

# **PHOTOCHEMISTRY OF (+)-CATECHIN AND (-)-EPICATECHIN**

Thesis submitted in fulfillment of the requirements for the degree

**Master of Science in Chemistry**

in the

**Department of Chemistry  
Faculty of Agricultural and Natural Science**

at the

**University of the Free State  
Bloemfontein**

by

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**January 2008**

## ACKNOWLEDGEMENTS

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

Prof. J.H. van der Westhuizen as supervisor and Dr. S.L. Bonnet as co-supervisor for their guidance, assistance, perseverance and invaluable advice;

The NRF, the University of the Free State and Mimosa Central Co-operative Ltd for financial support;

Mrs Anette Allemann for the recording of MS and IR data and Prof. T. van der Merwe from FARMOVS-PAREXEL for the recording of MS data;

To my father and mother, Andrè and Birgit, without your support, love, understanding and trust in me, I could not have completed this study. Thank you so much for giving me the opportunity to further my education;

To my sister, Monique and all my friends, especially Elanè, Hantie, Nerina and Tersia for their moral support and encouragement;

To my grandmother, Hannelore and my grandfather Boxer for their love and support;

The staff and fellow postgraduate students in the Chemistry department for their encouragement;

Above all I would like to thank my Heavenly Father for His guidance and the health He has bestowed upon my family and I.

Anke

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## SUMMARY

Despite the well known fact that photolysis of free phenolic catechins give rise to isomerisation at the C-2 position (e.g. (-)-*cis* epicatechin converts to the sterically less hindered (-)-*trans* isomer), researchers have failed to isolate any ring opened compounds via trapping of intermediates with nucleophiles such as methanol or ethanol and radical trap solvents such as 2-propanol. *Re*-closing of the ring was slow enough to allow bond rotation to yield the observed isomerisation at C-2 but too fast to allow trapping of the intermediate by methanol or 2-propanol. This is unexpected given that thermal ring opening under mild conditions with acid, base or BF<sub>3</sub> catalysis had resulted in the isolation of many ring opened species.

Our aim was to reinvestigate the photochemistry of free phenolic (+)-catechin, (-)-epicatechin and (+)-fisetinidol at 250 nm and to trap the putative ring opened intermediates with a soft carbon centred nucleophile such as phloroglucinol.

Photolysis of (+)-catechin in the presence of phloroglucinol with methanol as solvent resulted in the isolation of the optically active product 1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol with (1*S*,2*S*) absolute configuration and unreacted optically active starting material.

Photolysis of (-)-epicatechin under the same conditions resulted in the isolation of the optically active product 1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol with (1*R*,2*R*) absolute configuration, unreacted optically active starting material (-)-epicatechin, as well as (-)-*ent*-catechin.

The two above mentioned products are enantiomers and have identical NMR spectra, but mirror image CD spectra. The two starting materials, (+)-catechin and (-)-epicatechin, are diastereoisomers and do not have identical NMR spectra. Acetylated (-)-*ent*-catechin

from photolysis of (-)-epicatechin has the same NMR spectra as acetylated (+)-catechin but mirror image CD spectra.

Identification of the methoxy-trapped products, 2-((2*S*,3*R*)-2-acetoxy-3-(3',4'-diacetoxyphenyl)-3-methoxypropyl)benzene-2'',4'',6''-triyl triacetate and 2-((2*S*,3*S*)-2-acetoxy-3-(3',4'-diacetoxyphenyl)-3-methoxypropyl)benzene-2'',4'',6''-triyl triacetate, indicates an ionic mechanism, as a radical mechanism would result in a —CH<sub>2</sub>OH substituted product.

The absence of any coupling products in photolysis of (+)-3',4',5,7-tetra-*O*-methylcatechin, indicates that a free phenolic OH on the 1-position of the B-ring is essential to stabilize the carbocation intermediate long enough for condensation to take place via a quinone methide.

Remarkable is the complete stereoselectivity. This indicates that the 3-hydroxy group allows the bulky phloroglucinol group to attack the quinone methide from the *anti*-position only.

Photolysis of (+)-fisetinidol under the same conditions as irradiation of (+)-catechin, yielded the expected propan-2-ol, (1*S*,2*S*)-3-(2,4-dihydroxyphenyl)-1-(3,4-dihydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-propan-2-ol.

Our photolytic synthesized products, (1*S*,2*S*)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol and (1*R*,2*R*)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol, also have a diaryl chromophore in the 1-position.

We established an aromatic quadrant based rule to correlate the stereochemistry of the biaryl moiety on C-1 with the sign of the Cotton effect of the CD spectra. This rule is in agreement with previous rules established for 4-arylflavan-3-ols.

Photolysis of (+)-3',4',5,7-tetra-*O*-methylcatechin in methanol and in the presence of 5 eq. phloroglucinol gave no coupling. Isolation of 2-(2-hydroxy-2-methylpropyl)-3,5-dimethoxyphenol in the presence of acetone represents trapping of the *o*-quinone methide. Irradiation of (+)-3-*O*-tosyl-3',4',5,7-tetra-*O*-methylcatechin (better leaving group on C-3) at 300 nm gave (+)-3',4',5,7-tetra-*O*-methylcatechin.

Retention of the absolute configuration at C-3 indicates that fission of the O-S bond took place and not the C-O bond. We postulated that the sulfone group acted as chromophore of the photochemically active compound and not the aromatic rings.

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## OPSOMMING

Ten spyte van die feit dat fotoliese van vry fenoliese katesjien oorsprong gee aan isomerisasie op die koolstof-2 posisie (bv. (-)-*cis*-epikatesjien skakel om na die steriese minder verhinderde (-)-*trans* isomeer), kon navorsers nie daarin slaag om enige oop-ring verbindings te isoleer via intermediêre met nukleofiele soos metanol of etanol en radikaal-vang oplosmiddels soos propan-2-ol. Dit is onverwags, aangesien termiese ring opening onder matige toestande met suur, basis of BF<sub>3</sub> kataliese tot die isolering van baie oop-ring spesies gelei het.

Ons doel was om die fotochemie van vry fenoliese (+)-katesjien, (-)-epikatesjien en (+)-fisetinidol by 250 nm te ondersoek om sodoende die oop-ring intermediêre vas te vang met 'n koolstof gesentreerde nukleofiel soos floroglusinol.

Fotoliese van (+)-katesjien in die teenwoordigheid van floroglusinol met metanol as oplosmiddel het gelei tot die isolering van die opties aktiewe produk 1,3-di(2,4,6-trihidroksifeniel)-1-(3,4-dihidroksifeniel)propan-2-ol met (1*S*,2*S*) absolute konfigurasie en opties aktiewe, ongereageerde uitgangstof.

Fotoliese van (-)-epikatesjien onder dieselfde toestande het gelei tot die isolering van die opties aktiewe produk 1,3-di(2,4,6-trihidroksifeniel)-1-(3,4-dihidroksifeniel)propan-2-ol met (1*R*,2*R*) absolute konfigurasie, opties aktiewe, ongereageerde uitgangstof en (-)-*ent*-katesjien.

Bogenoemde twee produkte is enantiomere met identiese KMR spektra, maar spieëlbeeld CD spektra. Die twee uitgangstowwe, (+)-katesjien en (-)-epikatesjien, is diastereoisomere en het verskillende KMR spektra. Geasetileerde (-)-*ent*-katesjien vanaf die fotoliese van (-)-epikatesjien het dieselfde KMR spektrum as geasetileerde (+)-katesjien maar spieëlbeeld CD spektra.

Identifikasie van die metoksie-vasgevangde produkte 2-((2*S*,3*R*)-2-asetoksi-3-(3',4'

diasetoksifeniel)-3-metoksipropiel)benseen-2",4",6"-triyl triasetaat en 2-((2*S*,3*S*)-2-asetoksi-3-(3',4'-diasetoksifeniel)-3-metoksipropiel)benseen-2",4",6"-triyl triasetaat, dui op 'n ioniese meganisme, aangesien 'n radikaal meganisme tot 'n —CH<sub>2</sub>OH gesubstitueerde produk sou lei.

Die afwesigheid van enige koppelingsprodukte in die fotoliese van (+)-3',4',5,7-tetra-*O*-metielkatesjien, dui aan dat 'n vry fenoliese OH op die 1-posisie van die B-ring nodig is om die karbokatioon-intermediêr lank genoeg te stabiliseer vir kondensasie om via 'n kinoonmetied plaas te vind.

Die reaksie is hoogs stereoselektief en die aanval van die steriese verhinderde floroglusinol nukleofiel vind anti plaas op die kinoon metied.

Fotoliese van (+)-fisetinidol onder dieselfde kondisies as die fotoliese van (+)-katesjien, het gelei tot die vorming van die verwagte propan-2-ol nl., (1*S*,2*S*)-3-(2,4-dihidroksifeniel)-1-(3,4-dihidroksifeniel)-1-(2,4,6-trihidroksifeniel)-propan-2-ol en wys na die vorming van 'n kinoon metied intermediêr.

Ons fotolitiese produkte (1*S*,2*S*)-1,3-di(2,4,6-trihidroksifeniel)-1-(3,4-dihidroksifeniel)propan-2-ol en (1*R*,2*R*)-1,3-di(2,4,6-trihidroksifeniel)-1-(3,4-dihidroksifeniel)propan-2-ol, het ook 'n diariël chromofoor in die 1-posisie. Die diariël chromofoor is verskillend van diè in 4-ariëlflavan-3-ol (Ring versus oop sisteem, dus konformasie (ring) versus vry rotasie).

Ons het 'n aromatiese kwadrantreël vasgestel om die stereochemie van die diariël moïeteit op die koolstof-1 posisie te korreleer met die teken van die Cotton effek van die CD spectrum. Hierdie reël is in ooreenstemming met vorige reëls wat neergelê is vir 4-ariëlflavan-3-ole.

Fotoliese van (+)-3',4',5,7-tetra-*O*-metielkatesjien in metanol en in die teenwoordigheid van 'n oormaat floroglusinol het geen koppeling gelever nie. Isolering van 2-(2-

hidroksi-2-metielpropiel)-3,5-dimetoksifenol in die teenwoordighed van asetoon verteenwoordig die vasvang van die *o*-kinoonmetied.

Fotoliese van (+)-3-*O*-tosiel-3',4',5,7-tetra-*O*-metielkatesjien (beter verlatende groep op koolstof-3) by 300 nm lewer (+)-3',4',5,7-tetra-*O*-metielkatesjien.

Retensie van die absolute konfigurasie op koolstof-3, dui daarop dat splyting van die O-S binding by voorkeur plaasgevind het bo splyting van die C-O binding. Ons het gepostuleer dat die sulfoongroep van die fotochemies aktiewe verbinding as die chromofoor optree en nie die aromatiese ringe nie.

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# CHAPTER 1

## 1. GENERAL

### 1.1 Introduction

Despite a vast amount of research over a long period of time by a large number of dedicated scientists, progress in the industrial and pharmaceutical use of flavonoids are still hampered by a lack of knowledge about the chemistry of these compounds.

The deceptively simple C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> formula (one heterocyclic and two aromatic rings) of the monomeric building blocks gives rise to almost intractable complex extracts and many synthetic challenges remain to be resolved. This may be attributed to the following:

1. More than 5000 monomeric flavonoids of diverse chemical structures and characteristics have been described.
2. The large number of reactive positions on these monomers available for condensation results in extremely complex mixture of dimers, trimers, tetramers, oligomers and polymers linked at different positions. The possibility of branching and rearrangement of monomer subunits further increases the complexity.
3. The free phenolic nature of the constituent monomers renders these compounds prone to oxidation as is evident in the production of tea by fermenting tannin containing tea leaves. This increases the complexity of extracts and renders isolation of pure unoxidised free phenolic components difficult.
4. The free phenolic nature of constituent monomers renders chromatography difficult. Traditional silica based chromatography is not well suited for purification of free phenolic flavonoids and tannins because of the strong adsorption of these compounds on silica gel and subsequent low recovery rates. In fact, silica gel is used to remove flavonoids and tannins from plants extracts for biological screening.
5. The high chirality (three stereogenic centers on each monomeric building block except on the terminal unit) of the constituent monomers.

6. The high sensitivity of the conformation of the heterocyclic ring towards the substitution pattern and stereochemistry on the individual constituent carbons of this ring (three positions per monomeric unit).

The current crude oil shortages and political instability in the major oil producing countries have led to shortages and high crude oil prices. This has stimulated and renewed interest in sustainable agricultural based raw materials such as high tannin vegetable extracts for the chemical industry.

Increased safety and environmental concerns have stimulated a reversal in the world wide decline in the use of high tannin vegetable extracts as tanning materials. Leather tanned with vegetable extracts is biodegradable and can be recycled. Production of chromium tanned leather contaminates the environment and has to be correctly disposed of when these are no longer in use. For example, luxury cars that are marketed as environmentally friendly and fully recyclable cannot use chromium tanned leather.

A growing body of scientific evidence suggests that flavonoids have important biological effects including antitumor, antibacterial, antiviral, anti-oxidant, anti-allergic and anti-inflammatory effects. Epidemiological studies suggest that regular consumption of red wine has a beneficial effect on cardiovascular disease, cancer prevention and longevity. This so called "French Paradox" is mainly attributed to the high tannin content of red wine.

The increasing realisation that many of the beneficial health properties cannot be explained by previously believed non-specific enzyme inhibition and anti-oxidant activity, has stimulated a demand for free phenolic flavonoids for bio-assay screening by the drug discovery industry. The vast majority of known flavonoids have been isolated and purified as methyl ethers or acetates. Methylation and acetylation, whilst useful to isolate, purify and for structure elucidation, destroy the water solubility of these compounds and also the biological activity. The vast majority of published chemistry of

polyphenols applies to ethers and acetates and cannot be extrapolated reliably to the chemistry of the underivatized free phenolic entities and extracts.

This realisation and demand coincided with progressive improvement and availability of chromatographic methods more suitable to water soluble polar compounds (e.g. reverse phase HPLC and countercurrent chromatography) that promises access to compounds that was hitherto considered inaccessible or too laborious to pursue.

## 1.2 Reason for this study

Our investigation of the photochemistry of free phenolic catechin was prompted by the following:

1. The paucity of published results on the photochemistry of free phenolic flavonoids. A literature survey yielded only a few publications (cf. Literature Survey in Chapter 2).
2. The absence of investigations into the photochemistry of flavonoids at short wavelengths (250 nm) where free phenols normally absorb light. Most of the published photochemistry of flavonoids takes place from an  $n,\pi^*$ -excited state associated with a carbonyl chromophore conjugated with an aromatic ring that absorbs light at about 350 nm. Tannins and polyphenols often do not contain a carbonyl chromophore and requires light of 250 nm to obtain  $\pi,\pi^*$ -excited states.
3. The fact that phenols have a much lower pKa value in the excited state. Phenols are more acidic in the  $S_1$  than in the  $S_0$  state. This opens the way for new chemistry.
4. The realisation that B-ring quinone methides plays a major role in the chemistry of phenolic flavonoids and that photochemistry may offer a mild route to these intermediates.

5. In contrast with the radical type reactions associated with  $n,\pi^*$  triplet excited states,  $\pi,\pi^*$  singlet excited states may demonstrate ionic characteristics.

### 1.3 Aim of this study

Despite the well known fact that photolysis of free phenolic catechins gives rise to isomerisation at C-2 [e.g. (-)-*cis* epicatechin converts to the thermodynamically more stable (-)-*trans* isomer], there is only a single report documenting the trapping of intermediates in nucleophilic solvents such as methanol, ethanol or radical trap solvents such as 2-propanol. Recyclization of the ring was slow enough to allow bond rotation to yield the observed isomerisation at C-2 but too fast to allow trapping of the intermediate by methanol or 2-propanol. This is unexpected given that thermal ring opening under mild conditions with acid, base or  $\text{BF}_3$  catalysis had resulted in the isolation of many ring opened species (see Chapter 2).

Our aim was to reinvestigate the photochemistry of free phenolic (+)-catechin, (-)-epicatechin and (-)-fisetinidol at 250 nm, and to trap the putative acyclic intermediates with a soft carbon nucleophile such as phloroglucinol.

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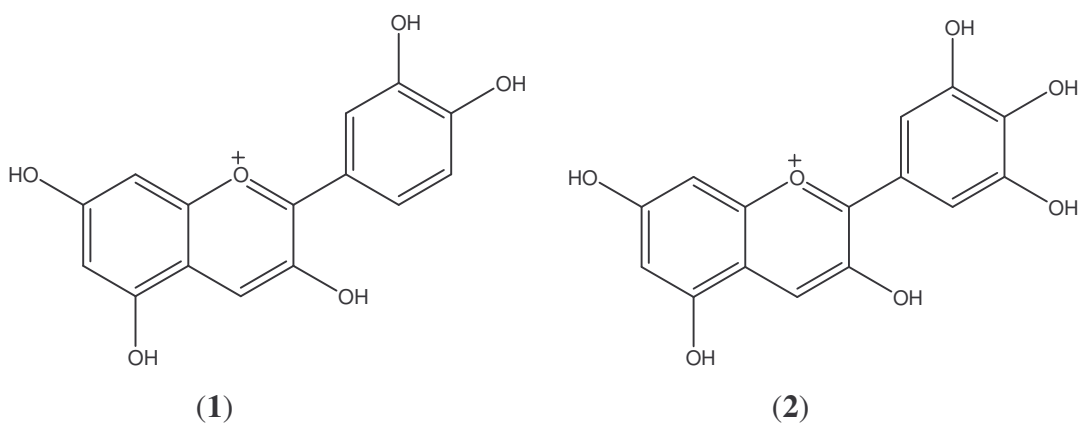
## CHAPTER 2

### 2. LITERATURE SURVEY

#### 2.1 Proanthocyanidins and Tannins

Proanthocyanidins are polyphenolic compounds that occur widely in woody and some herbaceous plants.<sup>1,2,3,4,5,6</sup> They are known as astringent, bitter-tasting plant polyphenols that bind and precipitate proteins.<sup>7,8</sup> They are flavan-3-ol oligomers that produce anthocyanidins e.g. (1) by cleavage of C-C-interflavanyl bonds on heating with mineral acids.<sup>1,9</sup> The prodelphinidins yield delphinidin (2) under the same conditions.

Proanthocyanidins are postulated to be formed by ionic coupling at C-4 of the C-ring of an electrophilic flavanyl unit, generated from a flavan-4-ol<sup>2</sup> or flavan-3,4-diol,<sup>3</sup> to the nucleophilic A-ring of another flavanyl moiety, usually a flavan-3-ol.



This is in contrast with the origin of the other major classes of complex C<sub>6</sub>-C<sub>3</sub>-C<sub>6</sub> secondary metabolites, bi- and triflavonoids.<sup>3,10</sup> They are postulated to be the products of oxidative coupling of monomers that possess a carbonyl group at C-4.



The leucoanthocyanidins, flavan-4-ols and flavan-3,4-diols, act as electrophilic chain-extender units in the synthesis of proanthocyanidins and the flavans and flavan-3-ols as nucleophilic chain-terminating units.<sup>2</sup>

The ability to complex with proteins via hydrogen bonds explains the use of tannins for leather tanning.<sup>11</sup> Tannin extraction from wattle bark (South Africa) and quebracho (South America) are important industries that supply raw materials for leather tanning.

Polyphenolic tannins are widely used as cold-set adhesives<sup>12,13,14,15,16</sup> for wood laminating. Tannins have reactive phenolic aromatic rings that react with formaldehyde to polymerize further. The reactive sites are, however, limited and much of the applied research on the chemistry of tannins have revolved around efforts to cleave the heterocyclic C-ring and create additional reactive sites for polymerization for cold set adhesive applications.<sup>16</sup> The unreactive tannin is activated by the addition of a limiting amount of formaldehyde in the presence of methanol. This prevents selfcondensation and provides hydroxymethyl moieties for polymerization with subsequently added resorcinol.

Tannins are responsible for much of the taste and flavour properties of tea. In Chinese or green tea, the fresh tea leaves are heated immediately after picking followed by drying to destroy enzymes. This tea contains mostly monomers of which epigallocatechin is the most important.<sup>17</sup> In Indian tea the freshly picked tea leaves are fermented. This leads not only to enhanced flavour but also to enzyme catalysed polymerisation of the monomeric phenols to a predominance of tannins (thearubigins), and by phenolate oxidative conversions to the theaflavins. The habit of adding milk to black Indian tea can probably be explained by the binding between milk proteins and tannins in the tea that ameliorates the excessive bitter taste.

Because tannin is a polymer that consists mainly of catechin monomers and because degradation studies mostly yield catechin or other flavan-3-ols, the chemistry of catechin was historically studied to obtain a better understanding of tannin chemistry. Due to the

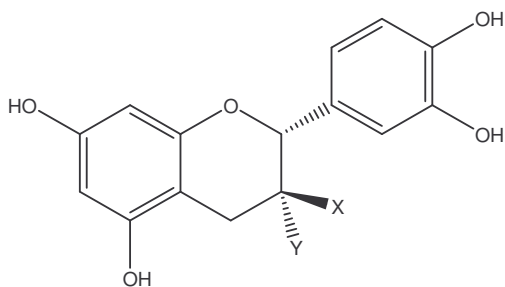
structure correspondence with condensed tannins, 4-arylflavan-3-ols have also been used as model compounds in the investigation of the chemical behaviour of tannins.

In recent years it has been realised that catechin is a major constituent of food with tantalizing health benefits and the chemistry of catechin has become important in its own right.

## 2.2 Catechins

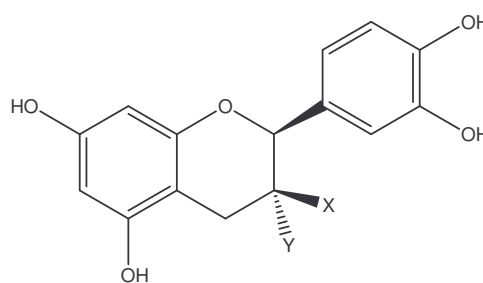
Flavan-3-ols, such as epicatechin, catechin and their oligomers, represent a major class of secondary polyphenolic plant metabolites.<sup>18,19</sup> Flavan-3-ols are present in most higher plants, and their high content in certain food plants, such as *Vitis vinifera* (grape wine), *Camellia sinensis* (tea), and *Theobroma cacao* (cocoa) are especially noteworthy in the context of human nutrition. They act as potent nucleophiles during the biosynthesis of oligomers.<sup>20</sup> 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 (**11**).<sup>20</sup>

The catechins are part of flavan-3-ols including (+)-catechin (**3**), (-)-epicatechin (**4**), (-)-*ent*-catechin (**5**), (+)-*ent*-epicatechin (**6**) and their derivatives (C-3-*O*-esters).<sup>21</sup>



(**3**) (+)-catechin): X=OH, Y=H

(**4**) (-)-(epicatechin): X=H, Y=OH



(**5**) (-)-*ent*-catechin): X=H, Y=OH

(**6**) (+)-*ent*-epicatechin): X=OH, Y=H

Because it is so common in vegetables and fresh fruit, catechins are important ingredients in our diet. The health benefits of catechins have been studied extensively in humans and in animal models. Catechins possess numerous important biological activities, such as

antimutagenic activity, antitumor activity and antioxidant properties.<sup>22,23,24</sup> Due to catechin's antioxidant properties, it exhibits protective effects against diseases involving oxidative stress such as cancers,<sup>25,26</sup> cardiovascular diseases<sup>27,28</sup> and neurodegenerative diseases.<sup>29</sup> This is supported by statistical and epidemiological investigations.<sup>25,26</sup>

(-)-Epicatechin plays a major role in the improvement of blood flow for cardiac health.<sup>30</sup> The epimers (-)-*ent*-catechin (**5**), (-)-*ent*-gallocatechin, and their gallates are effective in inhibiting cholesterol absorption.<sup>31</sup>

Before the agricultural era (hunter-gatherer era), modern man's diet supported a much higher catechin and flavonoid intake. Archeological and other evidence suggested man was much bigger and lived longer. Introduction of agriculture led to large populations being supported by relatively monotonous food sources. Today our fruits contain relatively low levels of catechin (low skin to mass ratio in commercial cultivars).

Flavonoids have a range of important functions in plants. These include structural components (lignin), protection against stress (antipathogenic phytoalexins, antioxidants and UV-absorbing compounds), pigments and signalling molecules.<sup>32</sup>

PAL (Phenylalanine Ammonium Lyase), CHS (Chalcone Synthase) and other branch point enzymes of the phenyl propanoid pathway are stimulated by solar radiation (UV-B). PAL catalyses the transformation of phenylalanine to *trans*-cinnamic acid that leads to the formation of complex phenolic compounds including flavonoids, tannins and lignin.<sup>33</sup> UV-B stimulates large increases of quercetin in the upper epidermis of *Vicia faba* (broad beans). A similar result was obtained with *Brassica napus*. It was considered essential to compare leaves at the same developmental stage, as flavonoid content generally decreases with leaf ageing.<sup>34,35</sup>

These results support the hypothesis that polyphenolics provided land plants with an internal filter against damaging solar UV-B and allowed land plants to evolve from marine and fresh water plant life. With the lack of ozone in the stratosphere early plant life without polyphenols was probably restricted to aquatic ecosystems where the filtering

of UV-B radiation (280 to 315 nm) by substances dissolved or suspended provided protection.<sup>34,35</sup>

## 2.3 Chemistry of catechins

### 2.3.1 Discovery and structure elucidation of catechins

The first catechin<sup>36</sup> [probably (-)-epicatechin (**4**)] was isolated by Runge<sup>37</sup> in 1821 from *Acacia catechu*. The first representation and stereochemistry of (+)-catechin (**3**) was, however, done by Freudenberg and coworkers.<sup>38</sup>

King and coworkers<sup>39</sup> as well as Whalley<sup>40</sup> determined the absolute configuration and structure of (+)-catechin (**3**) as (2*R*,3*S*)-3',4',5,7-tetrahydroxyflavan-3-ol. A further contribution was when Mayer and Bauni<sup>41</sup> made a correlation between the stereochemistry of (+)-gallocatechin and that of (+)-catechin (**3**).

Clark-Lewis and coworkers<sup>42</sup> showed by means of nuclear magnetic resonance that the heterocyclic rings of the catechins and the flavan-3,4-diols can adopt a five-point coplanar or half-chair conformation. The reasons for the difference in size of the coupling constants of the heterocyclic protons of the 2,3-*cis*- and the 2,3-*trans*-configurations became clear from this investigation:

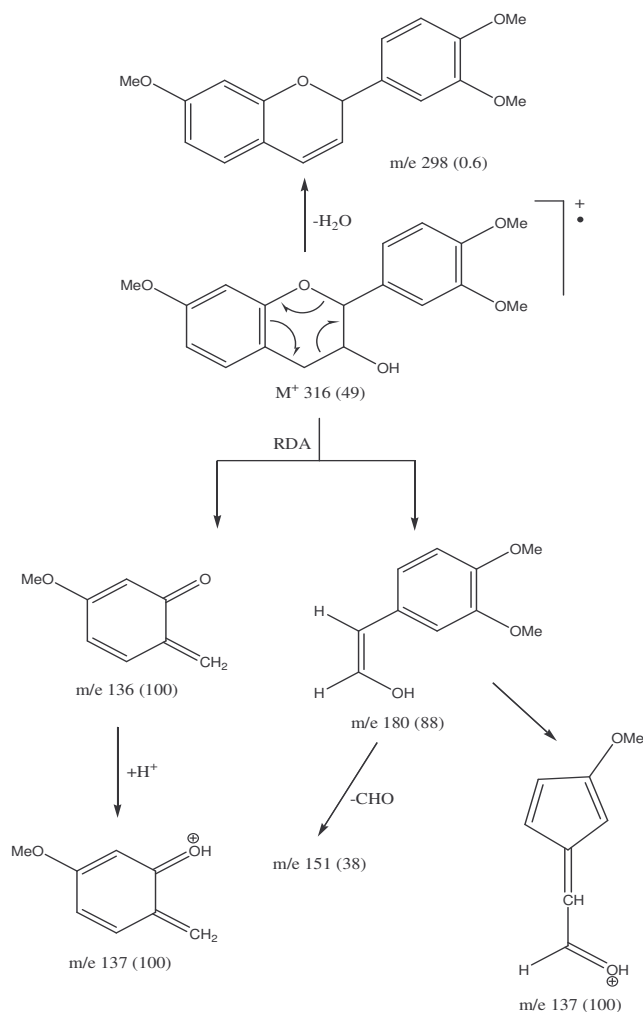
**Table A:** Coupling constants of heterocyclic protons of the 2,3-*cis*- and the 2,3-*trans*-flavan-3,4-diols.

	<b>J<sub>2,3</sub></b>	<b>J<sub>3,4</sub> (<i>cis</i>)</b>	<b>J<sub>3,4</sub> (<i>trans</i>)</b>
2,3- <i>trans</i> -3,4- <i>trans</i> -flavan-3-ol	8.2	5.6	9.0
2,3- <i>cis</i> -3,4- <i>trans</i> -flavan-3-ol	1.2	4.4	2.4

Van Rensburg and co-workers<sup>43</sup> reported that asymmetric dihydroxylation of a series of polyoxygenated 1,3-diarylpropenes with AD-mix- $\alpha$  or AD-mix- $\beta$  in the presence of methanesulfonamide and subsequent acid-catalysed cyclization afforded synthetic access to *trans*- and *cis*-flavan-3-ol derivatives, in excellent enantiomeric access and in good yields.

Mass spectra of (+)-3',4',7-tri-*O*-methylfisetinidol show an intense molecular ion [ $M^+$  316 (49)], but only low intensity ions of high mass. Ions that originate from Retro Diels-Alder (RDA)-fragmentation is dominant, while hydrogen-transfer to the primary A-ring fragment forms the base peak.<sup>44,45,46,47</sup> Drewes<sup>44</sup> postulated the following fragmentation patterns for flavan-3-ols: (Note that the *m/e* 137 fragment can form in two ways).

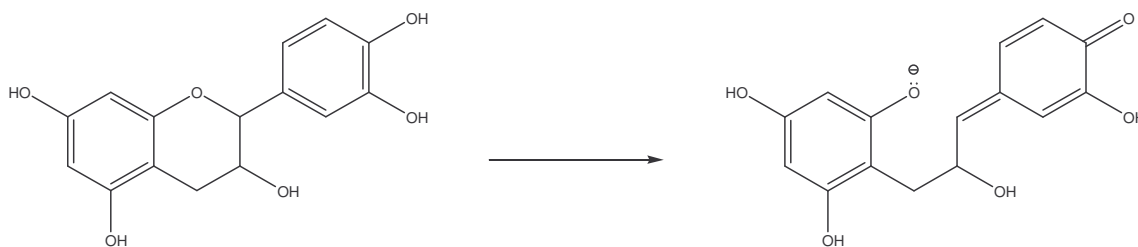
**(Scheme 1)**



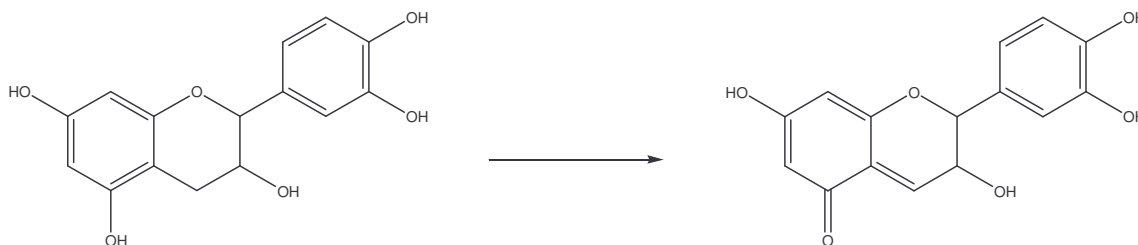
**Scheme 1**

### 2.3.2 Quinone methides

Much of the chemistry and photochemistry of catechin have been explained by formation of quinone methides. The quinone methides involved are either *p*-quinone methides from fission of the pyran ether bond (B-ring quinone methides) or *o*-quinone methides (A-ring quinone methides).<sup>48</sup>



*p*-quinone methides



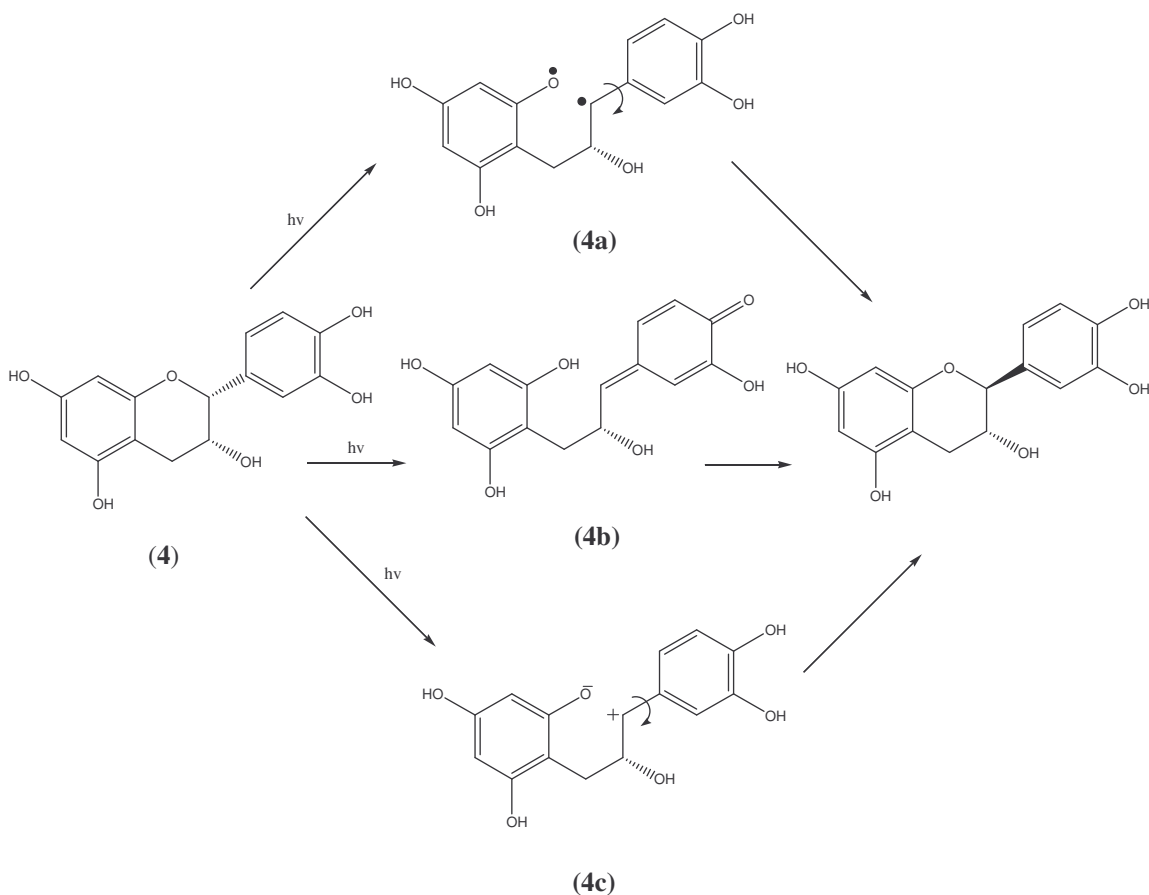
*o*-quinone methides

The *p*-quinone methide can be considered as a formally neutral benzylic carbocation at which there is limiting resonance stabilisation by electron donation from a *p*-oxygen anion substituent to the cationic benzylic carbon.<sup>49</sup> This strong interaction results in a high kinetic stability and large nucleophilic selectivities toward quinone methides.<sup>50,51</sup>

*Para*-quinone methides (*p*-QMs) are less polarised and more stable than their corresponding *o*-QMs and therefore are formed more readily than *o*-QMs. Hence formation of *o*-QMs is viable only if the *para*-position is unsubstituted; or substituted with a functional group that contains no  $\alpha$ -protons.<sup>52</sup>

Hemingway<sup>53</sup> postulated that procyanidin synthesis takes place via quinone methides and not via benzylic carbocations because base catalysis accelerates the reaction relative to

acid catalysis. The photochemical isomerization at C-2 of (-)-epicatechin (**4**) can take place via a radical (**4a**), quinone methide (**4b**) or ionic mechanism (**4c**).<sup>73</sup>



### Scheme 2

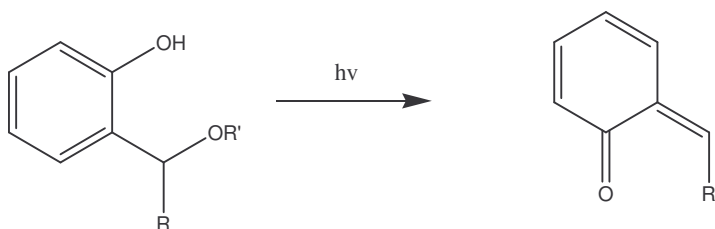
Phenols are more acidic in the photolytic excited state,  $S_1$  ( $pK_a \approx 4$ ) than in the ground state,  $S_0$  ( $pK_a \approx 10$ ). Water and methanol are sufficiently polar to mediate dissociation of excited-state phenols during the lifetime of the singlet state,  $S_1$ . Dissociation of the phenolic proton in  $S_1$  leads to an excited-state phenolate ion (adiabatic dissociation). Such excited state phenolates have their negative charge strongly delocalized into the aromatic ring.

It thus postulates that the intermediate in photochemical isomerisation of catechin at C-2 takes place via a quinone methide (**4b**). This is supported by the isolation of *p*-quinone methides from photolysis of benzylic alcohols (**Scheme 3**).<sup>48</sup>



### **Scheme 3**

Padwa and Wan demonstrated photolytic (room temperature, 254 nm) generation of *o*-QMs from *o*-hydroxybenzylalcohols (**Scheme 4**).<sup>54,55</sup>



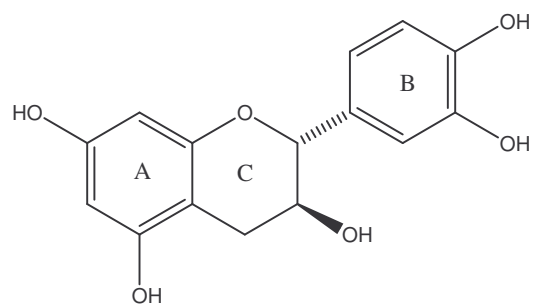
### **Scheme 4**

## **2.4 Cleavage of the heterocyclic ring of catechins**

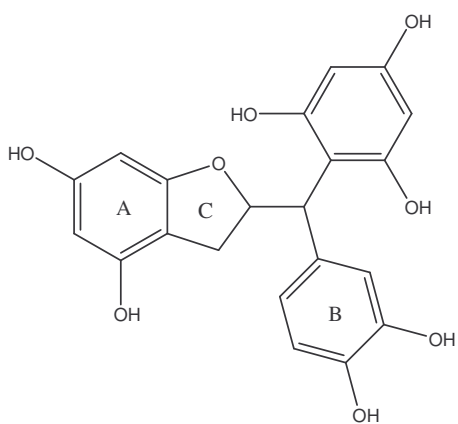
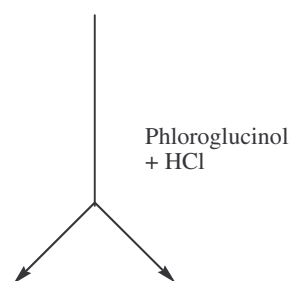
### **2.4.1 Acid catalysis**

Mayer and Merger<sup>56</sup> cleaved the heterocyclic ring of (+)-catechin in 1959 by means of HCl in the presence of excess phloroglucinol and assigned two possible structures (**7**) or (**8**) to the main product. (**Scheme 5**) The formation of (**7**) or (**8**) highlighted the electrophilic nature of C-2 of (**3**) under acidic conditions.

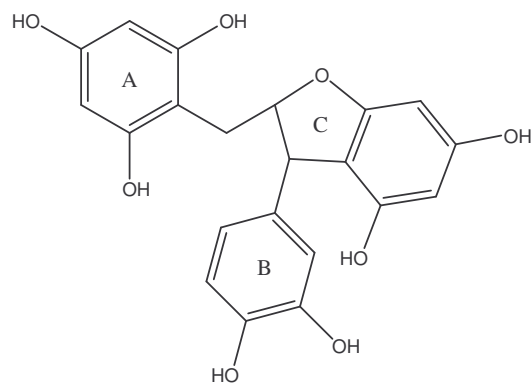




(3)



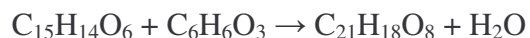
(7)



(8)

**Scheme 5**

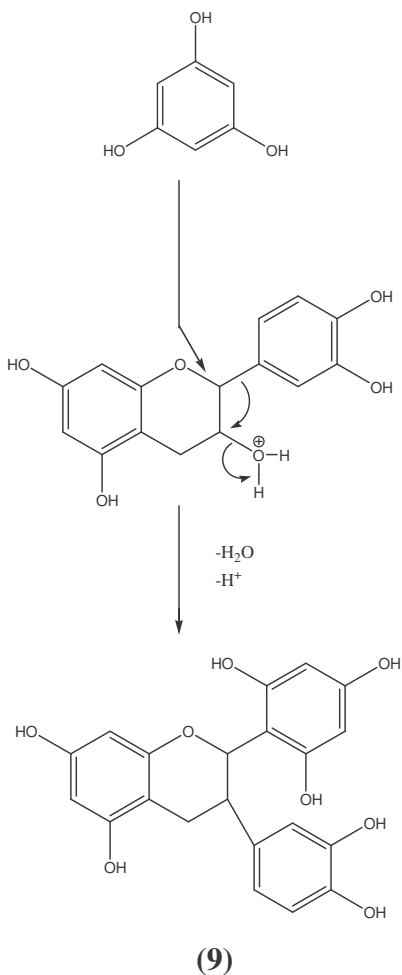
Mayer and Merger<sup>57</sup> showed in 1961 that catechins react with phloroglucinol in aqueous acidic medium according to the following equation:



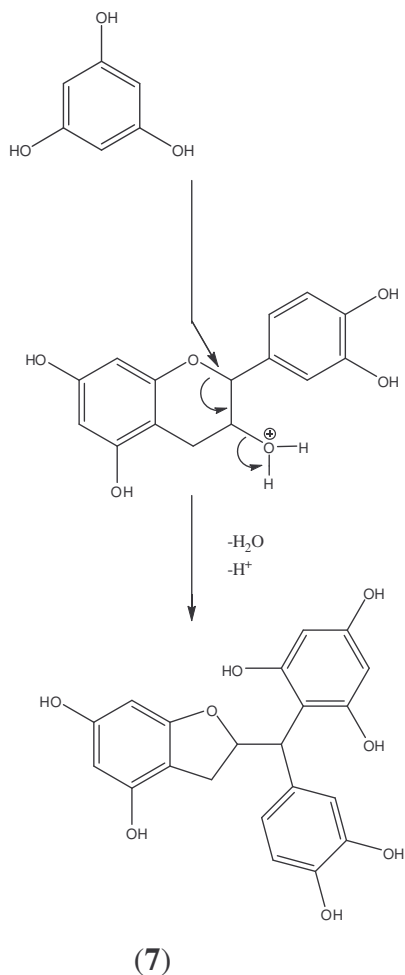
It was found that (+)-catechin (**3**) and (+)-*ent*-epicatechin (**6**) gave identical reaction products, however, (-)-*ent*-catechin (**5**) and (-)-epicatechin (**4**) gave enantiomers.

