Chapter - 3

Antioxidants based on Hindered phenol and Oxygen heterocyclics bridged by Chalcone, Pyrazoline And Phenyl pyrazoline

Benzylidene acetophenones constitute a class of naturally occurring pigments, which are often refereed to as chalcones. *Kostanecki and Tambor*[1] first used this term. During the past decade a number of reports have appeared in the literature describing the isolation of 1,3-disubstituted propenones (chalcones) from various parts of plants, roots[2], heartwood[3], buds[4], leaves[5], blossoms[6], inflorescences[7], flowers[8] and seeds[9]. These compounds exist in free state as chalcones or in the combined form as glycosides.

The presence of enone function in the chalcone molecule confers antibiotic activity[10]. This property is enhanced when substitution is made at α and β positions[11]. Some substituted chalcones and their derivatives including some of their heterocyclic analogues have been reported to possess some interesting biological properties. They show profound influence on the cardiovascular, cerebrovascular and neuromuscular systems[12].

Chalcones find applications as artificial sweeteners[13], scintillators[14], polymerisation catalyst[15] and organic brightening additive[16]. They also act as stabilisers against heat, visible light, ultraviolet light[17] and are useful in colour photography[18].

Chalcone forms the constituents of corrosion inhibiting lubricants suited for internal combustion engine containing silver and similar metal components[19]. It serves as suitable ultraviolet absorption additive in adhesive lacquers and plastics[20]. Chalcones constitute an important group of natural product and some of them possess a wide range of biological activities such as anti-bacterial[21], anti-inflammatory[22], anti-microbial[23], anti-tumour[24], anti-cancer[25], prostaglandin binding[26] and anti-feedant[27]. They also find use in the production of nematic liquid crystals[28], photosensitive polymers[29] and as antioxidants for oils[30].

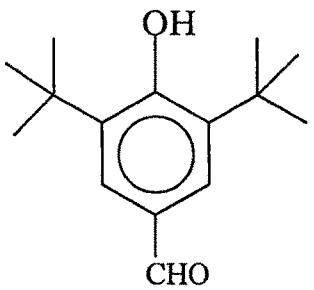
Benzo-α-pyrone, generally known as coumarin, is widely distributed in nature either in the free state or in the combined state. Coumarin and its derivatives have attracted considerable interest because of their various physiological and biochemical properties. Many plants, which belong to Rutaceae families, have been used as folk medicines having anti-microbial activity. This anti-microbial activity is due to coumarin present in it. The coumarin possesses wide range of pharmacological properties viz. anti-bacteiral[31], anticoagulant[32], vasodilatory[33] and anti-allergic[34]. Hydroxy coumarins are widely used as therapeutic agents. Several coumarin derivatives are present in various parts of plants and thus the plants are used for the treatment of disease in ayurveda.

Benzopyran-4[H]one (Chromone) and its derivatives play an important role in various biological systems. Several chromones have been reported to possess interesting physiological activities such as coronary spasmolytic[35], bronchodialatory[36] and as anti-allergic. They are also useful in the treatment of asthma[37]. Substituted chromones are useful as coronary vesodialatory[38] and anti-allergic drugs[39].

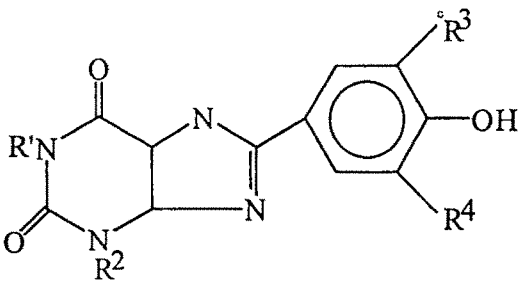
Pyrazolines are the important class of heterocyclic compounds associated with wide range of pharmaceutical properties such as anti-diabetic[40], anti-allergic[41], anti-bacterial[42] and anti-tubercular[43]. Many substituted pyrazolines are reported to possess insecticidal[44], herbicidal[45] and acaricidal activities[46]. 1-Phenyl-2-pyrazoline is found to be useful as antioxidant in polymers [47].

2,6-Di-tert-butyl-4-methylphenol (BHT, Butylated hydroxy toluene) prevents heart damage by extreme physical stress and improves cardiac resistance to acute overload as reported by *Golubeva et al.*[48] It also prevent 7,12-dimethyl benz[a] anthracene induced mammary carcinoma according to *Richard and his group*[49].

