REACTIONS OF POLYPHENOLS WITH α-KETO ACIDS

Thesis submitted in fulfillment of the requirements for the degree

Master of Science

in the

Department of Chemistry
Faculty of Agricultural and Natural Science

at the
University of the Free State
Bloemfontein

by

ROSINAH MAIYANE MONTSHO

Supervisor: Prof. J. H. van der Westhuizen
Co-Supervisor: Dr. S. L. Bonnet

January 2007

REACTION OF POLYPHENOLS WITH α-KETO ACIDS

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;

Dr. B. I. Kamara for her kindness, love and encouragement;

Prof. E. V. Brandt for assistance and encouragement.

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

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

Believers (Bible Believing Church) for their support, encouragement and prayers;

My parents Abednego and Bertha for their lifetime love, support and encouragement throughout my life and the education background they gave me;

My sisters, Shate, Mary, Zipporah and Martha, brothers Oupa and Patrick for their moral support and love;

Above all I thank God for His guidance and the health He has given me and my family.

R. M. Montsho

Table of contents

SUMMARY

Keywords: Methine bonds, diphenylmethane, antioxidant, α-dicarboxylic acid, benzofuran-2-one, 2-(2,4,6-trimethoxyphenyl)-acrylic acid, isoaurone, cyclopropane, phloroglucinol and pyruvic acid.

Novel methods of carbon-carbon bond formation are of considerable theoretical and practical interest to synthetic organic chemists. This work investigates the formation and synthetic potential of a methine bond (one carbon link) between two aromatic moieties to form diphenylmethane derivatives.

This methine link is of industrial importance when the aromatic moiety is hydroxylated. The colour stability of red wine is attributed to a methine bond that is the result of condensation between glyoxylic or pyruvic acid and an anthocyanidin. This bond may be formed spontaneously during the ageing of wine. Wattle extract based adhesives rely on the reaction between formaldehyde and polyphenols to form methine linked polymers. Patented antioxidants rely on the availability of a benzylic proton on a methine link, *ortho* to a hydroxy group (Irganox®HP-136).

The proximity of the two carbonyl double bonds in α-dicarbonyl compounds enhances the reactivity of each other towards nucleophiles. In the case of α-keto acids the α-keto group is more electrophilic than the carboxylic group and susceptible to attack by nucleophiles.

The hydroxy groups of phloroglucinol and other polyhydroxybenzenes donate electrons to the aromatic ring to increase the nucleophilicity of the aromatic carbons. Polyphenols thus become ambident nucleophiles that can react either via oxygen or carbon and have the ability to form new carbon-carbon bonds with suitable electrophiles.

As part of our ongoing investigation into the importance of *p*-quinone methides in flavonoid chemistry the reaction of a variety of polyhydroxyphenols with α-keto acids were investigated.

Addition of an aromatic ring to a carbonyl group creates a benzylic hydroxy group. With strongly nucleophilic aromatic rings this benzylic substituent is replaced with a second aromatic ring to yield the anticipated methine linked biaryl compound.

Phloroglucinol reacts with pyruvic acid to give 4,6-dihydroxy-3-methyl-3-(2,4,6-trihydroxyphenyl)-1-benzofuran-2(3H)-one and with glyoxylic acid to yield 4,6-dihydroxy-3-(2,4,6-trihydroxyphenyl)-1-benzofuran-2(3H)-one. These products are lactones between the phenolic- and carboxylic acid moiety of an intermediate biaryl organic acid. With oxaloacetic acid a 4,5′,6,7′-tetrahydroxy-2H-spiro[benzofuran-3,4′-chroman]-2,2′-dione is isolated.

With unreactive aromatic nucleophiles the benzylic hydroxy group is eliminated before substitution can take place if hydrogen is available in the α-position. Tri-o-methylphloroglucinol reacts with pyruvic acid to give methyl-2-(2,4,6-trimethoxyphenyl)-acrylate via the elimination of water. This acrylic acid reacts with ozone to form methyloxo-(2,4,6-trimethoxyphenyl)-acetate and with diazomethane to form 2-methoxy(2,4,6-trimethoxyphenyl)-4,5-dihydrofurane.

To demonstrate the potential of this reaction we reacted resorcinol with *p*-hydroxyphenylpyruvic acid and obtained both the Z and E isomers of 6-hydroxy-3-(4-hydroxybenzylidene)-3H-benzofuran-2-one. This isoaurone synthesis represents an improvement on the recently published synthesis of this natural product.

We have developed a novel reaction to form carbon-carbon bonds and synthesize methine linked diaryl compounds. We have developed this reaction into a new procedure to synthesize free phenolic 3-substituted benzofuran-2-ones. We adapted this reaction to improve a recently published method to synthesize a free phenolic isoaurone. We can use our reaction to synthesize acrylic acids with a phenolic substitutuent in the α-position and have started to explore the potential of this α,β-unsaturated carboxylic acid as intermediates for various synthetic procedures.

CHAPTER 1

1.1 INTRODUCTION

Polyphenolic diarylmethanes are ubiquitous in nature. These compounds play important roles as red wine pigments, natural and synthetic antioxidants and tannin based adhesives. Many biologically active, naturally occurring compounds and synthetic pharmaceuticals are diarylmethane derivatives.

Diarylmethanes are characterized by a methine bond (one carbon link) between two aromatic moieties. Formation of this bond is of considerable theoretical and practical interest, particularly when the aromatic moiety is a phenol or flavonoid.

The following survey highlights examples from the literature where these bonds are formed from reactions between carbonyl electrophiles and phenolic nucleophiles to form compounds that act as red wine pigments, antioxidants and adhesives.

The methine bond under discussion is also important in benzofuran-2-ones, isoaurones and α-aryl carboxylic acids. Here follows a literature review of synthetic methods towards these compounds.

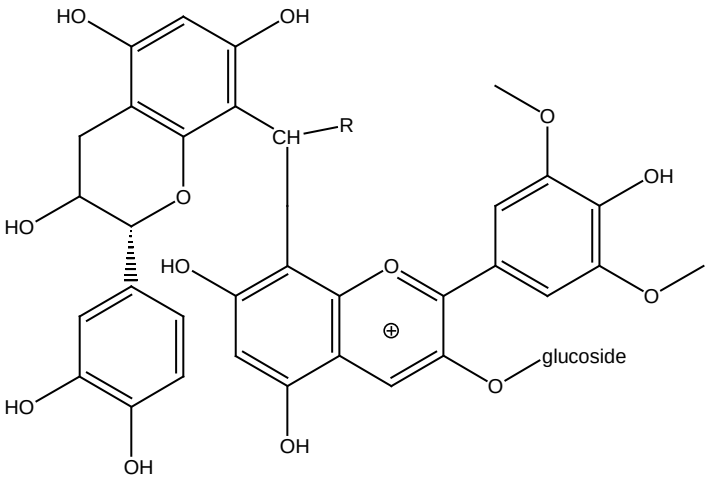
1.2 RED WINE PIGMENTS

During ageing the colour of wine gradually changes from the red-purple of the young wine towards more stable orange-like hues. These changes are attributed to chemical changes of the original unstable grape anthocyanins. It has been suggested that the long term colour stability of red wine pigments is the result of condensation reactions between anthocyanidin and other monomeric flavonoid units via methine bridges.

1.2.1. Acetaldehyde derived methine bridges:

The high reactivity of flavonoids towards aldehydes is well known[1]. In enology the reaction between anthocyanins and tannins, mediated by acetaldehyde, has received considerable attention[2,3,4]. Acetaldehyde is formed from ethanol by oxidation[5].

Pissarra and coworkers studied coloured pigments in red wine by LC/MS analysis and characterized an oligomer consisting of (+)-catechin and malvidin-3-glucoside linked by an acetaldehyde derived methine bridge[6] **(1)**.

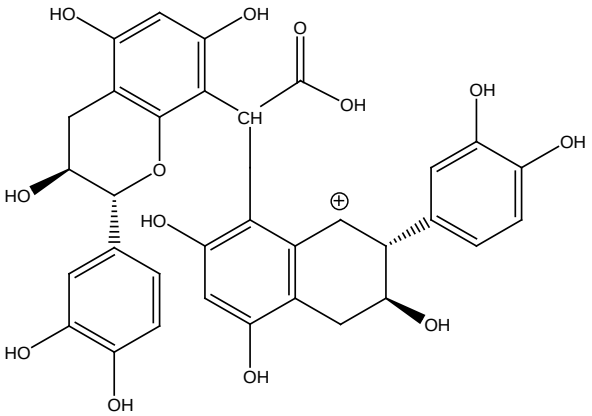


(1)

1.2.2 Glyoxylic aldehyde and glyoxylic acid derived methine bridges:

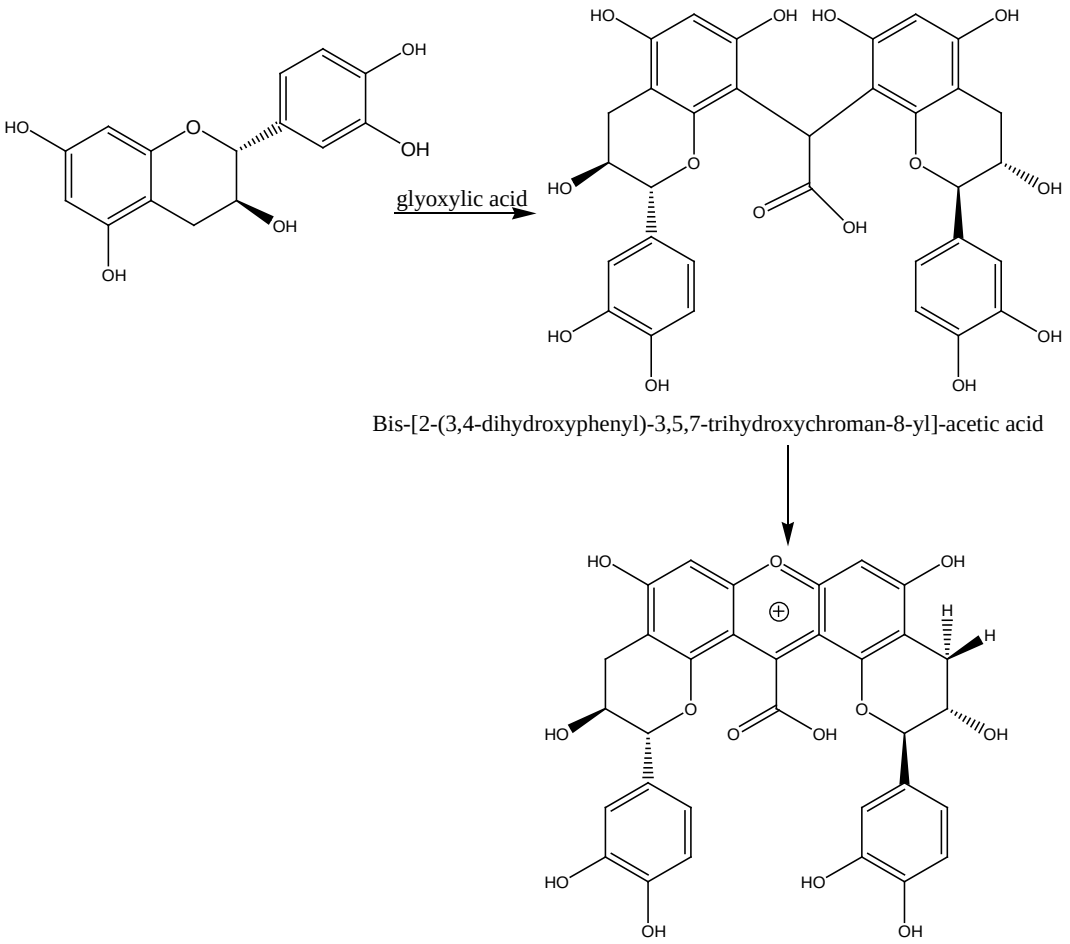
Iron has been found to catalyze wine oxidation[7]. It has been postulated that iron oxidizes tartaric acid to glyoxylic aldehyde and glyoxylic acid, and that these very reactive aldehydes are involved in the polycondensation of catechin and other wine tannins during wine ageing[8].

The carboxy-methine linked dimer of catechin, (bis-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-8-yl]-acetic acid) (**2**) has been synthesized and isolated from the reaction between catechin and glyoxylic acid[7].



(2)

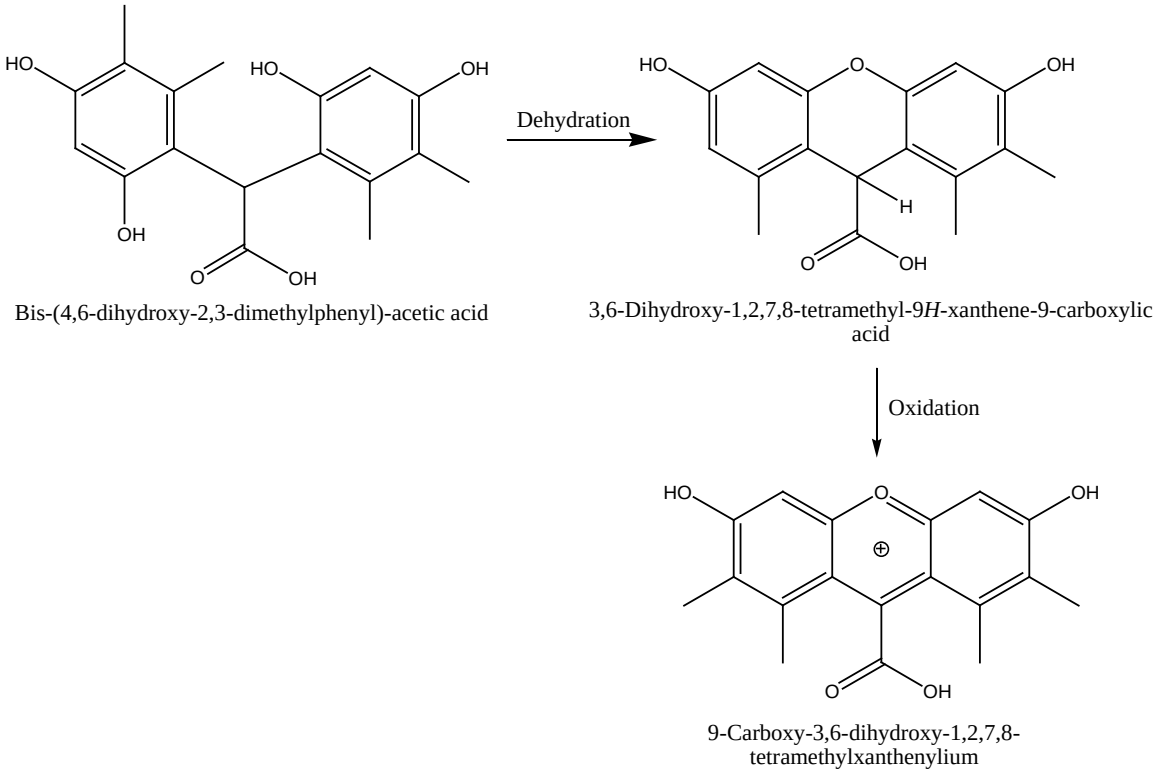
Es-Safi and co-workers isolated and characterised a xanthylium salt from the glyoxylic acid mediated dimerisation of (+)-catechin[9] (**Scheme 1**).



Bis-[2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxychroman-8-yl]-acetic acid

Scheme 1

Formation of this compound is explained in terms of cyclisation of the colourless dimer to a xanthene as described for 9-methylxanthene[10], followed by oxidation of the activated double benzylic methine carboxylic bridge to form the yellowish xanthylium salt (**Scheme 2**).



Bis-(4,6-dihydroxy-2,3-dimethylphenyl)-acetic acid

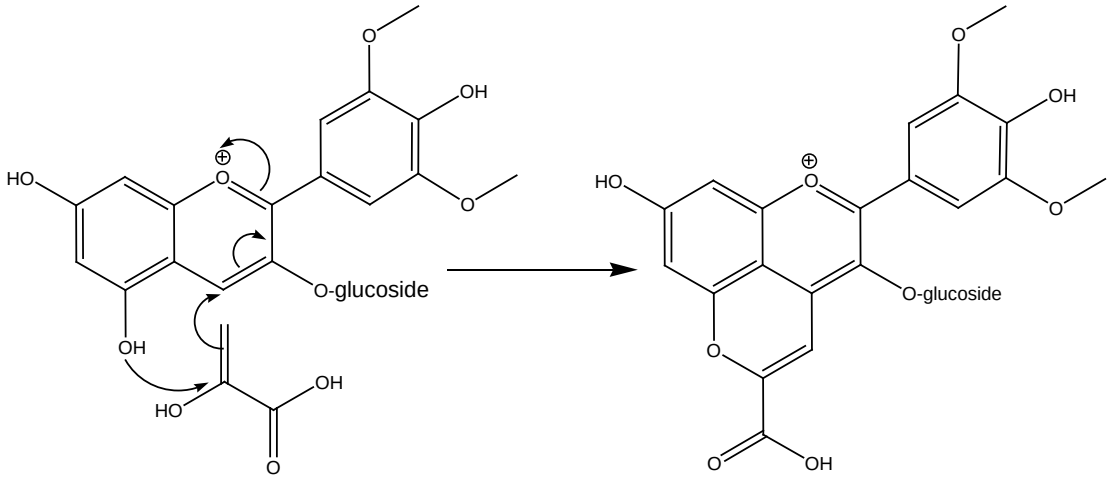
3,6-Dihydroxy-1,2,7,8-tetramethyl-9*H*-xanthene-9-carboxylic acid

9-Carboxy-3,6-dihydroxy-1,2,7,8-tetramethylxanthenylium

Scheme 2

1.2.3 Pyruvic acid derived methine bridges:

Pyruvic acid is a natural ingredient of wine[11]. Anthocyanin pyruvates, from the reaction between anthocyanins and pyruvic acid, are major constituents of wine pigments. After one to two years of ageing, the anthocyanin content decreases significantly and is replaced by new wine pigments. The anthocyanin-pyruvic acid adducts are the main precursors of new wine pigments via reactions with flavanols[12]. Fulcrand and co-workers explained the formation of a stable pigment that originated from malvidin-3-monoglucoside and pyruvic acid by coupling with the enol of pyruvic acid, followed by dehydration and re-aromatization to form pyrano-anthocyanins (**Scheme 3**).



Scheme 3

All vinifera anthocyanins posses a free 5-OH in the A ring[13]. A comparison of the products obtained from two flavylium salts, one with a phloroglucinol A-ring and the other with a resorcinol A-ring, leads to the conclusion that a phloroglucinol A-ring is essential in the formation of genuine wine pigments like xanthylium salts and acetaldehyde bridged anthocyanin-tannin structures[14].

1.3 ANTIOXIDANTS

Epidemiological and other studies suggest that polyphenols, which are relatively abundant in food, have important beneficial effects on human health. These beneficial effects have been attributed to nonspecific radical scavenging properties[15,16].

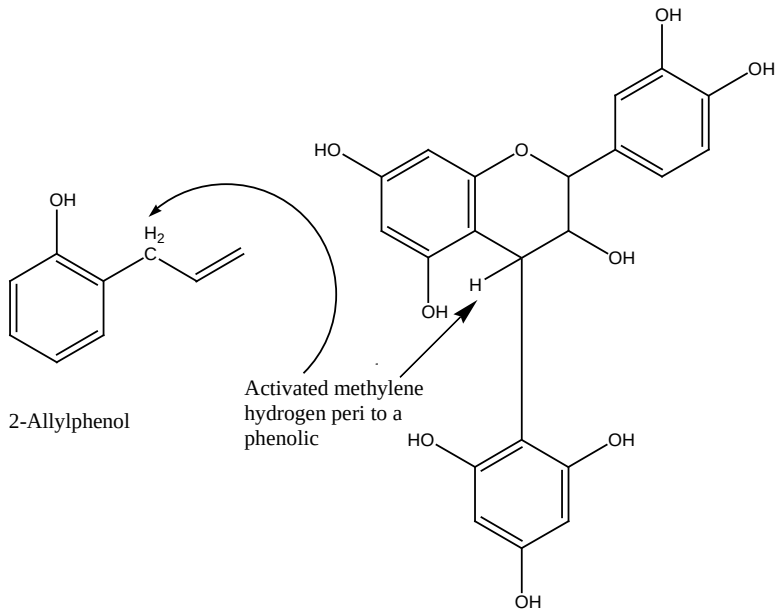
The low incidence of coronary heart disease in France compared to other countries with a comparable diet, has been called the “French paradox”. There is a strong believe that the lower risk of heart disease is due to the higher red wine consumption and associated high antioxidant (flavonoids)[17] intake. It was pointed out that the French paradox has probably more to do with red wine pigments formed during wine maturation than with grape pigments and tannins in young wines[18].

Both alkyl (unpaired electron on carbon, R•) and peroxy radicals (unpaired electron on oxygen, R-O-O•) act as chain carriers in radical chain reactions. To prevent autoxidations and radical damage both these carriers need to be trapped by radical

scavengers. Traditional radical scavengers, including phenols can trap peroxy radicals, but alkyl radicals react too fast and can not be trapped.

Benzylic and allylic hydrogens, other than the hydrogen of hydroxy and amino groups, play an important role in antioxidant activity of phenols and amines[19].

Ohkatsu and coworkers[20] described an *ortho*-substituent effect that allows rapid regeneration of phenols used as radical traps. On the basis of this effect they have proposed and tested several phenols with high performance as radical traps. Some of these phenols were dramatically active against peroxy radicals. By chance they also discovered these very active phenols to be able to trap alkyl radicals[21]. Both the *ortho*-effect and ability to trap alkyl radicals seem to depend on the availability of an activated methylene group *ortho* to the phenolic hydroxy group. It was assumed that the o-methylene group on the *ortho* position acts as a hydrogen donor to regenerate the phenol (**Scheme 4**).

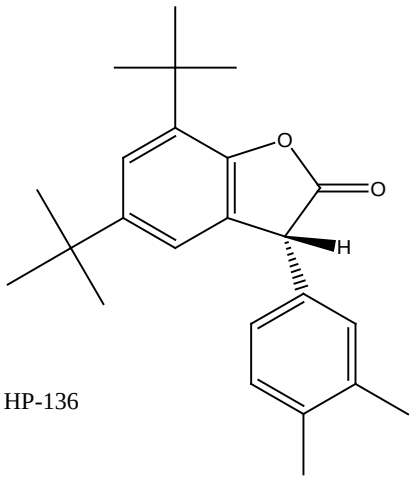


Scheme 4

Activation of the methylene group was provided by an allyl group. 2-Allylphenols proved to be effective alkyl radical trapping agents. Surprisingly it was found that an electron donating substituent such as a methoxy group *para* to the phenol enhances the radical trapping ability and that an electron withdrawing substituent such as acetyl in the *para* position completely destroys the antioxidant activity.

This suggests that the free electron of the phenoxy radical exists as a π-electron instead of a σ-electron, allowing resonance distribution of this electron into the para position. This also suggests that conjugation plays a role in transferring the methylene hydrogen to the phenoxy radical to regenerate the phenol.

It was recently reported that 5,7-di-tert-butyl-3-(3,4-dimethylphenyl)-3H-benzofuran-2-one (**3**) traps both alkoxy and peroxy radicals[22]. This substituted benzofuran-2-one is now commercially available as a powerful radical scavenger and is used in combination with phenolic compounds and phosphites to provide protection polymers against radical degradation (Ciba lactone, Irganox® HP-136)[23].



HP-136

5,7-di-tert-butyl-3-(3,4-dimethylphenyl)-3H-benzofuran-2-one

(3)

Their stabilizing effect is explained by the formation of stable benzofuranonyl radicals by cleavage of the weakly bonded double benzylic hydrogen atom and reaction of these radicals with alkyl radicals to terminate chain reactions[24].

These results are of immense importance to flavanoids and particularly 4-aryl substituted flavonoids (the backbone of tannins) that contain a methylene group activated by a second aryl substituent and an ortho phenolic hydrogen on the A ring. Quinone methides (π –systems) has been postulated to be involved in a variety of reactions of these flavonoids.

1.4 ADHESIVES

Mimosa extract is produced by aqueous leaching of freshly stripped *Acacia mearnsii* bark. The extract has a polyphenolic content of more than 75%. The Mimosa extract is treated with formaldehyde to form adhesives. Formaldehyde reacts with the 6- or 8-position of 5,7-dihydroxyflavonoids to form a methine derivative that will react with another 6- or 8-position of another flavonoid to form a dimer, and eventually a polymer, where the aryl moieties of the monomers are linked by methine bridges. The flavonoid units behave as typical phenols in their reactions with formaldehyde. Reaction takes place mainly on the A-ring (**6**- and **8**-position). Pyrogallol or catechol type B-rings are relatively unreactive[25].

1.5 SYNTHESIS OF BENZOFURAN-2-ONES

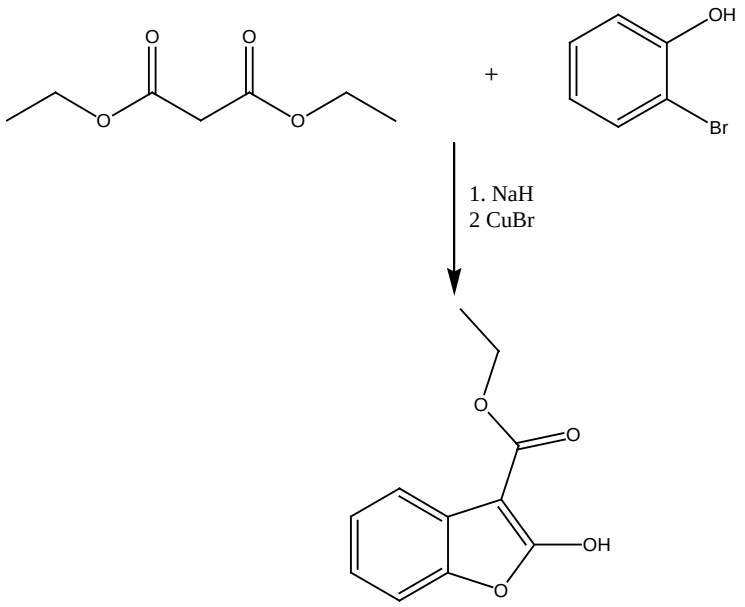
Benzofuran and benzodihydrofuran derivatives are found in several species of higher plants. The vast majority of the few hundred naturally occurring examples were detected in the Asteraceae[26].

Benzofuran derivatives exhibit various physiological activities in living organisms including antibacterial properties, toxicity against fish, allergenic activity, plant growth inhibitory activity, and trembles in cattle and milk sickness in humans[25].

Benzofuran-2-ones are five membered lactones. Of particular interest in terms of antioxidant activity is the 3-hydrogen which is both benzylic and α to a carbonyl group. Benzofuran-2-ones can be envisaged as resulting from the condensation of a phenolic unit with an α-keto acid.

The following synthetic procedures have been described:

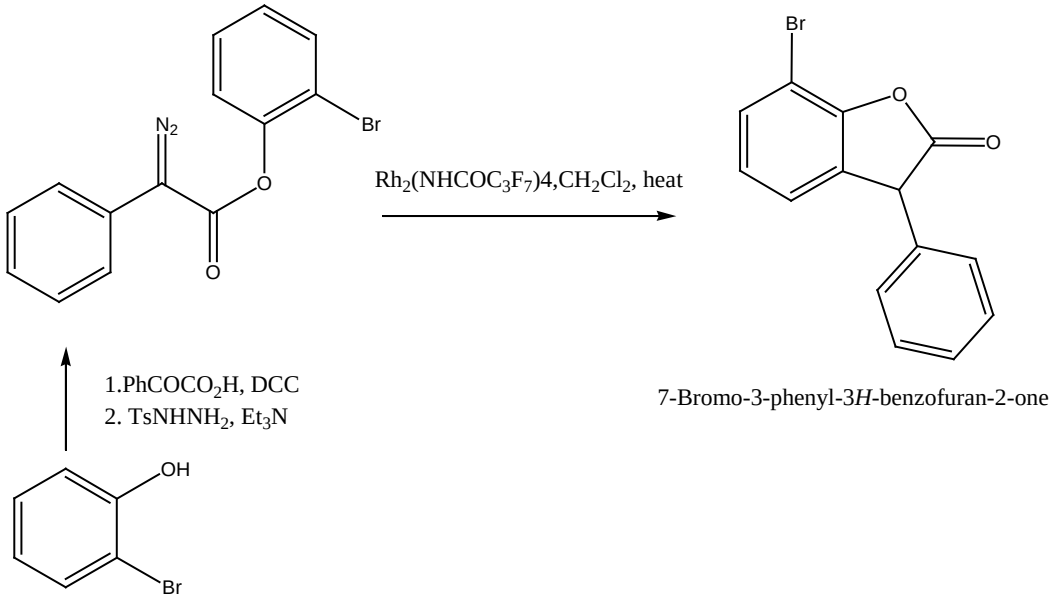
Setsune and co-workers[27] reacted *o*-bromophenoxide with sodium salts of active methylene compounds (diethyl malonate, ethyl acetoacetate and ethyl cyanoacetate) in the presence of copper(I)bromide in dioxane and obtained benzofuran-2-one derivatives. The reaction is accelerated by the phenoxide group of *o*- and *p*-bromophenols (**Scheme 5**).



Ethyl-2-hydroxybenzofuran-3-carboxylate

Scheme 5

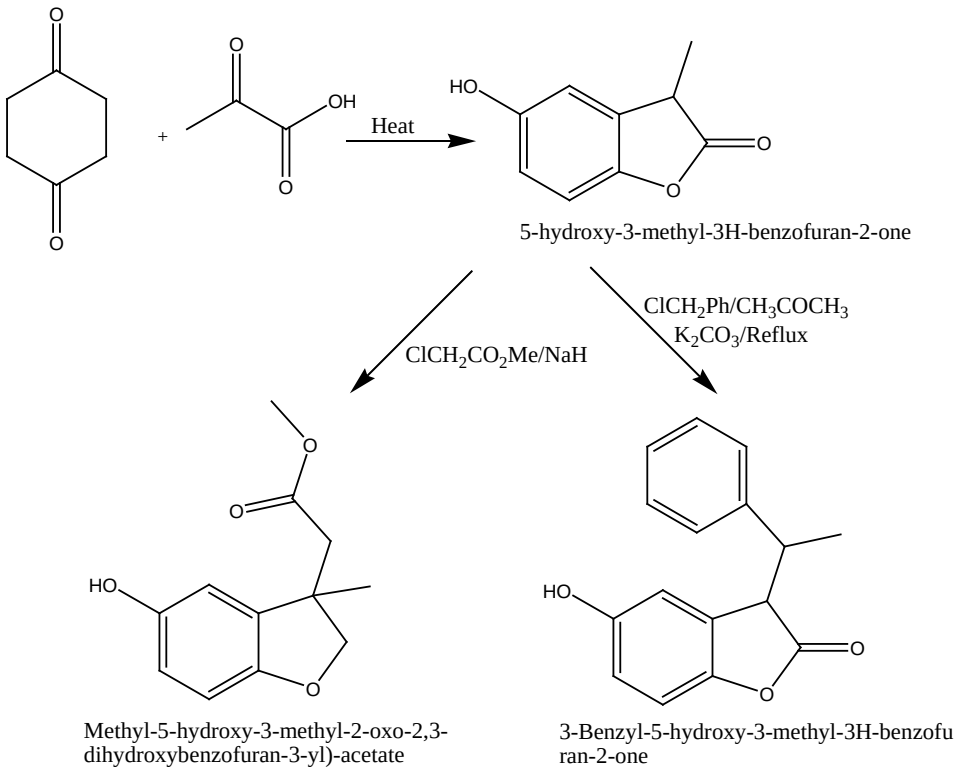
Moody and co-workers[28] used rhodium (II) mediated intramolecular aromatic C-H insertion (from diazocarbonyl compounds) to synthesize benzofuran-2-ones (**Scheme 6**).



7-Bromo-3-phenyl-3*H*-benzofuran-2-one

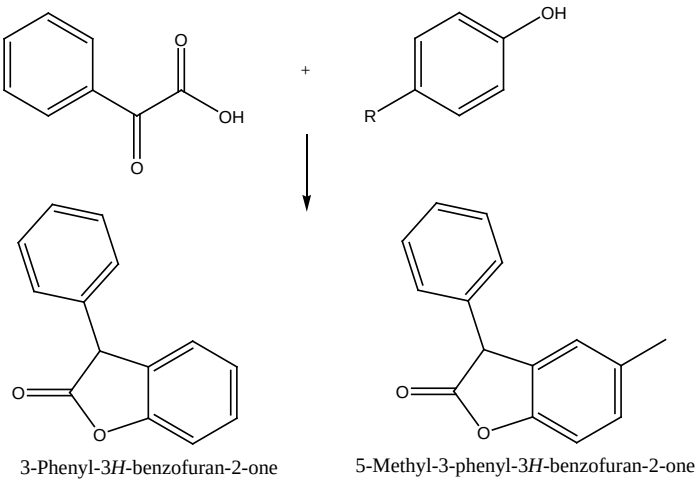
Scheme 6

Luo and coworkers[29] synthesized 5-hydroxy-3-methyl-3H-benzofuranone and 3C-alkylated derivatives from pyruvic acid and 1,4-cyclohexanedione (**Scheme 7**).



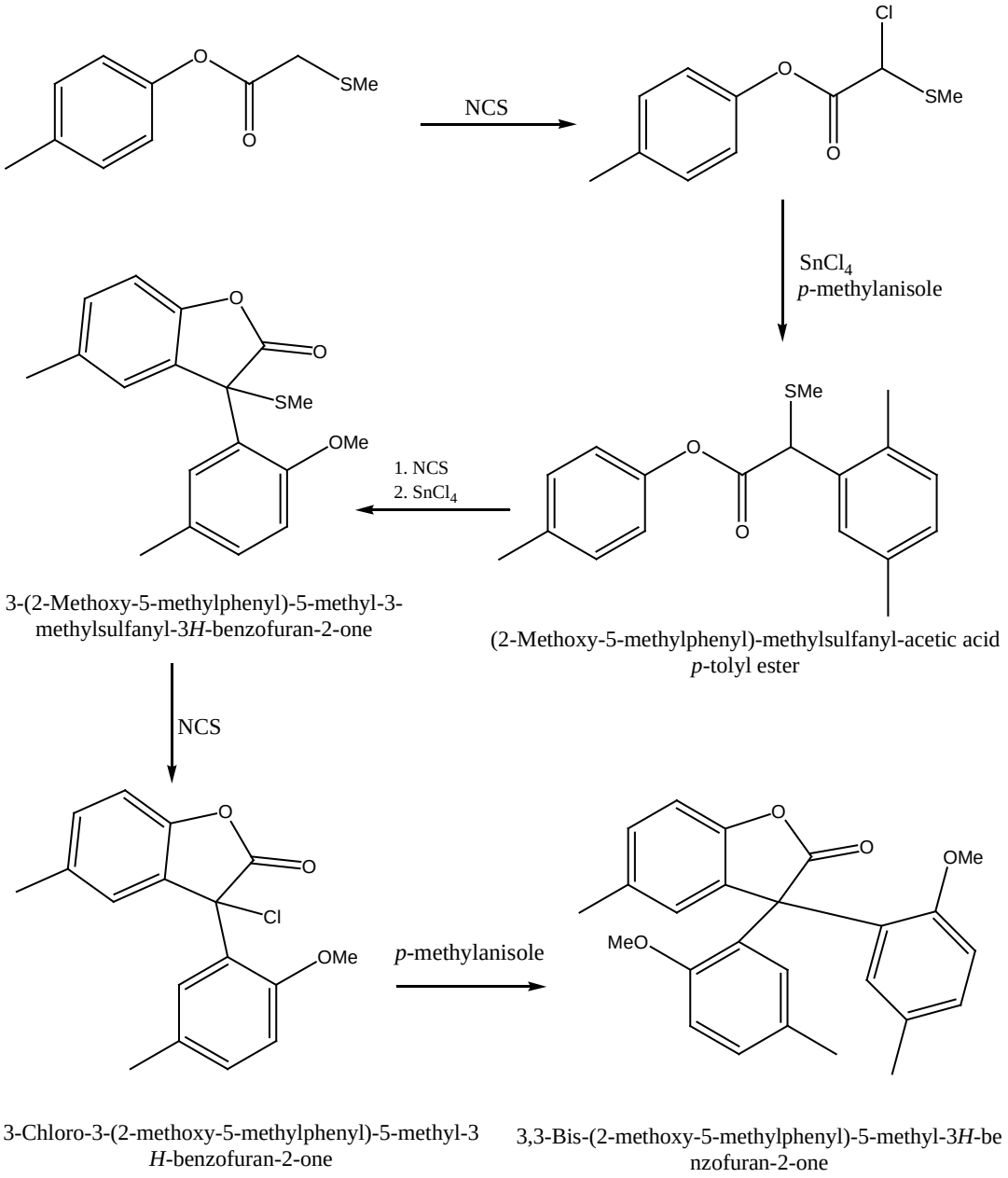
Scheme 7

Magnus and co-workers[30] used the reaction between mandelic acid and phenol to synthesise 3-phenyl-3H-benzofuran-2-one. *p*-Cresol yielded 5-methyl-3-phenyl-3H-benzofuran-2-one (**Scheme 8**).



Scheme 8

Magnus and co-workers coupled a second phenyl group in the 3-position to obtain a bis-3-phenylbenzofuran-2-one (**Scheme 9**).