Based on the mechanism, two possible structures (**9**) and (**7**) were assigned to the product. (Mechanisms A and B respectively, **Scheme 6**) Both structures and mechanistic approaches were however later proved incorrect by the same researchers.<sup>58,59</sup>

### Mechanism A

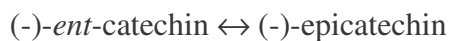
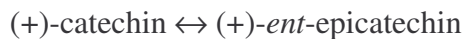


### Mechanism B

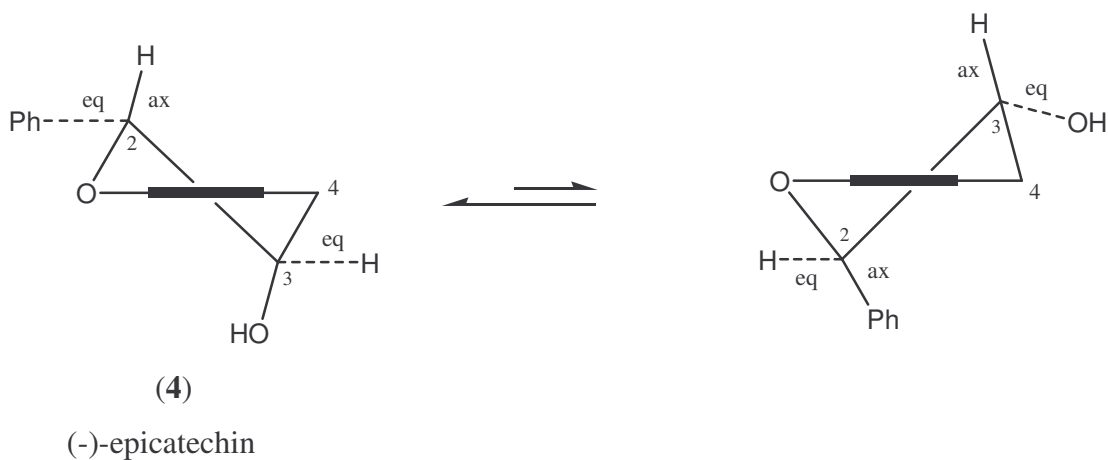
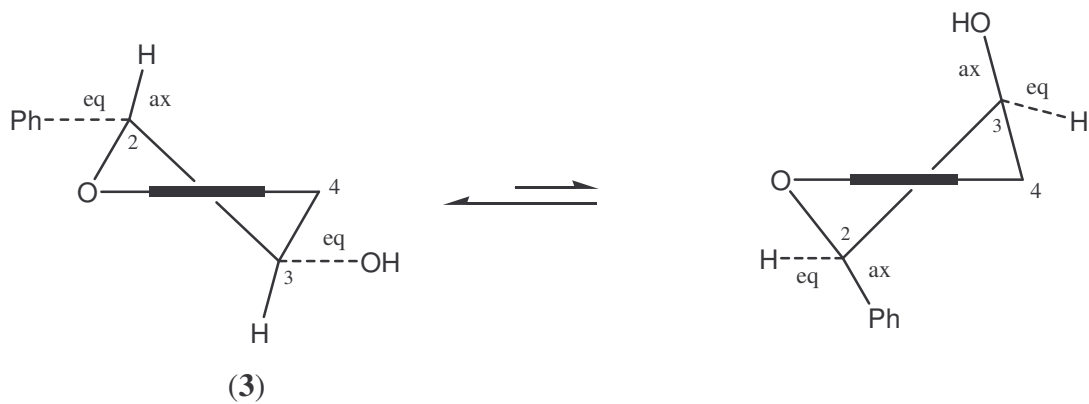


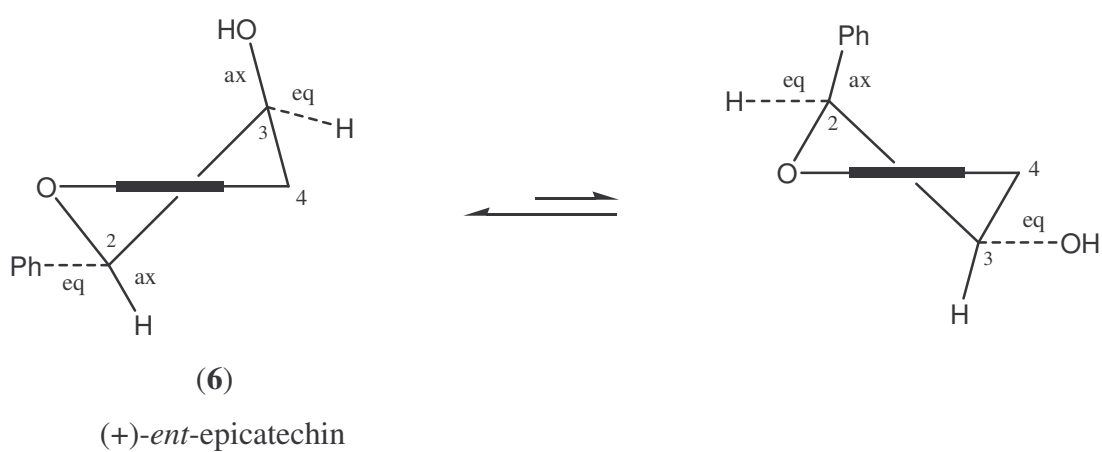
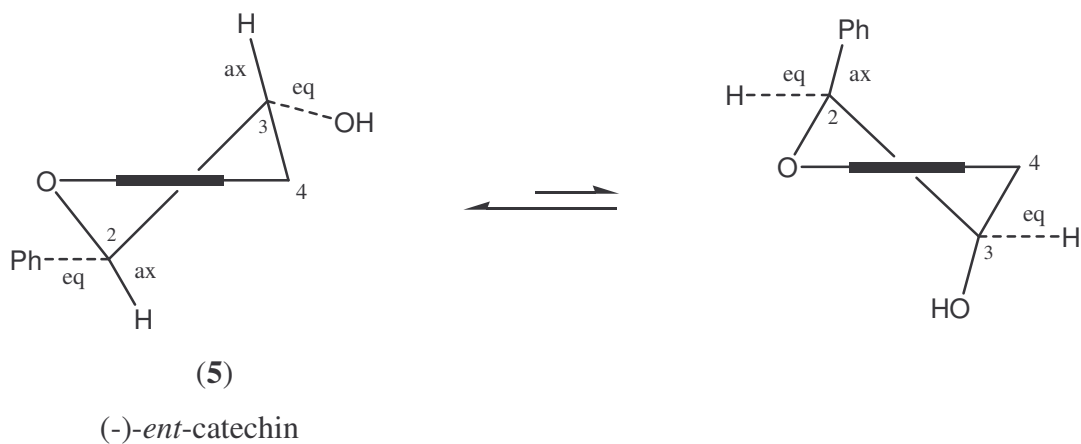
### Scheme 6

If the conformation of catechins (**3** and **5**) and epicatechins (**4** and **6**) are investigated and by assuming that the following epimerization-equilibria are possible,

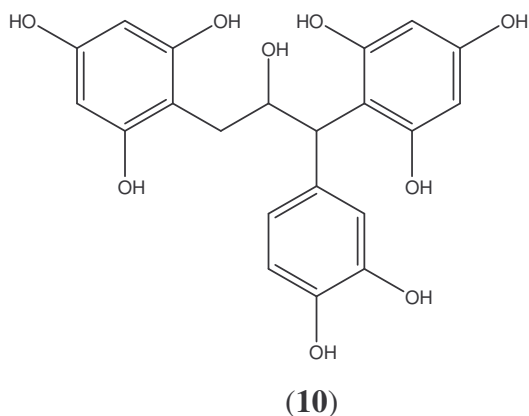


then it is clear why (+)-catechin (**3**) and (+)-ent-epicatechin (**6**) generates a single reaction product and that (-)-ent-catechin (**5**) and (-)-epicatechin (**4**) give an identical product, excluding the optical rotation.



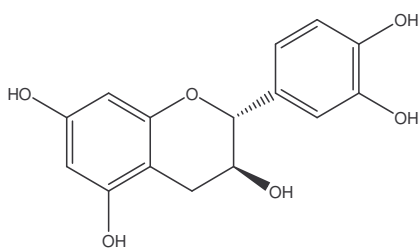


Mayer and coworkers<sup>58</sup> however, reported during 1963 that the main product of both (+)-catechin (3)/(+)-ent-epicatechin (6) and (-)-ent-catechin (5)/(-)-epicatechin (4) condensations with phloroglucinol probably has a common origin in the intermediate product (10).

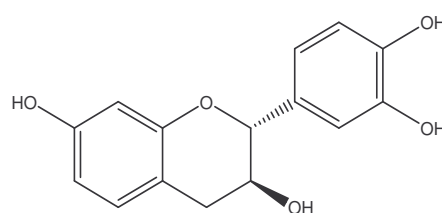


Structure **(10)** was not considered as a transition product in terms of the formation of di-catechin according to Freudenberg and Weinges.<sup>60</sup>

Botha and coworkers<sup>61</sup> reported that some model tannin building blocks, namely (+)-catechin, **(3)** and (-)-fisetinidol, **(11)** can be activated by means of acid-catalysed fission of their heterocyclic rings with simultaneous grafting of nucleophilic phenolic species such as phloroglucinol and resorcinol A-rings at the 2-positions. This does not only activate existing phloroglucinol and resorcinol units present in the flavonoid molecule through cleavage of the heterocyclic ether bond, but also furnishes new reactive positions on the grafted phenolic units, enabling the modified molecule to react spontaneously with formaldehyde at both ends.

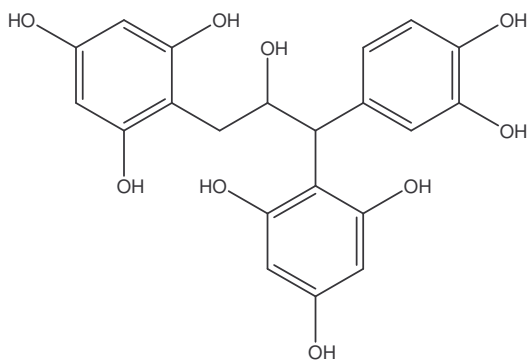


**(3)**

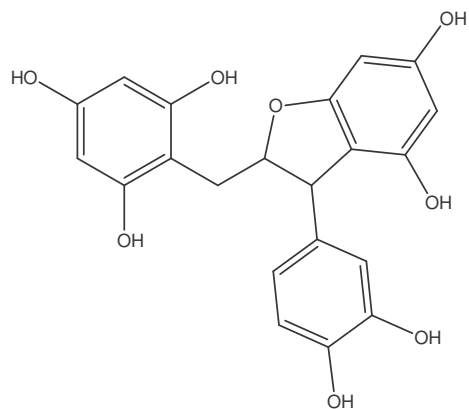


**(11)**

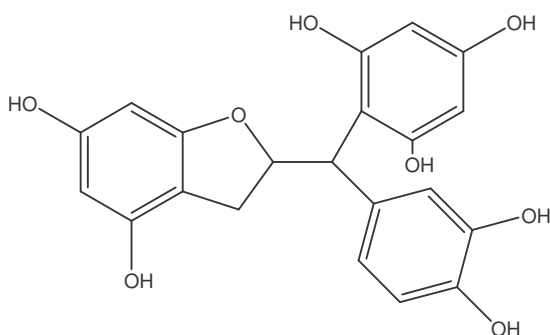
The acid-catalysed, nucleophilic condensations between the flavan-3-ols **(3)** and **(11)** and phloroglucinol or resorcinol, respectively gives without exception a 1,1,3-triphenylpropanol **(12)** which, depending on the structure and presence of a 'free' phloroglucinol moiety in the grafted molecule, will undergo cyclization to a 2-benzyl-3-phenyl-2,3-dihydrobenzofuran **(13)** or a 2-diphenylmethyl-2,3-dihydrobenzofuran **(14)**.



(12)



(13)



(14)

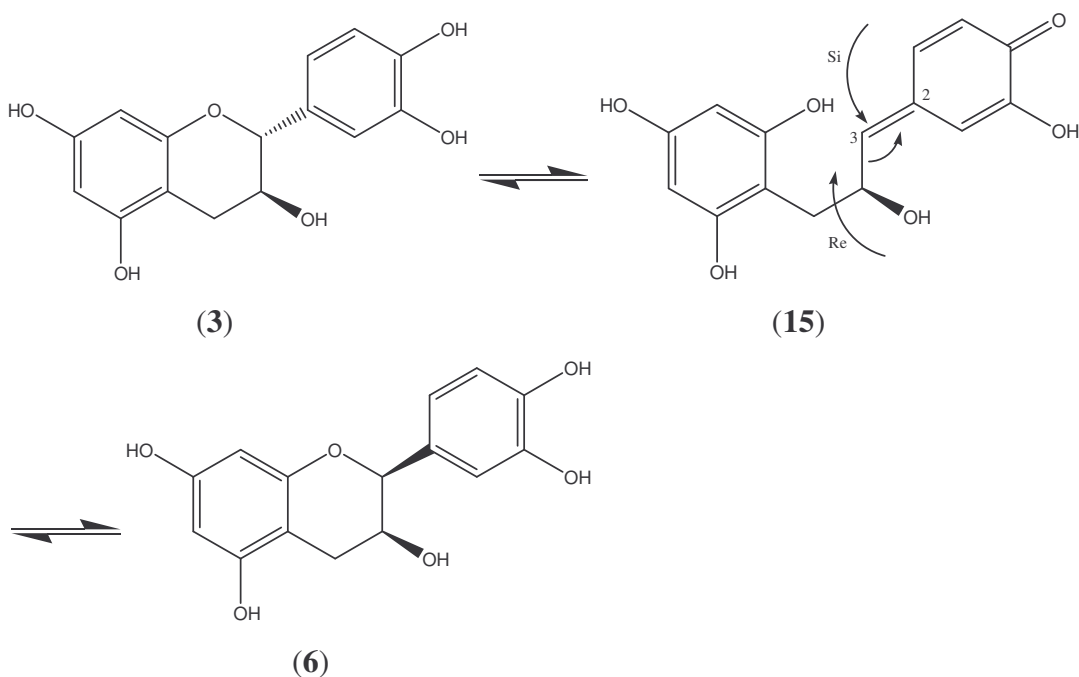
Where the attacking nucleophile is phloroglucinol, cyclization occurs *via* intramolecular water elimination between a hydroxy group of the grafted phloroglucinol and the only available aliphatic hydroxy at C-2 of the 1,1,3-triarylpropan-2-ol with formation of a 2-benzyl-3-phenyl-2,3-dihydrobenzofuran (13).<sup>62</sup>

When the flavan-3-ol itself presents a phloroglucinol unit after heterocyclic ring opening, and where the attacking nucleophile is resorcinol, then water elimination occurs *via* a hydroxy group of the original phloroglucinol unit and the aliphatic hydroxy to deliver a 2-diphenylmethyl-2,3-dihydrobenzofuran (14). No cyclization of the formed 1,1,3-triphenylpropan-2-ol (12) takes place without a phloroglucinol moiety.

Peng, Conner & Hemmingway<sup>63</sup> treated catechin with mineral acids (H<sub>2</sub>SO<sub>4</sub>, HCl or BF<sub>3</sub>.H<sub>2</sub>O) in the presence of phenol and obtained products consistent with the opening of the pyran ring and nucleophilic attack at C-2 by the *para*-position of phenol as described by Mitsunaga.<sup>64,65,66</sup> (See 2.4.3)

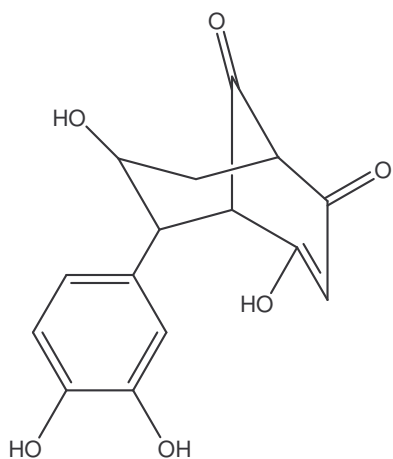
## 2.4.2 Base catalysis

When (+)-catechin (**3**) is dissolved in an alkaline solution under mild conditions (ambient temperature, pH 10.5) the pyran ring cleaves to give a quinone methide (**15**) that recondenses with the phloroglucinol A-ring to form a mixture of (+)-catechin (**3**) (*Si*-face attack) and (+)-*ent*-epicatechin (**6**) (*Re*-face attack) in a 3.5 to 1 ratio (**Scheme 7**).<sup>67</sup>



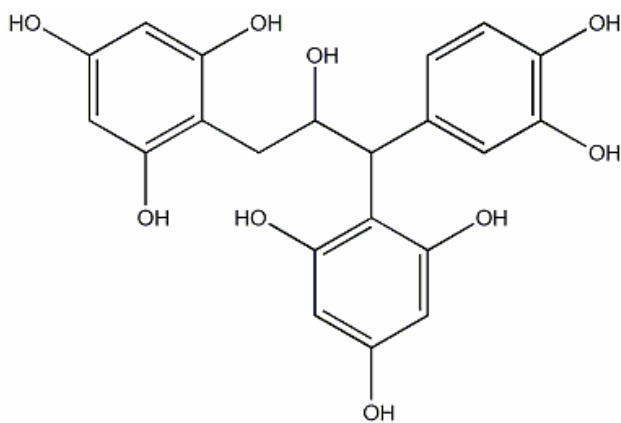
### **Scheme 7**

Under more drastic conditions (0.5% NaOH, reflux for 45 minutes), the phloroglucinol A-ring attacks the quinone methide via carbon with subsequent rearrangement to form catechinic acid (**16**)<sup>68</sup> [the enol of 6-(3,4-dihydroxyphenyl)-7-hydroxy-2,4,9-bicyclo[3,3,1]nonatrione] in higher than 90% yield. The absolute configuration was determined by X-ray analysis and is consistent with *Re*-face attack of the phloroglucinol A-ring on the quinone methide (**15**).



(16)

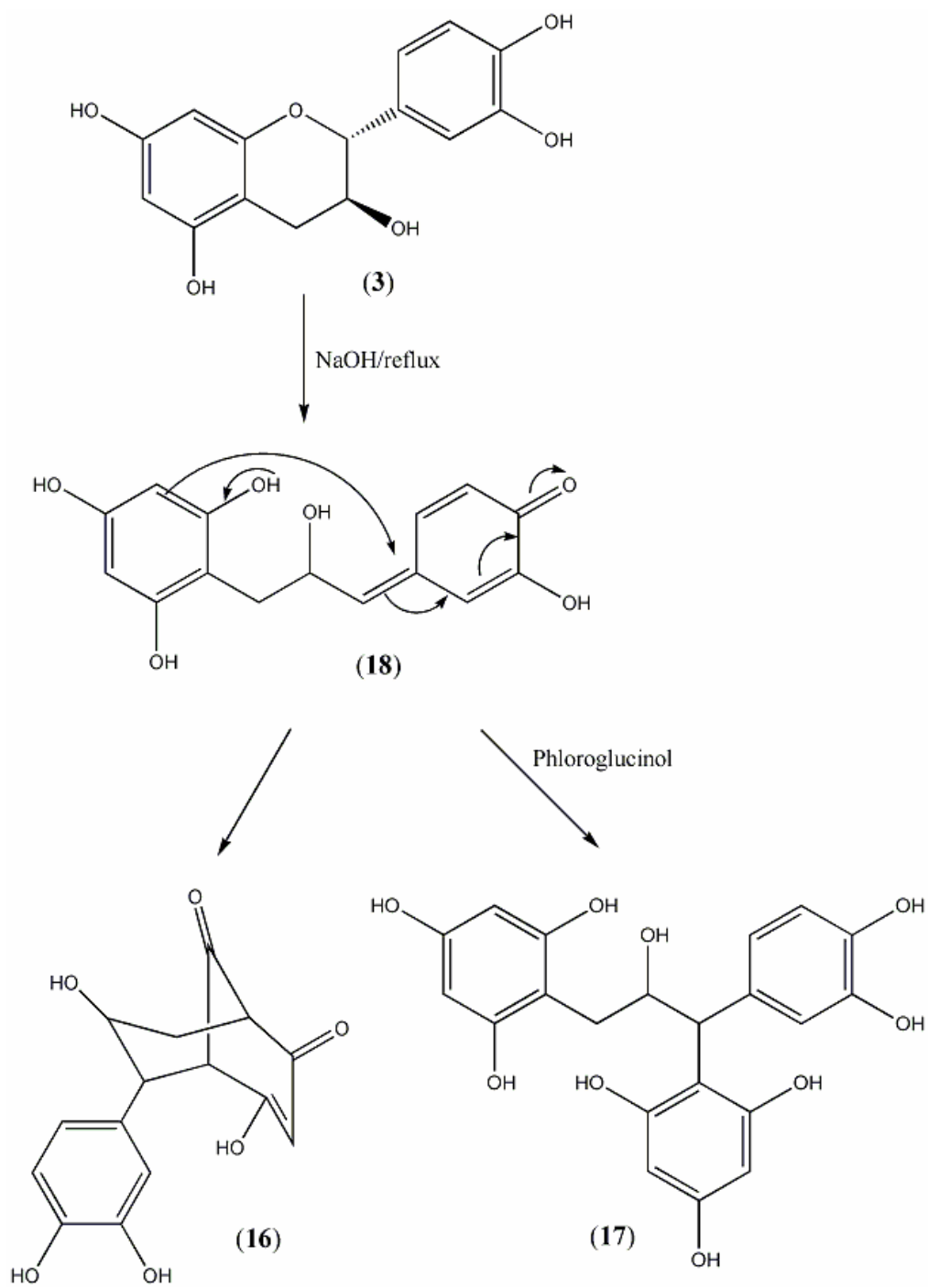
In the presence of phloroglucinol (pH = 12, ambient temperature) Laks and coworkers<sup>67,69</sup> isolated both catechinic acid (16) and a phloroglucinol adduct with unspecified absolute configuration (17).



(17)

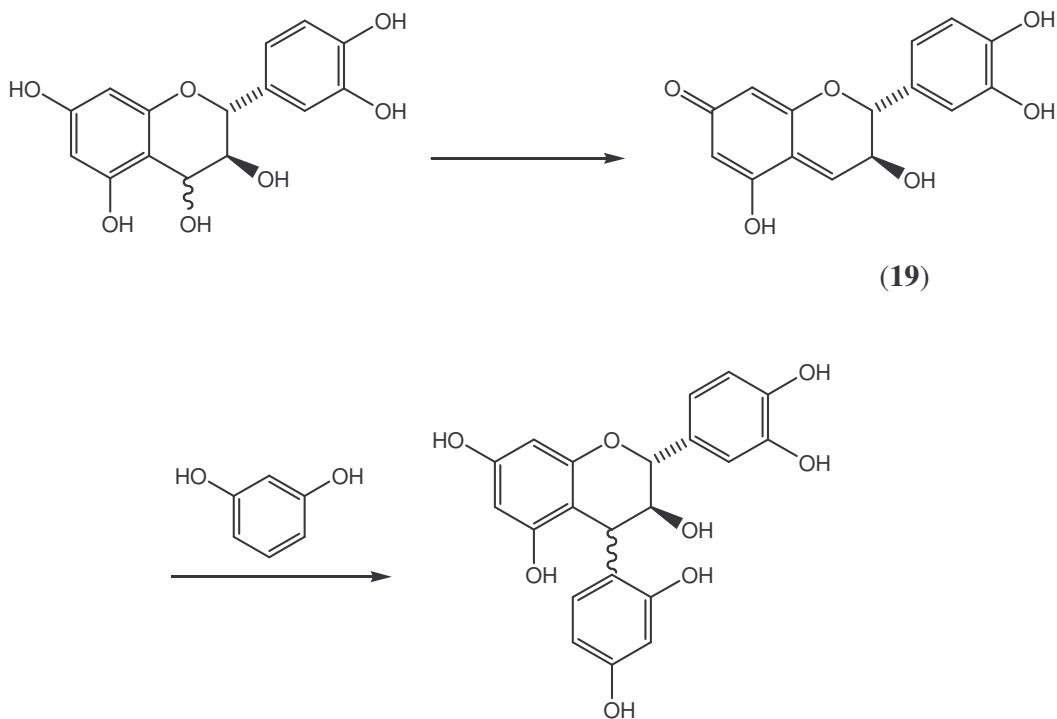


Formation of **(16)** and **(17)** was postulated to take place via a quinone methide intermediate **(18)**.



**Scheme 8**

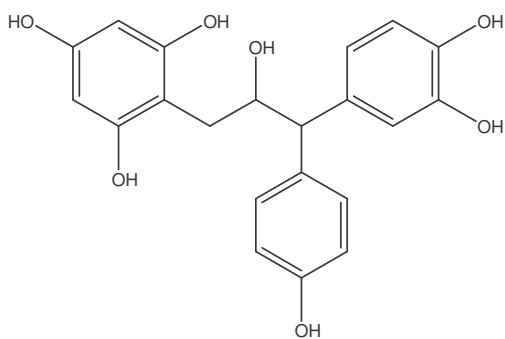
Flavan-3-ol derivatives with a good leaving group at C-4 (*e.g.* flavan-3,4-diols) give an A-ring quinone methide (**19**) without cleavage of the pyran ring.<sup>62</sup> (Scheme 9)



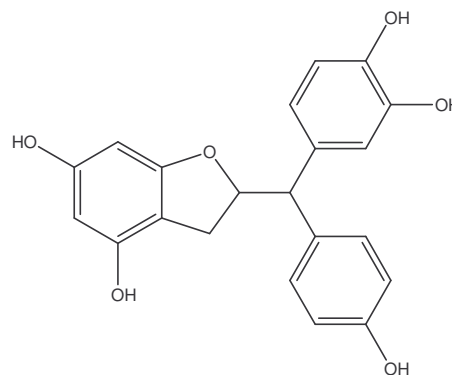
### Scheme 9

#### 2.4.3 BF<sub>3</sub> catalysis

Mitsunaga and co-workers<sup>64,65,66</sup> reported a boron trifluoride catalyzed condensation between phenol and (+)-catechin (**3**). They isolated two products, a ring opened phenolated product, 2-[3-(3,4-dihydroxyphenyl)-2-hydroxy-3-(4-hydroxyphenyl)-propyl]benzene-1,3,5-triol (**20**), and a dehydration product, 4,6-dihydroxy-2-(3',4',4''-trihydroxydiphenylmethane)coumaran (**21**).



(20)



(21)

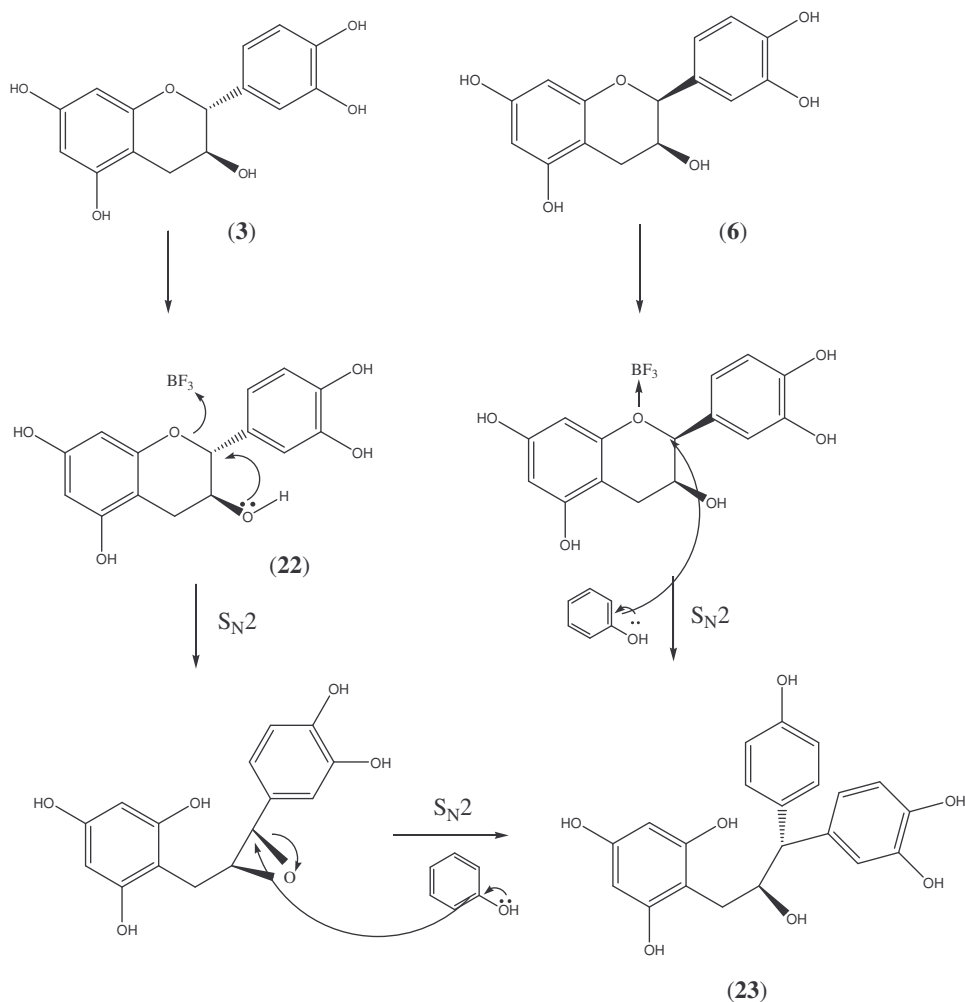
The phenolation mechanism was proposed as follows by Peng and coworkers:<sup>63</sup>

1.  $\text{BF}_3$  coordinates to the nonbonding electron pair on the pyran ring oxygen. This coordination weakens the heterocyclic ether bond and allows phenol to attack via its carbon at C-2 of the pyran ring to form (20).
2. Dehydration between the C-2 hydroxy group and the aromatic hydroxy group on the A-ring subsequently gives the coumaran (21).

The highest yield was obtained in aromatic solvents (benzene, toluene, xylene, anisole).  $\text{BF}_3$  is stable in aromatic solvents because it forms weak  $\pi$ -complexes. Yields were slightly lower in chlorinated solvents (dichloromethane, trichloromethane and tetrachloromethane). Yields in protic solvents (methanol, cyclohexanol) and aprotic solvents with oxygen (ethylene glycol dimethylether, dioxane) were very low, because coordination of  $\text{BF}_3$  to the oxygen atoms of the solvent competes with coordination to the pyran ring oxygen. These solvents may solvate and capture the nucleophile and reduce its reactivity. Catechin and its phenolated products did not dissolve well in these solvents and had to be suspended in the reaction mixture.

Subsequently Peng and coworkers<sup>63</sup> established that (+)-*ent*-epicatechin (6) gave the same product under the same conditions, albeit in a lower yield. They postulated that (+)-*ent*-epicatechin (6) reacts via a direct  $\text{S}_{\text{N}}2$  mechanism at the 2-position and that (+)-

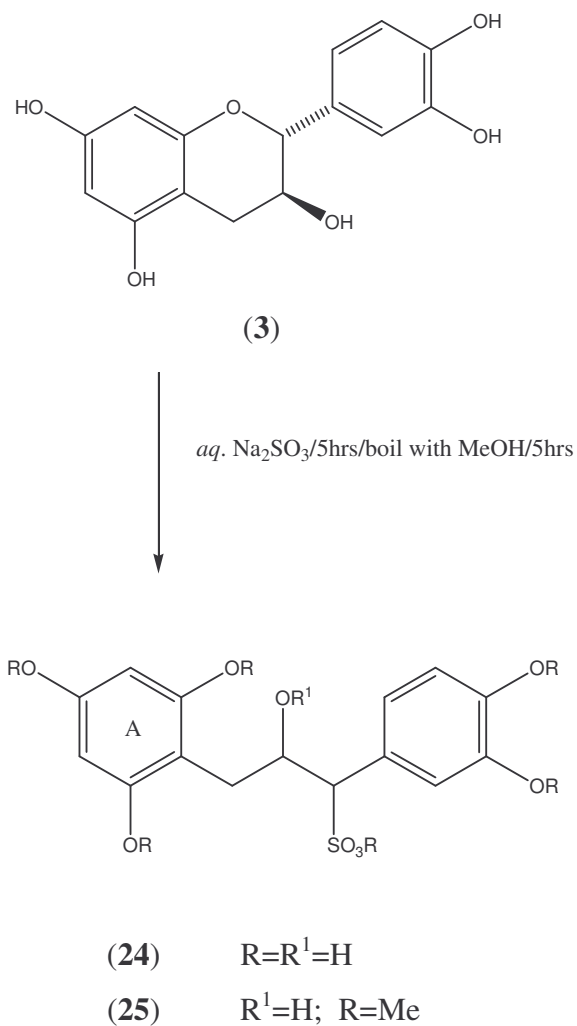
catechin (**3**) reacts indirectly via an epoxide intermediate (**22**). (Scheme 10) The neighbouring group effect accelerates the reaction and is possible because the hydroxy group at the C-3 position is located in the *anti*-position relative to the ether bond in the pyran ring. This allows attack from the back. Neighbouring group participation via an  $S_N2$  mechanism for cleavage of the pyran ring is not possible in the case of (+)-*ent*-catechin (**6**) because the hydroxyl group on C-3 is in a *cis*-position relative to the leaving group. Peng and coworkers<sup>63</sup> postulated from the above mechanistic considerations that the absolute configuration of C-1 in the phenolated product (**23**) is *R* assuming that the stereochemistry of (+)-catechin (**3**) at C-2 is maintained during the reaction.



**Scheme 10**

#### 2.4.4 Sodium sulphite catalysis

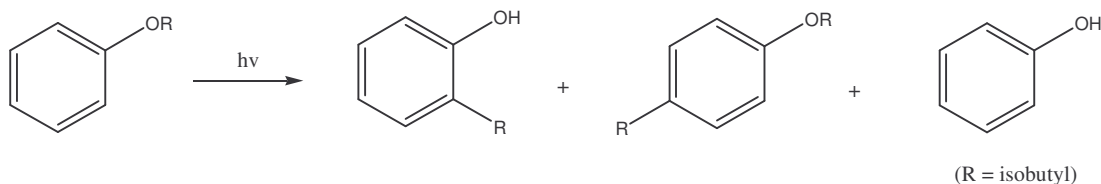
(+)-Catechin (**3**) undergoes extremely facile opening of the ring as a result of nucleophilic attack by the sulphite group at C-2, affording 1,3-diphenylpropan-2-ol-1-methylsulphonate (**24**), isolated as the full methyl ether (**25**) following ion exchange and methylation. The reaction probably represents an  $S_N2$ -mechanism with attack by the lone pair electrons of the sulfur atom and a phenoxide ion as leaving group.<sup>63,70</sup> (**Scheme 11**)



**Scheme 11**

## 2.5 Photochemistry of catechins

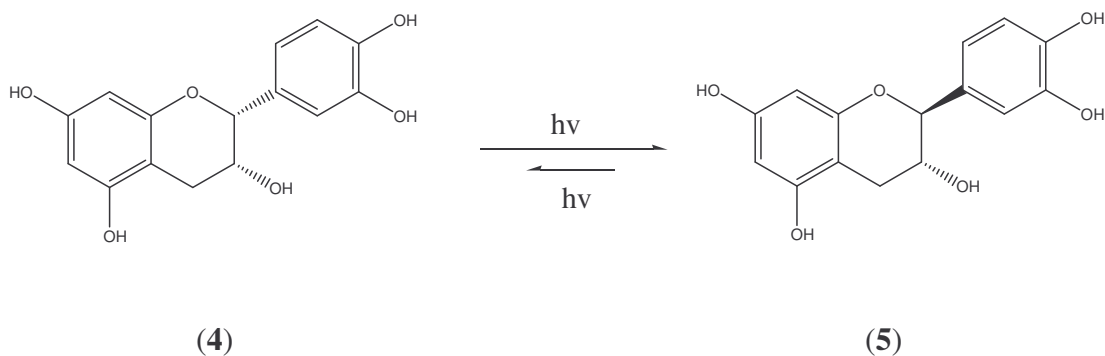
Alkylethers of phenols undergo photofragmentation, usually with migration of the alkylgroup to the *ortho*- or *para*-position (Photo-Fries rearrangement).<sup>71</sup> (Scheme 12) If the alkylgroup is very stable (e.g. isobutyl) the unsubstituted phenol can be isolated. The usefulness of the reaction is restricted by the short wavelength where light is absorbed (<250 nm) and the high S<sub>1</sub> and T<sub>1</sub> energy levels that makes sensitisation difficult. Electron donating substituents may move the light absorption wavelength to a slightly higher region (>250 nm).<sup>63,68,72</sup>



**Scheme 12**

Forest and coworkers<sup>73</sup> investigated the photochemistry of catechin as a model tannin to obtain a better understanding of the environmental photochemistry of humic substances in aquatic systems. Catechin was found to undergo reversible photoisomerisation to epicatechin. This enables catechin to act as a natural sunscreen and attenuate light energy through non-destructive techniques.

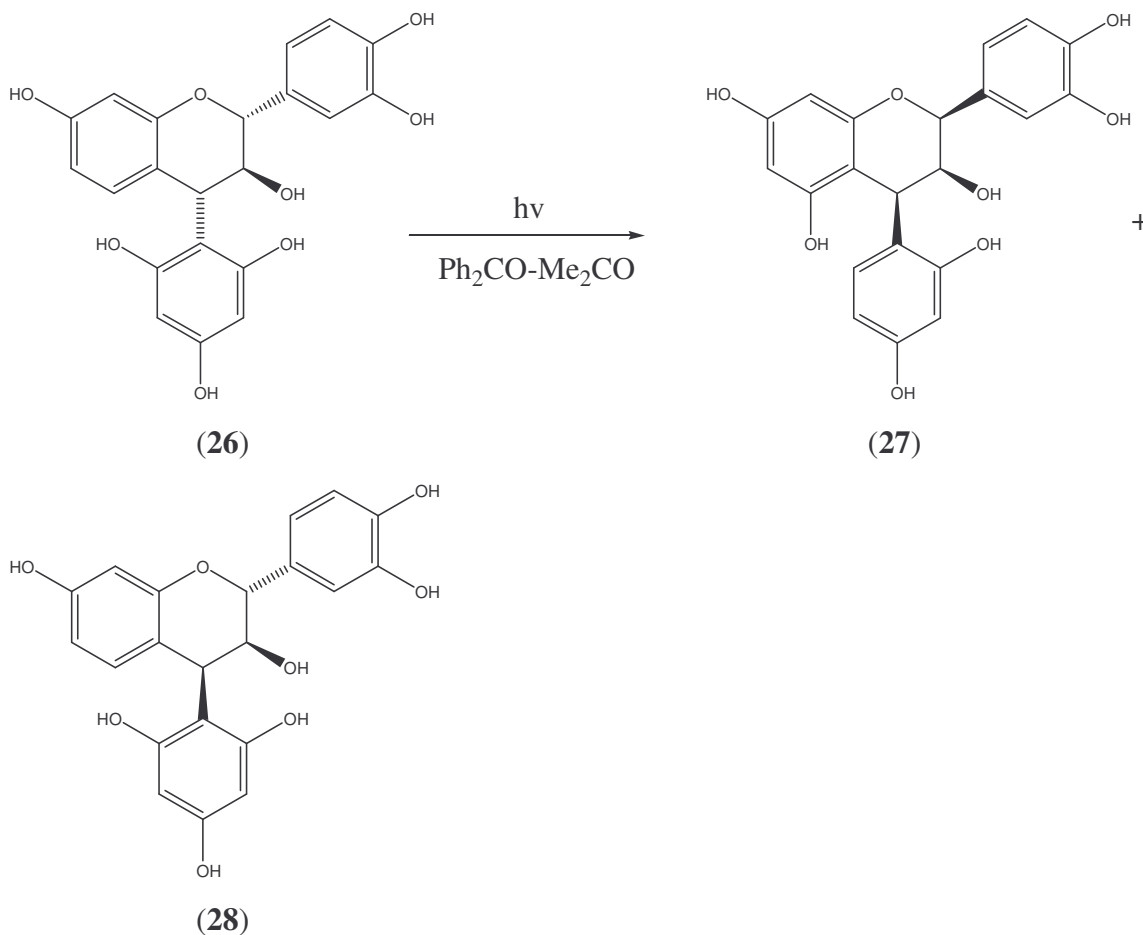
Photolysis at 254 nm of the naturally produced (-)-*cis* isomer, epicatechin (**4**) in a 1:1 mixture of CH<sub>3</sub>CN and H<sub>2</sub>O gave conversion to the thermodynamically more stable (-)-*trans* isomer (**5**). (Scheme 13) Forest and coworkers<sup>73</sup> could not separate the two isomers by conventional silica gel chromatography and conversion was determined by <sup>1</sup>H NMR and optical rotation. 90% Conversion was achieved after 8 minutes. Irradiation at 300 nm gave similar results. The reverse isomerisation, from (**5**) to (**1**) never exceeded 5% under similar conditions. Photolysis of (+)-tetra-*O*-methylcatechin gave conversion to the corresponding *cis* isomer in a comparable 5% yield.



### **Scheme 13**

The photochemical isomerization can take place via an ionic (**c**), radical (**a**) or quinone methide mechanism (**b**) (**Scheme 2**) involving homolytic or heterolytic cleavage of the pyran O-C ring. Forest and coworkers<sup>73</sup> failed to isolate any ring opened compounds via trapping of intermediates by nucleophiles such as methanol and radical trap solvents such as 2-propanol. Re-closing of the ring was slow enough to allow bond rotation to yield the observed isomerisation but too fast to allow trapping of the intermediate by methanol or 2-propanol. Given that the tetramethyl ether that is not capable of yielding a quinone methide intermediate undergoes a similar isomerization, reaction via the biradical or zwitterionic intermediate was considered a more likely mechanism.

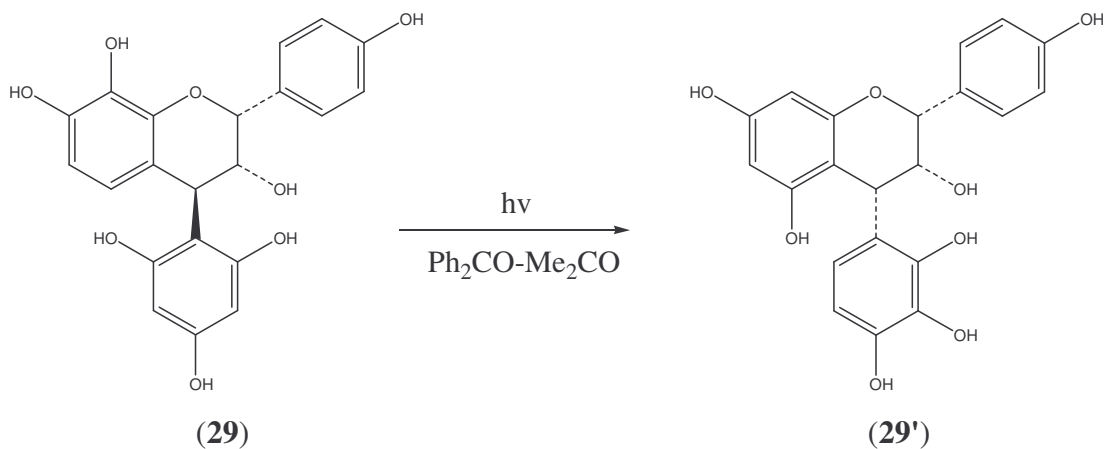
Van der Westhuizen and coworkers<sup>71</sup> investigated the photochemistry of 4-arylsubstituted catechins. Sensitized photolysis (0.05 M benzophenone) of (2*R*,3*S*,4*S*)-2,3-*trans*-3,4-*trans*-4-(2,4,6-trihydroxyphenyl)flavan-3-ol (**26**) gave (2*S*,3*S*,4*S*)-2,3-*cis*-3,4-*cis*-4-(2,4-dihydroxyphenyl)flavan-3-ol (**27**) and trace amounts of 2,3-*trans*-3,4-*cis*-4-(2,4,6-trihydroxyphenyl)flavan-3-ol (2*R*,3*S*,4*R*) (**28**). (**Scheme 14**)



### Scheme 14

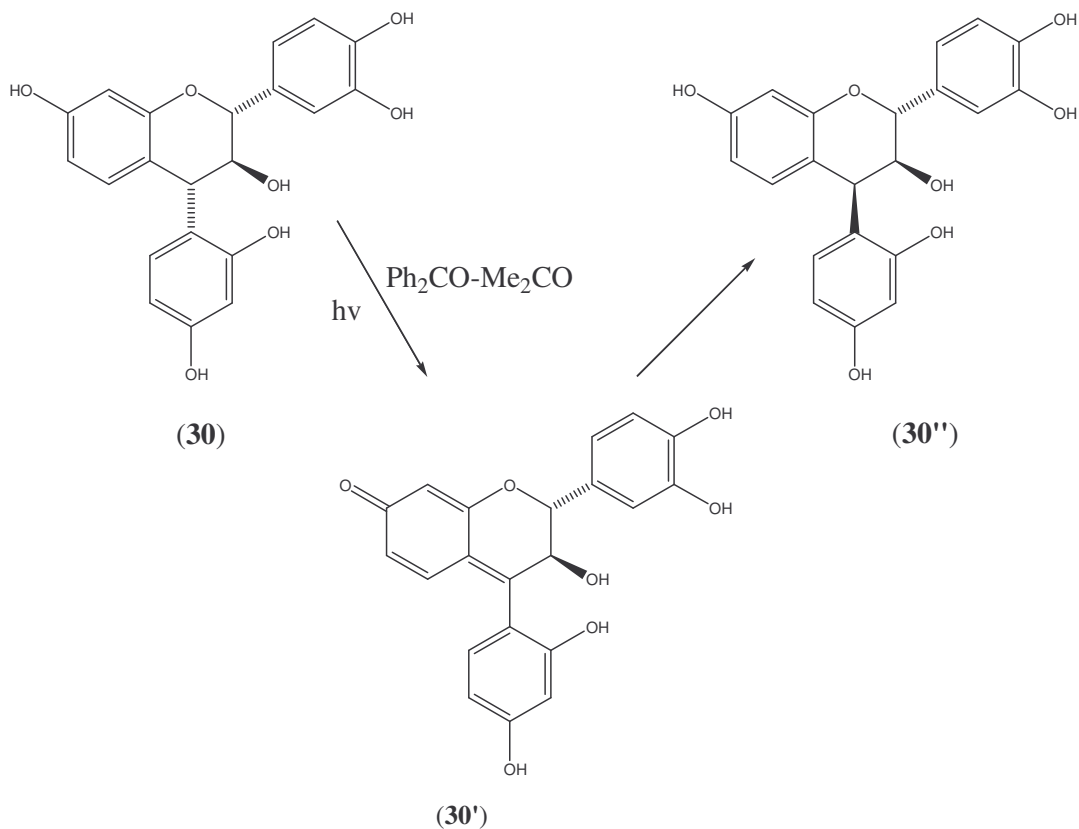
The transformation requires heterolytic cleavage of the ether bond followed by intramolecular re-cyclisation with the stronger nucleophilic hydroxy group of the phloroglucinol D-ring. Inversion at C-4 is due to ca. 180° rotation about the C-3-C-4 bond of the intermediate zwitterion. Involvement of a quinone methide intermediate is not ruled out. Inversion or retention of configuration at C-2 (to yield either **27** or **28**) depends on whether recyclization with the A-ring takes place from the top or bottom of the intermediate zwitterion or quinone methide (+180 or -180° rotation). Similar results were obtained with the 2,3-*cis*-3,4-*trans* isomer (**29**). (**Scheme 15**)





### **Scheme 15**

This reaction does not take place if the 2,4,6-trihydroxyphenyl substituent (phloroglucinol moiety) in the 4-position is replaced with a 2,4-dihydroxy substituent (resorcinol moiety) (**30**). Resorcinol is not a strong enough nucleophile to replace phloroglucinol at the 2-position of flavan-3-ols. This observation indicated that an  $S_N2$  mechanism may be responsible for the photochemical rearrangements. Inversion of configuration at C-4 was the only transformation observed when (**30**) was photolyzed, probably via an A-ring quinone methide. (**Scheme 16**)



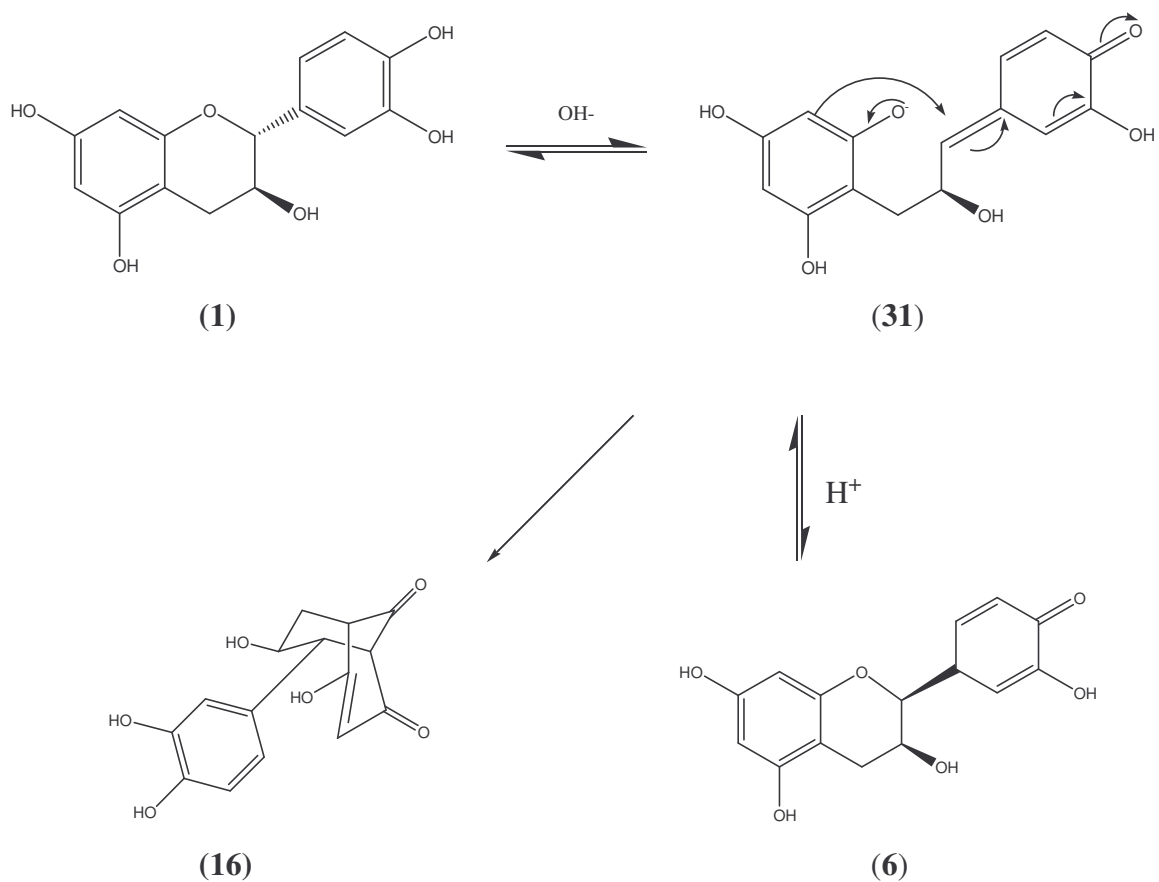
**Scheme 16**

## 2.6 Epimerization of (+)-catechin

Epimerization of (+)-catechin (**3**) in hot water or dilute caustic solution to (+)-*ent*-epicatechin (**6**) is well-known.<sup>74</sup> It was shown by Sears and coworkers<sup>75</sup> that (+)-catechin (**3**) undergoes rearrangement to catechinic acid (**16**) in hot alkaline solution. The quinone methide (**31**) suggested by Mehta & Whalley<sup>76</sup> is a logical intermediate in both processes. (Scheme 17)

The rates of epimerization of (+)-catechin (**3**) to (+)-*ent*-epicatechin (**6**) and of (-)-epicatechin (**4**) to (-)-*ent*-catechin (**5**) in aqueous solution were measured over the pH range 5.4-11.0 and the temperature range 34-100 °C. The rate of conversion of (+)-catechin (**3**) to catechinic acid (**16**) was also measured under these conditions. First-order kinetics was observed for all three processes. At low pH,  $k(\text{epimerization}) \gg k(\text{rearrangement})$ , and epimerization approached an equilibrium in which (+)-catechin (**3**)

predominated over (+)-epicatechin (**6**). Near pH 11 and at elevated temperatures,  $k(\text{epimerization})$  was only slightly greater than  $k(\text{rearrangement})$ , and the rapid, irreversible formation of catechinic acid (**16**) under these conditions determined product composition. The epimerization of (+)-catechin (**3**) and its rearrangement to catechinic acid (**16**) can be rationalized in terms of a quinone methide intermediate.