Hirota et al.[50] have synthesised compound **2** (R^1=R^2 =Me, R^3=R^4=CMe_3) by the condensation of 3,5-di-tert-butyl-4-hydroxybenzaldehyde **1** and 1,3-dimethyl-5,6-di amino uracil in ethanol. Salt of compound **2** is reported to be pharmaceutically acceptable as asthma inhibitor.



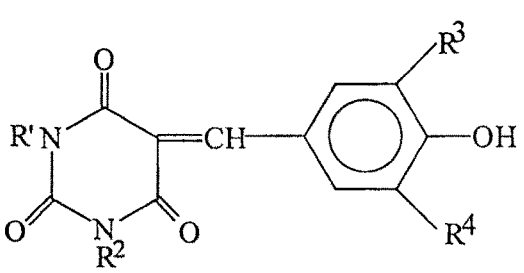
[1]



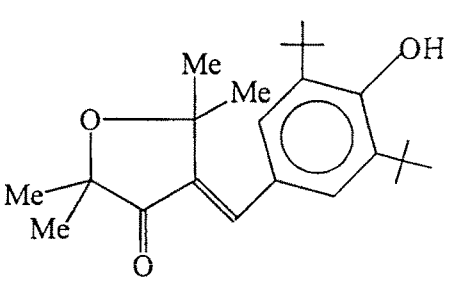
[2]

Pyrimidine and its derivatives **3** (R^1=R^2=H, alkyl, substituted phenyl, cyclohexyl, R^3=R^4=alkyl, alkoxy) obtained by condensation of aldehyde **1** with 1-methyl-3-phenyl pyrimidine-2,4,6-trione in alcohol are known to possess anti-inflammatory activity. They also act as an agent for the treatment of respiratory tract disorder and cardiovascular disorder according to *Hiroyuki et al.*[51]

4-Benzylidene tetrahydro furan-3-one and its analogue **4** in combination with aldehyde **1** is used as sunscreen, antioxidant and skin anti-inflammatory as reported by *Luppi Bernadette* and research group[52].



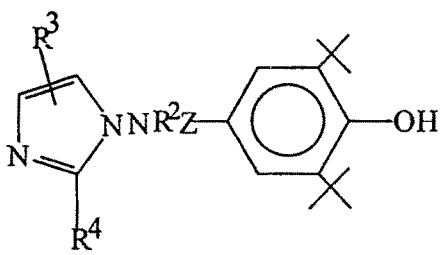
[3]



[4]

Nicolas et al.[53] have synthesised compound **5** (R^2, R^3 = H, alkyl; Z = CH_2; R^4= alkylthio) from benzoyl amino imidazole derivatives and aldehyde **1.** The synthesised compounds act as anti-inflammatory and anti-edema agents.

2,6-Di-tert-butyl phenol with heterocyclic moiety like benzoxazole, benzthiazole, indole, imidazole[1,2,α] pyrimidine at the 4th position were synthesised by *Kubokazuo et al.*[54] and they were screened for anti-inflammatory activity. Compound **6** was synthesised from *o*-amino phenol and 4,3,5-OH-$(Me_3C)_2C_6H_2COCl$. Benzoxazole **6** and 2-(3,5-di-tert-butyl-4-hydroxyphenyl) indole showed potent anti-inflammatory activity.



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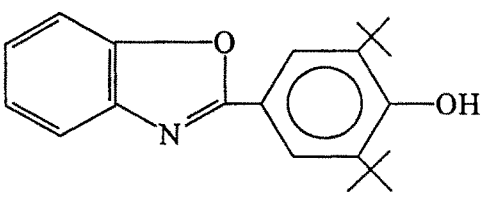
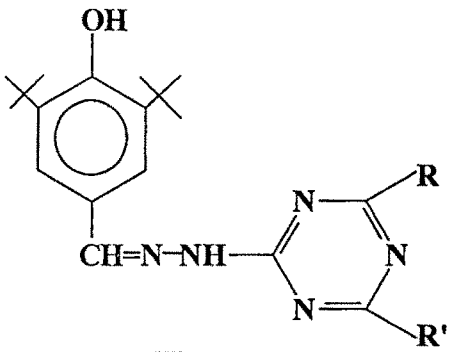


[6]

Kurt and Guenther[55] have synthesised 6-[3,5-di-tert-butyl-4-hydroxy benzylidene] hydrazino-2,4-morpholino-s-triazine **7** (R=R^1=acylamino or morpholino) and used as antioxidant for polypropylene. Compound **7** was synthesised by refluxing 2,4-di-morpholino-6-hydrazino-s-triazine and aldehyde **1** in benzene.