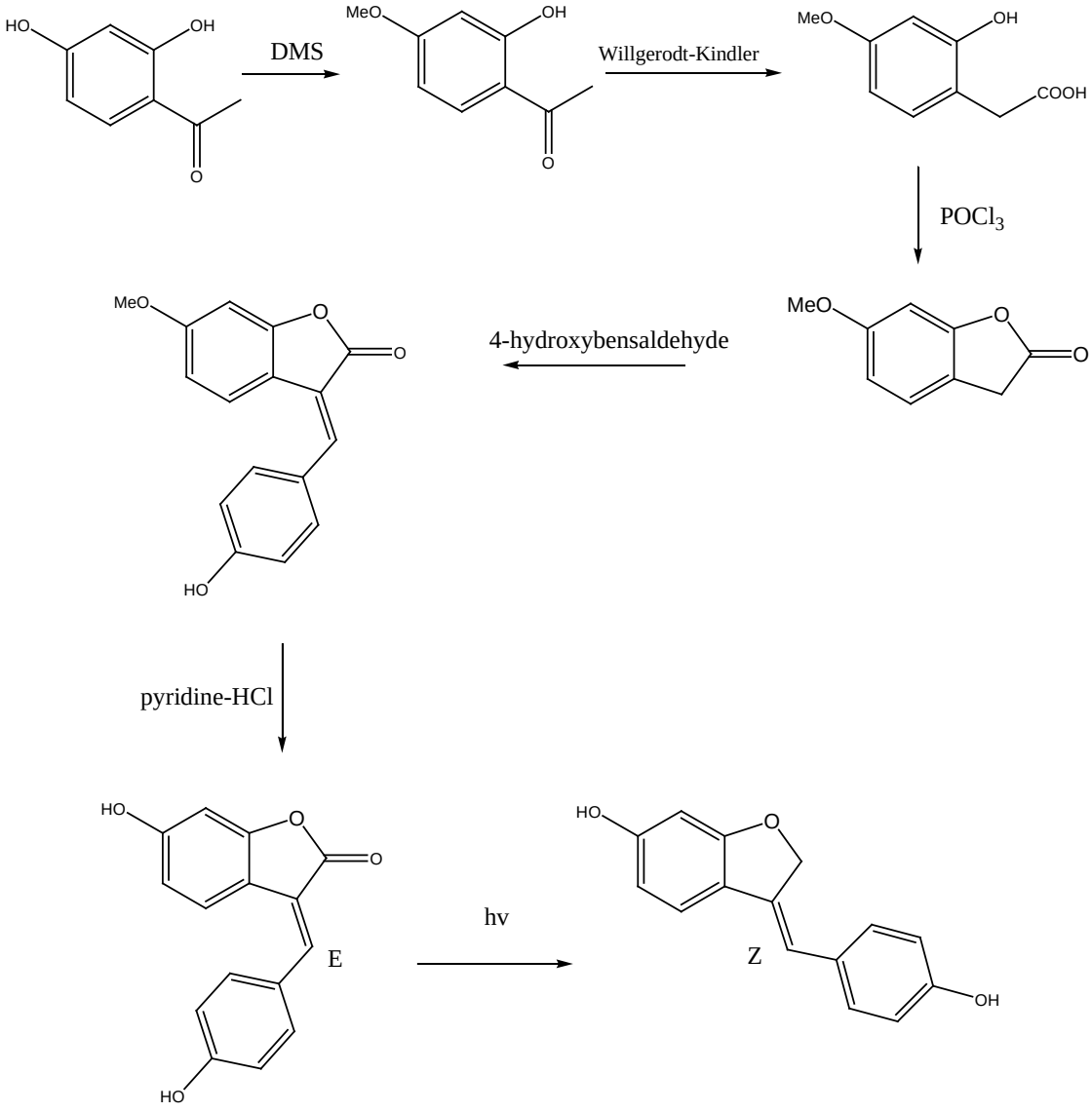


Scheme 9

1.6. ISOAURONES

Aurones and isoaurones are yellow pigments of plants that are structurally related to flavonoids and benzofurans [31]. Venkateswarlu and co-workers[32] synthesized 6-hydroxy-3-[(4-hydroxyphenyl) methylene]benzo[b]furan-2-one (isoaurone) from 6-

methoxy-2(3H)-benzofuranone (**Scheme 10**). The authors proved that isoaurostatin, a novel topoisomerase-I inhibitor does not contain an isoaurone structure as had been reported previously[33], but is in fact daidzein, an isoflavone. Isoaurones show a strong IR carbonyl absorption at 1750 cm^{-1} compared to 1630 cm^{-1} in the case of isoflavones.



Scheme 10

^{1}H NMR analysis showed the product to be a mixture of the E- and Z-isomers in a 90:10 ratio. This ratio was confirmed with HPLC. The method requires demethylation to obtain the free phenolic isoaurone. Photolysis of the pure Z-isomer yielded a

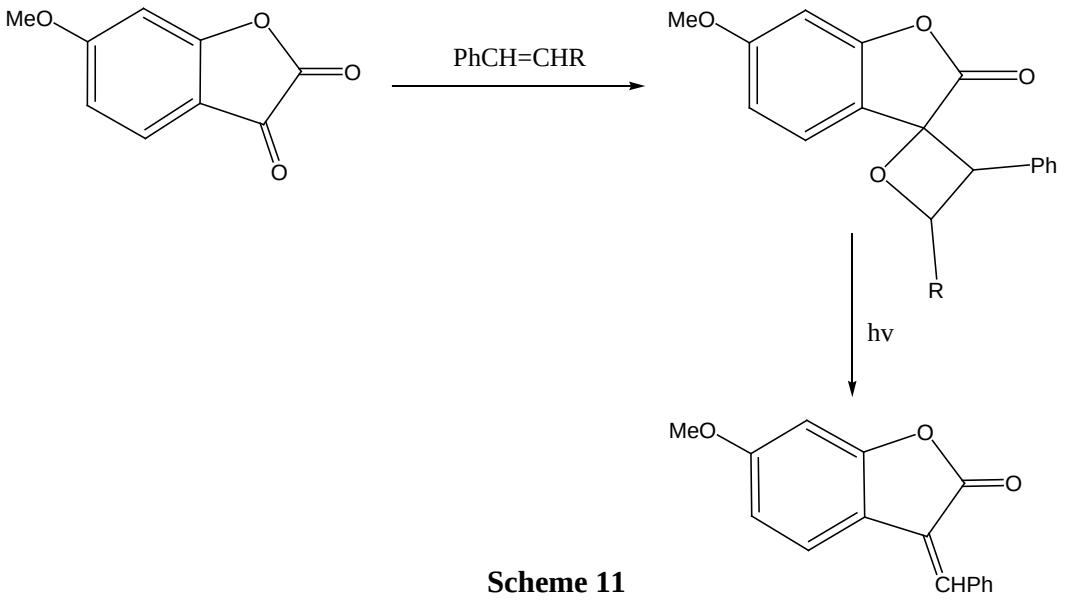
photostationary mixture of the two isomers in the ratio of Z/E of 90/10. The Z and E configuration was assigned on the premise that the *ortho* protons (H-2' and H-6') of the pendant aryl group in the Z-isomer will be deshielded by the carbonyl group and will give downfield resonances relative to the E-isomer[34] (Table 1).

Chemical shifts of H-2' and H-6' in the NMR spectra of Z- and E-isomers of isoaurones:

Table 1: ^{1}H NMR of Z and E-isomer

ISOMER	CHEMICAL SHIFT (δ)/ DMSO-d_6	CHEMICAL SHIFT (δ)/ DMSO-d_6
	H-10	H-2'/ H-6'
Z	7.72 (s)	8.16 (d)
E	7.50 (s)	7.65 (d)

Irradiation of 6-methoxybenzofuran-2,3-dione in a benzene solution with excess styrene or β-ethoxystyrene gave 6-methoxyisoaurone. The reaction was postulated to take place via [2+2] cycloaddition of styrene to the 3-carbonyl group to give an oxetane intermediate (**Scheme 11**).



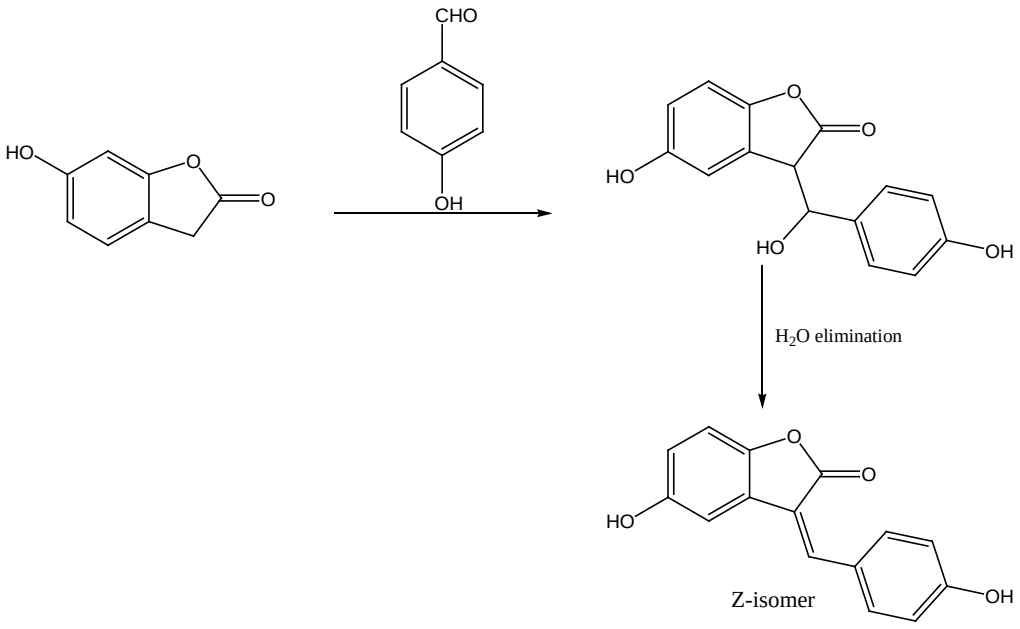
Scheme 11

The ^{13}C NMR resonance of the carbonyl carbon was used diagnostically by Gray and co-workers to distinguish the isoaurone from other flavonoids (Table 2).

Table 2: ^{13}C NMR chemical shifts of carbonyl carbon of flavonoids ($CDCl_3$)

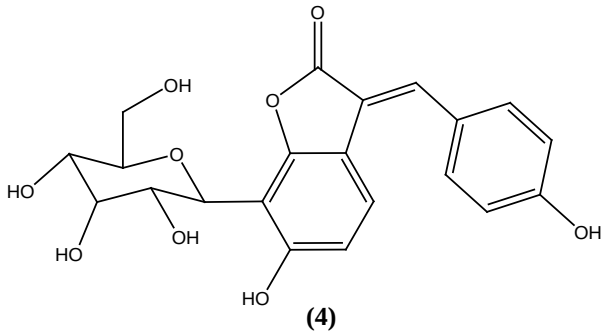
Flavonoid	^{13}C absorption of carbonyl group (δ)
Flavone	175-177
Isoflavone	175
Coumarin	160
Aurone	183
Isocoumarin	162
Isoaurone	170

Schildknecht and coworkers[35] isolated marginalin, an isoaurone from the water beetle, *Dytiscus marginalis*. Barbier[36] synthesized Z-marginalin by KOH catalyzed addition of *p*-hydroxybenzaldehyde to the methylene group of 5-hydroxybenzofuran-2-one. Consequently, the isoaurone isolated by Schildknecht, who reported no stereochemical data, is the E-isomer. Barbier postulated that the E-isomer was the thermodynamically more stable isomer. He attributed the fact that he isolated only the Z-isomer to the 5-OH group, which likely directed the elimination of the intermediary secondary alcohol to give only this isomer.



Scheme 12

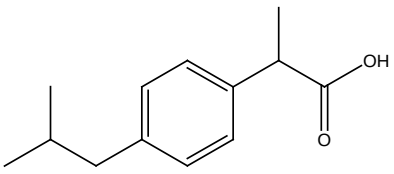
Pterocarposiden (**4**), an isoaurone glucoside, was isolated from the hardwood of *Pterocarpus marsupium*[37]. The structure has been arrived at using spectroscopic data which is in agreement with the data discussed here (IR 1740 cm^{-1} isoaurone carbonyl), ^{13}C NMR (CD_3OD) δ 170.6 (isoaurone carbonyl) ^{1}H NMR (CD_3OD) δ 7.57 (olefinic proton). The authors did not make an explicit stereochemical assignment. The H-2'/ H-6' resonances at δ 7.61 indicate E-configuration (see Table 1).



(4)

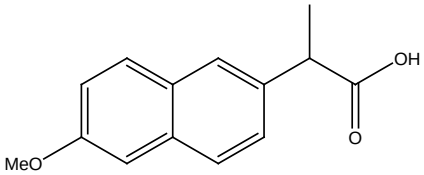
1.7. α-ARYL CARBOXYLIC ACIDS AND ESTERS

Benzofuran-2-ones are lactones of *o*-hydroxy α-aryl acids. Synthesis of acyclic α-aryl carboxylic acids and their derivatives are of interest in medicinal chemistry. These acids are structural components of pharmaceutical compounds that are used widely to treat pain and inflammatory diseases. Commercial examples are ibuprofen (**5**), ketoprofen (**7**), naproxen (**6**) and flurbiprofen (**8**). These compounds act by inhibition of the cyclooxygenase system[38] .



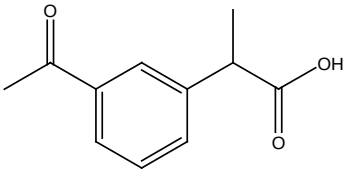
Ibuprofen

(5)



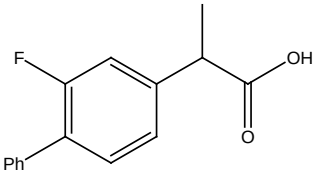
Naproxen

(6)



Ketoprofen

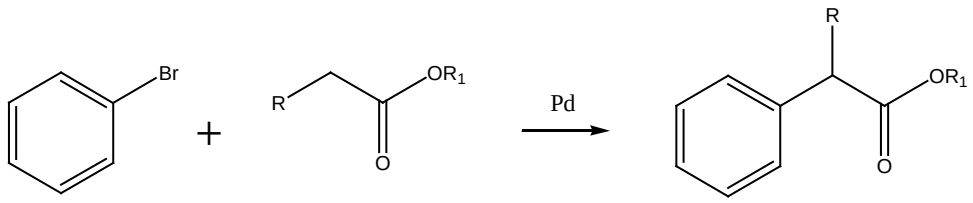
(7)



Flubiprofen

(8)

Buchwald and coworkers[39] and Hartwig and coworkers[40] independently developed palladium-catalyzed procedures for α-arylation of esters. No examples of free phenolic aryl substituents were reported. The reaction takes place via the enol of the ester.



Scheme 13

1.8. REFERENCES

1. Hillis, W. E.; Urbach, G. *Journal of Applied Chemistry* **1959,** 9, 474.
2. Timberlake, C. F.; Bridle, P. *American Journal of Enology and Viticulture* **1959**, 27, 97.
3. Bakker, J.; Picinelli, A.; Bridle, P *Vitis* **1993**, 32, 111.
4. Rivas-Gonzalo, J. C.; Bravo-Haro, S.; Santos-Buelga, C. *Journal of Agriculture and Food Chemistry* **1996**, 43, 1444.
5. Dallas, C.; Ricardo-da-Silva, J. M.; Laureano, O. *Journal of the Science of Food and Agriculture* **1996**, 70, 493.
6. Pissarra, J.; Rivas-Gonzalo, J.; De Freitas, V. *Journal of Food Science* **2003**, 68, 476-481.
7. Cacho, J.;Castells, J. E.; Esteban, A.; Laguna, B.; Sagrista, N. *American Journal of Enology and Viticulture **1995***, 46, 380.
8. Fulcrand, H.; Cheynier, V.; Oszmianski, J.; Moutounet, M. *Phytochemistry* **1997**, 46, 223.
9. Es-Safi, N.; Le Guerneve, C.; Labarbe, B.; Fulcrand, H.; Cheynier, V.; Moutounet, M. *Tetrahedron Letters* **1999**, 40, 5869.
10. White, J.; Foo, L. Y. *Tetrahedron Letters* **1990**, 31, 2789.
11. Fulcrand, H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. *Phytochemistry* **1998**, 47, 1401.

12. Mateus, N.; Oliveira, J.; Pissarra, J.; Gonzales-Paramas, Rivas-Gonzalo, J. C.; Santos-Buelga, C.; Silva, A. M. S.; De Freitas, V. A. P. *Food Chemistry* **2006**, 97, 689.
13. Brouillard, R.; Chassaing, A.; Fougerousse, A. *Phytochemistry* **2003**, 64, 1179.
14. Escribano-Bailon, T.; Dangles, O.; Brouillard, R. *Journal of Agriculture and Food Chemistry* **1996**, 41, 1583.
15. Soleas, G. J.; Grass, L.; Josephy, P. D.; Goldberg, D. M.; Diamandis, E. P. *Clinical Biochemistry* **2002**, 35(2), 119-124.
16. Penny, M. et. al.; *American Journal of Medicine* **2002**, 113(3B), 71.
17. St. Leger, A. S.; Cochrane, A. L.; Moore, F. *Lancet* **1979**, 1, 1017.
18. Brouillard, R.; George, F.; Fougerousse, A. *Biofactors* **1997**, 6, 403.
19. Nakamura. T.; Nishi, H.; Kokusenya, Y.; Hirotu, K.; Miura, Y. *Chemical Pharmaceutical Bulletin* **2000**, 48, 235.
20. Ohkatsu, Y.; Ishikawa, S.; Tobita, E.; *Polymer Degradation Stability* **2000**, 67, 542.
21. Ohkatsu, Y.; Matsuura, T; Yamamoto, M. *Polym Degradation Stability* **2003**, 81, 151.
22. Kroehnke, C. *Polyolefins* **1997**, 10, 555.
23. Pauquet, P. *Journal of Macromoecular Science Pure Applied Chemistry* **1999**, A35, 1717.
24. Mar'in, A.; Greci, L.; Dubs, P. *Degradation Stability* **2002**, 76, 489.
25. Pizzi, A.; Scharfetter, H. O. *Journal of Applied Polymer Science* **1978**, 22, 1745.
26. Proksch, P.; Rodriques, E. *Phytochemistry* **1983**, 22, 2335.
27. Setsune, J.; Matsukawa, K.; Kitao, T. *Tetrahedron Letters* **1982**, 23, 663.
28. Moody, C. J.; Doyle, K. J.; Elliot, M. C.; Mowlem, *Journal of Pure & Applied Chemistry* **1994**, 66, 2107.
29. Luo, W.; Yu, Q.; Holloway, H. W.; Parrish, D.; Greig, N. H.; Brossi, A. *Journal of Organic Chemistry* **2005**, 70, 6171.
30. Magnus, P.; Venable, J. D.; Shen, L.; Lynch, V. *Tetrahedron Letters* **2005**, 46, 707.
31. Harborne, J. B. *The Flavonoids, advances in Research since **1986***, Chapman and Hall, London, 340.

32. Venkateswarlu, S.; Panshagnula, G. K.; Guraiah, B. G.; Subbaraju, G. V. *Tetrahedron* **2005**, 61, 3013-3017.
33. Suzuki, K.; Yahara,S.; Maehata, K.; Uyeda, M. *Journal of Natural Products* **2001**, 64, 204.
34. Marathe, K. G.; Byrne, M. J.; Vidwans, R. N. *Tetrahedron* **1966**, 22, 1789.
35. Schildknecht, H.; Koerning, W.;Siewerdt, D.; Krauss, D. *Liebigs Annalen Chemie* **1970**, 734, 116.
36. Barbier, M. *Liebigs Annalen Chemie* **1987**, 545.
37. Handa, S. S.; Singh, R.; Maurya, R.; Satti, N. K.;Suri, K. A.; Suri, O. P. *Tetrahedron Letters* **2000**, 41, 1579.
38. Vanne, J. R.; Botting, R. M. *Inflamm Res* **1995**, 44, 1.
39. Moradi, A. W.; Buchwald, S. L. *Journal of American Chemical Society* **2001**, 123. 7996.
40. Jørgensen, M.; Lee, S,; Liu, X.; Wolkowski, J. P.; Hartwig, J. F. *Journal of American Chemical Society* **2002**, 124, 12557.

1. Hillis, W. E.; Urbach, G. *Journal of Applied Chemistry* **1959,** 9, 474

2 Timberlake, C. F.; Bridle, P. *American Journal of Enology and Viticulture* **1959**, 27, 97

3 Bakker, J.; Picinelli, A.; Bridle, P *Vitis* **1993**, 32, 111

4 Rivas-Gonzalo, J. C.; Bravo-Haro, S.; Santos-Buelga, C. *Journal of Agriculture and Food Chemistry* **1996**, 43, 1444

5 Dallas, C.; Ricardo-da-Silva, J. M.; Laureano, O. *Journal of the Science of Food and Agriculture* **1996**, 70, 493

6 Pissarra, J.; Rivas-Gonzalo, J.; De Freitas, V. *Journal of Food Science* **2003**, 68, 476-481

7 Cacho, J.;Castells, J. E.; Esteban, A.; Laguna, B.; Sagrista, N. *American Journal of Enology and Viticulture **1995**, 46, 380*

8 Fulcrand, H.; Cheynier, V.; Oszmianski, J.; Moutounet, M. *Phytochemistry* **1997**, 46, 223-227

9 Es-Safi, N.; Le Guerneve, C.; Labarbe, B.; Fulcrand, H.; Cheynier, V.; Moutounet, M. *Tetrahedron Lett.* **1999**, 40, 5869-5872

10 White, J.; Foo, L. Y. *Tetrahedron Lett.* **1990**, 31, 2789-2792.

11Fulcrand, H.; Benabdeljalil, C.; Rigaud, J.; Cheynier, V.;Moutounet, M. *Phytochemistry* **1998**, 47, 1401-1407

12Mateus, N.; Oliveira, J.; Pissarra, J.; Gonzales-Paramas, Rivas-Gonzalo, J. C.; Santos-Buelga, C.; Silva, A. M. S.; De Freitas, V. A. P. *Food Chemistry* **2006**, 97, 689-695

13Brouillard, R.; Chassaing, A.; Fougerousse, A. *Phytochemistry* **2003**, 64, 1179-1186

14 Escribano-Bailon, T.; Dangles, O.; Brouillard, R. *J Agric Food Chem* **1996**, 41, 1583-1592

15Soleas, G. J. et. al. *Clinical Biochemistry* **2002**, 35(2), 119-124., Penny, M. et. al.; *Am. J. Med*. **2002**, 113(3B), 71-88

[16] Penny, M. et

17 St. Leger, A. S.; Cochrane, A. L.; Moore, F. *Lancet* **1979**, 1, 1017-10200

18Brouillard, R.; George, F.; Fougerousse, A. *Biofactors* **1997**, 6, 403-410

19. Nakamura. T.; Nishi, H.; Kokusenya, Y.; Hirotu, K.; Miura, Y. *Chem. Pharm. Bull.* **2000**, 48, 235

20Ohkatsu, Y.; Ishikawa, S.; Tobita, E.; *Polym Degrad Stab* **2000**, 67, 542-545

21 Ohkatsu, Y.; Matsuura, T; Yamamoto, M. *Polym Degrad Stab* **2003**, 81, 151-156

22 Kroehnke, C. *Polyolefins* **1997**, 10, 555

23Pauquet, P. *J Macromol Sci Pure Appl Chem* **1999**, A35, 1717

24 Mar'in, A.; Greci, L.; Dubs, P. *Polym Degrad Stab* **2002**, 76, 489-494

25 Pizzi, A.; Scharfetter, H. O. *J Applied Polymer Science* **1978**, 22, 1745

26 Proksch, P.; Rodriques, E. *Phytochemistry* **1983**, 22, 2335-2348

27 Setsune, J.; Matsukawa, K.; Kitao, T. *Tetrahedron Letters* **1982**, 23, 663-666

28 Moody, C. J.; Doyle, K. J.; Elliot, M. C.; Mowlem, *J. Pure & Appl Chem* **1994**, 66, 2107-2110

29 Luo, W.; Yu, Q.; Holloway, H. W.; Parrish, D.; Greig, N. H.; Brossi, A. *J Org Chem* **2005**, 70, 6171-6176

30 Magnus, P.; Venable, J. D.; Shen, L.; Lynch, V. *Tetrahedron Lett.* **2005**, 46, 707-710

31 Harborne, J. B. *The Flavonoids*; Chapman and Hall, London, **1988**, 340.

32 Venkateswarlu, S.; Panshagnula, G. K.; Guraiah, B. G.; Subbaraju, G. V. *Tetrahedron* **2005**, 61, 3013.

33 Suzuki, K.; Yahara,S.; Maehata, K.; Uyeda, M. *J Nat Prod* **2001**, 64, 204-207.

34Marathe, K. G.; Byrne, M. J.; Vidwans, R. N. *Tetrahedron* **1966**, 22, 1789-1795

35Schildknecht, H.; Koerning, W.;Siewerdt, D.; Krauss, D. *Liebigs Ann Chem* **1970**, 734, 116

36Barbier, M. *Liebigs Ann Chem* **1987**, 545-546

37 Handa, S. S.; Singh, R.; Maurya, R.; Satti, N. K.;Suri, K. A.; Suri, O. P. *Tetrahedron Letters* **2000**, 41, 1579-1581

38Vanne, J. R.; Botting, R. M. *Inflamm Res* **1995**, 44, 1-10

39 Moradi, A. W.; Buchwald, S. L. *J Am Chem Soc* **2001**, 123. 7996-8002

40 Jørgensen, M.; Lee, S,; Liu, X.; Wolkowski, J. P.; Hartwig, J. F. *J Am Chem Soc*, **2002**, 124, 12557-12565

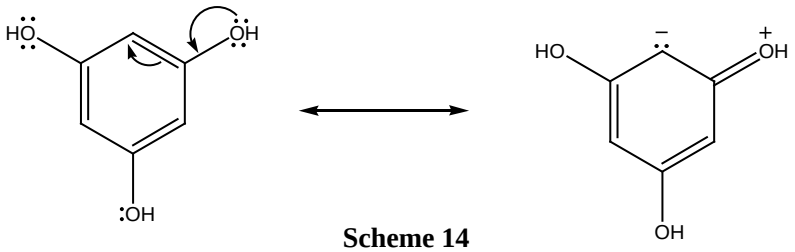
CHAPTER 2

2.1 INTRODUCTION

This work investigates the formation of α-keto acid derived methine bonds (one carbon atom link) between polyphenolic aromatic moieties. As indicated in the literature review, a better understanding of the reaction between electrophilic carbonyl groups and nucleophilic aromatic groups to form polyphenolic diaryl derivatives is of importance to a variety of practical and theoretical applications including formation of wine pigments, adhesives, antioxidants, benzofuran-2-ones, isoaurones and α-aryl carboxylic acids.

2.1.1 CARBANION BEHAVIOUR OF PHENOLS

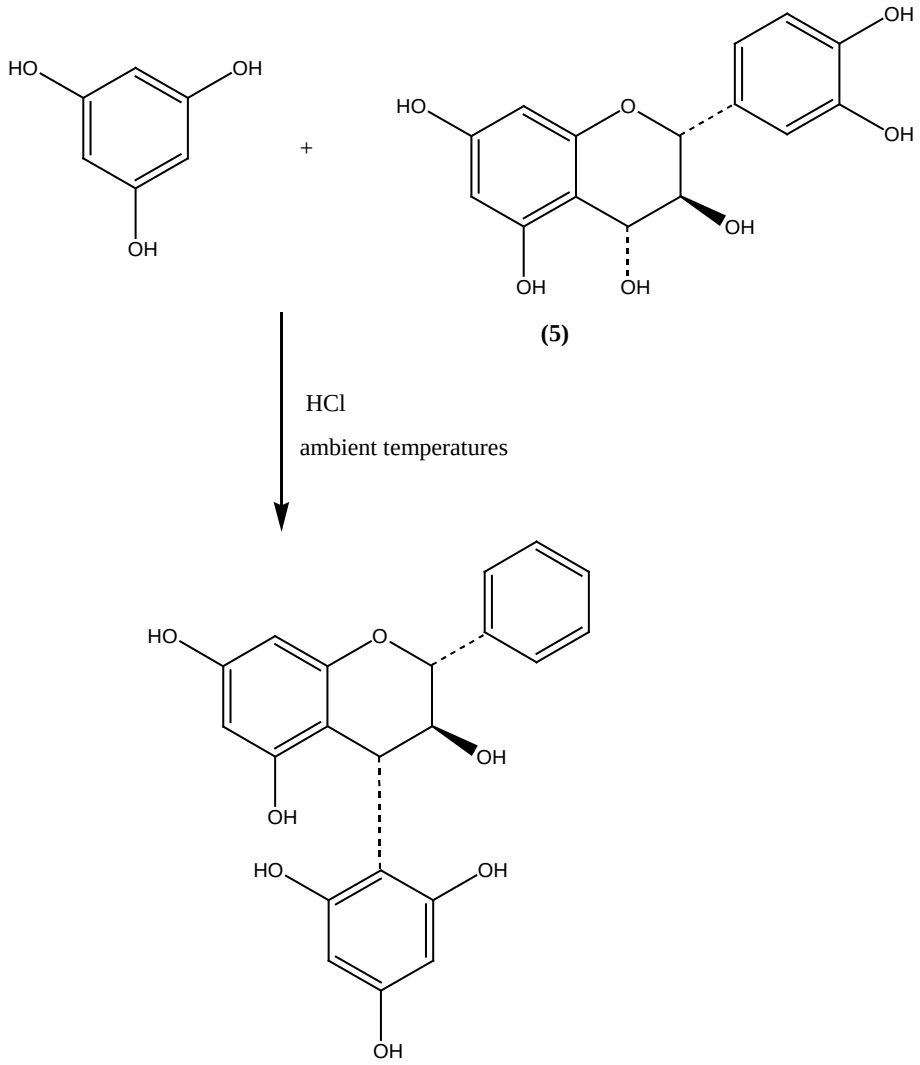
The hydroxy groups of phloroglucinol and other polyhydroxybenzenes donate electrons to the aromatic ring to increase the nucleophilicity of the aromatic carbons. Polyphenols thus become ambident nucleophiles that can react either via oxygen or carbon and have the ability to form new carbon-carbon bonds with suitable electrophiles. Electron flow from the hydroxy group into the ring, places a partial negative charge in the ring to increase its nucleophilicity[1] (**Scheme14**). The ring carbons can thus react like carbanions with electrophiles.



Scheme 14

As expected, polyhydroxybenzenes and their ethers are extremely reactive towards electrophiles. Roux and co-workers[2] developed a biomimetic synthesis of 4-arylflavan-3-

ols (**2.1**), biflavonoids and triflavonoids based upon the acid catalyzed generation of 4-carbocations from flavan-3,4-diols (**5**) of known absolute configuration followed by stereoselective attack by the strongly nucleophilic rings of polyhydroxylated aromatic rings (**Scheme 15**).

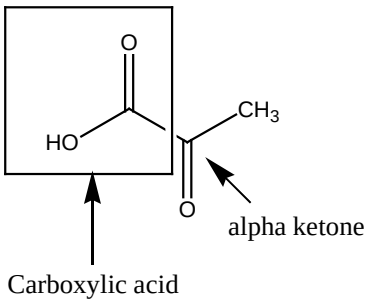


Scheme 15

2.1.2 CHEMISTRY OF α-KETO ACIDS

2.1.2.1 Electrophilicity of α-keto acids:

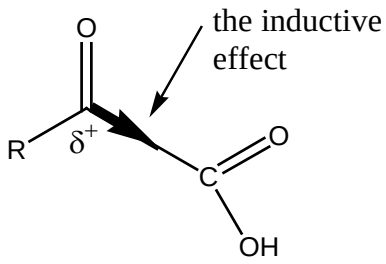
α-Keto- or α-oxo acids have contiguous ketone and carboxylic acid functional groups (**9**).



(9)

The enhanced reactivity of α-dicarbonyl compounds towards nucleophilic attack[3] derives from the proximity of two carbonyl double bonds.

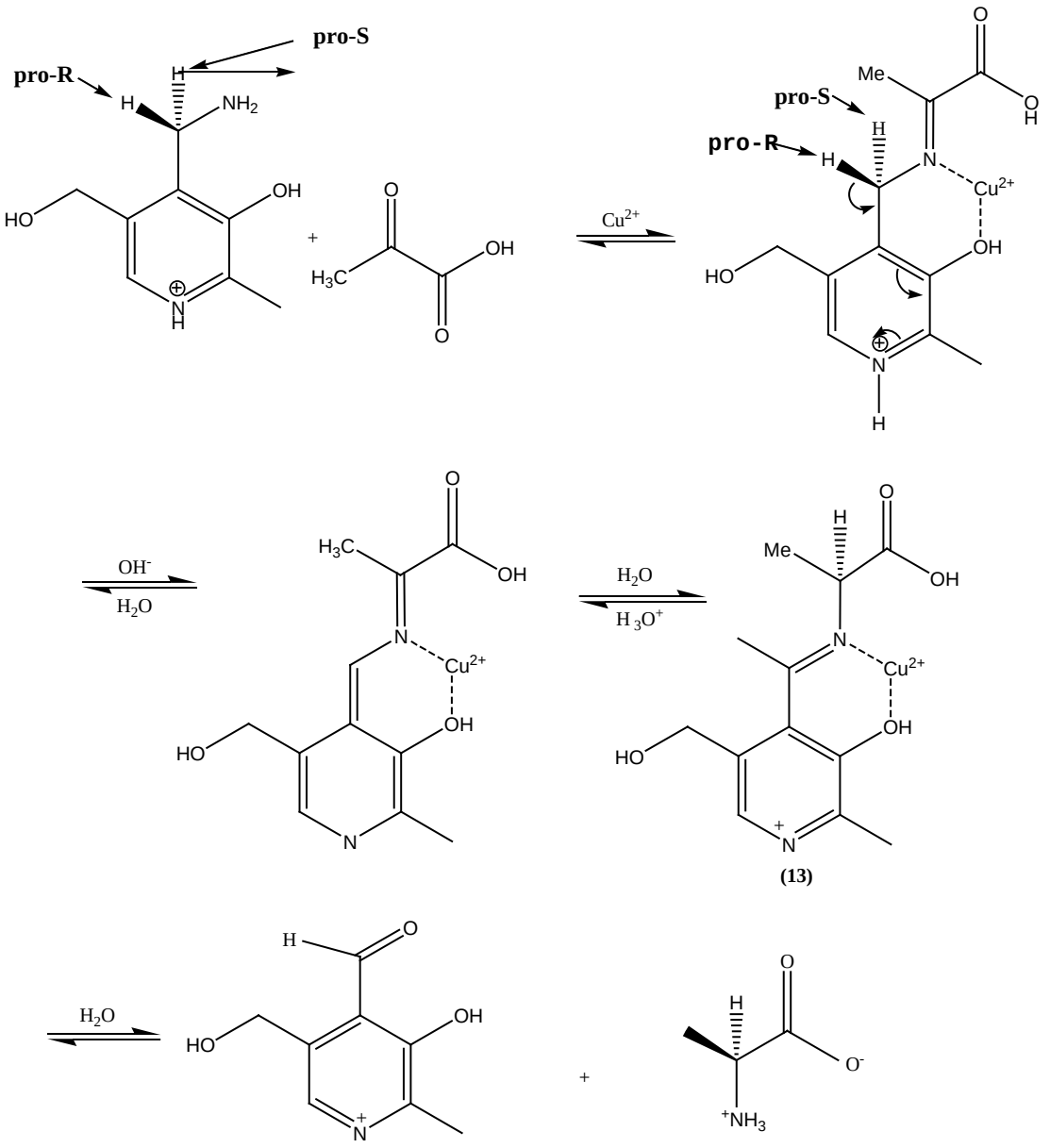
The carboxylic group withdraws carbonyl oxygen electrons from the α-carbonyl carbon causing electron deficiency and enhanced reactivity (electrophilicity) towards nucleophiles (**10**).



(10)

Addition of Grignard reagents to α-keto esters occur selectively at the carbonyl group and hydride reducing agents show comparable selectivity[4].

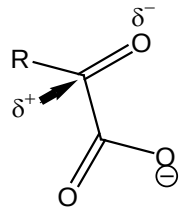
The reactivity of the carbonyl group in pyruvic acid plays an important role in amino acid synthesis. Condensation of the α-carbonyl group with the primary amino group of pyridoxamine followed by a stereospecific enzyme mediated proton pro-transfer and hydrolysis of the resulting imine to an amino acid is shown below[5] (**Scheme 16**).



Scheme 16

2.1.2.2 Acidity of α-keto acids:

The α-oxo oxygen of α-keto acids stabilizes the carboxylate negative charge through induction (**11**):



(11)

The acidity of oxo carboxylic acids is thus increased by the electron withdrawing effect of the oxo-group. Pyruvic acid (pKa 2.5) is more acidic than acetic acid (pKa 4.8) due to this inductive effect.

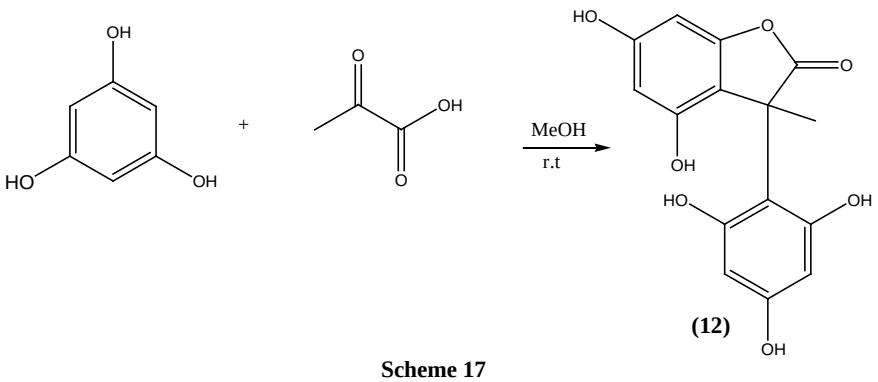
On the other hand, the pKa of a dicarboxylic acid is generally less than the dissociation constant of acetic acid. The presence of an α-carboxylate ion reduces the acidity of an acid. This effect is associated with electrostatic repulsion of the two negative charges on two adjacent carboxylate ions.

2.2 REACTIONS OF PHLOROGLUCINOL WITH α-KETO ACIDS

As part of our ongoing investigation into the importance and synthetic potential of the reactions between polyphenols and α-keto acids, we investigated the reaction between phloroglucinol and a variety of α-keto acids that occur naturally.