### Scheme 17

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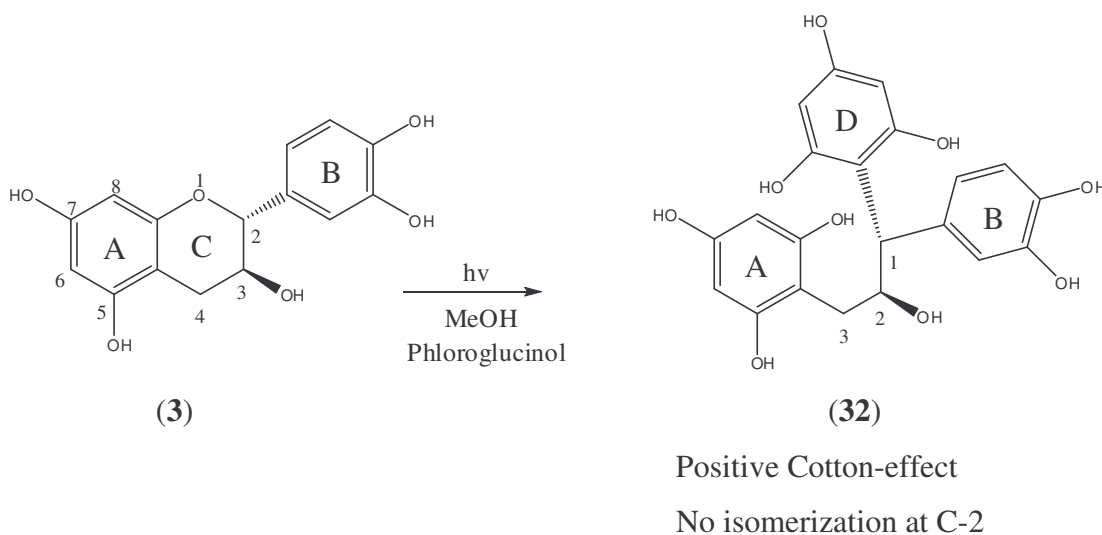
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## CHAPTER 3

### 3.1 Results

#### 3.1.1 Photolysis of (+)-catechin and (-)-epicatechin

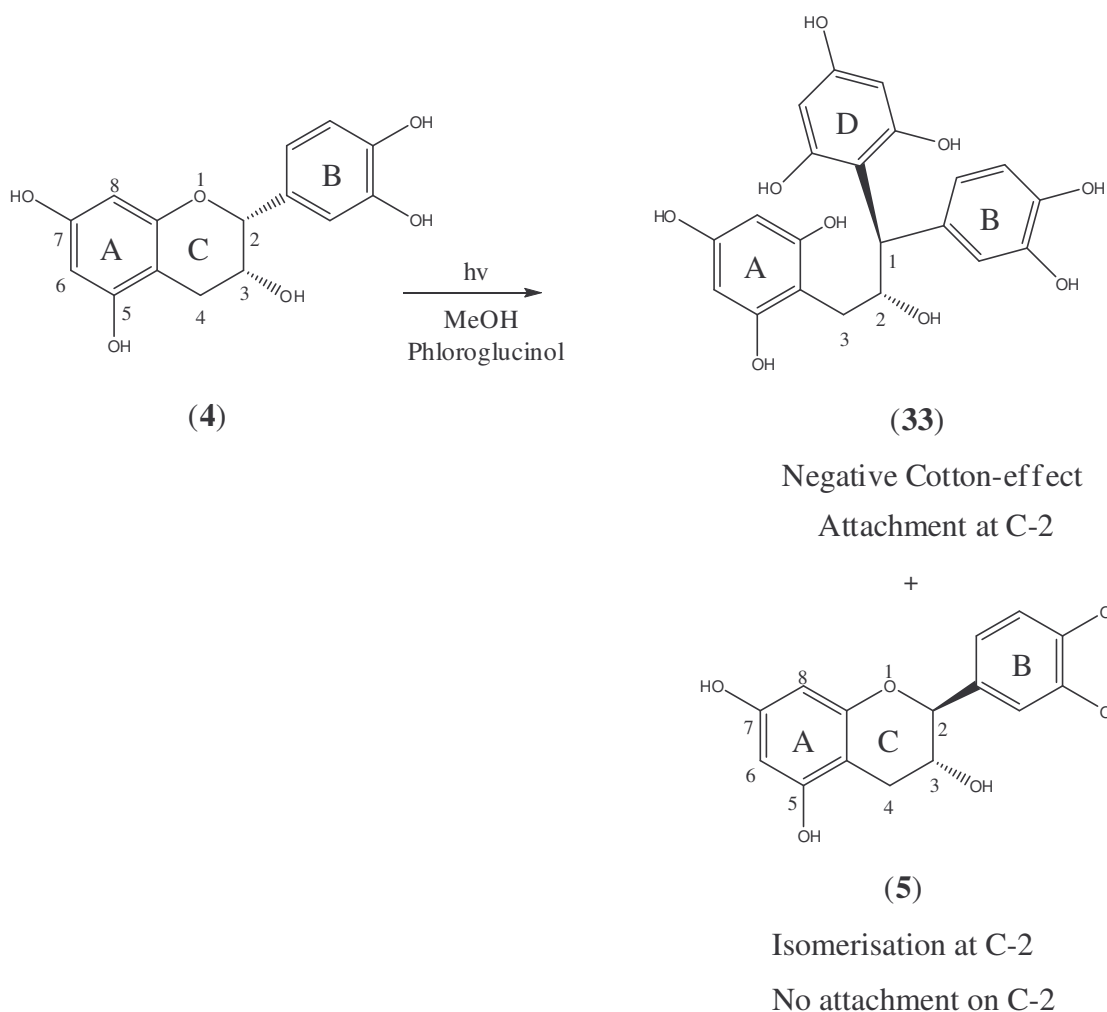
Photolysis of (+)-catechin (**3**) at 250 nm in the presence of phloroglucinol with methanol as solvent resulted in the isolation of the optically active product (**32**) (11 % yield) with a (1*S*,2*S*) configuration and unreacted optically active starting material (**3**).



#### Scheme 1

Photolysis of (-)-epicatechin (**4**) at 250 nm under the same conditions resulted in the isolation of the optically active product (**33**) (8 % yield) with a (1*R*,2*R*) configuration, unreacted optically active starting material (-)-epicatechin (**4**) as well as (-)-*ent*-catechin (**5**) in a 2:1 ratio.

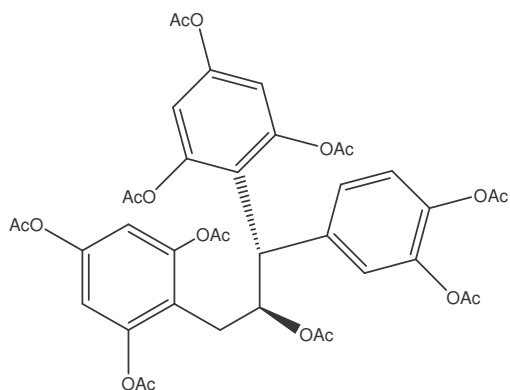




## Scheme 2

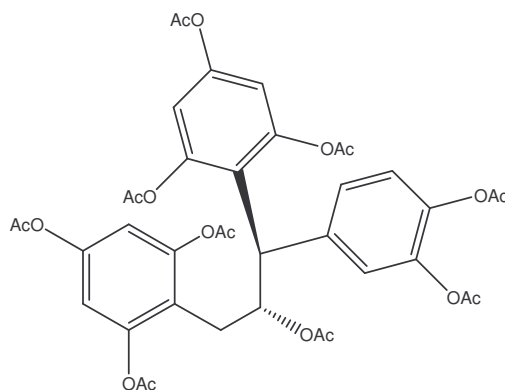
The two products are enantiomers (32) and (33) and have identical NMR spectra (Plate 1a,b and Plate 2a,b) but mirror image CD spectra (CD-plate 3). CD-plates 1 and 2 show the individual Cotton effects of each product, (32) and (33) respectively, as well as their  $[\theta]$  values. The two starting materials, (+)-catechin (3) and (-)-epicatechin (4), are diastereoisomers and do not have identical NMR spectra. Acetylated (-)-*ent*-catechin (35') from photolysis of (-)-epicatechin (4) has the same NMR spectra as acetylated (+)-catechin (3') but mirror image CD spectra (CD-plate 6). CD-plates 4 and 5 show the individual Cotton effects of each product, (3') and (35') respectively, as well as their  $[\theta]$  values.

Acetylation of the free phenolic products (**32**) and (**33**) yielded the two nona-acetate products (**34**) and (**35**) with identical NMR spectra (**Plate 5a-e**) and mirror image CD spectra (**CD-plate 9**). **CD-plate 7** and **8** show the individual Cotton effects of each product, (**34**) and (**35**) respectively, as well as their  $[\theta]$  values.



Positive Cotton-effect

(**34**)



Negative Cotton-effect

(**35**)

(-)-Epicatechin (**4**) also undergoes isomerisation at C-2 to give a non-coupled product (**5**) whilst (+)-catechin (**3**), does not undergo inversion at C-2.

A complete structure elucidation of the products (**32**) and (**33**) is given under Section 5.1.1.

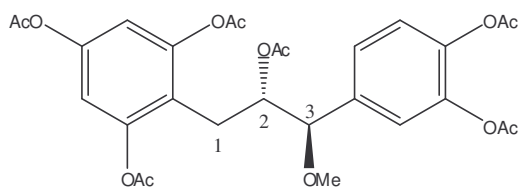
The following salient features apply:

The presence of nine *O*-acetyl groups, respectively, indicates fission of the heterocyclic ring and addition of a phloroglucinol moiety. One acetate resonance is in the aliphatic region at  $\delta = 1.72$ .

As expected from previous work on flavonoids with diaryl polyphenol moieties,<sup>1</sup> the phloroglucinol unit (D-ring) experiences rotational isomerism and its aromatic protons resonate as a two proton singlet.

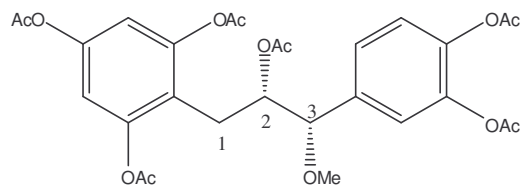
Purification of the free phenolic starting material on silica leads to low yields of the phenolic products, as phenols are normally strongly absorbed on the silica gel.

Direct acetylation of the reaction mixture from (+)-catechin (**3**) before TLC, resulted in much improved yields of the nona-acetate (**34**) as well as the identification of an additional reaction product (**36**) accompanied by its diastereoisomer (**37**).



(2*S*,3*R*-isomer)

**(36)**



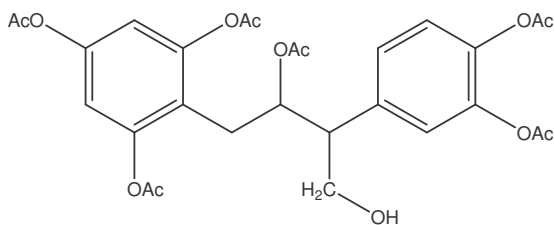
(2*S*,3*S*-isomer)

**(37)**

However, the two diastereoisomers could not be separated. High resolution NMR permitted assignment of all the protons in the two diastereoisomers (ratio 1:4) (**Plate 4a,b**). We assumed that the major isomer (**36**) is from attack *anti* to the 2-OH. The decreased bulk of MeOH in comparison with phloroglucinol allows the formation of some product from *syn* attack (**37**).

As demonstrated in **Scheme 2** (Chapter 2), photolytic ring opening of the heterocyclic ring and isomerisation at C-2, can take place via a radical (**a**), ionic (**c**) or quinone methide mechanism (**b**).<sup>3</sup>

Identification of the methoxy-trapped products (**36** and **37**), indicates an ionic mechanism, as a radical mechanism would result in a —CH<sub>2</sub>OH substituted product (**38**).<sup>4</sup>



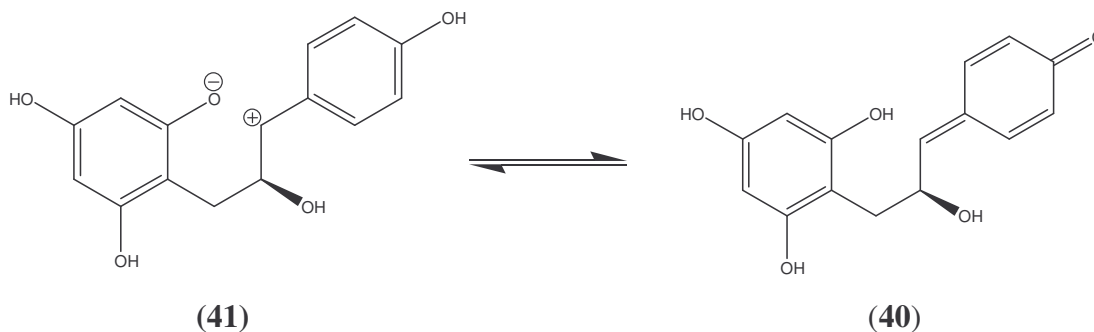
Radical mechanism product

(38)

Singlet excited  $\pi,\pi^*$ -states are associated with ionic reaction products and  $\pi,\pi^*$ -triplet states with radical reaction products.<sup>4</sup>

We could not distinguish unequivocally between the quinone methide (40) and the ionic intermediate (41) mechanisms, but because they are tautomers, there is not much difference between a *para*- or *ortho*-hydroxy stabilized benzylic cation and the quinone methide in terms of reactivity to nucleophiles. (Scheme 3)

The absence of any coupling products in photolysis of (+)-tetra-*O*-methylcatechin (39), indicates that a free phenolic OH at C-4' of the B-ring is essential to sufficiently stabilize the carbocation for condensation to take place via a quinone methide.<sup>5,6,7</sup>

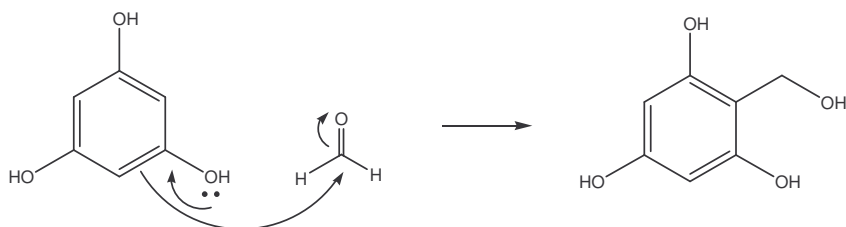


### Scheme 3

The total stereoselectivity is attributed to the 3-hydroxy group that allows the bulky phloroglucinol group to attack the quinone methide from the *anti*-position only or stereospecific involvement of the epoxy intermediate (41a). Phloroglucinol is a well

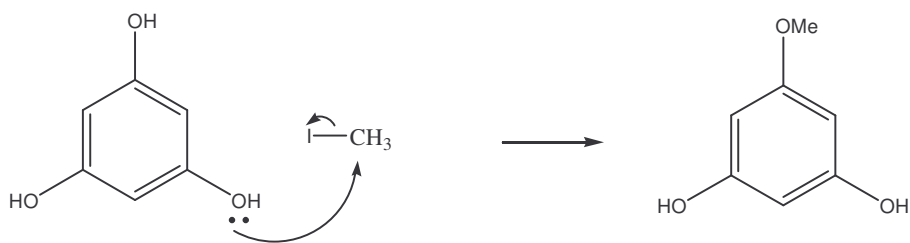
known ambident nucleophile<sup>8</sup> which can react either via oxygen (**Scheme 4**) or carbon (**Scheme 5**).

Via carbon:



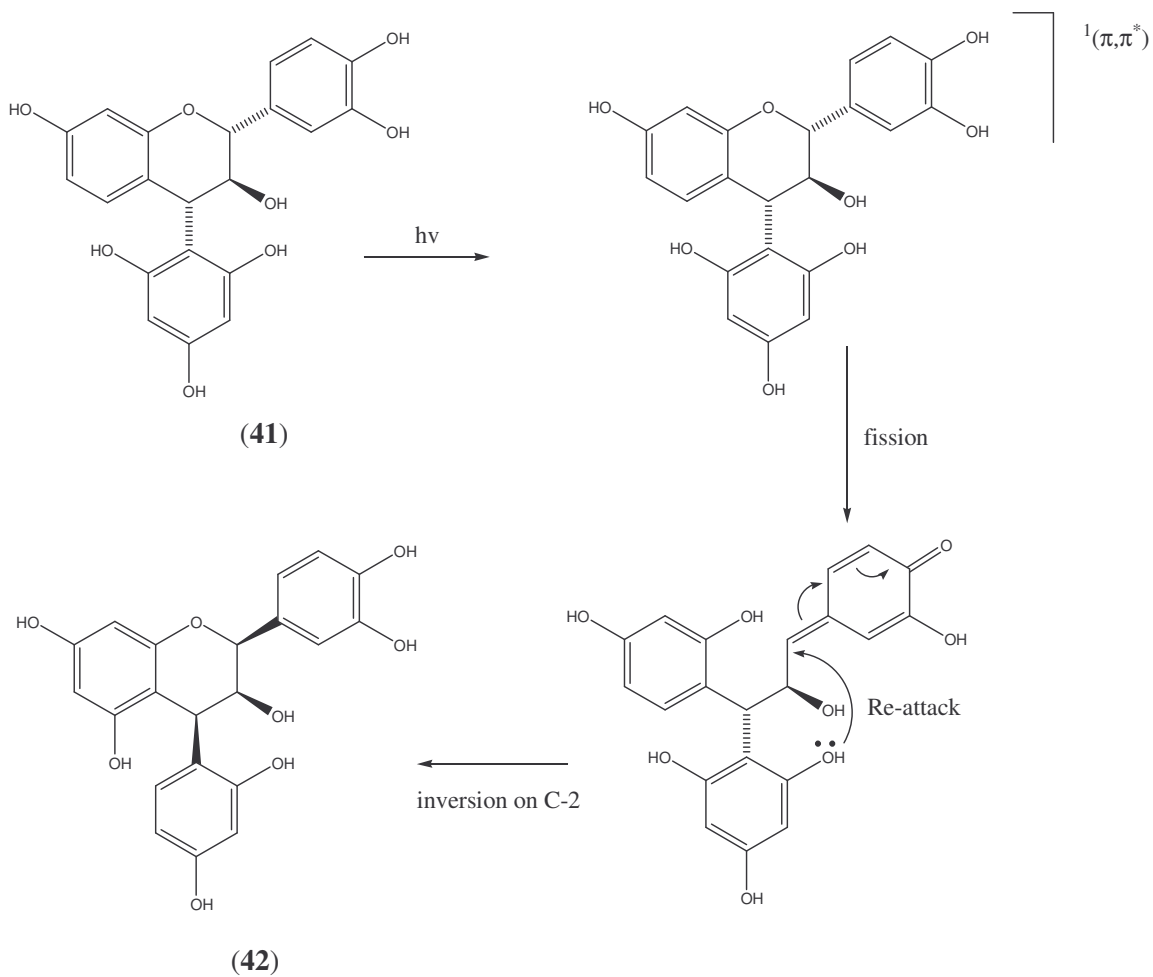
**Scheme 4**

Via oxygen:



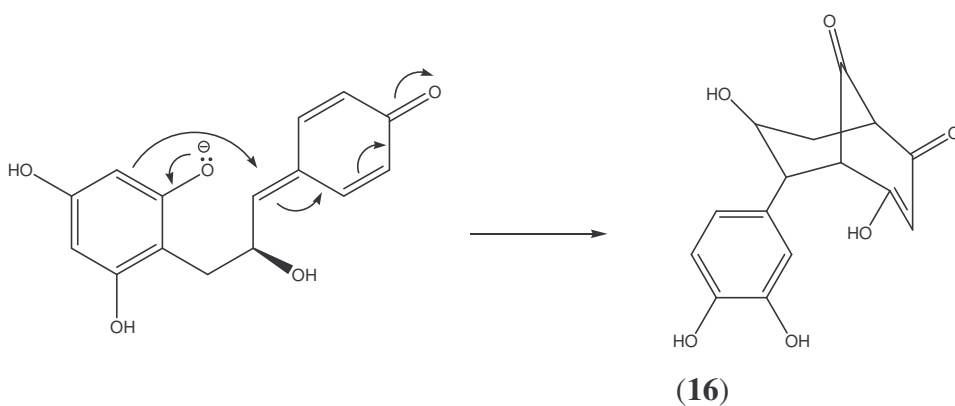
**Scheme 5**

Photolytic transformation of 2,3-*trans*-3,4-*trans*-4-arylflavan-3-ol (**41**) to 2,3-*cis*-3,4-*cis*-4-arylflavan-3-ol (**42**)<sup>9</sup> (**Scheme 6**) represents an example where the 4-phloroglucinol moiety reacts with the quinone methide via oxygen.



**Scheme 6**

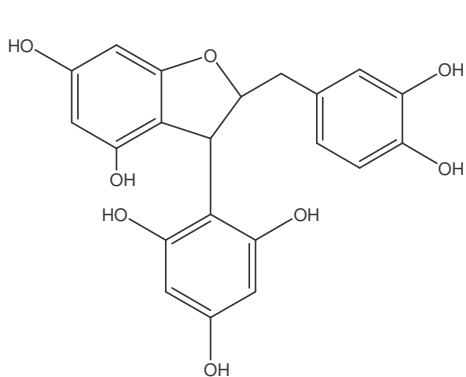
Base catalyzed transformation of (+)-catechin (**3**) to catechinic acid<sup>10</sup> (**16**) represents a reaction where the A-ring phloroglucinol moiety attacks the quinone methide via carbon.



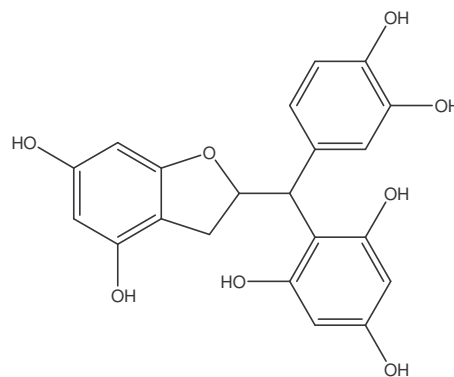
**Scheme 7**

Our work represents the first example where phloroglucinol reacts with a photolytically generated quinone methide via carbon. It is the photolytic equivalent of acid, base and  $\text{BF}_3$  catalytic addition of (+)-catechin (**3**) to C-4 of phloroglucinol (see Chapter 2).

Acid- or  $\text{BF}_3$  catalysed addition of phloroglucinol is followed by cyclisation to yield benzofurans (**13**) and (**7**), while the base catalyzed reaction also results in the formation of catechic acid (**16**).



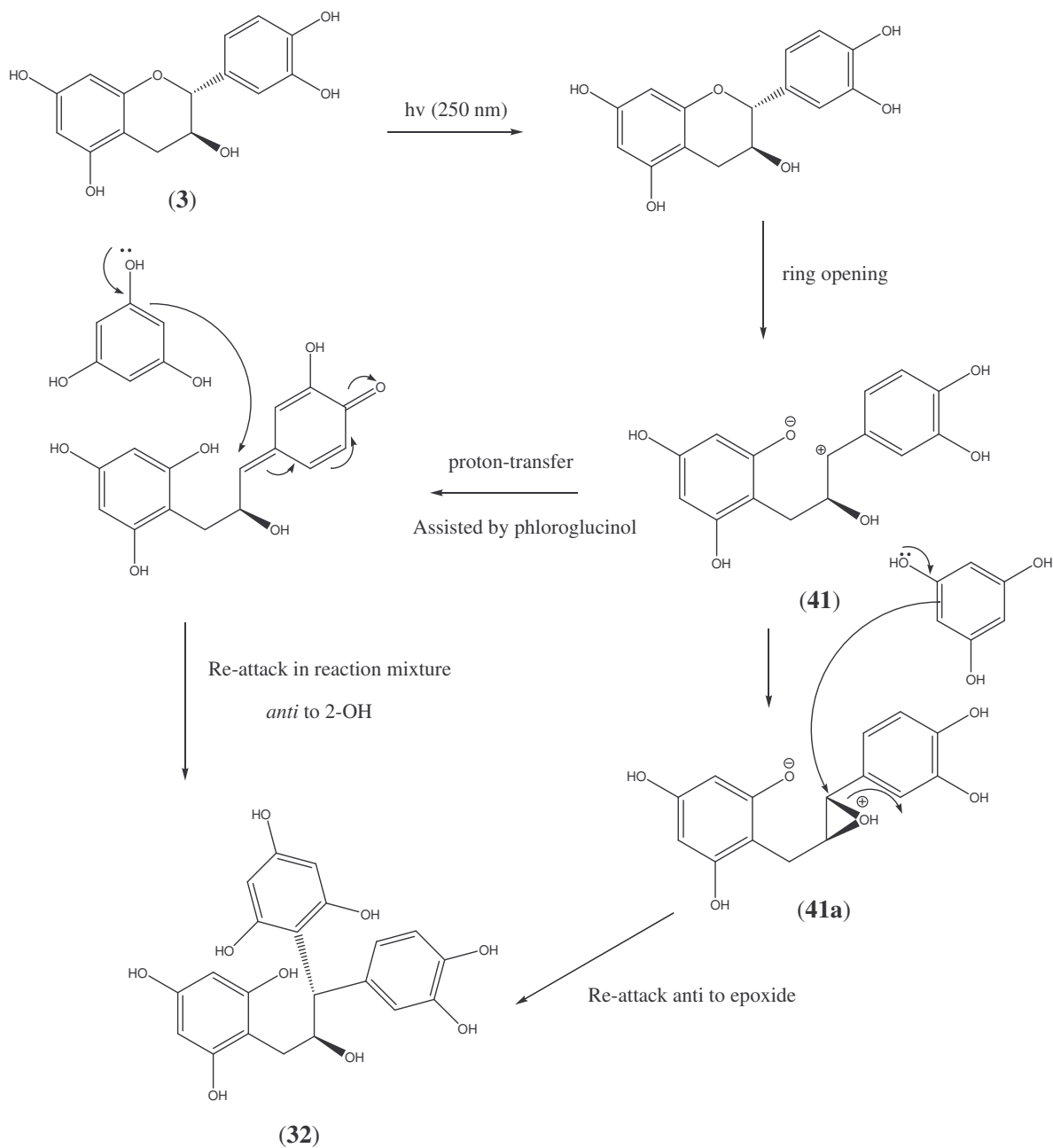
(13)



(7)

Photolytic conditions do not favour formation of (**13**) and (**7**) and give the ring opened product exclusively in good yields with the aliphatic OH intact.

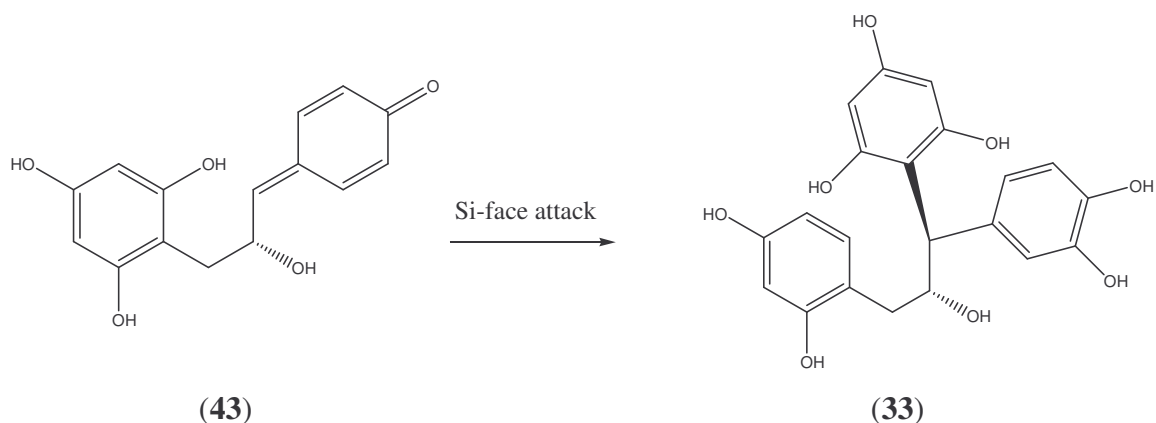
We summarized our reaction mechanism as follows:



### Scheme 8

The same mechanism accounts for the reaction of (-)-epicatechin (4), but the stereochemistry at C-3 forces attack from the *Si*-face. *Re*-face attack is sterically inhibited by the axial 3-OH group on the quinone methide derived from (-)-epicatechin (4).



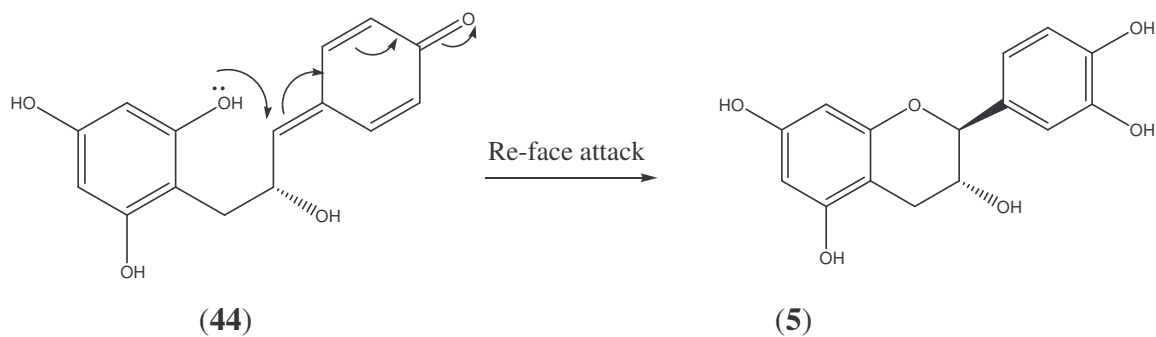


### Scheme 9

*Re*-face attack of the A-ring via oxygen explains the formation of (-)-*ent*-catechin (5) from (-)-epicatechin (4). (Scheme 10)

Arguably, steric hindrance allows *Si*-face attack only to take place at C-2. Coincidentally, this is also the thermodynamically more stable product (2,3-*trans* is more stable than 2,3-*cis*).<sup>7</sup>

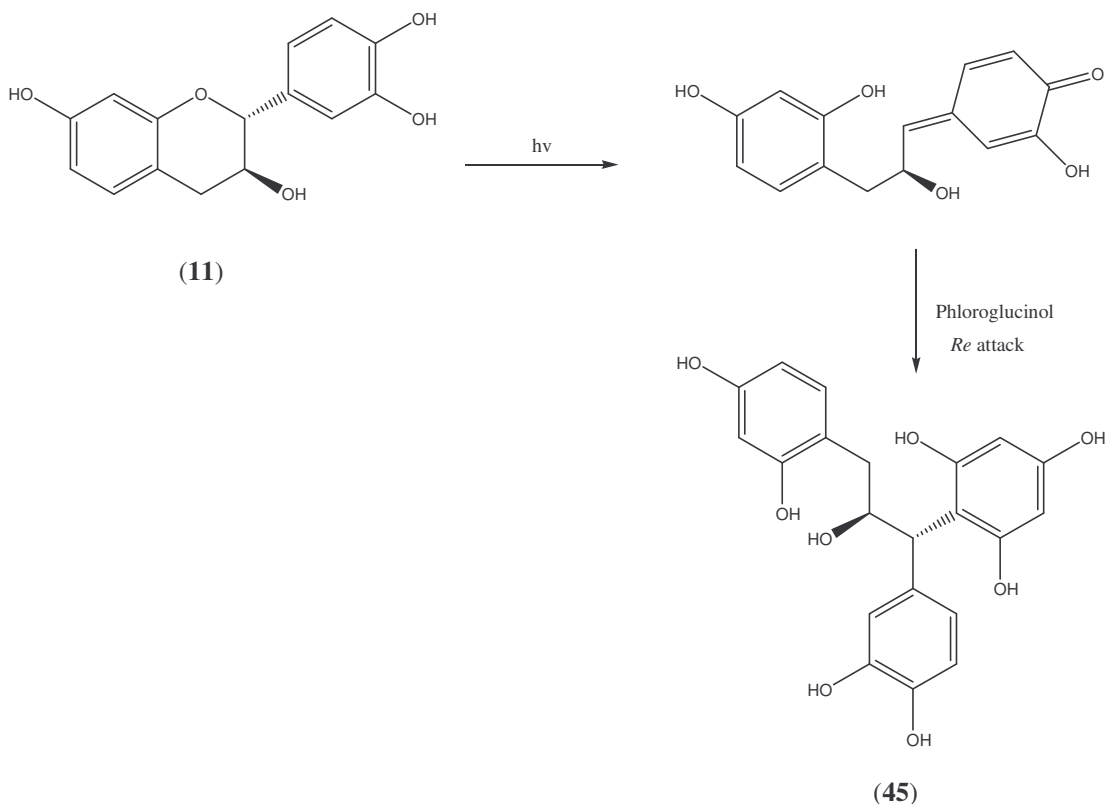
Application of this argument to (+)-catechin (3) (from ring closure from the sterically less hindered position *anti* to the 3-OH) results in reformation of the starting material. The 5% isomerisation reported by Forest and coworkers<sup>7</sup> from NMR on the reaction mixture was inadequate to allow isolation of the product. We could not find any evidence of C-2 isomerisation from (+)-catechin (3) on TLC.



### Scheme 10

### 3.1.2 Photolysis of (-)-fisetinidol

Photolysis of (-)-fisetinidol (**11**) under the same conditions as applied to (+)-catechin (**3**), yielded the expected 1,1,3-triarylpropan-2-ol (**45**) (11 % yield) from addition of phloroglucinol to the quinone methide intermediate. (**Scheme 11**)



#### Scheme 11

Nucleophilic attack of phloroglucinol to the *Re*-face, gives *2S*-absolute configuration. Similar to the formation of (**32**) and (**33**) from (+)-catechin (**3**) and (-)-epicatechin (**4**), the absolute configuration of the C-3 position of the starting material determines absolute configuration on C-1 of the product (**45**).

The CD curve (**CD-plate 10**) shows the expected positive Cotton effect, in agreement with the rule we developed for (**32**) and (**33**) and is also in agreement with the rules established by Van der Westhuizen and coworkers.<sup>5</sup>

Acetylation of the condensation product (**45**) (**Plate 8a-c**) showed eight acetate resonances which correspond with a ring opened product (**46**). The resonance at  $\delta = 2.04$  clearly corresponds to two acetates that are assigned to the H-2"/H-6" acetate groups on the phloroglucinol ring. The acetate resonance at  $\delta = 1.75$  is shifted upfield from the other acetates and is assigned to the aliphatic position.

In contrast to the products from (+)-catechin (**3**) and (-)-epicatechin (**4**), no rotational isomerisation is present and no heating was required to observe all the NMR resonances.

### 3.1.3 Circular dichroism (CD)

Circular dichroism (CD) is a spectroscopic technique which reveals information about a molecule's chirality or "handedness". This technique has been used for many years to study and quantify optically active compounds and their interactions. The information content of steady state CD spectra can be used to uniquely identify chiral compounds and their configurations, predict the secondary structure of proteins and other biological macromolecules, and in kinetic mode as a probe to monitor the structural changes accompanying protein folding or unfolding. CD can also be used to monitor and quantify ligand binding processes and is an increasingly important tool in chiral drug development.<sup>11</sup> CD gives less specific structural information than e.g. X-ray crystallography or protein NMR spectroscopy. However CD spectroscopy is a quick method, which does not require large amounts of material and extensive data processing. Thus CD can be used to survey a large number of solvent conditions, varying temperature, pH, salinity and the presence of various cofactors.

CD methods have been used as tools in establishing the absolute configuration at C-4 of flavanoids.<sup>12</sup> It has been used systematically in the studies of flavanones,<sup>13</sup> flavan-3-ols,<sup>14</sup> 4-arylflavan-3-ols<sup>1,15</sup> and dimeric proanthocyanidins.<sup>16,17</sup> Defining the heterocyclic ring conformation is the prerequisite for unequivocal assessment of absolute configuration at C-4 as it influences the sign of the Cotton effect in the transition state (200-240 nm) in the CD spectra of 4-arylflavan-3-ol,<sup>15</sup> biflavonoids<sup>1</sup> 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 stereogenic centre is positive for 4*R*- and negative for 4*S*-configurations, in agreement with the aromatic quadrant rule.<sup>5,18</sup>

### **3.1.4 CD analysis of the reaction products from photolysis of (+)-catechin, (-)-epicatechin and (-)-fisetinidol**

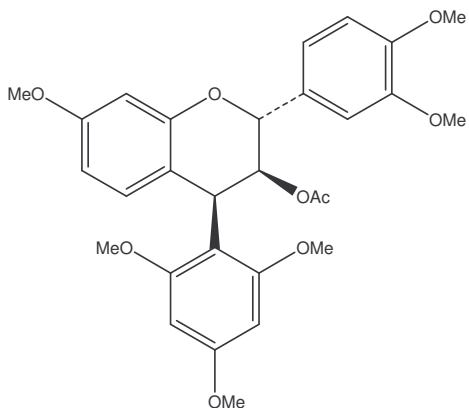
Botha and co-workers<sup>1</sup> studied a series of 4-arylflavan-3-ols and concluded that the absolute configuration of the diaryl moiety at the 4-position determines the sign of the high amplitude Cotton effect (220 to 240 nm). They concluded that configuration at C-2 and C-3 played a lesser role.

Based on the abovementioned results they established the following rule regarding the absolute configuration at C-4 of 4-aryl-flavan-3-ols, biflavonoids and triflavonoids:

A positive Cotton effect reflects a  $\beta$ -orientated C-4 aryl flavanyl substituent.

A negative Cotton effect reflects an  $\alpha$ -orientated C-4 aryl flavanyl substituent.

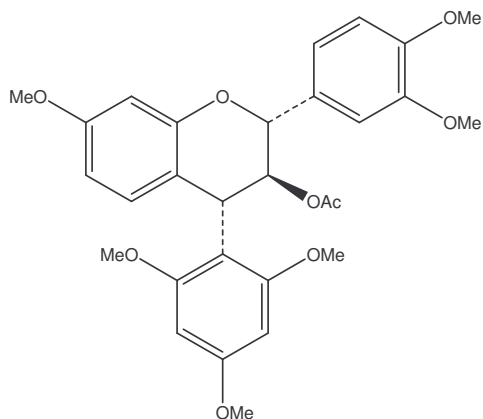
The absolute configuration at C-4 can thus be determined by means of circular dichroism.



(47)

Positive Cotton-effect (4*R*)

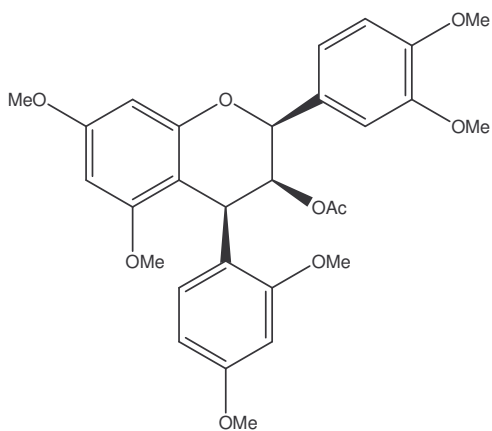
$J_{3,4}$  (*cis*) = 9.5 Hz (chair conformation)



(48)

Negative Cotton-effect (4*S*)

Van der Westhuizen and co-workers<sup>5</sup> found 2,3-*cis*-3,4-*cis*-4-arylflavan-3-ols that deviate from this rule. They concluded that the rule only applies if the C-ring is in a chair conformation. Coupling constants for the hydrogens on the C-ring of the 4-arylflavan-3-ols that does not follow the rule supports a boat conformation.

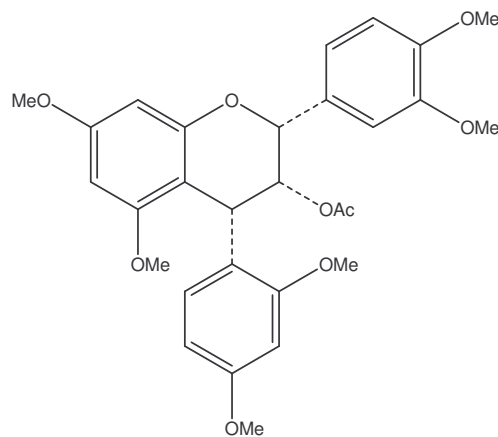


(49)

Found: negative Cotton-effect

Expected: positive Cotton-effect

$J_{3,4}$  (*cis*) = 6.5 Hz (boat conformation)



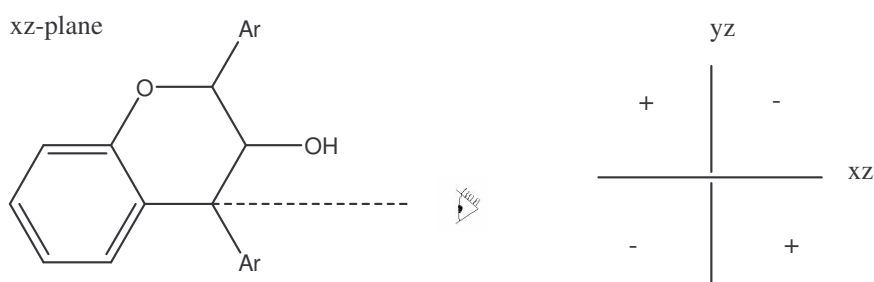
(50)

Found: positive Cotton-effect

Expected: negative Cotton-effect

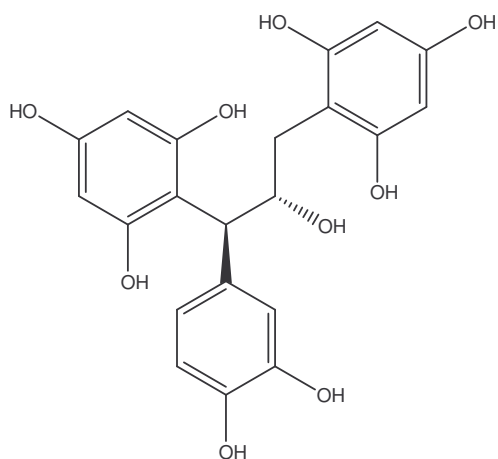
The aromatic quadrant rule<sup>19</sup> was adapted so that it could be applied to the determination of the absolute configuration at C-4 of flavanols, provided the conformation of the C-ring was known.<sup>5</sup>

For this purpose two planes, one through the six carbon atoms of the A-ring (xz-plane) and one perpendicular to that plane, through the benzylic carbon (C-4) (yz-plane) is taken. The molecule is then divided into four quadrants with the xz-plane horizontal and the yz-plane vertical. If the A-ring is viewed from the benzylic 4-carbon, then the substituents in the top right and bottom left quadrant contribute towards a negative Cotton-effect. Substituents in the top left and bottom right quadrants will contribute towards a positive Cotton-effect.



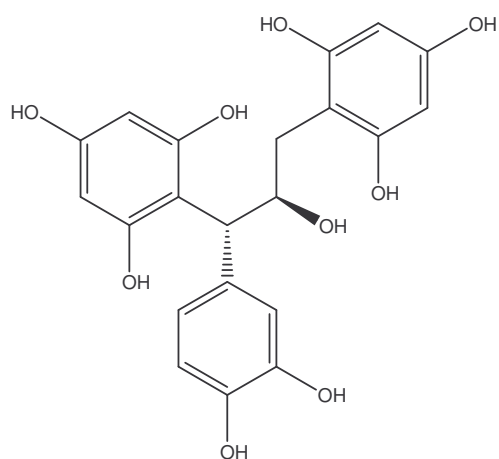
The 4-arylsubstituent gives the largest contribution to the sign of the high amplitude short wavelength Cotton-effect due to the fact that it is directly linked to the C-4 stereogenic asymmetric centre.

Our photolytic synthesized products (**32**) and (**33**) also have a diaryl chromophore equivalent to a diarylmoiety in the 4-position of a flavan-3-ol. Redrawing of our products (**32'**) and (**33'**) with phlorglucinol on the A-ring position, allows us to make a positive correlation between van der Westhuizen's CD rule<sup>5</sup> or the quadrant rule<sup>18,19</sup> and our structure assignments.