Hideki et al.[56] reported the synthesis of α-cyano-3,5-di-tert-butyl-4-hydroxy cinnamide derivatives **8** (R=H, Et; X=CH_2, O, CH_2CH_2) from cyanoacetopiperidine and aldehyde **1.** Compound **8** is used as U.V.absorber for polypropylene.



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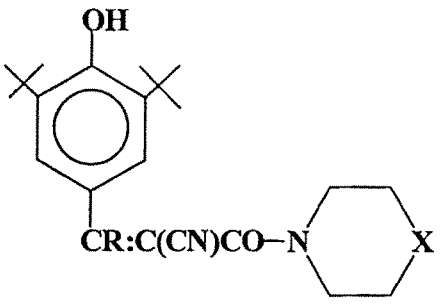
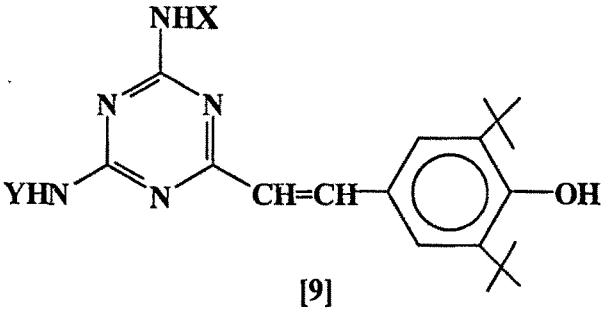


[8]

2,4-Di-amino-1,3,5-triazine and its derivatives **9** (X,Y = H, alkyl) are useful for the treatment of allergic disorder and inflammation as reported by *Kunio et al*[57]. They have synthesised compound **9** by heating 2,4-diamino-6-methyl-1,3,5- triazine and aldehyde **1** in formic acid.

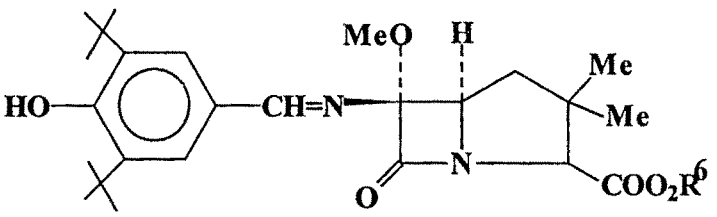


[9]

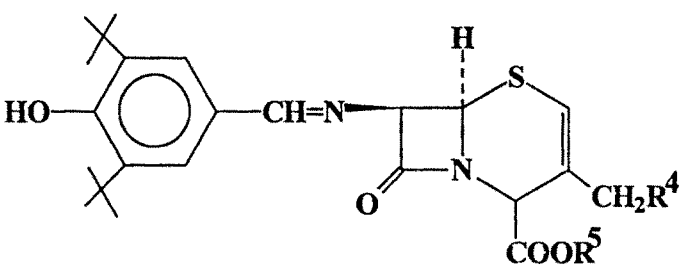
Hideo and co-workers[58] have synthesised β-lactum antibiotics **10** and **11** by condensing amino cephalosporanate derivatives with aldehyde **1**. (R^4 =H, OAc. R^5=CH_2CCl_3, $CHPh_2$, R^6=CH_2CCl_3, $CH_2OCOCMe_3$, $CHPh_2$).

Jagdish et al.[59] reported the synthesis and anti-inflammatory activity of [3,5-Di-tert-butyl-4-hydroxyphenyl) methylidene] dihydroindole. They have synthesised compound **12** (R^1=H, $C_{1\text{-}6}$alkyl, $CONHR^3$, R^3=H, $C_{1\text{-}6}$ alkyl; R^2=H, $C_{1\text{-}6}$ alkyl,

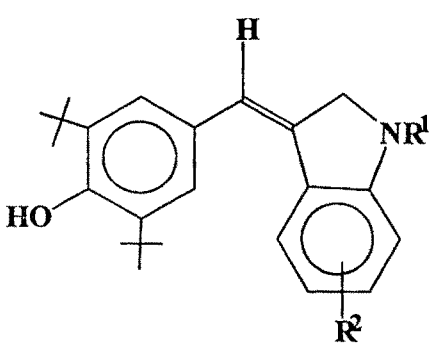
alkoxy, halo, OH, CF_3, $COOR^4$, R^4= H, C_{1-6}alkyl) by reaction between 5-methyl-2-oxindole and aldehyde **1** using sodium acetate in glacial acetic acid.