2.2.1 Pyruvic acid

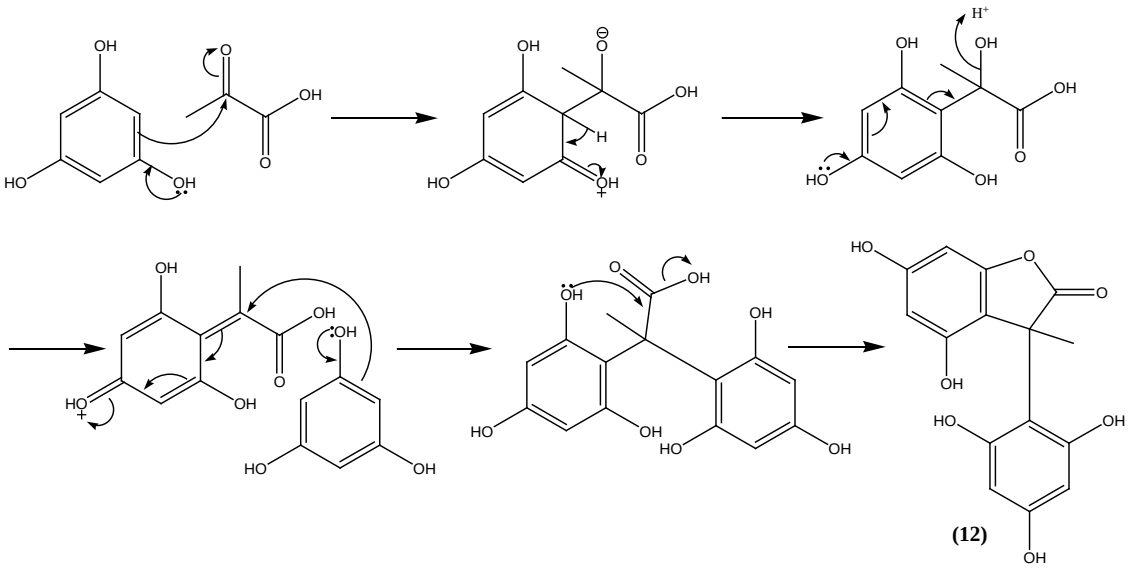
Phloroglucinol reacts with pyruvic acid in methanol to give 4, 6-dihydroxy-3-methyl-3-(2, 4, 6-trihydroxyphenyl)-1-benzofuran-2(3H)-one (**12**) (**Scheme 17**). Pyruvic acid is a strong enough acid to catalyze the reaction.



Scheme 17

The mechanism is given in **Scheme 18**. The salient features are:

1) Attack of phloroglucinol via carbon at the α-carbonyl group of pyruvic acid to give a 2-hydroxy-2-aryl pyruvic acid derivative with a benzylic hydroxyl group.
2) Substitution of the benzylic hydroxyl group by a second phloroglucinol group, again via carbon to form a 2, 2-diaryl pyruvic acid derivative. The reaction may take place via quinone methide after elimination of water (S_N1 mechanism) or via direct S_N2 substitution. Benzylic hydroxyl groups are known to be unstable and difficult to isolate under acidic conditions.
3) Intramolecular esterification of the 2, 2-diaryl acid to form a five membered lactone ring.



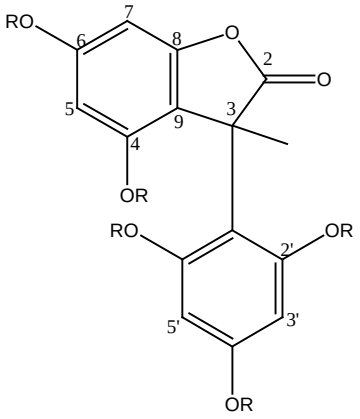
Scheme 18

The final product is a free phenolic 3-aryl substituted benzofuran-2-one that can only be prepared with difficulty and in low yields with existing methods. No protection and deprotection is required. Yields are generally good (Table 3). At high temperatures polymerisation reduces the yield, even when limiting the amount of pyruvic acid.

Table 3

Solvent	Yield, results
MeOH (r.t)	53% yield
EtOH (reflux)	Polymerized
H_2O (r.t)	20% yield

Methylation yielded the expected penta-o-methyl derivative (**13**) with the diagnostic five methyl groups.



R = H (12)
R = CH_3 (13)

Table 4: ^{1}H NMR data of (**12**) and (**13**)

Proton (s)	(**12**), acetone-d_6 , 298 K	(**13**), chloroform-d, 298K
7-H	6.01 (s)	6.35 (d, J 2.0 Hz)
5-H	6.01 (s)	6.14 (d, J 2.0 Hz)
5′-H/3′H	6.01 (s)	6.13 (s)
-CH_3	1.98 (s)	1.98 (s)
5x-OCH_3		3.65, 3.73, 3.79 and 3.81.

The ^{1}H NMR spectrum of the free phenolic lactone (**12**) shows the equivalence of aromatic protons which resonate at δ 6.01 and the $–CH_3$ that resonates at δ 1.98.

The ^{1}H NMR spectrum of the methyl ester derivative (**13**) shows a m-doublet at δ 6.35 (J 2.0 Hz) representing 7-H. The 5-H and 3′-H/5′-H overlap at δ 6.13. The $–CH_3$ resonates as a singlet at δ 1.98.

The five methoxy resonances are well defined in the ^{1}H NMR spectrum of (**13**) and establish the number of free hydroxy groups and confirm the fact that one hydroxy group has reacted intramolecularly with the carboxylic acid to form a lactone.

The proton decoupled ^{13}C NMR spectrum of the methylated product (**13**) shows the expected 15 carbon resonances. This proofs that two phloroglucinol units have condensed with pyruvic acid. The following is notable:

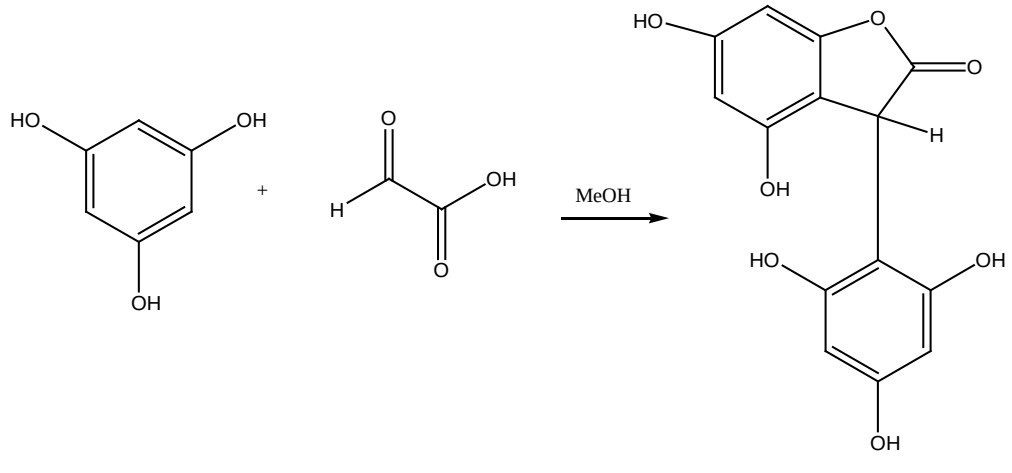
1) The carbonyl resonance at δ 182.05 is diagnostic of a five membered lactone carbonyl[6]. The chemical shift and low intensity of the resonance at δ 48.19 is indicative of a quaternary carbon (C-3).
2) The CH_3 group resonates at δ 24.46. This assignment is supported by an HMQC experiment that shows strong coupling with the CH_3 resonance at δ 1.98 (^{1}H NMR plate 1d)
3) The four hydrogen methine bonded aromatic carbons resonate at δ 94.69 (C-5), δ 92.82 (C-3′ and C-5′, equivalent due to free rotation) and 89.32 (C-7). These assignments are supported by HMQC correlations to the corresponding hydrogens at δ 6.35 (H-7), 6.14 (H-5) and δ 6.13 (H-3′ and H-5′). The correlation with the two proton singlet at δ 6.13 proves that the carbon at δ 92.82 represents two carbon atoms. (^{1}H NMR plate 1d)
4) The six oxygen bonded aromatic carbons are represented by resonances at δ 161.05, 160.66 (x2), 156.37 and 154.52.
5) The two carbon bonded aryl carbons resonate at δ 114.11 (C-9) and δ 109.35 (C-1′).

6) An HMBC analysis of (**13**) clearly shows 2 and 3 bond correlation of the aliphatic methyl protons with the carbonyl group (C-2), the quarternary carbon (C-3) and the two aryl atoms (C-9 and C-1′)

The low-resolution electron impact mass spectrum has the expected M^+ of 374.3251. (Calculated for $C_{20}H_{22}O_7$ = 374.3355). The fragmentation pattern (**Scheme A**) is in agreement with the proposed structure and support the 5-membered lactone ring.

2.2.2 Glyoxylic acid

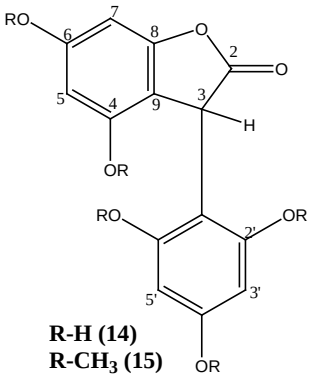
Glyoxylic acid reacts with phloroglucinol in THF to give 4, 6-dihydroxy-3-(2, 4, 6-trihydroxyphenyl)-1-benzofuran-2(3H)-one (**14**) (**Scheme 19**). Glyoxylic acid is a strong enough acid to catalyze the reaction.



(14)

Scheme 19

At high temperature the reaction occurs faster than at room temperature. Acetylation and methylation yielded the expected penta-acetate (**16**) and pentamethyl (**15**) derivatives with the diagnostic five acetate and five methyl groups, respectively.



R-H (14)
R-CH_3 (15)
R-OAc (16)

Table 5: ^{1}H NMR data of (**14**), (**15**) and **(16)**

Proton(s)	(**14**), acetone-d_6, 298K	(**15**),chloroform-d, 298 K	(**16**),chloroform-d, 298 K
7-H	δ 6.01 (s)	δ 6.35 (d, J 2.0 Hz)	δ 7.15 (d, J 2.0 Hz)
5-H	δ 6.01 (s)	δ 6.05 (d, J 2.0 Hz)	δ 6.60 (d, J 2.0 Hz)
5′-H	δ 6.01 (s)	δ 6.14 (d, J 2.0 Hz)	δ 6.91 (d, J 2.0 Hz)
3′-H	δ 6.01 (s)	δ 6.20 (d, J 2.0 Hz)	δ 7.01 (d, J 2.0 Hz)
3-H	δ 5.65	δ 5.28	δ 5.28
5x -OMe		δ 3.60, 3.71 and 3.82	
5x-OAc			δ 1.8-2.4

The ^{1}H NMR spectrum of the free phenolic analogue (**14**) reflects the equivalence of the aromatic protons which resonate as one singlet at δ 6.01. A one-proton singlet (δ 5.65) is associated with the presence of 3–H on the heterocyclic ring.

The doublets ($^4J_{HH}$ J 2.0 Hz, 7-H and 5-H) present in the aromatic region of the ^{1}H NMR spectrum of the methylated and acetylated lactones (**15** and **16**) exhibit the expected AB-spin system. 3′-H and 5′-H resonate as doublets (J 2.0 Hz) which indicated their non-equivalence due to the slow rotation of the ring on the NMR spectrum time scale. The five methoxy and acetoxy resonances are well defined in the NMR spectrum of the derivatives (**15** and **16**) and establishes the number of free hydroxy groups and confirm

that one hydroxy group has reacted intramolecularly with the carboxylic acid to form a lactone.

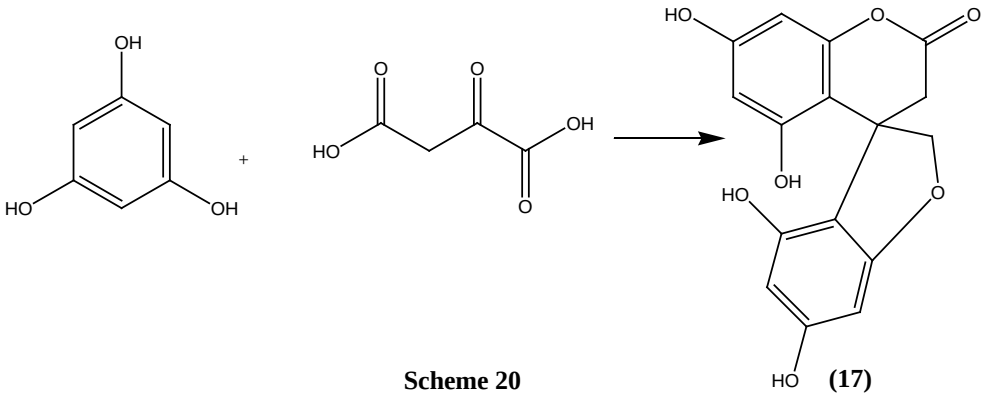
As expected the $–CH_3$ resonance observed at δ 1.92 in the pyruvic acid derivative is replaced by a 3–H resonance (δ 5.28) in the glyoxylic acid derivative. The aromatic protons appear as a four proton singlet in the free phenolic derivative (δ 6.0) and as four doublets (J 2Hz) in the methyl ester and acetate. This indicates restricted rotation about the C-3-C-1′ σ-bond. The C-H bond in **15** and **16** introduces restricted rotation because in **12** and **13** you may indeed see only one atropisomer, i.e. rotation is completely inhibited by the presence of the methyl groups.

MS analysis gave the expected M^+ m/e 360.2563 (Calculated for $C_{19}H_{20}O_7$ = 360.3636). The fragmentation pattern corresponds with that of the pyruvic acid derived product (**12**). (**Scheme B**)

The strong infra red absorption at 1730 cm^{-1} is diagnostic for a five membered lactone carbonyl.

2.2.3 Oxaloacetic acid

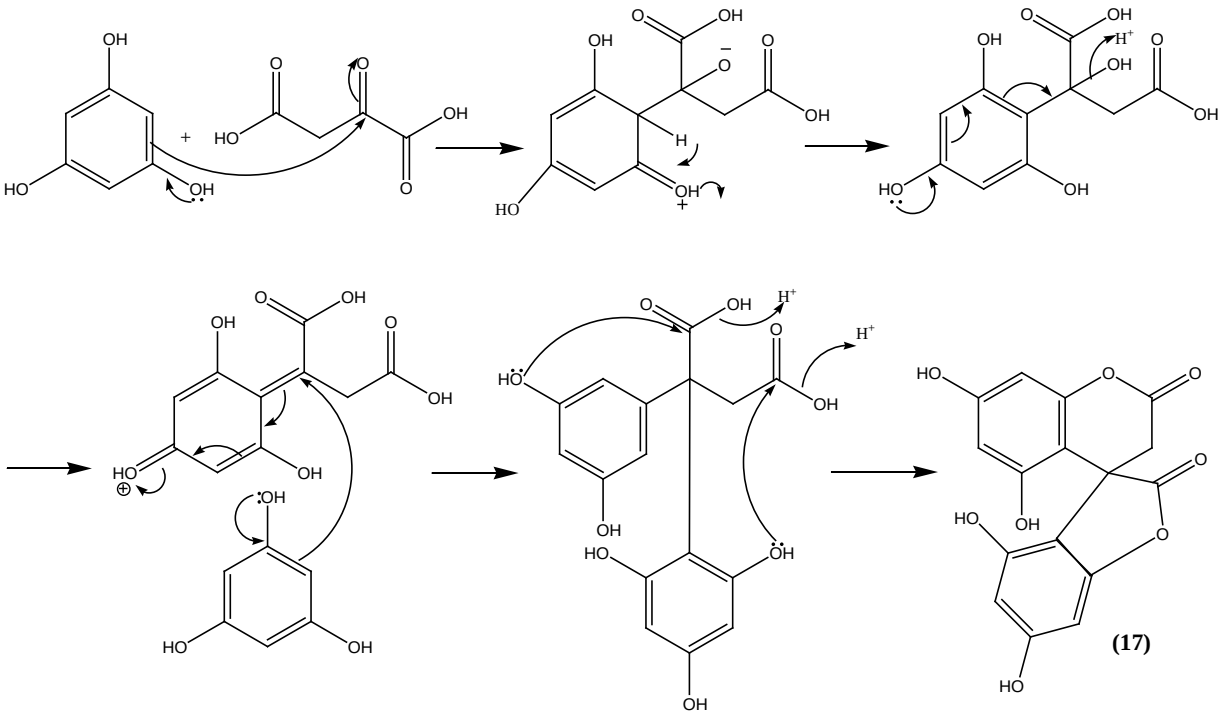
Acid catalyzed reaction of oxaloacetic acid and phloroglucinol gives 4,5′,6,7′-tetrahydroxy-2H-spiro[benzofuran-3,4′-chroman]-2,2′-dione (**17**) (**Scheme 20**).



Scheme 20 **(17)**

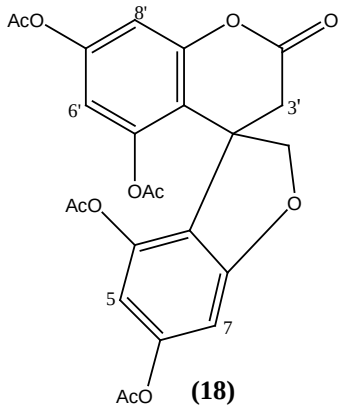
The mechanism is given in **Scheme 21**: The salient features are:

1) Attack of phloroglucinol via carbon at the α-carbonyl group of oxaloacetic acid to give a 2-hydroxy-2-aryl oxaloacetic acid derivative with a benzylic hydroxyl group.
2) Substitution of the benzylic hydroxyl group by a second phloroglucinol group, again via carbon to form a 2,2-diaryl oxaloacetic acid derivative.
3) Dual intermolecular cyclisation of the 2,2-diaryl acid to form both γ- and δ-lactone functionalities



Scheme 21

Acetylation of the reaction mixture improves yields considerably as the free phenol decomposes on TLC. The tetraacetate was isolated in yields of up to 46%.



(18)

Table 6: ^{1}H NMR data of (**18)**

Proton (s)	(**18**) chloroform-d, 298K
8'-H	6.92 (d, J 2.0 Hz)
6'-H	6.96 (d, J 2.0 Hz)
5-H	7.03 (d, J 2.0 Hz)
7-H	6.99 (d, J 2.0 Hz)
3'-H_{eq}	3.35 (d, J 16.0 Hz)
3'-H_{ax}	3.02 (d, J 16.0 Hz)
4x OAc	2.19-2.32 (s)

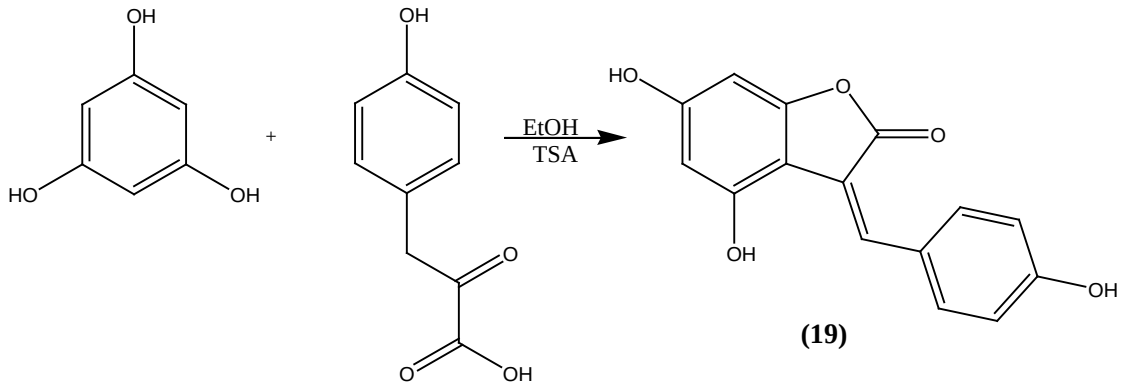
The ^{1}H NMR spectrum of the acetate (**18**) exhibits an AB-spin system (J 2.0 Hz) representing the 8' and 6' protons which resonate at δ 6.92 and 6.96. The 7-H and 5-H also resonate as doublets (J 2.0 Hz) at δ 6.99 and 7.03 which indicated their non-equivalence due to the lactone ring. The diastereotopic methylene protons (3′–CH_2) resonate as geminal doublets (J 16 Hz) at δ 3.02 and 3.35.

The proton decoupled ^{13}C NMR spectrum of the acetylated product shows the expected 24 carbon resonances. The following is notable:

1) The two carbonyls resonating at δ 174.72 (C-2) and 168.45 (C-2') are diagnostic of γ- and δ- lactone carbonyls respectively[6]. The four acetate carbonyls resonate at δ 174.72, 168.45, 168.19, and 162.90.
2) The chemical shift and low intensity of the resonance at δ 45.91 indicates a quaternary carbon (C-4').
3) The prochiral carbon resonates at δ 36.97 (C-3')
4) The four -OCH_3 groups resonate at δ 21.06, 21.03, 20.68 and 20.25.
5) The four hydrogen attached aromatic carbons resonate at 113.01 (C-5), 112.32 (C-3), 108.12 (C-6') and 102.85 (C-8').
6) The four oxygen bonded aromatic carbons resonate at δ 152.46, 152.40, 152.15 and 151.75.
7) The four carbon bonded aryl carbons resonate at δ 147.69(C-8), 147.55 (C-9), 116.47 (C-9) and 108.73 (C-10).

2.2.4 *p*-Hydroxyphenylpyruvic acid and phloroglucinol

p-Hydroxyphenylpyruvic acid reacts with phloroglucinol in ethanol to give (Z)-6-hydroxy-3-(4-hydroxybenzylidene)-1-benzofuran-2(3H)-one [**19,** (**Scheme 22**)]. The reaction requires acid catalysis (*p*-toluenesulfonic acid) and an elevated temperature (refluxing ethanol).

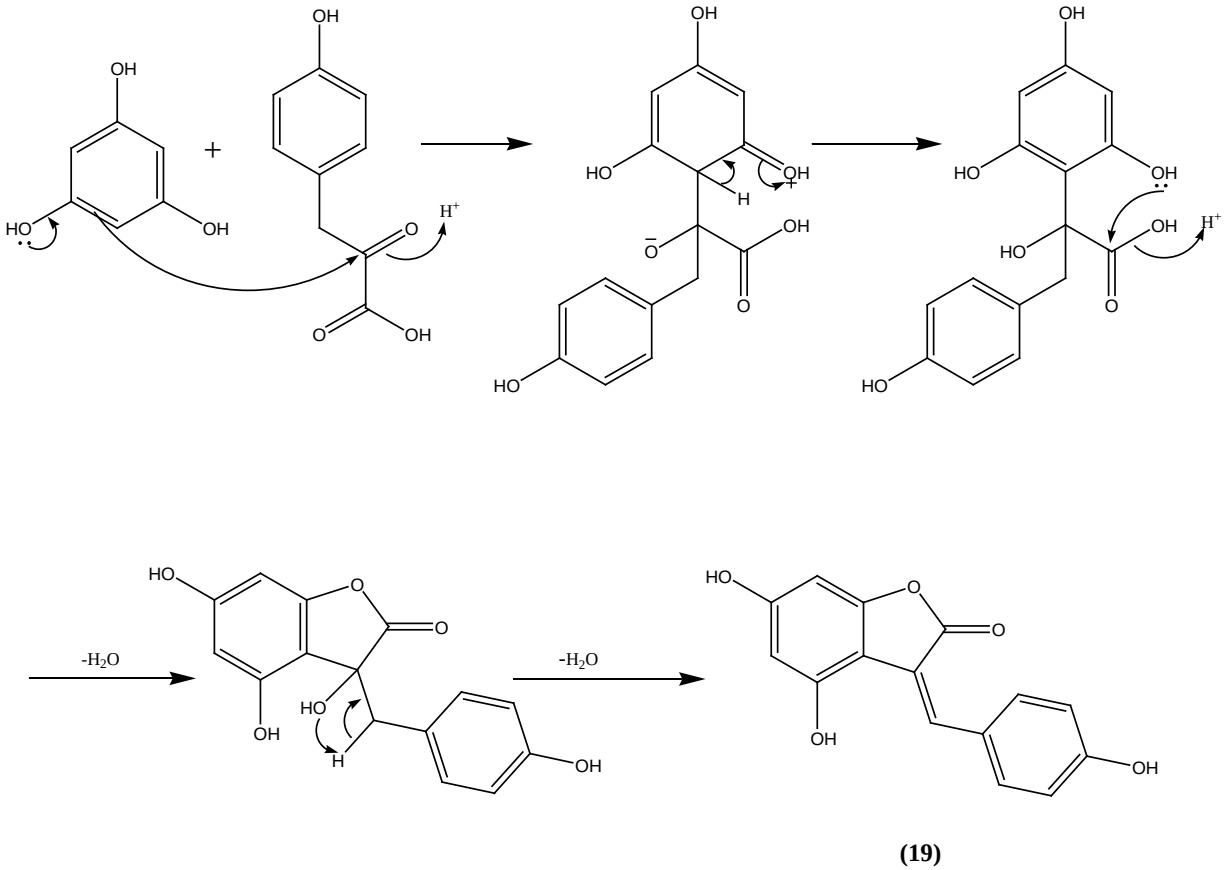


(19)

Scheme 22

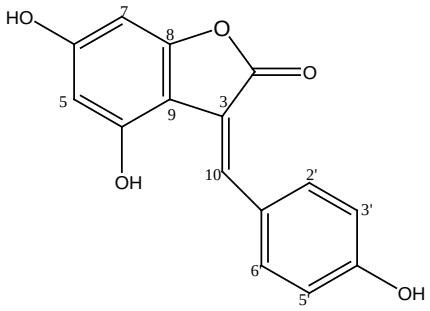
The mechanism is given in **Scheme 23**. The salient features are:

1) Attack of phloroglucinol via carbon at the α-carbonyl group of *p*-hydroxyphenylpyruvic acid to give the 2-hydroxy-2-aryl *p*-hydroxyphenylpyruvic acid intermediate with a benzylic hydroxy group.
2) Internal cyclisation of the 2, 2-diaryl acid to form a γ-lactone.
3) Dehydration to form a double bond. It is not certain whether water elimination takes place before or after lactonization. It however competes successfully with attack of a second phloroglucinol nucleophile on the benzylic position and prevents formation of a 2,2-diaryl derivative.



(19)

Scheme 23



(19)

Table 7: ^{1}H NMR data of (**19**)

Proton(s)	(**19**) acetone-d_6, 298k
7-H	δ 6.26 (d, J 2.0 Hz)
5-H	δ 6.19 (d, J 2.0 Hz)
10-H	δ 7.98 (s)
2′-H/6′-H	δ 8.11 (d, J 9.0 Hz)
3′-H/5′-H	δ 6.92 (d, J 9.0 Hz)

The ^{1}H NMR-spectrum exhibits the expected *m*-coupled one proton doublets ($^{4}J_{HH}$ 2.0 Hz) on the A-ring and an AA′BB″ spin system (J 9 Hz) associated with the *para* substituted B-ring (see table 6). Diagnostic is the deshielded 10-H proton corresponding to the β-proton of an α,β-unsaturated ester (δ 7.98). The chemical shift of 2′-H and 6′-H (δ 8.11) indicates deshielding by the carbonyl group that is only possible in the Z-isomer[7]. As indicated in the literature survey the 2′-H and 6′-H resonances of the E isomer should be at ca δ 7.70 and the 10-H absorption at δ 7.50 (δ 7.7 for the Z-isomer).

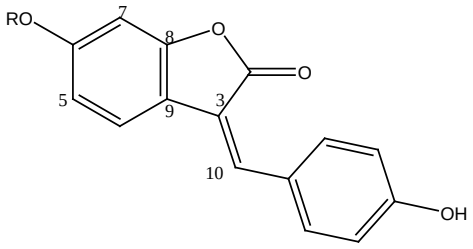
The proton decoupled ^{13}C NMR spectrum shows the expected 16 carbons.
The following are notable:

1) The carbonyl resonance at δ 166.4 is diagnostic of a γ-lactone carbonyl.

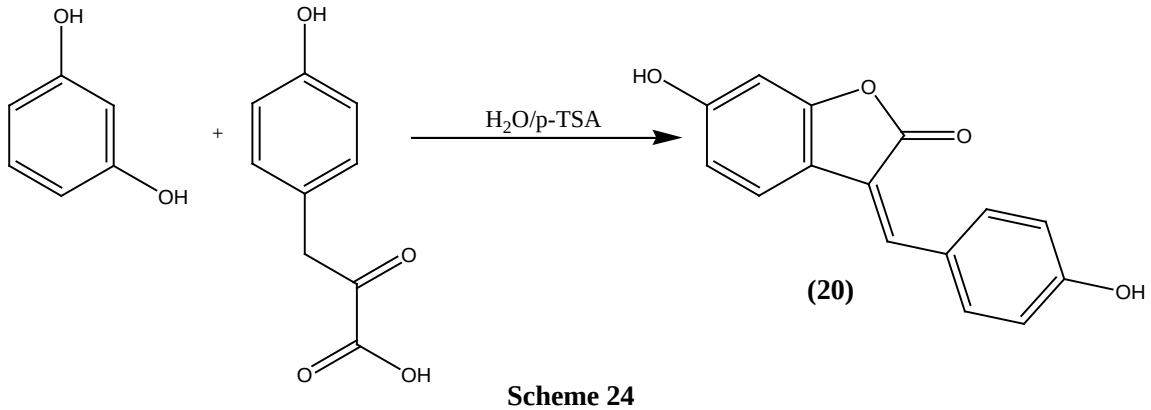
2) The low intensity of the resonance at δ 104.9 is indicative of quaternary carbon (C-3).
3) The six non oxygen bonded aromatic carbons resonate at higher field at δ 90.3 (C-7), 98.3 (C-5), 115.2 (C-3′/5′) and 133.9 (C-2′/6′) than the four oxygen bonded aromatic carbons at δ 159.5 (C-8), 159.4 (C-5), 154.4 (C-4′) and 154.2 (C-4)
4) The two carbon bonded aryl carbons resonate at δ 126.7 (C-1') and 117.6 (C-9)
5) C-10 resonates at δ 139.0.

2.2.5 ***p*-Hydroxyphenylpyruvic acid and resorcinol (novel synthesise of a naturally occurring isoaurone).**

Acid catalyzed reaction of p-hydroxyphenylpyruvic acid with resorcinol in H_2O gives a naturally occurring isoaurone[8] (**20**) (**Scheme 24**)



R=H (20)
R=Ac (21)



(20)

Scheme 24

Table 8: ^{1}H NMR data of (**20**) and (**21**)

Proton(s)	(**20**), acetone-d_6, 298K	**(21),** chloroform-d, 298K
3′/5′-H	δ 7.01 (d, J 9.0 Hz)	δ 7.26 (d, J 9.0 Hz)
2’/6’-H	δ 7.70 (d, J 9.0 Hz)	δ 7.70 (d, J 9.0 Hz)
10–H	δ 7.50 (s)	δ 7.82 (s)
5-H	δ 6.64 (dd, J 2.0, 9.0 Hz)	δ 6.80 (dd, J 2.0, 9.0 Hz)
4-H	δ 7.70 (dd, J 9.0 Hz)	δ 7.75 (d, J 9.0 Hz)
7-H	δ 6.68 (d, J 2.0 Hz)	δ 6.75 (d, J 2.0 Hz)
2xOAc		2.33-2.37 (s)

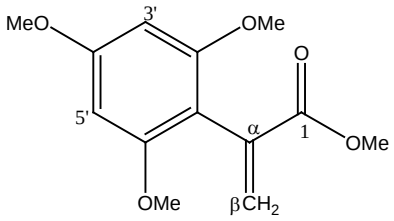
The Z-configuration of the olefinic bond was based on the chemical shifts of the olefinic proton (H-10) and the *ortho* protons (H-2' and H-6') of the aryl unit. These protons are deshielded by the carbonyl and are expected to give a downfield resonance[7]. In the E-isomer H-2', H-6' of the aryl unit appear as a doublet in the range of δ 7.0-7.8 whereas in the Z-isomer the corresponding protons appear in the range of δ 8.0-8.2. The chemical shift of synthetic (**20**), gave a doublet at δ 7.65 (H-2'/H-6') supporting the E-configuration. The olefinic protons (H-10) in this isomer showed a singlet at δ 7.50. The ^{1}H NMR of the acetate (**21**) shows the expected two acetates at δ 2.33 and 2.37.

2.3 REACTIONS OF 1,3,5-TRIMETHOXYBENZENE WITH PYRUVIC ACID

Replacement of free phenolic phloroglucinol with its tri-o-methylated analogue (1,3,5-trimethoxybenzene) leads to a change in the reaction mechanism to yield α-substituted acrylic acids. The reaction requires acid catalysis (sulfuric acid) and elevated temperature (refluxing ethanol). Lewis acid catalysis is also possible (ytterbium (III) trifluoro-methanesulfonate).

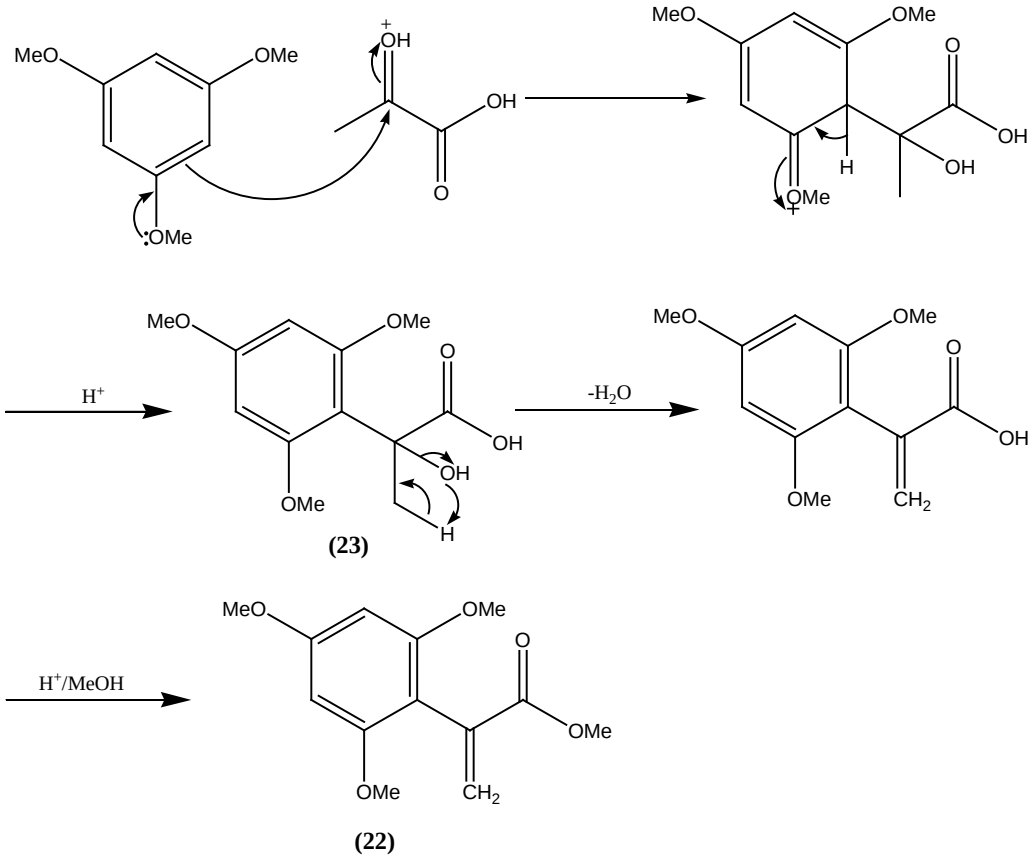
2.3.1 H_2SO_4 as a catalyst.

Acid catalyzed reaction of trimethoxybenzene and pyruvic acid in methanol gives methyl 2-(2,4,6-trimethoxyphenyl)acrylate (**22**).



(22)

The mechanism is given in **Scheme 25**

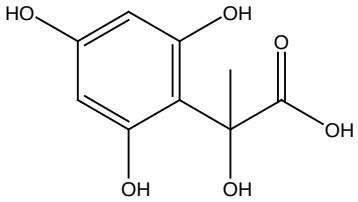


(23)

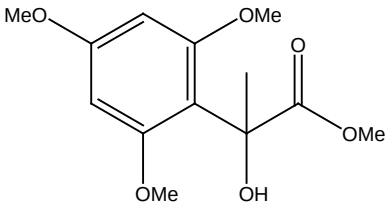
(22)

Scheme 25

In contrast with the free phenolic intermediate (**24**), which is formed when the coupling is perfomed with the phenolic phloroglucinol, the tri-o-methyl intermediate (**23**) does not react with a second molecule of the aromatic nucleophile. It is dehydrated instead to form the α,β-unsaturated acrylic ester (**22**).



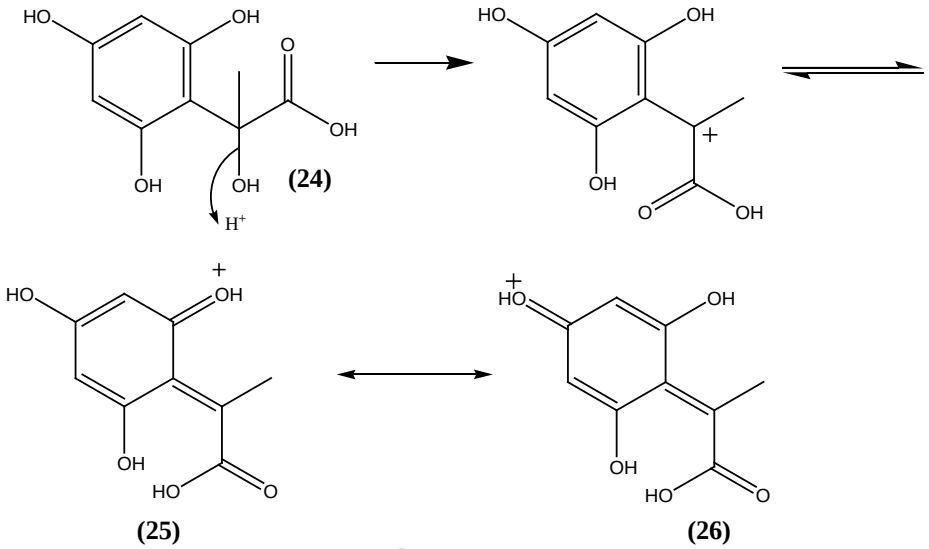
(24)



(23)

Salient features of the mechanism are:

1) The tri-o-methyl phloroglucinol is not a strong nucleophile and is less inclined to replace the benzylic hydroxy with a trimethoxy aryl group.
2) The stability of the phloroglucinol benzylic carbocation is probably enhanced by a *para* and *ortho* quinone methide (**25** or **26**). This stability is not available to the methylated product, causing it to lose water rapidly instead of waiting for a second phloroglucinol molecule to attack.