(32')

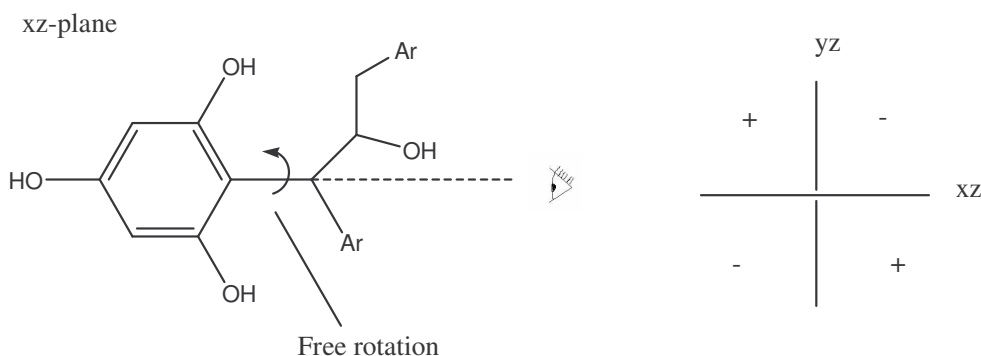
Positive Cotton-effect (3S)



(33')

Negative Cotton-effect (3R)

We would not expect a free rotational system to agree with the rule derived for fixed configurations.

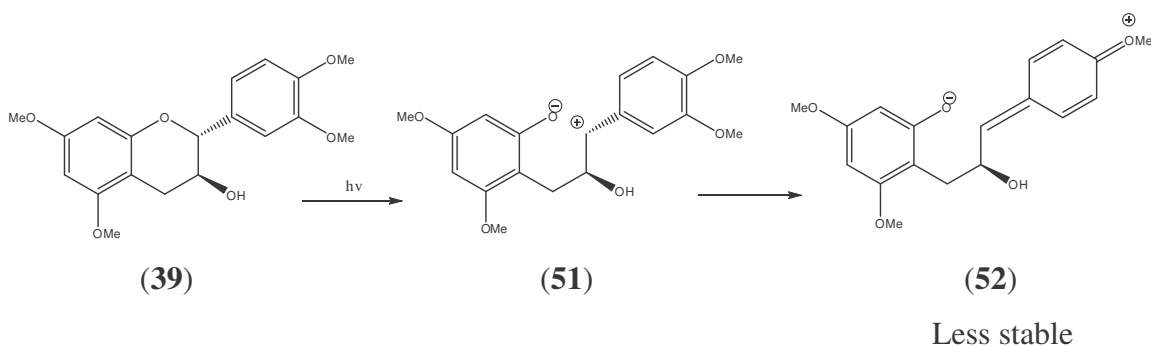


The similarities between the CD spectra of 4-arylflavan-3-ols and our series of compounds (**32**, **33**) are conspicuous. In the former class of compounds, the sign of the high amplitude Cotton effect in the 220-240 nm region is determined by the orientation of the 4-aryl substituent in accordance with the aromatic quadrant rule.<sup>5,19</sup> Although the aromatic quadrant rule is only applicable to aromatic systems with conformationally rigid adjoining ring systems, the remarkable similarities in the CD spectra of the two classes of compounds may reflect a conformational preference for the acyclic analogues in which the orientation of the C-1 substituent (corresponding to the C-2 position in the starting

material) is approaching that in the corresponding 4-arylflavan-3-ol. Such conformational congruence may then feasibly explain the very similar chiroptical properties of the two classes of compounds.

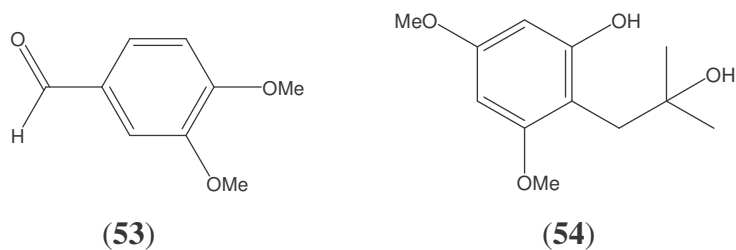
### 3.1.5 Photolysis of (+)-3',4',5,7-tetra-*O*-methylcatechin

Photolysis of (+)-3',4',5,7-tetra-*O*-methylcatechin (**39**) in MeOH and in the presence of 5 eq. phloroglucinol gave no coupling. This is expected as the intermediary carbocation (**51**) from fission of the heterocyclic ring does not have access to free phenolic quinone methide stabilization intermediate, but relies on (**52**) for stabilization. (**Scheme 12**)



### Scheme 12

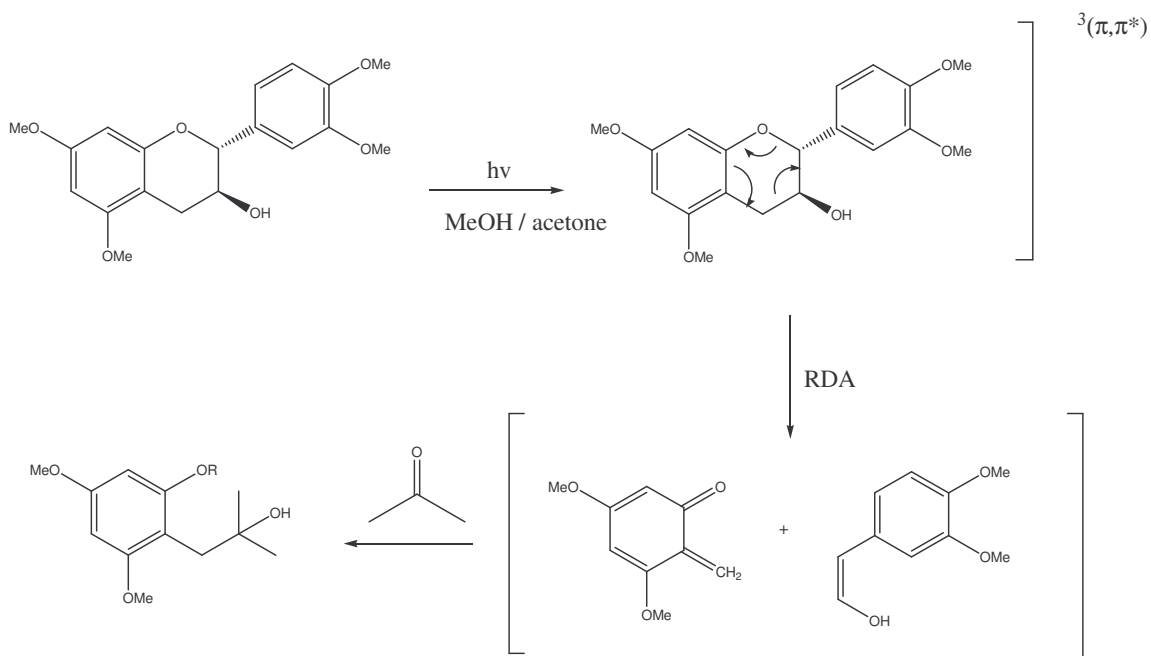
In the presence of acetone we isolated products (**53**) and (**54**) in low yields.



Product (**54**) was acetylated to yield (**55**).



The mechanism is postulated as follows: **(Scheme 13)**



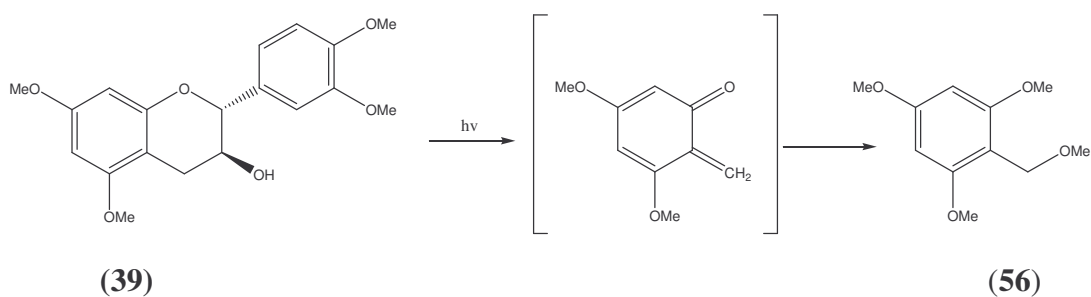
R = H (**54**)

R = Ac (**55**)

RDA = Retro Diels-Alder

### Scheme 13

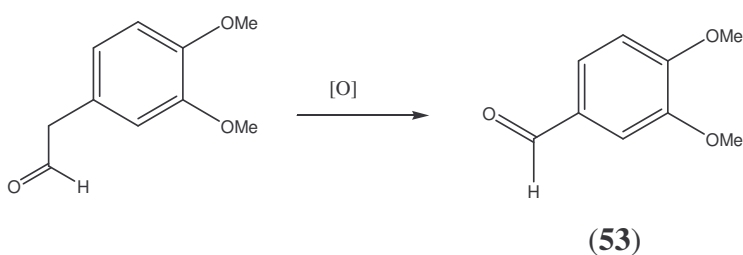
This reaction is in agreement with the work of Fourie and coworkers<sup>6</sup> who isolated product (**56**) in methanol from a similar *ortho*-quinone methide intermediate: **(Scheme 14)**



### Scheme 14

We could not detect (**56**) in our reaction mixture, probably due to the presence of phloroglucinol. Phloroglucinol may act as a hydrogen donor. Van der Westhuizen and coworkers<sup>5</sup> reported that the yield increases in the photolytic deoxygenation of flavanols in the presence of phloroglucinol and attributed this to the hydrogen donation of phloroglucinol.

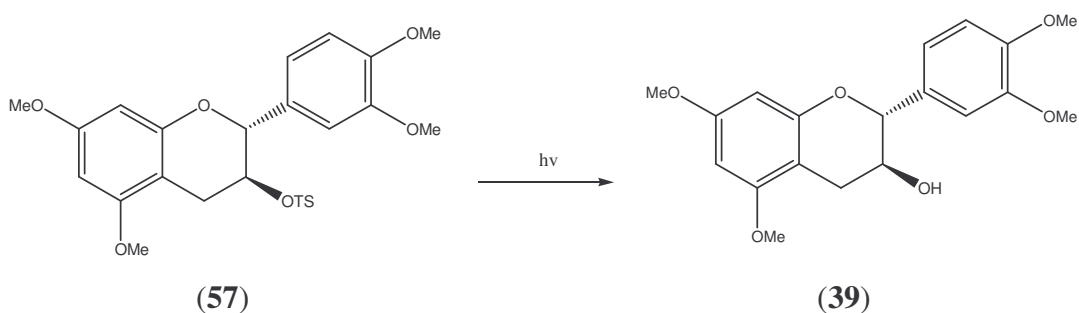
The aldehyde (**53**) is probably the result of photo-oxidation due to traces of singlet oxygen in our reaction.



**Scheme 15**

### 3.1.6 Photolysis of (+)-3-*O*-tosyl-3',4',5,7-tetra-*O*-methylcatechin

Irradiation of (+)-3-*O*-tosyl-3',4',5,7-tetra-*O*-methylcatechin (**57**) (better leaving group at C-3) at 300 nm gave (+)-3',4',5,7-tetra-*O*-methylcatechin (**39**).



**Scheme 16**

Retention of 2,3-*trans* configuration and no inversion at C-3 indicates that fission of the O-S bond took place and not the C-O bond. We postulated that the sulfonyl group now acted as chromophore of the photochemically active compound and not the aromatic rings.

Photolysis of (+)-catechin (**3**), (-)-epicatechin (**4**) and (-)-fisetinidol (**11**) with resorcinol at 250 nm was also investigated. These three reactions showed no coupling. Only addition of phloroglucinol afforded the desired coupling products.

## 3.2 Structure elucidation

### 3.2.1 (1*S*,2*S*)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (32)

The <sup>1</sup>H-NMR spectra of the free phenolic product from (+)-catechin (**3**) is fully assigned in **Plates 1a and b**. The following salient features can be seen:

- Seven aromatic hydroxy group resonances, which disappeared with the addition of D<sub>2</sub>O.
- The characteristic ABX system between  $\delta = 6.48 - 6.64$ .
- The two protons of the free rotating phloroglucinol moiety appear as a singlet at  $\delta = 5.84$ . The two protons from the restricted rotational phloroglucinol moiety appear as two humps at  $\delta = 5.90$  and  $\delta = 5.82$ . At 120 °C these two humps coalesce into a sharp singlet at  $\delta = 5.85$  of the same intensity as the free rotating phloroglucinol resonance at  $\delta = 5.84$ .
- H-2 and H-1 overlaps at  $\delta = 4.46$  at room temperature, however, these two resonances become visible as a doublet at  $\delta = 4.58$  and a multiplet at 4.54, respectively at 120 °C.
- One of the H-3 protons appears as a doublet of doublets at  $\delta = 2.43$  and the other proton overlaps with DMSO at  $\delta = 2.50$ .

- f) Heating of the sample to 90 °C resulted in movement of the DMSO signal and the doublet of H-3 $\alpha$  became visible at  $\delta = 2.70$  as a broad doublet.

### 3.2.2 (1R,2R)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (33)

The  $^1\text{H-NMR}$  spectra of the free phenolic product from (-)-epicatechin (**4**) is fully assigned in **Plate 2a** and **2b**. The following salient features can be seen:

- a) Seven aromatic hydroxyl group resonances, which disappeared with the addition of  $\text{D}_2\text{O}$ .
- b) The characteristic ABX system between  $\delta = 6.48 - 6.64$ .
- c) The two protons on the free rotating phloroglucinol moiety appear as a singlet at  $\delta = 5.84$ . The two protons from the restricted rotational phloroglucinol moiety appear as two humps at  $\delta = 5.90$  and  $\delta = 5.82$ . At 120 °C these two humps coalesce into a sharp singlet at  $\delta = 5.85$  of the same intensity as the free rotating phloroglucinol resonance at  $\delta = 5.84$ .
- d) H-2 and H-1 overlaps at  $\delta = 4.46$  at room temperature, however, these two resonances become visible as a doublet at  $\delta = 4.58$  and a multiplet at 4.54, respectively at 120 °C.
- e) The one H-3 proton appears as a doublet of doublets at  $\delta = 2.37$  and the other proton overlaps with DMSO at  $\delta = 2.50$ .
- f) Heating of the sample to 90 °C resulted in movement of the DMSO signal and the doublet of H-3 $\alpha$  became visible at  $\delta = 2.70$  as a broad doublet.

Product (**32**) and (**33**) has identical  $^1\text{H-NMR}$  spectra and could only be distinguished by means of CD spectra. (**CD-Plate 1-3**)

### 3.2.3 (2R,3S)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate (3') (Plate 3)

- The characteristic ABX system is seen between  $\delta = 7.30 - 7.15$ .
- Two doublets of doublets are seen at  $\delta = 2.87$  and  $\delta = 2.66$ , which integrates for one proton each and are assigned to the two methylene protons on C-4.
- Five acetyl groups are present which indicates that the starting material, (+)-catechin (**3**), was completely acetylated.

### 3.2.4 2-((2S,3R)-2-acetoxy-3-(3',4'-diacetoxyphenyl)-3-methoxypropyl)benzene-2'',4'',6''-triyl triacetate (36) and 2-((2S,3S)-2-acetoxy-3-(3',4'-diacetoxyphenyl)-3-methoxypropyl)benzene-2'',4'',6''-triyl triacetate (37) (Plate 4a and 4b)

- The characteristic ABX system is seen between  $\delta = 7.30 - 7.00$ .
- Two doublets of doublets are seen at  $\delta = 2.85$  and  $\delta = 2.61$  for the major product, and at  $\delta = 3.00$  and  $\delta = 2.53$  for its isomer, which integrates for one proton each and are assigned to the two protons on C-1.
- The methoxy group on C-3 is proof of an open ring product.
- Six acetoxy groups are present. The resonance at  $\delta = 1.85$  is more shielded and represents the aliphatic acetoxy on C-2.

### 3.2.5 (1S,2S)-1,3-di(2,4,6-triacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propan-2-ol (34)

#### <sup>1</sup>H – NMR (Plate 5a)

The <sup>1</sup>H-NMR has the following salient features:

- The characteristic ABX system of the B-ring, is similar to that of (+)-catechin (**3**),  $\delta$  7.19 (1H, d, J = 2.0 Hz, H-2'''), 7.17 (1H, dd, J = 2.0, 8.5 Hz, H-6''') and 7.10 (1H, d, J = 8.5 Hz, H-5''').

- 2) A two proton singlet at  $\delta = 6.85$  assigned to the free rotating C-3 phloroglucinol unit. The singlet indicates that we have a ring opened product. (Two doublets,  $J = 2.0$  Hz, would be expected as a phloroglucinol moiety that is part of the heterocyclic ring system). An additional two proton broad singlet at  $\delta = 6.82$  is assigned to the new phloroglucinol moiety on C-1 (H-3' and H-5'). Broadening is due to restricted rotation.
- 3) Concerning the four aliphatic protons:
- A broad singlet at  $\delta = 5.85$  assigned to H-2. This proton is coupled to H-3 $\alpha$  / H-3 $\beta$  and the double benzylic proton H-1. It is deshielded by the OAc group.
  - A doublet at  $\delta = 4.7$  ( $J = 9.07$  Hz) is assigned to H-1. This proton is coupled to H-2 only and is deshielded by the two aryl groups. The H-3 $\alpha$  and H-3 $\beta$  protons (two doublets of doublets at  $\delta = 3.19$  and  $\delta = 3.00$ ) on C-3 are coupled to each other ( $J = 14.4$  Hz, geminal coupling) and to H-2 ( $J = 4.6$  and  $J = 5.7$  Hz, respectively). The non-equivalence of the two protons is due to chirality on C-1.
- 4) Nine resonances at  $\delta = 2.27, 2.26, 2.25, 2.23, 2.04$  (two),  $2.00$  (two),  $1.72$  are assigned to the acetoxy methyl groups.
- This indicates that we have nine acetoxy groups which confirms that the ring has not closed. The upfield acetate at  $\delta = 1.72$  is assigned to the aliphatic acetate C-2.

### **Carbon ( $^{13}\text{C}$ ) (Plate 5b)**

$^{13}\text{C}$  has the following resonances:

- Seven acetate carbonyls at  $\delta = 167.9, 168.0, 168.35$  (two),  $168.58$  (two), and  $170.5$ . (Two missing acetate groups).
- Nine acetate methyl groups at  $\delta = 21.1, 21.0, 20.8, 20.6$  (two),  $20.5$  (two) and  $20.4$  (two).

In addition to the hydrogens attached to carbon that is identified below with HSQC (**Plate 5d**) the following additional resonances are seen in  $^{13}\text{C}$ .

- a) Nine acetate resonances between  $\delta = 167$  and  $171$ .
- b) The large singlet (two carbon resonance) at  $\delta = 150.5$  is assigned to C-3''/C-5'' on the free rotational phloroglucinol unit.
- c) The two carbon humps at  $\delta 149.6$  is assigned to C-3'/C-5' on the rotational phloroglucinol unit with restricted rotation.
- d) The deshielded resonances at  $\delta = 149.3, 149.2, 142.0$  and  $140.6$  are assigned to the remaining oxygen bonded carbons at C-3''', C-4' (phloroglucinol), C-4'' (phloroglucinol) and C-4''' (on the resorcinol moiety), respectively.
- e) The remaining small resonances at  $\delta = 138.2, 123.0$  and  $118.8$  are assigned to the remaining quaternary carbons on the aromatic rings (C-1', C-1'' and C-1''').
- f) The nine methyl groups of the acetates appear between  $\delta 20.3$  and  $\delta 21.1$ .

### **COSY (Plate 5c)**

- 1) The COSY experiment confirms the expected strong coupling between H-3 $\alpha$  and H-3 $\beta$  (strong correlation due to geminal nature).
- 2) COSY also confirms that H-2,  $\delta = 5.84$  is coupled to both H-3 $\alpha$  and H-3 $\beta$ , as well as to H-1 ( $\delta = 4.75$ ).
- 3) H-1 is only coupled to H-2 (coupling to H-3 $\alpha$  and H-3 $\beta$  is absent).
- 4) H-3 $\alpha$  and H-3 $\beta$  are only coupled to H-2 (coupling to H-1 is absent).

### **HSQC (Plate 5d)**

- 1) The following correlations apply:
  - a) Between H-3 $\alpha$  and H-3 $\beta$  ( $\delta 3.19$  and  $3.00$ ) and C-3 ( $\delta = 26.18$ ).
  - b) Between H-1 ( $\delta 4.75$ ) and C-1 ( $\delta = 26.1$ ).

- c) Between H-3''/H-5'' ( $\delta = 6.85$ ) and C-3''/C-5'' ( $\delta = 150.5$ ) of the free rotational phloroglucinol unit.
- d) Between H-3' and H-5' ( $\delta = 6.82$ ) and the hump at 114.3, which allowed us to identify the phloroglucinol unit with restricted rotation (C-2'/C-6').

### **HMBC (Plate 5e)**

The HMBC spectrum shows the expected correlations. Of particular importance is the correlation between H-1 ( $\delta$  4.76) and C-1' that allows us to prove that the additional phloroglucinol is attached on the C-1 position.

### **3.2.6 (1R,2R)-1,3-di(2,4,6-triacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propan-2-ol (35) and (2S,3R)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate (35')**

The acetylated products, (**34**) and (**35**), obtained from (+)-catechin (**3**) and (-)-epicatechin (**4**) gave identical NMR spectras. These products could only be distinguished by means of circular dichroism. (See **CD-plate 7-9**)

The acetylated starting material, (**3'**) obtained from photolysis of (+)-catechin (**3**) and the acetylated product (**35'**) from (-)-epicatechin (**4**), gave identical NMR-spectra. These products could only be distinguished by means of circular dichroism. Product (**3'**) and product (**35'**) gave a mirror image CD spectrum. (See **CD-plate 4-6**)

### **3.2.7 (1S,2S)-3-(2,4-dihydroxyphenyl)-1-(3,4-dihydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-propan-2-ol (45) (Plate 7a-7c)**

An HMBC correlation between C-3 ( $\delta = 36.77$ ) and H-6'' ( $\delta = 6.82$ ) made it possible to differentiate between the two ABX systems in the structure.



### 3.2.8 2-(2-hydroxy-2-methylpropyl)-3,5-dimethoxyphenol (54) and 3,4-dimethoxybenzaldehyde (53) (Plate 9a and 9b)

The NMR spectra of 2-(2-hydroxy-2-methylpropyl)-3,5-dimethoxyphenol (**54**) has the following salient features:

- 1) The ABX resonance system associated with the B-ring of the starting material has disappeared. This is proof that the B-ring was indeed lost.
- 2) The six proton resonances at  $\delta_{\text{H}}$  1.28 coupled to a carbon at  $\delta_{\text{C}}$  in the HSQC indicated two identical  $\text{CH}_3$ -groups and that acetone has been incorporated. This conclusion is supported by the quaternary carbon (C-2') at  $\delta_{\text{C}}$  75.25.
- 3) The A-ring remains intact as shown by the two doublets at  $\delta_{\text{H}}$  6.18 and 6.08.
- 4) The two methoxy groups, at  $\delta_{\text{H}}$  3.77 and 3.75, supports the statement in (3).
- 5) The two proton singlet at  $\delta_{\text{H}}$  2.82 is attributed in what remains of the C-4 on the catechin ring ( $\delta_{\text{C}}$  91.17) and is attributed to C-1' of our product. The resonance corresponding with C-2 and the proton coupled to C-1' (on the aromatic ring) in the HMBC indicates that it is directly attached to the aromatic ring.
- 6) Absence of carbonyl absorption indicates that the acetone carbonyl is not present.
- 7) The two one proton resonances at  $\delta_{\text{H}}$  8.48 and  $\delta_{\text{H}}$  1.94 disappeared when  $\text{D}_2\text{O}$  was added, indicating that we indeed have two OH-groups in our product. This conclusion is supported by the appearance of one acetate group in the acetylated derivative. (**Plate 10a**) The tertiary OH group is not expected to be acetylated under our conditions.

The aldehyde (**53**) has the following salient features in the  $^1\text{H-NMR}$  spectra (**Plate 11**)

- 1) The ABX system associated with the B-ring.
- 2) Characteristic aldehyde resonance at  $\delta_{\text{H}} \pm 10$  ppm.

### 3.3 Future Work

Future work in this area may be summarized as follows:

- a) Increase the yields by varying photolytical conditions (e.g. more polar solvents and aprotic solvents etc.)
- b) Investigate alternative chromatography (e.g. reverse phase HPLC, Sephadex and counter current chromatography) to increase the recovery of free phenolic reaction products that are absorbed on silica gel.
- c) Purification of the 3-methoxy derivatives which could not be separated in this study.
- d) Investigation of the previous claims that benzophenone increases the yield of inversion at C-2 not by excitation transfer but via radical abstraction.

### 3.4 Conclusion

- a) We developed a novel method to open the heterocyclic ring of flavan-3-ols via photolytic cleavage of the ether bond.
- b) We trapped the intermediates with methanol and phloroglucinol to obtain phloroglucinol grafted derivatives of flavan-3-ols.
- c) We demonstrated that the trapping mechanism was stereoselective and under control of the stereochemistry of the 3-hydroxy moiety of the flavan-3-ol.
- d) By judicious choice of starting material we could obtain both the (*R,R*) and (*S,S*) enantiomers of the grafted phloroglucinol products.
- e) We adapted existing CD rules to enable us to assign stereochemistry of the grafted unit at the C-2 position.

- f) We proved that the *para*-hydroxy free phenol was essential for grafting to take place and no coupling takes place if this position is derivatized.
- g) We isolated a product from the *ortho*-quinone methide derivatized from the A-ring proving that *ortho*-quinone methides is an alternative mechanism if the formation of the B-ring quinone methide is not possible.
- h) This work advances our understanding of the chemistry and photochemistry of flavan-3-ols, provides novel flavonoid derivatives for testing in bio-assays and will be of interest to the adhesive industry.

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## **CHAPTER 4**

### **STANDARD EXPERIMENTAL TECHNIQUES**

The following general techniques were used during this study.

#### **4.1 CHROMATOGRAPHIC TECHNIQUES**

##### **4.1.1 Thin Layer Chromatography**

Qualitative thin layer chromatography was performed on Merck aluminium sheets (silica gel 60 F<sub>254</sub>, 0.25 mm). Preparative thin layer chromatography was performed on glass plates (20x20 cm), covered with a layer (1.0 mm) Kieselgel PF<sub>254</sub> (100 g Kieselgel in 230 ml distilled water per 5 plates). The plates were dried at room temperature and used unactivated. The plates were loaded with a maximum of 25 mg material per plate. After development in the appropriate eluent the plates were dried in fast stream of air and the bands identified by either UV-light (254 nm) or by the appropriate spraying reagent. The bands were eluted with acetone and the acetone removed under reduced pressure.

Small-scale separations were conducted on Merck Precoated (0.25 mm) TLC Plates Silica Gel 60 F<sub>254</sub> with each plate charged with 3-5 mg of crude product.

##### **4.1.2 Centrifugal Chromatography**

Chromatography was performed in a thin layer of silica gel coated on a circular piece of glass called a rotor. A motor drives the rotor at a constant speed by a shaft passing through an opening in the centre. The compound to be separated was applied as a solution in the centre of the pre-cast rotor by means of a hand-held syringe. The chosen solvent system was then pumped to the centre. The solvent is forced by centrifugal forces through the adsorbent layer effectively separating the individual components as a result of the different affinities to the solvent mixture. As the individual rings reach the outer rim of the rotor they are spun off the edge of the glass together with the solvent. A solvent channel collects the elute and brings it to the output tube where the fractions are collected in test tubes.

Centrifugal chromatography was performed with an Analtech Cyclograph<sup>TM</sup> with commercially available Analtech rotors (1 mm, 2mm and 4 mm depending on the mass of material to be separated).

#### 4.1.3 Column Chromatography

Separations on Sephadex LH-20 from Pharmacia and Kieselgel from Merck (Art 773, 170-230 mesh) were performed with various column sizes and at differing flow rates. Fractions were collected in test tubes.

#### 4.1.4 Spraying Reagents

All TLC plates were sprayed with a 2% (v/v) solution of formaldehyde (40%) in concentrated sulphuric acid and subsequently heated to 110 °C for maximum colour development.

### 4.2 SPECTROSCOPIC METHODS

#### 4.2.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

A 600MHz Bruker spectrometer were used to record the <sup>1</sup>H NMR, NOE, COSY, HMBC, HMQC (600 MHz) and <sup>13</sup>C, APT (150 MHz) experiments. The solvents used were chloroform-d<sub>1</sub> (CDCl<sub>3</sub>), acetone-d<sub>6</sub> and methanol-d<sub>4</sub> (MeOD) with TMS (tetramethylsilane) as internal standard. Chemical shifts were expressed as parts per million (ppm) on the delta (δ) scale and coupling constants (J) are accurate to 0.01 Hz.. The following abbreviations are used:

s	singlet	dd	doublet of doublets
d	doublet	t	triplet
q	quartet	m	multiplet

#### 4.2.2 Circular dichroism (CD)

CD spectra were recorded on Jasco J-710 spectropolarimeter in spectrophotometric grade Methanol (~1 mg/10 ml MeOH). The formula used to calculate the molecular ellipticity  $[\theta]$  was:

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

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

#### 4.2.3 Mass Spectrometry (MS)

The samples were injected via a Rheodyne valve into the carrier solvent pumped at a flow rate of 50 $\mu$ l/minute by a Perkin Elmer series 200 micro pump. The sample was thus delivered into the electro spray ionization source of a Waters Quattro Ultima triple quadrupole mass spectrometer, operated in the positive mode. The capillary voltage was 3.5kV, the source temperature 80°C and the desolvation temperature was 150°C. The cone voltage was at 40V and all other settings were adjusted for maximum detection of the ions. Data was acquired by scanning the mass range from m/z = 100 to m/z = 1000 at a scan rate of 3 seconds/scan. A representative mass spectrum of the sample was produced by addition of the spectra across the injection peak and subtraction of the background.

#### 4.2.4 Infrared (IR)

Solid state FR-IR spectra were recorded as KBr pellets on a Bruker Tensor 27 spectrometer in the range of 3000 - 600  $\text{cm}^{-1}$ .

## 4.3 CHEMICAL METHODS

### 4.3.1 Acetylation

Dried material was dissolved in minimum of pyridine. Two drops of acetic anhydride per one drop of pyridine was added and left overnight. The reaction mixture was poured onto crushed ice and the resulting precipitate was filtered and repeatedly washed with distilled water to remove excess pyridine and acetic anhydride. Precipitate was redissolved in acetone and dried.

### 4.3.2 Tosylation

Dried material was dissolved in dry pyridine and excess of *p*-toluene sulfonic anhydride and left overnight. The reaction mixture was poured onto crushed ice and the resulting precipitate was filtered and repeatedly washed with distilled water to remove excess pyridine and acetic anhydride. Precipitate was redissolved in acetone and dried.

### 4.3.3 Methylation using dimethylsulphate

Phenolic material was dissolved in dry acetone (25 ml), dry potassium carbonate (8 equiv.) was added and dimethylsulphate (10 equiv.) was subsequently added drop-wise over a period of 30 minutes under N<sub>2</sub>. The reaction mixture was refluxed for 1 hour, filtered and acetone was removed under vacuum. Excess dimethylsulphate was destroyed by addition of dilute ammonia solution and extraction with ethyl acetate.

## 4.4 ANHYDROUS SOLVENTS AND REAGENTS

Acetone was left over dry K<sub>2</sub>CO<sub>3</sub> (oven-dried, 24 hours, 200 °C) for 24 hours. The K<sub>2</sub>CO<sub>3</sub> was filtered off and the solvent distilled over 3 Å molecular sieves and stored under N<sub>2</sub>.



Dichloromethane and dimethylformamide were refluxed over  $\text{CaH}_2$  under  $\text{N}_2$  for 12 hours with subsequent fresh distillation under  $\text{N}_2$  before use.

#### 4.5 FREEZE-DRYING

Phenolic material in aqueous solution was freeze-dried using a Vacutex Freezemobile.

#### 4.6 PHOTOCHEMICAL REACTIONS

All photochemical reactions were carried out inside the photochemical reactor RAYON manufactured by SOUTHERN N. E. ULTRAVIOLET Co. Middletown, Connecticut, USA, equipped with RAYONET PHOTOCHEMICAL REACTOR lamps CAT. NO. RPR-2537 Å, 3000 Å and 3500 Å respectively.

#### 4.7 ABBREVIATIONS

The following abbreviations were used to describe the solvent system and protective groups in this study:

A	acetone
Ac	acetate
$\text{CDCl}_3$	chloroform-d
EtOAc	ethyl acetate
MeOH	methanol
EtOH	ethanol
H	hexane
Me	methyl
T	toluene
RDA	Retro Diels-Alder

## CHAPTER 5

### 5.1 PHOTOLYSIS OF (+)-CATECHIN AND (-)-EPICATECHIN

#### 5.1.1 Synthesis of (1S,2S)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (32)

(+)-Catechin (**3**) (209 mg, 0.7 mmol) and phloroglucinol (401 mg; 3.2 mmol) were irradiated in MeOH (25 ml) at 250 nm for 28 hours. Column chromatography (T/A 4:6) yielded two bands with  $R_f = 0.33$  and  $R_f = 0.52$ .

The fraction  $R_f = 0.33$  yielded (1S,2S)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (32) (25 mg, 11%).  $^1\text{H NMR } \delta$  (600 MHz, DMSO,  $\text{Me}_4\text{Si}$ ) 8.93 (1H, s, 1xOH), 8.87 (1H, s, 1xOH), 8.80 (2H, s, 2xOH), 8.74 (1H, s, 1xOH), 8.47 (1H, s, 1xOH), 8.38 (1H, s, 1xOH), 6.61 (1H, d,  $J = 2.0$  Hz, H-2''), 6.53 (1H, d,  $J = 8.3$  Hz, H-5''), 6.50 (1H, dd,  $J = 2.0, 8.3$  Hz, H-6''), 5.85 (2H, s, H-3', H-5'), 5.84 (2H, s, H-3'', H-5''), 4.58 (1H, d,  $J = 4.3$  Hz, H-2), 4.54 (1H, m, H-1), 2.70 (1H, d,  $J = 14.3$  Hz, H-3 $\alpha$ ), 2.43 (1H, dd,  $J = 9.6$  Hz, 14.3 Hz, H-3 $\beta$ ). (**Plate 1a** and **Plate 1b**)

Found (ES)  $[\text{M}+\text{H}]^+$ , 417.1189, ( $\text{C}_{21}\text{H}_{20}\text{O}_9 + \text{H}^+$ ) requires  $m/z$  417.1185.

IR (KBr):  $\nu_{\text{max}} = 3418.92, 1614.14, 1151.31, 1022.76, 988.53 \text{ cm}^{-1}$ .

CD:  $[\theta]_{286.6} 8.334 \times 10^2$ ,  $[\theta]_{262.6} 6.266 \times 10^2$ ,  $[\theta]_{239.0} -2.178 \times 10^2$ ,  $[\theta]_{220.4} 3.868 \times 10^3$ ,  $[\theta]_{207.8} -3.378 \times 10^2$ ,  $[\theta]_{201.6} 3.273 \times 10^2$ ,  $[\theta]_{194.6} -1.676 \times 10^2$ ,  $[\theta]_{190.4} 1.700 \times 10^2$ ,  $[\theta]_{184.6} -7.611 \times 10^2$ . (**CD-plate 1**)

The fraction  $R_f = 0.52$  yielded unreacted starting material, (+)-catechin (**3**) (50 mg). After acetylation, the  $^1\text{H-NMR}$  spectrum of (**3**) corresponded to that of (**3'**). (See **Plate 3**)

### 5.1.2 Synthesis of (1*R*,2*R*)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (33)

(-)-Epicatechin (**4**) (218 mg, 0.8 mmol) and phloroglucinol (421 mg; 3.3 mmol) were irradiated in MeOH (25 ml) at 250 nm for 28 hours. Column chromatography (T/A 4:6) yielded two bands with  $R_f = 0.26$  and  $R_f = 0.45$ .

The fraction  $R_f = 0.26$  yielded (1*R*,2*R*)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (33) (17 mg, 8%).  $^1\text{H NMR } \delta$  (600 MHz, DMSO,  $\text{Me}_4\text{Si}$ ) 8.95 (1H, s, 1xOH), 8.89 (2H, s, 2xOH), 8.81 (1H, s, 1xOH), 8.75 (1H, s, 1xOH), 8.49 (1H, s, 1xOH), 8.40 (1H, s, 1xOH), 6.62 (1H, d,  $J = 2.0$  Hz, H-2'''), 6.53 (1H, d,  $J = 8.3$  Hz, H-5'''), 6.50 (1H, dd,  $J = 2.0, 8.3$  Hz, H-6'''), 5.85 (2H, s, H-3', H-5'), 5.84 (2H, s, H-5'', H-7''), 4.58 (1H, d,  $J = 4.3$  Hz, H-2), 4.54 (1H, m, H-1), 2.37 (1H, dd,  $J = 9.6$  Hz, 14.3 Hz, H-3 $\beta$ ), 2.09 (1H, d,  $J = 14.3$  Hz, H-3 $\alpha$ ). (**Plate 2a** and **2b**)

Found (ES)  $[\text{M}+\text{H}]^+$ , 417.1189, ( $\text{C}_{21}\text{H}_{20}\text{O}_9 + \text{H}^+$ ) requires  $m/z$  417.1185.

IR (KBr):  $\nu_{\text{max}} = 3418.92, 1614.14, 1151.31, 1022.76, 988.53 \text{ cm}^{-1}$ .

CD:  $[\theta]_{344.2} 3.036 \times 10^2$ ,  $[\theta]_{293.8} 6.836 \times 10^2$ ,  $[\theta]_{276.6} 8.644 \times 10^2$ ,  $[\theta]_{260.0} -2.638 \times 10^2$ ,  $[\theta]_{238.2} 6.911 \times 10^2$ ,  $[\theta]_{222.2} -3.818 \times 10^3$ ,  $[\theta]_{206.8} 1.237 \times 10^3$ ,  $[\theta]_{199.8} -4.058 \times 10^2$ ,  $[\theta]_{188.0} -5.125 \times 10^2$ . (**CD-plate 2**)

The  $R_f = 0.45$  yielded unreacted starting material, (-)-epicatechin (**4**) (52 mg). After acetylation, the  $^1\text{H-NMR}$  spectrum of (**4**) corresponded to that of (**4'**). (See **Plate 6**)

### 5.1.3 Synthesis of 2-((2*S*,3*R*)-2-acetoxy-3-(3',4'-diacetoxyphenyl)-3-methoxypropyl)benzene-2'',4'',6''-triyl triacetate (36) and (1*S*,2*S*)-1,3-di(2,4,6-triacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propan-2-ol (34)

(+)-Catechin (**3**) (350 mg, 1.2 mmol) and phloroglucinol (675 mg, 5.4 mmol) were irradiated at 250 nm in MeOH (25 ml) under nitrogen for 14 hours. Acetylation followed by column chromatography (silica gel, T:A 8.5:1.5) gave four fractions;  $R_f = 0.55$  (brown

with spray reagent),  $R_f$  0.47 (orange yellow with spray reagent),  $R_f$  0.42 (orange yellow with spray reagent) and  $R_f$  0.25 (yellow with spray reagent).

The fraction  $R_f$  0.55 yielded (2*R*,3*S*)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate {acetylated (+)-catechin}, (**3'**), (80 mg, 18%).  $^1\text{H NMR } \delta$  (600 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ) 7.25 (1H, dd,  $J = 8.3, 2.0$  Hz, H-6'), 7.19 (1H, d,  $J = 8.3$  Hz, H-5'), 7.16 (1H, d,  $J = 2.0$  Hz, H-2'), 6.66 (1H, d,  $J = 2.0$  Hz, H-6), 6.60 (1H, d,  $J = 2.0$  Hz, H-8), 5.25 (1H, k,  $J = 6.24$  Hz, H-3), 5.14 (1H, d,  $J = 6.24$  Hz, H-2), 2.87 (1H, dd,  $J = 5.1, 16.7$  Hz, H-4 $\alpha$ ), 2.66 (1H, dd,  $J = 6.24, 16.7$  Hz, H-4 $\beta$ ), 2.28 (6H, s, 2xOAc), 2.27 (3H, s, 1xOAc), 2.26 (3H, s, 1xOAc), 2.00 (3H, s, 1xOAc). (**Plate 3**)

Found (ES)  $[\text{M}+\text{H}]^+$ , 501.1405, ( $\text{C}_{25}\text{H}_{24}\text{O}_{11} + \text{H}^+$ ) requires  $m/z$  501.1396.

IR (KBr):  $\nu_{\text{max}} = 1772.53, 1371.97, 1203.96, 1124.35, 1040.49$   $\text{cm}^{-1}$ .

CD:  $[\theta]_{441.8} 1.437 \times 10^3$ ,  $[\theta]_{365.2} 1.017 \times 10^3$ ,  $[\theta]_{300.6} 1.245 \times 10^3$ ,  $[\theta]_{270.0} -1.622 \times 10^3$ ,  $[\theta]_{229.4} 5.749 \times 10^3$ ,  $[\theta]_{212.4} -5.020 \times 10^2$ ,  $[\theta]_{197.6} 8.358 \times 10^2$ . (**CD-plate 4**)

The fraction  $R_f$  0.47 yielded 2-((2*S*,3*R*)-2-acetoxy-3-(3',4'-diacetoxyphenyl)-3-methoxypropyl)benzene-2'',4'',6''-triyl triacetate, (**36**), (64 mg, 14%).  $^1\text{H NMR } \delta$  (600 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ), 7.10 (1H, d,  $J = 8.2$  Hz, H-5'), 7.06 (1H, d,  $J = 2.0$  Hz, H-2'), 7.03 (1H, dd,  $J = 2.00, 8.20$  Hz, H-6'), 6.87 (2H, s, H-3'', H-5''), 5.06 (1H, ddd,  $J = 3.66, 6.72, 8.20$  Hz, H-2), 4.12 (1H, d,  $J = 3.66$  Hz, H-3), 3.23 (3H, s, 1xOMe), 2.85 (1H, dd,  $J = 6.72, 13.75$  Hz, H-1 $\alpha$ ), 2.61 (1H, dd,  $J = 8.20, 13.75$  Hz, H-1 $\beta$ ), 2.20 (3H, s, 1xOAc), 2.21 (3H, s, 1xOAc), 2.19 (6H, s, 2xOAc), 2.18 (3H, s, 1xOAc), 1.85 (3H, s, 1xOAc). (**Plate 4a**)

Found (ES)  $[\text{M}+\text{H}]^+$ , 575.1769, ( $\text{C}_{28}\text{H}_{30}\text{O}_{13} + \text{H}^+$ ) requires  $m/z$  575.1764.

Isomerization of above product was seen in a ratio of 1:4. The fraction  $R_f$  0.47 also yielded 2-((2*S*,3*S*)-2-acetoxy-3-(3',4'-diacetoxyphenyl)-3-methoxypropyl)benzene-2'',4'',6''-triyl triacetate, (**37**) in very low yield. The reaction was repeated but the smaller isomer could not be separated, therefore identification was done on the same  $^1\text{H-NMR}$  spectra.  $^1\text{H NMR } \delta$  (600 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ) 7.21 (1H, dd,  $J = 2.0, 8.3$  Hz, H-6'), 7.15 (1H, d,  $J = 2.0$  Hz, H-2'), 7.13 (1H, d,  $J = 8.3$  Hz, H-5'), 6.75 (2H, s, H-3'', H-5''), 5.00

(1H, ddd, J = 3.06, 6.48, 8.20 Hz, H-2), 4.33 (1H, d, J = 3.54, H-3), 3.28 (3H, s, 1xOMe), 3.00 (1H, dd, J = 10.9, 14.3 Hz, H-1 $\alpha$ ), 2.53 (1H, dd, J = 2.90, 14.3 Hz, H-1 $\beta$ ), 2.16 (3H, s, 1xOAc), 2.15 (3H, s, 1xOAc), 2.07 (3H, s, 1xOAc), 2.05 (6H, s, 2xOAc), 2.03 (3H, s, 1xOAc). (**Plate 4b**)

Found (ES) [M+H]<sup>+</sup>, 575.1769, (C<sub>28</sub>H<sub>30</sub>O<sub>13</sub> + H<sup>+</sup>) requires m/z 575.1764.

The fraction R<sub>f</sub> 0.42 yielded (1S,2S)1,3-di(2,4,6-triacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propan-2-ol, (**34**) (80 mg, 20%). <sup>1</sup>H NMR  $\delta$  (600 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) 7.19 (1H, d, J = 2.0 Hz, H-2'''), 7.17 (1H, dd, J = 2.0, 8.5 Hz, H-6'''), 7.10 (1H, d, J = 8.5 Hz, H-5'''), 6.85 (2H, s, H-3'', H-5''), 6.82 (2H, broad s, H-3', H-5'), 5.84 (1H, s, H-2), 4.75 (1H, d, J = 9.07 Hz, H-1), 3.19 (1H, dd, J = 5.7, 14.4 Hz, H-3 $\alpha$ ), 3.00 (1H, dd, J = 4.6, 14.4 Hz, H-3 $\beta$ ), 2.27 (3H, s, 1xOAc), 2.26 (3H, s, 1xOAc), 2.25 (3H, s, 1xOAc), 2.23 (3H, s, 1xOAc), 2.04 (6H, broad s, 2xOAc), 2.00 (6H, s, 2xOAc), 1.72 (3H, s, 1xOAc). (**Plate 5a**)

<sup>13</sup>C NMR  $\delta$  (150 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) 170.6-168.0 (9xO-C(=O)CH<sub>3</sub>), 150.5 (C-3''/C-5''), 149.6 (C-3'/C-5'), 149.3 (C-3'''), 149.2 (C-4'), 142.0 (C-4''), 140.6 (C-4'''), 138.2 (C-1'), 126.2 (C-2'''), 123.5 (C-5'''), 123.0 (C-1''), 122.8 (C-6'''), 118.8 (C-1'''), 114.3 (C-2'/C-6'), 113.7 (C-2''/C-6''), 71.2 (C-2), 42.1 (C-3 $\alpha$ / $\beta$ ), 26.1 (C-1), 21.1-20.3 (9xO-COCH<sub>3</sub>). (**Plate 5b**)

Found (ES) [M+H]<sup>+</sup>, 795.2139, (C<sub>39</sub>H<sub>38</sub>O<sub>18</sub> + H<sup>+</sup>) requires m/z 795.2135.

IR (KBr):  $\nu_{\max}$  = 1773.54, 1371.41, 1192.99, 1024.57 cm<sup>-1</sup>.

