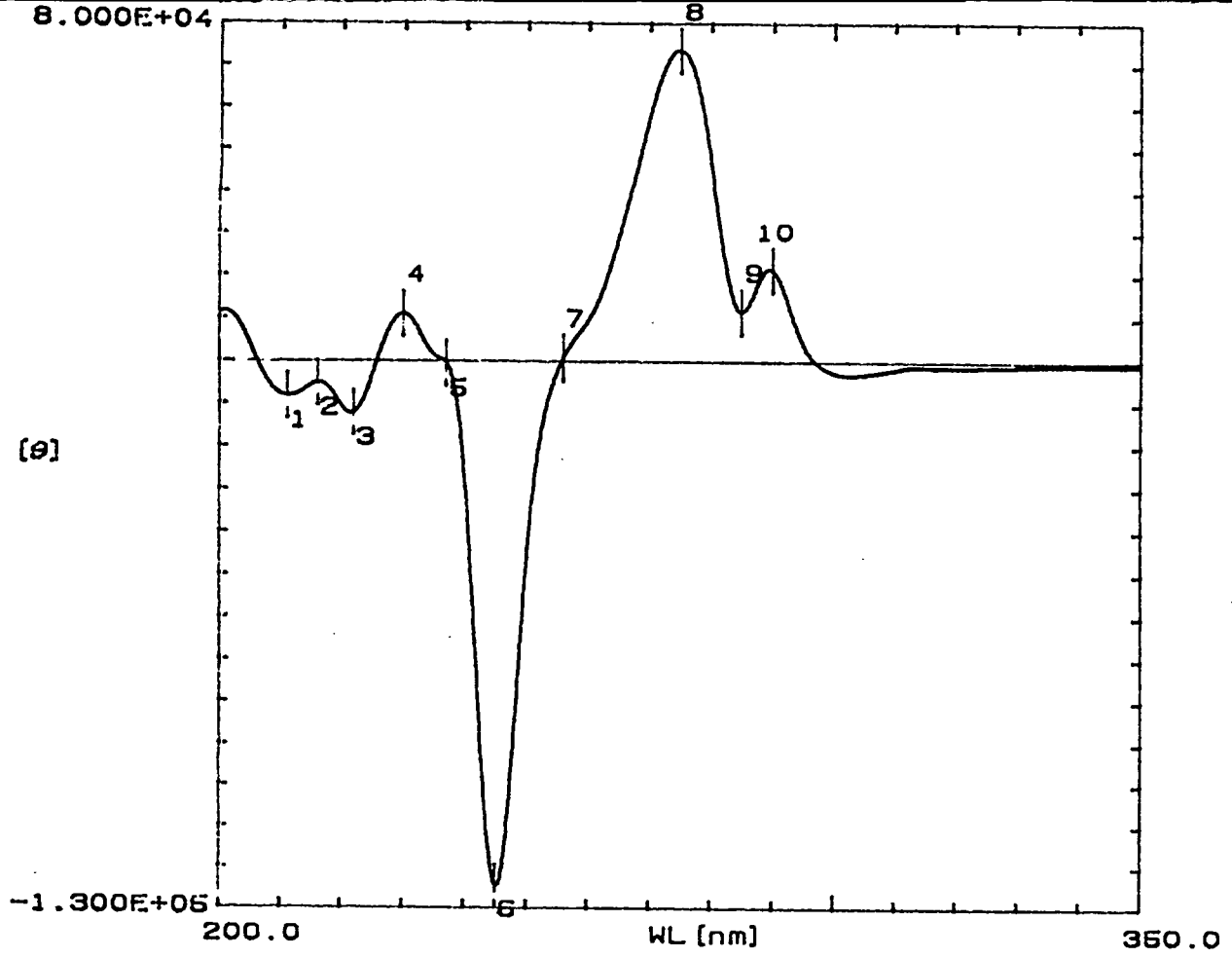


No.	Wavelength	Value
1	206.00 nm	1.564E+04
2	210.00 nm	1.120E+04
3	217.00 nm	2.662E+04
4	222.00 nm	-4.904E+03
5	234.00 nm	-1.144E+05
6	262.00 nm	-7.732E+02

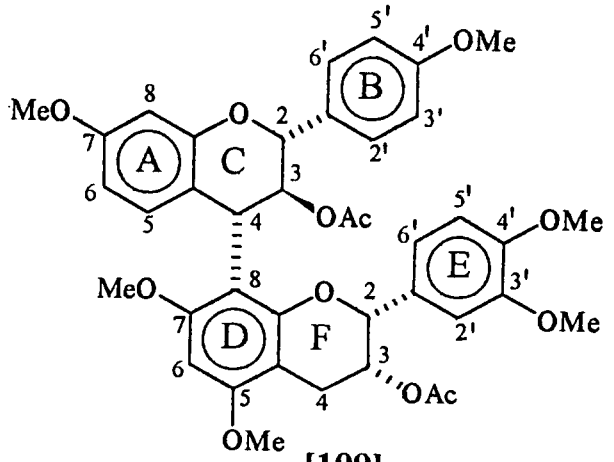


[108]

Plate 6

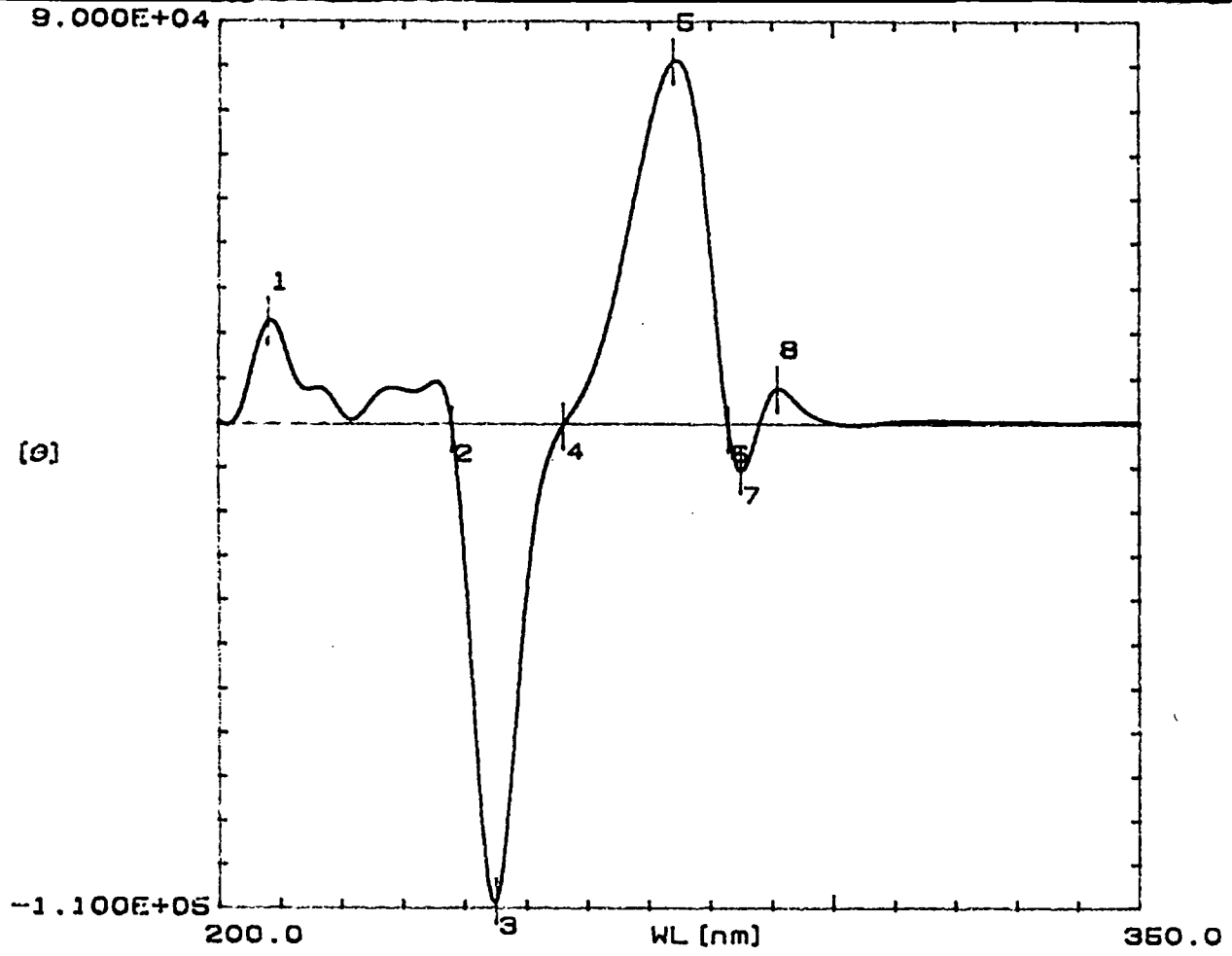


No.	Wavelength	Value
1	211.00 nm	-8.067E+03
2	216.00 nm	-5.030E+03
3	222.00 nm	-1.206E+04
4	230.00 nm	1.096E+04
5	237.00 nm	-5.583E+02
6	245.00 nm	-1.243E+05
7	256.00 nm	6.898E+02
8	275.00 nm	7.347E+04
9	285.00 nm	1.146E+04
10	290.00 nm	2.119E+04

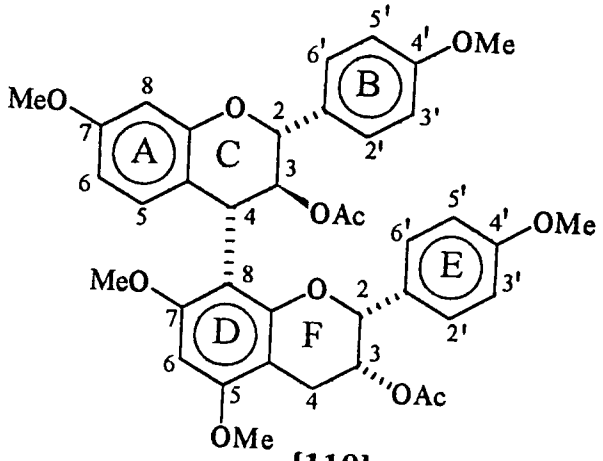


[109]