[10]



[11]



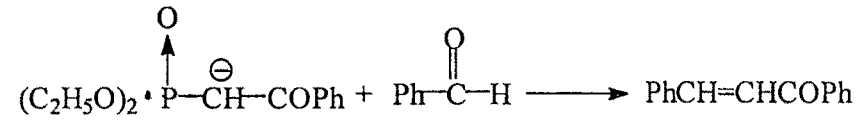
[12]

Above survey of earlier research publications on 3,5-di-tert-butyl-4-hydroxybenzaldehyde in combination with heterocyclic compounds reveals that they have remarkable potential to act as biologically active agents. Moreover it has been observed that chalcones, pyrazolines, phenyl pyrazolines, chromones and coumarins are biologically active compounds and they are not studied in combination with 3,5-di-tert-butyl-4-hydroxybenzaldehyde **1**. It was therefore thought of interest to synthesise new compounds having combination of hindered phenol and oxygen heterocyclics bridged by chalcone, pyrazoline and phenyl pyrazoline and to screen for anti-bacterial activity against various bacteria.

Different methods are reported for the synthesis of chalcones.

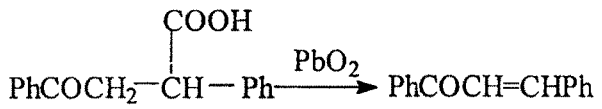
1. Debromination of chalcone α,β-dibromide with one mole of tri alkyl phosphine[60], chromous chloride[61] or by the action of potassium hydroxide[62] produces chalcone in excellent yield.

2. Phosphorane, of the general formula $Me_nPh_{(3-n)}P{=}ChCOPh$ (n=0,1,2,3), is reported to react with benzaldehyde to give chalcone in good yields[63]. Chalcone has also been obtained by the reaction of benzaldehyde with phosphonate carbanion[64].



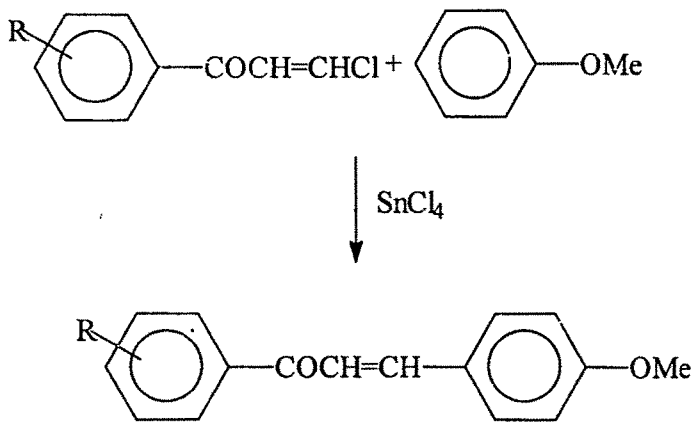
Alternatively, potassium derivatives of diethylphenyl phosphonate reacts with an aromatic aldehyde to yield desired chalcone.[65]

3. Chalcone can also be synthesised by the oxidative decarboxylation of 3-benzoyl-2-phenyl propanoic acid in the presence of lead dioxide[66].



4. Subsituted β-chloro vinyl ketones have been condensed with phenolic ethers in the presence of stannic chloride to give chalcone in fair yield[67].

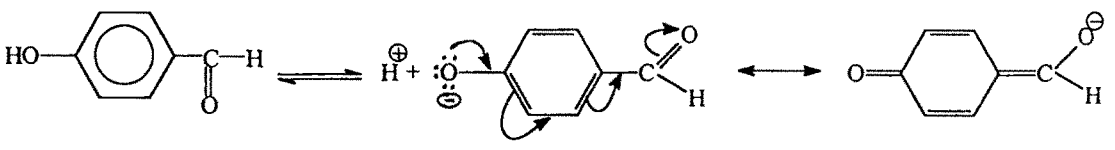
   β-Chloro vinyl ketone on reaction with aromatic hydrocarbons and alkyl halides under the influence of $SnCl_4$ gave corresponding chalcones.



The most convenient method for the synthesis of chalcone is the Claisen-Schmidt condensation of equimolar quantities of substituted acetophenone with substituted aldehyde in the presence of alcoholic alkali[68]. In the synthesis of poly hydroxy chalcones by Claisen-Schmidt reaction, higher concentration of alkali as a condensing agent is desirable. In general the electron donating substituents in aldehydic component and electron withdrawing substituents in the ketone favour

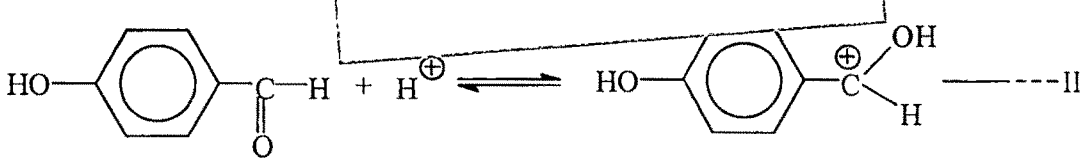
Claisen-Schmidt condensation in presence of HCl. The reaction of *p*-hydroxybenzaldehyde with substituted acetophenone claimed to proceed better in acid than in alkaline medium[69].