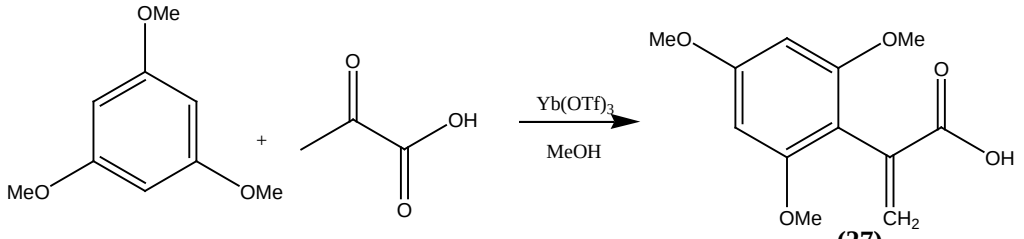
Scheme 26

The three methoxy groups appear as two singlets, the 2- and 6-OMe being equivalent due to free rotation. The two β-protons of the α,β-unsaturated acid resonate at the expected deshielded position δ 5.75 and 6.6. The small coupling ($^2J_{HH}$ 2.0 Hz) is characteristic of geminal alkene protons.

Table 9: ^{1}H NMR data of (**22**)

Proton(s)	(**22**) chloroform-d, 298K
3-H/5-H	6.00 (s)
β-H	6.60 (d, J 2.0 Hz)
	5.75 (d, J 2.0 Hz)
4x –OMe	3.74, 3.78 and 3.85

2.3.2 Ytterbium (III) trifluoromethanesulfonate hydrate as a catalyst

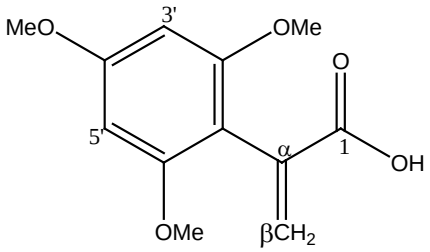


(27)

Scheme 27

Ytterbium (III) trifluoromethanesulfonate hydrate[9] is a Lewis acid and not a strong enough acid to catalyze formation of the methyl ester which is isolated when H_2SO_4 is used**.**

The final product is the unmethylated α-aryl substituted acrylic acid that can be prepared at low temperatures. Yields are generally good (48%).



(27)

Table 10: ^{1}H NMR data of (**27**)

Proton(s)	(**27**), chloroform-d_6 , 298K
3'/5'-H	δ 6.18 (s)
β-H	δ 6.60 (d, J 2.0 Hz)
	δ 5.70 (d, J 2.0 Hz)
3x-OMe	δ 3.70, 3.80

The ^{1}H NMR spectrum of (**27**) displays the equivalence of the aromatic protons which resonate as a singlet at δ 6.18.

The ^{13}C NMR spectrum of (**27**) shows the expected 12 carbons.

The following is notable:

1) The carbonyl carbon resonates at δ 173.16 (C-1)
2) The low intensity of the resonance at δ 107.70 is indicative of the unsaturated α-carbon.
3) The two non oxygen bonded aromatic carbons resonate at higher field at δ 92.3 (C-3'/5') than the three oxygen bonded carbons at δ 161.69 (C-4'), 159.04 (C-2'/6').
4) The $-CH_2$ resonates at δ 131.46 (C-β) and the carbon bonded to the aryl carbon at δ 133.41 (C-1').

2.4. REACTIONS OF THE 2-(2,4,6-TRIMETHOXYPHENYL)-ACRYLIC ACID METHYL ESTER

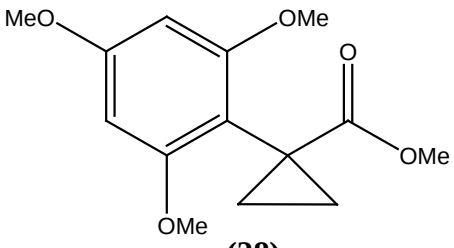
2.4.1 Diazomethane

Our novel method to produce acrylic acid derivatives with a polyphenolic substituent in the α-position opens the door to a variety of new reactions and synthetic methods. We expect these products to demonstrate biological activities.

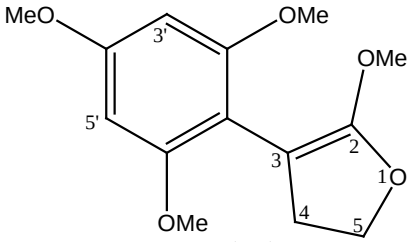
The introduction of an α,β-unsaturated carboxylic acid moiety into polyphenols promises a plethora of new synthetic routes to flavonoids and related compounds. A preliminary investigation into the potential of this reaction yielded the following results:

(a) Carbene insertion with diazomethane (synthesis of 4-methoxy-3-(2′,4′,6′-trimethoxyphenyl)-2H-oxete) (**27**).

Diazomethane typically reacts with alkenes via carbene insertion to form cyclopropanes **(28)**[10].

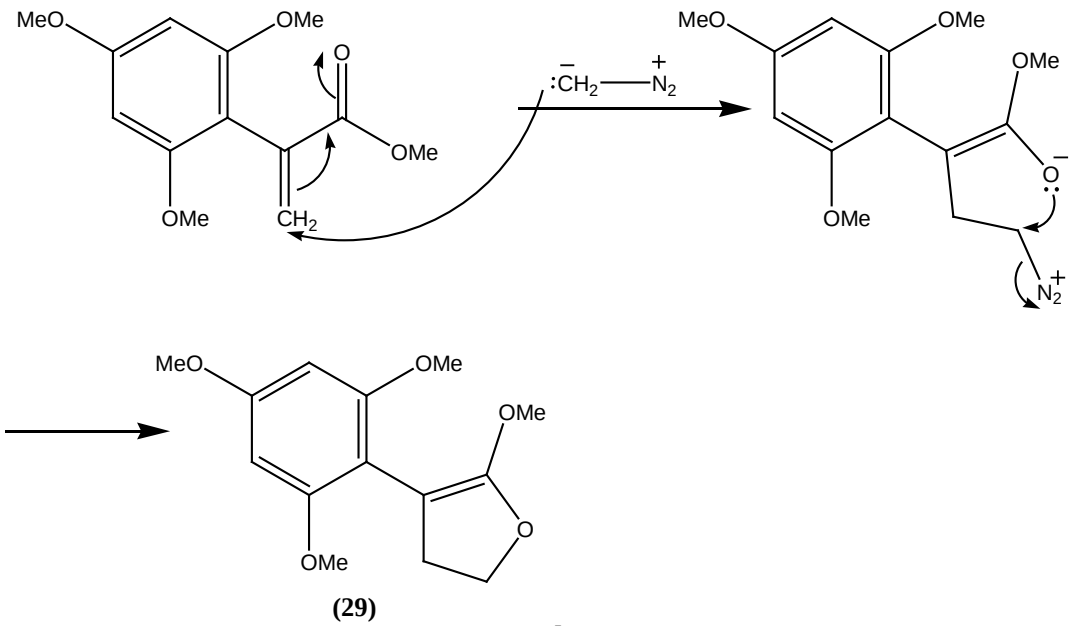


(28)



(29)

However, treatment of acrylic ester (**22**) with diazomethane leads to almost quantitative isolation of the 2-methoxy-3-(2,4,6-trimethoxyphenyl)-4,5-dihidrofuran (**29**). Although the formation of the cyclopropane structure **(28)** is the prefered product (“soft-soft” interaction), the high strain in the three membered ring hinders this reaction and the cyclopentene ring structure **(29)** [“hard-soft” interaction] is preferably formed **(Scheme 28).**



(29)

Scheme 28

Table 11: ^{1}H NMR data of (**29**)

Proton(s)	(**29**) chloroform-d_6 , 298K
3'-H/5'-H	6.05 (s)
4-H_{eq}	4.79 (dd, J 10, 4 Hz)
4-H_{ax}	4.51 (dd, J 10, 8 Hz)
5-H_{eq}	2.59 (dd, J 10, 4 Hz)
5-H_{ax}	1.58 (dd, J 8, 10 Hz)
4x –OMe	3.72, 3.74 and 3.81

The ^{1}H NMR spectrum of (**29**) displays equivalence of the aromatic protons which resonate as a singlet at δ 6.05. The doublet of doublets at δ 4.79 and 4.51 were assigned to 4-H_{eq} (dd, J 10, 4 Hz) and 4-H_{ax} (dd J 10, 8 Hz). The cyclopentene structure leads to the non-equivalence of the 4-H_{ax}, 5-H_{ax}, 4-H_{eq}, and 5-H_{eq} protons.

A COSY experiment proves that the four aliphatic hydrogens are either geminal or vicinal, supporting our cyclopentene structure (NMR plate 8e).

The ^{13}C NMR spectrum indicates the presence of C-2 at δ 176.3. The three oxygen bonded aromatic carbons resonate at lower field δ 161.5 (C-4') and δ 158.9 (C-2'/6') than the two non oxygen bonded carbons at δ 92.2 (C-3′/5′). The carbon bonded to the aryl group resonates at δ 109.5 and the four methoxy groups are well defined, resonating at δ 56.4, 55.8 and 53.1.

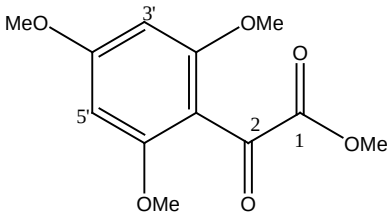
A DEPT-135 experiment proves that the carbons at δ 27.6 and 77.6 are associated with the cyclopentene CH_2 groups (inverted, NMR plate 8c). An HMQC experiment demonstrates that the two protons at δ 4.79 and 4.51 and the two protons at δ 2.59 and 1.58 are attached to the same carbons, respectively [(δ 77.6) and (δ 27.6), respectively]. (NMR plate 8d). This correlation proves the presence of two $–CH_2$ carbons with unequivalent protons on each.

The mass spectrum (M^+ m/e 266.1155) proves addition of a CH_2 group to the methyl acrylate (**22**) (M^+ m/e 252). The fragmentation pattern is in agreement with the proposed structure (**Scheme C).**

Consecutive coupling displayed by the COSY spectrum (COSY NMR plate 8e) together with the ^1H NMR spectrum are used to assign the cyclopentene ring protons. They are assigned as follows: 4-H_{eq} (δ 4.79, dd, J 10, 4 Hz), 4-H_{ax} (δ 4.51, dd, J 10, 8 Hz), 5-H_{eq} (δ 2.59, dd, J 10, 4 Hz) and 5-H_{ax} (δ 1.58, dd, J 8, 10 Hz).

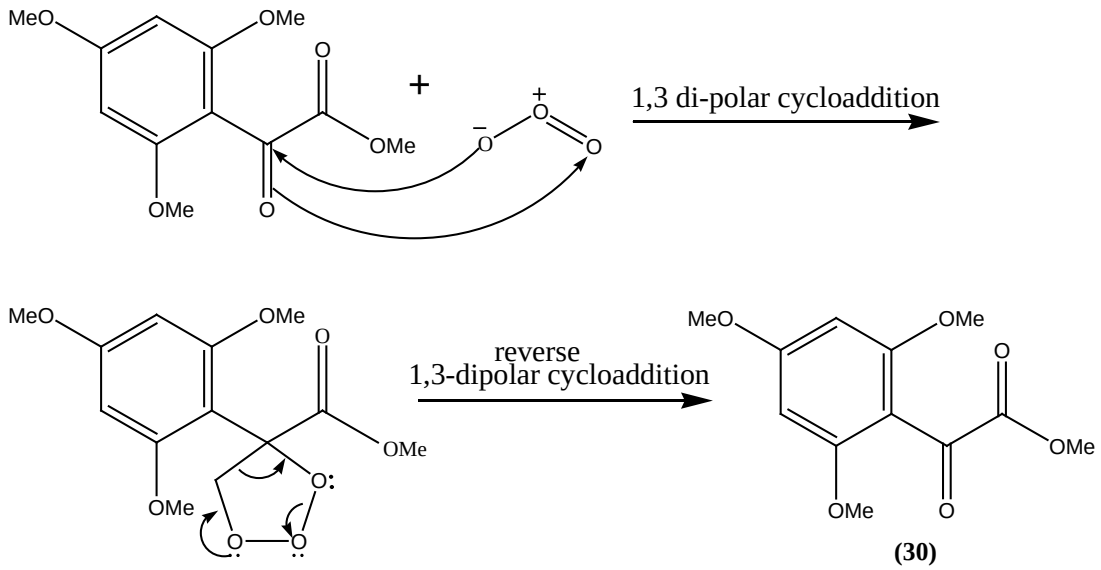
2.4. 2. <u>Ozonolysis</u>

Treatment of methyl 2-(2,4,6-trimethoxyphenyl)acrylate (**22**) with ozone in EtOAc at -78 ºC gives methyl oxo (2,4,6-trimethoxyphenyl) acetate (**30**).



(28)

Ozonolysis usually starts with a 1,3-dipolar cycloaddition of ozone (O_3) to the double bond. The intermediate ozonide is unstable with a weak O-O bond. It decomposes by a reverse 1,3-dipolar cycloaddition to replace the original alkene double bond with a carbonyl bond. This represents a method of cleaving a π-bond oxidatively to two carbonyl group.



(30)

Scheme 30

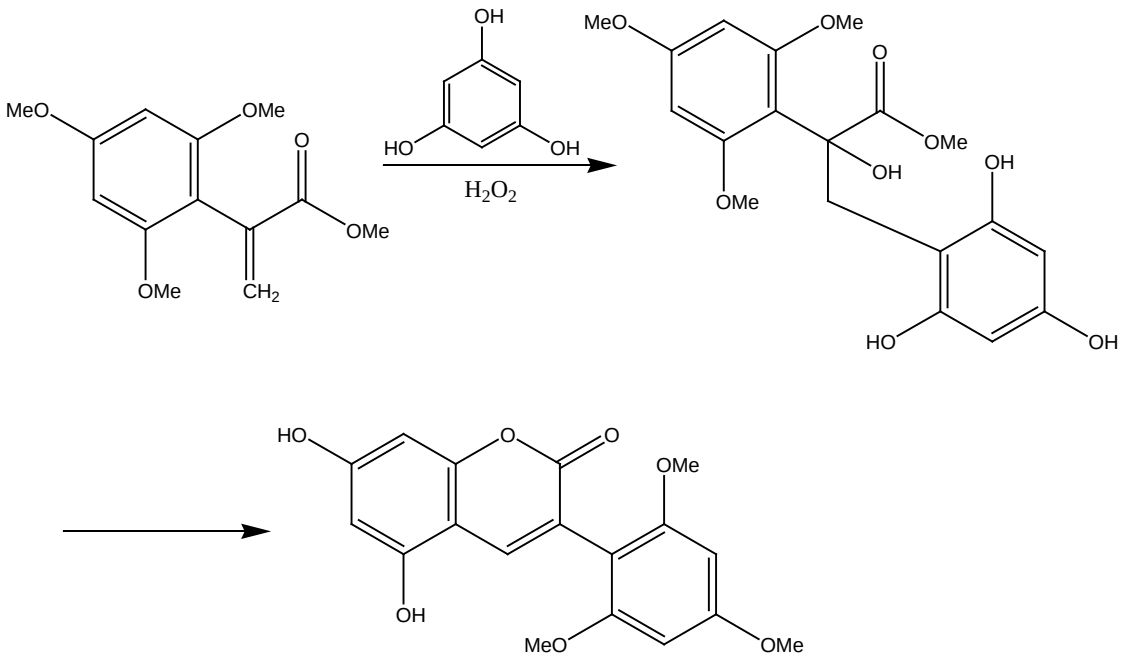
Table 12: ^{1}H NMR data of **(30)**

Proton(s)	(**30**), chloroform-d, 298K
3'/5'-H	δ 6.00 (s)
4x -OMe	δ 3.7-3.8

The ^{1}H NMR spectrum shows equivalence of aromatic protons at δ 6.00 and four methoxy protons at δ 3.71 (x2) and 3.80 (x2) The ^{13}C NMR spectrum indicates the presence of two carbonyl groups at δ 184.0 and 162.6. The oxygen bonded aromatic carbons resonate at δ 162.0 and 160.5 and non oxygen bonded carbons at δ 93.0. Four methoxy groups are evident at δ 56.2 and 55.6.

2.4.3. Epoxidation

Epoxidation of the α-substituted acrylic acid followed by coupling of a phenolic moiety to the β-position[11,12,13] would give an elegant synthesis of $C_6C_3C_6$ compounds (flavonoids) starting with naturally occuring pyruvic acid (**Scheme 31**).



Scheme 31

Efforts to epoxidise the double bond with conventional epoxidation agents (*m*CPBA) failed, probably because of the polarized nature of the α,β-unsaturated alkene. An initial effort to use nucleophilic epoxidising agents (alkaline hydrogen peroxide) hydrolyzes the ester.

2.5. Evaluation of 4,6-dihydroxy-3-(2,4,6-trihydroxyphenyl)-1-benzofuran-2(3H)-one (14) for antioxidant activity

As discussed in the literature review (page 1) 4,6-dihydroxy-3-(2,4,6-trihydroxyphenyl)-1-benzofuran-2(3H)-one (**14**) has the benzylic hydrogen on a methine group adjacent to an *ortho* hydroxy of the aromatic moiety[19] required for antioxidant activity. This compound turns spontaneously red when exposed to sunlight, similar to catechin and wattle bark extract. Compound (**14**) was evaluated in the DPPH[14,15] based anti-oxidant assay. This colorimetric DPPH assay is a method for evaluation of the radical scavenging capacity (RSC) of a compound or plant extract. The results are given in Table 13.

Table 13: Evaluation of (**14**) for anti-oxidant activity

Concentration, ppm	**Extract Number**	**%Radical Scavenging**	**Results**
100	Green Tea Vital	95.79	Moderate
50		95.66	
25		95.79	
12.5		70.81	
6.25		40.60	
100	Compound (**14**)	75.82	Inactive
50		62.98	
25		50.61	
12.5		39.54	
6.25		29.76	
100	Standard	96.47	Active
10		95.79	
1		61.31	
0.1		7.82	
0.01		7.64	

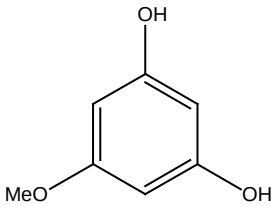
It is unclear why we obtained a negative test in the DPPH based anti-oxidant assay. We plan further tests with other antioxidant assays.

2.6. INCOMPLETE RESULTS

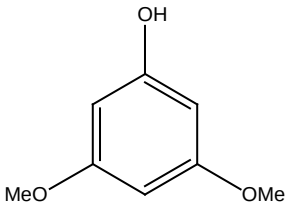
Various conditions and reagents were investigated during the development of the chemistry discussed above. In many cases results were poor or complex reaction mixtures were obtained that could not be purified with our limited chromatographic facilities. In some cases complete polymerisation of the starting material was observed.

All efforts to react pyruvic acid with catechin resulted in polymeric products with very low Rf values, too complex to investigate. It seems that catechin is more reactive than phloroglucinol, possibly indicating that the B-ring becomes involved in the reaction via opening of the heterocyclic C-ring. We plan to repeat our work with phloroglucinol at lower concentrations and temperatures.

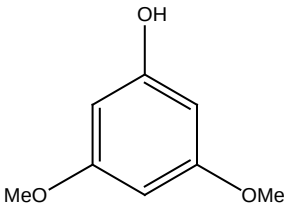
Our initial investigation into the reactions with the series phloroglucinol, mono-o-methylphloroglucinol (**30**) di-o-methylphloroglucinol (**31**) and tri-o-methyl-phloroglucinol (**32**) to establish at what stage the mechanism changes from substitution of the benzylic hydroxyl group to elimination was temporarily abandoned. Mono- and dimethylated phloroglucinol gave mixtures of products that could not be purified with TLC.



(31)



(32)

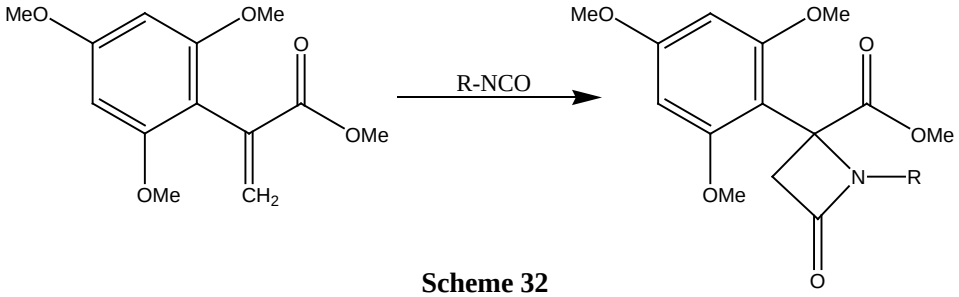


(33)

2.7. FUTURE WORK

We plan the following:

1. Epoxidation of the α-substituted acrylic acid or ester to develop new synthetic procedures for flavonoids. Protection of the o-hydroxy group will also enable us to synthesize lactones.
2. Investigation of the potential of [2+2] cycloaddition of chlorosulfonylisocyanate to the α,β-unsaturated to synthesize novel β-lactams (**Scheme 32**).



Scheme 32

2.8. REFERENCES

1. J. McMurry, *Organic Chemistry* **1999**, Brookes and Cole, USA, 608.
2. Botha, J. J; Ferreira, D; Roux, D. G. *Chemical Communications* **1978**, 698.
3. Vollhardt, K. P. C; Schore N. E. *Organic Chemistry* **1987** 925.
4. Witiak, D. T; Patel D. B.; Lin, Y. *Journal of Chemical Society* **1967**, 89, 1908.
5. Massicot, J.; Marthe, J. P.; Heitz, S. *Bulletin Society of Chemistry* **1963**, 2712.
6. Pretsch, E.; Buhlmann, P.; Affolter, C. *Structure Determination of Organic Compounds* **2000**, 3rd edition, 139.
7. Venkateswarlu, S.; Panchagnula, G. K.; Guraiah, M. B.; Subbaraju, G. V. *Tetrahedron* **2005**, 61, 3013.
8. Marathe, K. G.; Byrne, M.J.; Vidwans, R. N. *Tetrahedron* **1966**, 22, 1789.
9. Kobayashi, S.; Sudiura, M.; Kitagawa H.; Lam, W. W, L. *Chemical Reviews* **2002**, 102, 2227.

10. Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry* **2001**, Oxford University, USA 1063.
11. Volsteedt, F. du R.; Ferreira, D.; Roux, D. G. *Journal of Chemical Society, Perkin Transaction* 1 **1977**, 134.
12. Fourie, T. G.; Ferreira, D.; Roux, D. J. *Journal of Chemical Society, Perkin Transaction* 1 **1977**, 134.
13. Ferreira, D.; Roux, D. G. *Journal of Chemical Society, Perkin Transaction* 1 **1981**, 12220.
14. du Toit, R.; Volsteedt, Y.; Apostolides, Z. *Toxicology* **2001**, 166, 63.
15. Soler-Rivas, C.; Espin, J. C.; Wichers, H. J, *Phytochemistry Analysis*, II, **2000**, 330.

REFERENCES

1. J. McMurry, Organic Chemistry, Brookes and Cole, USA, **1999**, 5thed, 608.

2. Botha, J. J; Ferreira, D; Roux, D. G *Chem. Comm* **1978**, 698.

3. Vollhardt, K. P. C; Schore N. E. *Organic Chemistry*, **1987** 925.

4. Witiak, D. T; Patel D. B.; Lin, Y. *J. Chem. Soc* **1967**, 89, 1908.

5. Massicot, J.; Marthe, J. P.; Heitz, S. *Bull. Soc. Chim*, 1963, 2712.

6. Pretsch, E.; Buhlmann, P.; Affolter, C. *Structure Determination of Organic Compounds* **2000**, 3rd edition, 139.

[7] Marathe, K. G.; Byrne, M.J.; Vidwans, R. N. *Tetrahedron* **1966**, 22, 1789

7. Venkateswarlu, S.; Panchagnula, G. K.; Guraiah, M. B.; Subbaraju, G. V. *Tetrahedron*, **2005**, 61, 3013.

8. Kobayashi, S.; Sudiura, M.; Kitagawa H.; Lam, W. W, L. *Chem.Rev*., **2002**, 102, 2227.

[10] Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry* Oxford University, USA, **2001**, 1063

9. Volsteedt, F. du R.; Ferreira, D.; Roux, D. G. *J. Chem. Soc.*, Perkin Trans. 1, **1977**, 134.

10. Fourie, T. G.; Ferreira, D.; Roux, D. J. *J. Chem. Soc.*, Perkin Trans . 1, **1977**, 134.

11. Ferreira, D.; Roux, D. G. *J. Chem. Soc.*, Perkin Trans. 1, **1981**, 12220.

[14] du Toit, R.; Volsteedt, Y.; Apostolides, Z. *Toxicology*, **2001**, 166, 63

[15] Soler-Rivas, C.; Espin, J. C.; Wichers, H. J, *Phytochem. Anal*. 11, 330

CHAPTER 3

3. EXPERIMENTAL

STANDARD EXPERIMENTAL PROCEDURES

The following general experimental techniques were used in this study.

3.1. CHROMATOGRAPHIC TECHNIQUES

3.1.1. Thin Layer Chromatography

Qualitative thin layer chromatography was performed on Merck aluminium sheets (silica gel 60 F_{254}, 0.25 mm). Preparative thin layer chromatography was perfomed on glass plates (20x20 cm), covered with a layer (1.0 mm) Kieselgel PF_{254} (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 to a maximum of 25 mg material per plate. After development the plates were dried at room temperature in a fast stream of air and the different bands were distinguished under UV light (254 nm), scraped off and eluted with acetone.

3.1.2 Centrifugal Chromatography

Chromatography was performed in a thin layer of silica gel coated on a circular piece of glass called a rotor. A motor drove the rotor at a constant speed by a shaft passing through a hole in the centre. The compound to be separated was applied as a solution at the centre of the pre-cast rotor by way of a solvent pump or hand held syringe. The chosen solvent mixture 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 their different affinities for the layer and solvent mixture. As

the individual rings reached the outer rim of the rotor they were spun off the edge of the glass together with the solvent. A solvent channel collected the elute and brought it to the output tube where the fractions were collected.

Centrifugal chromatography was performed with an Analtech Cyclograph^{TM} with commercially available Analtech rotors (4mm).

3.1.3. 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.

3.1.4 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 ºC for maximum colour development.

3.2. SPECTROSCOPIC METHODS

3.2.1 Nuclear Magnetic Resonance Spectroscopy (NMR)

300MHz and 600MHz Bruker spectrometer were used to record the ^{1}H NMR and ^{13}C NMR spectra. The solvents used were chloroform-d ($CDCl_3$) and acetone-d_6 with TMS (tetramethylsilane) as internal standard. Chemical shifts were expressed as parts per million (ppm) (δ scale) and coupling constants were measured in Hz. The following abbreviations are used:

s	singlet
d	doublet
dd	doublet of doublet

3.2.2 Mass Spectrometry

High resolution mass spectra were recorded at 70eV on a VG SEQ mass spectrometer with a MASPEC II data system.

3.3 CHEMICAL METHODS

3.3.1. Acetylation

Dried material was dissolved in a minimum amount of pyridine. Acetic anhydride (two drops per drop of pyridine) was added and the resulting solution stirred at room temperature for 8 hours. 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.

3.3.2 Methylation with Diazomethane

Dried material (100mg) was dissolved in methanol (20mℓ) and cooled to -10ºC. Diazomethane generated by the reaction of NaOH (16 ml, 30% in a 95% v/v ethanol solution), with N-methyl-N-nitroso-*p*-toluenesulphonamide (Diazald, 5g) in ether (100mℓ) and glycol (12.5 mℓ) as co-solvent was distilled slowly into the sample. The reaction mixture was stored at -10ºC for 48 hours and the excess diazomethane was removed at room temperature in a fast moving air current.

3.3.3. Methylation using dimethylsulphate

A dried sample (1 equiv) was dissolved in dry acetone (25cm^3) and dry potassium carbonate (8 equiv) was added. Dimethylsulfate (10 equiv) was subsequently added dropwise over a period of 30 minutes. The reaction mixture was refluxed for 1 hour, the mixture was filtered and the acetone removed under vacuum. Excess dimethyl sulphate was destroyed with 1M ammonia and the resulting mixture extracted with ethyl acetate.

ABBREVIATIONS

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

A	acetone
Ac	acetyl
$CDCl_3$	chloroform-d
EtOAc	ethyl acetate
MeOH	methanol
EtOH	ethanol
H	hexane
Me	methyl
T	toluene
THF	tetrahydrofuran

CHAPTER 4

4. REACTION OF PHLOROGLUCINOL AND α-KETO ACIDS

4.1.1. Synthesis of 4,6-dihydroxy-3-methyl-3-(2,4,6-trihydroxyphenyl)-1-benzofuran-2(3H)-one (12).

Phloroglucinol (1g, 8 mmol, 1 eq) and pyruvic acid (1g, 12 mmol, and 1.5 eq) were dissolved in methanol (150mℓ) and stirred at room temperature for 72 hours. The methanol was removed under vacuum. Column chromatography (silica gel, T:A:EtOAc 7:2:1) gave the two fractions, R_f 0.58 (0.45g, yellow orange with the spray reagent) and Rf 0.70 (0.5g, red brown with the spray reagent).

The fraction R_f 0.70 yielded unreacted phloroglucinol as white needles from acetone, mp. 214-217°C (Lit[1] 218°C), M^+ 126, δ (acetone $-d_6$) 5.9 (s, aromatic protons).

The R_f 0.58 fraction gave 4,6-dihydroxy-3-methyl-3-(2,4,6-trihydroxyphenyl)-1-benzofuran-2(3H)-one (**12**), (1.3g, 53%). δ_H (300MHz, acetone-d_6, Me_4Si) 6.01 (1H, d, J 2.0 Hz, 7-H,), 6.01 (3H, d, 5-H, 5′/3′-H), 1.98 (s-CH_3). (^{1}H NMR plate 1a).

Methylation of the benzofuran-2-one (**12**) with dimethylsulfate yielded 4, 6-dimethoxy-3-methyl-3-(2,4,6-trimethoxyphenyl)-1-benzofuran-2-(3H)-one (**13**), R_f 0.61 (T: A: EtOAc 7:2:1), red-brown with the spray reagent, as an amorphous solid. δ_H (300MHz, $CDCl_3$, $MeSi_4$) 6.35 (1H, d, J 2.0 Hz, 7-H), 6.14 (1H, d, J 2.0 Hz, 5-H) , 6.13 (2H, s, 3′-H, 5′H), 3.65, 3.73, 3.79, and 3.81 (5x-OMe), 1.98 (s,-CH_3); δ_C (150 MHz, $CDCl_3$) 182.05 (C=O), 48.19 (C3) , 154.52 (C4) , 94.69 (C5) , 156.37 (C6) , 89.32 (C7), 114.10 (C9), 109.35 (C1′), 160.66 (C2′/6′), 92.82 (C3′/5′), 161.05 (C4′); 24.46 (-CH_3) m/z 374 (M^+, 85%), 315 (100), 221(35). Found: M^+, 374.3251. $C_{20}H_{22}O_7$ requires 374.3355. (^{1}H NMR plate 1b, ^{13}C NMR. plate 1c, mass scheme A).

4.1.2. Synthesis of 4,6-dihydroxy-3-(2,4,6-trihydroxyphenyl)-1-benzofuran-2(3H)-one (14).

Phloroglucinol (0.9g, 3.9mmol, 5 eq) and glyoxylic acid (0.1g, 1.3mmol, 1 eq) were dissolved in THF (10 mℓ) and stirred at room temperature for 12 hours. The methanol was removed under vacuum. Column chromatography (Sephadex, Ethanol) gave two fractions, R_f 0.52 (0.5g, yellow orange with spray reagent) and R_f 0.66 (300 mg, yellow-orange).

The fraction R_f 0.66 yielded unreacted phloroglucinol as white needles from acetone, mp. 214-217°C (Lit[1] 218°C), M^+ 126, δ (acetone $-d_6$) 5.9 (s, aromatic protons).

The R_f 0.52 fraction gave 4,6-dihydroxy-3-(2,4,6-trihydroxyphenyl)-1-benzofuran-2(3H)-one (15), (0.3g, 64 %). δ_H (300 MHz, acetone-d_6, Me_4Si) 6.01 (4H, s, 5-H, 7-H, 5′-H, 3′ overlapping), 5.65 (1H, s, 3-H). (^{1}H NMR plate 2a)

Methylation of the benzofuran-2-one (**14**) with dimethylsulfate yielded 4, 6-dimethoxy-3-methyl-3-(2, 4, 6-trimethoxyphenyl)-1-benzofuran-2-(3H)-one (**15**) (R_f 0.43 (T: A 9.5: 0.5), δ_H (300 MHz, $CDCl_3$, Me_4Si) 6.35 (1H, d, J 2.0 Hz, 7-H,), 6.20 (1H, d, J 2.0 Hz, 3'-H), 6.14 (1H, d, J 2.0 Hz, 5'-H,), 6.05 (1H, d, J 2.0 Hz, 5-H), 5.28 (1H, s, 3-H) and 3.60, 3.71 and 3.82 (5x-OMe) red-brown with the spray reagent. Found, M^+ 360.2563, $C_{19}H_{20}O_7$ requires 360.3636, υ_{maks} ($CDCl_3$) 1730 cm^{-1} (lactone carbonyl absorption) (^{1}H NMR plate 2b, mass scheme B).

Acetylation of benzofuran-2-one (**14**) yielded 4,6-diacetoxy-3-(2,4,6-triacetoxyphenyl)-1-benzofuran-2(3H)-one (**16**) as white amorphous material. δ_H (300 MHz, $CDCl_3$, $MeSi_4$) 7.15 (1H, d, J 2.0 Hz, 7-H,), 6.60 (1H, d, J 2.0 Hz, 5-H,), 6.90 (1H, d, J 2.0 Hz, 5'-H), 7.01 (1H, d, J 2.0 Hz, 3'-H) and 5.28 (1H, s, 3-H); δ_C (150 MHz, $CDCl_3$, $MeSi_4$) 172.6 (C=O), 168.5 (C4′), 168.2 (C5), 167.8 (C2′/6′), 154.1 (C7), 154.1 (C8), 151.1 (C4′), 150.6 (C5), 149.6 (C2′/6′), 116.1 (C9), 114.9 (C1′), 114 (C4), 113.6 (C6), 112.0 (C3′), 103.1 (C5′), 39.8 (C), 30.8 (C3), 21.0-19.8 (5x-OAc).

4.1.3. Synthesis of 4,5′,6,7′-tetrahydroxy-2H-spiro[benzofuran-3,4′-chroman]-2,2′-dione (17).

Phloroglucinol (0.32g, 2.5mmol, 2 eq) and oxaloacetic acid (0.165g, 1.25mmol, and 1 eq) were dissolved in MeOH (20mℓ). Hydrochloric acid (3M, 1mℓ) was added and the reaction mixture was stirred at room temperature for 72hrs. Water was added and the reaction mixture was extracted with ethyl acetate and solvent removed under vacuum.
Acetylation of the crude reaction mixture followed by PTLC gave 4 bands at R_f 0.59 (25 mg, yellow brown), 0.32 (0.25g, red brown with the spray reagent) and 0.25 (0.01g, yellow), 0.17 (0.001g, dark brown).

The R_f 0.32 fraction gave 4,5′,6,7′-tetrahydroxy-2H-spiro[benzofuran-3,4′-chroman]-2,2′-dione (**17**) (250 mg, 46%). δ_H (600MHz, $CDCl_3$, $SiMe_4$) 6.92 (1H, d, J 2.0 Hz, 8'-H), 6.96 (1H, d, J 2.0 Hz, 6'-H), 7.03 (1H, d, J 2.0 Hz, 5-H), 6.99 (1H, d, J 2.0 Hz, 7-H), 3.35 (1H, d, J 16.0 Hz, 3'-H_{eq}), 3.0 (1H, d, J 16 Hz, 3'-H_{ax}), 2.1- 2.4 (4x-OAc). δ_C (150MHz**,** $CDCl_3$, $SiMe_4$)174.72 (C-=O), 168.45 (C=O), 45.91 (C-4'), 36.97 (C-3'), 113.01 (C-5), 112.32 (C-7), 108.12 (C-6'), 102.85 (C-8'), 147.69 (C-8), 147.55 (C-9), 116.47 (C-9)and 108.72 (C-10), 152.545 (C-6), 152.40 (C-4), 152.15 (C-5'), 151.75 (C-7') and 21.06-20.25 (4x-OAc). (^{1}H NMR. plate 3a, ^{13}C NMR plate 3b, APT plate 3c).

4.1.4. Synthesis of 4,6-hydroxy-3-(4-hydroxybenzylidene)-1-benzofuran-2(3H)-one (19).

Phloroglucinol (0.1g, 0.8mmol, 1eq) and *p*-hydroxyphenylpyruvic acid (0.143g, 0.8mmol, and 1eq) were dissolved in EtOH. Toluene sulfonic acid (0.005g) was added and the mixture was stirred at room temperature for 72 hours. Ethanol was removed under vacuum. PTLC (T:A:EtOAc 7:2:1) gave two fractions, R_f 0.45 (0.065g, blue-green with the spray reagent) and R_f 0.61 (0.01g, red brown with the spray reagent).

The fraction R_f 0.61 yielded unreacted phloroglucinol as white needles from acetone, mp. 214-217°C (Lit[1] 218°C), M^+ 126, δ (acetone –d_6) 5.9 (s, aromatic protons).

The fraction R_f 0.45 gave the 3-(4-hydroxybenzylidene)-benzofuran-2-one (**19**) as a yellow powder (0.65g, 33%). δ_H (600MHz, acetone-d_6, $SiMe_4$) 6.27 (1H, d, J 2.0 Hz, 7-H), 6.19 (1H, d, J 2.0 Hz, 5-H), 7.98 (1H, s, 10-H), 8.11 (2H, d, J 9.0 Hz, 2′/6′-H), 6.92 (2H, d, J 8.0 Hz, 3′/5′-H). δ_C (300MHz, acetone-d_6, $MeSi_4$)166.43 (C=O), 104.91 (C3), 154.16 (C4), 98.29 (C5), 90.34 (C7), 159.53 (C8), 133.87 (C2′/6′), 115.21 (C3′/5′), 154.36 (C4′), 126.65 (C1′), 117.58 (C9), 139.08 (C-10) .Found 270.5. $C_{15}O_5H_{10}$ requires 270.2414. (^{1}H NMR plate 4a and ^{13}C NMR plate 4b).