Plate 7

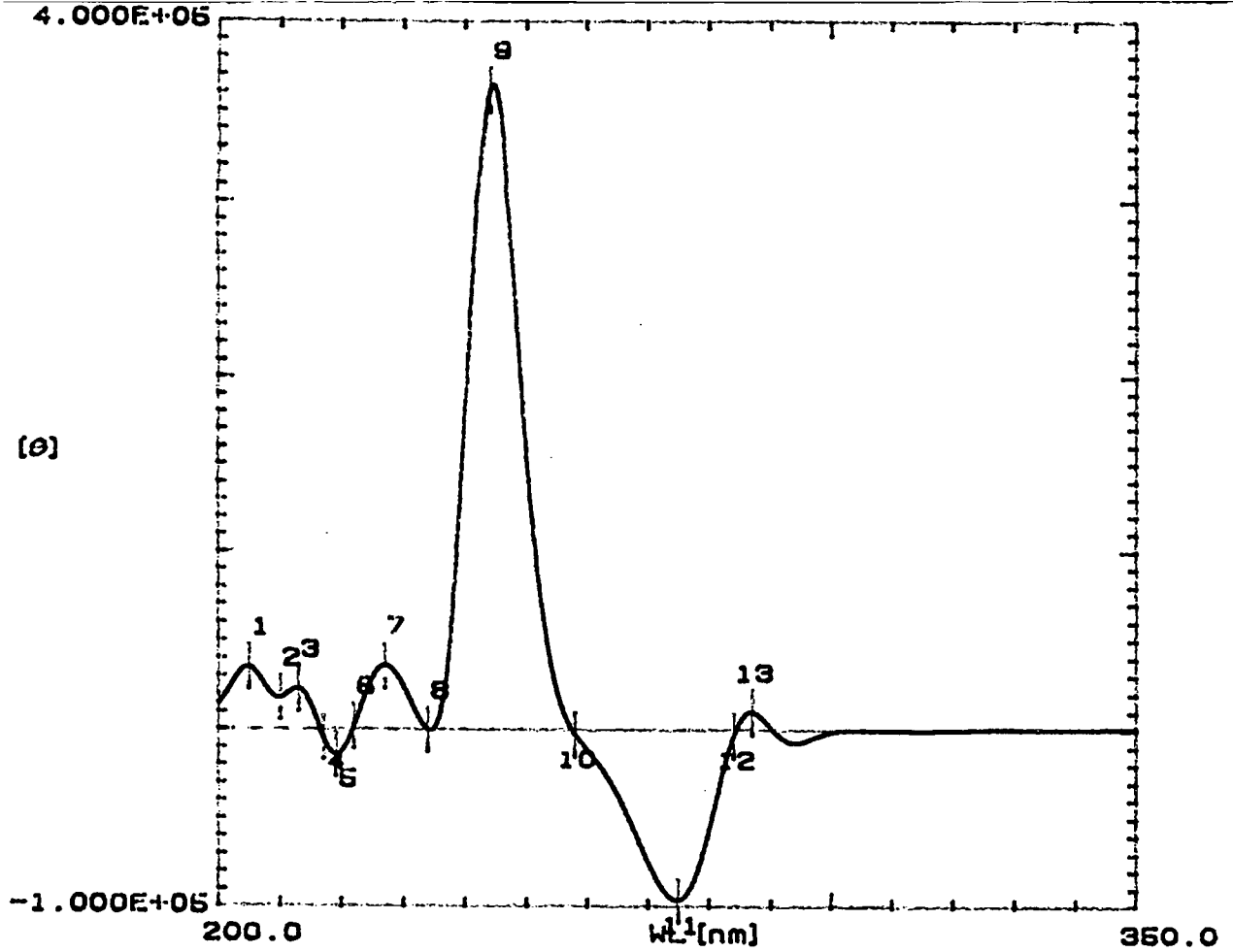


No.	Wavelength	Value
1	208.00 nm	2.267E+04
2	238.00 nm	-1.297E+03
3	245.00 nm	-1.082E+05
4	256.00 nm	-6.313E+02
5	274.00 nm	8.110E+04
6	283.00 nm	-1.287E+03
7	285.00 nm	-1.066E+04
8	291.00 nm	7.723E+03

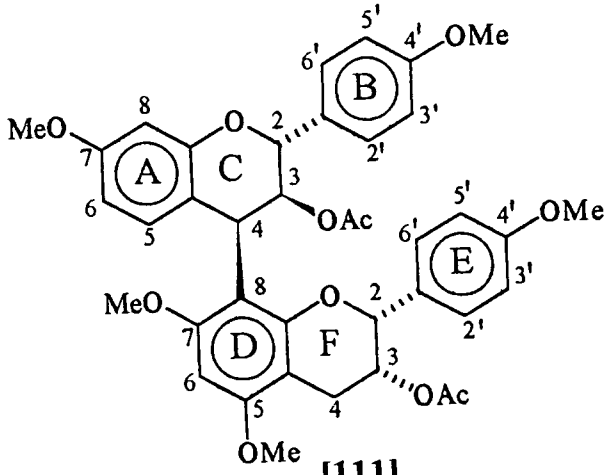


[110]

Plate 8

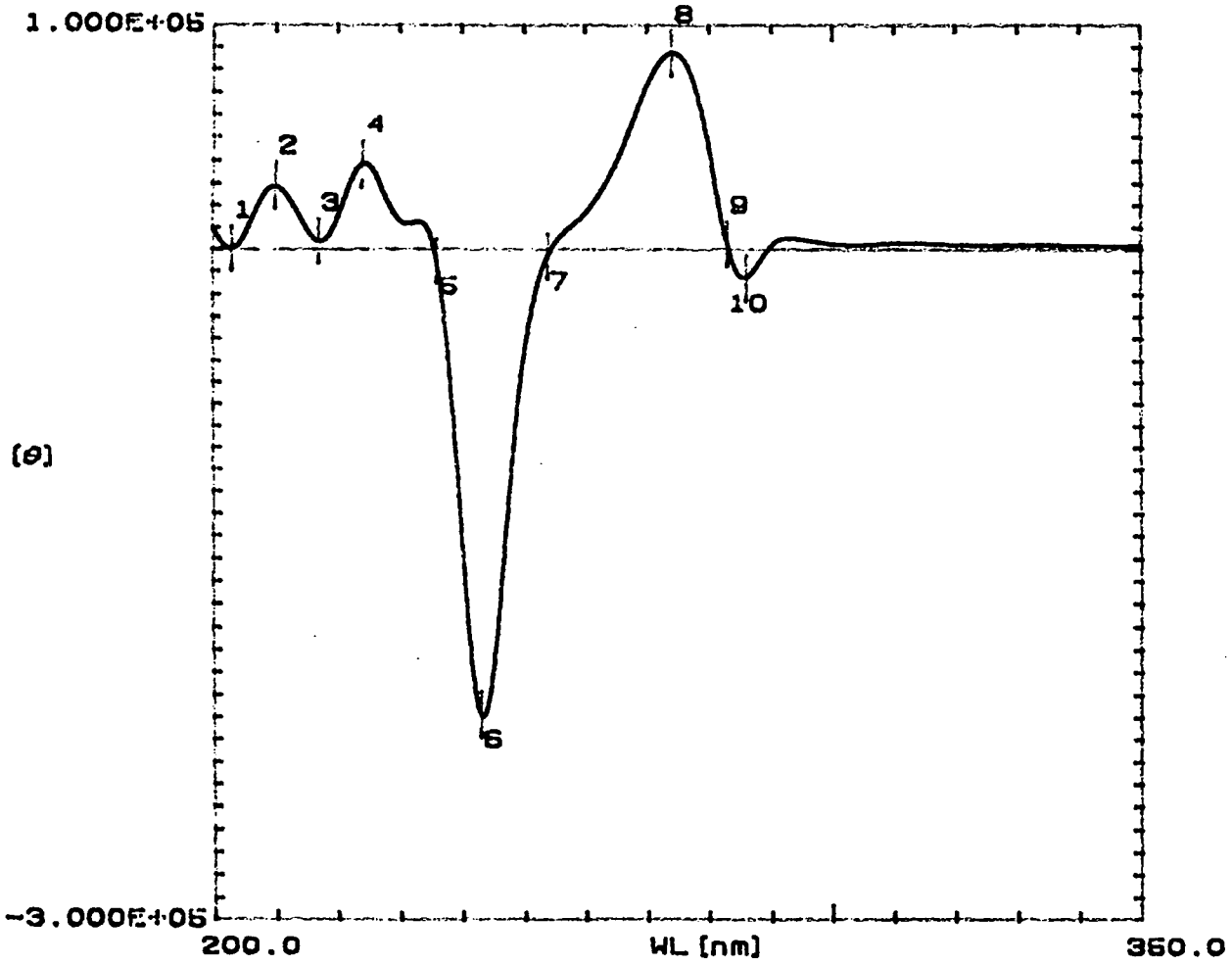


No.	Wavelength	Value
1	205.00 nm	3.472E+04
2	210.00 nm	1.780E+04
3	213.00 nm	2.275E+04
4	217.00 nm	-4.473E+03
5	219.00 nm	-1.423E+04
6	222.00 nm	2.147E+03
7	227.00 nm	3.584E+04
8	234.00 nm	7.860E+00
9	244.00 nm	3.618E+05
10	258.00 nm	-2.643E+03
11	276.00 nm	-9.804E+04
12	284.00 nm	-3.011E+03
13	287.00 nm	1.035E+04

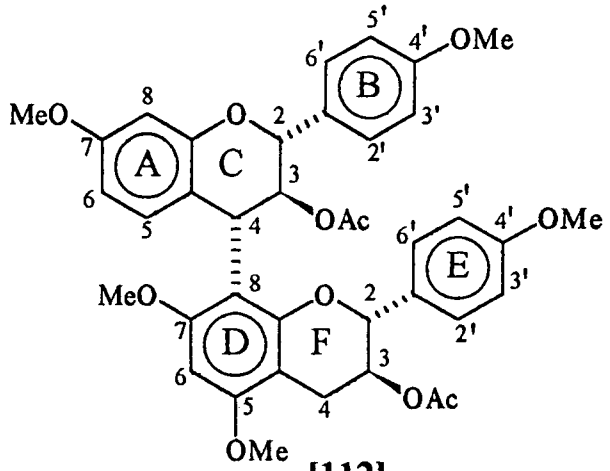


[111]