CD: [ $\theta$ ]<sub>347.4</sub> -3.892 x 10<sup>1</sup>, [ $\theta$ ]<sub>278.4</sub> 2.398 x 10<sup>3</sup>, [ $\theta$ ]<sub>209.2</sub> 8.739 x 10<sup>3</sup>, [ $\theta$ ]<sub>193.0</sub> -9.367 x 10<sup>1</sup>, [ $\theta$ ]<sub>187.0</sub> 1.543 x 10<sup>3</sup>. (**CD-plate 7**)

The fraction R<sub>f</sub> 0.25 yielded acetylated phloroglucinol (223 mg, 33%). <sup>1</sup>H NMR  $\delta$  (600 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) 6.84 (3H, s, aromatic protons), 2.26 (6H, s, COCH<sub>3</sub>). The fraction is identical to the commercial sample (Merck 8.18887.0100 100g), before acetylation.

**5.1.4 Synthesis of (1*R*,2*R*)-1,3-di(2,4,6-triacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propan-2-ol (35) and (2*S*,3*R*)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate (35')**

(-)-Epicatechin (**4**) (286 mg, 1.0 mmol) and phloroglucinol (640 mg, 5.0 mmol) were irradiated at 250 nm in MeOH (25 ml) under nitrogen for 14 hours. Acetylation followed by column chromatography (silica gel, T:A 8.5:1.5) gave four fractions;  $R_f$  0.37 (brown with spray reagent),  $R_f$  0.31 (orange yellow with spray reagent),  $R_f$  0.23 (orange yellow with spray reagent) and  $R_f$  0.14 (yellow with spray reagent).

The fraction  $R_f$  0.37 yielded (1*R*,2*R*)-1,3-di(2,4,6-triacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propan-2-ol, (**35**) (36 mg, 13%).  $^1\text{H NMR } \delta$  (600 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ) 7.20 (1H, d,  $J = 2.0$  Hz, H-2'), 7.18 (1H, dd,  $J = 2.0, 8.5$  Hz, H-6'), 7.11 (1H, d,  $J = 8.5$  Hz, H-5'), 6.86 (2H, s, H-5, H-7), 6.83 (2H, broad s, H-4'', H-6''), 5.84 (1H, s, H-2), 4.76 (1H, d,  $J = 9.07$  Hz, H-3), 3.20 (1H, dd,  $J = 5.76, 14.41$  Hz, H-1 $\alpha$ ), 3.02 (1H, dd,  $J = 4.62, 14.41$  Hz, H-1 $\beta$ ), 2.27 (3H, s, 1xOAc), 2.26 (3H, s, 1xOAc), 2.25 (3H, s, 1xOAc), 2.23 (3H, s, 1xOAc), 2.04 (6H, broad s, 2xOAc), 2.00 (6H, s, 2xOAc), 1.72 (3H, s, 1xOAc). (See **Plate 5a – e**)

Found (ES)  $[\text{M}+\text{H}]^+$ , 795.2139, ( $\text{C}_{39}\text{H}_{38}\text{O}_{18} + \text{H}^+$ ) requires  $m/z$  795.2135.

IR (KBr):  $\nu_{\text{max}} = 1773.54, 1371.41, 1192.99, 1024.57 \text{ cm}^{-1}$ .

CD:  $[\theta]_{292.4} 2.262 \times 10^3$ ,  $[\theta]_{251.4} 8.697 \times 10^2$ ,  $[\theta]_{230.4} -2.726 \times 10^3$ ,  $[\theta]_{209.6} -9.031 \times 10^3$ ,  $[\theta]_{195.8} 2.344 \times 10^3$ . (**CD-plate 8**)

The fraction  $R_f$  0.31 yielded acetylated phloroglucinol, (230 mg, 34%).  $^1\text{H NMR } \delta$  (600 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ) 6.86 (3H, s, aromatic protons), 2.16 (9H, s, 3xOAc).

The fraction  $R_f$  0.23 yielded (2*R*,3*R*)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate {acetylated (-)-epicatechin (**4'**) (71 mg, 17%).  $^1\text{H NMR } \delta$  (600 MHz,  $\text{CDCl}_3$ ,  $\text{Me}_4\text{Si}$ ) 7.36 (1H, d,  $J = 2.3$  Hz, H-2'), 7.27 (1H, dd,  $J = 8.3, 2.7$  Hz, H-6'), 7.21 (1H, d,  $J = 8.3$  Hz, H-5'), 6.68 (1H, d,  $J = 2.0$  Hz, H-6), 6.58 (1H, d,  $J = 2.0$  Hz, H-8), 5.40 (1H, m, H-3), 5.12 (1H, broad s,  $J < 1$  Hz, H-2), 2.98 (1H, dd,  $J = 4.5, 17.7$  Hz, H-4 $\alpha$ ), 2.90 (1H,

dd,  $J = 2.0, 17.7$  Hz, H-4 $\beta$ ), 2.30 (3H, s, 1xOAc), 2.29 (6H, s, 2xOAc), 2.28 (3H, s, 1xOAc), 1.92 (3H, s, 1xOAc). (**Plate 6**)

Found (ES)  $[M+H]^+$ , 501.1402, ( $C_{25}H_{24}O_{11} + H^+$ ) requires  $m/z$  501.1396.

IR (KBr):  $\nu_{\max} = 1770.74, 1372.08, 1211.15, 1022.14$   $cm^{-1}$ .

The fraction  $R_f$  0.14 yielded (2*S*,3*R*)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate {acetylated (-)-catechin} (**35'**) (40 mg, 10%).  $^1H$  NMR  $\delta$  (600 MHz,  $CDCl_3$ ,  $Me_4Si$ ), 7.25 (1H, dd,  $J = 2.00, 8.20$  Hz, H-6'), 7.19 (1H, d,  $J = 8.2$  Hz, H-5'), 7.17 (1H, d,  $J = 2.0$  Hz, H-2'), 6.66 (1H, d,  $J = 2.0$  Hz, H-6), 6.60 (1H, d,  $J = 2.0$  Hz, H-8), 5.25 (1H, m, H-3), 5.14 (1H, d,  $J = 6.3$  Hz, H-2), 2.87 (1H, dd,  $J = 5.1, 16.7$  Hz, H-4 $\alpha$ ), 2.66 (1H, dd,  $J = 6.6, 16.7$  Hz, H-4 $\beta$ ), 2.28 (6H, s, 2xOAc), 2.27 (3H, s, 1xOAc), 2.25 (3H, s, 1xOAc), 1.99 (3H, s, 1xOAc). (See **Plate 3**)

Found (ES)  $[M+H]^+$ , 501.1402, ( $C_{25}H_{24}O_{11} + H^+$ ) requires  $m/z$  501.1396.

IR (KBr):  $\nu_{\max} = 1771.89, 1371.07, 1194.11, 1122.11, 1024.43$   $cm^{-1}$ .

CD:  $[\theta]_{286.0} 3.328 \times 10^3$ ,  $[\theta]_{275.2} 3.342 \times 10^3$ ,  $[\theta]_{228.6} -2.289 \times 10^3$ ,  $[\theta]_{215.0} 1.122 \times 10^2$ ,  $[\theta]_{203.2} -9.267 \times 10^3$ ,  $[\theta]_{184.8} 3.559 \times 10^3$ . (**CD-plate 5**)

## 5.2 PHOTOLYSIS OF (-)-FISSETINIDOL

### 5.2.1 Synthesis of (1*S*,2*S*)-3-(2,4-dihydroxyphenyl)-1-(3,4-dihydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-propan-2-ol (45)

(+)-Fisetinidol (**11**) (304 mg, 1.1 mmol) and phloroglucinol (631 mg, 5.0 mmol) were irradiated in MeOH (25 ml) at 250 nm for 5 hours. Column chromatography (T/A 4:6) gave one product with  $R_f = 0.45$

The fraction  $R_f$  0.45 yielded (1*S*,2*S*)-3-(2,4-dihydroxyphenyl)-1-(3,4-dihydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-propan-2-ol (**45**) (25 mg, 11%).  $^1H$  NMR  $\delta$  (600 MHz, Acetone- $d_6$ ,  $Me_4Si$ ) 6.81 (1H, d,  $J = 8.3$  Hz, H-6'''), 6.78 (1H, d,  $J = 2.0$  Hz, H-3'''), 6.69 (1H, dd,  $J = 2.0$  Hz, 8.3 Hz, H-5'''), 6.65 (1H, d,  $J = 8.3$  Hz, H-6'''), 6.34 (1H, d,  $J = 2.4$  Hz, H-2'''), 6.24 (1H, dd,  $J = 2.4, 8.3$  Hz, H-5'''), 5.99 (2H, s, H-3', H-5'), 4.75 (2H, s, H-1, H-

2), 3.32 (2H, s, 2xOH), 2.74 (1H, dd, J = 3.4, 14.3 Hz, H-3 $\alpha$ ), 2.69 (1H, dd, J = 8.3, 14.3 Hz, H-3 $\beta$ ). (**Plate 7a**)

$^{13}\text{C}$  NMR  $\delta$  (150 MHz, Acetone- $d_6$ , Me $_4$ Si) 157.62 (C-2',C-6'), 157.22 (C-3'''), 157.04 (C-2''), 156.29 (C-4'), 144.37 (C-4''), 142.89 (C-4'''), 134.60 (C-1'), 132.04 (C-6''), 119.75 (C-6'''), 116.89 (C-1''), 115.78 (C-2'''), 114.66 (C-5'''), 106.54 (C-5''), 105.51 (C-1'''), 102.801 (C-3'), 95.32 (C-3''/C-5''), 75.36 (C-2), 45.11 (C-1), 36.77 (C-3). (The spectrum was divided into **Plate 7b** and **7c**)

Found (ES)  $[\text{M}+\text{H}]^+$ , 401.1240, (C $_{21}$ H $_{20}$ O $_8$  + H $^+$ ) requires m/z 401.1236.

IR (KBr):  $\nu_{\text{max}}$  = 1623.23, 1261.47, 802.19 cm $^{-1}$ .

CD:  $[\theta]_{301.6}$  1.000 x 10 $^4$ ,  $[\theta]_{291.8}$  1.027 x 10 $^4$ ,  $[\theta]_{225.0}$  -3.555 x 10 $^3$ ,  $[\theta]_{205.6}$  -1.373 x 10 $^4$ ,  $[\theta]_{198.0}$  2.259 x 10 $^4$ ,  $[\theta]_{190.4}$  -1.522 x 10 $^3$ ,  $[\theta]_{184.4}$  1.595 x 10 $^3$ . (**CD-plate 10**)

Above fraction was acetylated according to the procedure on page 69 and yielded 2-((1S,2S)-2-acetoxy-3-(2,4-diacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propyl)benzene-1,3,5-triyl triacetate (46).  $^1\text{H}$  NMR  $\delta$  (600 MHz, CDCl $_3$ , Me $_4$ Si) 7.20 (1H, d, J = 2.0 Hz, H-2'''), 7.15 (1H, dd, J = 2.0, 8.3 Hz, H-6'''), 7.13 (1H, d, J = 8.3 Hz, H-6''), 7.11 (1H, d, J = 9.2 Hz, H-5'''), 6.94 (1H, dd, J = 2.0, 11.4 Hz, H-5''), 6.88 (1H, d, J = 2.3 Hz, H-3''), 6.81 (2H, s, H-3', H-5'), 6.00 (1H, m, H-2), 4.62 (1H, d, J = 9.7 Hz, H-1), 3.09 (1H, dd, J = 5.5, 15.3 Hz, H-3 $\alpha$ ), 3.06 (1H, dd, J = 5.5, 15.3 Hz, H-3 $\beta$ ), 2.29 (3H, s, 1xOAc), 2.28 (3H, s, 1xOAc), 2.27 (3H, s, 1xOAc), 2.24 (3H, s, 1xOAc), 2.06 (6H, s, 2xOAc), 1.94 (3H, s, 1xOAc), 1.77 (3H, s, 1xOAc). (**Plate 8a**)

$^{13}\text{C}$  NMR  $\delta$  (150 MHz, CDCl $_3$ , Me $_4$ Si) 170.26-167.98 (8xO-C $\underline{\text{O}}$ CH $_3$ ), 149.90-140.67 (7xCO), 137.85 (C-1'), 132.23 (C-6''), 126.49 (C-5''), 125.54 (C-1''), 123.46 (C-5'''), 123.00 (C-3''), 122.61 (C-1'''), 118.87 (C-6'''), 116.17 (C-2'''), 114.17 (C-3'/C-5'), 71.07 (C-2), 42.08 (C-1), 31.34 (C-3), 21.16-20.25 (8xO-CO $\underline{\text{C}}$ H $_3$ ), ((The spectrum was divided into **Plate 8b** and **8c**) Found (ES)  $[\text{M}+\text{H}]^+$ , 737.2085, (C $_{37}$ H $_{36}$ O $_{16}$  + H $^+$ ) requires m/z 737.2081.



## 5.3 PHOTOLYSIS OF (+)-3',4',5,7-TETRA-*O*-METHYLCATECHIN

### 5.3.1 Synthesis of 2-(2-hydroxy-2-methylpropyl)-3,5-dimethoxyphenol (54)

(+)-3',4',5,7-tetra-*O*-methylcatechin (**39**) (50 mg; 0.14 mmol) and phloroglucinol (82 mg; 0.65 mmol) in a mixture of methanol (90%) and acetone (10%) were irradiated at 300 nm for 2½ hours. Column chromatography (silica gel, T:A 7:3) gave two fraction; R<sub>f</sub> 0.60 (yellow orange with spray reagent) and R<sub>f</sub> 0.45 (brown with spray reagent).

The fraction R<sub>f</sub> 0.60 yielded 2-(2-hydroxy-2-methylpropyl)-3,5-dimethoxyphenol (54) (7 mg, 14%). <sup>1</sup>H NMR δ (600 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) 8.48 (1H, s, OH-1), 6.18 (1H, d, J = 2.4 Hz, H-3), 6.08 (1H, d, J = 2.4 Hz, H-5), 3.77 (3H, s, OMe), 3.75 (3H, s, OMe), 2.82 (2H, s, CH<sub>2</sub>), 1.94 (1H, s, OH-2'), 1.28 (6H, s, 2x-CH<sub>3</sub>). (**Plate 9a**)

<sup>13</sup>C NMR δ (150 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) 159.84 (C-2), 159.29 (C-4), 157.54 (C-6), 106.05 (C-1), 94.56 (C-3), 91.17 (C-5), 75.25 (C-2'), 55.53, 55.25 (2x-OCH<sub>3</sub>), 35.74 (C-1'), 29.26 (C-3'). (**Plate 9b**)

Found (ES) [M+H]<sup>+</sup>, 227.1287, (C<sub>12</sub>H<sub>18</sub>O<sub>4</sub> + H<sup>+</sup>) requires m/z 227.1283.

The fraction R<sub>f</sub> 0.60 was acetylated and yielded 2-(2-hydroxy-2-methylpropyl)-3,5-dimethoxyphenyl acetate (55). <sup>1</sup>H NMR δ (600 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) 6.38 (1H, d, J = 2.4 Hz, H-3), 6.27 (1H, d, J = 2.4 Hz, H-5), 3.81 (3H, s, OMe), 3.78 (3H, s, OMe), 2.72 (2H, s, CH<sub>2</sub>), 2.53 (1H, s, OH-2'), 2.29 (3H, s, 1xOAc), 1.20 (6H, s, 2xCH<sub>3</sub>). (**Plate 10a**)

<sup>13</sup>C NMR δ (150 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) 169.34 (C=O), 159.20 (C-3/C-5), 150.78 (C-1), 111.68 (C-2), 99.69 (C-4), 96.75 (C-6), 72.12 (C-2'), 55.50 (2xOCH<sub>3</sub>), 36.89 (C-1'), 29.51 (2xCH<sub>3</sub>), 21.11 (COCH<sub>3</sub>). (**Plate 10b**)

Found (ES) [M+H]<sup>+</sup>, 269.1394, (C<sub>14</sub>H<sub>20</sub>O<sub>5</sub> + H<sup>+</sup>) requires m/z 269.1389.

IR (KBr): ν<sub>max</sub> = 1261.24, 1094.14, 1019.94, 799.90 cm<sup>-1</sup>.

The fraction R<sub>f</sub> 0.45 yielded 3,4-dimethoxybenzaldehyde (53) (4 mg, 8%). <sup>1</sup>H NMR δ (600 MHz, CDCl<sub>3</sub>, Me<sub>4</sub>Si) 9.86 (1H, s, 1xCHO), 7.47 (1H, dd, J = 2.0, 8.3 Hz, H-6), 7.42

(1H, d, J = 2.0 Hz, H-2), 6.99 (1H, d, J = 8.3 Hz, H-5), 3.98 (3H, s, 1xOMe), 3.95 (3H, s, 1xOMe). Identical to a commercial sample (Aldrich 44,375-8). (**Plate 11**)

Found (ES)  $[M+H]^+$ , 167.0711, ( $C_9H_{10}O_3 + H^+$ ) requires  $m/z$  167.0708.

### 5.3.2 Synthesis of (+)-3-O-Tosyl-3',4',5,7-Tetra-O-methylcatechin (57)

(+)-3',4',5,7-tetra-*O*-methylcatechin (**39**) (50 mg; 0.14 mmol) was tosylated according to the procedure on page 69. The product, (+)-3-*O*-tosyl-3',4',5,7-tetra-*O*-methylcatechin (**57**) was radiated under  $N_2$  at 250 nm in acetone for 14 hours. Column chromatography (silica gel, T:A 7:3) gave two fraction;  $R_f$  0.55 (yellow orange with spray reagent) and  $R_f$  0.40 (brown with spray reagent).

$R_f$  0.55 yielded (+)-3',4',5,7-tetra-*O*-methylcatechin (39) (11 mg, 90%).  $^1H$  NMR  $\delta$  (600 MHz,  $CDCl_3$ ,  $Me_4Si$ ) 7.00 (1H, dd, J = 8.3, 2.0 Hz, H-6'), 6.98 (1H, d, J = 2.0 Hz, H-2'), 6.91 (1H, d, J = 8.3 Hz, H-5'), 6.14 (1H, d, J = 2.3 Hz, H-6), 6.11 (1H, d, J = 2.3 Hz, H-8), 4.67 (1H, d, J = 8.3 Hz, H-2), 4.09-4.04 (1H, m, H-3), 3.90 (6H, s, 2xOMe), 3.81 (3H, s, 1xOMe), 3.76 (3H, s, 1xOMe), 3.08 (1H, dd, J = 5.7, 16.3 Hz, H-4 $\alpha$ ), 2.60 (1H, dd, J = 9.1, 16.3 Hz, H-4 $\beta$ ). (**Plate 12**)

Found (ES)  $[M+H]^+$ , 347.1499, ( $C_{19}H_{22}O_6 + H^+$ ) requires  $m/z$  347.1494.

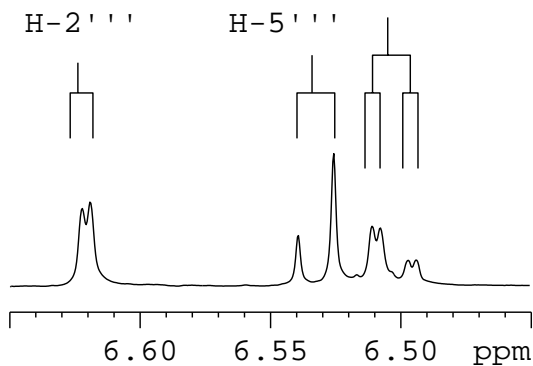
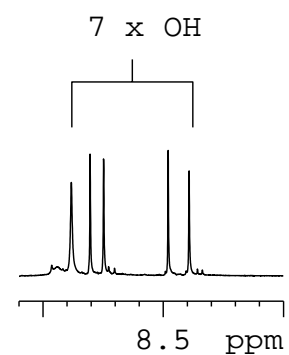
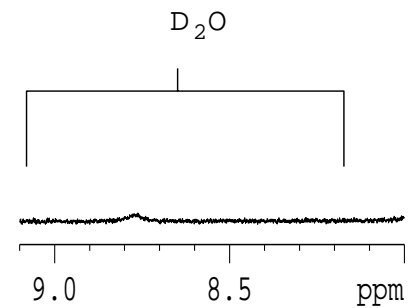
$R_f$  0.40 yielded unreacted starting material, (2S,3R)-2-(3',4'-dimethoxyphenyl)-5,7-dimethoxychroman-3-ol (57) [(+)-3-*O*-tosyl-tetra-*O*-methylcatechin] (35 mg).  $^1H$  NMR  $\delta$  (600 MHz,  $CDCl_3$ ,  $Me_4Si$ ) 7.29 (2H, d, J = 8.3 Hz, H-2'', H-6''), 7.03 (2H, d, J = 8.0 Hz, H-3'', H-5''), 6.71 (1H, dd, J = 2.0 Hz, 8.3 Hz, H-6'), 6.64 (1H, d, J = 8.3 Hz, H-5'), 6.46 (1H, d, J = 2.0 Hz, H-2'), 6.02 (2H, k, H-6, H-8), 4.76 (1H, d, J = 8.1 Hz, H-2), 4.64-4.59 (1H, m, H-3), 3.82 (3H, s, 1xOMe), 3.71 (3H, s, 1xOMe), 3.67 (3H, s, 1xOMe), 3.62 (3H, s, 1xOMe), 3.21 (1H, dd, J = 5.8, 16.7 Hz, H-4 $\alpha$ ), 2.83 (1H, dd, J = 8.3, 16.7 Hz, H-4 $\beta$ ). (**Plate 13**)

Found (ES)  $[M+H]^+$ , 501.1589, ( $C_{26}H_{28}O_8S + H^+$ ) requires  $m/z$  501.1583.

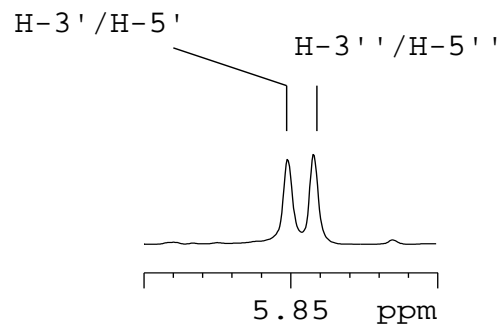
CD:  $[\theta]_{423.2}$   $1.279 \times 10^5$ ,  $[\theta]_{360.8}$   $1.101 \times 10^5$ ,  $[\theta]_{293.2}$   $1.775 \times 10^5$ ,  $[\theta]_{274.6}$   $-8.739 \times 10^3$ ,  $[\theta]_{260.0}$   $7.208 \times 10^4$ ,  $[\theta]_{249.6}$   $9.070 \times 10^4$ ,  $[\theta]_{234.0}$   $-2.912 \times 10^5$ . (**CD-plate 11**)

Plate 1a (DMSO, 298 K)

Disappearance of OH groups in D



Sharp singlet at 120 C



Two humps seen at 25 C

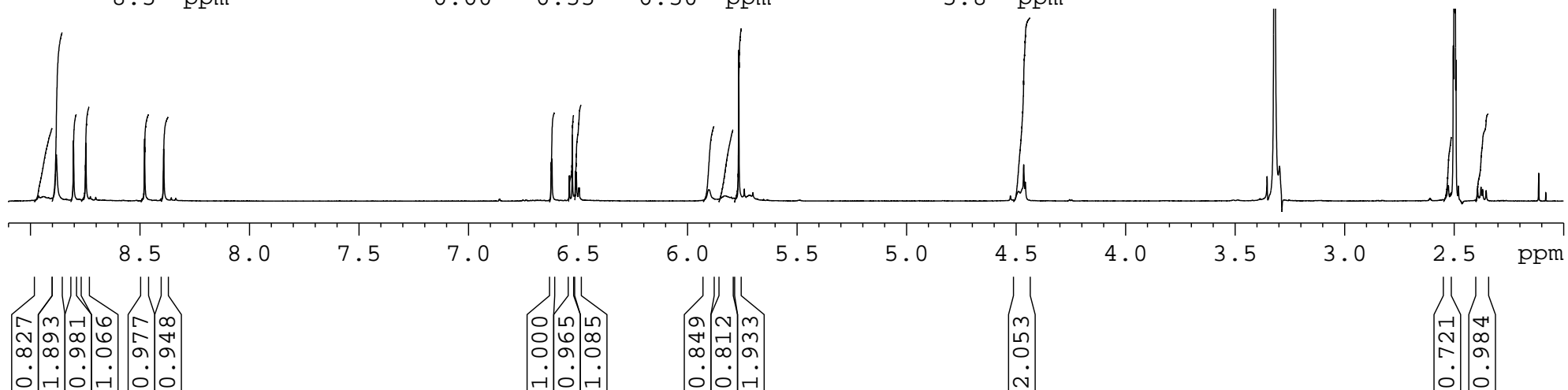
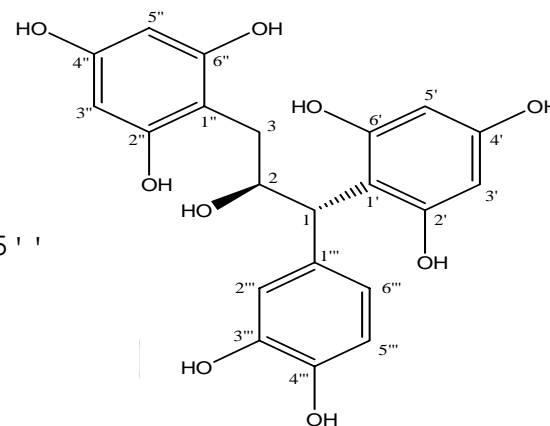
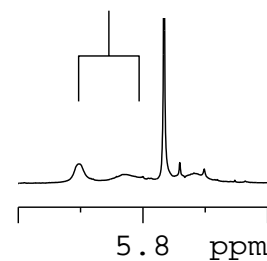
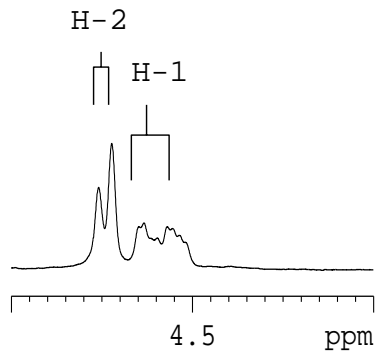
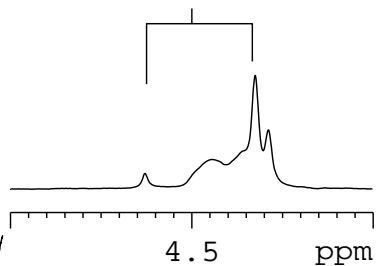


Plate 1b (DMSO, 298 K)

H-2 and H-1 no longer overlap at 120 C

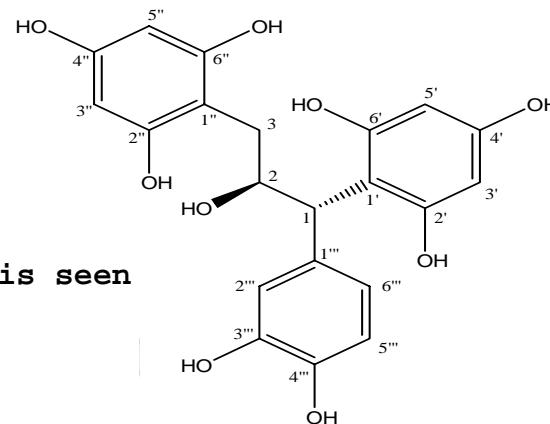
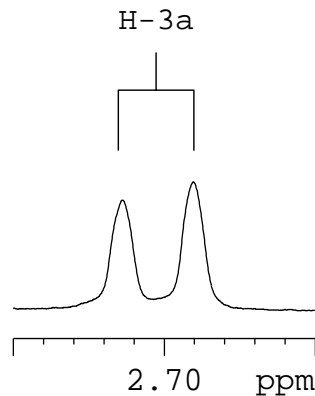
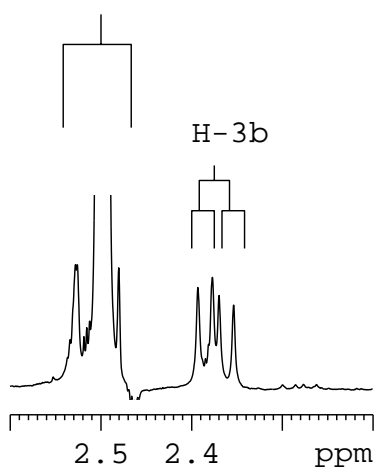


H-2 and H-1 overlap



At 90 C a broad doublet is seen

H-3a overlaps with DMSO



(32)

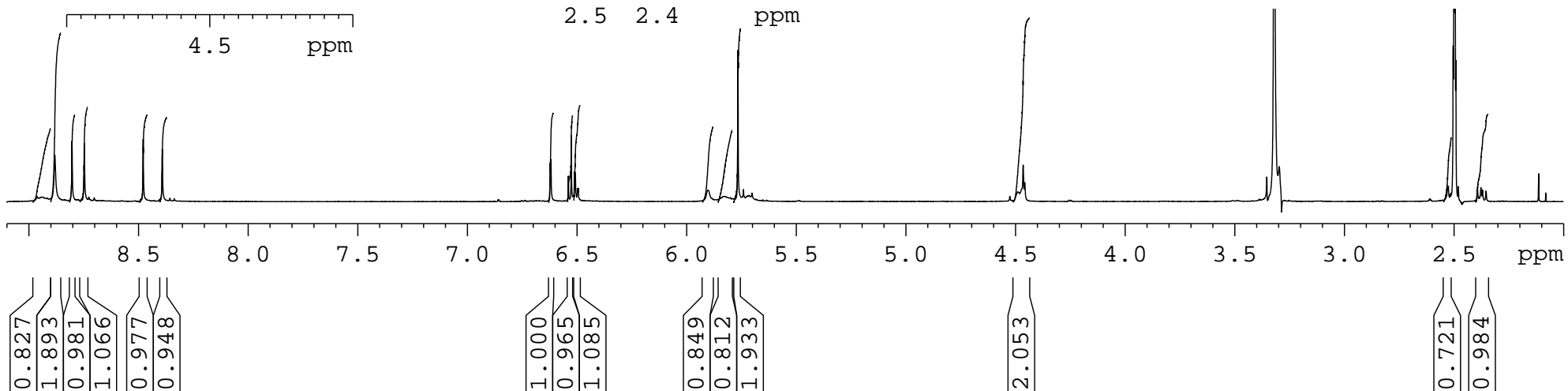
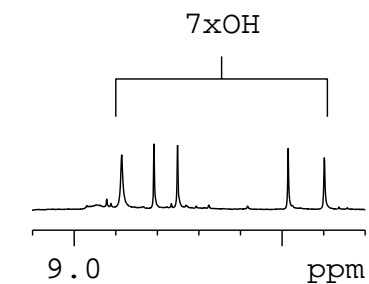
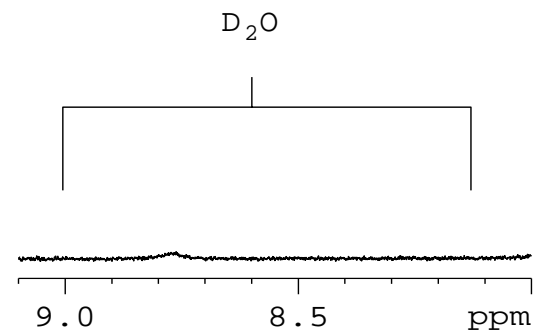
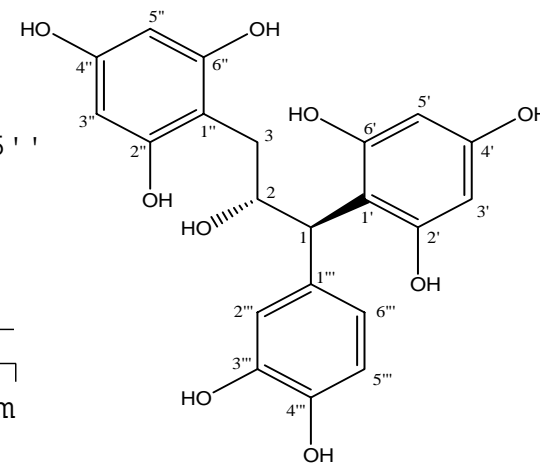
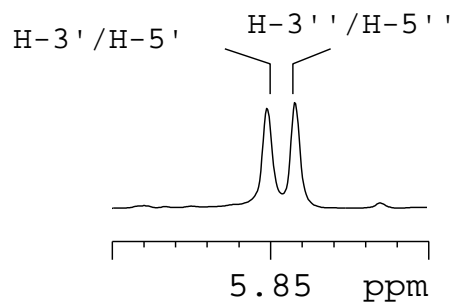


Plate 2a (DMSO, 298 K)

Disappearance of OH groups in



Sharp singlet at 120 C



Two humps seen at 25 C

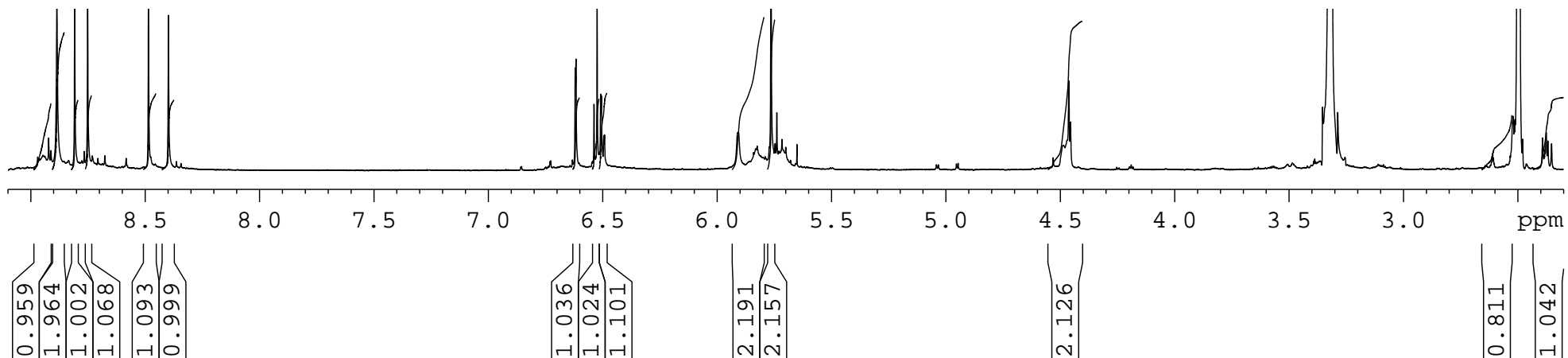
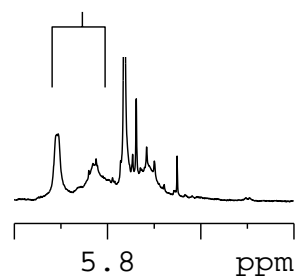
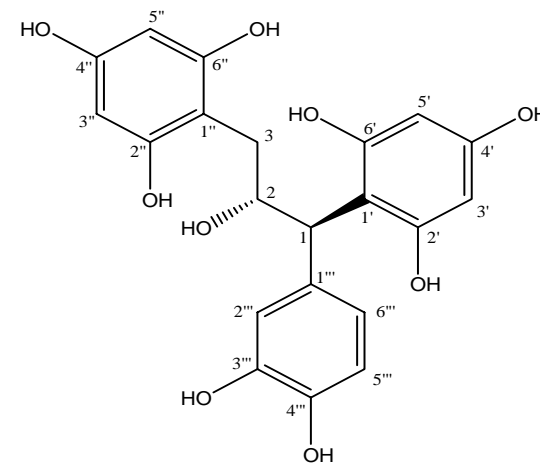
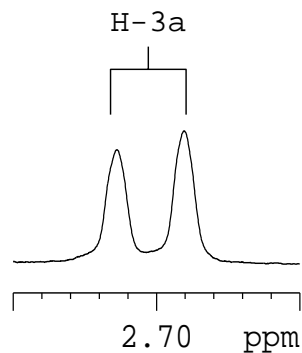
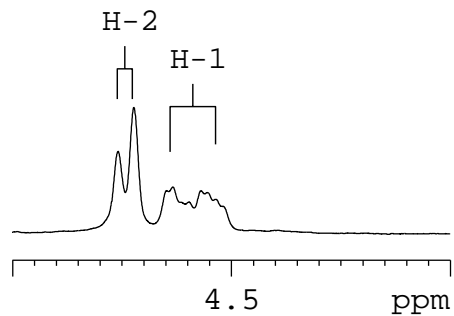


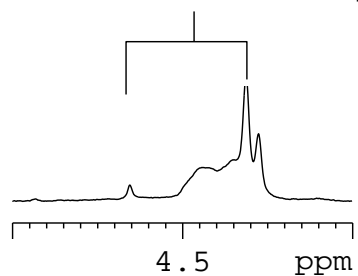
Plate 2b (DMSO, 298 K)

At 90 C a broad doublet is seen

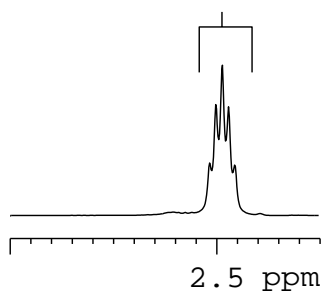
H-2 and H-1 no longer overlap at 120 C



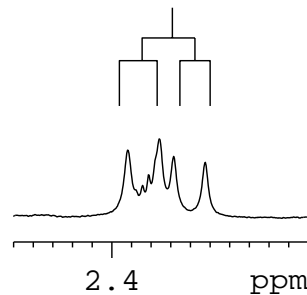
H-2 and H-1 overlap



H-3a overlaps with DMSO



H-3b



(33)

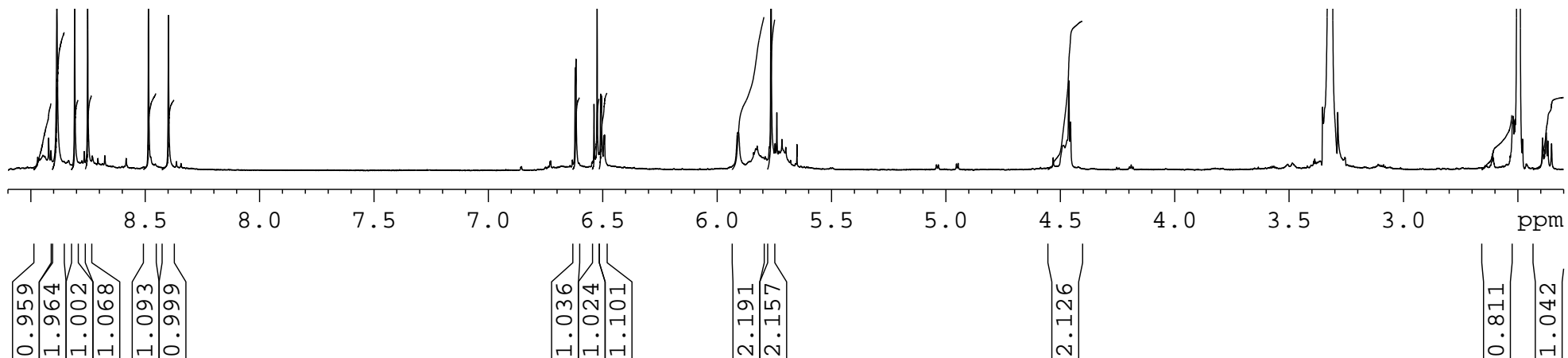


Plate 3 (CDCl<sub>3</sub>, 298 K)

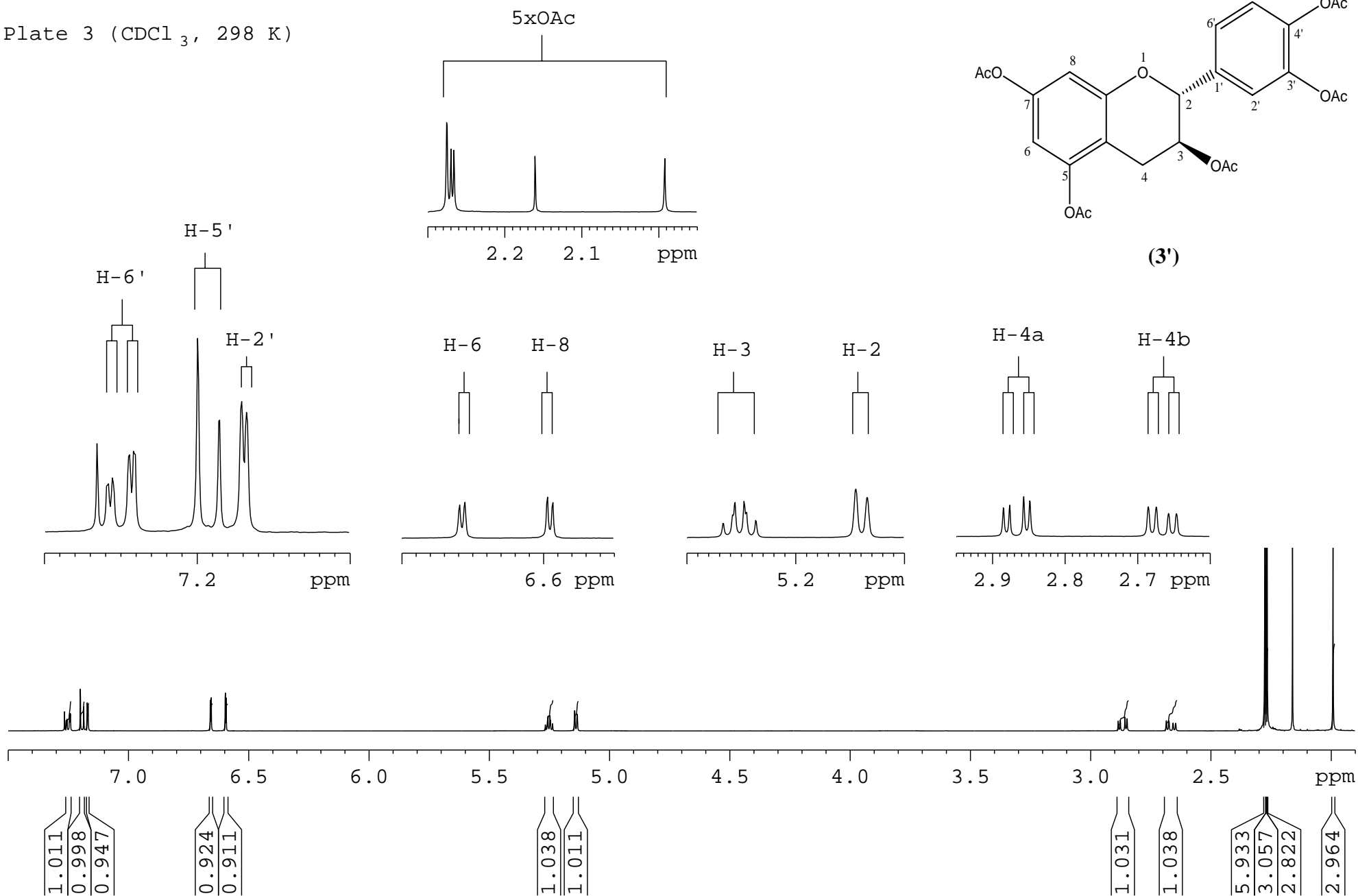
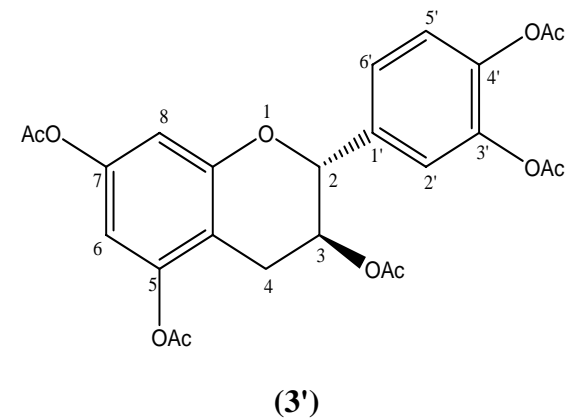
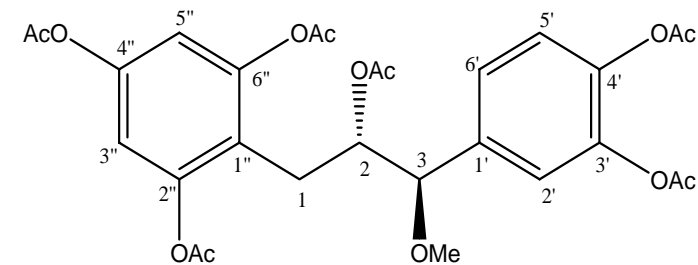


Plate 4a (CDCl<sub>3</sub>, 298 K)



(36)