4.1.5. Synthesis of 6-hydroxy-3-(4'-hydroxybenzylidene)-1-benzofuran-2(3H)-one (20).

Resorcinol (0.1g, 0.9 mmol, 1eq) and *p*-hydroxyphenylpyruvic acid (0.163mg, 0.9 mmol, 1eq) were dissolved in H_2O. Toluene sulfonic acid was added and the mixture was refluxed for 96 hours. The mixture was extracted with ethyl acetate and the solvent removed under vacuum. PTLC (Hexane:EtOAc 6:4) gave two fractions, R_f 0.6 (blue-green with the spray reagent) and R_f 0.40 (red-brown).

The fraction R_f 0.60 yielded unreacted resorcinol as white needles from acetone, mp. 107-109°C (Lit[1] 110°C), M^+ 110, δ (acetone –d_6) 6.3 (s, aromatic protons).

The fraction R_f 0.4 yielded the isoaurone (**20**) as yellow crystals (0.025g, 11 %), mp 257-160 (Lit[7] 258-260) δ_H (600MHz, acetone, $SiMe_4$) 7.01 (2H, d, J 9.0 Hz, 3′/5′-H), 7.70 (2H, d, J 9.0 Hz, 2′/6′-H), 7.5 (1H, s, 10-H), 6.64 (1H, dd, J 9.0 Hz, 2, 5-H), 7.7 (1H, d, J 9.0 Hz, 4-H), 6.68 (1H, d, J 2.0 Hz, 7-H) [Lit[7] ^{1}H NMR δ (400MHz, DMSO-d_6, $SiMe_4$) 6.92 (d, J 9.0 Hz, 3′/5′-H), 7.65 (d, J 9.0 Hz, 2′/6′-H), 7.50 (s, 10-H), 7.69 (dd, J 9.0 Hz, 4-H), 6.58 (d, J 2Hz, 5-H), 6.64 (d, J 2Hz, 7-H)]. Found M^+ 254.5.$C_{15}H_{10}O_4$ requires 254.242. (^{1}H NMR plate 5a).

Acetylation gave the diacetate **(21)**, R_f 0.5 (T:EtOAc 9:1) as an amorphous yellow compound. Found ^{1}H NMR δ ($CDCl_3$) 7.26 (d, J 9.0, 3'/5'-H), 7.70 (d, J 9.0, 2'/6'-H), 7.82

(s, 10-H), 6.80 (dd, J 2.0 Hz, 4-H), 6.96 (d, J 9.0, 2.0 Hz, 5-H), 7.75 (d, J 9.0 Hz, 7-H) and 2x-OAc (2.33-2.37). (^{1}H NMR plate 5b)

4.2.1. Synthesis of Methyl 2-(2, 4, 6-trimethoxyphenyl) acrylate (22).

1,3,5-Trimethoxybenzene (0.25g, 1.5mmol, 1eq) and pyruvic acid (0.25g, 3mmol, 2 eq) were dissolved in MeOH (250mℓ). H_2SO_4 (1M, 20 drops) was added and the reaction mixture was refluxed for 72 hrs. The methanol was removed under vacuum. Centrifugal chromatography (4mm rotor, T:EtOAc 7:3) gave the two fractions, R_f 0.72, (blue with a spray reagent) and R_f 0.60 (purple with a spray reagent).

The fraction R_f 0.60 gave unreacted 1,3,5-trimethoxybenzene as transparent crystals (88.6 mg) from methanol, mp 49-50 °C (Lit[1] 51-53°). M^+ 168, δ ($CDCl_3$) 6.1 (s, aromatic protons).

The fraction R_f 0.72 gave methyl 2-(2,4,6-trimethoxyphenyl)acrylate (**22**) as white crystals (350 mg, 93%), mp 123 °. δ_H (600MHz, $CDCl_3$, $SiMe_4$) 6.6 (1H, d, J 2.0 Hz, β-H), 5.75 (2H, s, 3/5-H), 5.75 (1H, d, J 2.0 Hz, β-H), 3.74, 3.78 and 3.85 (4x-OCH_3). δ_C 168.37 C=O), 107.98 (Cα), 161.17 (C4'), 158.63 (C2′/6′), 90.86 (C3′/5′), 133.45 (C1′) and 55.89, 55.34 and 51.96 (4x-Ome). (^{1}H NMR plate 6a, ^{13}C NMR plate 6b, DEPT 135 plate 6c).

4.2.2. Synthesis of 2-(2, 4, 6-trimethoxyphenyl) acrylic acid (27).

1,3,5-Trimethoxybenzene (0.25g, 0.15mmol, 1eq) and pyruvic acid (0.025g, 0.3 mmol, 2eq) were dissolved in methanol (50mℓ). Ytterbium(III)trifluoromethanesulfonate-hydrate (0.005g) was added and the reaction mixture was stirred at room temperature for 48 hours. The methanol was removed under vacuum. Chromatography (Preparative TLC in T:EtOAc 7:3) gave two bands R_f 0.4 (blue with the spray reagent) and R_f 0.6 (purple with the spray reagent).

The fraction R_f 0.4 gave 2-(2,4,6-trimethoxyphenyl) acrylic acid (**27**) as white crystals (0.017g, 48 %), mp 70-72 °C. δ_H (600MHz, $CDCl_3$, $SiMe_4$) 6.6 (1H, d, J 2.0 Hz, β-H), 6.1 (2H, s, 3/5-H), 5.7 (1H, d, J 3.0 Hz, β-H), 3.8,3.6 (3x-OMe). δ_C (150MHz, acetone d_6 , $MeSi_4$) 173.2 (C=O), 107.7 (Cα), 133.4 (C-1′), 91.2 (C-3′/5′), 161.7 (C-4′), 159.0 (C-2/6) and 129.0 (–C_β). (^{1}H NMR plate 7a and ^{13}C NMR plate 7b).

4.2.3. Synthesis of 2-methoxy-3-(2,4,6-trimethoxyphenyl)-4,5-dihydrofurane (29).

Methyl 2-(2, 4, 6-trimethoxyphenyl)acrylate (**22**) (0.05g, 0.2mmol, 1eq) was dissolved in MeOH (5mℓ) and cooled to ~10ºC. Methylation with diazomethane and PTLC gave two fractions R_f 0.7 (blue with the spray reagent) and R_f 0.6 (brown).

The fraction R_f 0.6 was identified with ^{1}H NMR as unreacted acrylate (**22**).

The fraction R_f 0.6 was identified as 2-methoxy-3-(2,4,6-trimethoxyphenyl)-4,5-dihydrofurane (**29**) as a white amorphous solid (0.045g, 85%). δ_H (600MHz, $CDCl_3$, $SiMe_4$) 6.05 (2H, s, 3′/5′-H), 4.7 (1H, dd, J 10, 4 Hz, 2 H_{eq}), 4.5 (1H, dd, J 10, 8 Hz, 2 -H_{ax}), 2.59 (1H, dd, J 10, 4 Hz, 3-H_{eq}), 1.58 (1H, dd, J 8, 10 Hz, 3-H_{ax}), δ 3.57-4.10 (4x –OCH_3). δ_C 176.3 (C-5), 160.9 (C4′), 159.0 (C2′/6′), 92.2 (C3′/5′), 109.5 (C-1), 100.0 (C1′), 77.6 (C-2). 27.6 (C-3).m/z 266 (M^+, 100%), 266 (100), 235 (15), 207 (75). (Found M^+ 266.1155, $C_{14}H_{18}O_5$ requires 266.1154) (^{1}H NMR plate 8a and ^{13}C NMR plate 8b, Dept 135 plate 8c, HMQC plate 8d, COSY plate 8e, mass scheme C).

4.2.4 Synthesis of Methyl oxo-(2, 4, 6-trimethoxyphenyl) acetate (30).

Methyl 2-(2, 4, 6-trimethoxyphenyl) acrylate (0.02g) was dissolved in EtOAc (10mℓ) and cooled to -78 ºC. O_3 gas was bubbled through the solution for 10 minutes and cooled again for 20 minutes. Ethyl acetate was removed under vacuum. PTLC gave a R_f 0.54 fraction, red with the spray reagent.

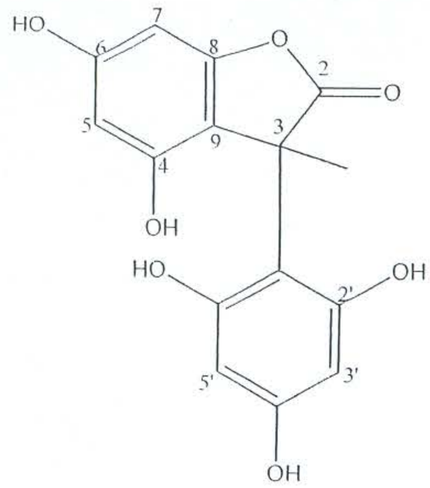
The R_f 0.54 fraction was identified as methyl oxo-(2,4,6-trimethoxyphenyl)acetate (**30**), (0.005g, 25%). δ_H (300MHz, $CDCl_3$, $MeSi_4$) 6.0 (2H, s, 3-H/5-H) and 4x-OMe. ^{13}C NMR

δ_C (150MHz, $CDCl_3$, $MeSi_4$) 180.0 and 162.64 (C=O), 162.0 (C1′/6′), 160 (C4′-160) and 4x-OMe (^{1}H NMR plate 9a and ^{13}C NMR plate 9b)

4.3 REFERENCE

1. Lewis R. J; Sr. *Hazardous Chemicals*, **1993**, International Thompson, USA, 1025.

Plate 1a (CDCl 3, 298K)



(12)

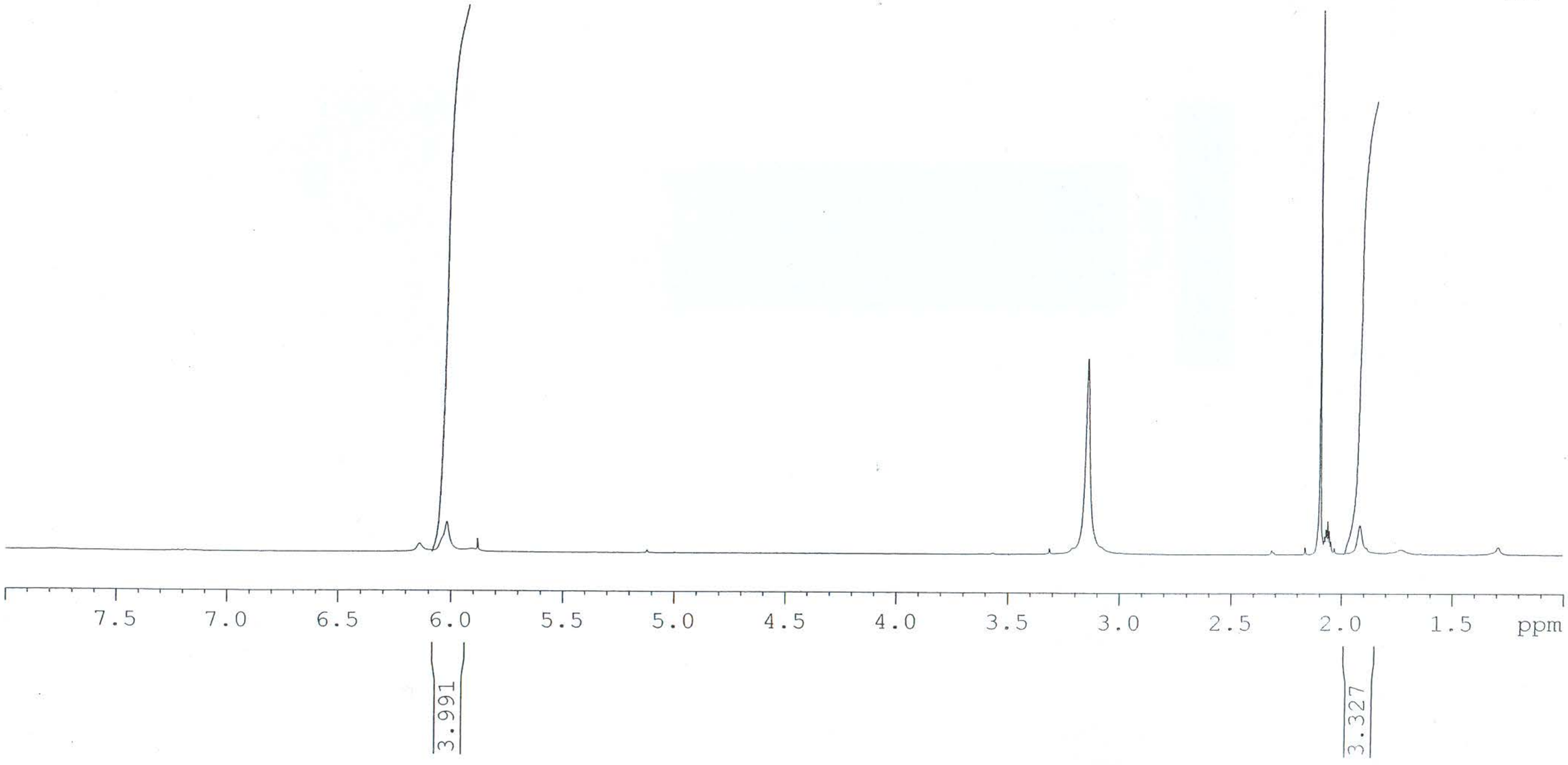


Plate 1c ($CDCl_3$, 298K)

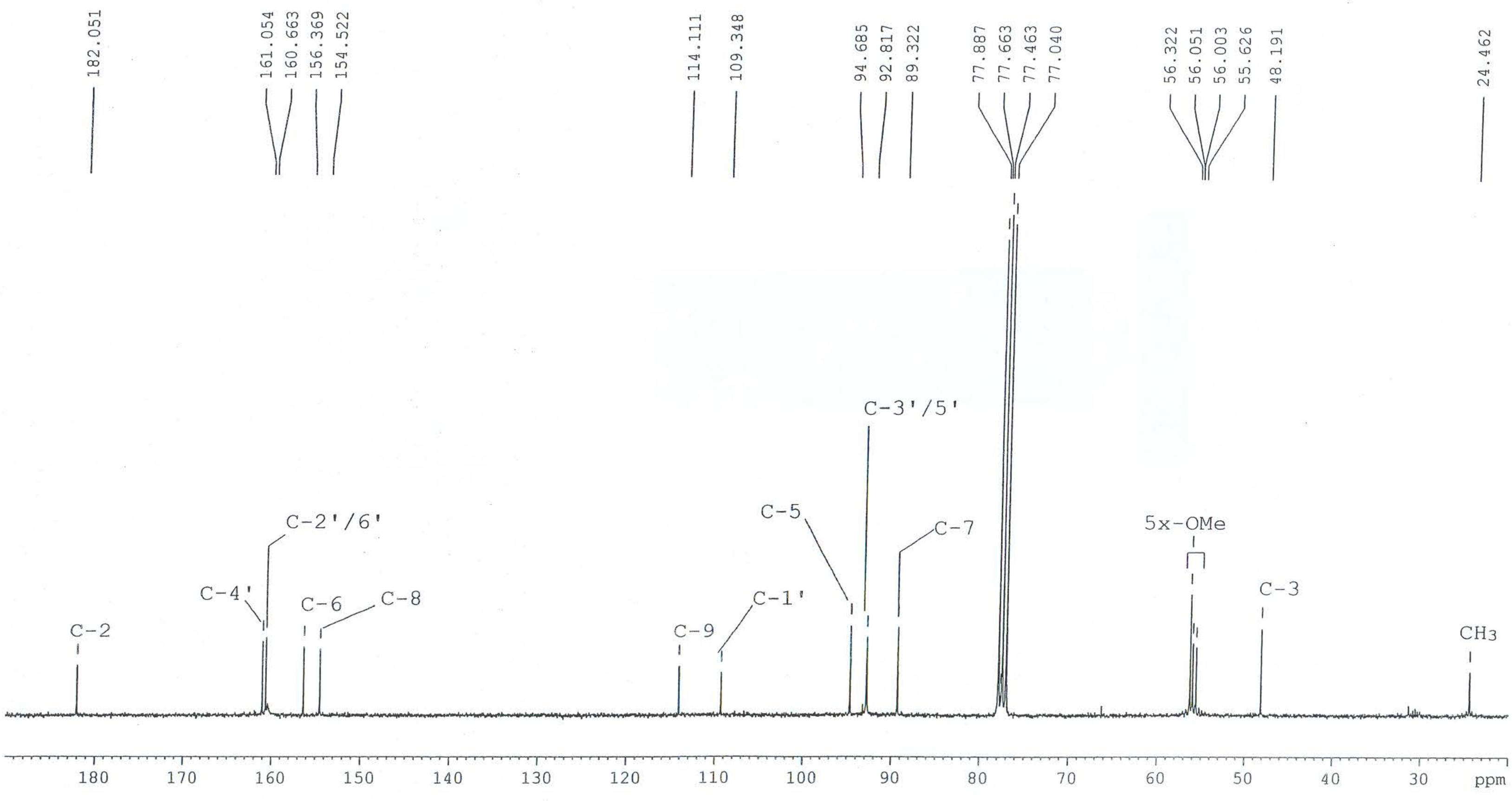


Plate 1d ($CDCl_3$, 298K)

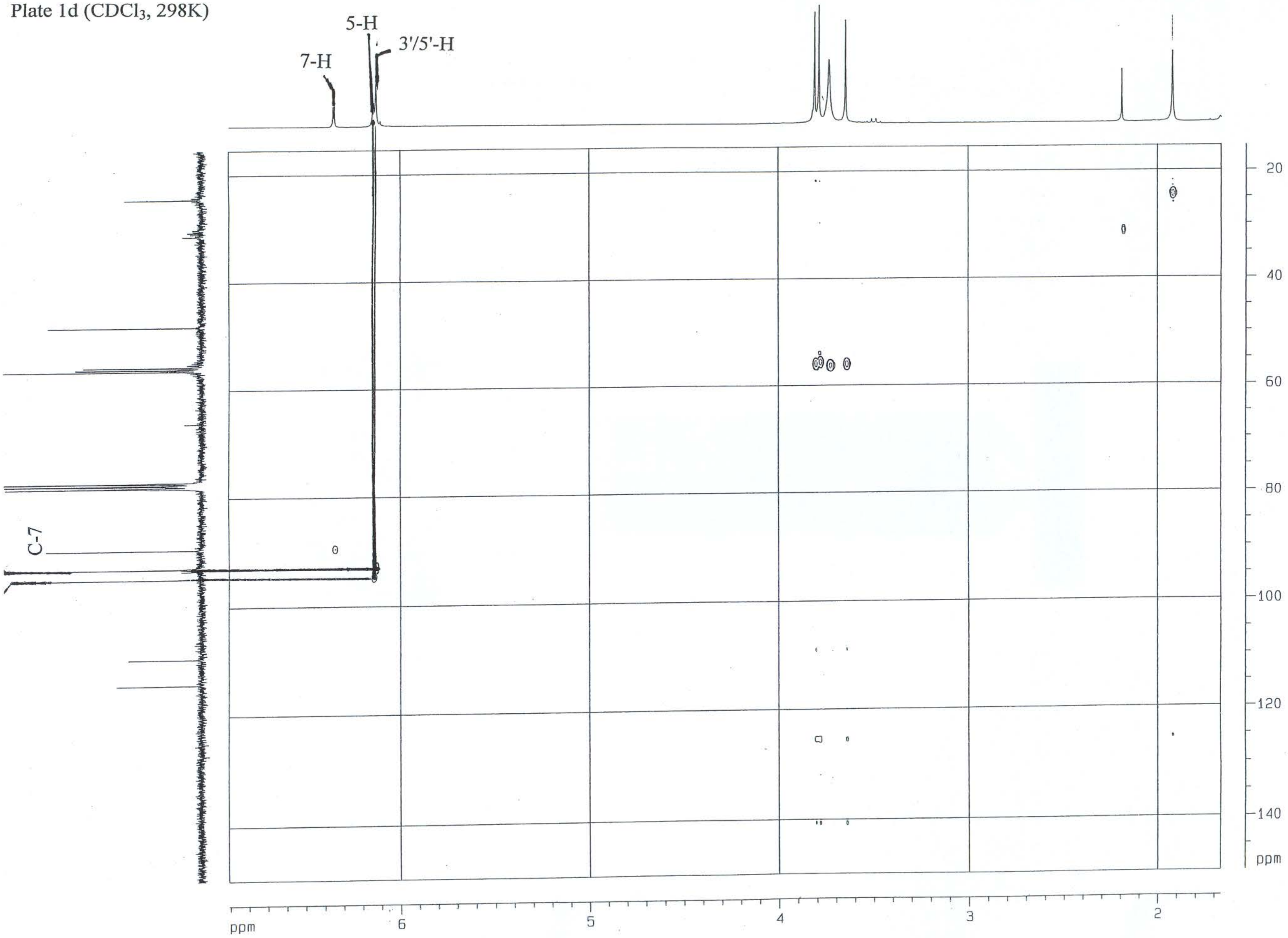


Plate 1e ($CDCl_3$, 298K)

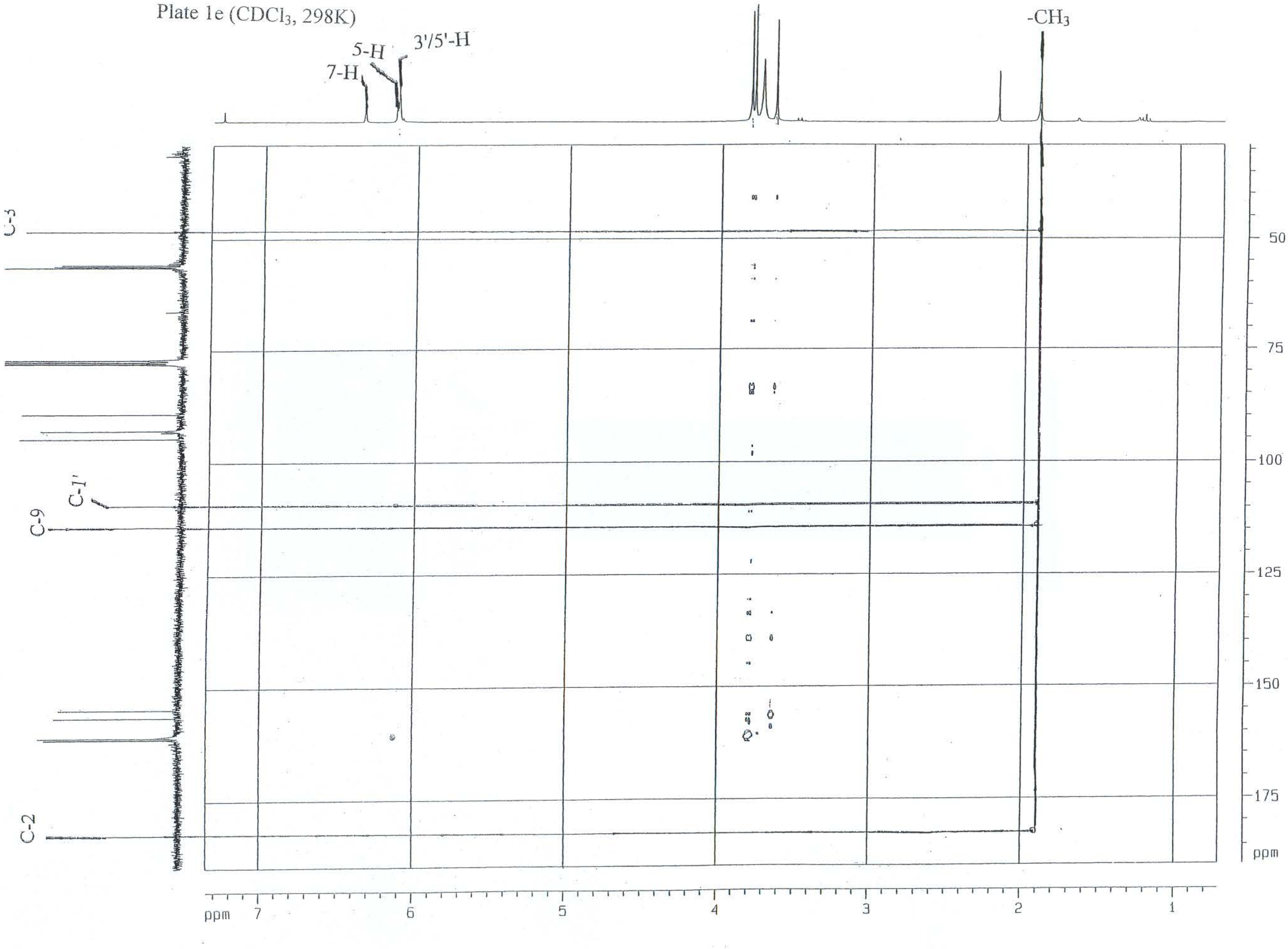
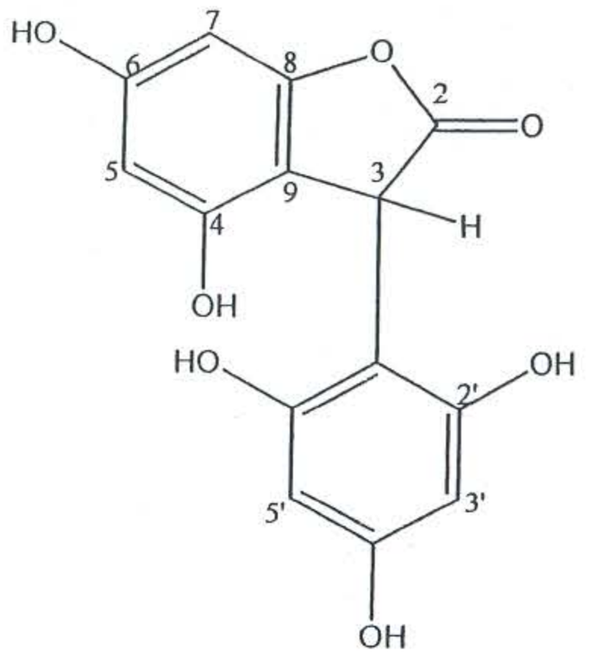


Plate 2a (Acetone, 298K)



(14)

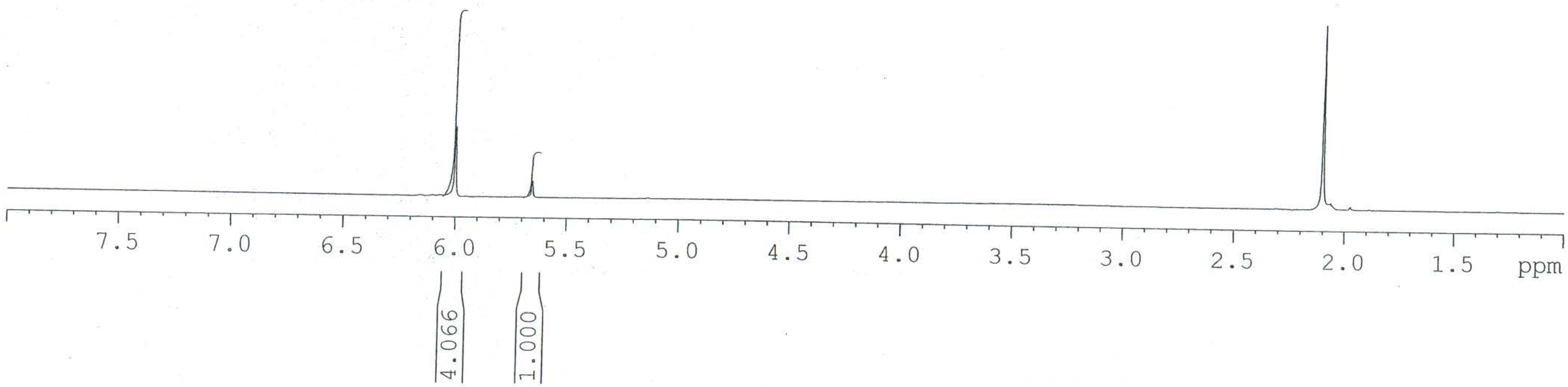
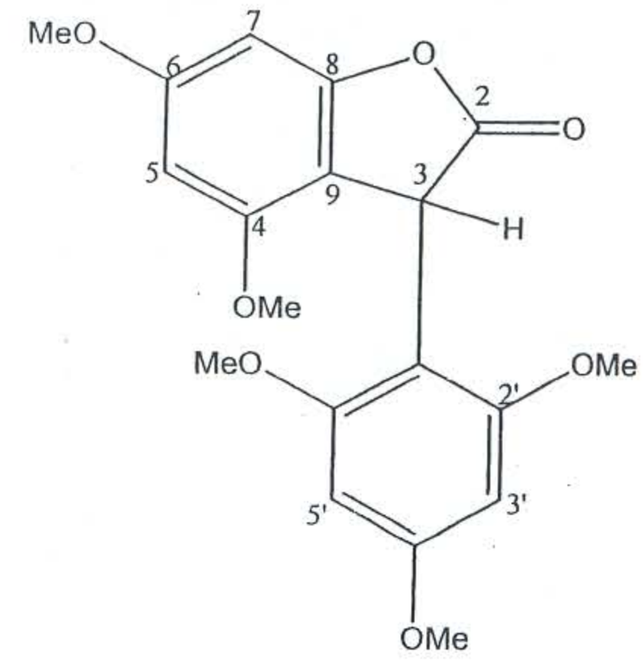
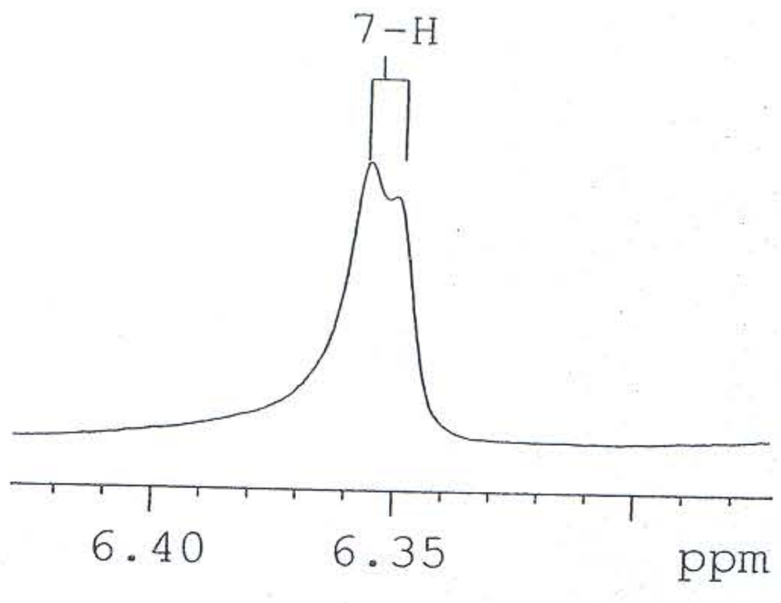
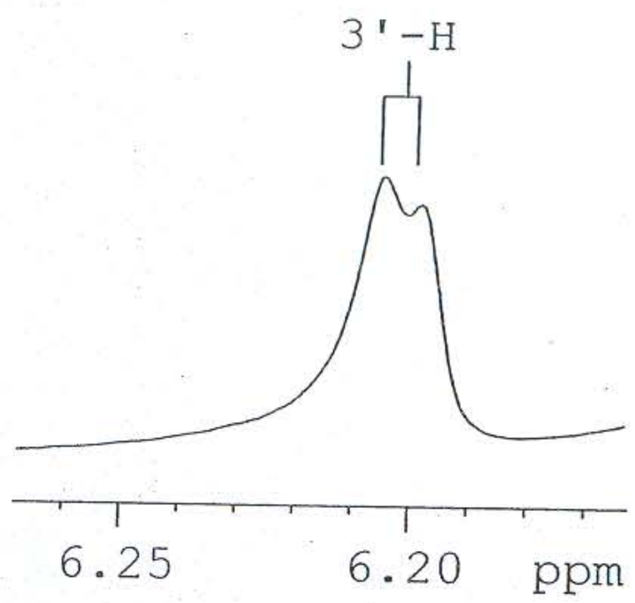


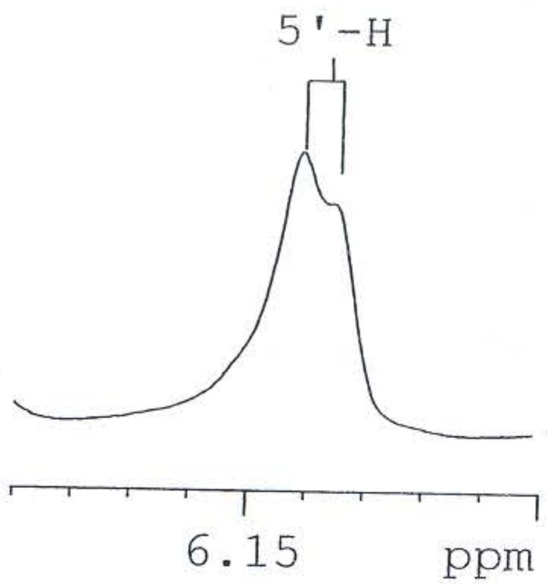
Plate 2b ($CDCl_3$, 298K)

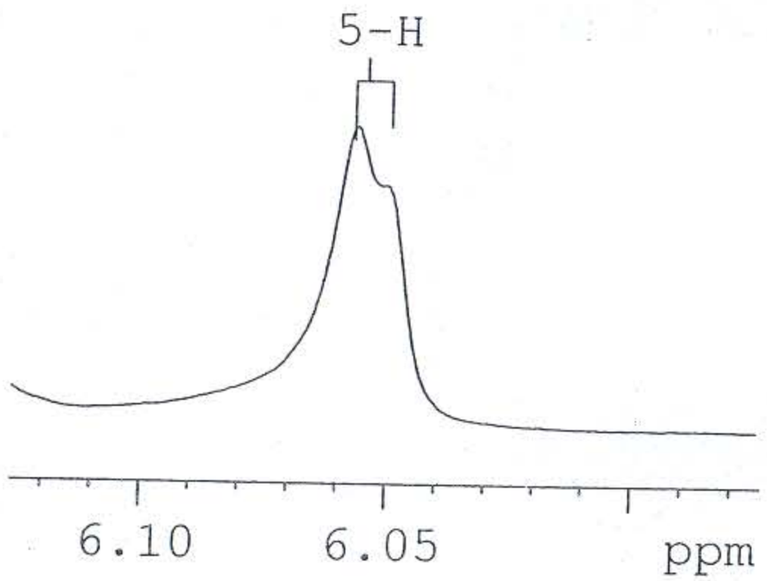


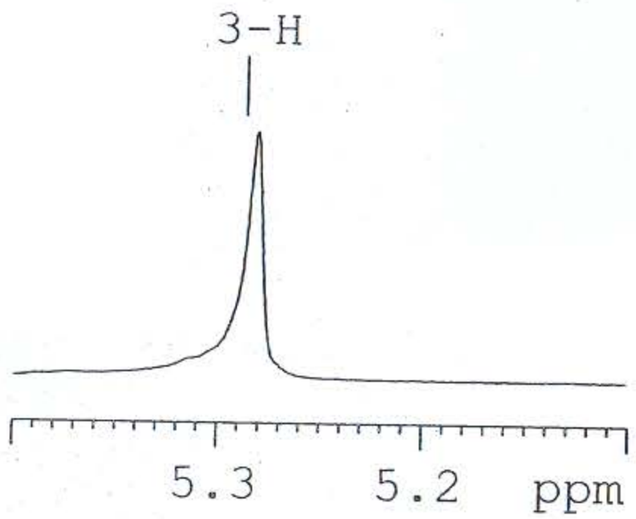
(15)