Plate 9

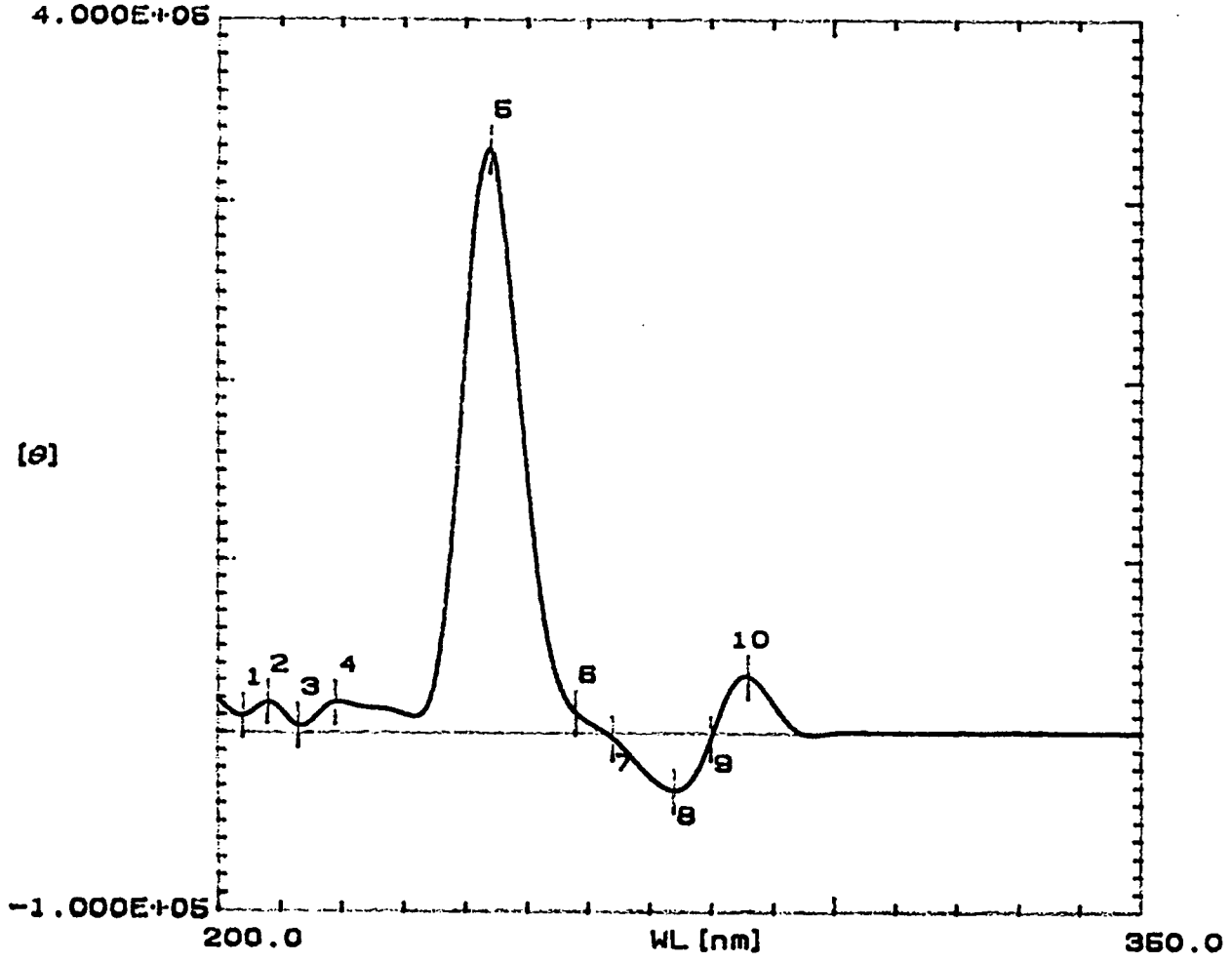


No.	Wavelength	Value
1	203.00 nm	3.736E+02
2	210.00 nm	2.867E+04
3	217.00 nm	3.737E+03
4	224.00 nm	3.830E+04
5	236.00 nm	-5.192E+03
6	243.00 nm	-2.086E+05
7	254.00 nm	-2.878E+03
8	274.00 nm	8.743E+04
9	283.00 nm	2.896E+03
10	286.00 nm	-1.273E+04

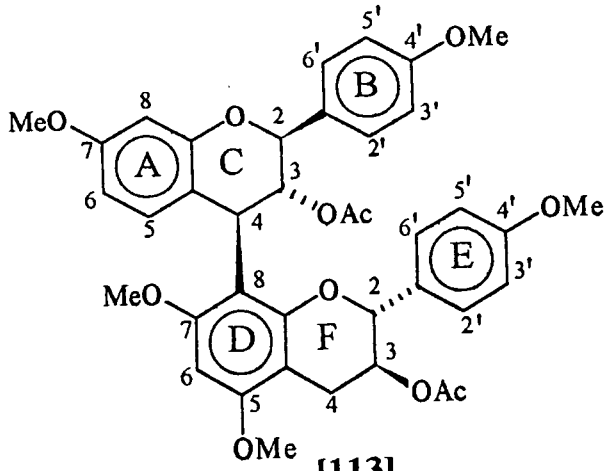


[112]

Plate 10

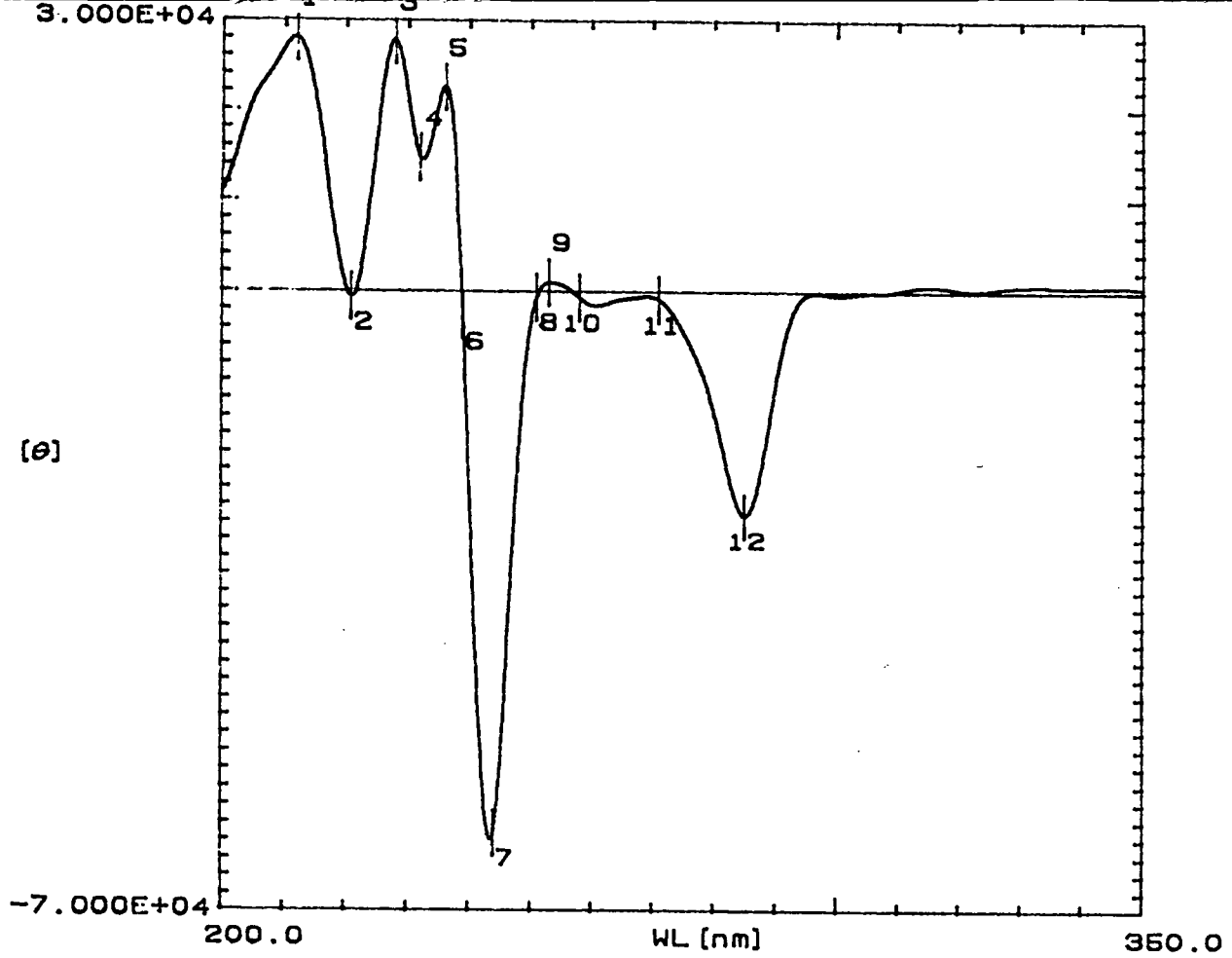


No.	Wavelength	Value
1	204.00 nm	8.608E+03
2	208.00 nm	1.715E+04
3	213.00 nm	4.075E+03
4	219.00 nm	1.755E+04
5	244.00 nm	3.286E+05
6	258.00 nm	1.202E+04
7	264.00 nm	-2.462E+03
8	274.00 nm	-3.217E+04
9	280.00 nm	-2.816E+03
10	286.00 nm	3.288E+04



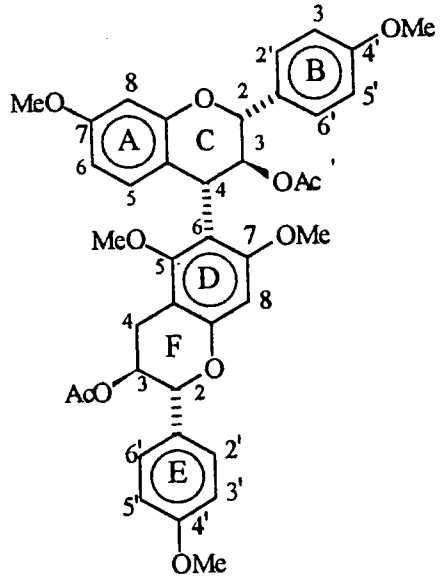
[113]