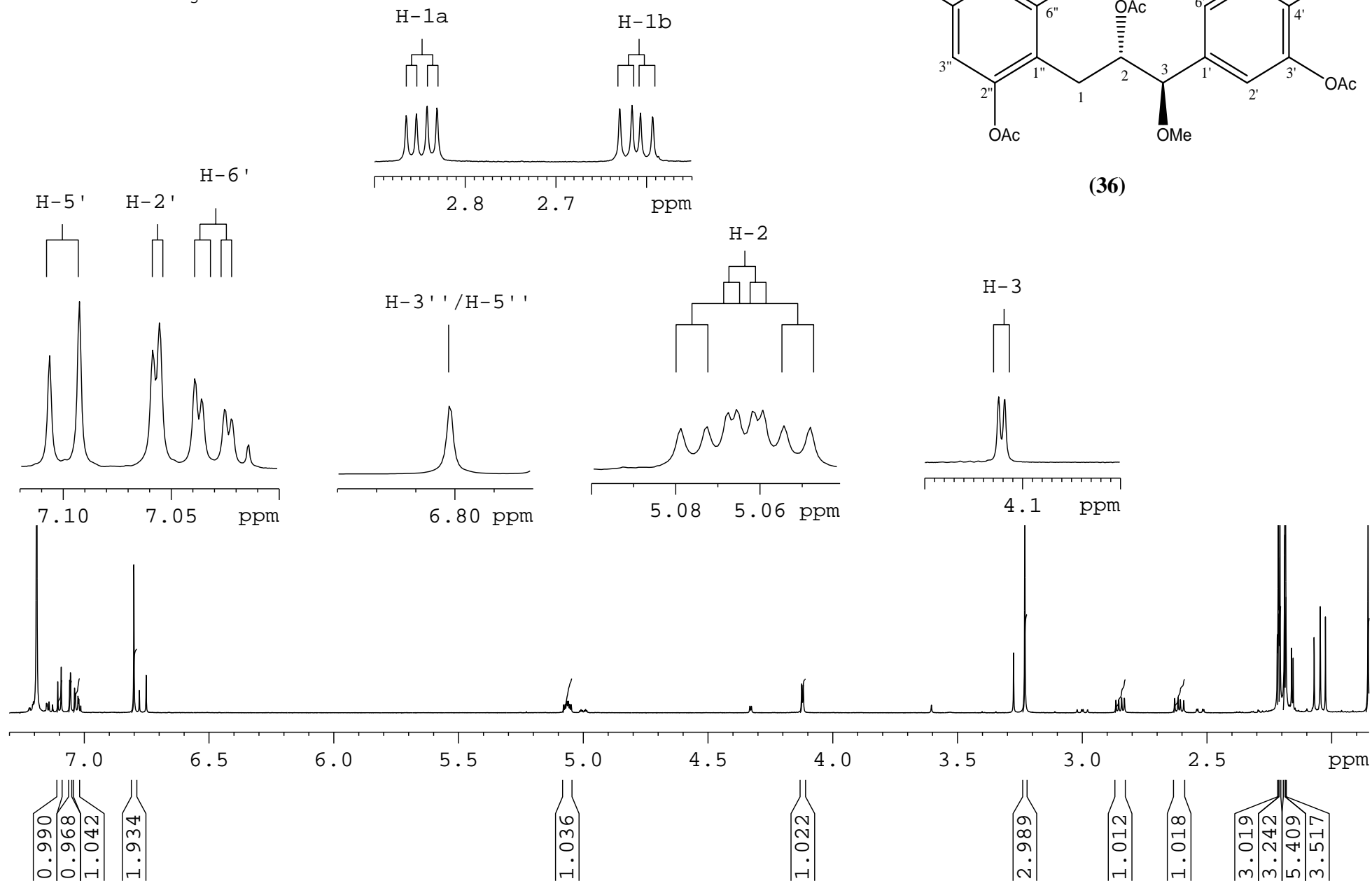
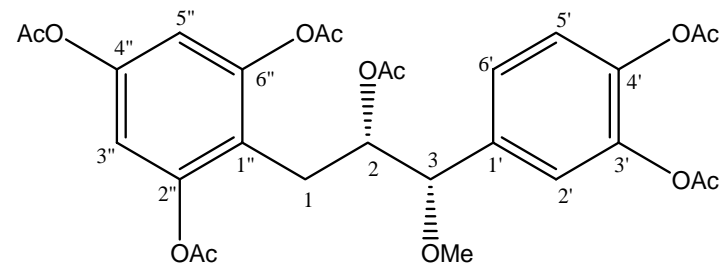




Plate 4b (CDCl<sub>3</sub>, 298 K)



(37)

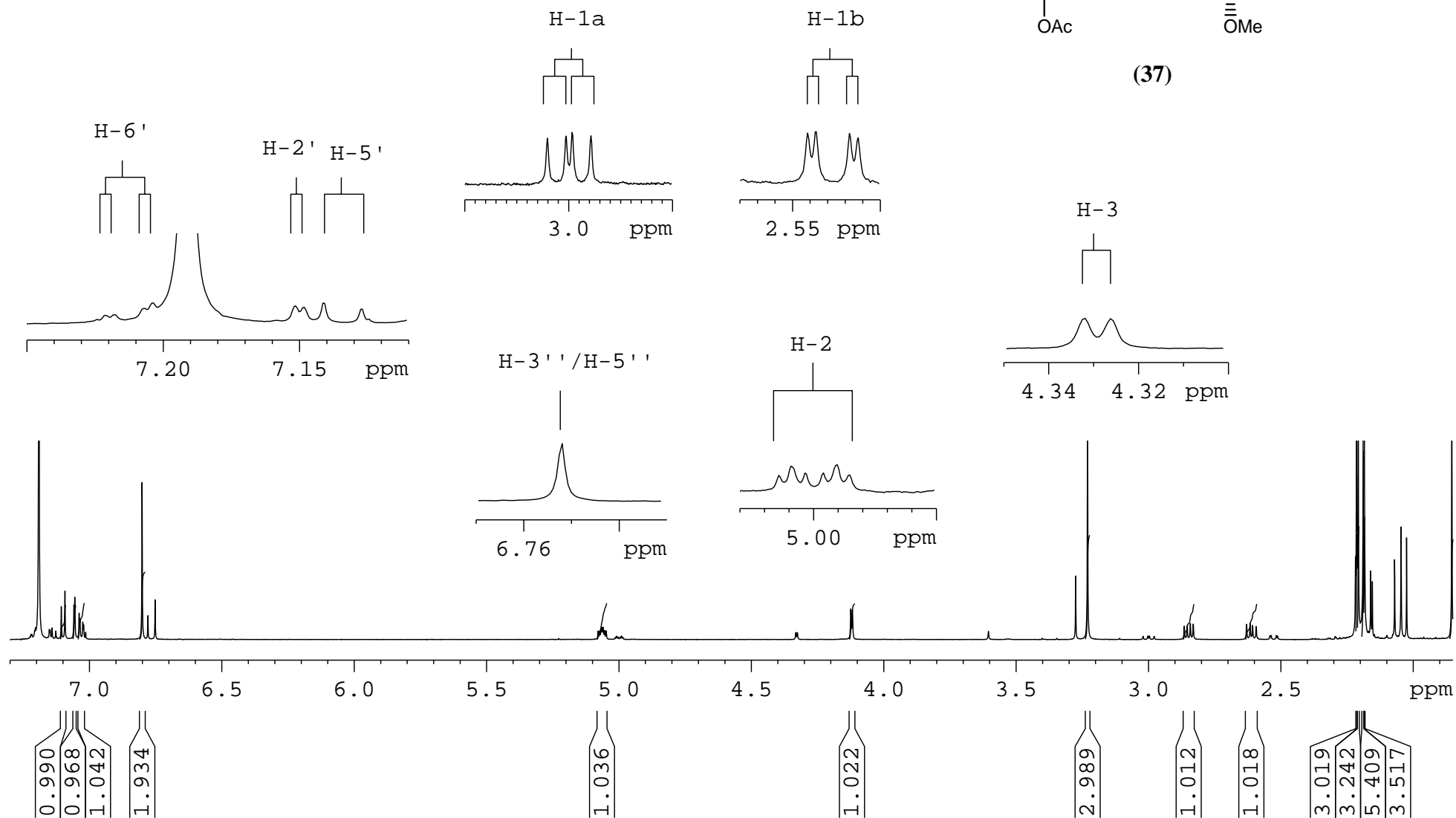


Plate 5a (CDCl<sub>3</sub>, 298 K)

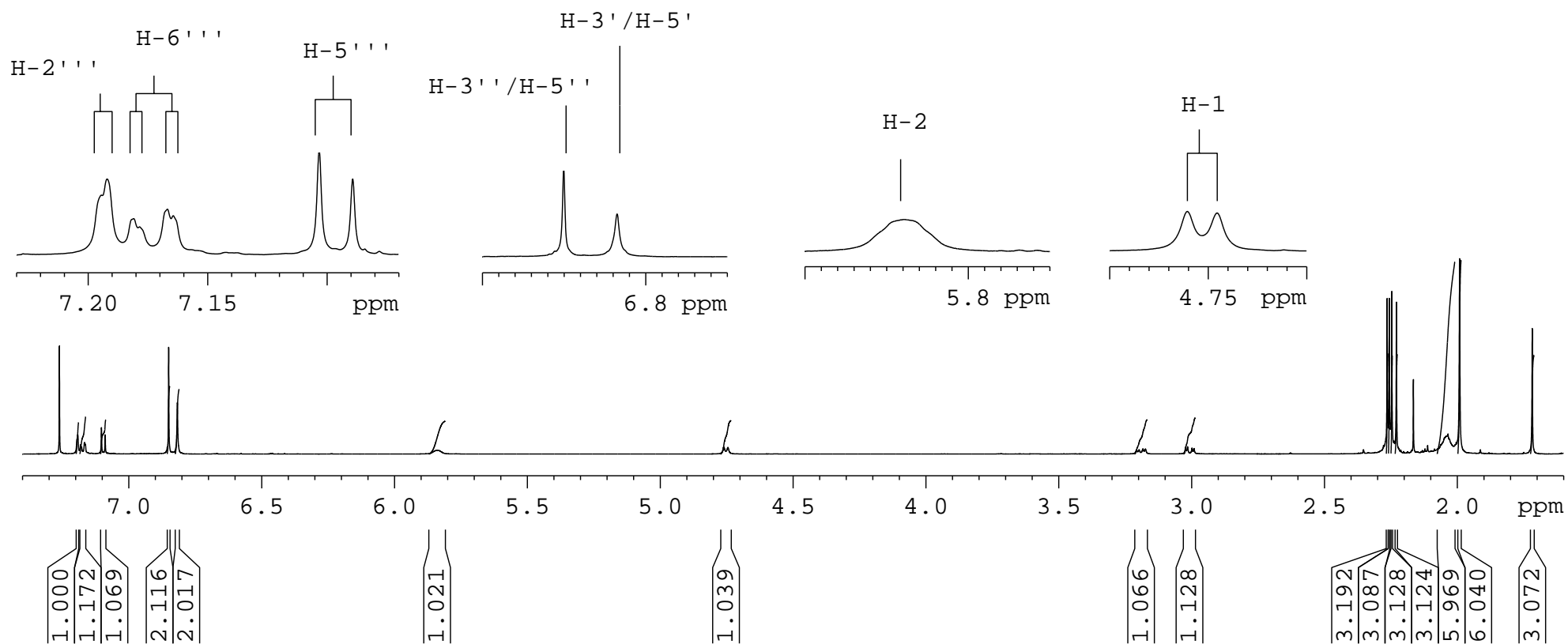
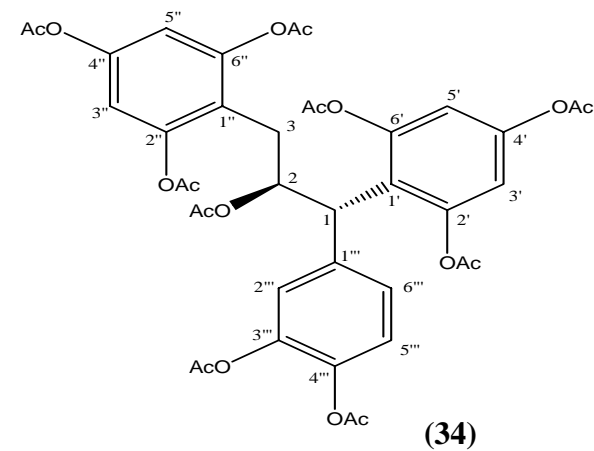
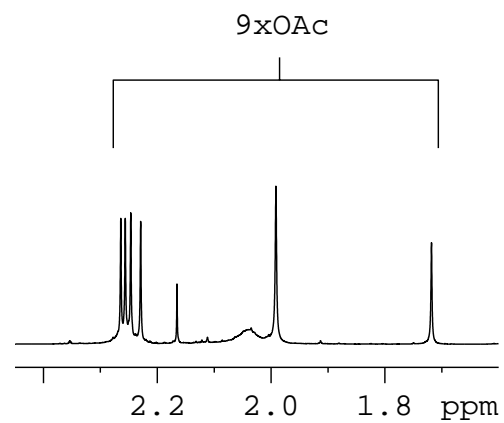
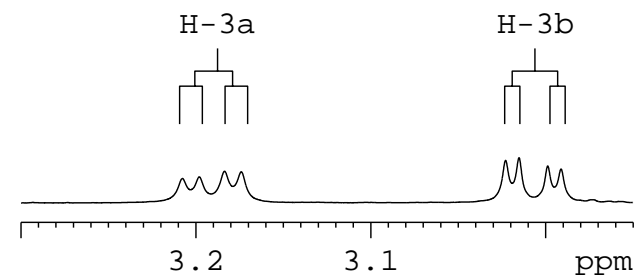


Plate 5b (CDCl<sub>3</sub>, 298 K)

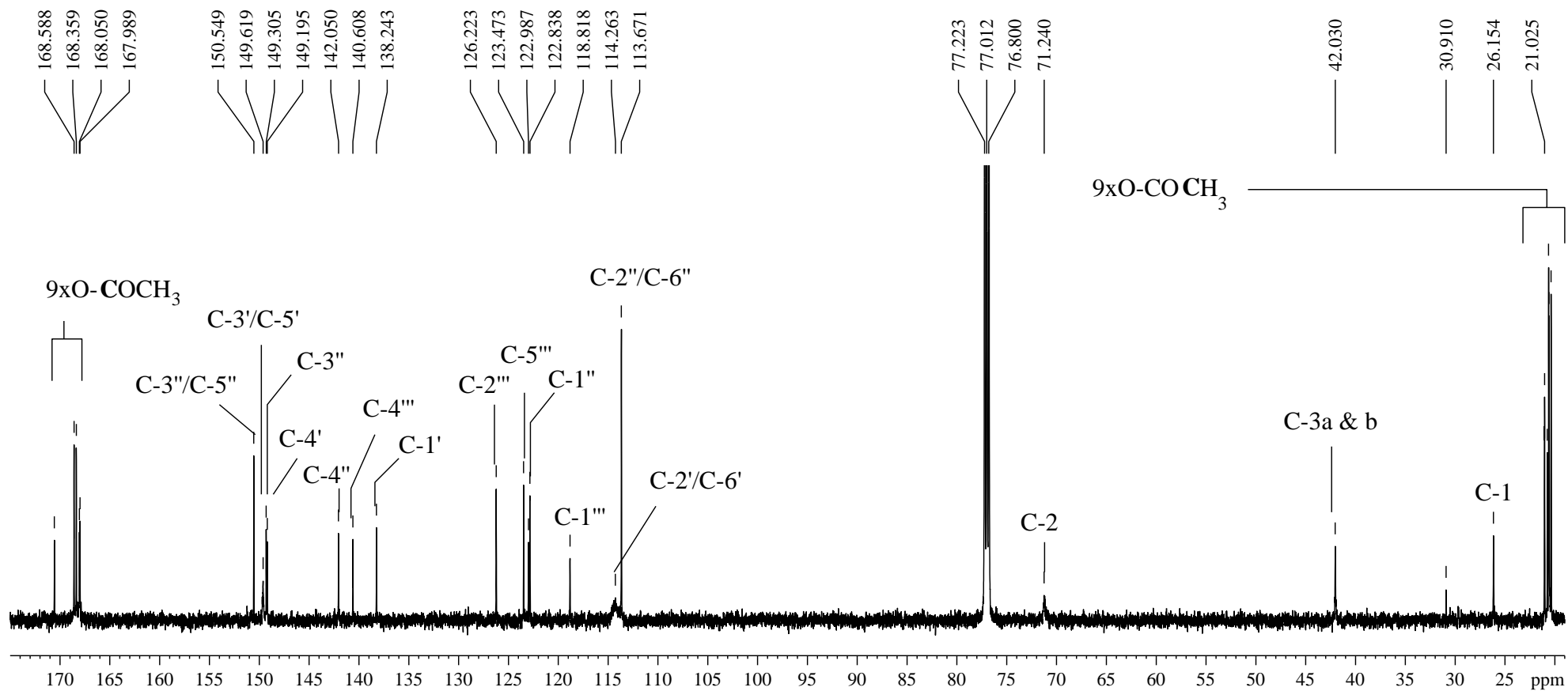
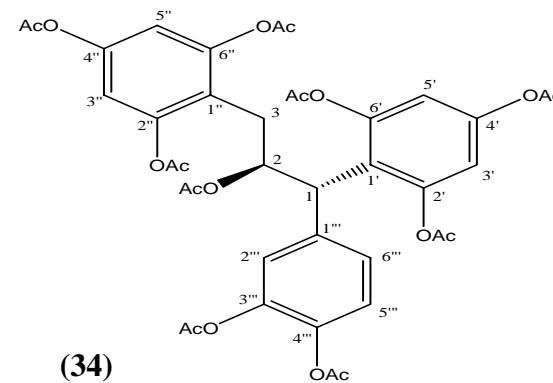
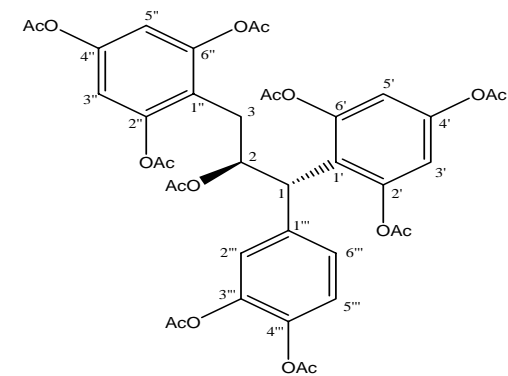
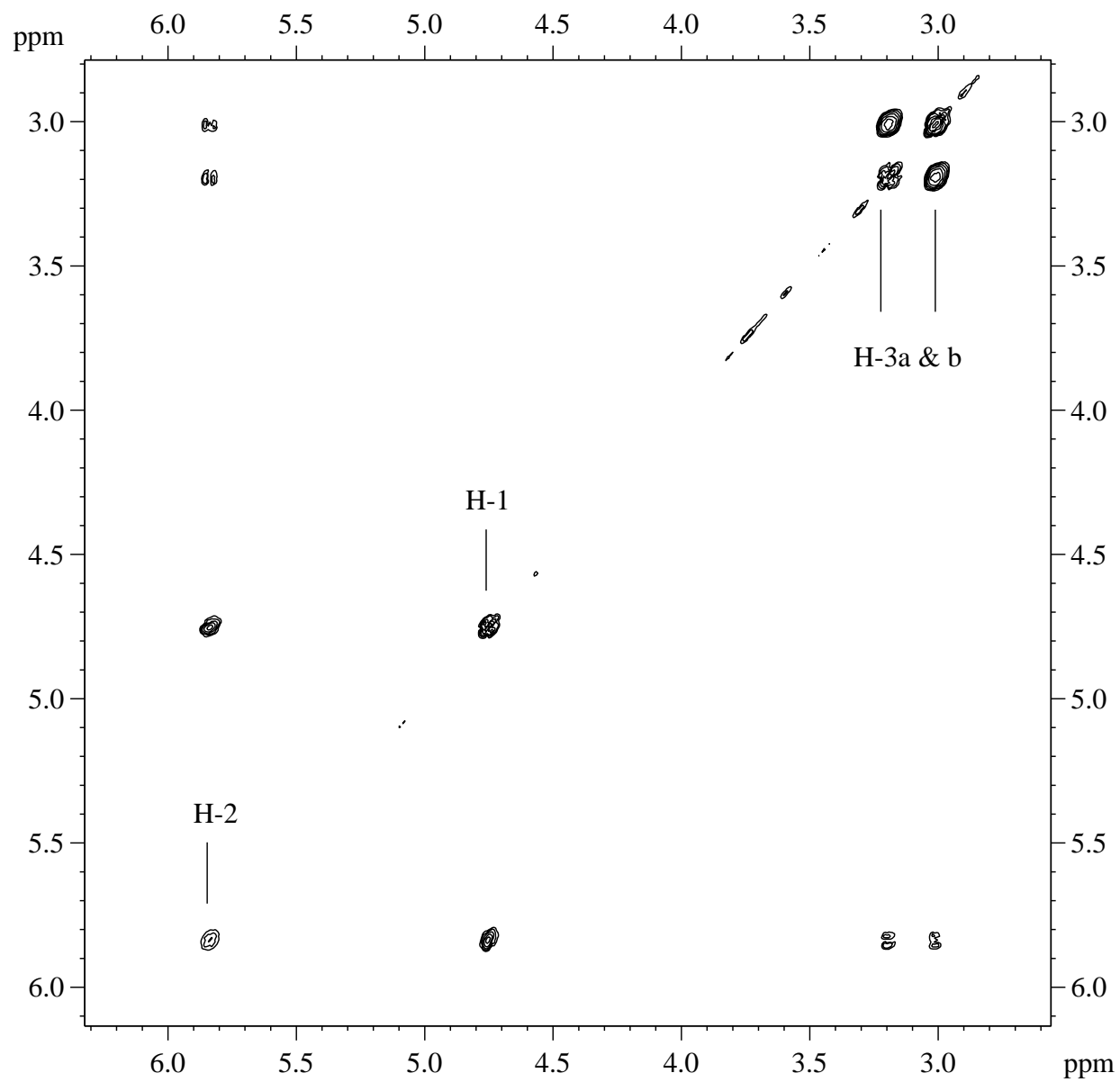
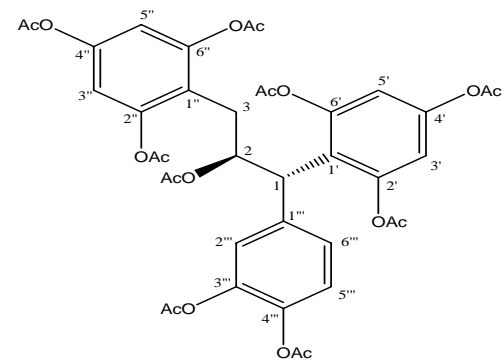
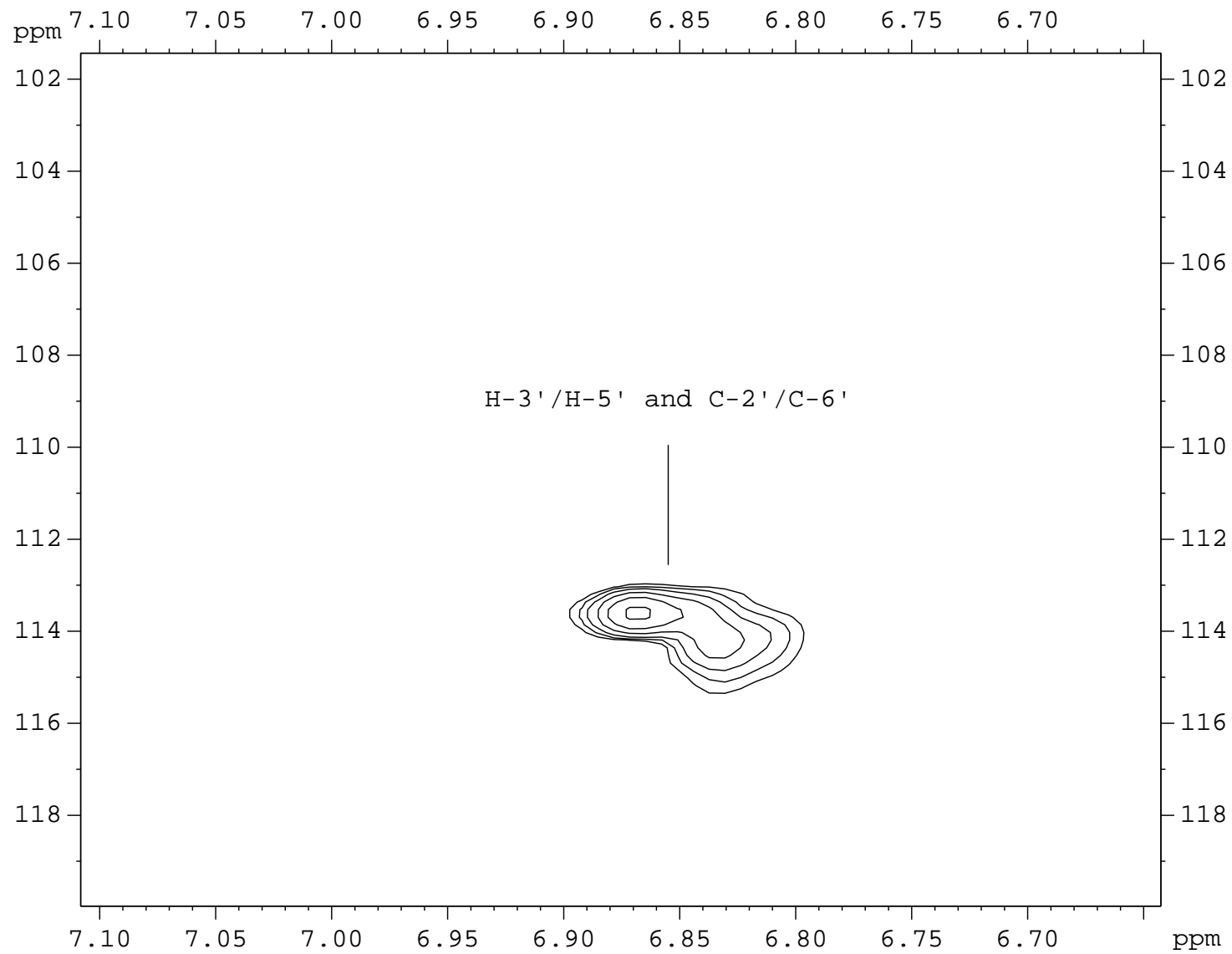


Plate 5c (CDCl<sub>3</sub>, 298 K)



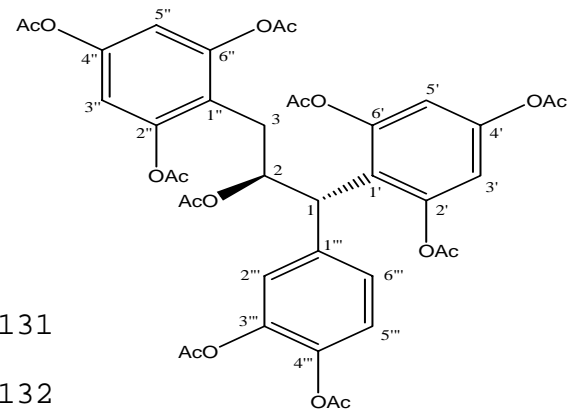
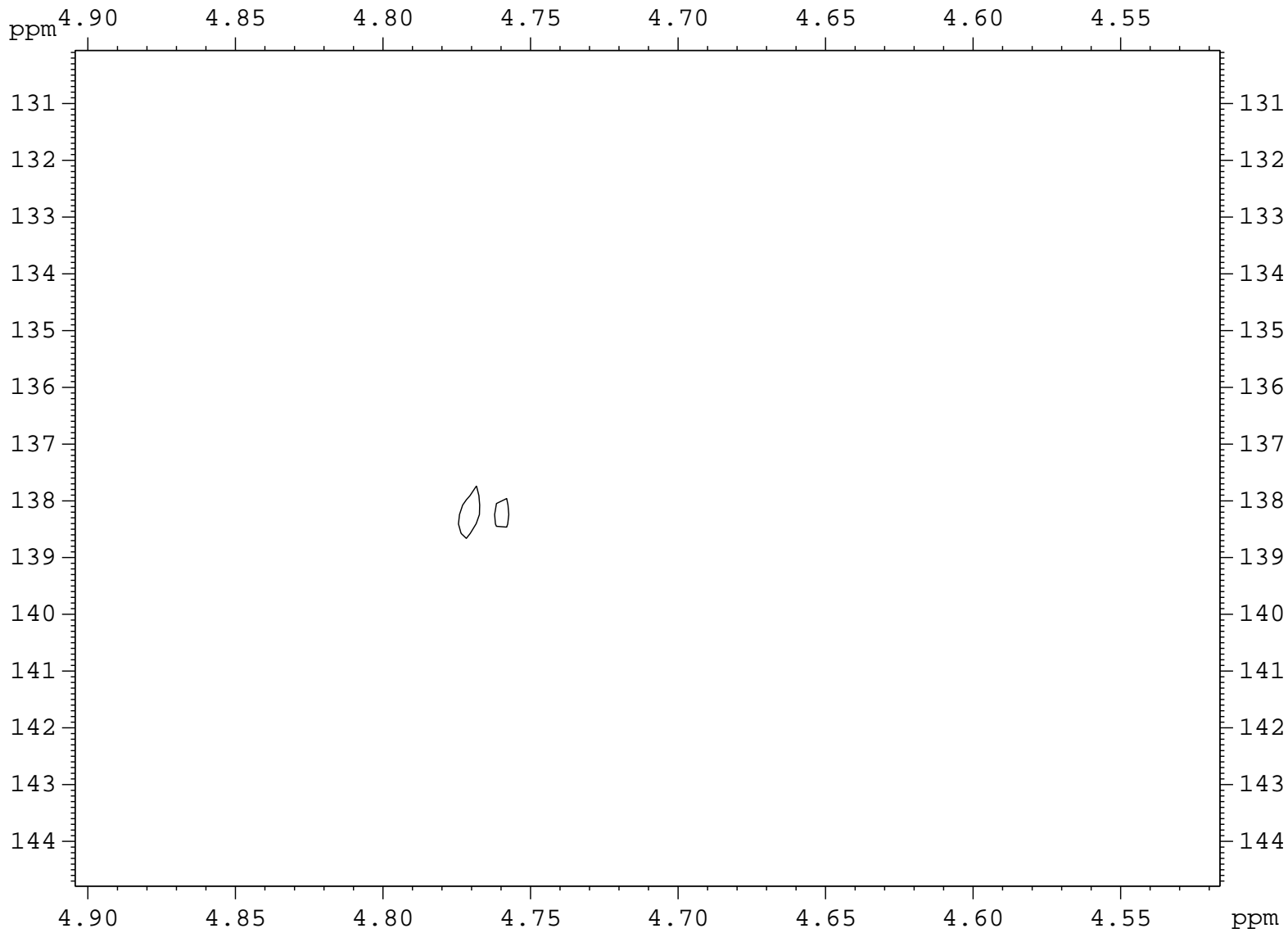
(34)

Plate 5d (CDCl<sub>3</sub>, 298 K)



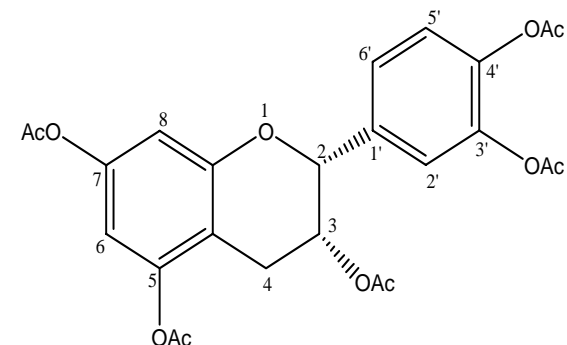
**(34)**

Plate 5e (CDCl<sub>3</sub>, 298 K)

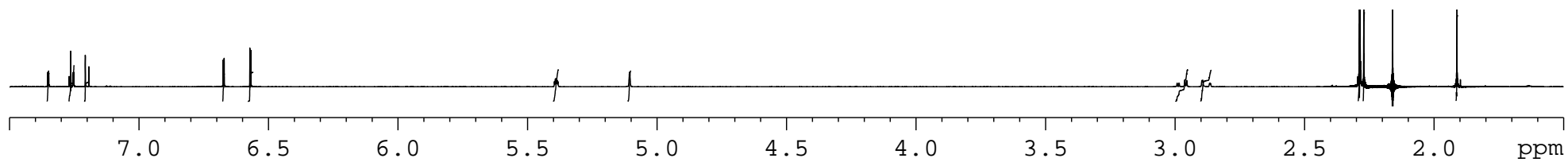
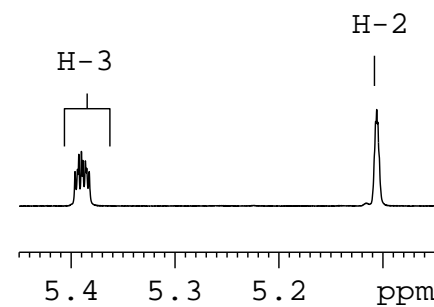
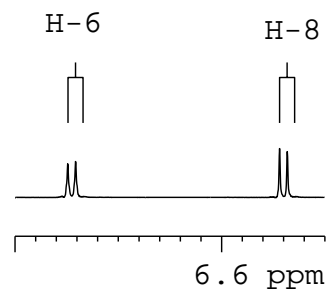
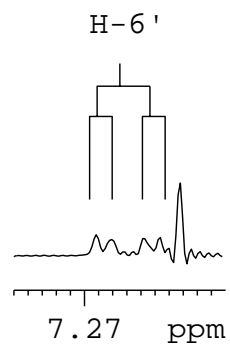
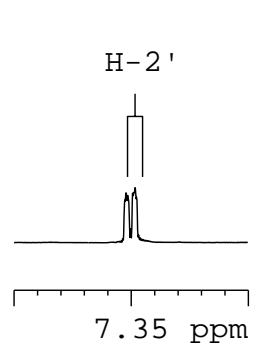
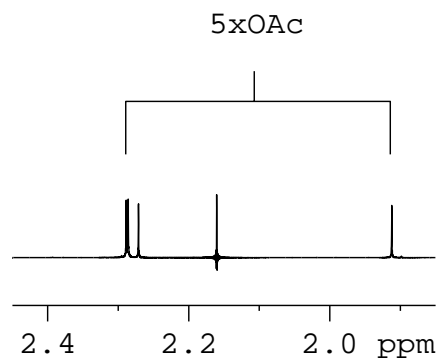
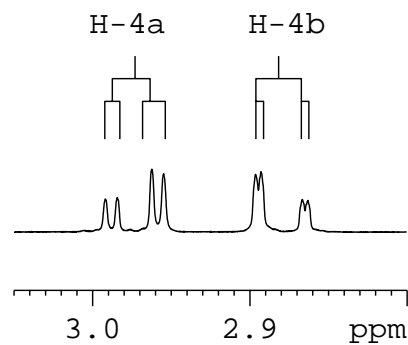


**(34)**

Plate 6 (CDCl<sub>3</sub>, 298 K)



(4')



0.952  
1.202  
0.996

0.929  
0.964

1.051

1.018

1.048  
1.034

2.930  
5.801  
2.974

2.856

Plate 7a (Acetone-d<sub>6</sub>, 298 K)

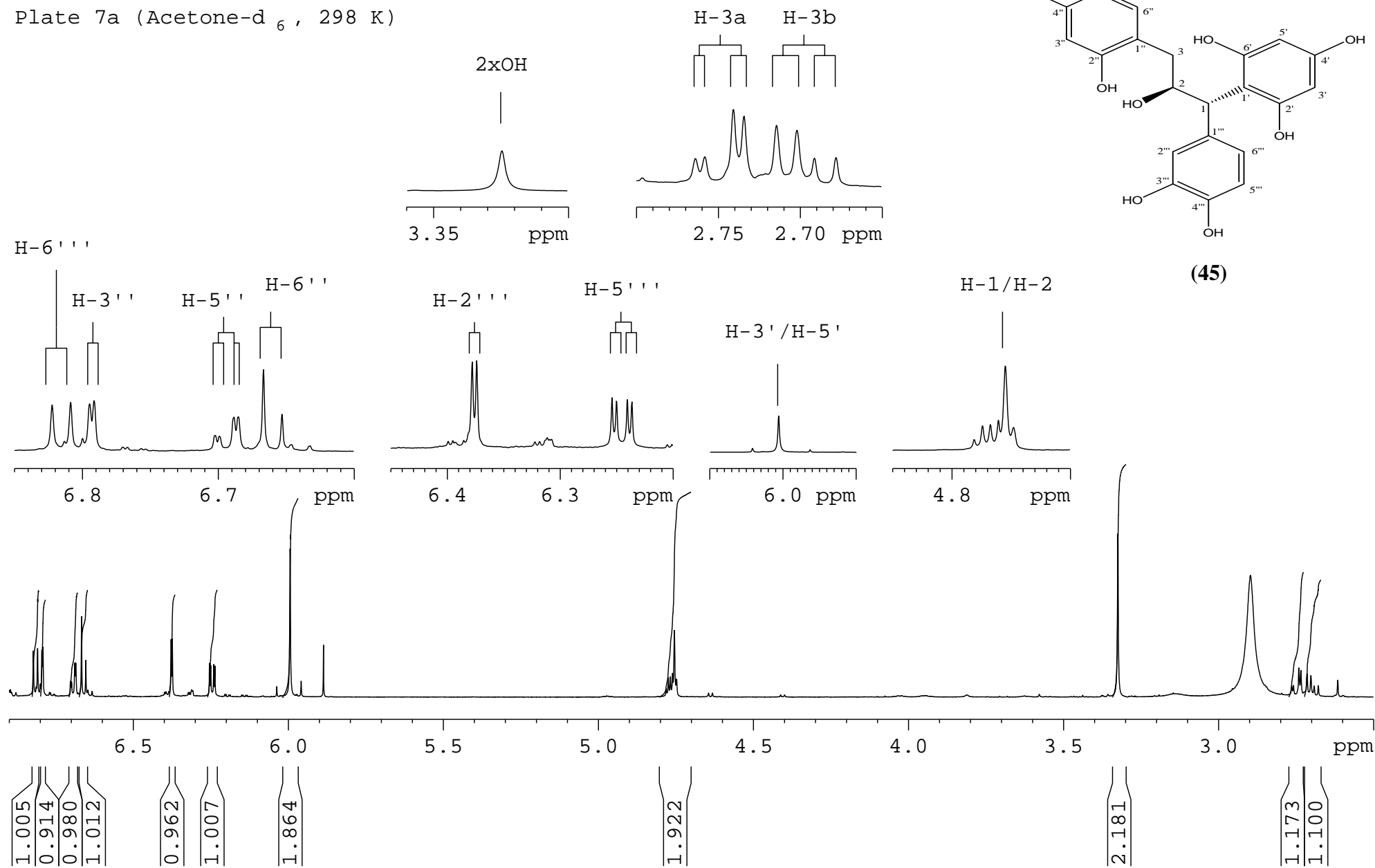




Plate 7b (Acetone-d<sub>6</sub>, 298 K)

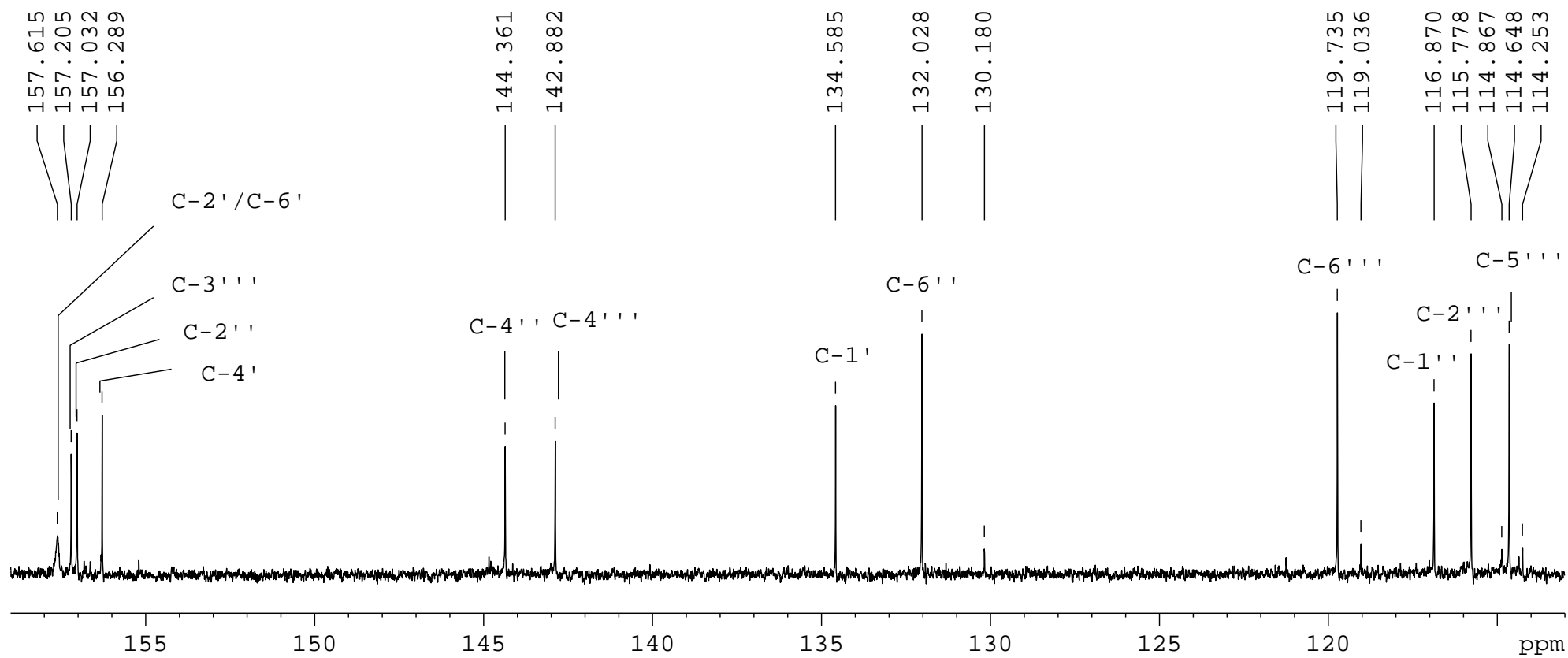
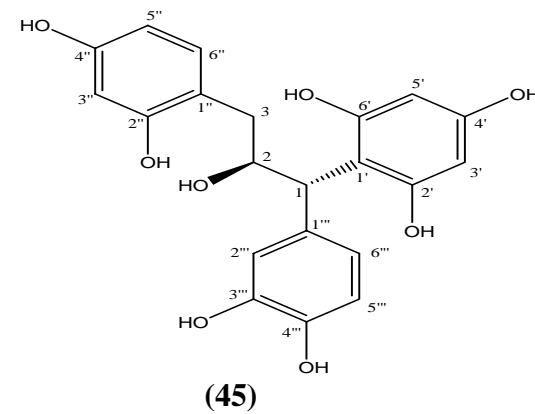


Plate 7c (Acetone-d<sub>6</sub>, 298 K)

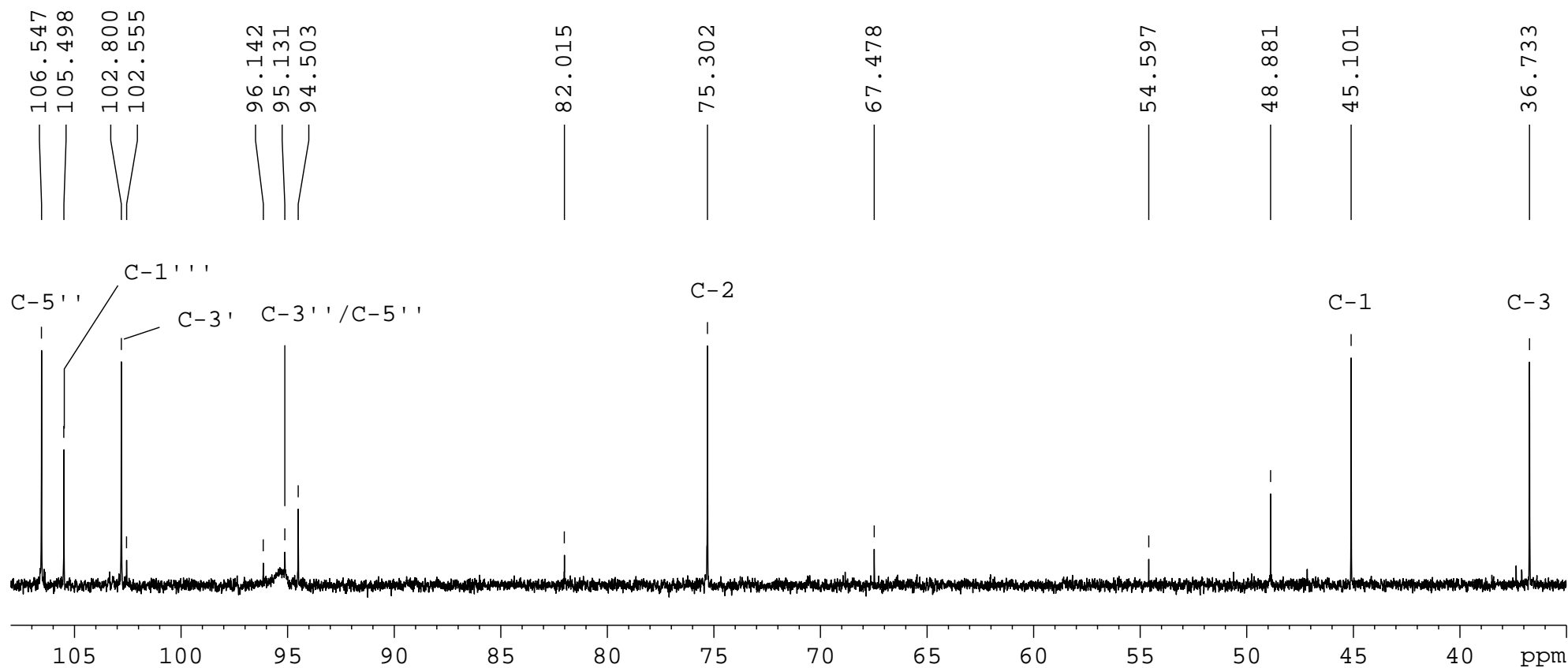
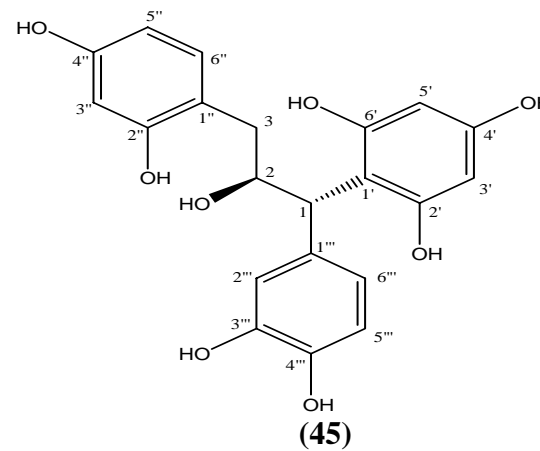


Plate 8a (CDCl<sub>3</sub>, 298 K)