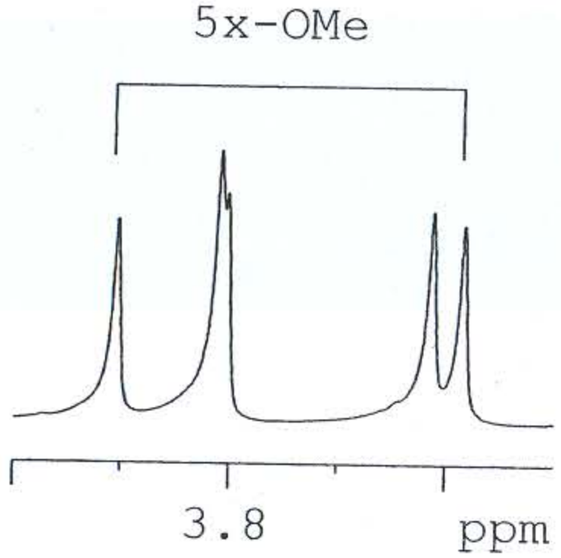












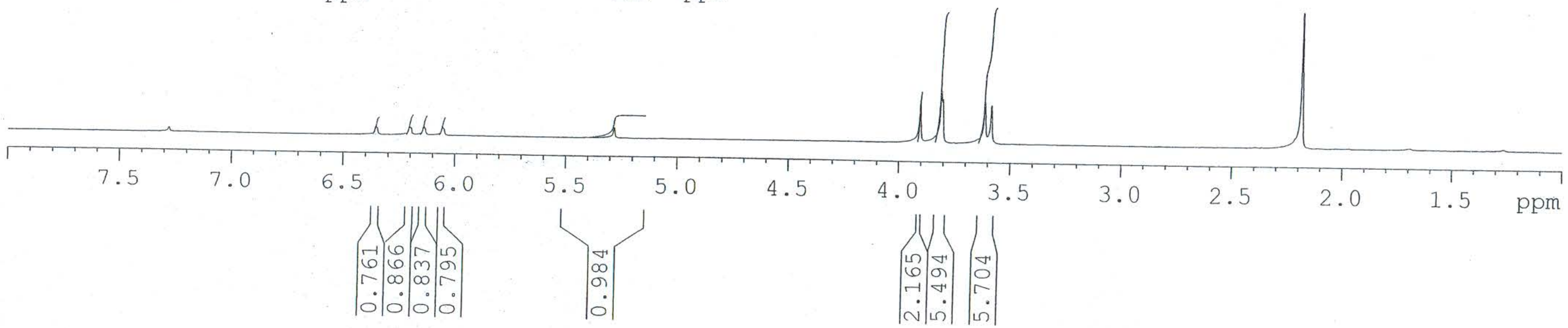
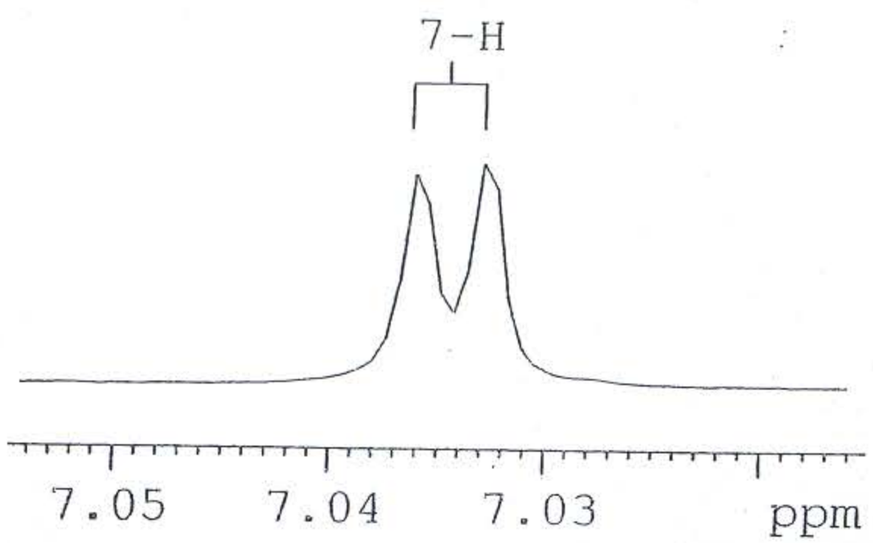
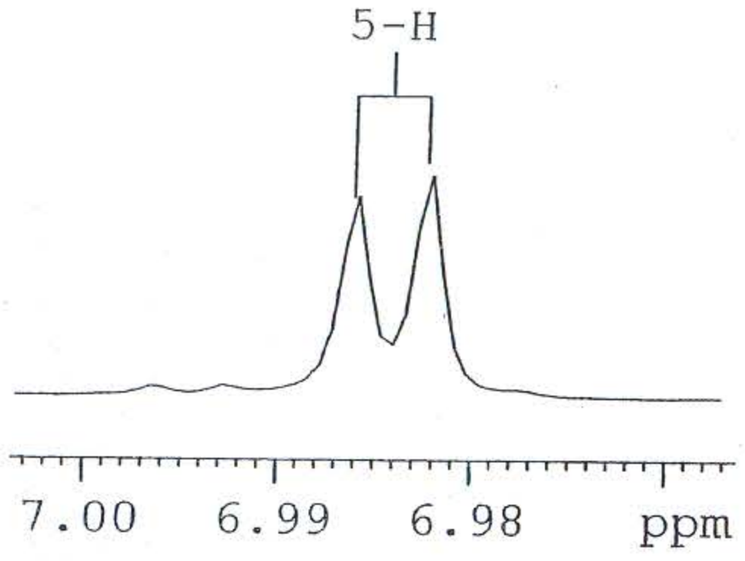
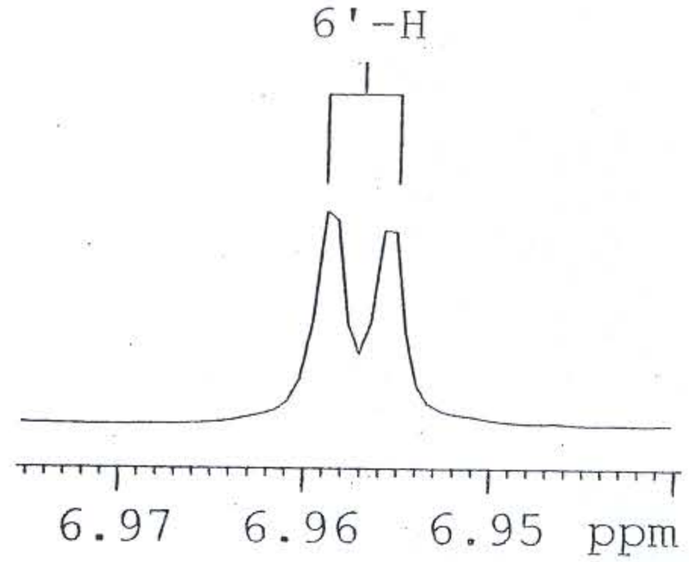
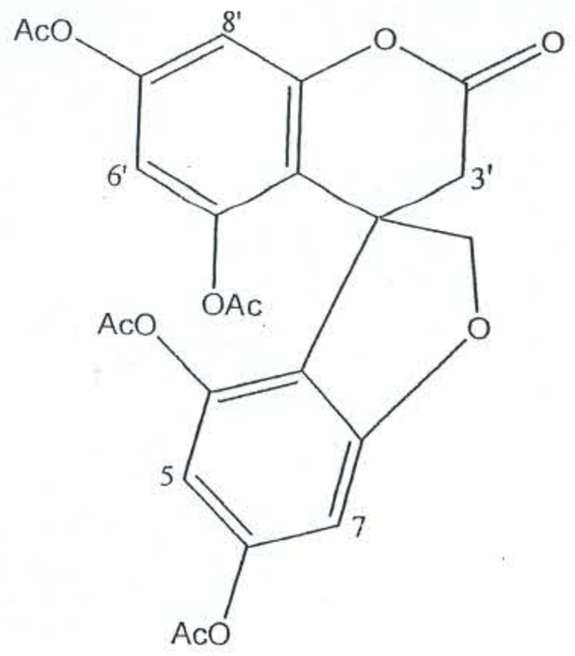


Plate 3a ($CDCl_3$, 298K)

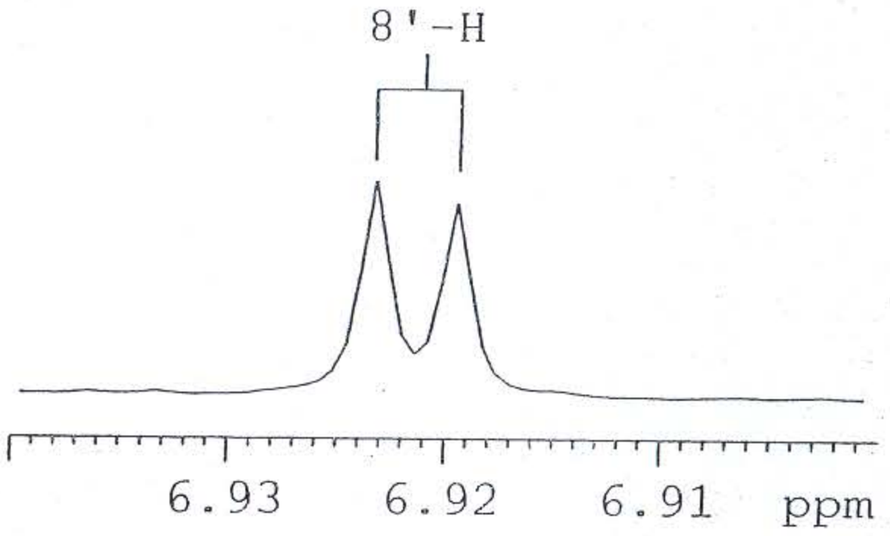


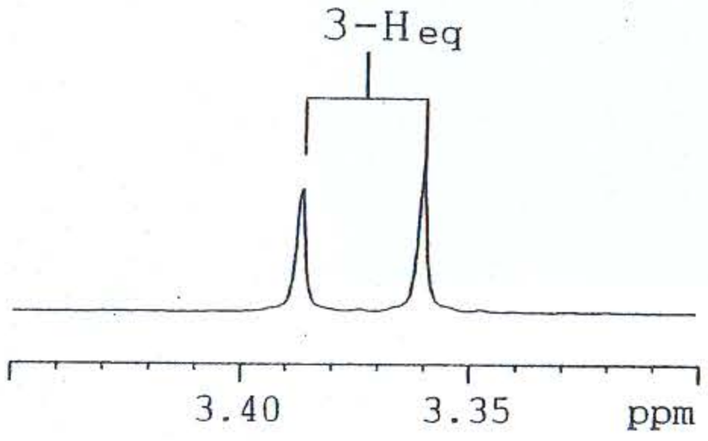


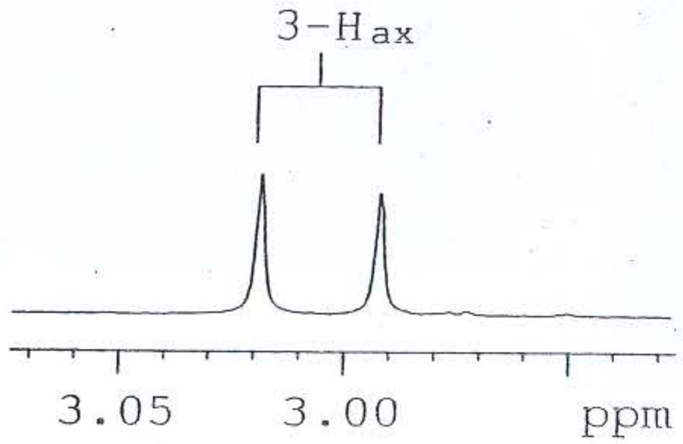


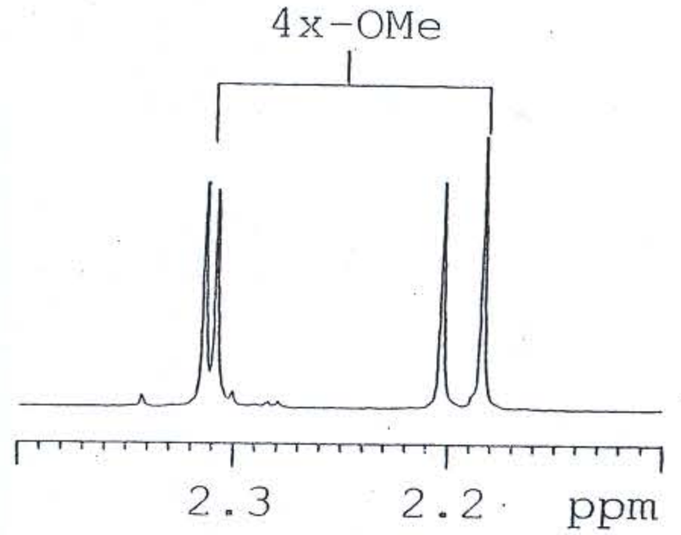


(18)









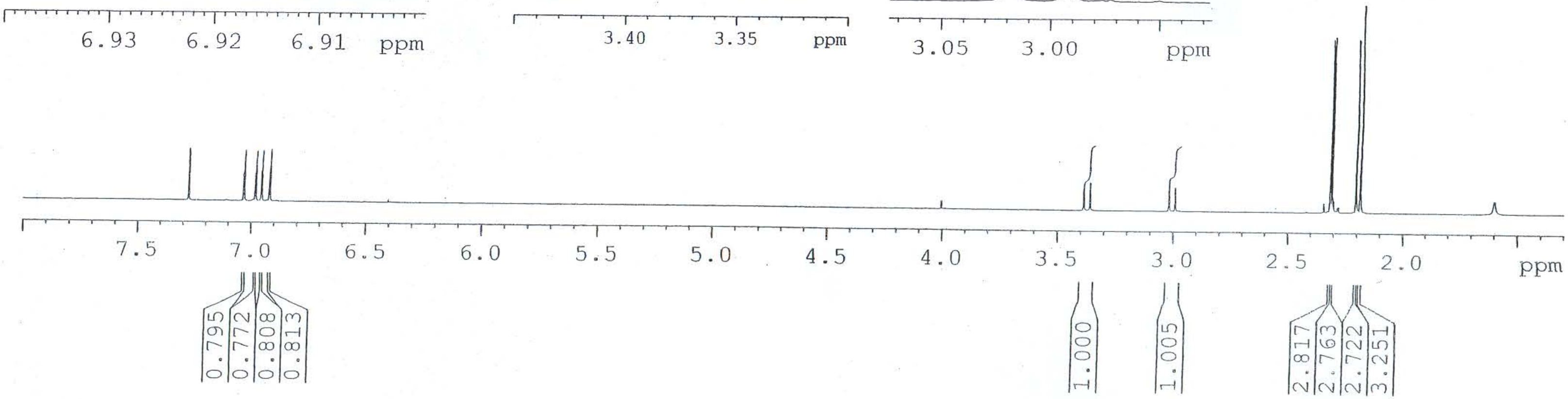


Plate 3b ($CDCl_3$, 298K)

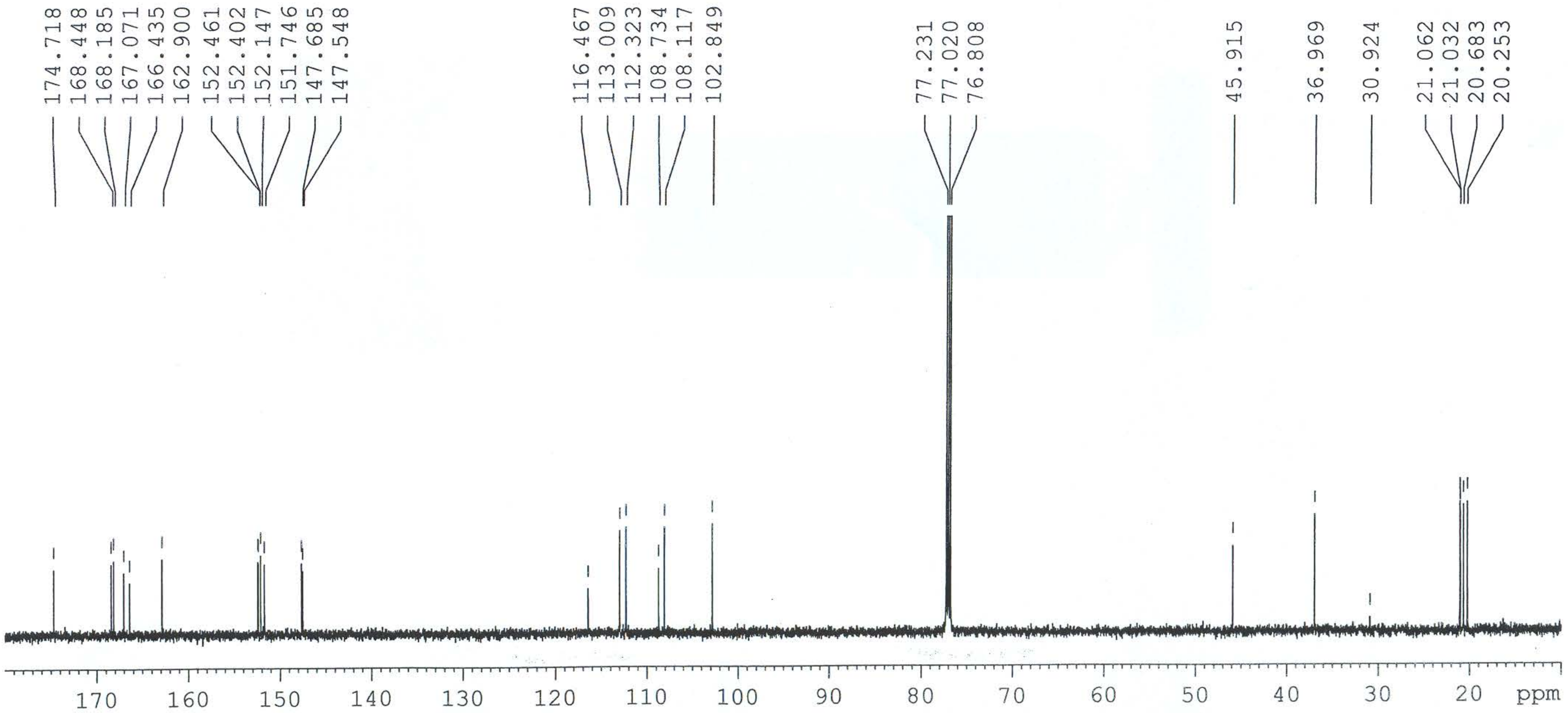


Plate 3c ($CDCl_3$, 298K)

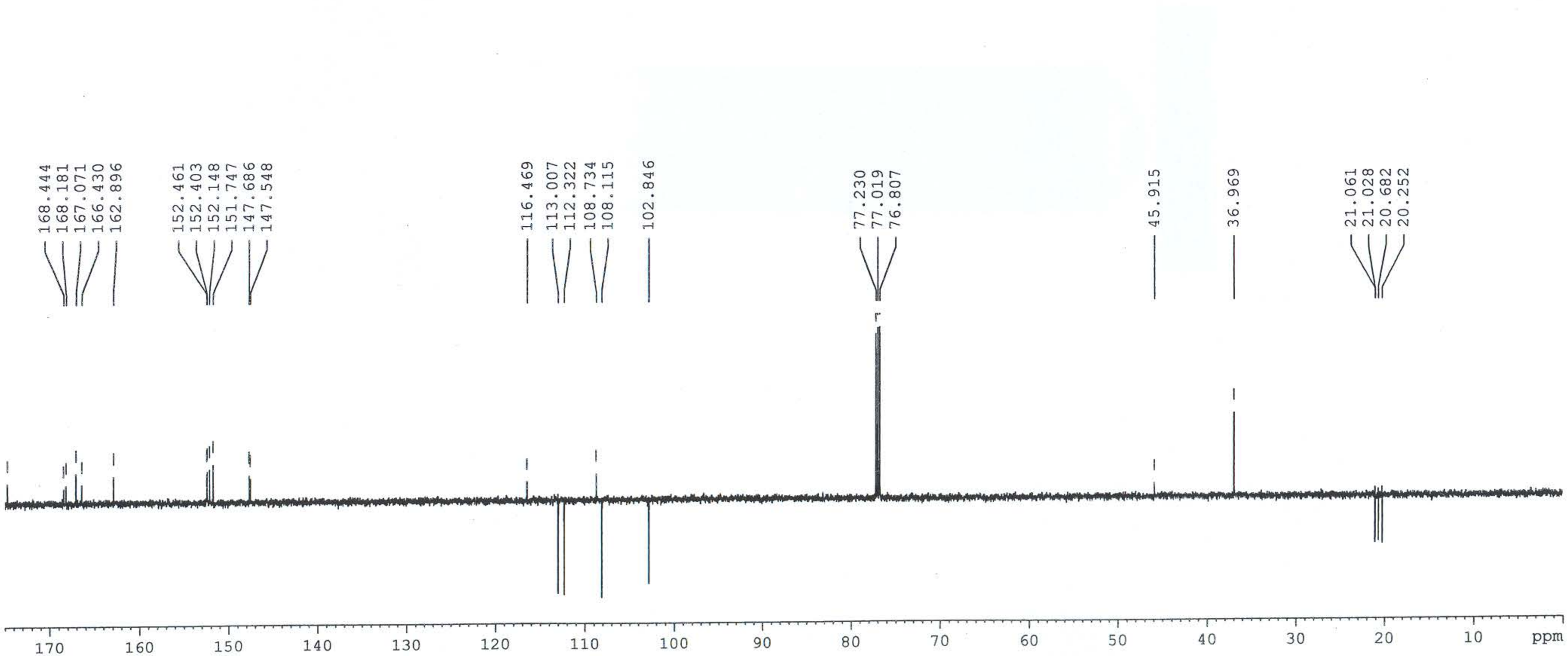
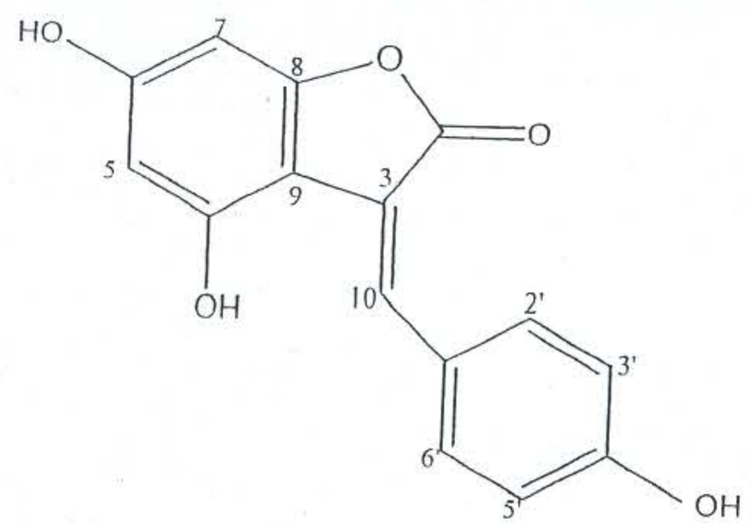


Plate 4a (Acetone, 298K)



(19)

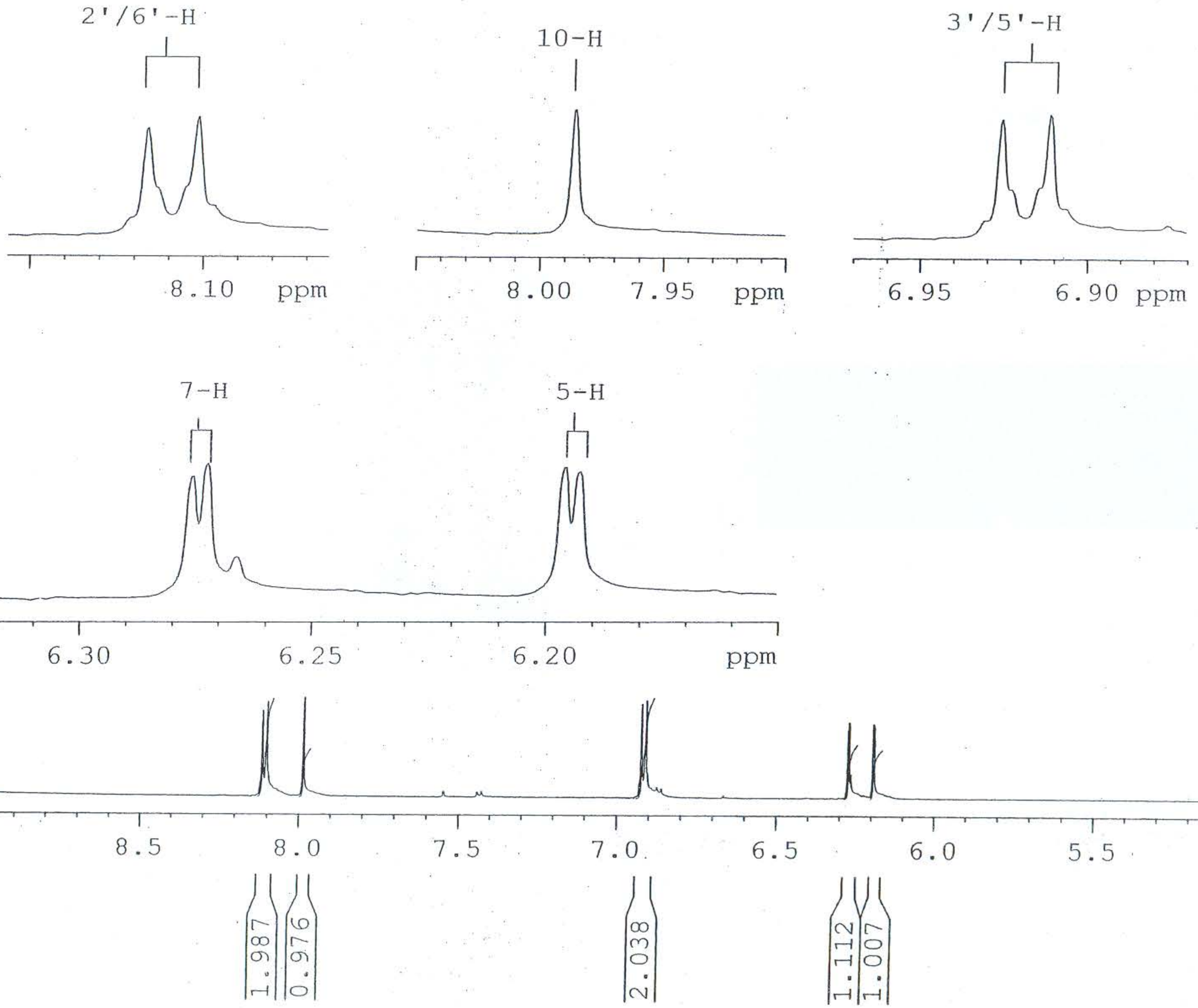


Plate 4b (Acetone,298K)

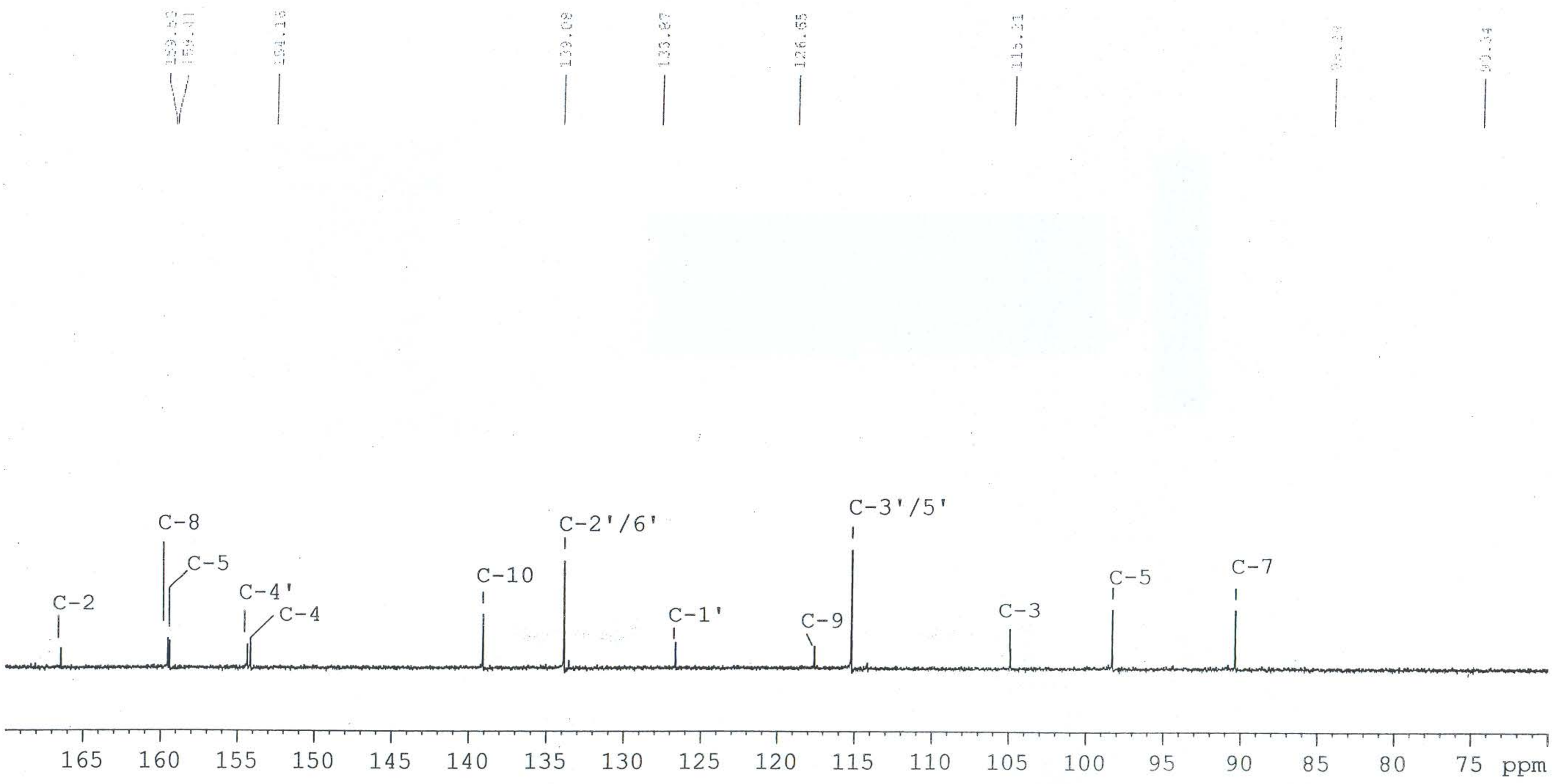
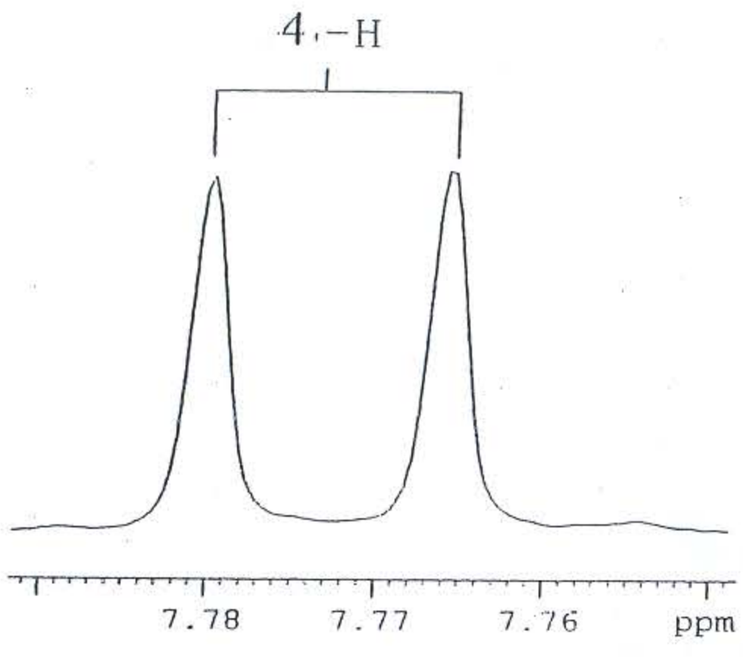
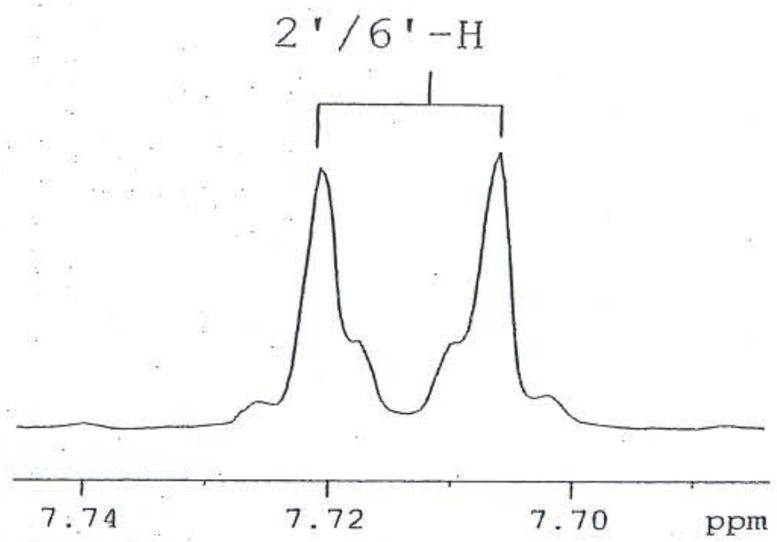
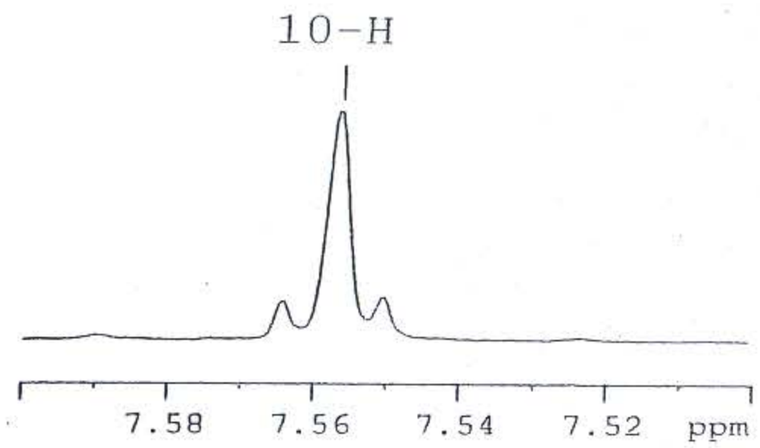
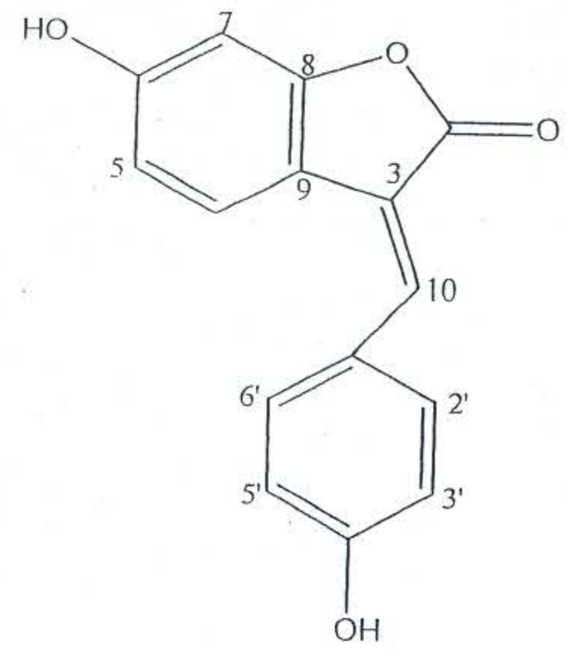


Plate 5a (Acetone, 298K)

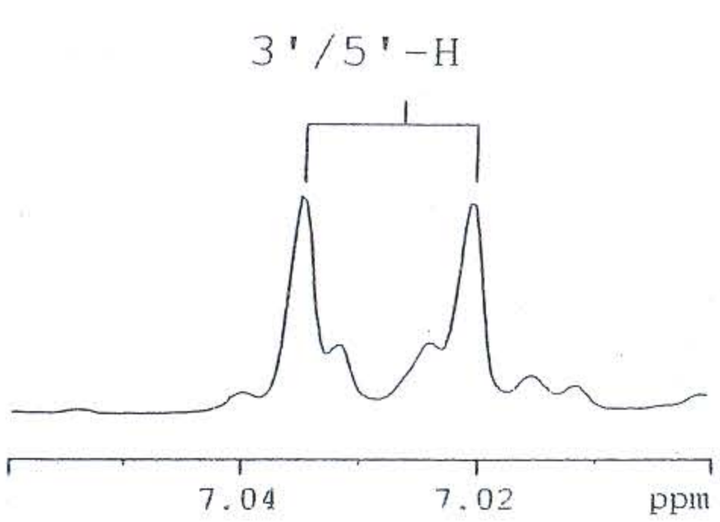


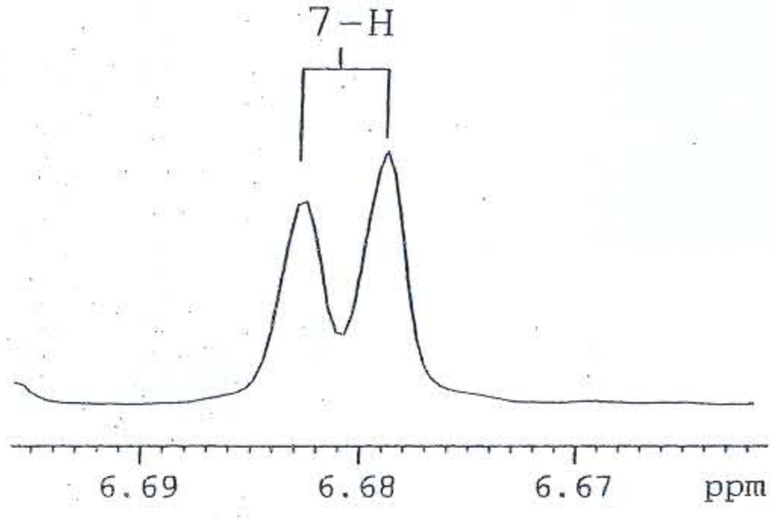


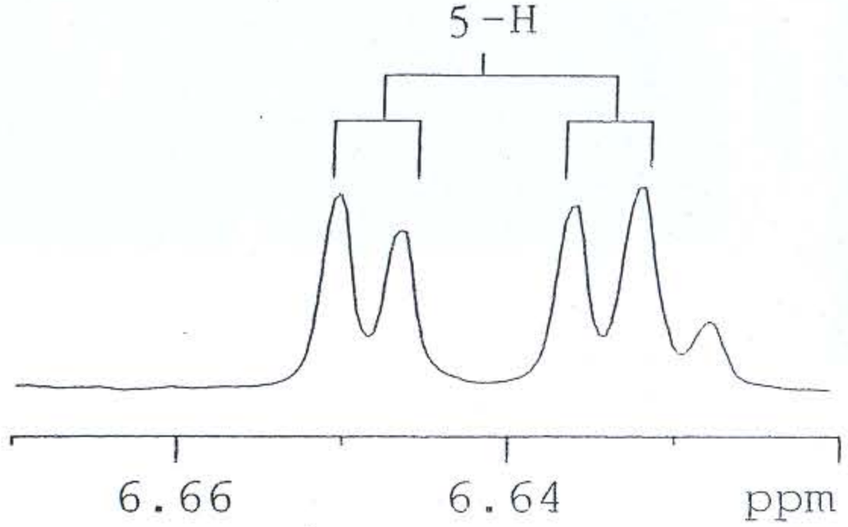




(20)