Plate 11

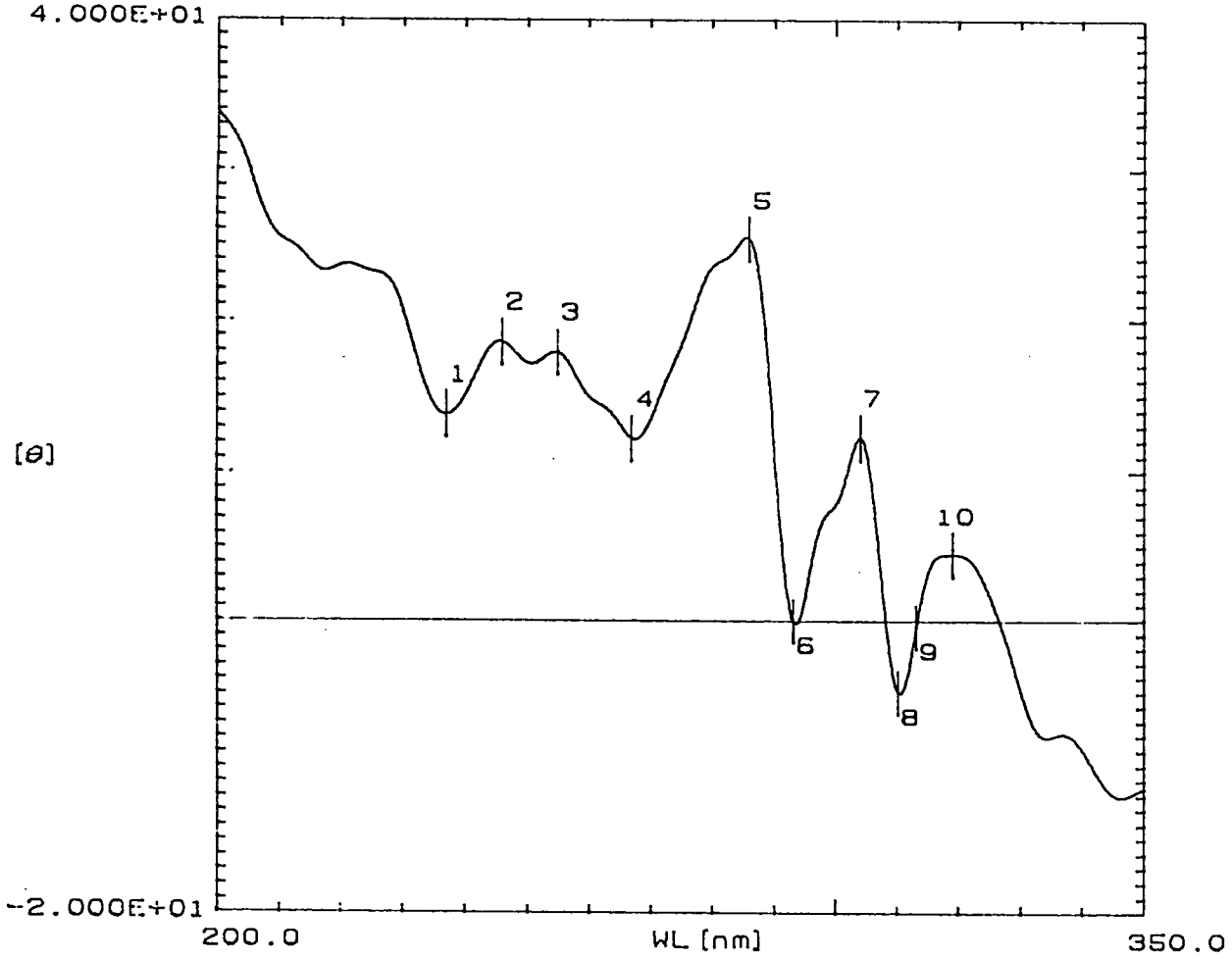


No.	Wavelength	Value
1	212.00 nm	2.798E+04
2	221.00 nm	-6.738E+02
3	228.00 nm	2.758E+04
4	232.00 nm	1.472E+04
5	236.00 nm	2.250E+04
6	239.00 nm	-2.807E+03
7	244.00 nm	-6.122E+04
8	251.00 nm	-6.485E+02
9	253.00 nm	8.954E+02
10	258.00 nm	-7.466E+02
11	271.00 nm	-8.448E+02
12	285.00 nm	-2.533E+04

Plate 12



[114]



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

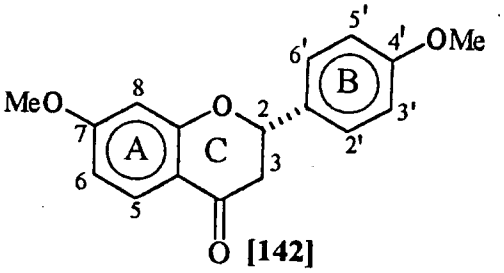
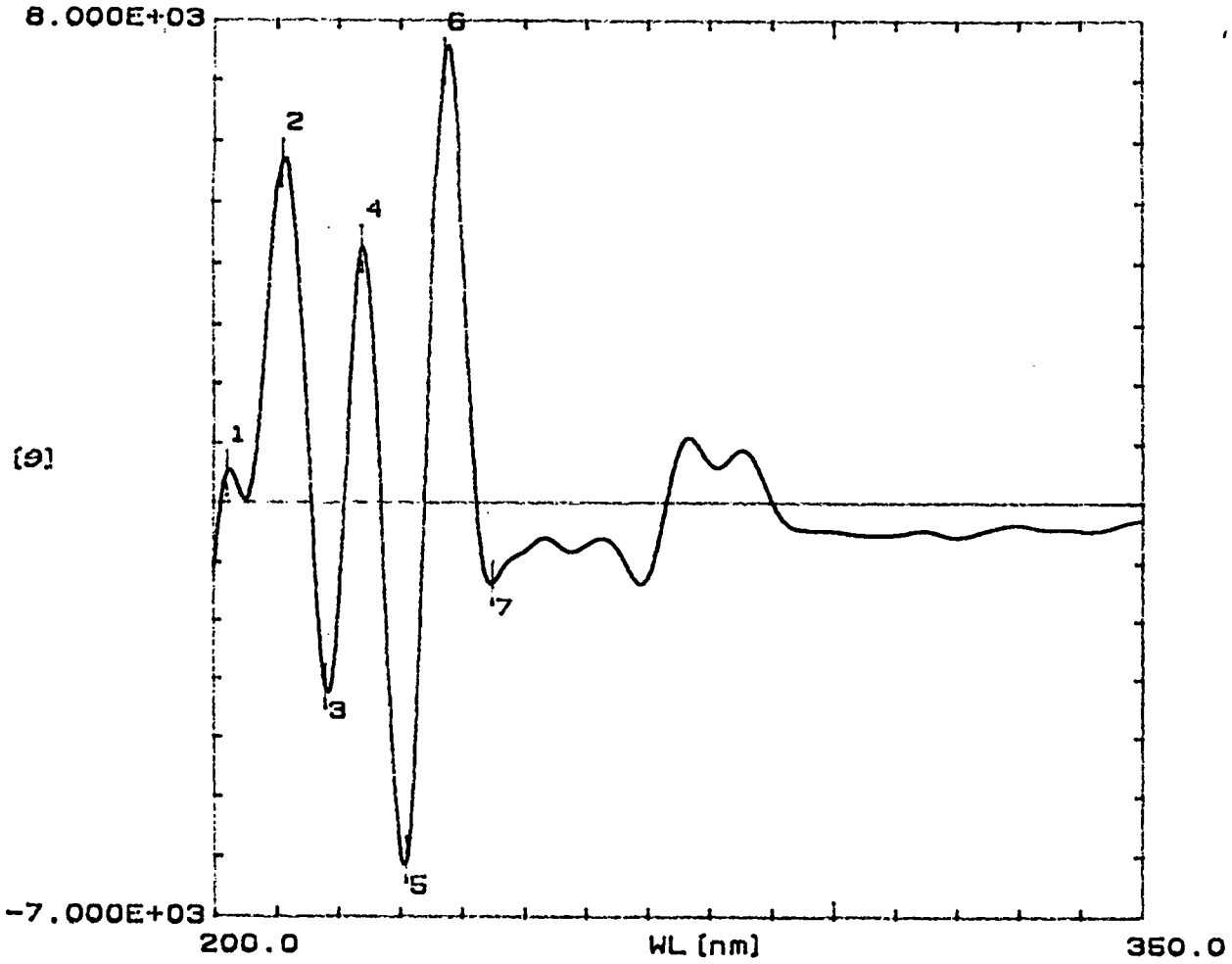


Plate 18



No.	Wavelength	Value
1	202.00 nm	5.010E+02
2	211.00 nm	5.662E+03
3	218.00 nm	-3.130E+03
4	224.00 nm	4.240E+03
5	231.00 nm	-6.062E+03
6	237.00 nm	7.326E+03
7	245.00 nm	-1.349E+03

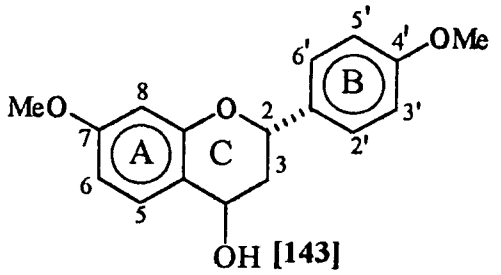
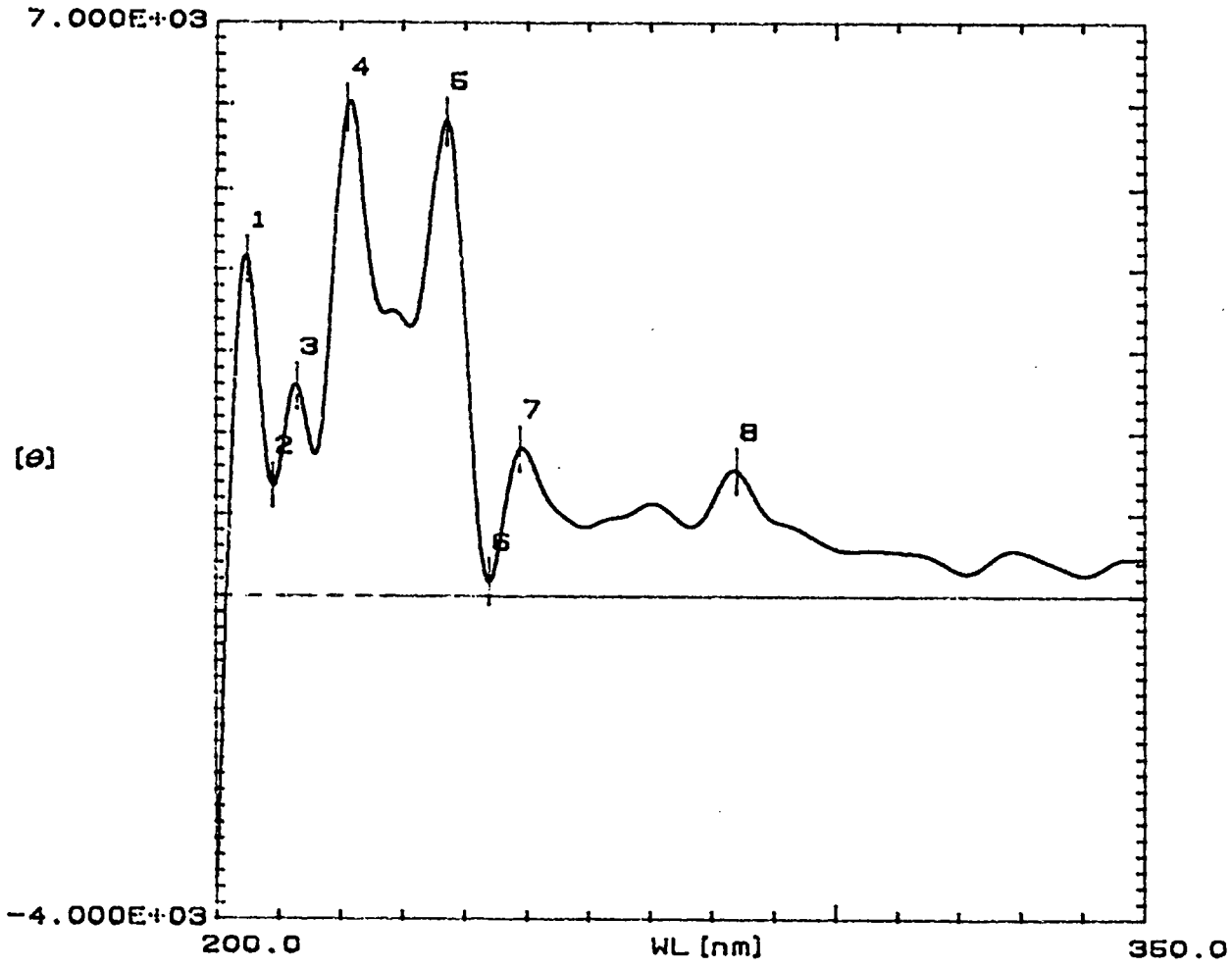
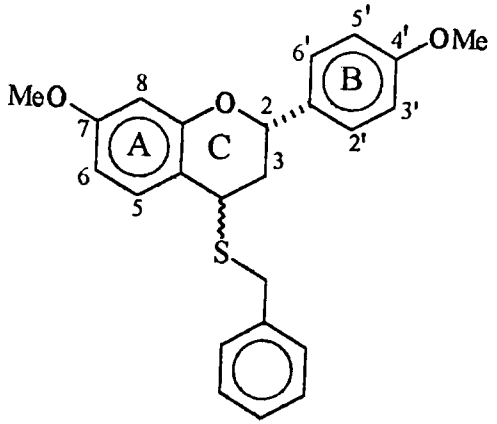