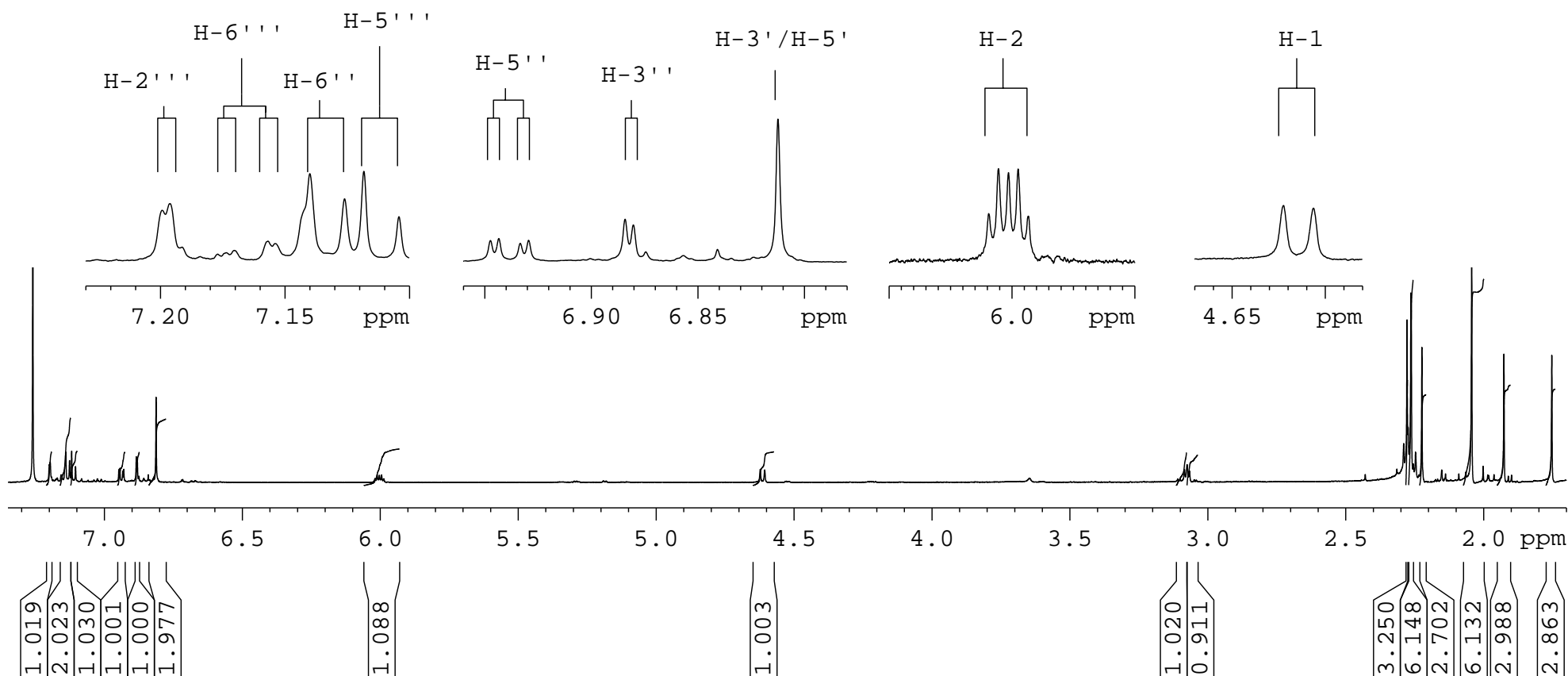
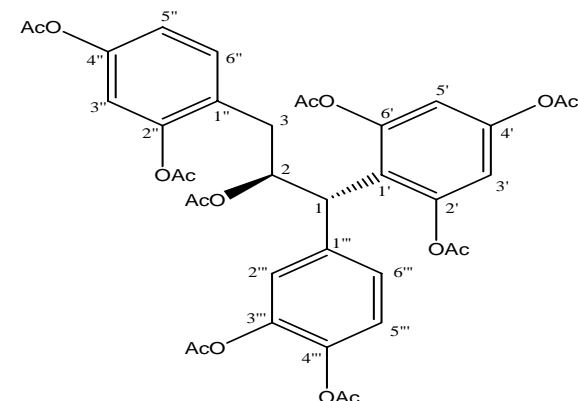
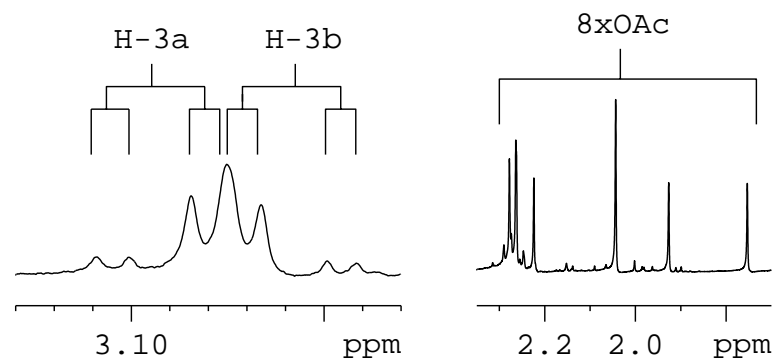


Plate 8b (CDCl<sub>3</sub>, 298 K)

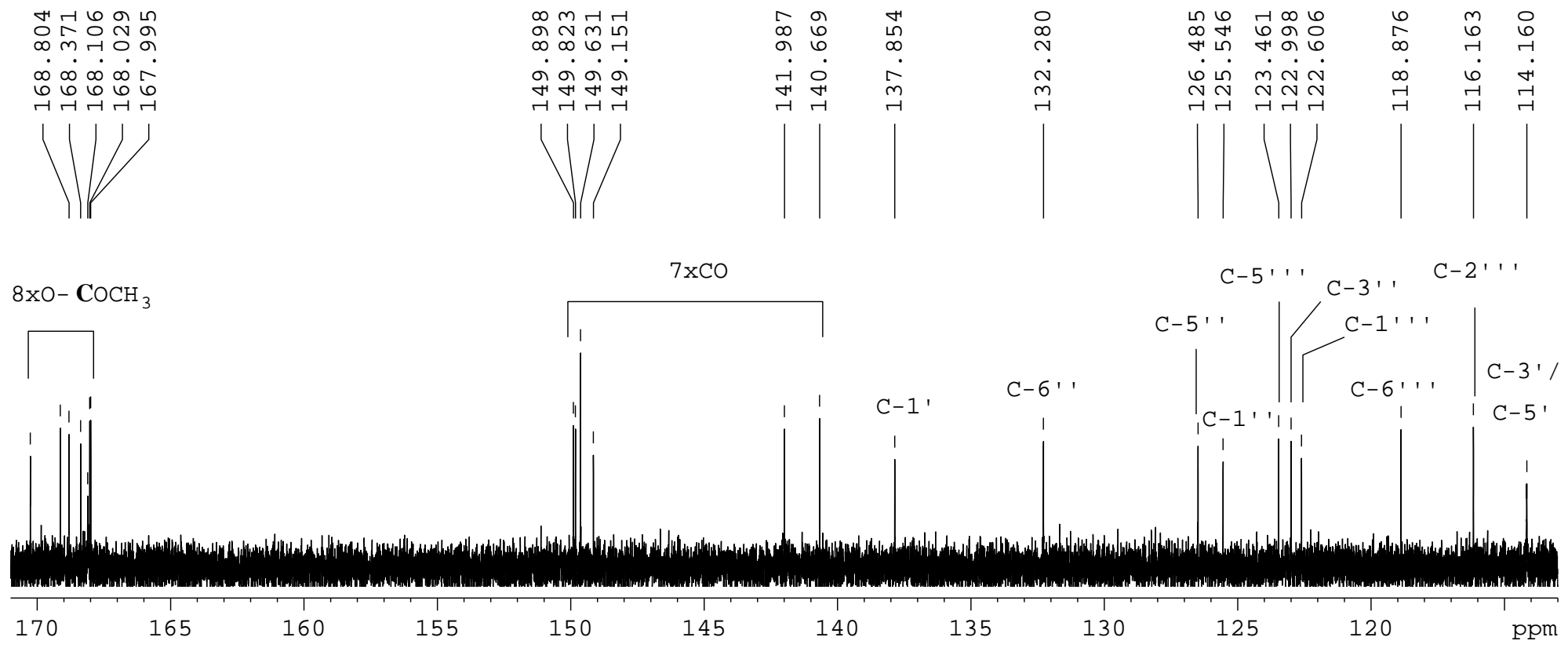
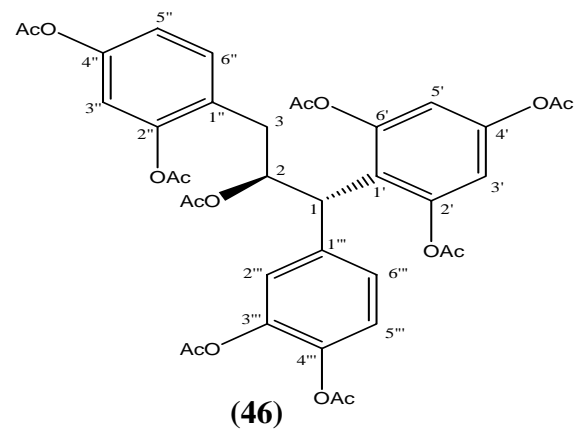
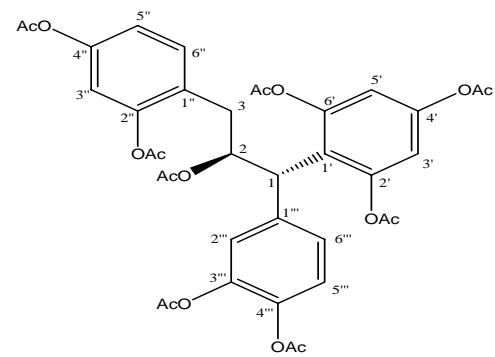


Plate 8c (CDCl<sub>3</sub>, 298 K)



(46)

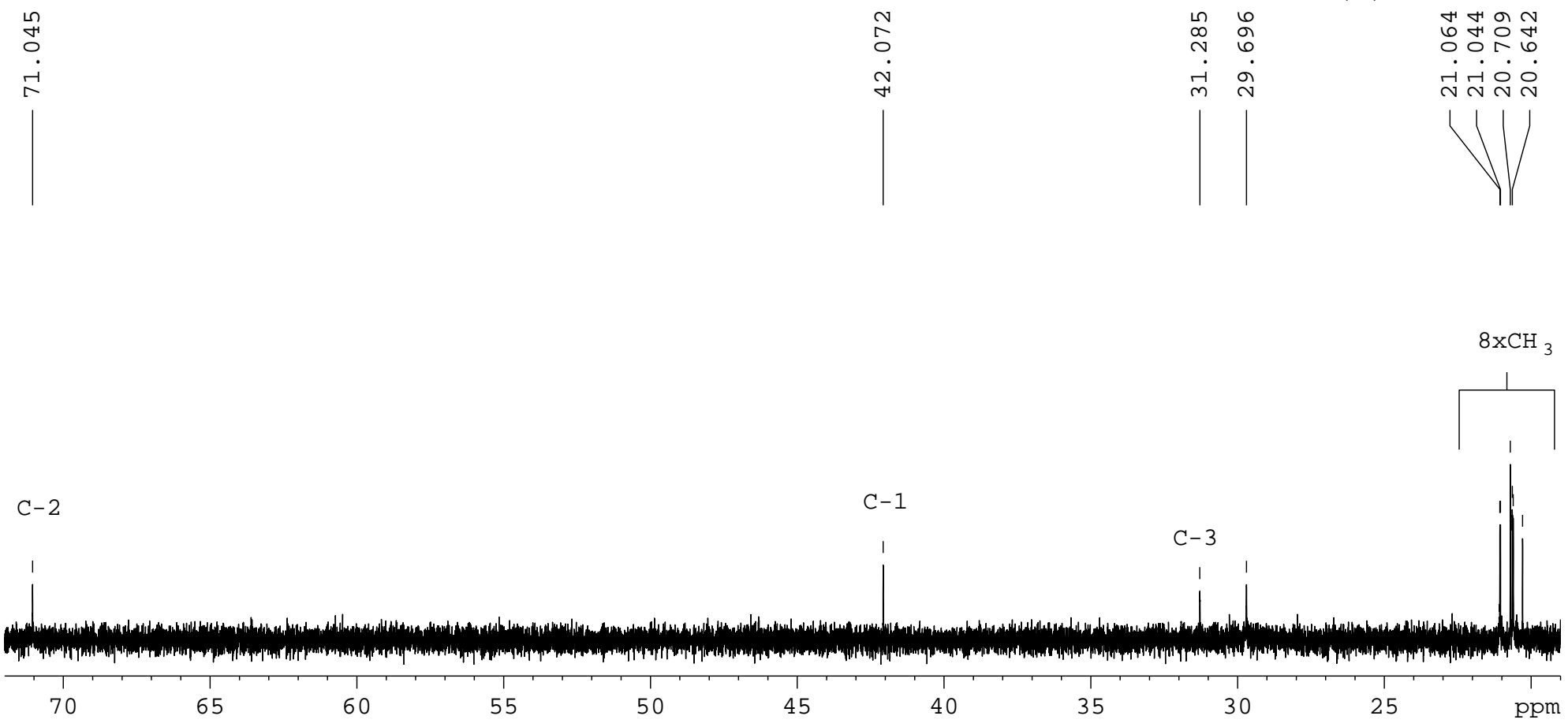
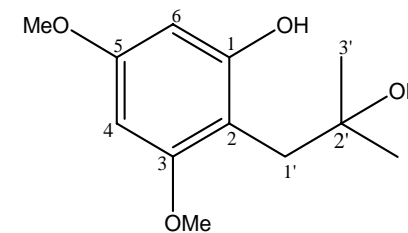


Plate 9a (CDCl<sub>3</sub>, 298 K)



(54)

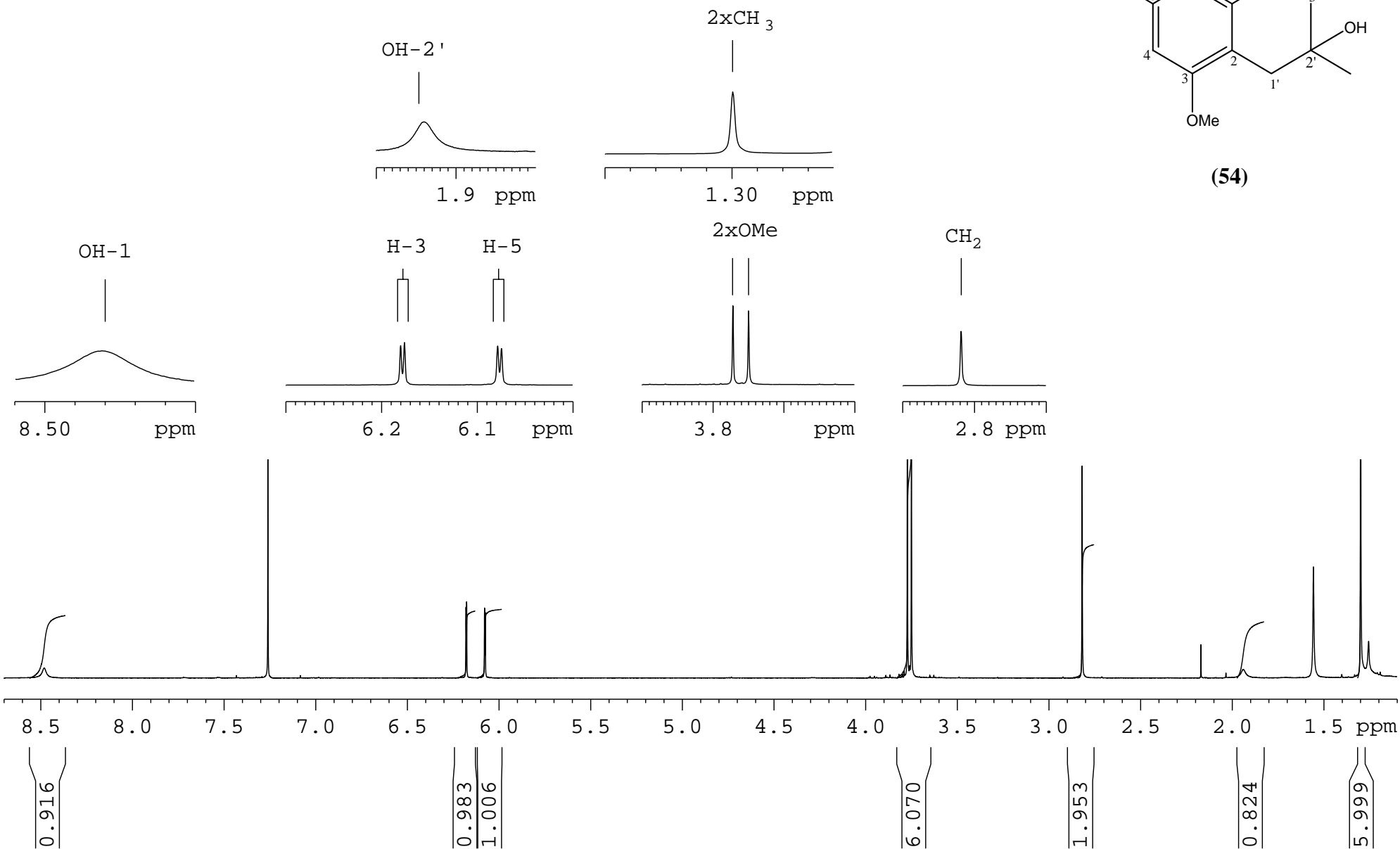
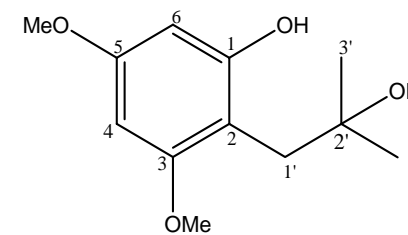


Plate 9b (CDCl<sub>3</sub>, 298 K)



(54)

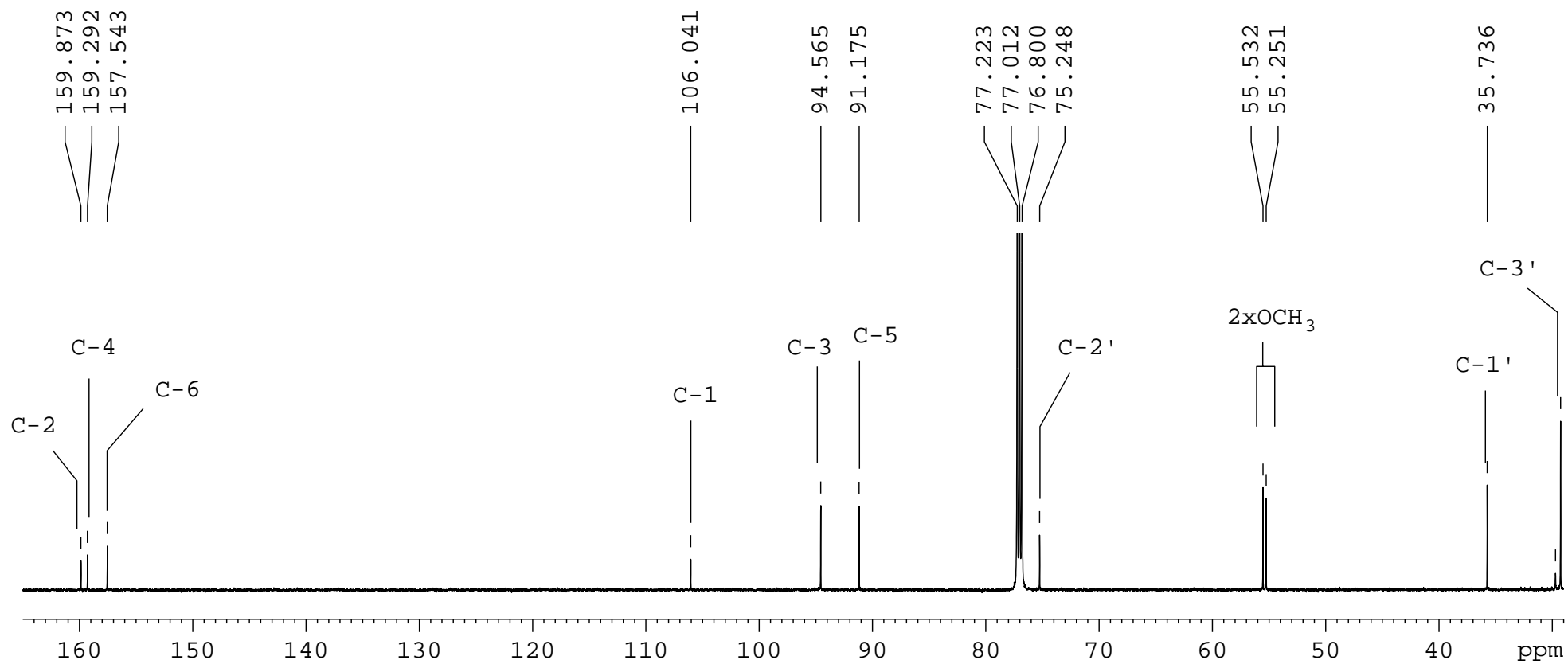
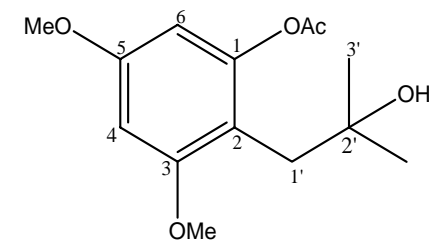


Plate 10a (CDCl<sub>3</sub>, 298 K)



(55)

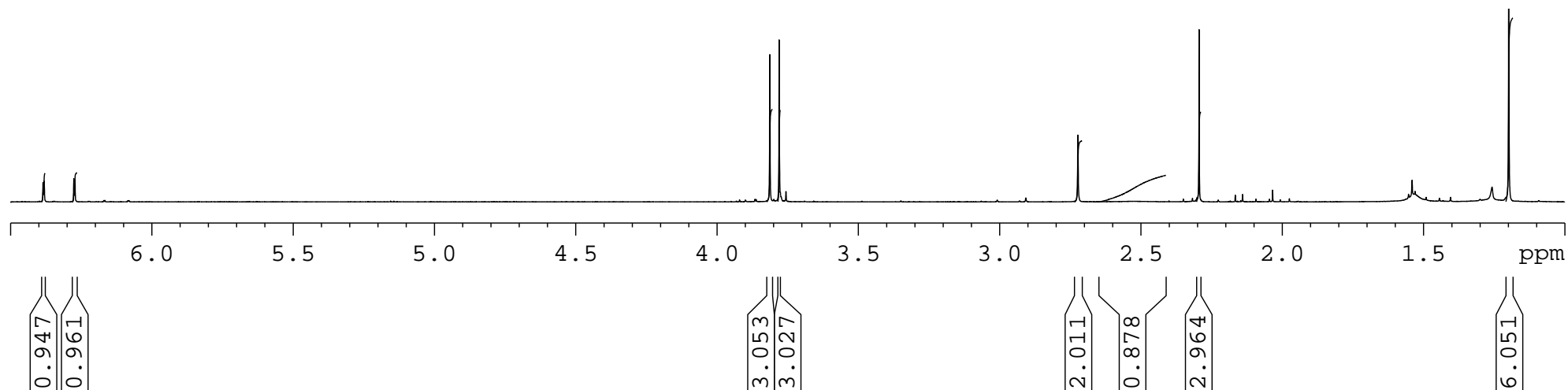
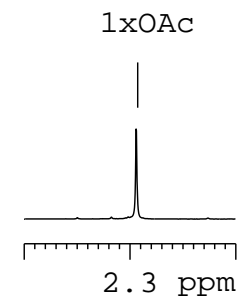
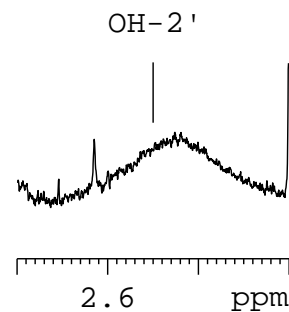
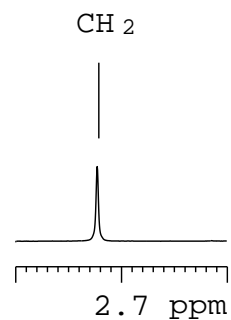
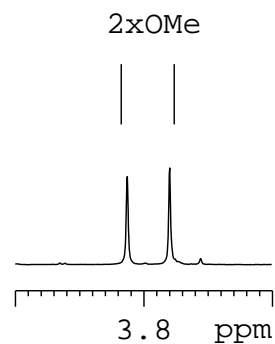
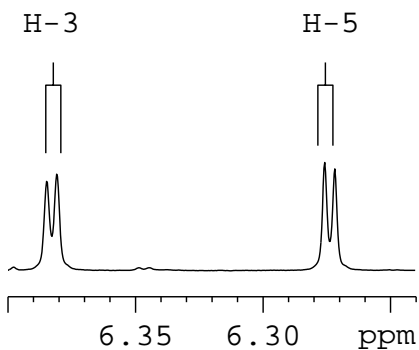
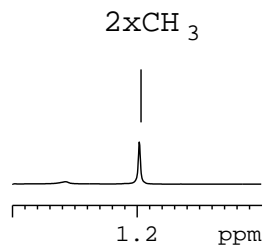
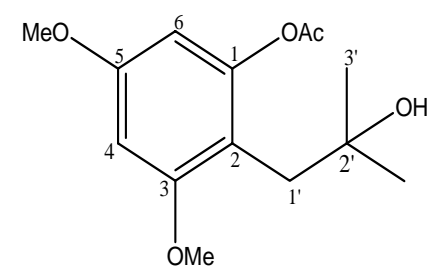




Plate 10b (CDCl<sub>3</sub>, 298 K)



(55)

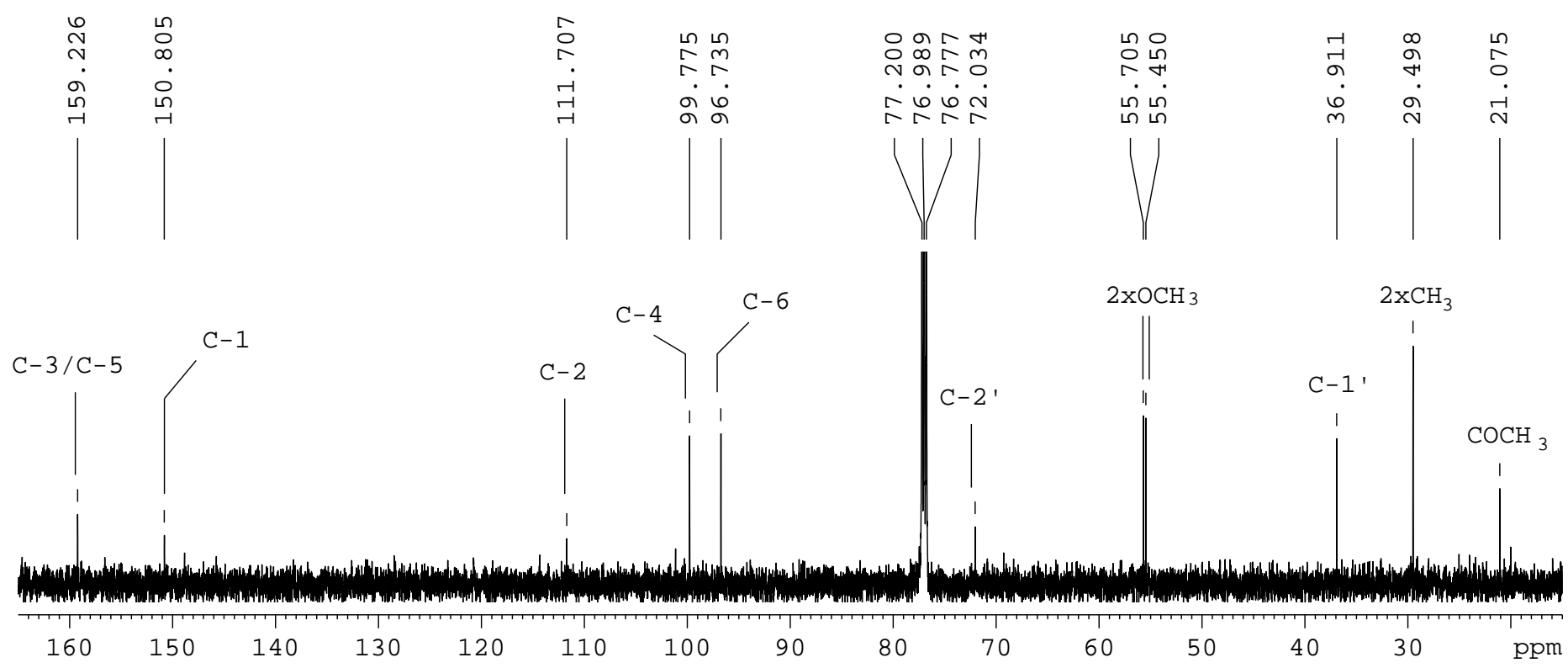
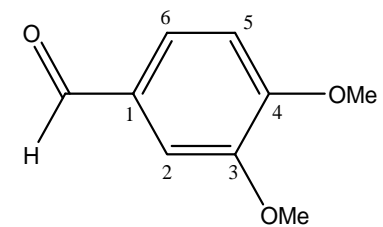


Plate 11 (CDCl<sub>3</sub>, 298 K)



(53)

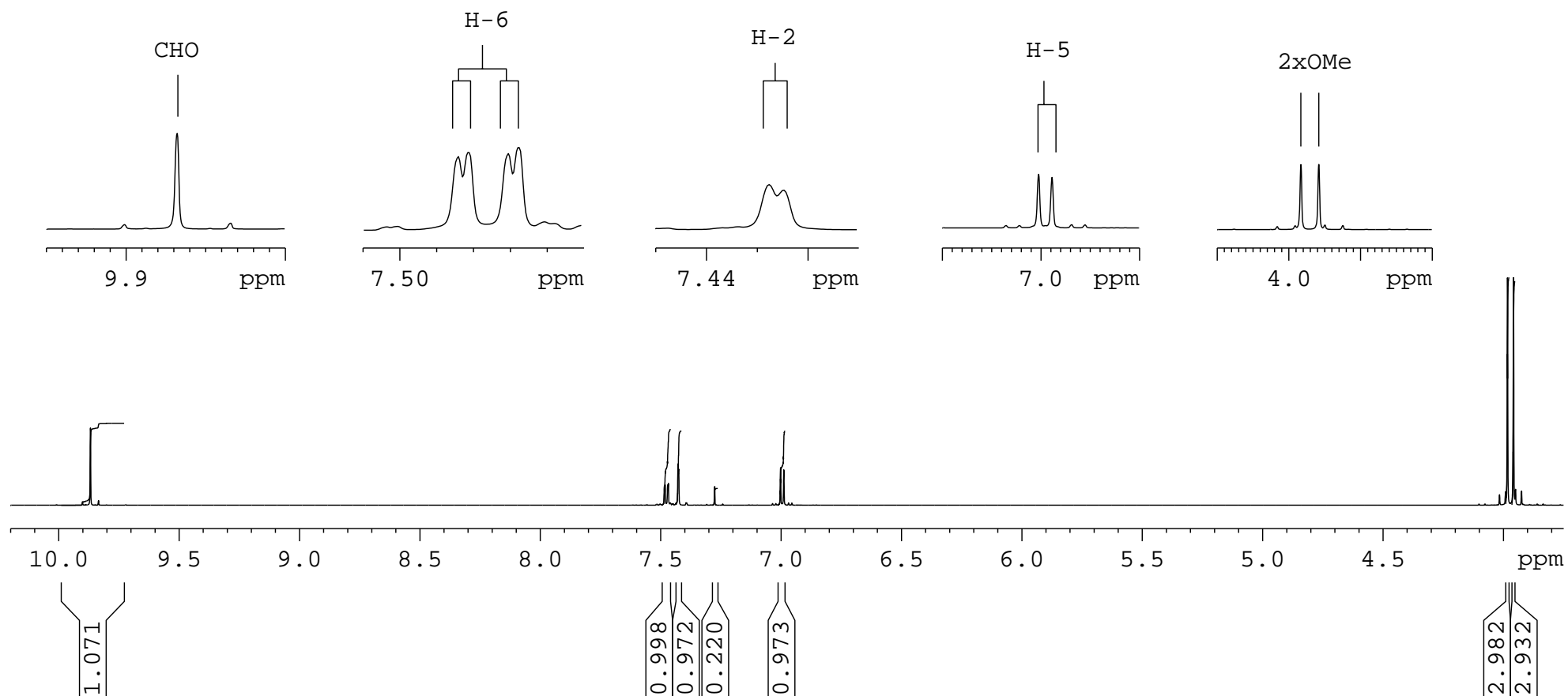
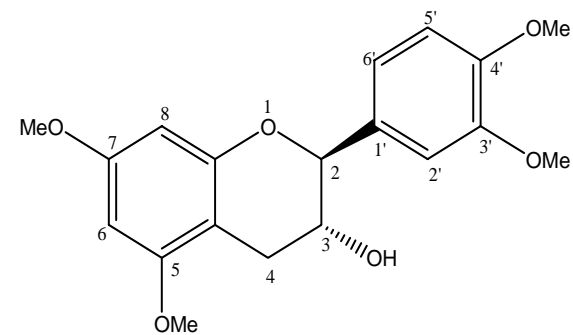


Plate 12 (CDCl<sub>3</sub>, 298 K)



(39)

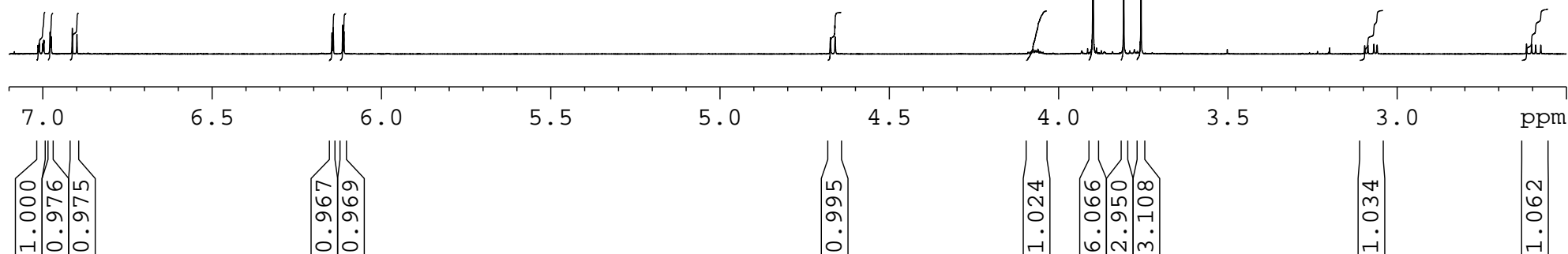
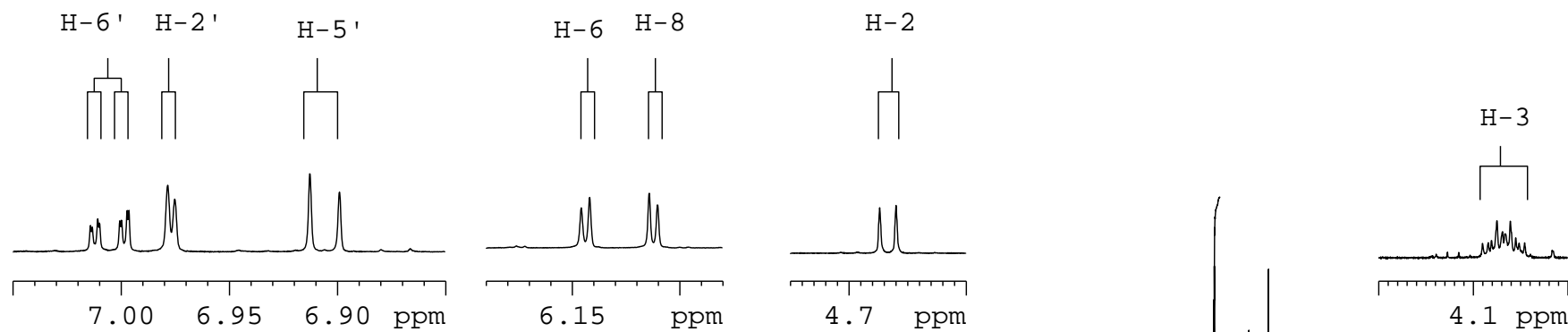
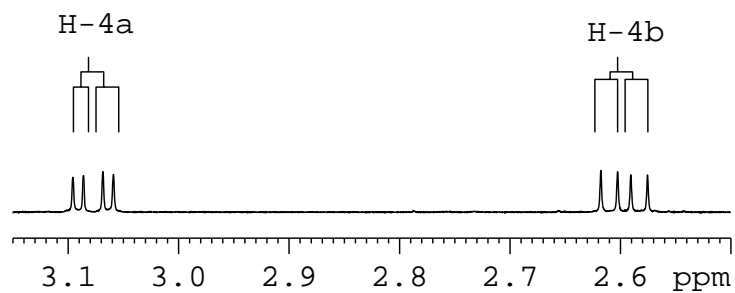
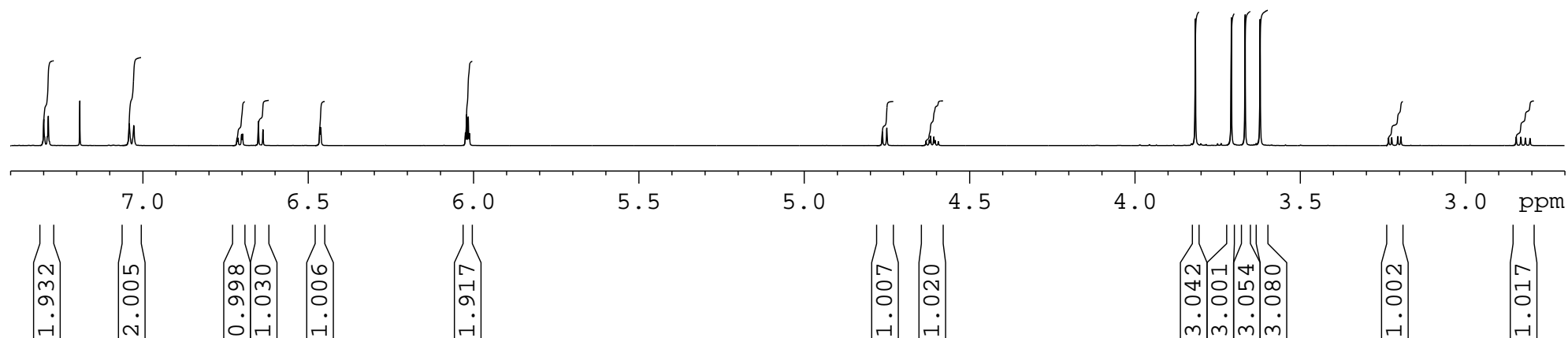
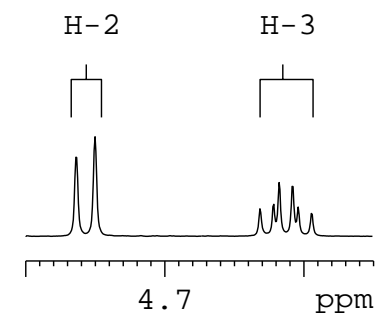
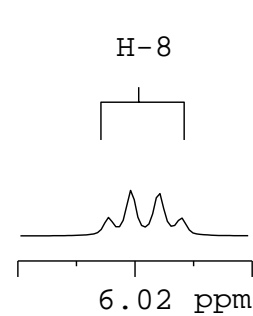
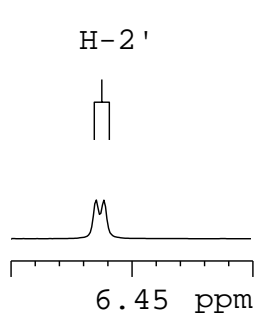
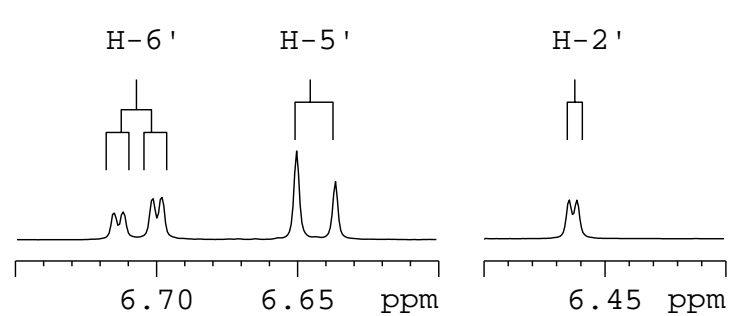
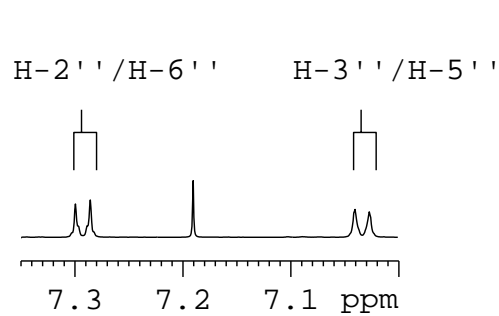
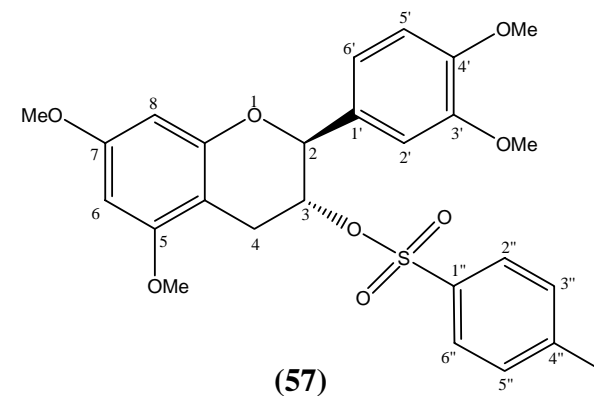
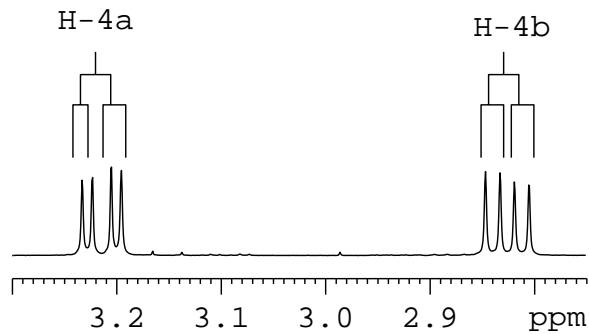
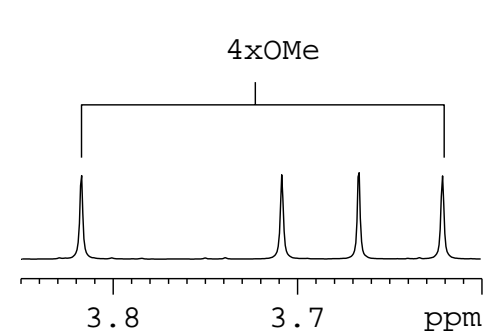
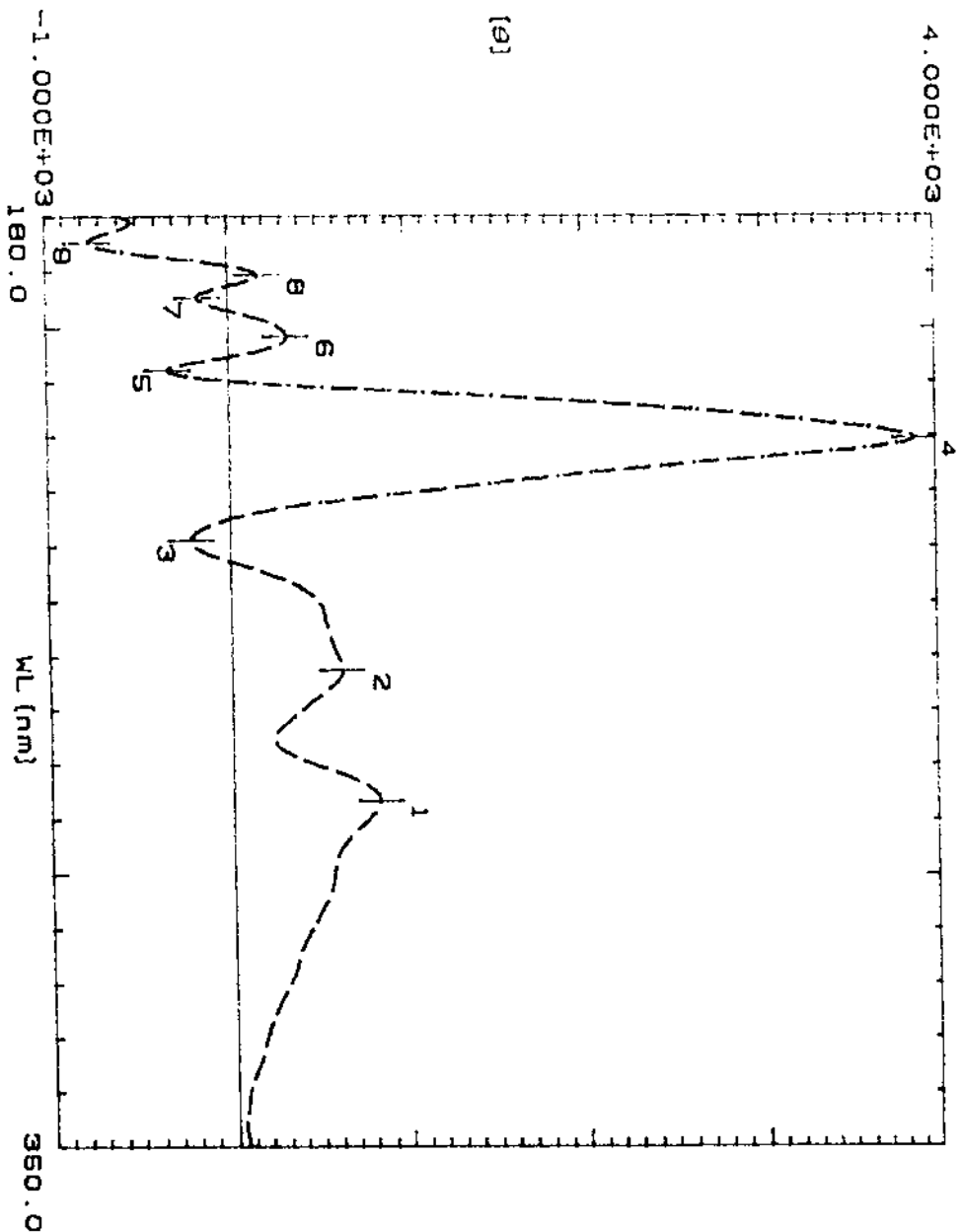
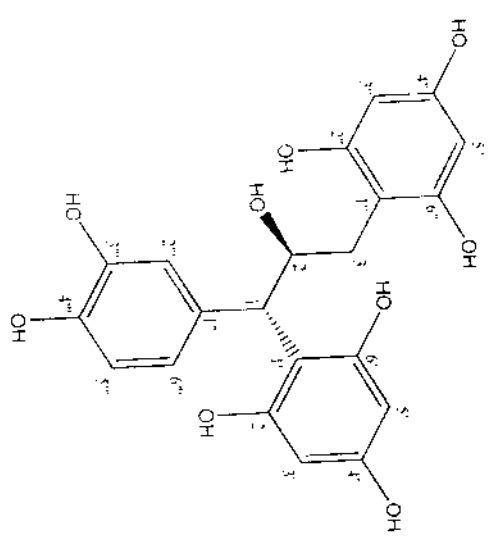


Plate 13 (CDCl<sub>3</sub>, 298 K)