In alkaline medium, reaction (I) is favoured, which obviously lowers the reactivity of the carbonyl carbon and hence does not lead to chalcone formation.



I

In acid medium, howe estricted and the formation of carbonium erts a favourable influence on chalcone fo



II

Acidic medium

The mechanism of chalcone formation in acidic medium is shown in Figure 1.

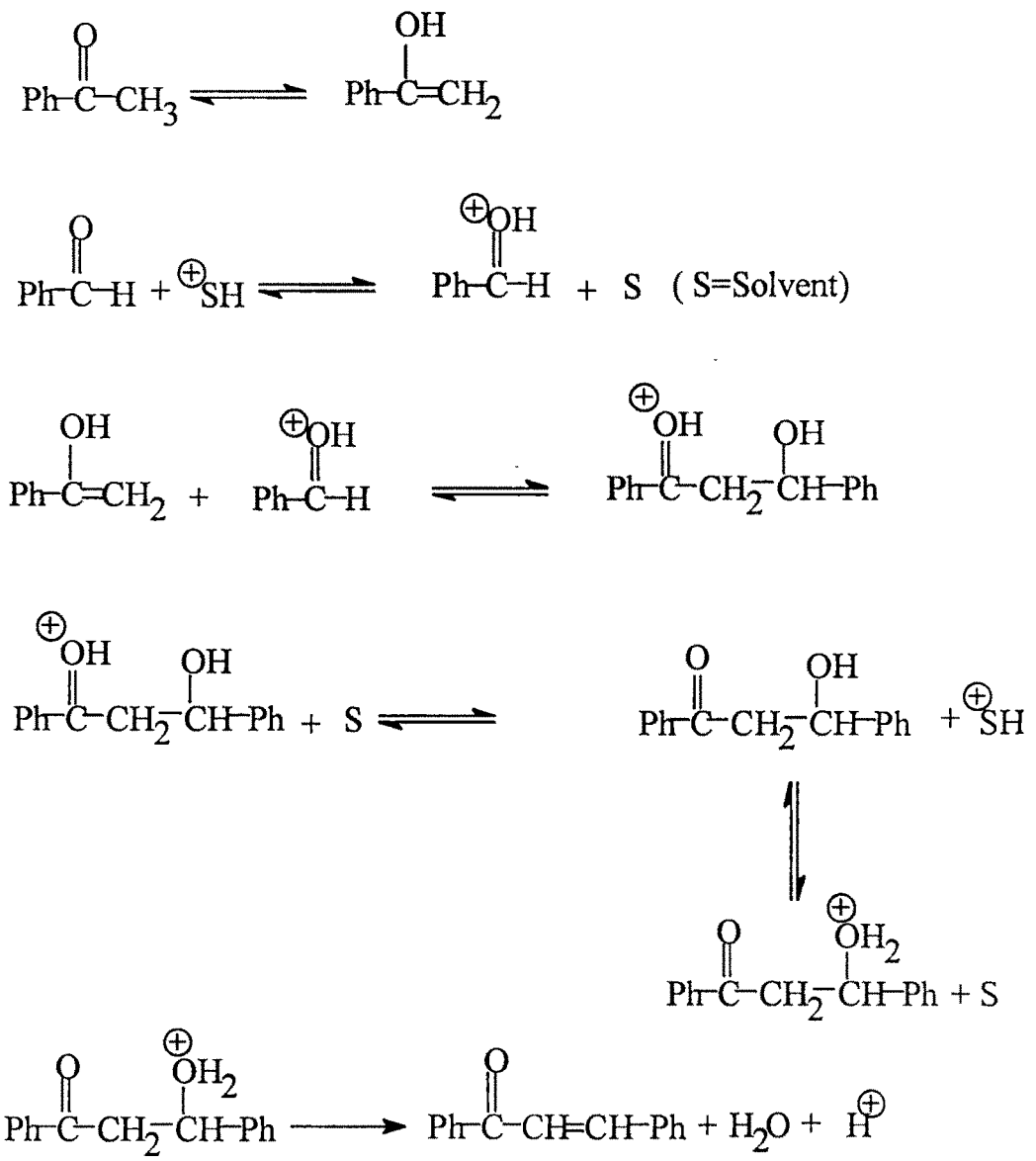


Figure 1.