Plate 19

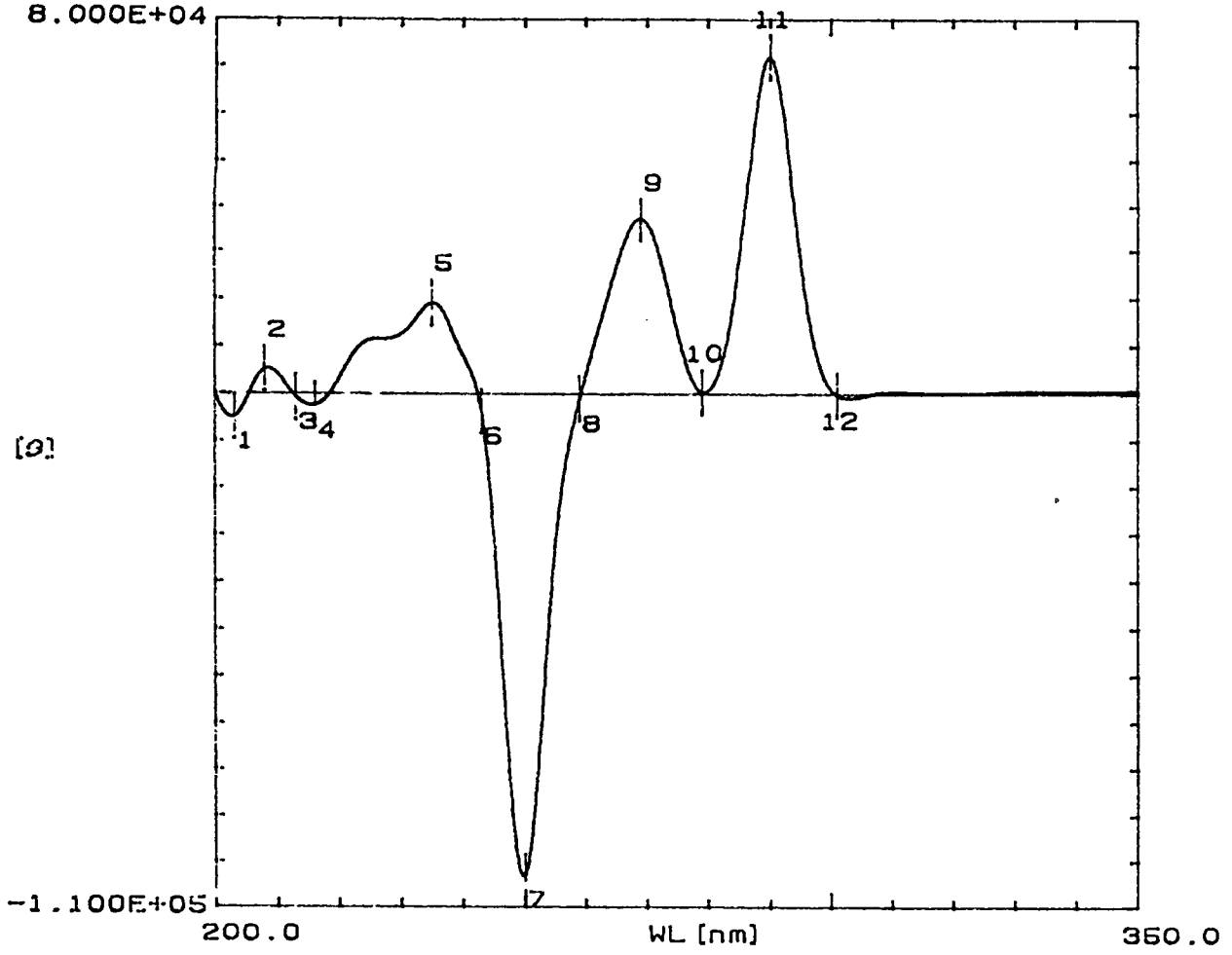


No.	Wavelength	Value
1	205.00 nm	4.133E+03
2	209.00 nm	1.362E+03
3	213.00 nm	2.579E+03
4	221.00 nm	5.873E+03
5	237.00 nm	5.800E+03
6	244.00 nm	1.756E+02
7	249.00 nm	1.812E+03
8	284.00 nm	1.545E+03

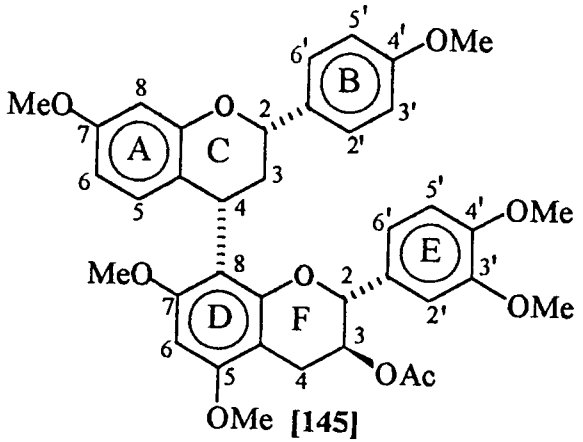


[144]

Plate 20

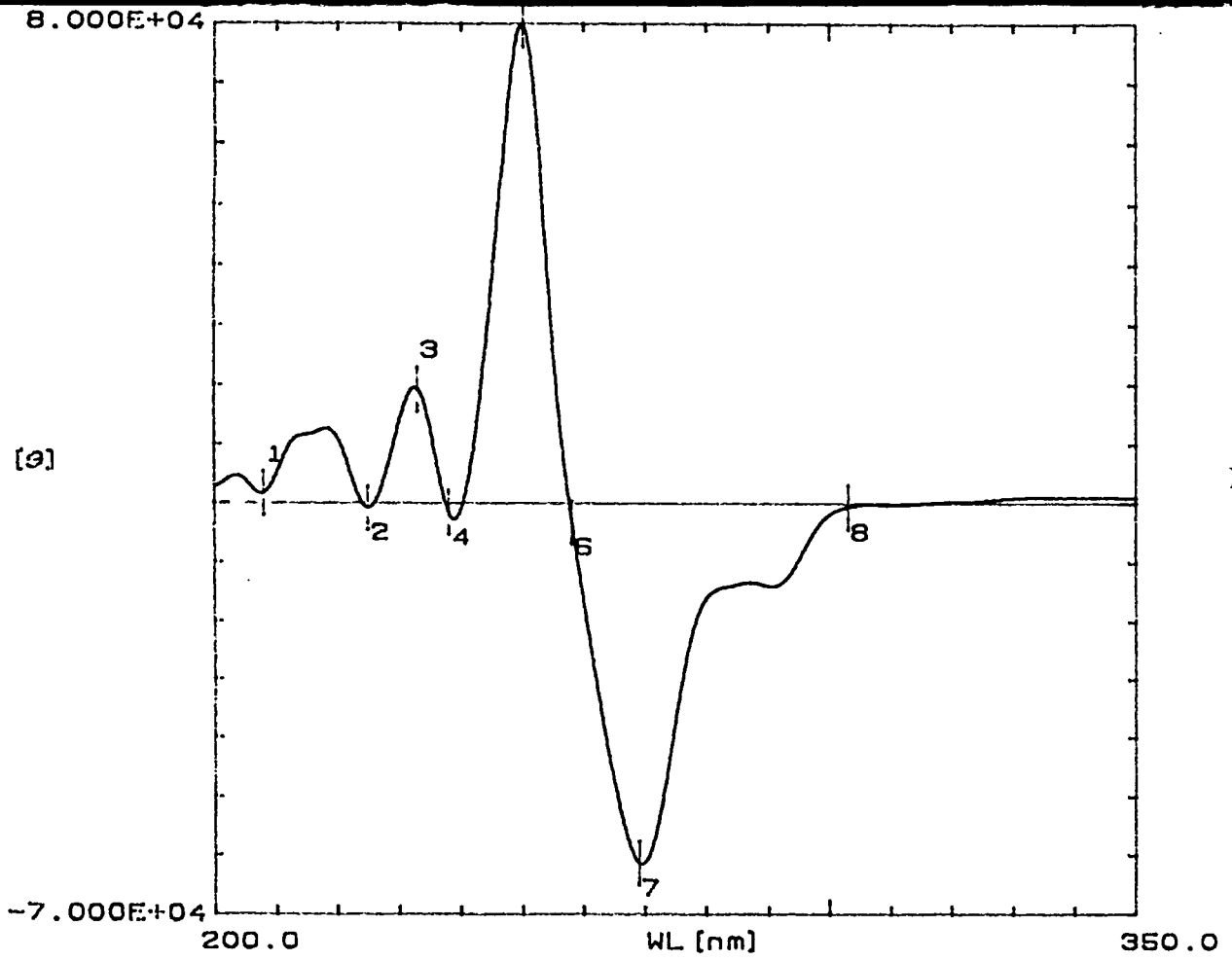


No.	Wavelength	Value
1	203.00 nm	-4.910E+03
2	208.00 nm	5.105E+03
3	213.00 nm	-7.453E+02
4	216.00 nm	-2.434E+03
5	235.00 nm	1.916E+04
6	243.00 nm	-3.700E+03
7	250.00 nm	-1.029E+05
8	259.00 nm	-1.008E+03
9	269.00 nm	3.721E+04
10	279.00 nm	2.794E+02
11	290.00 nm	7.188E+04
12	301.00 nm	-3.669E+02