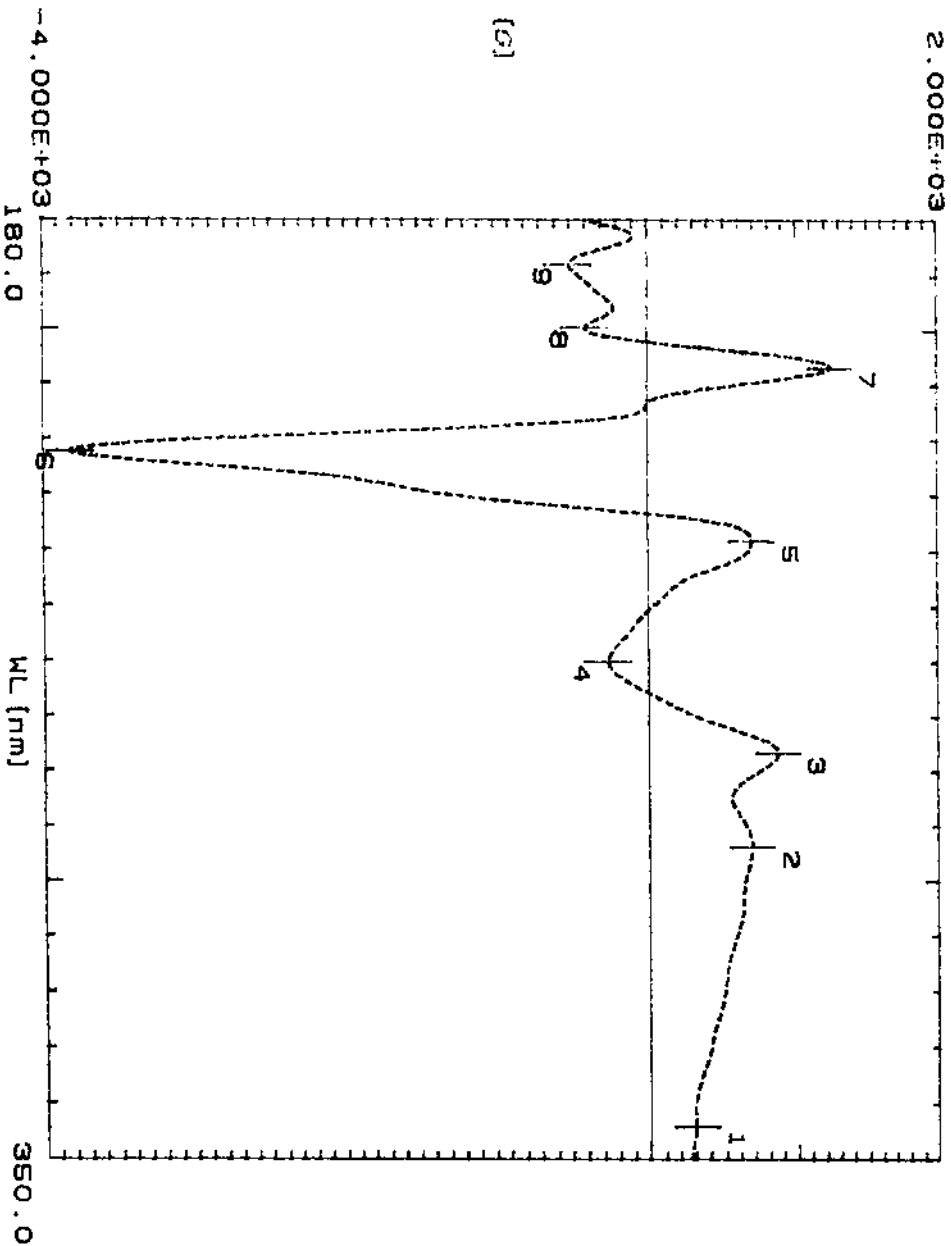


CD-plate 1: (1S,2S)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (32) (1.06 mg/10 ml MeOH)

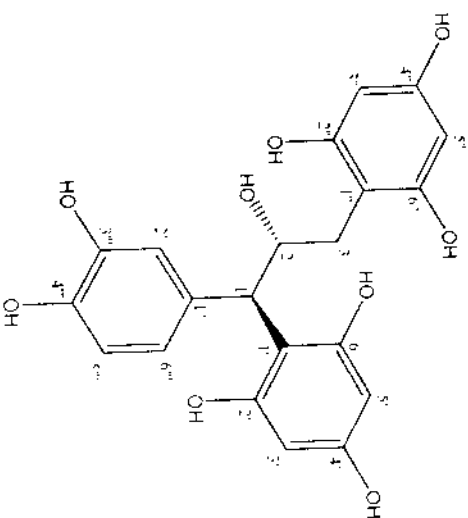


(32)

No.	Wavelength	Value
1	286.60 nm	8.334E+02
2	262.60 nm	6.266E+02
3	239.00 nm	-2.178E+02
4	220.40 nm	3.868E+03
5	207.80 nm	-3.378E+02
6	201.60 nm	3.273E+02
7	194.60 nm	-1.676E+02
8	190.40 nm	1.700E+02
9	184.60 nm	-7.611E+02

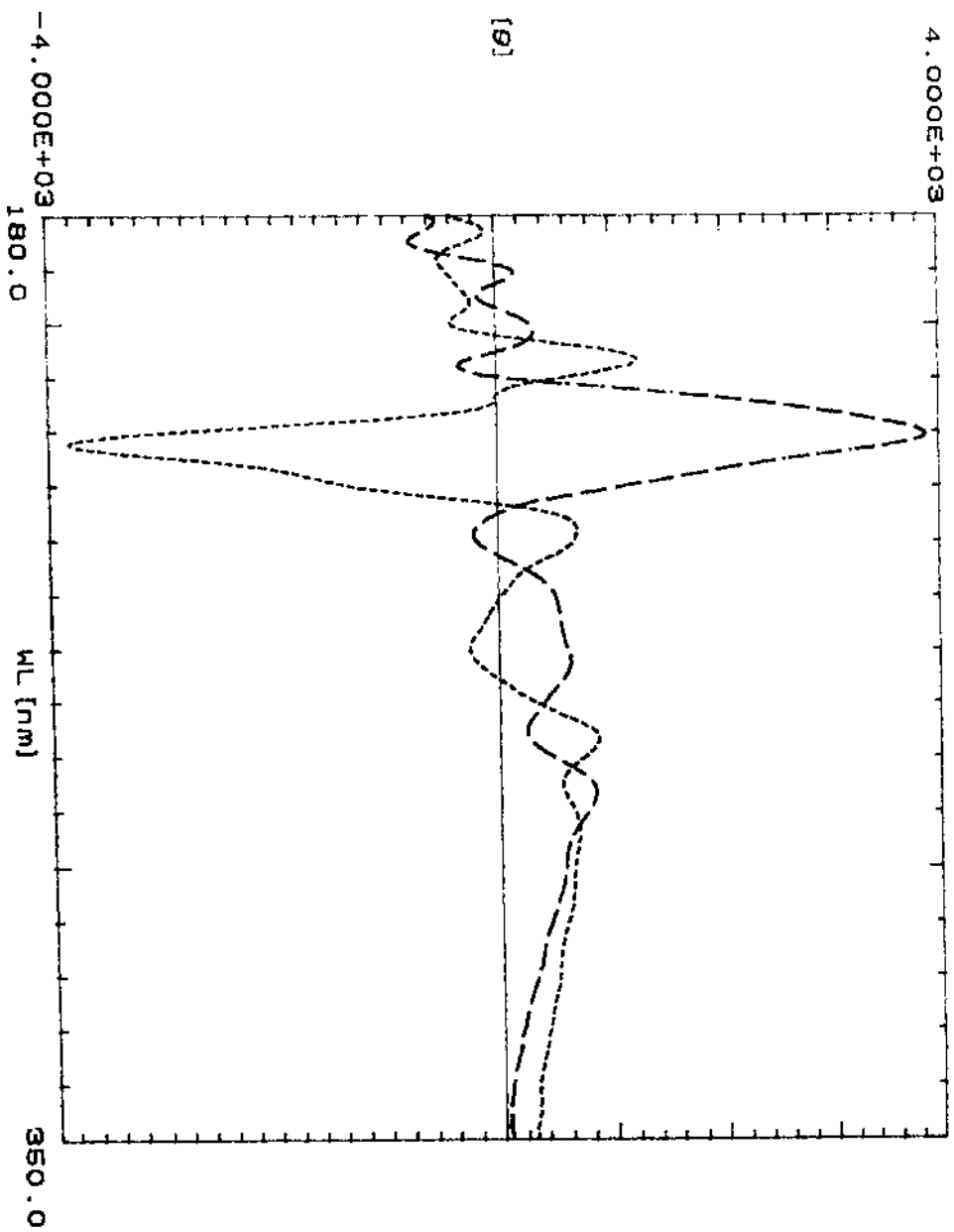


CD-plate 2: (1R,2R)-1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (33) (1.10 mg/10 ml MeOH)

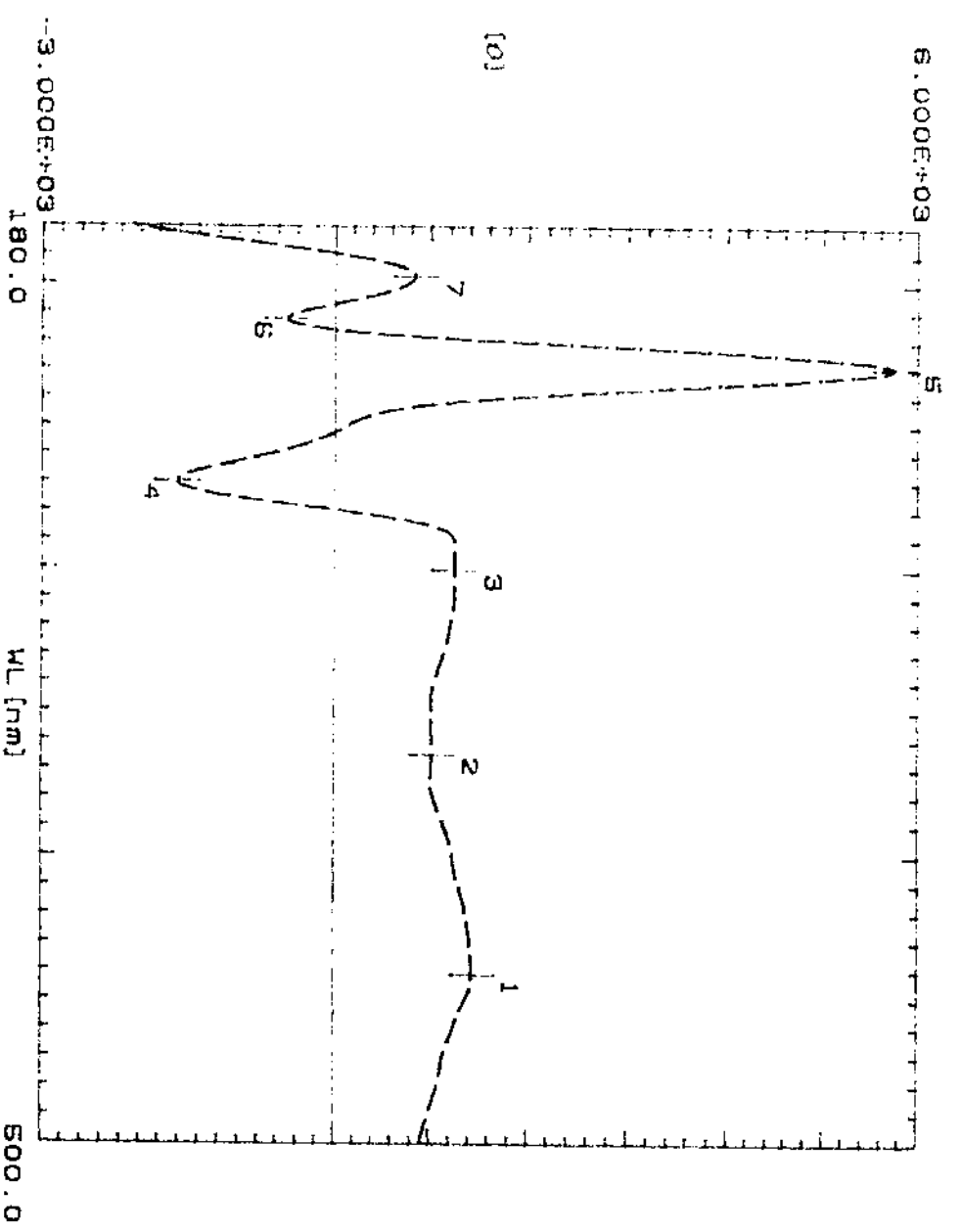


(33)

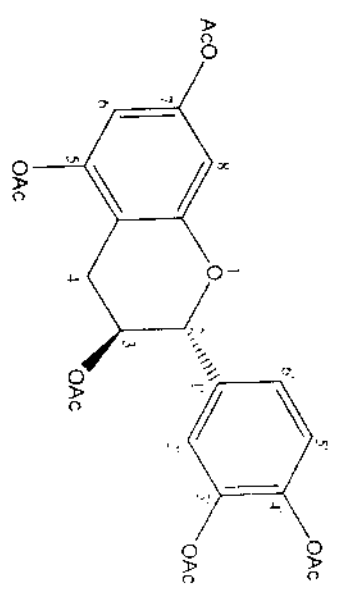
No.	Wavelength	Value
1	344.20 nm	3.036E+02
2	293.80 nm	6.836E+02
3	276.60 nm	8.644E+02
4	260.00 nm	-2.638E+02
5	238.20 nm	6.911E+02
6	222.20 nm	-9.818E+03
7	206.80 nm	1.237E+03
8	189.60 nm	-4.058E+02
9	188.00 nm	-5.125E+02



**CD-plate 3:** Mirror image CD spectrum of (1S,2S)1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (**32**) and (1R,2R)1,3-di(2,4,6-trihydroxyphenyl)-1-(3,4-dihydroxyphenyl)propan-2-ol (**33**)

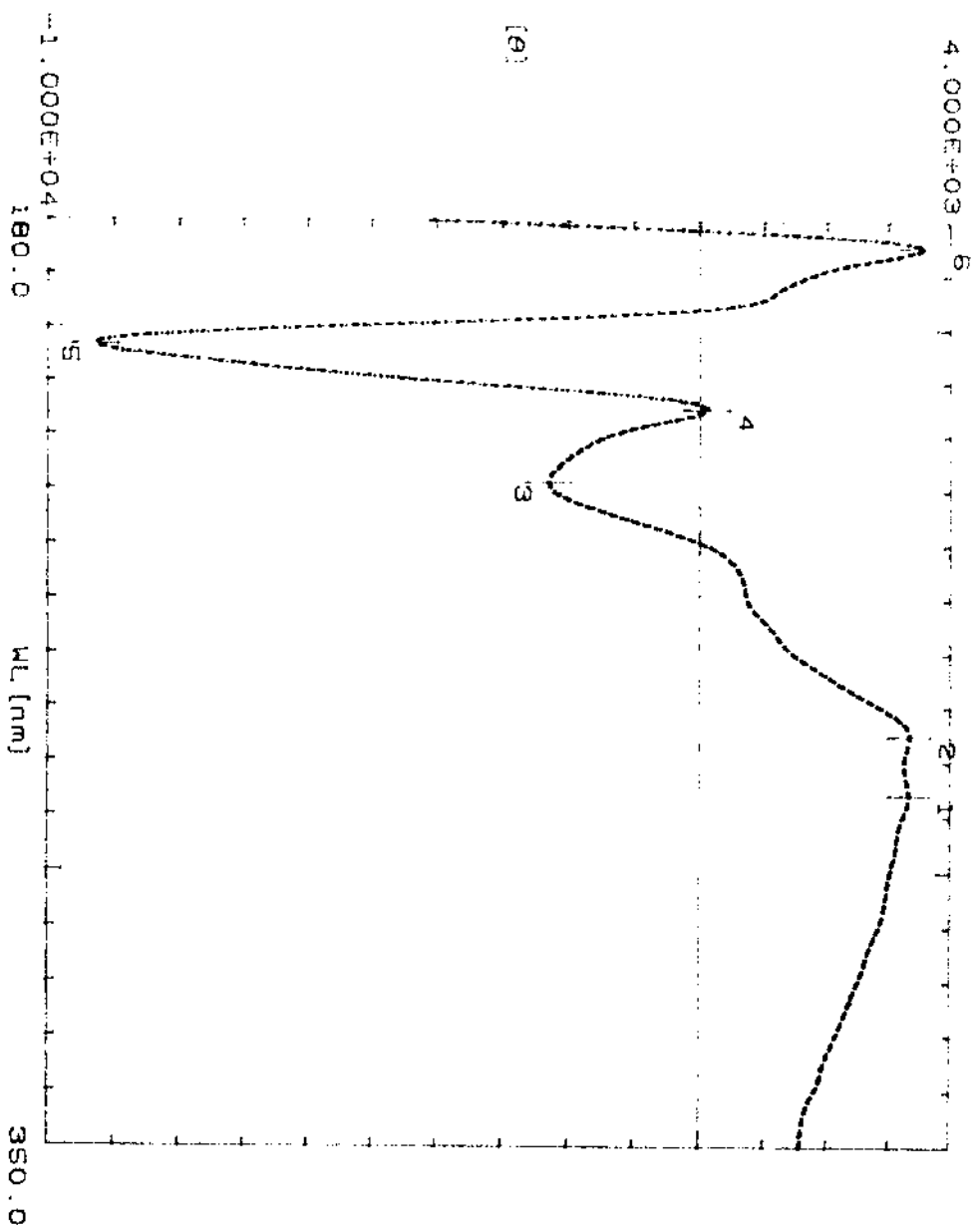


CD-plate 4: (2R,3S)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate  
 {acetylated (-)-Catechin} (3') (1.05 mg/10 ml MeOH)

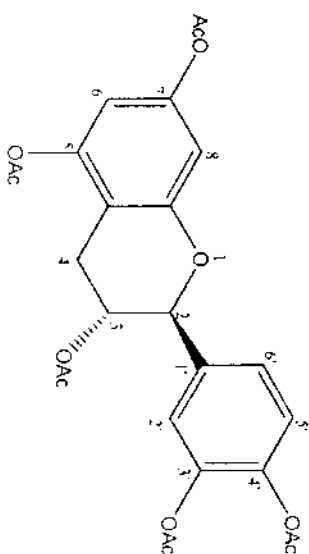


No.	Wavelength	Value
1	441.80 nm	1.437E+03
2	365.20 nm	1.017E+03
3	300.60 nm	1.245E+03
4	270.00 nm	-1.622E+03
5	229.40 nm	5.749E+03
6	212.40 nm	-5.020E+02
7	187.60 nm	8.358E+02



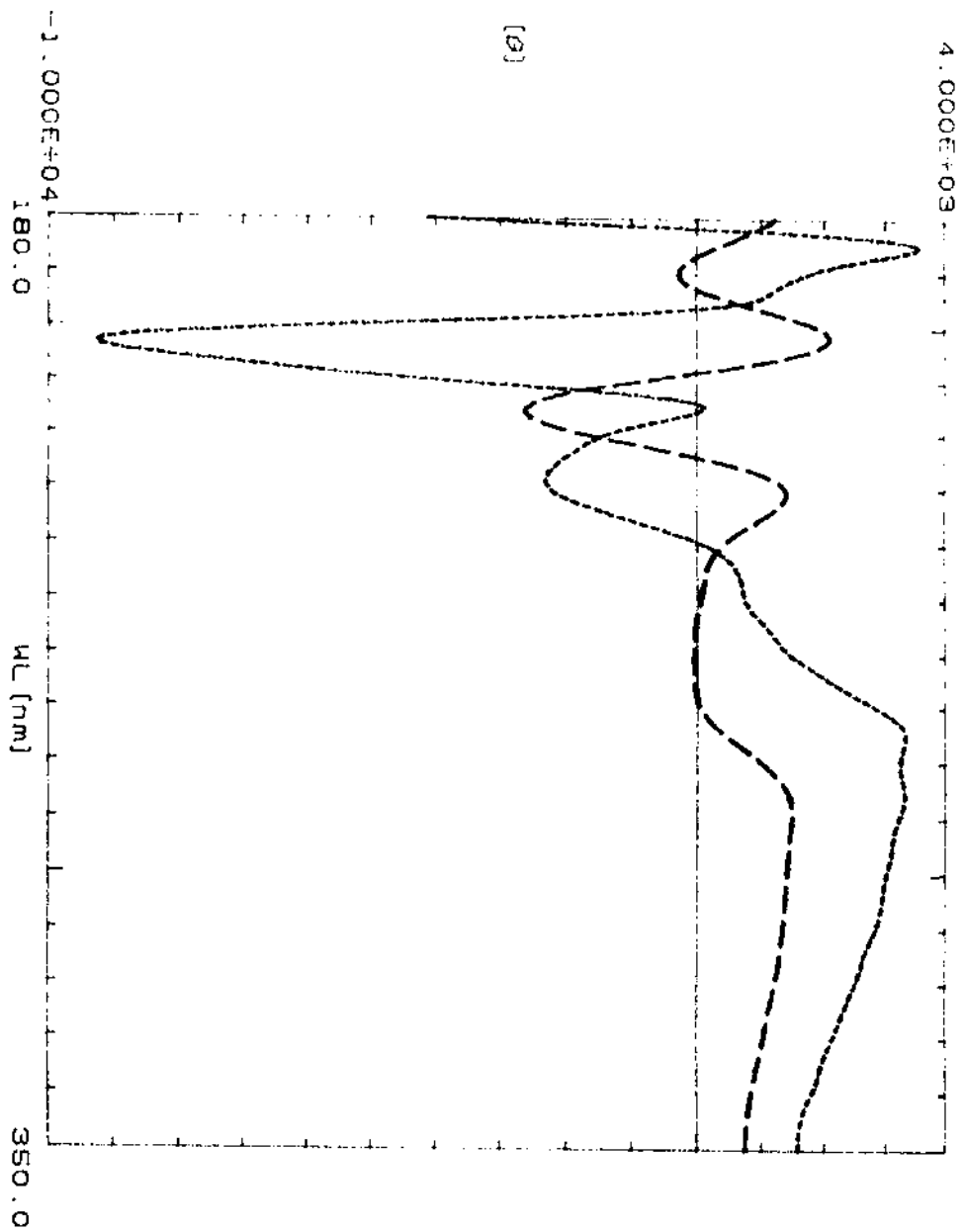


**CD-plate 5:** (2S,3R)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate  
 {acetylated (-)-catechin (35')} (0.99 mg/10 ml MeOH)

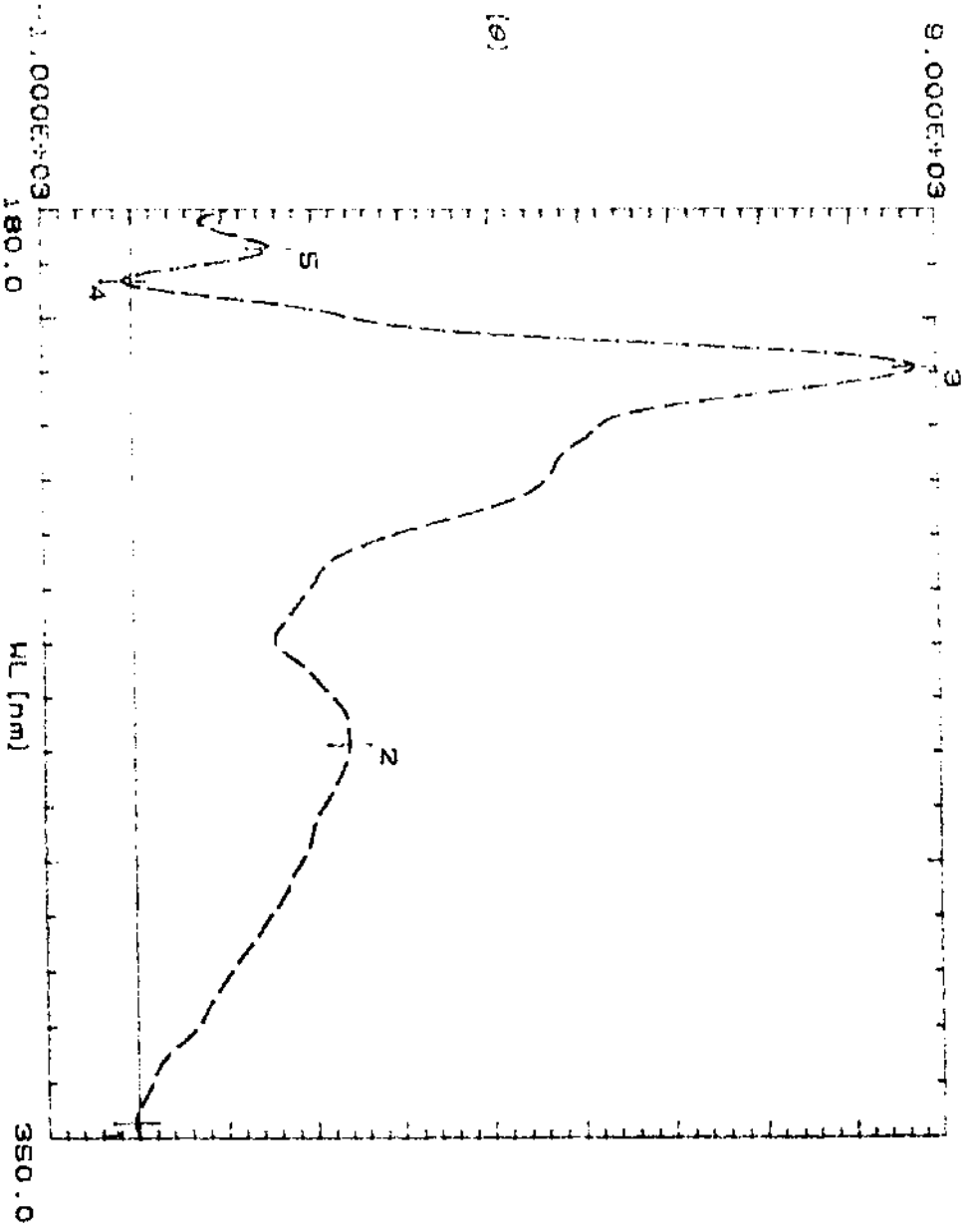


(35')

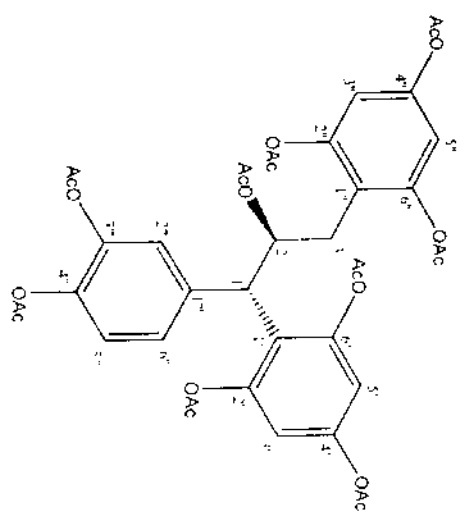
No.	Wave length	Value
1	286.00 nm	3.328E+03
2	275.20 nm	-3.342E+03
3	228.60 nm	-2.289E+03
4	215.00 nm	1.122E+02
5	203.20 nm	-9.267E+03
6	184.80 nm	3.559E+03



**CD-plate 6:** Mirror image CD spectrum of (2R,3S)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate {acetylated (+)-Catechin} (3') and (2S,3R)-2-(3',4'-diacetoxyphenyl)chroman-3,5,7-triyl triacetate {acetylated (-)-catechin (35')}

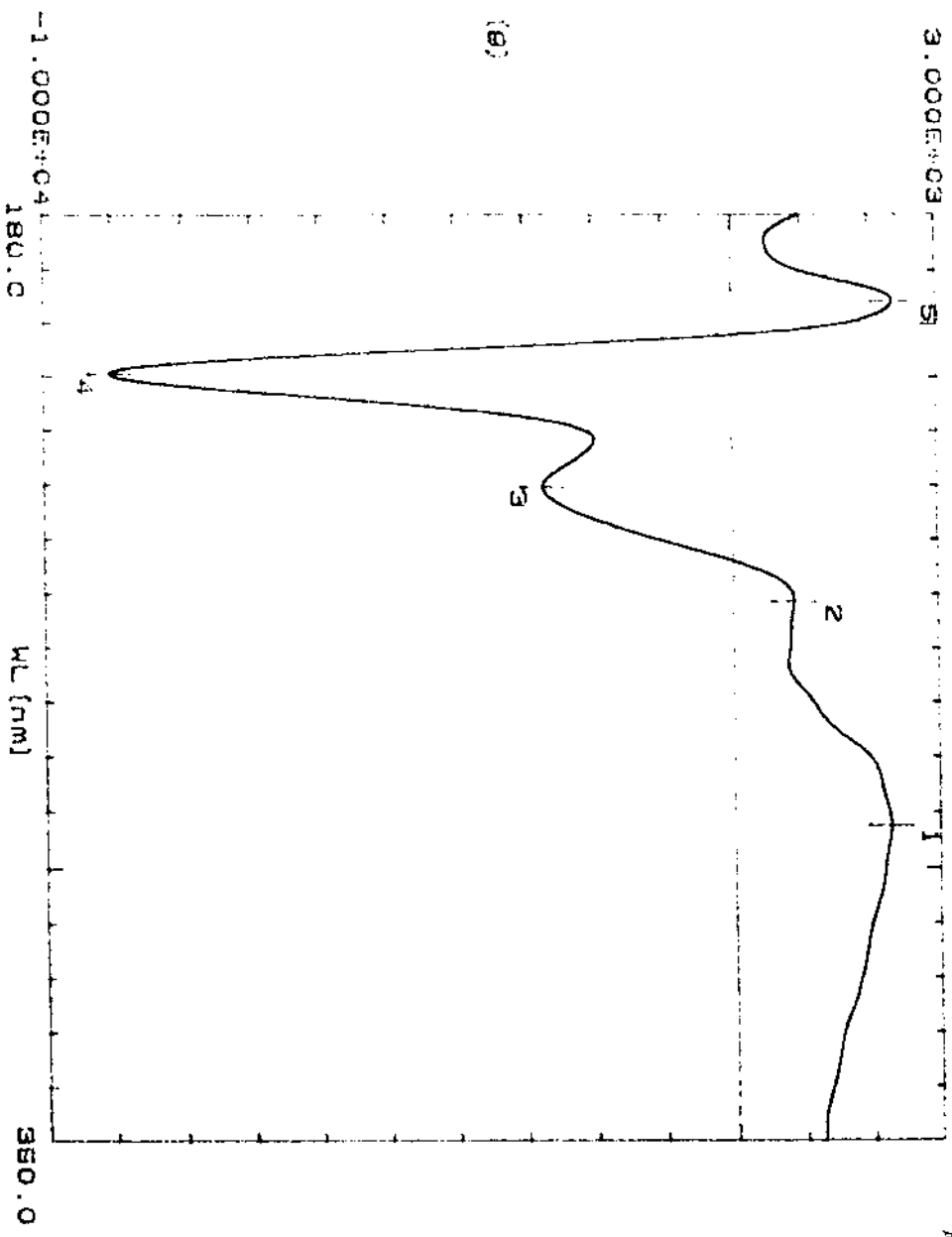


CD-plate 7: (1S,2S)-1,3-di(2,4,6-triacetoxylphenyl)-1-(3,4-diacetoxylphenyl)propan-2-ol (**34**) (1.10 mg/10 ml MeOH)

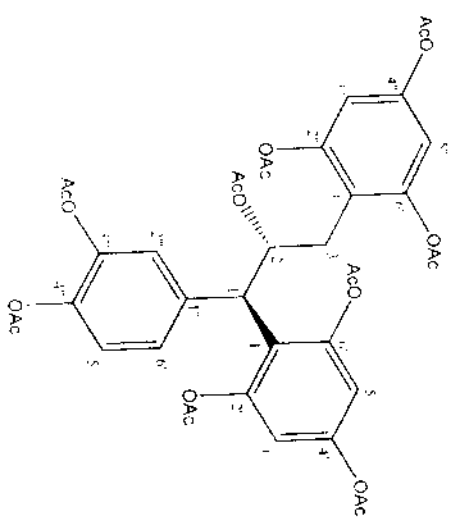


(34)

No.	Wavelength [nm]	Value
1	347.40 nm	-3.882E+01
2	278.60 nm	2.398E+03
3	209.20 nm	8.739E+03
4	193.00 nm	-9.367E+01
5	187.00 nm	1.549E+03

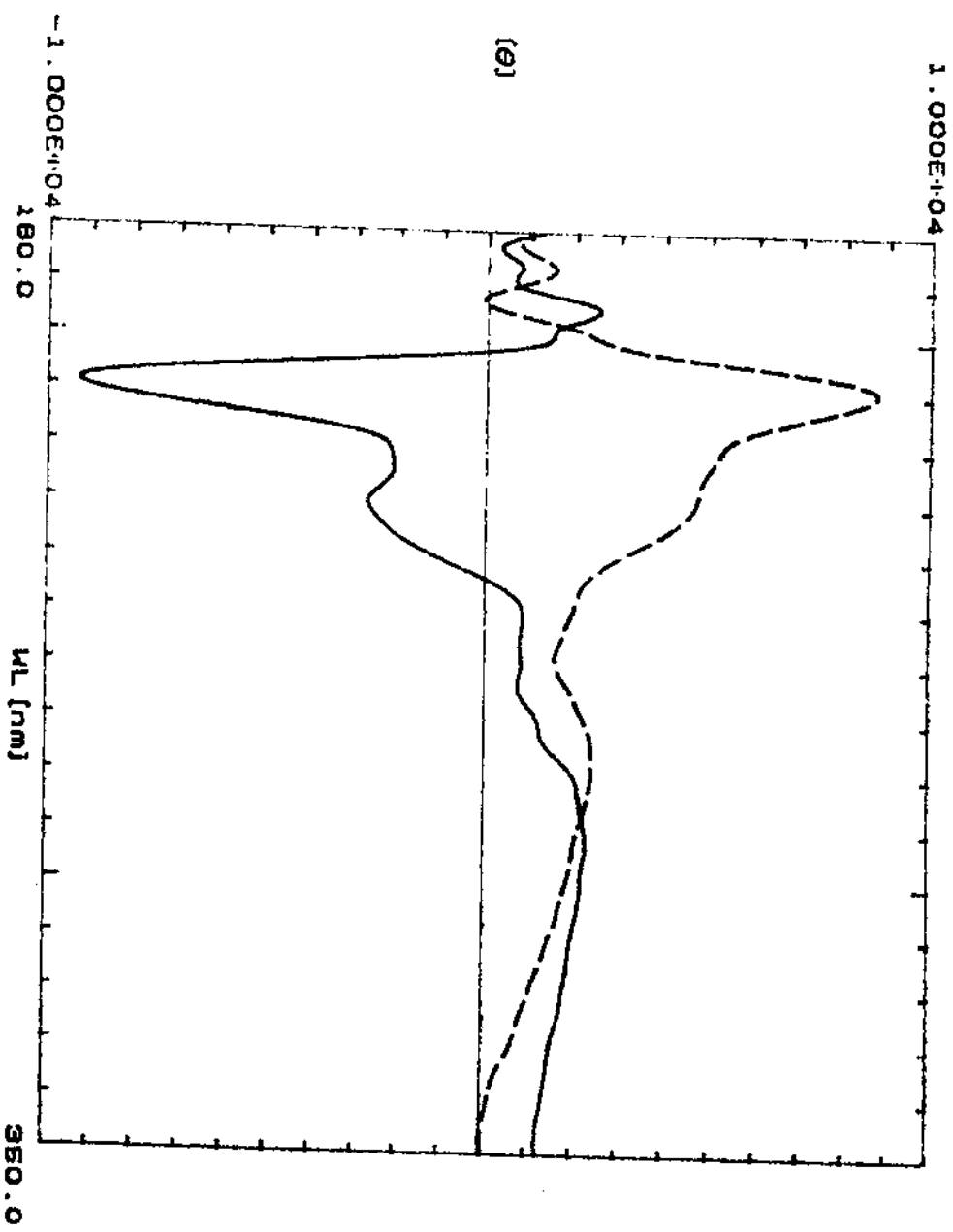


CD-plate 8: (1R,2R)-1-(3-di(2,4,6-triacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propan-2-yl (35) (1.05 mg/10 ml MeOH)

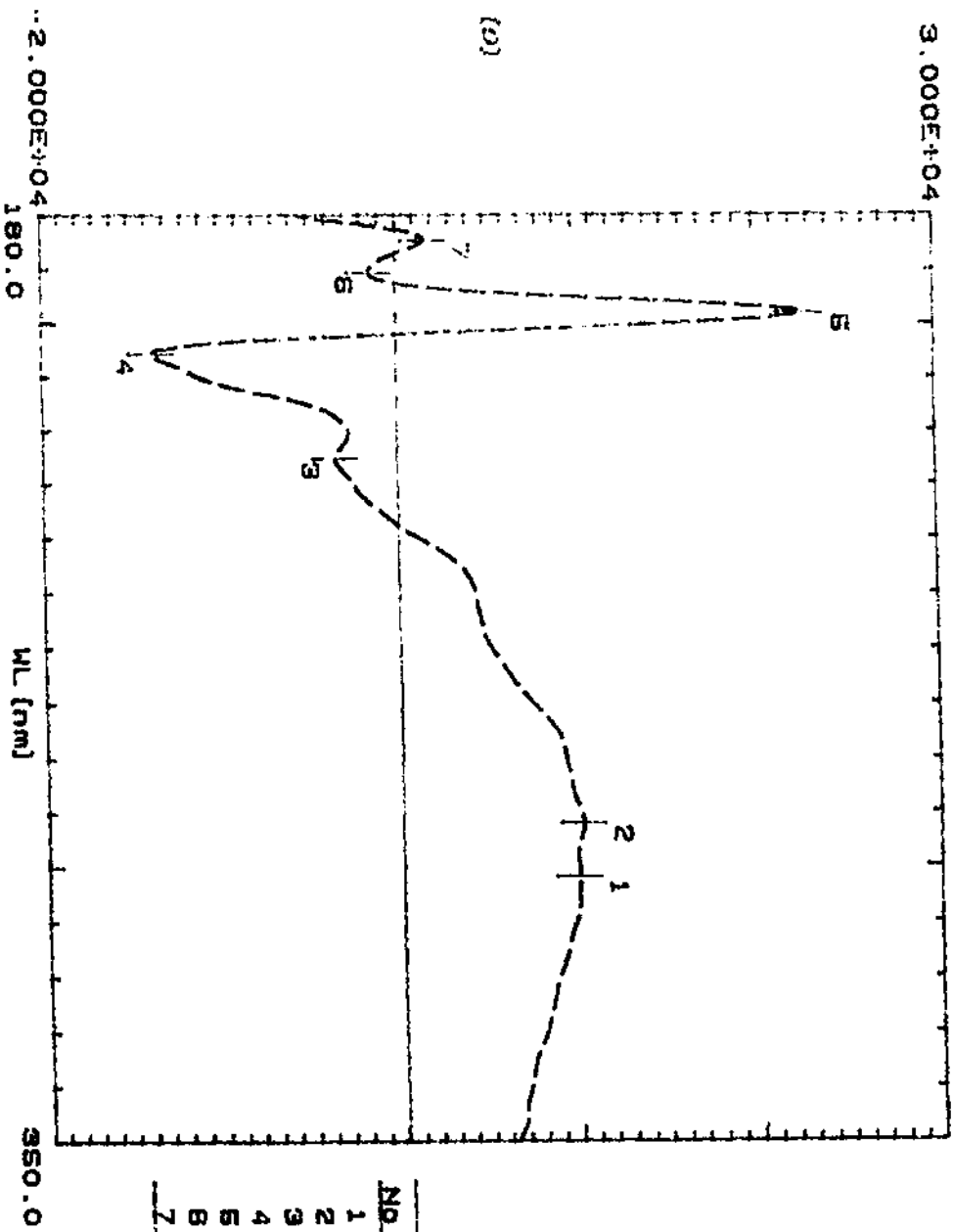


(35)

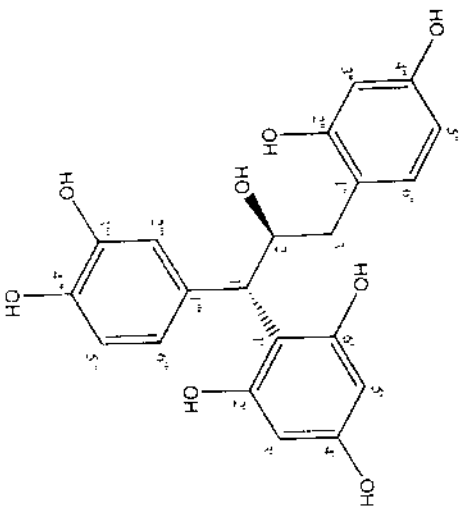
No.	Wavelength [nm]	Value
1	282.40 nm	2.262E+03
2	251.40 nm	8.697E+02
3	230.40 nm	-2.726E+03
4	209.60 nm	-9.091E+03
5	195.80 nm	2.344E+03



CD-plate 9: Mirror image CD spectrum of (1*S*,2*S*)-1,3-di(2,4,6-triacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propan-2-ol (**34**) and (1*R*,2*R*)-1,3-di(2,4,6-triacetoxyphenyl)-1-(3,4-diacetoxyphenyl)propan-2-ol (**35**)

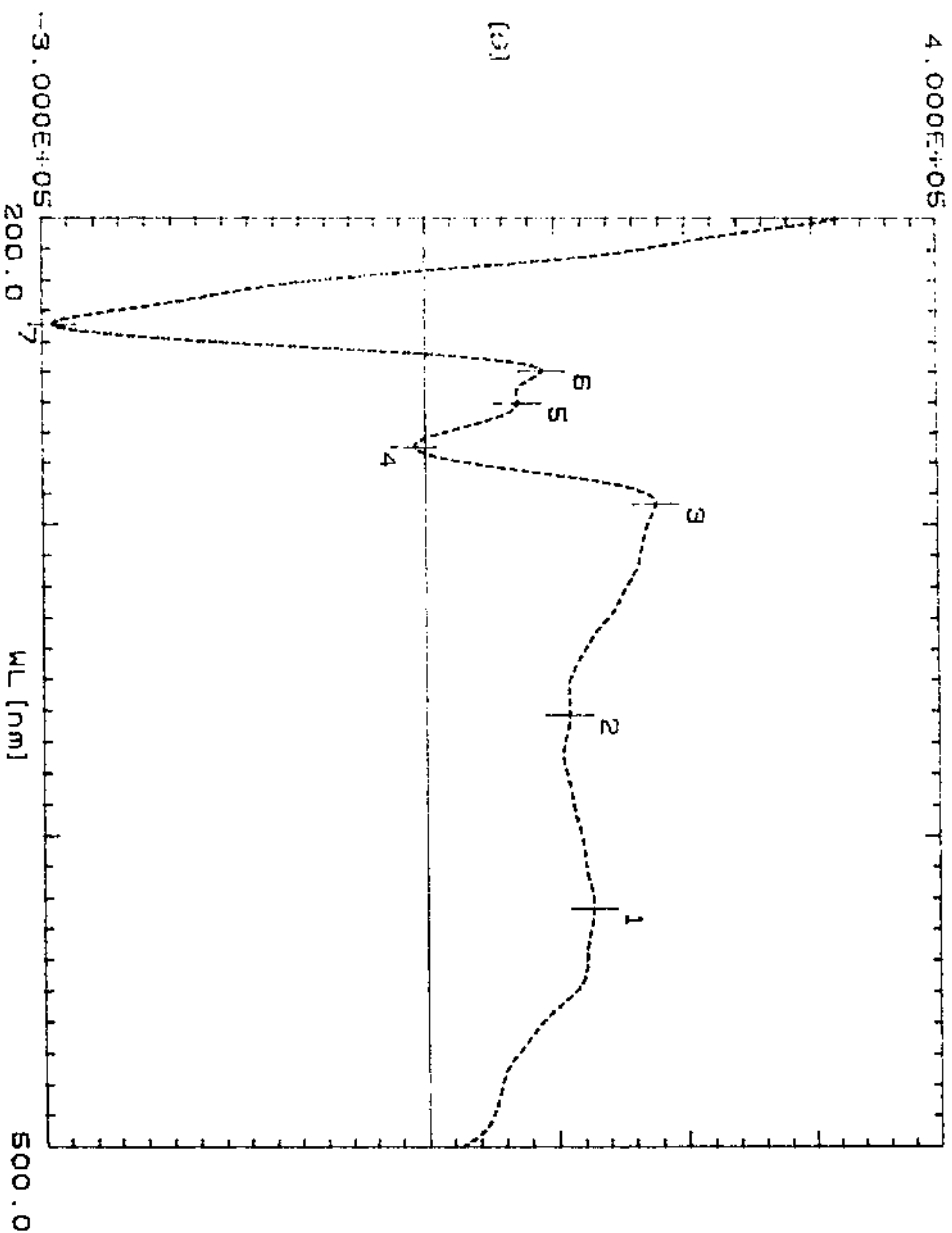


CD-plate 10: (1S,2S)-3-(2,4-dihydroxyphenyl)-1-(3,4-dihydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)-propan-2-ol (45) (1.06 mg/10 ml MeOH)

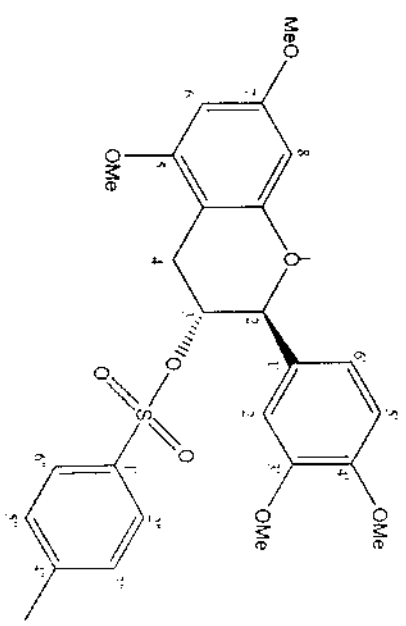


(45)

No.	WaveLength	Value
1	301.80 nm	1.000E+04
2	281.80 nm	1.027E+04
3	225.00 nm	-9.555E+03
4	205.80 nm	-1.979E+04
5	198.00 nm	2.259E+04
6	190.40 nm	-1.522E+03
7	184.40 nm	1.595E+03



CD-plate 11: (2*S*,3*R*)-2-(3',4'-dimethoxyphenyl)-5,7-dimethoxychroman-3-ol (**57**) [(+)-3-*O*-tosyl-tetra-*O*-methylcatechin] (1.12 mg/10 ml MeOH)



(**57**)

No.	Wavelength	Value
1	423.20 nm	1.279E+05
2	360.80 nm	1.101E+05
3	293.20 nm	1.775E+05
4	274.60 nm	-8.739E+03
5	260.00 nm	7.208E+04
6	249.60 nm	9.070E+04
7	234.00 nm	-2.912E+05