Present work*

3,5-Di-tert-butyl-4-hydroxybenzaldehyde **1** was prepared by the oxidation of 2,6-di-tert-butyl-4-methyl phenol with bromine in tert-butyl alcohol according to the reported method[70]. Phenolic and oxygen heterocyclic ketones were synthesised by known methods.

2,5-Dihydroxyacetophenone **b** was prepared from hydroquinone. Hydroquinone was converted in to hydroquinone diacetate, which on Fries migration in the presence of Lewis acid gave corresponding ketone **b** in good yield[71].

2,4-Dihydroxyacetophenone **c** was synthesised from resorcinol using acetic acid and zinc chloride by the application of *Nencki* reaction.

2-Methyl-3-acetyl-7-hydroxy benzopyran-4-[H]one **e** and 2-methyl-3-acetyl-6-hydroxy benzopyran-4-[H]one **f** were synthesised from ketone **c** and ketone **b** respectively in the presence of acetic anhydride and sodium acetate by using *Kostanecki-Robinson acylation*[72].

2,4-Dihydroxybenzaldehyde was synthesised from resorcinol using DMF and $POCl_3$ in excellent yield (*Vilsmeyer-Hack* reaction). 3-Acetyl-7-hydroxy benzopyran-2-[H]one **d** was synthesised from 2,4-dihydroxybenzaldehyde by the application of *Knoevenagel* condensation using ethylacetoacetate in the presence of piperidine.[73]. Ketones **a** and **g** were used directly for the reaction.

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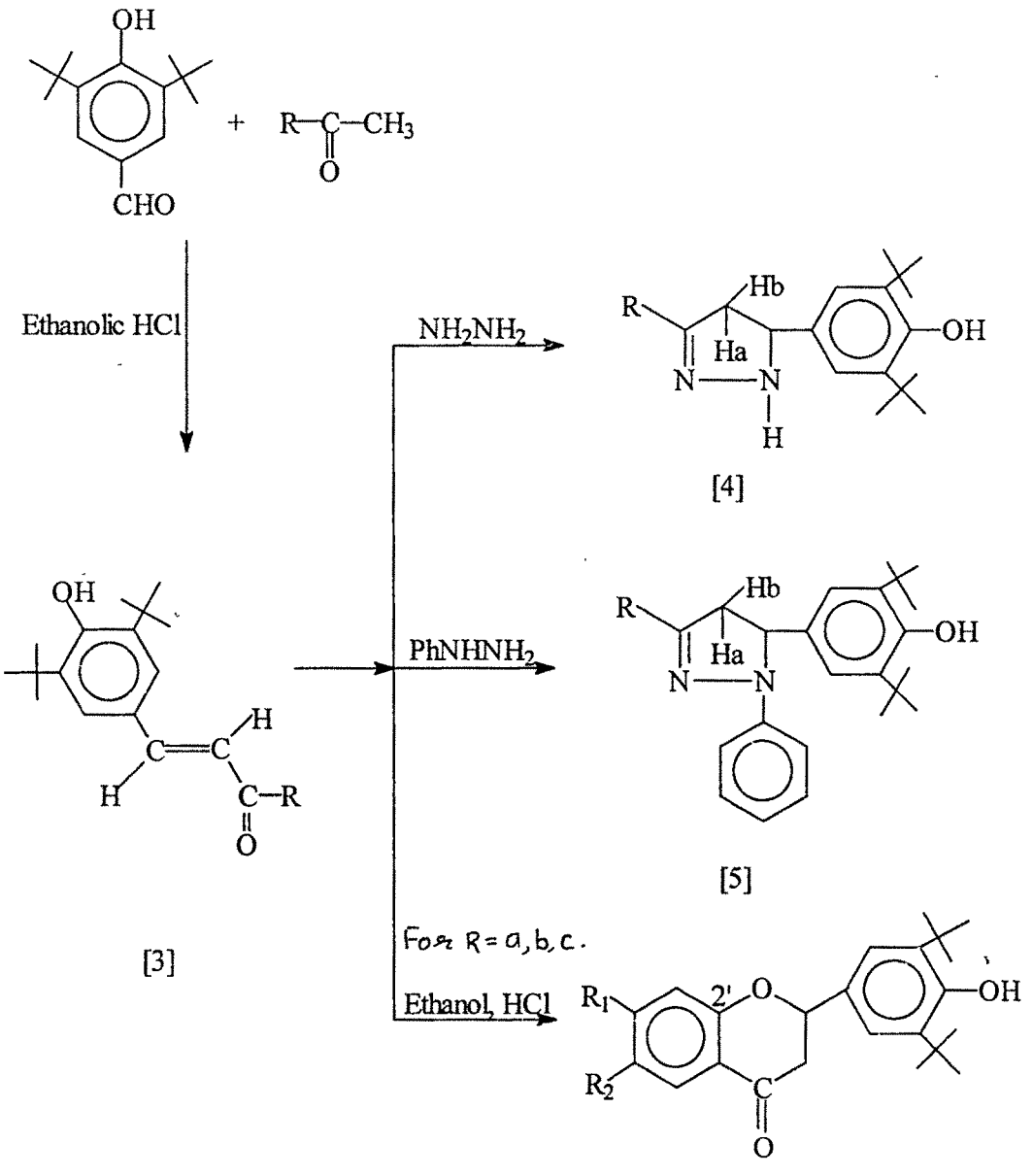
*Published [84]