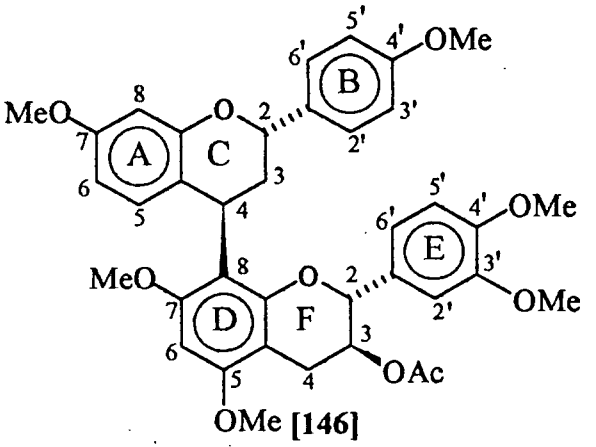


[145]

Plate 21

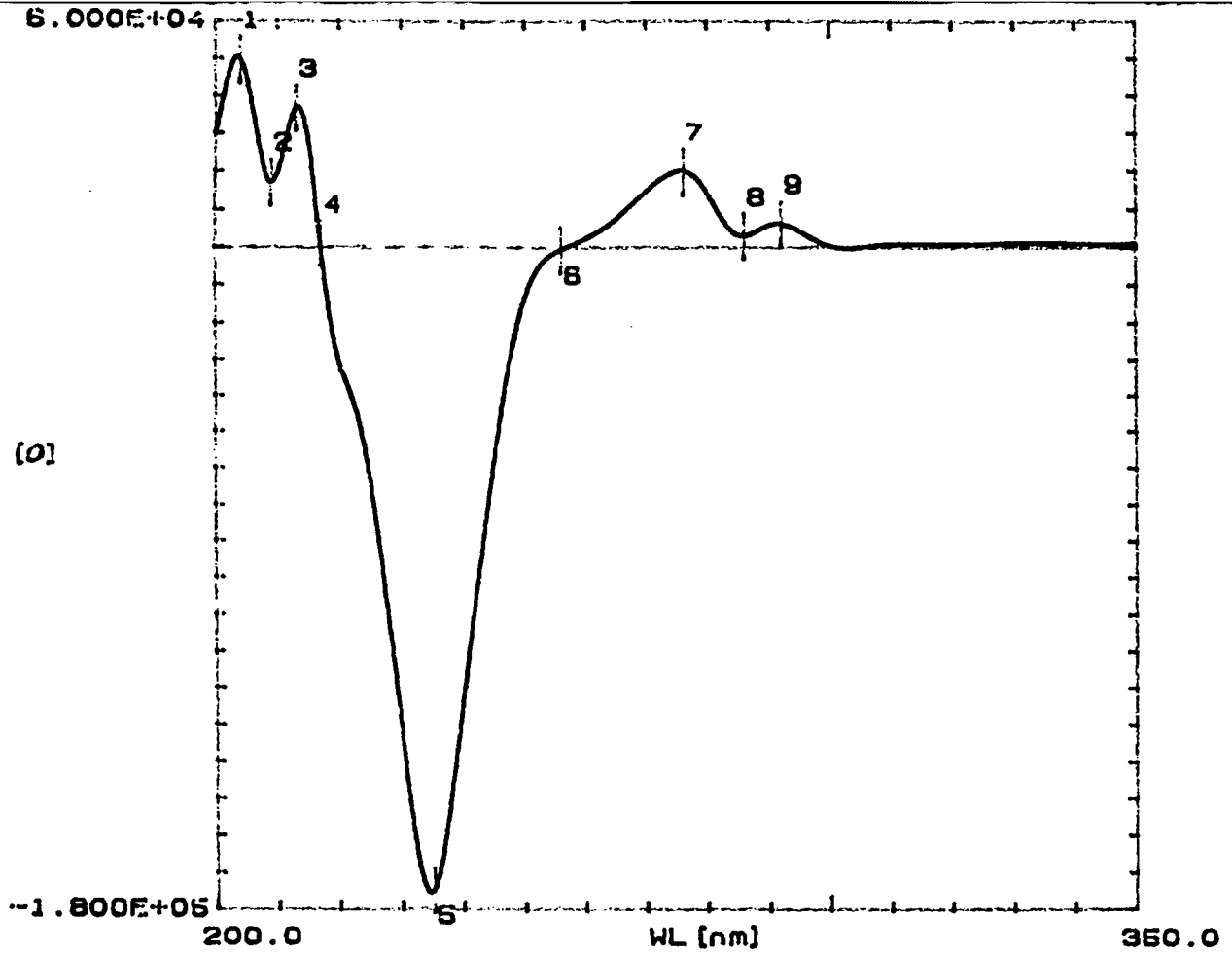


No.	Wavelength	Value
1	208.00 nm	1.823E+03
2	225.00 nm	-6.757E+02
3	233.00 nm	1.937E+04
4	238.00 nm	-1.446E+03
5	250.00 nm	7.980E+04
6	258.00 nm	-3.125E+03
7	269.00 nm	-6.125E+04
8	303.00 nm	-5.249E+02

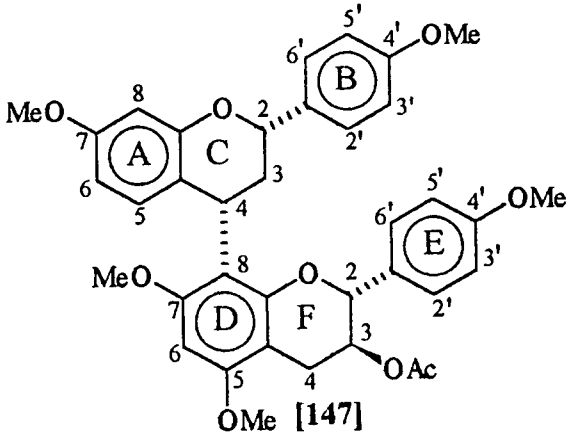


OMe [146]

Plate 22

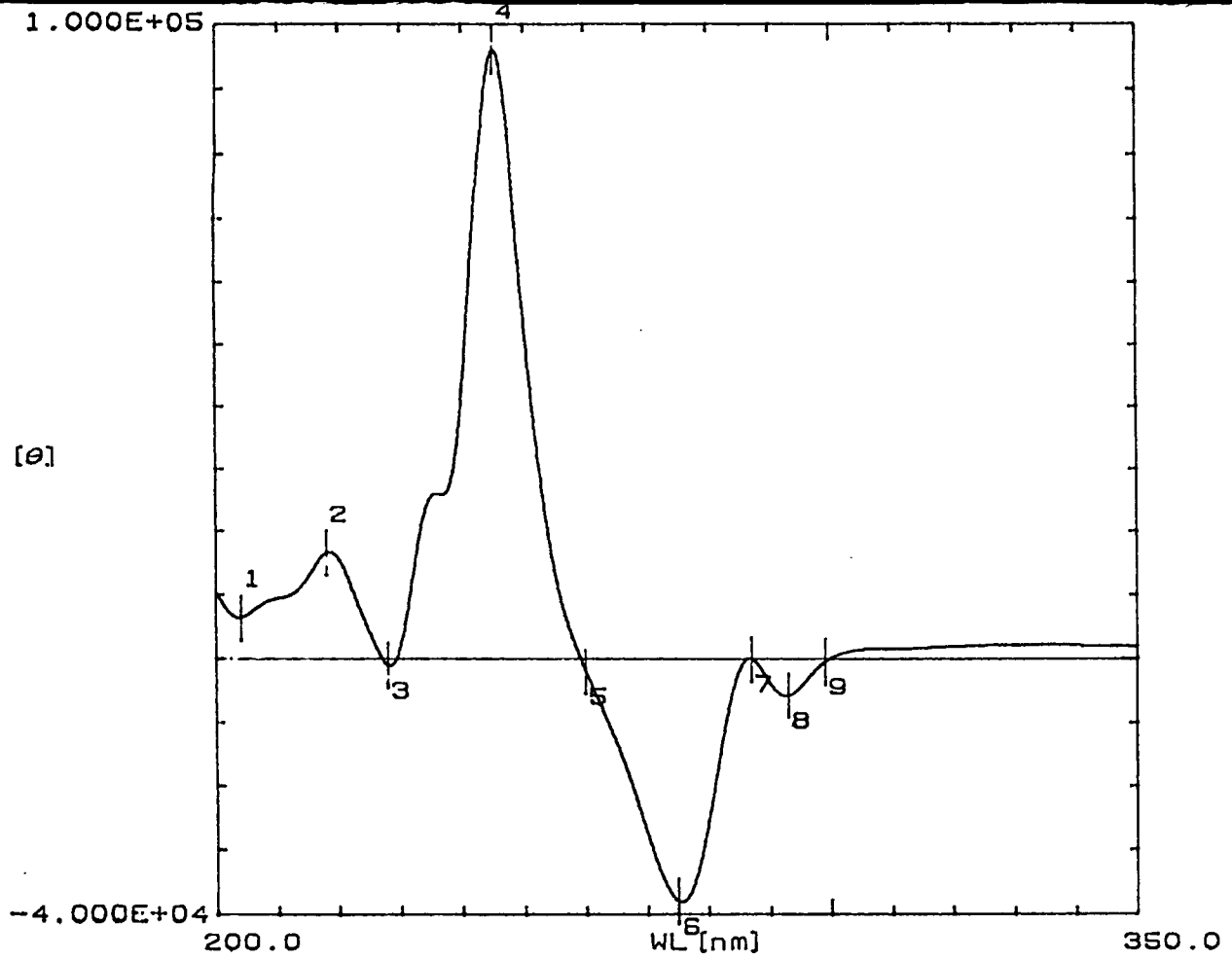


No.	Wavelength	Value
1	204.00 nm	4.985E+04
2	209.00 nm	1.726E+04
3	213.00 nm	3.695E+04
4	217.00 nm	1.270E+03
5	235.00 nm	-1.746E+05
6	256.00 nm	-7.261E+02
7	276.00 nm	2.019E+04
8	286.00 nm	3.091E+03
9	292.00 nm	6.330E+03