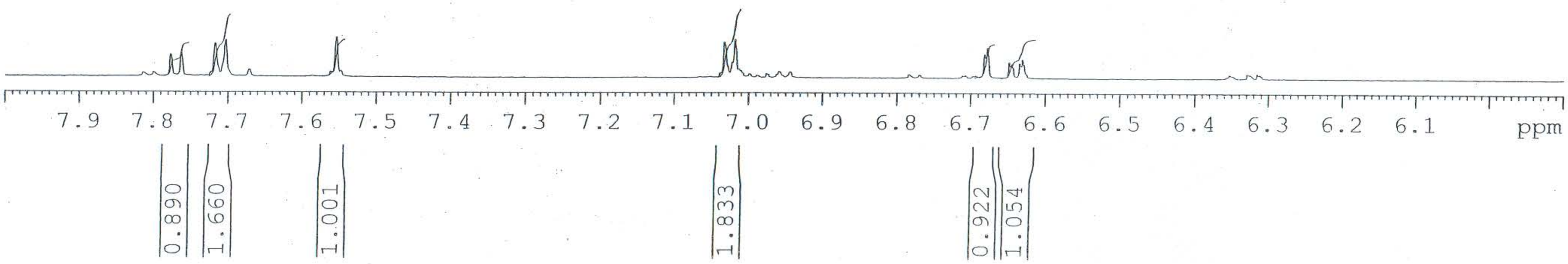
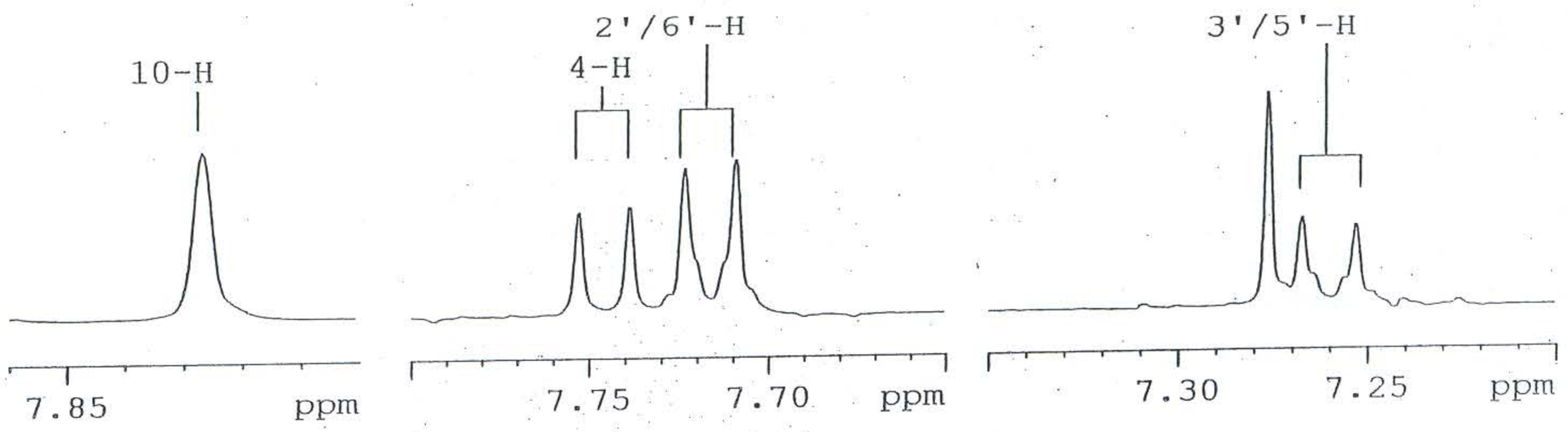
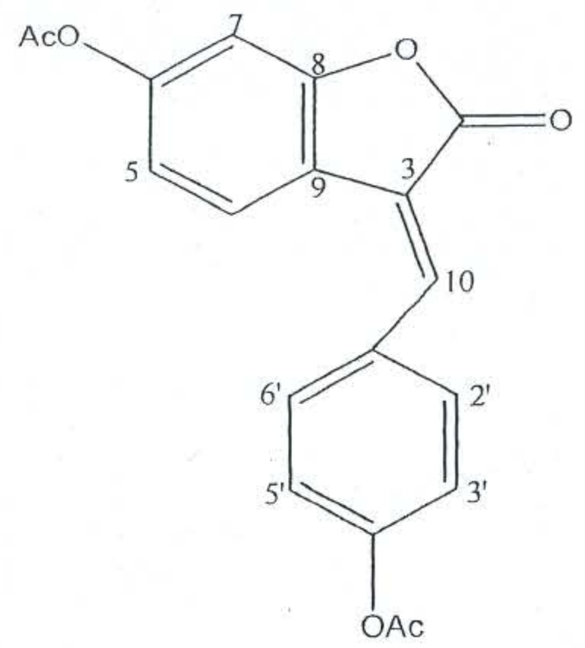


Plate 5b ($CDCl_3$, 298K)





(21)

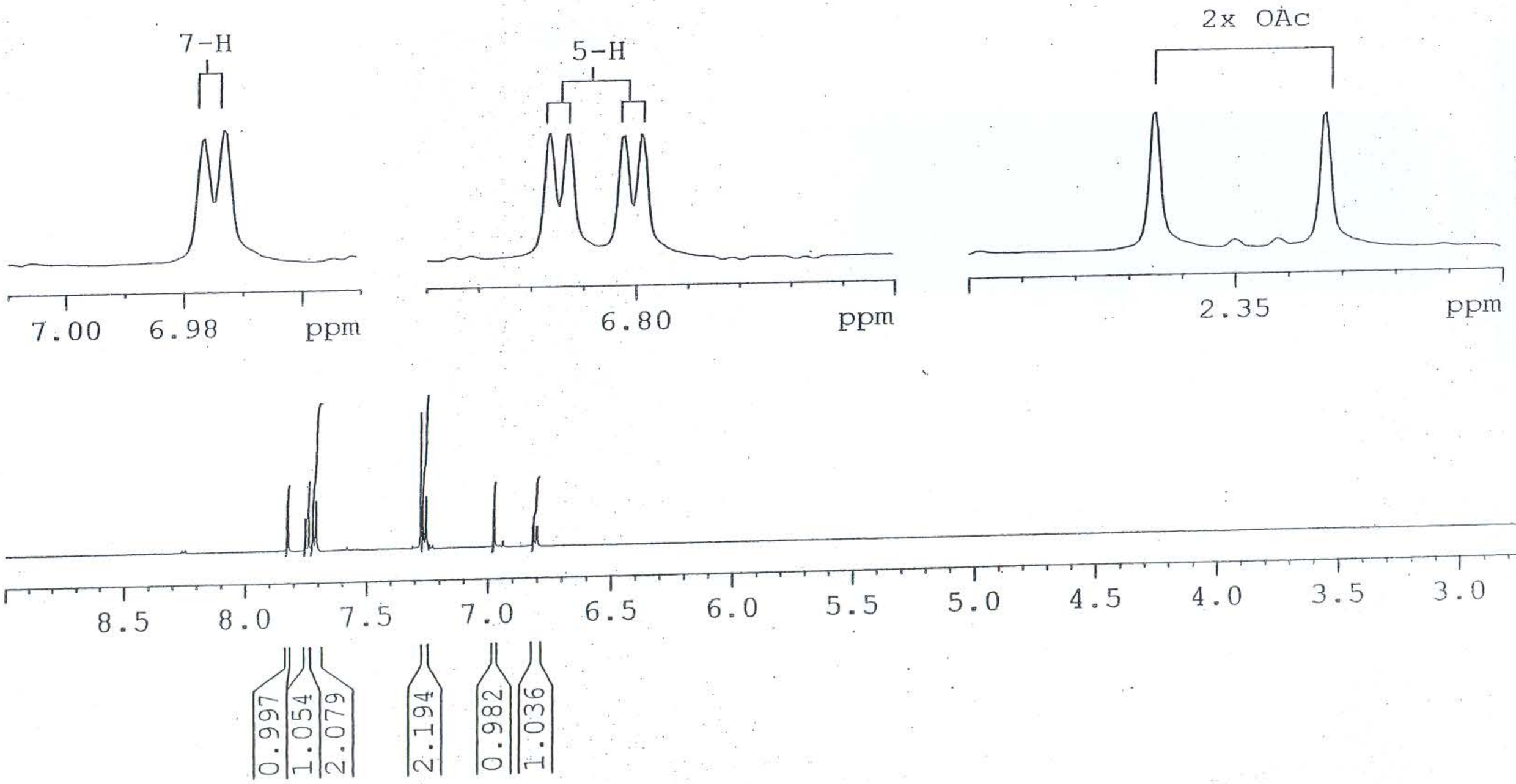
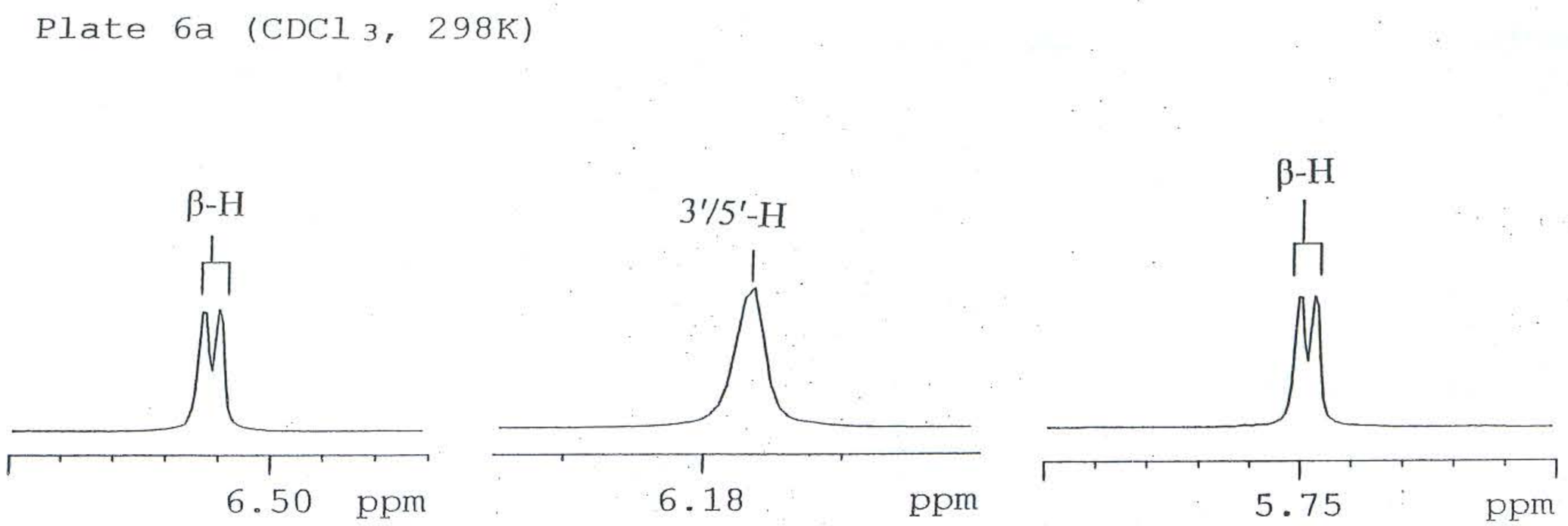
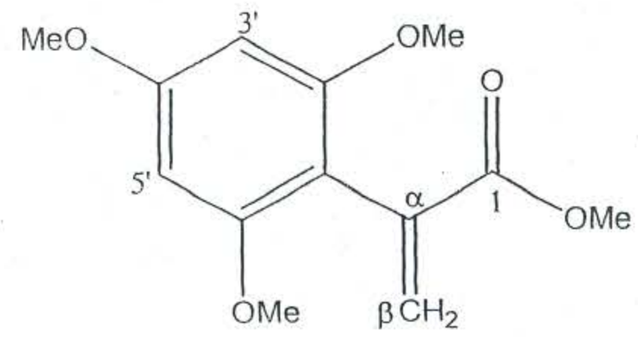


Plate 6a ($CDCl_3$, 298K)





(22)

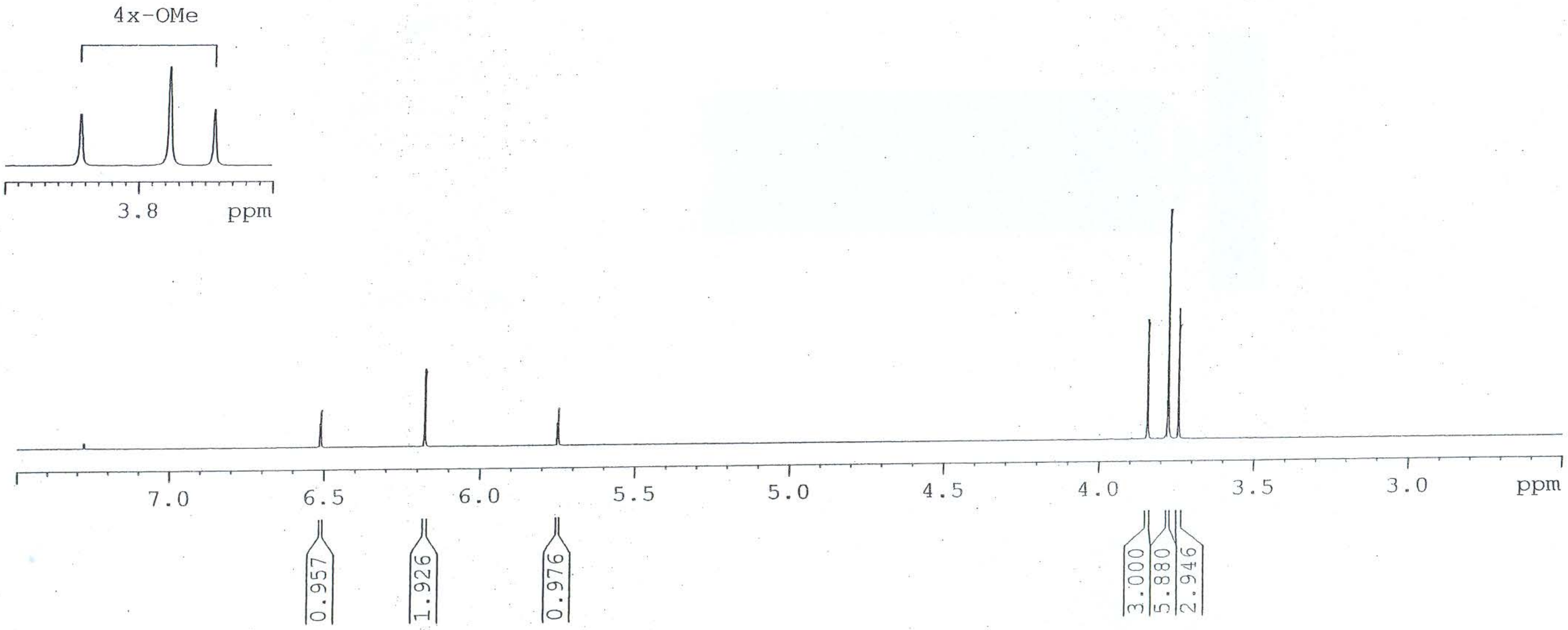


Plate 6b (CDCl 3,298K)

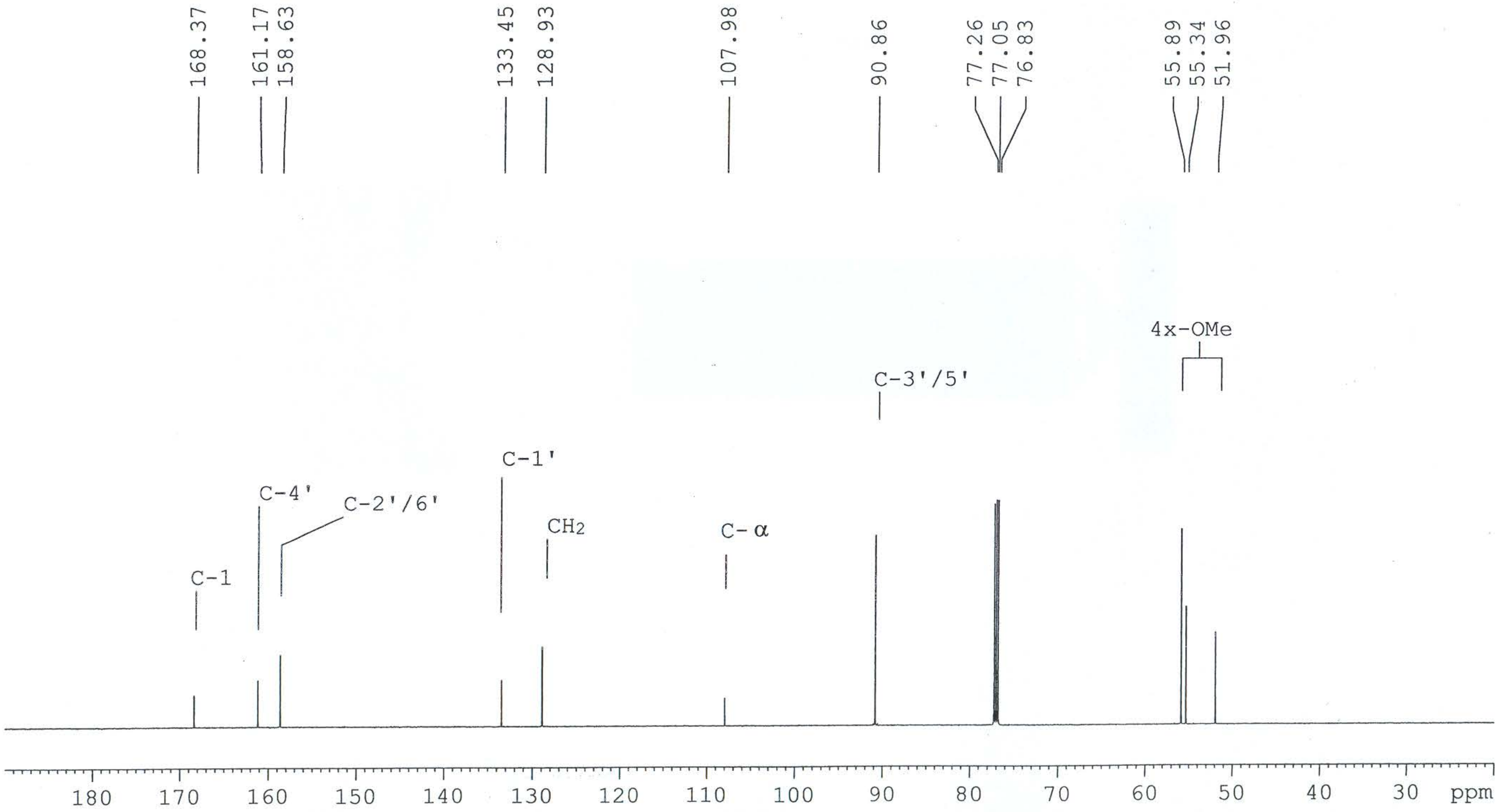


Plate 6c ($CDCl_3$, 298K)

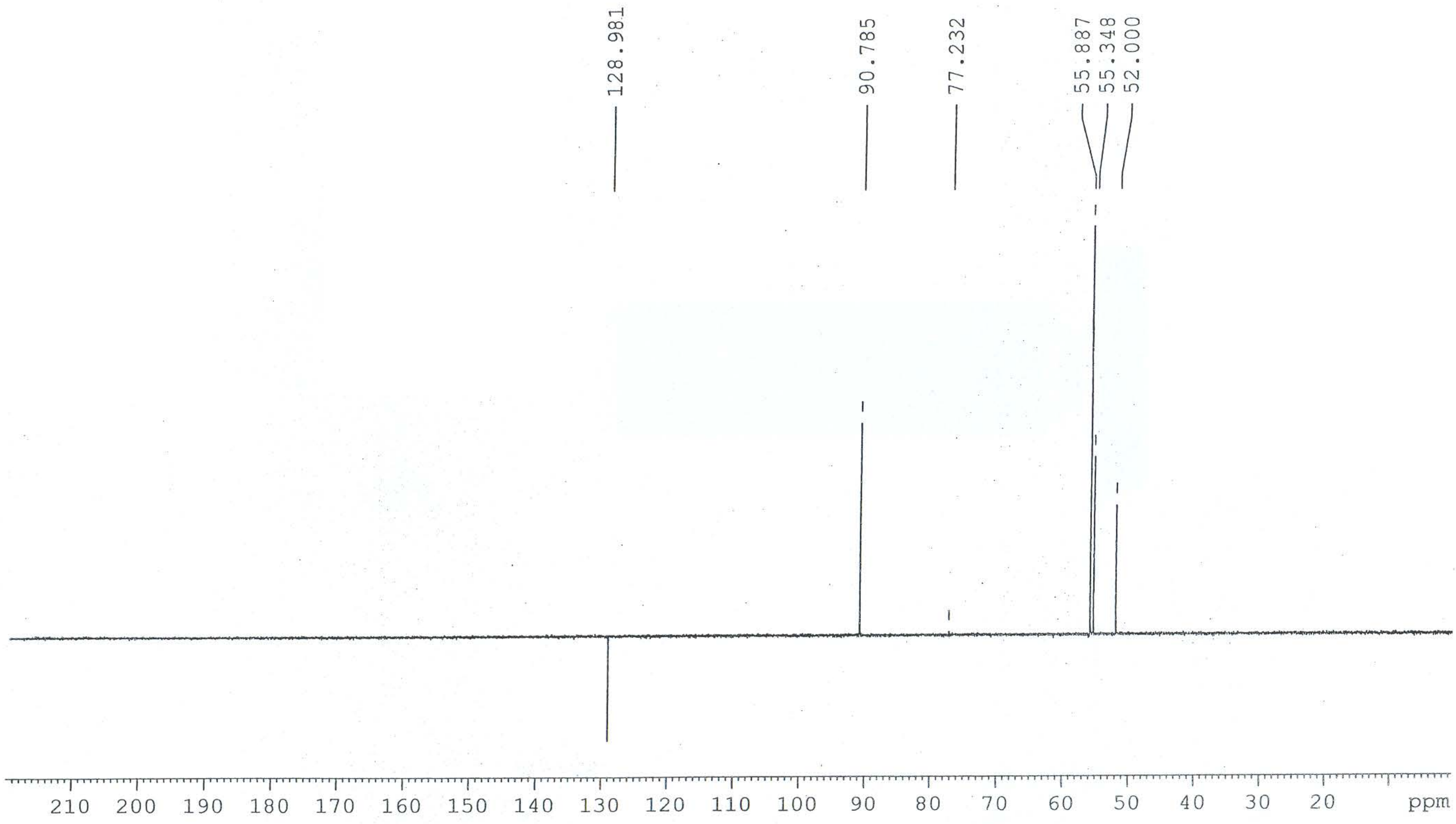
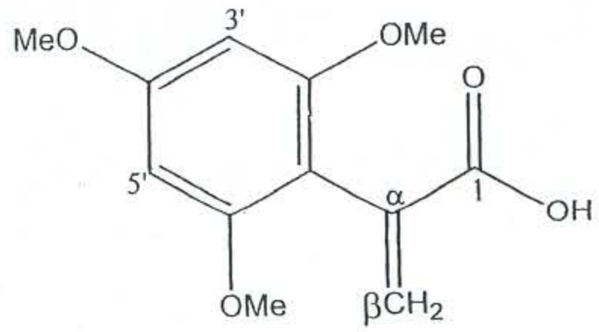
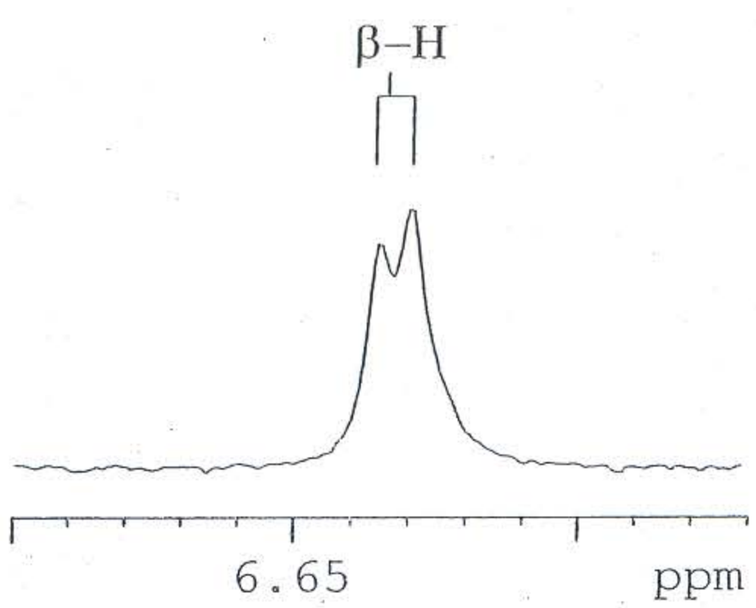
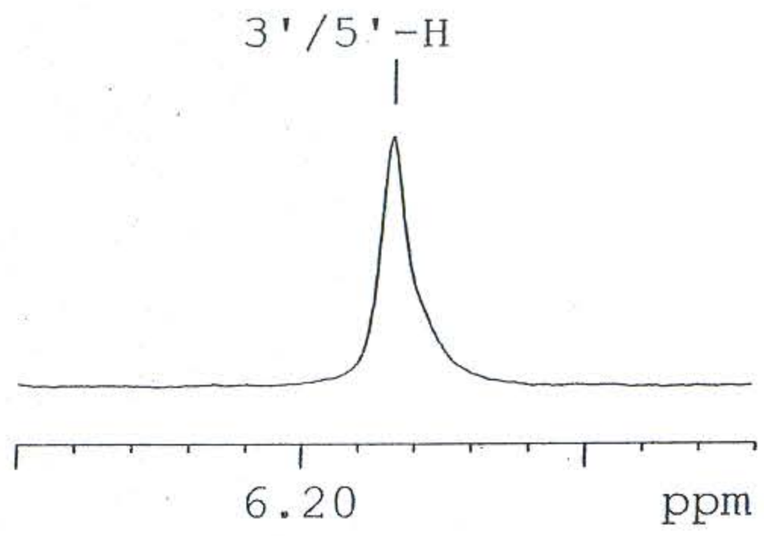


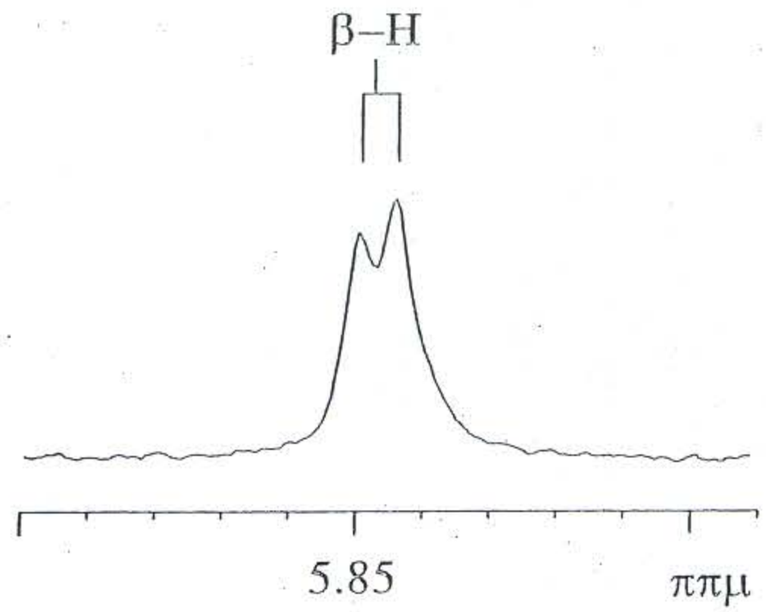
Plate 7a (CDCl 3, 298K)



(27)







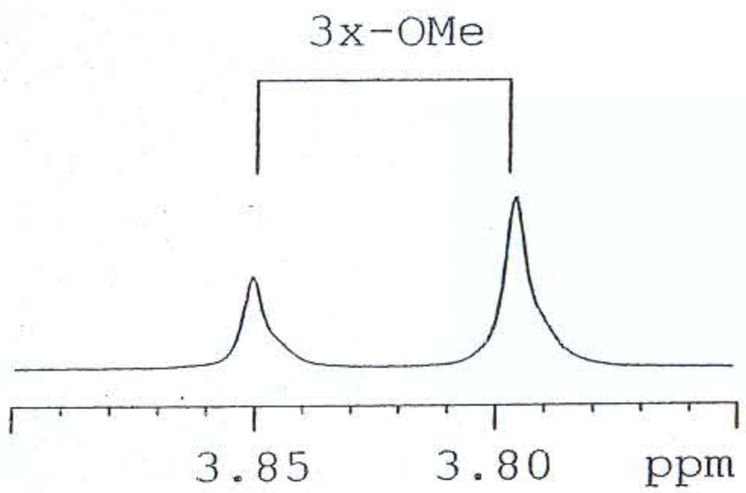


Plate 7b ($CDCl_3$, 298K)

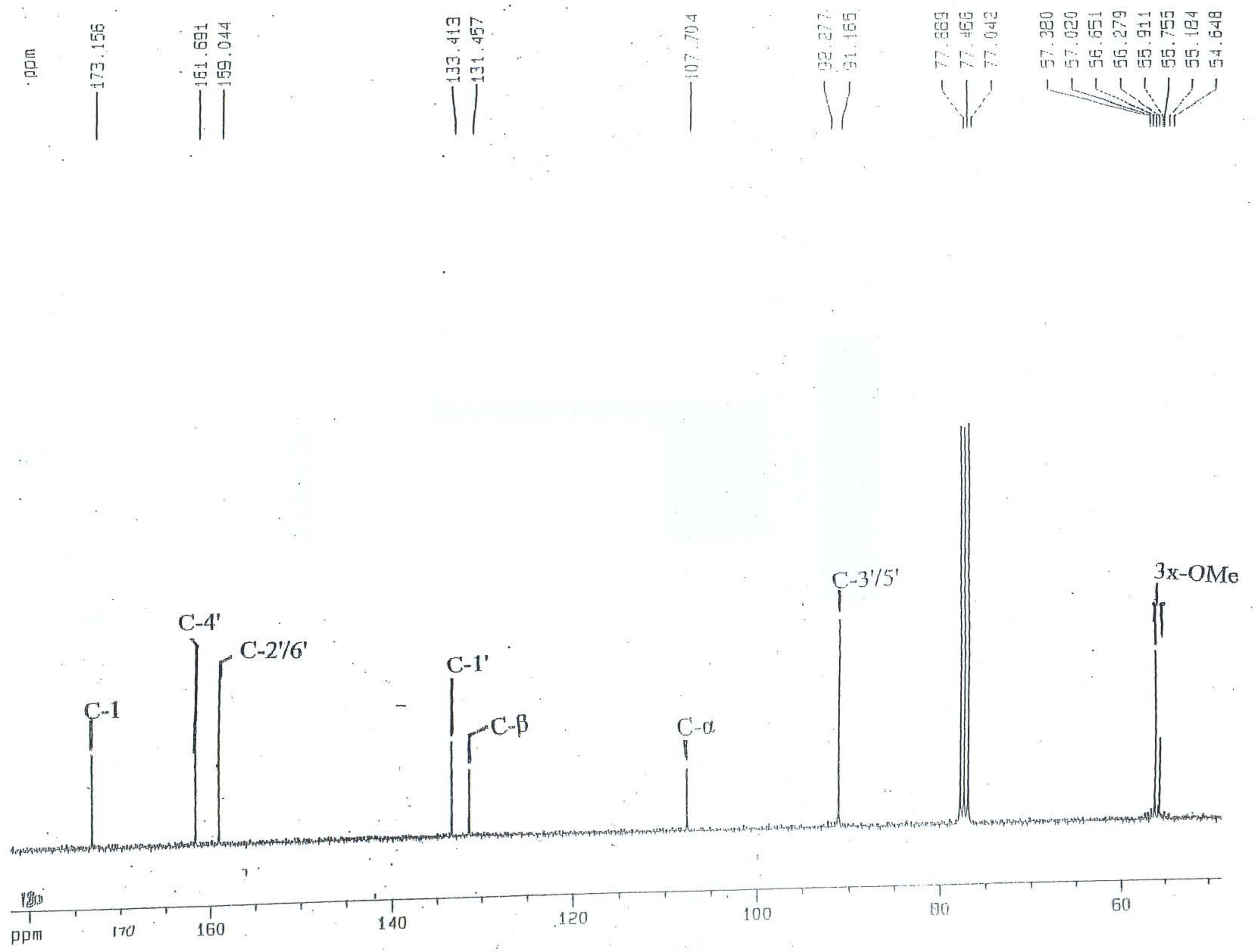
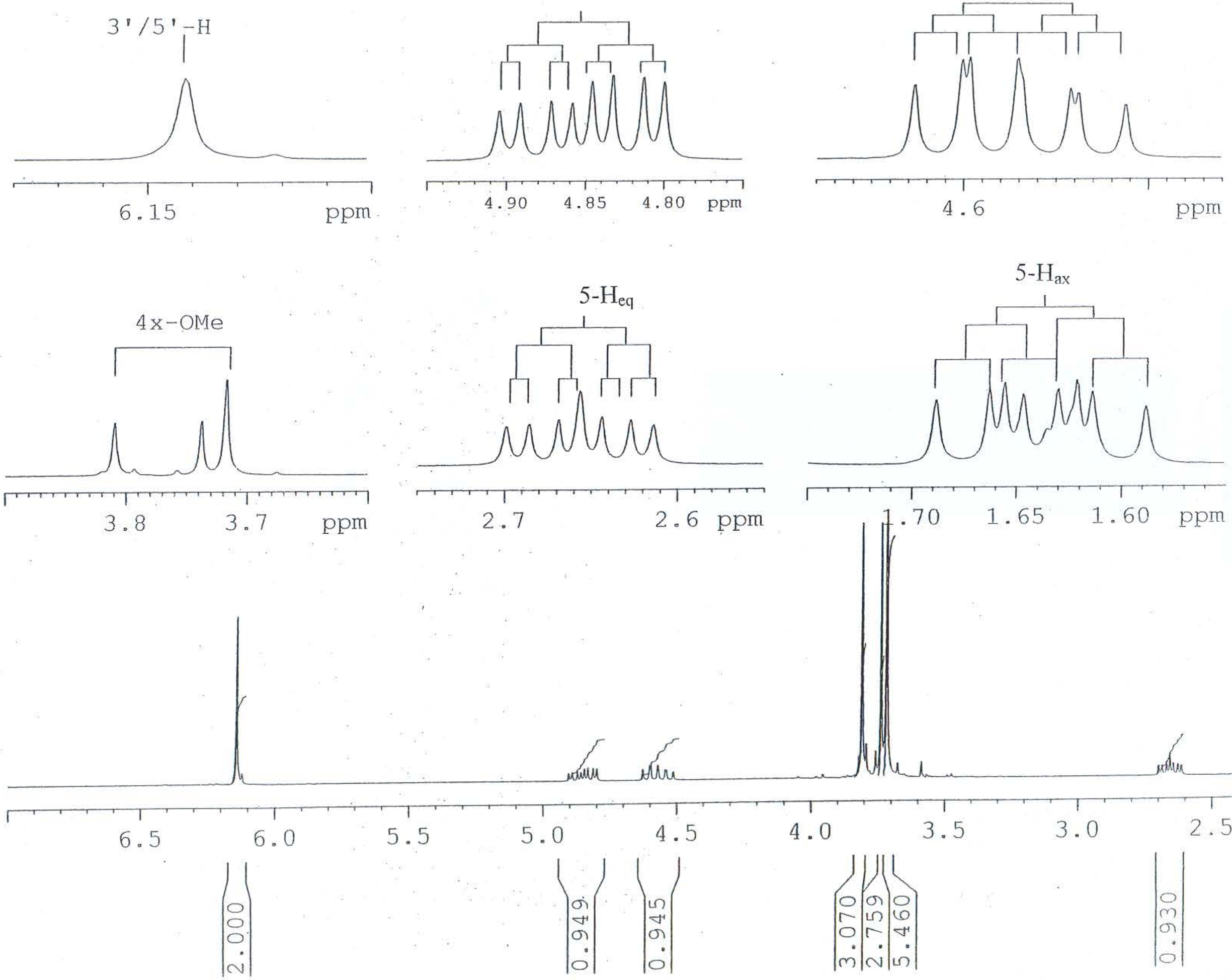
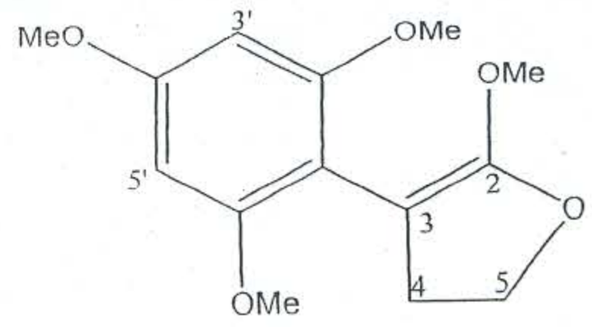


Plate 8a ($CDCl_3$, 298K)





(29)

Plate 8b ($CDCl_3$, 298K)

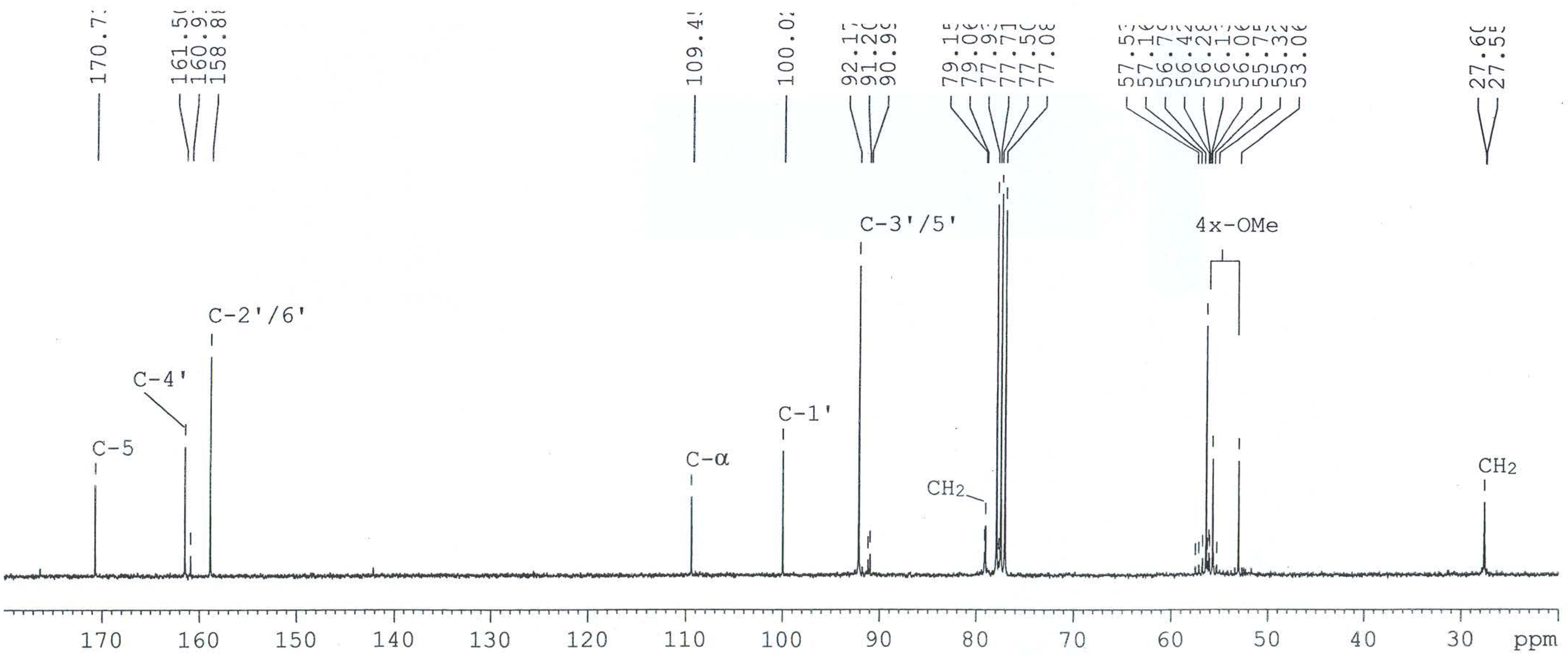


Plate 8c ($CDCl_3$, 298K)