*Synthesis of 1-(2',5'-dihydroxyphenyl)-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propenone **3b**.*

Condensation of aldehyde **1** with ketone **2b** afforded chalcone **3b** and product **I** (Scheme 1). Compounds obtained were purified through column chromatography.

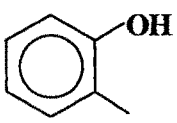
Structure of the chalcone **3b** was established on the basis of elemental analysis, IR, NMR and CMR spectral studies. IR spectrum of chalcone **3b** (Fig. 1) recorded in Nujol showed band at 3620 cm^{-1} due to OH stretching frequency of hindered phenolic group. Band observed at 3580 cm^{-1} is due to hydroxyl group at position $C_{2'}$ and band at 3250 cm^{-1} is due to presence of free hydroxyl group at position $C_{5'}$. Band observed in the lower region for $C_{2'}$ hydroxyl group compared to $C_{5'}$ is due to the intramolecular hydrogen bonding with oxygen of carbonyl group of chalcone. ^1H NMR spectrum (Fig. 2) recorded in $CDCl_3$ using TMS as internal standard showed singlets at δ 1.28 & 1.35 correspond to eighteen protons for two tert-butyl groups. Singlets at δ 5.19 & 5.50 indicated the presence of hydroxyl proton. H_α proton of chalcone appeared at δ 6.96 and δ 7.00 with J value 15.6Hz. Two singlets at δ 7.26 and δ 7.28 for two protons observed are due to presence of aromatic protons of di-tert-butyl phenol ring. Multiplet in the region of δ 7.10 to 7.31 for three protons is due to $H_{3'}$, $H_{5'}$ and $H_{6'}$ of aromatic ring. Doublets at δ 7.40 and 7.46 with J value 15.6 Hz for one proton indicated H_β proton of chalcone. NMR data indicated that compound exists in two isomeric forms E and Z. Integral calculation has shown that concentration of one of the isomers in the reaction mixture was 43% and the other 57%. R_f value of both these isomers were found to be same on TLC (benzene : alcohol :: 9:1).

The IR spectrum (Fig. 2a) of the compound **I** showed bands at 3617, 3446, 2958, 1666, 1643 cm^{-1}. ^1H NMR spectrum (Fig. 2b) recorded in $CDCl_3$ using TMS as internal standard showed singlet at δ 1.50 for eighteen protons. Singlet was also observed at δ 5.55 for one proton. Two doublets were observed at δ 6.93 and at

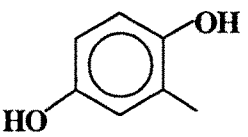

OH
CHO
+
R—C—CH3
O
Ethanolic HCl
NH2NH2
PhNHNH2
For R = a, b, c.
Ethanol, HCl
Hb
Ha
R
OH
N
N
H
[3]
[4]
[5]
[6]
R1
R2
2'
O
C
O
R1 = R2 = H
R1 = H, R2 = OH
R1 = OH , R2 = H

Scheme 1

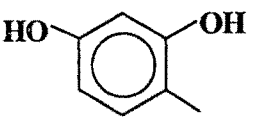
Where R in scheme 1



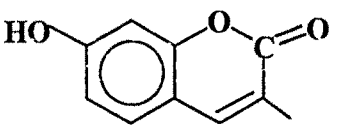
[a]