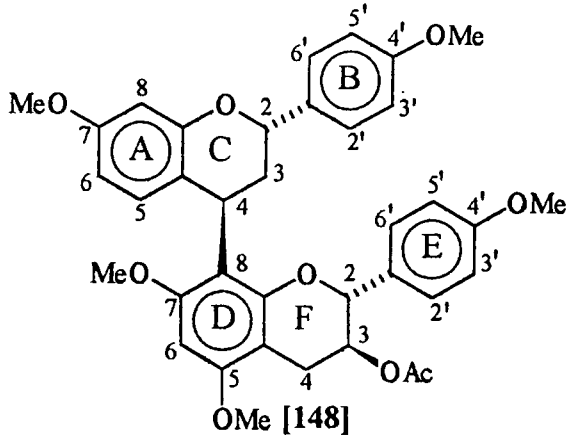


[147]

Plate 23

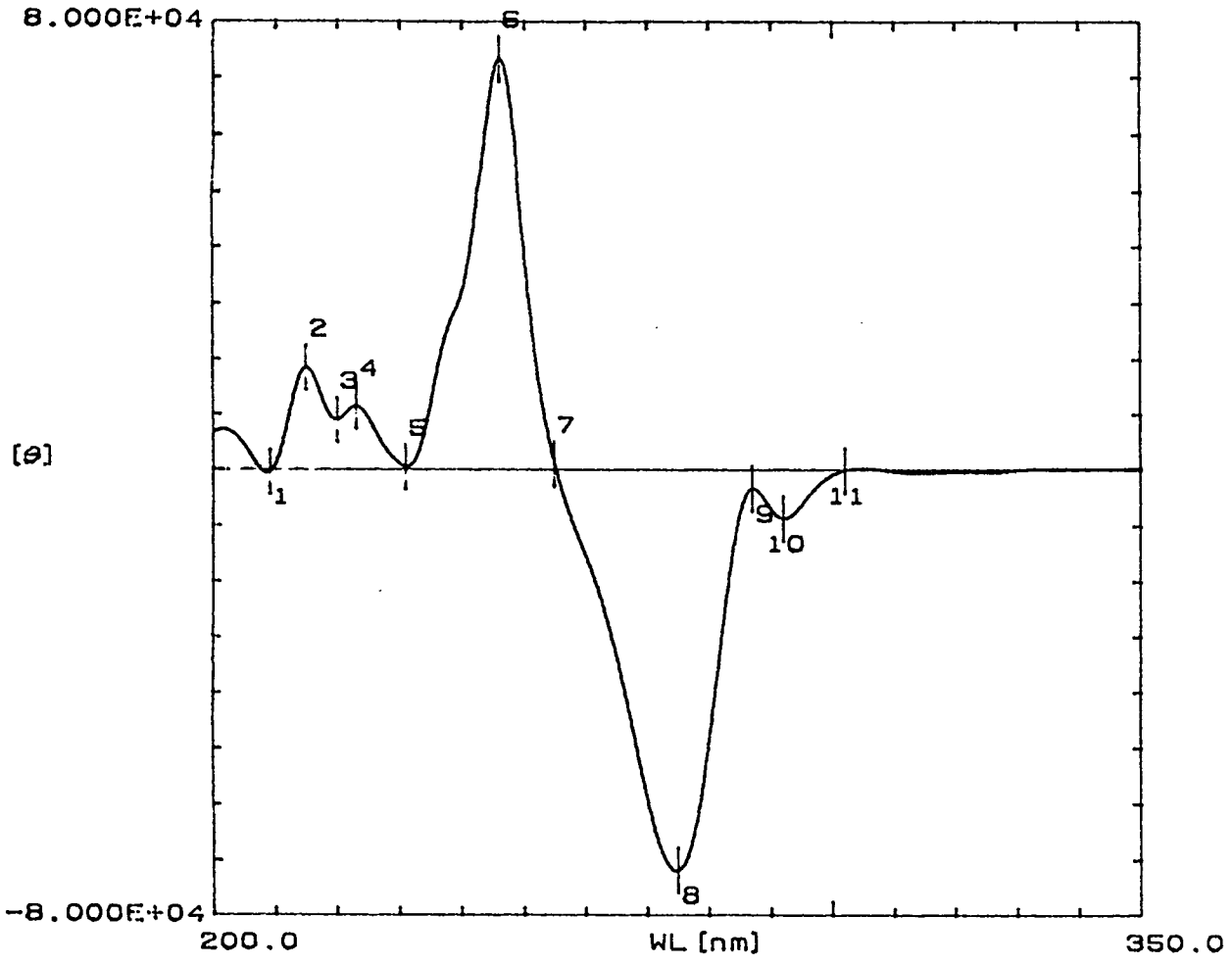


No.	Wavelength	Value
1	204.00 nm	6.427E+03
2	218.00 nm	1.664E+04
3	228.00 nm	-1.040E+03
4	245.00 nm	9.595E+04
5	260.00 nm	-2.022E+03
6	275.00 nm	-3.785E+04
7	287.00 nm	-7.490E+00
8	293.00 nm	-5.771E+03
9	299.00 nm	-4.077E+02

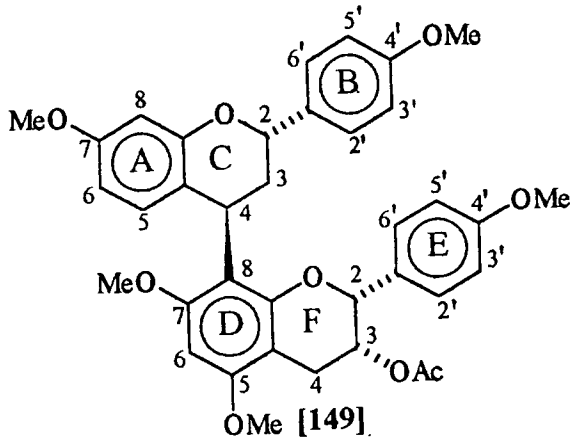


[148]

Plate 24

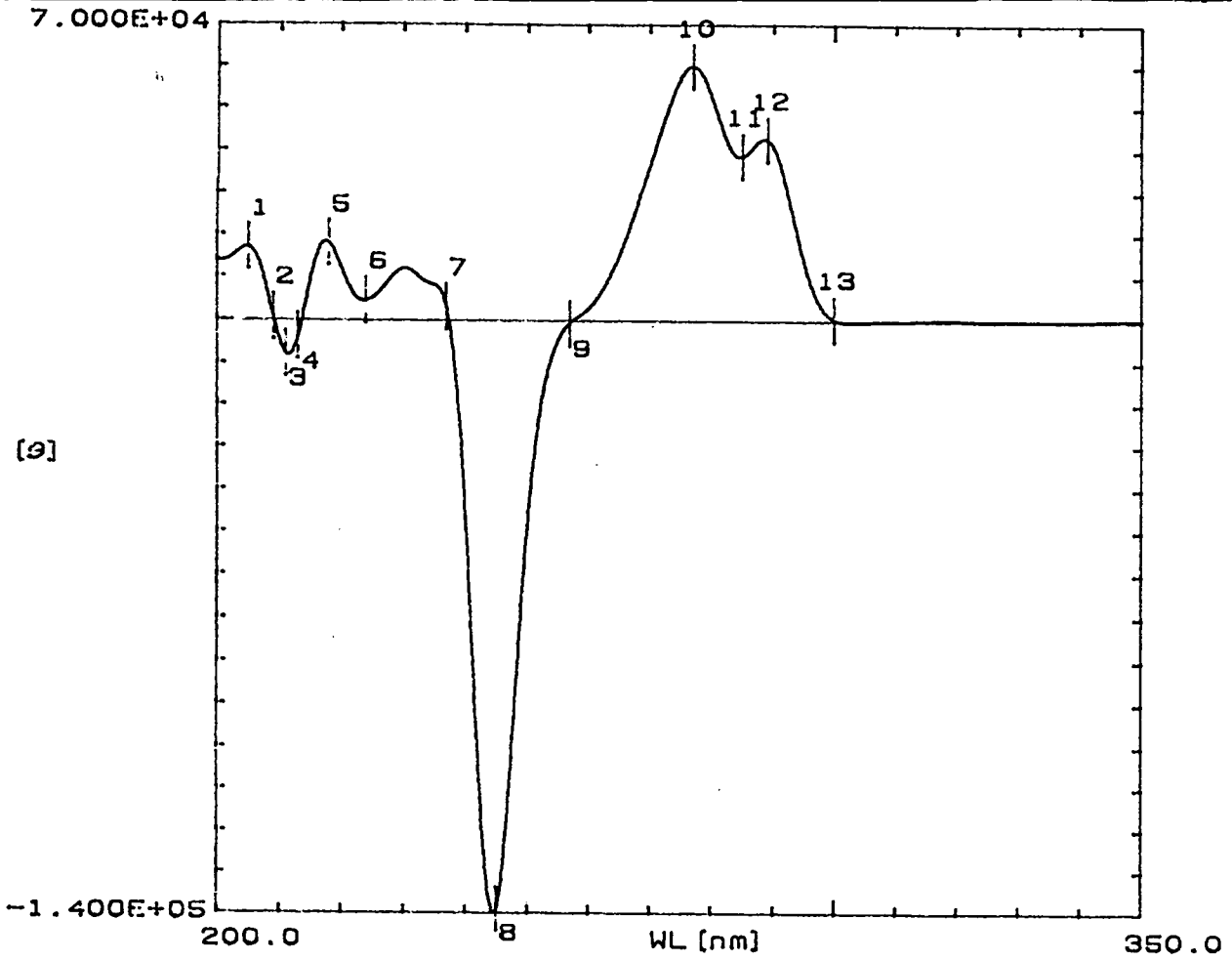


No.	Wavelength	Value
1	209.00 nm	-3.798E+02
2	215.00 nm	1.848E+04
3	220.00 nm	9.046E+03
4	223.00 nm	1.147E+04
5	231.00 nm	5.820E+02
6	246.00 nm	7.336E+04
7	255.00 nm	1.111E+03
8	275.00 nm	-7.175E+04
9	287.00 nm	-3.310E+03
10	292.00 nm	-8.706E+03
11	302.00 nm	-5.473E+01

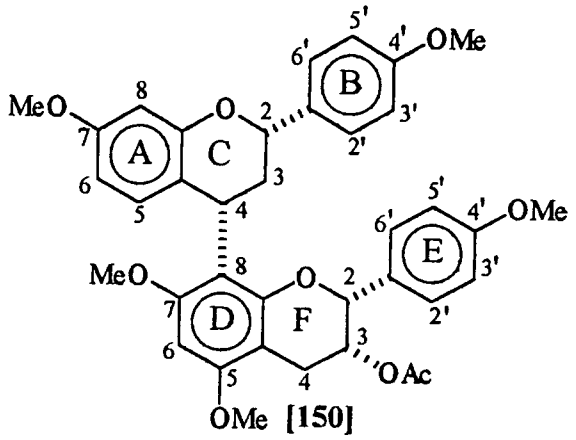


[149]