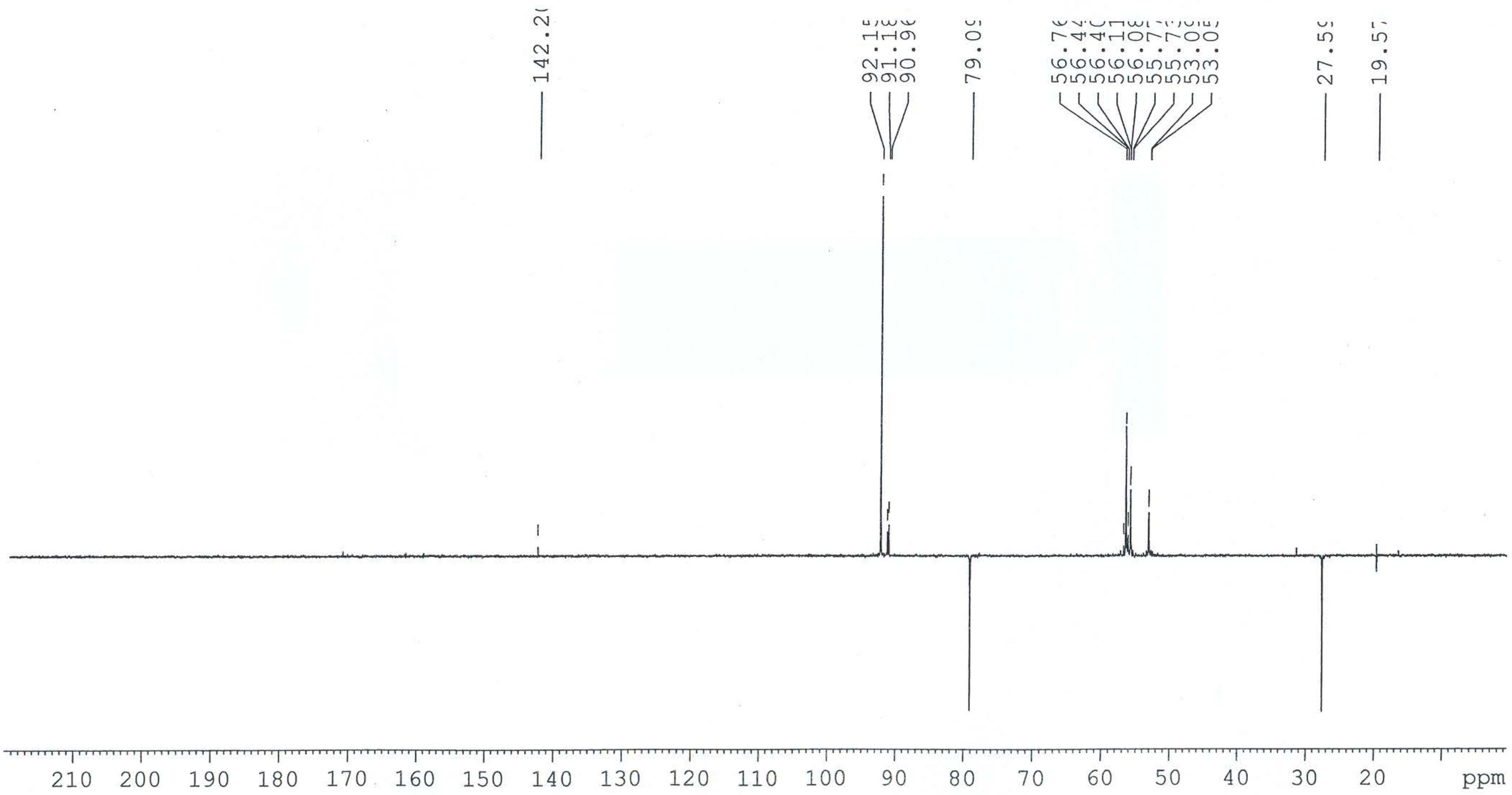


Plate 8d ($CDCl_3$, 298K)

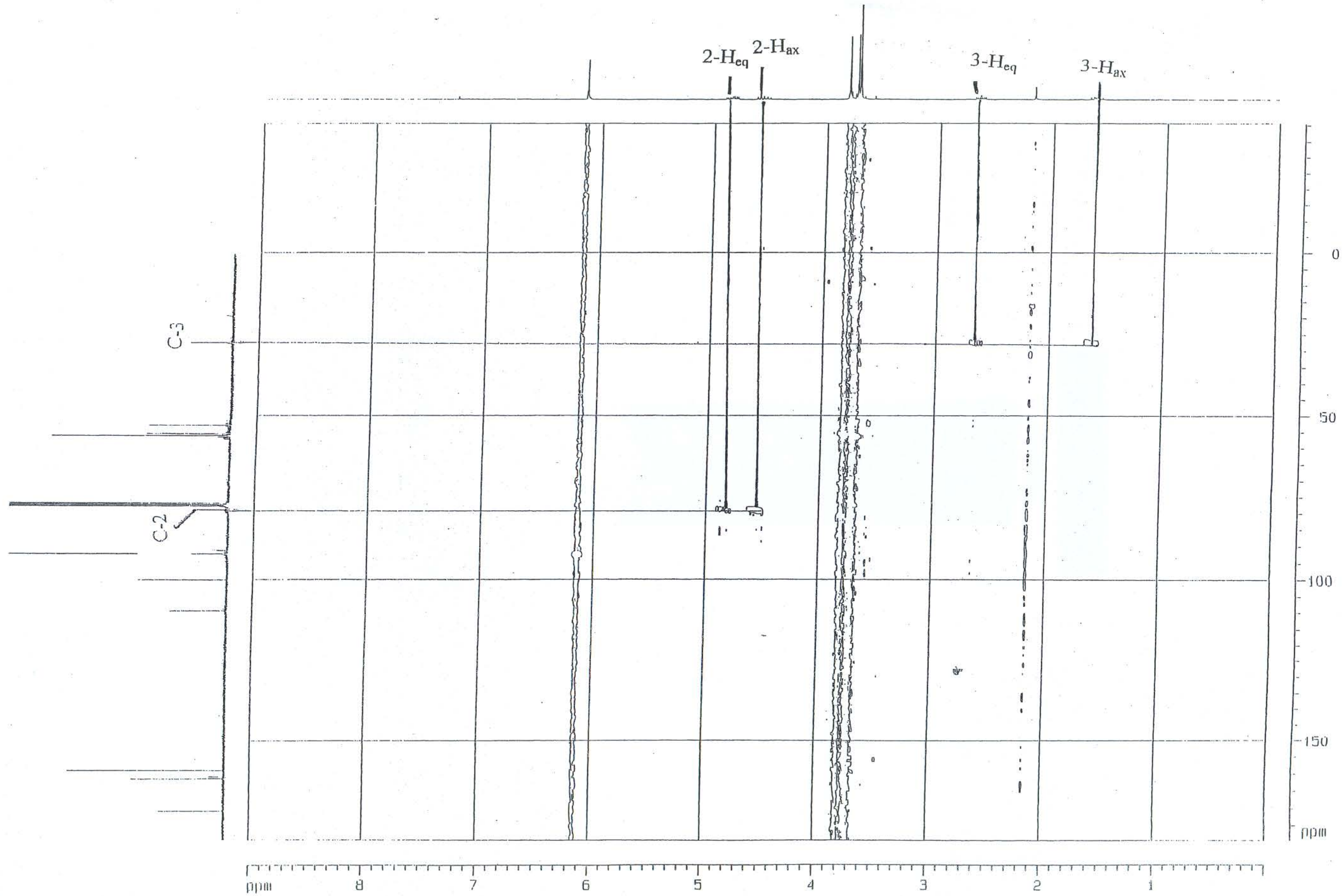
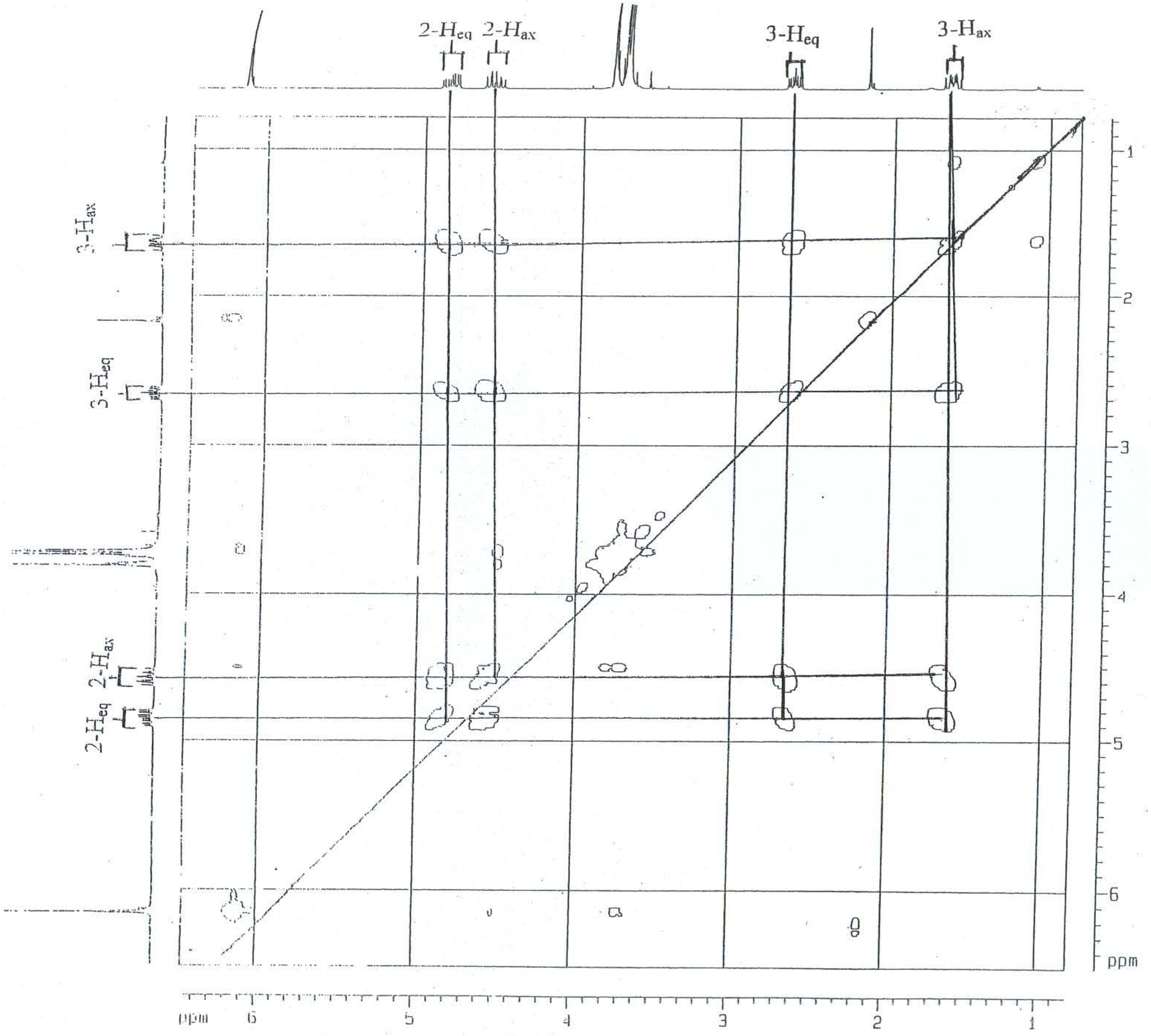
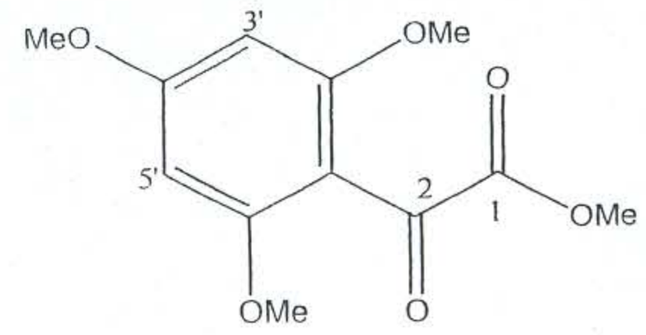


Plate 8e ($CDCl_3$, 298K)



COSY45

Plate 9a (CDCl 3, 298K)



(30)

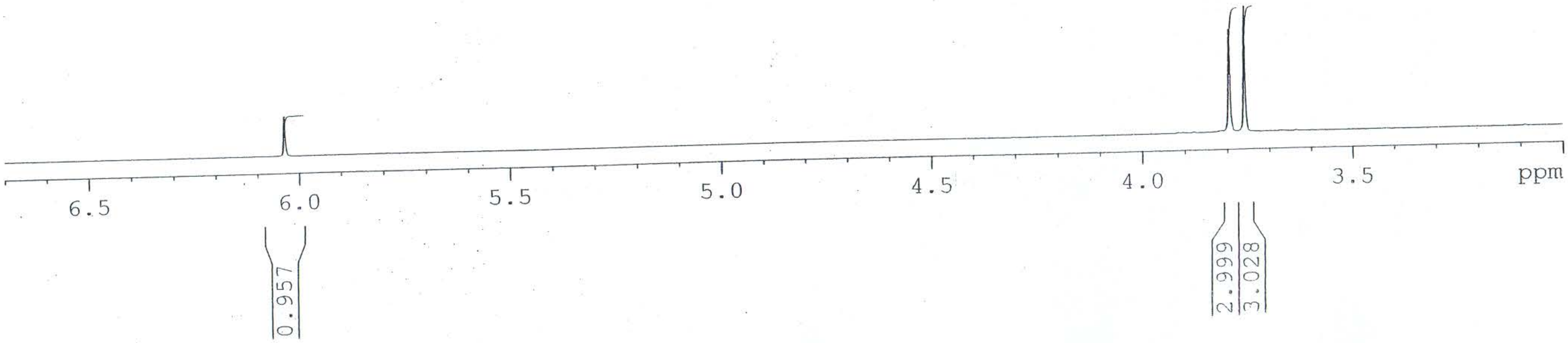


Plate 9b ($CDCl_3$, 298k)

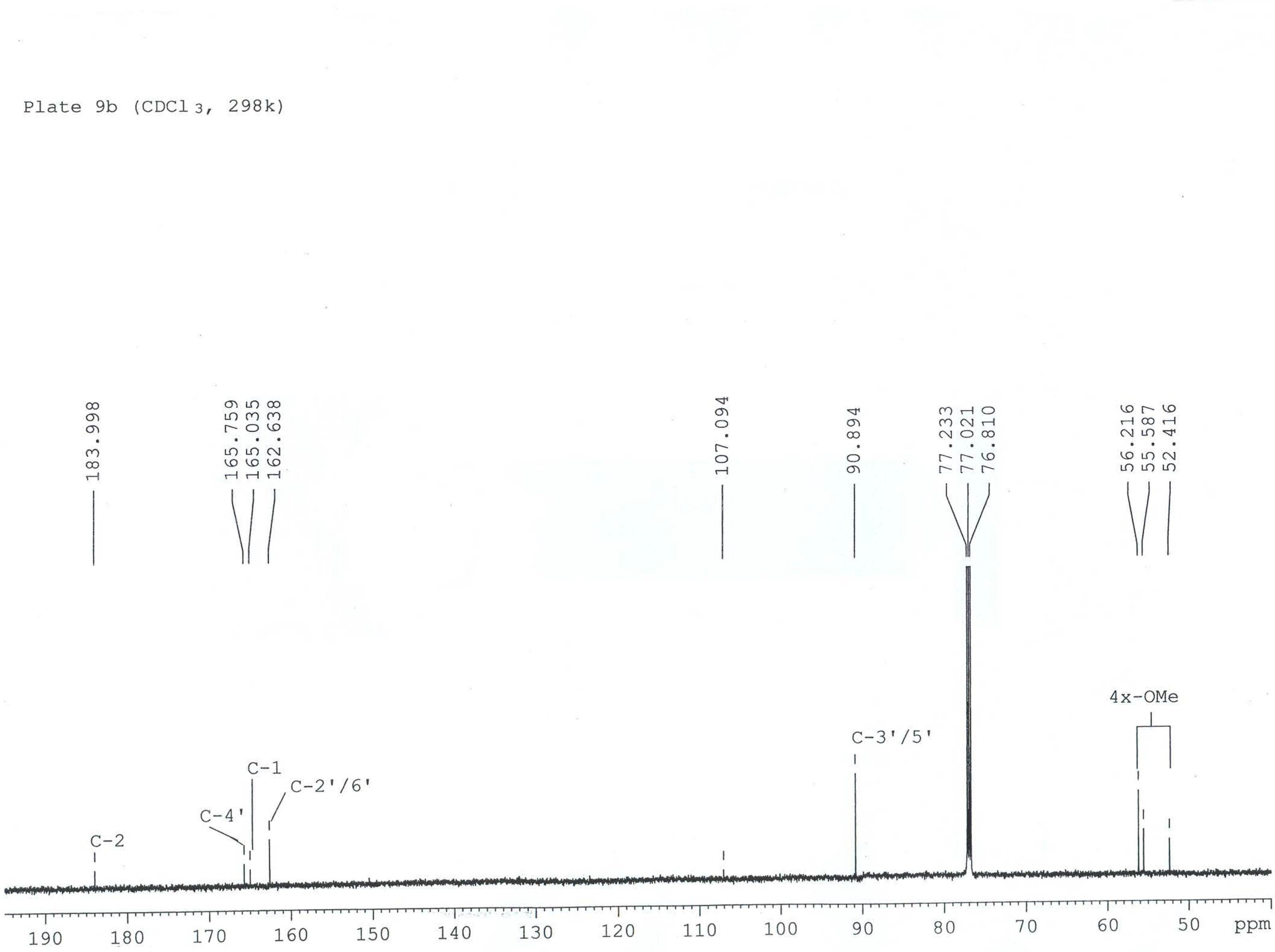
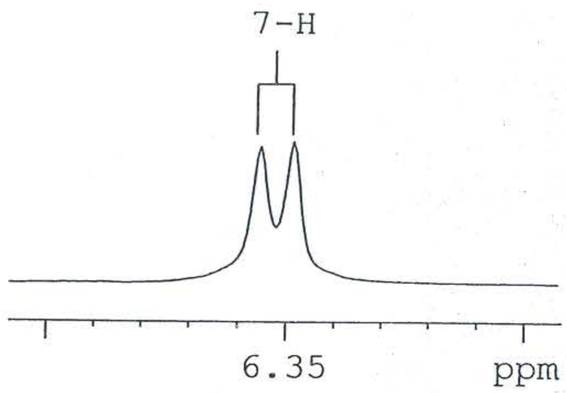
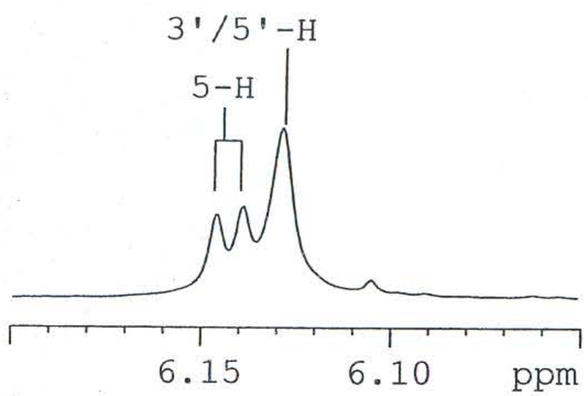
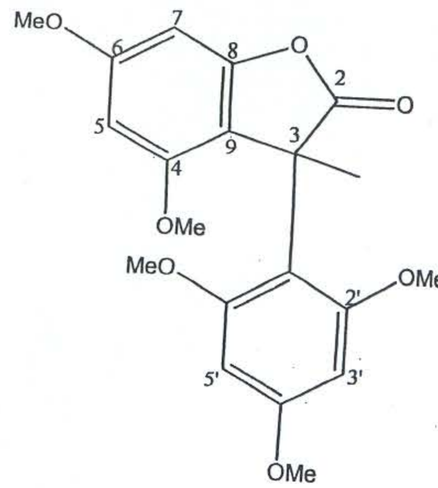


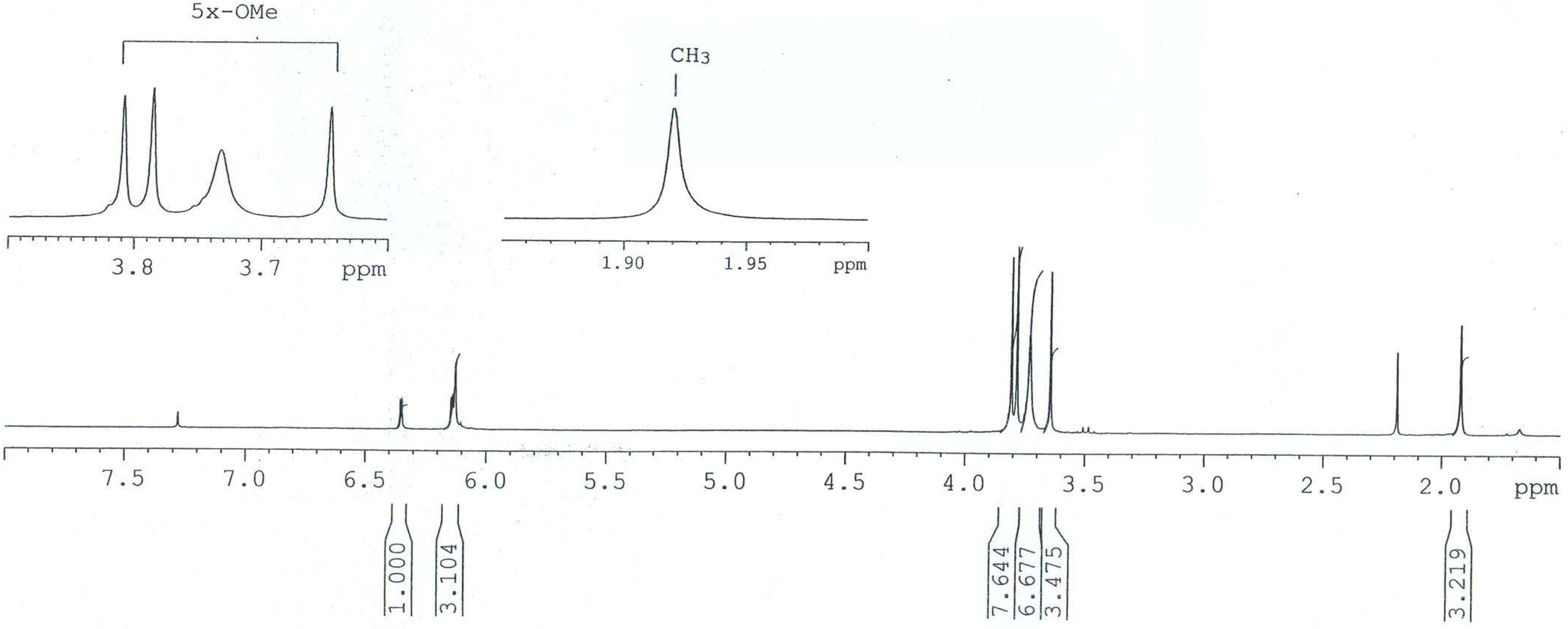
Plate 1b ($CDCl_3$, 298K)



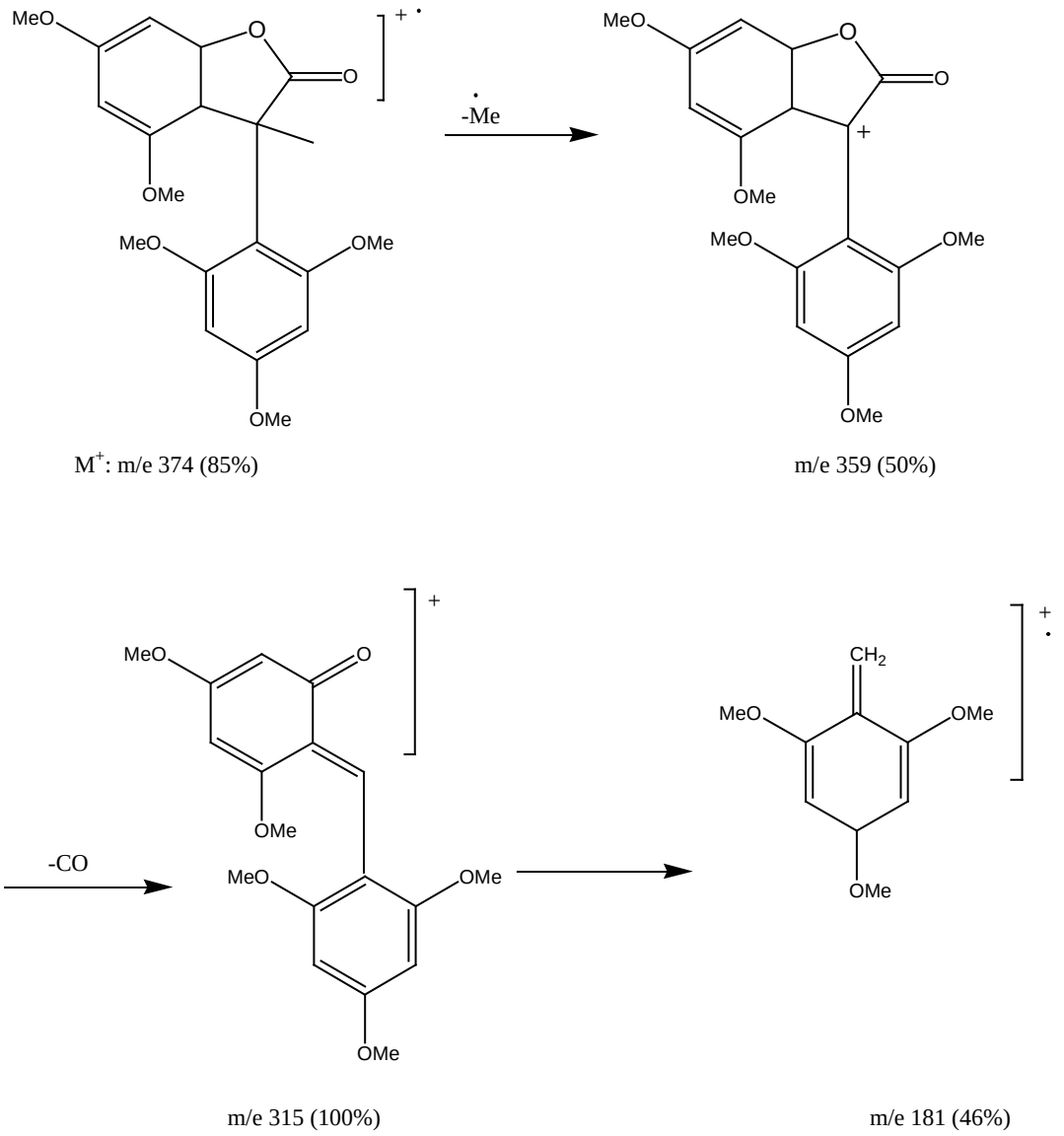




(13)

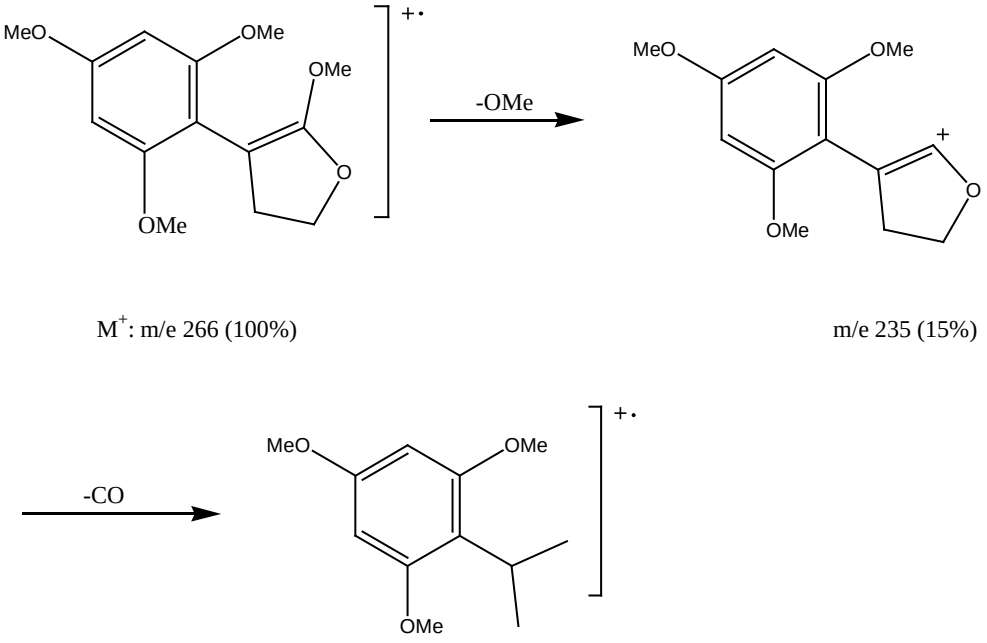


MS Fragmentation of 4, 6-dihydroxy-3-methyl-3-(2, 4, 6-trihydroxyphenyl)-1-benzofuran-2(3H)-one. (**12**)



Scheme A

MS Fragmentation of methyl 2-methoxyoxo-(2, 4, 6-trimethoxyphenyl)-4,5-dihydrofurane (**29**)



M^+: m/e 266 (100%)

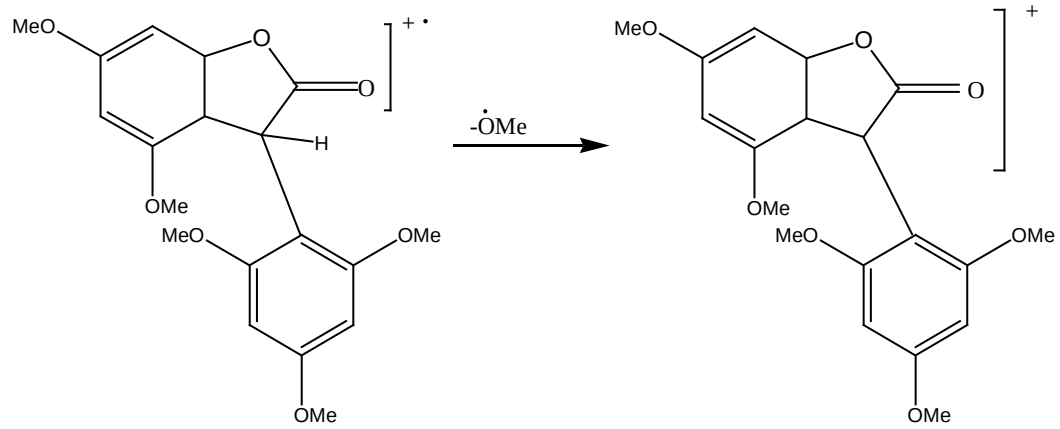
m/e 235 (15%)

m/e 207 (75%)

Scheme C

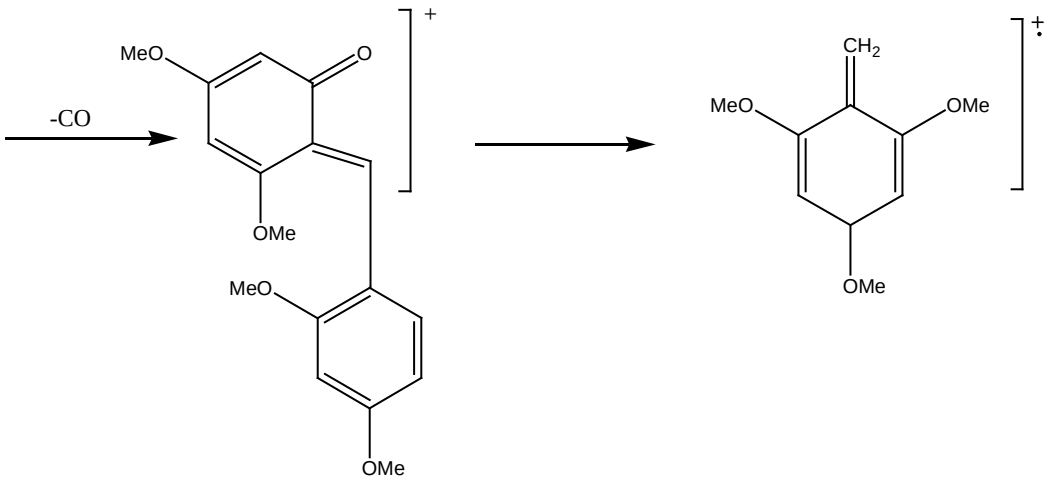
.

MS Fragmentation of 4,6-dihydroxy-3-(2,4,6-trihydroxyphenyl)-1-benzofuran-2(3H)-one(**14**)



M^+: m/e 360 (100%)

m/e 329 (5%)



m/e 301 (100%)

m/e 181 (46%)

Scheme B

OPSOMMING

Nuwe metodes om koolstof-koolstof bindings te vorm is van beduidende teoretiese en praktiese belang vir sintetiese organiese chemici. Hierdie werk ondersoek die vorming van ’n metienbinding (enkel koolstof verbinding) om difenielmetaan-verbindings te vorm asook die sintetiese potensiaal van hierdie verbinding.

Metienverbindings is van industriële belang indien die aromatiese gedeelte poligehidroksileer is. Die kleurstabiliteit van rooiwyn word aan ‘n metienbinding tussen antosianidiene toegeskryf. Hierdie bindings ontstaan spontaan tydens veroudering van rooiwyn vanaf pirodruiwesuur of glioksielsuur. Wattelbasekstrak word gebruik om kleefstof te vervaardig. Kleefstofvorming berus op die koppeling van polifenole met metienbindings afkomstig vanaf formaldehied. Gepatenteerde antioksidante bevat ‘n bensiliese waterstof op ‘n metiengroep *orto* tot ’n aromatiese hidroksielgroep (Irganox®HP-136).

Twee karbonielgroepe op aangrensende koolstofatome verhoog mekaar se reaktiwiteit teenoor nukleofiele aanval. In die geval van α-keto karboksielsure is die α-ketoon-groep baie meer reaktief as die karboksielgedeelte en geneig tot reaksie met elektrofiele.

Die hidroksielgroep van floroglusinol en polihidroksi-arielverbindings skenk elektrone aan die aromatiese ring om die nukleofiliteit van die aromatiese koolstowwe te verhoog. Polifenole is dus ambidente nukleofiele wat via suurstof of via koolstof kan reageer. Polifenole kan dus koolstof-koolstofbindings met geskikte elektrofiele (soos α-keto karboksielsure) vorm.

Hierdie verhandeling behels ’n ondersoek na die reaksies van floroglusinol met ’n verskeidenheid α-keto karboksielsure en die sintetiese potensiaal van hierdie produkte.

Addisie van ’n aromatiese ring aan ’n karbonielgroep skep ’n bensiliese hidroksiegroep. In die geval van reaktiewe nukleofiele aromatiese ringe word hierdie

bensiliese hidroksiegroep met ’n tweede aromatiese groep vervang om die verwagte metiengekoppelde biarielverbinding te vorm.

Floroglusinol reageer met pirodruiwesuur om 4,6-dihidroksi-3-metiel-3-(2,4,6-trihidroksifeniel)-1-bensofuraan-2(3H)-oon te vorm en met glioksielsuur om 4,6-dihidroksi-3-(2,4,6-trihidroksifeniel)-1-bensofuraan-2(3H)-oon te vorm. Hierdie produkte is laktone tussen die karboksielsuur- en fenolgedeelte van die diarielverbinding. Floroglusinol vorm ’n spirobilaktoon, 4,5',6,7'-tetrahidroksi-2H-spiro[bensofuran-3,4'-chroman]-2,2' dioon met oksaalasynsuur.

Met minder reaktiewe aromatiese nukleofiele vind eliminasie van die bensiliese hidroksiegroep plaas, voordat substitusie met ’n tweede aromatiese nukleofiel kan plaasvind, op voorwaarde dat ’n waterstofatoom in die α-posisie beskikbaar is. Trimetoksiefloroglusinol reageer met pirodruiwesuur in metanol om metiel-2-(2, 4, 6-trimetoksifeniel)-akrilaat via eliminasie van water te lewer. Hierdie akrilaat reageer met osoon om okso-(2,4,6-trimetoksifeniel)-asynsuur metielester te vorm en met diasometaan om 2-metoksie-3-(2,4,6-trimetoksiefeniel)-4,5-dihidrofuraan te vorm.

Om die potensiaal van hierdie reaksie te demonstreer is resorsinol met *p*-hidroksifenielpirodruiwesuur gereageer om die Z-isomeer van 6-hidroksi-3-(4-hidroksibensilideen)-3H-bensofuraan-2-oon te vorm. Hierdie isoauroonsintese is ’n verbetering van ’n onlangs gepubliseerde sintese van hierdie natuurlike produk.

Ons het dus ’n nuwe metode ontwikkel om koolstof-koolstof bindings en metiengekoppelde diarielverbindings te vorm. Hierdie reaksie is ontwikkel tot ’n nuwe prosedure om vry fenoliese 3-gesubstitueerde bensofuraan-2-one te sintetiseer. Die reaksie is aangepas om ’n onlangs gepubliseerde sintese van ’n vry fenoliese isoauroon te verbeter. Die reaksie is gebruik om akrielsure met ’n fenoliese substituent in die α-posisie te sintetiseer en daar is begin om die sintetiese potensiaal van hierdie nuwe metode, om α,β-onversadigde sure te sintetiseer, te ondersoek.