Plate 25

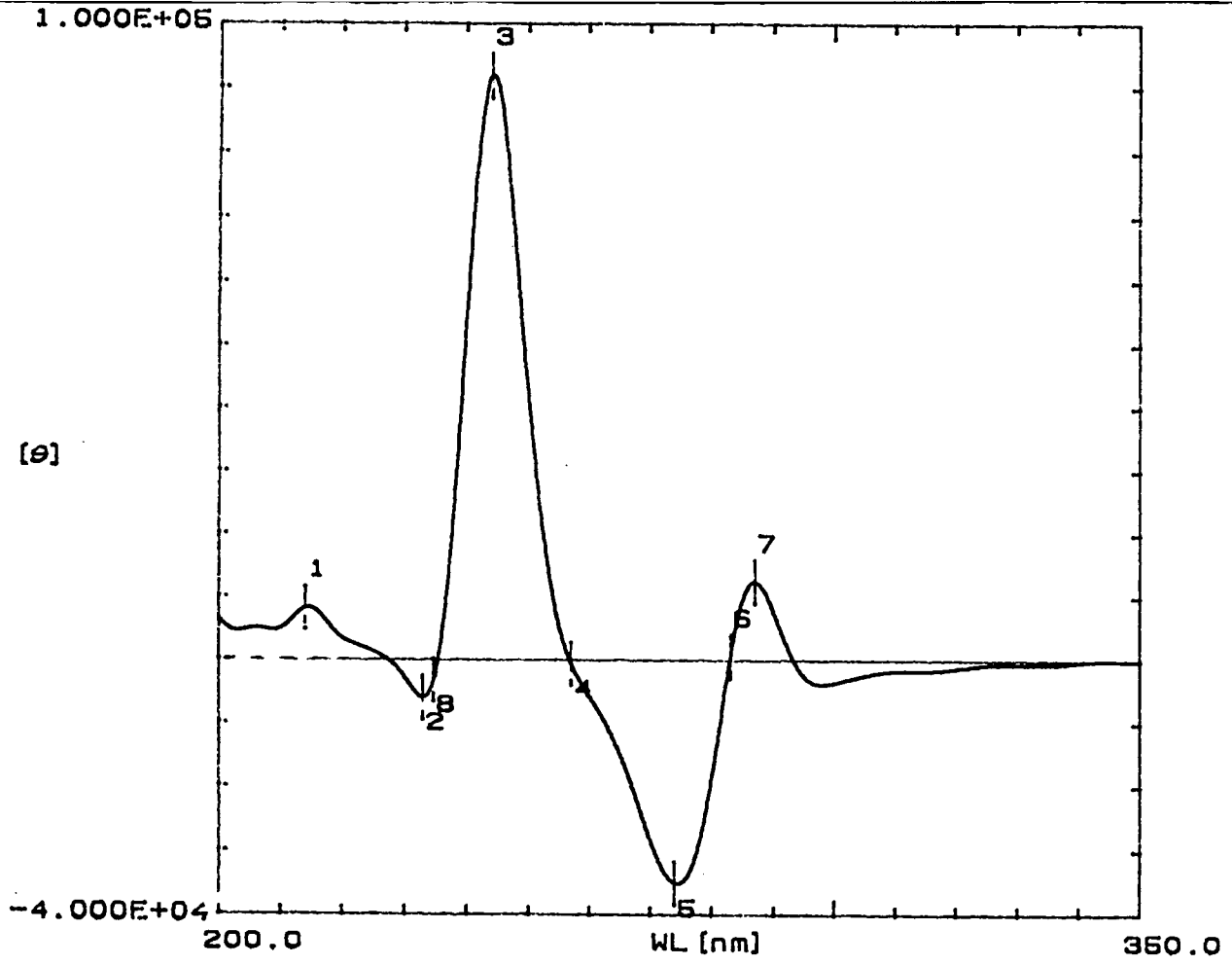


No.	Wavelength	Value
1	205.00 nm	1.701E+04
2	209.00 nm	7.910E+02
3	211.00 nm	-7.828E+03
4	213.00 nm	-3.722E+03
5	218.00 nm	1.809E+04
6	224.00 nm	4.512E+03
7	237.00 nm	3.081E+03
8	245.00 nm	-1.388E+05
9	257.00 nm	-6.318E+02
10	277.00 nm	6.013E+04
11	285.00 nm	3.880E+04
12	289.00 nm	4.271E+04
13	300.00 nm	3.950E+02

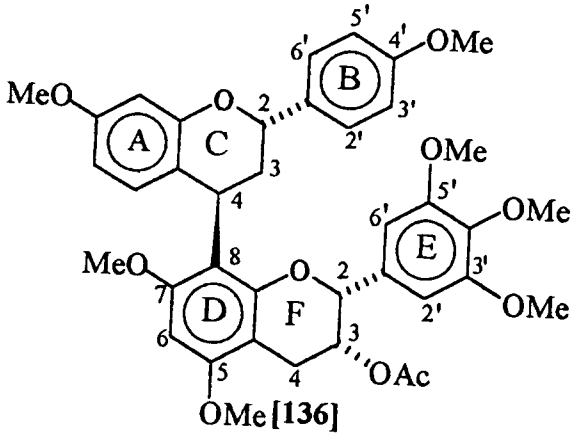


[150]

Plate 26

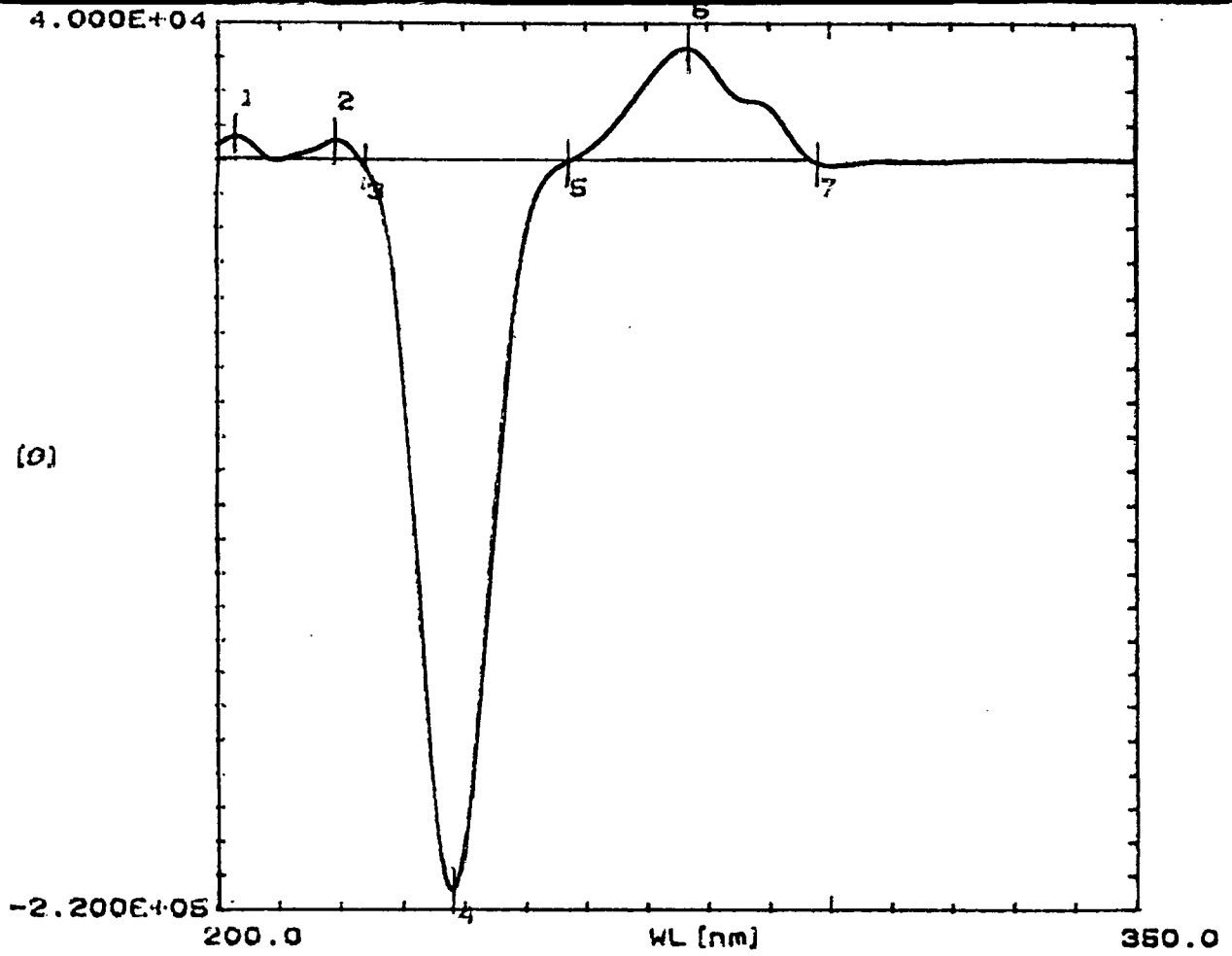


No.	Wavelength	Value
1	214.00 nm	8.206E+03
2	233.00 nm	-6.016E+03
3	244.00 nm	9.187E+04
4	257.00 nm	-5.887E+02
5	274.00 nm	-3.500E+04
6	283.00 nm	6.098E+02
7	287.00 nm	1.253E+04
8	234.70 nm	-3.254E+03

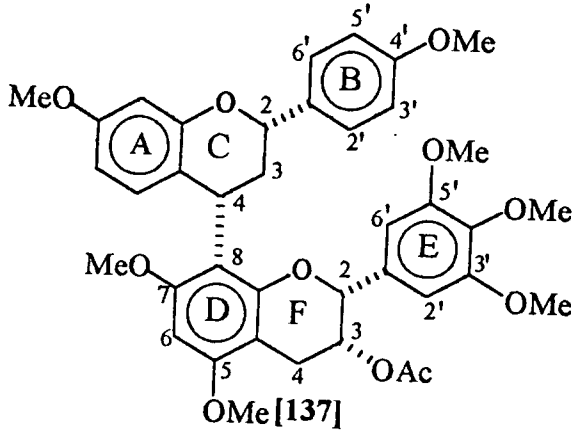


[136]

Plate 27



No.	Wavelength	Value
1	203.00 nm	5.782E+03
2	219.00 nm	5.729E+03
3	224.00 nm	-1.739E+03
4	238.00 nm	-2.138E+05
5	257.00 nm	-6.674E+02
6	277.00 nm	3.240E+04
7	298.00 nm	-8.072E+02



[137]

Plate 28

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