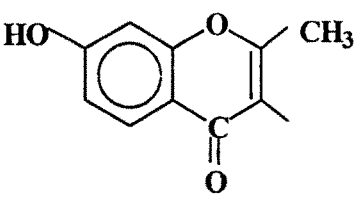
[b]



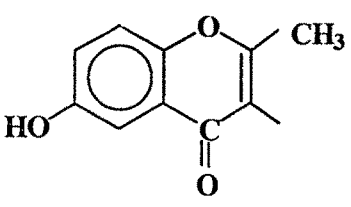
[c]



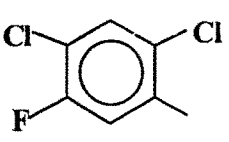
[d]



[e]



[f]



[g]

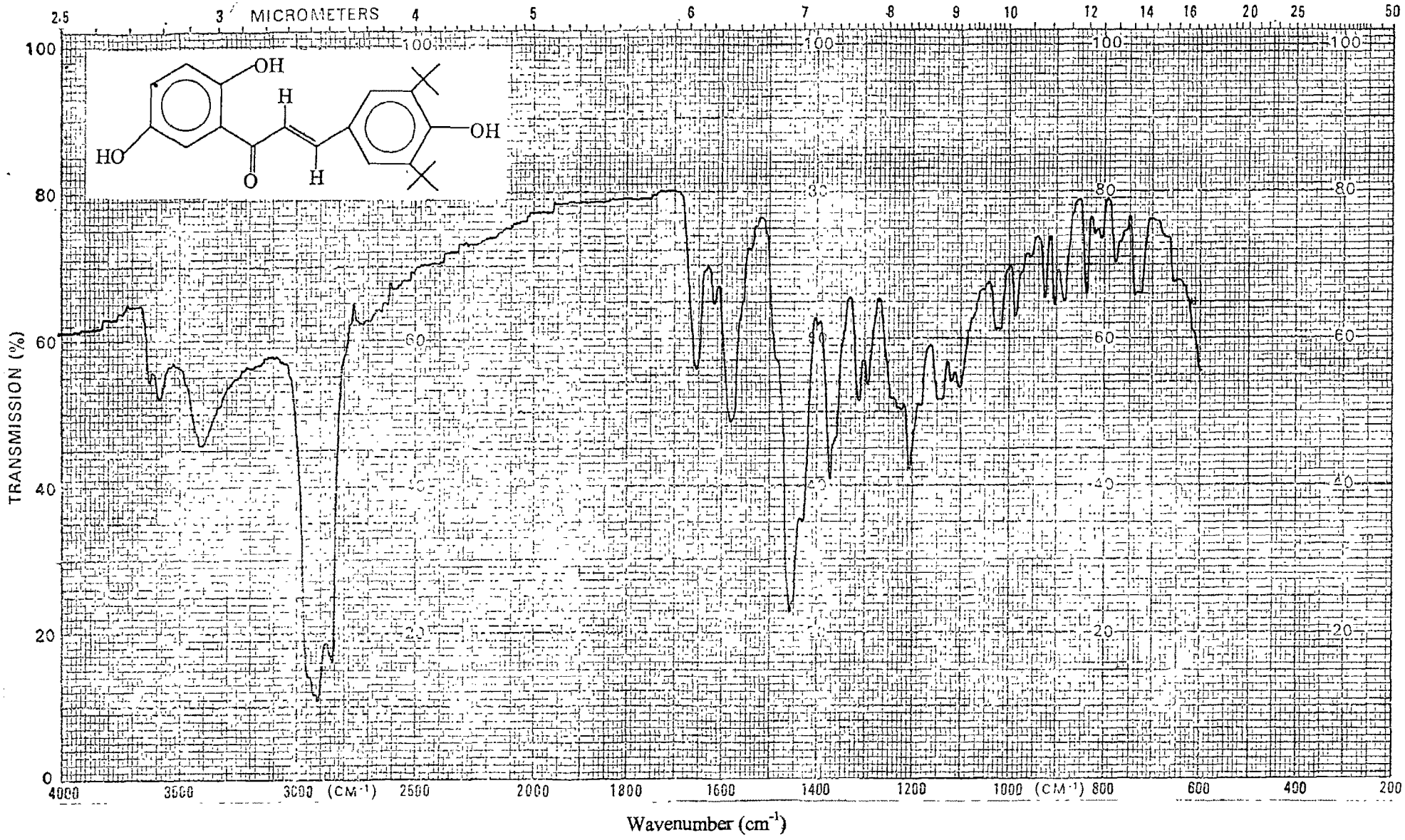


Figure 1

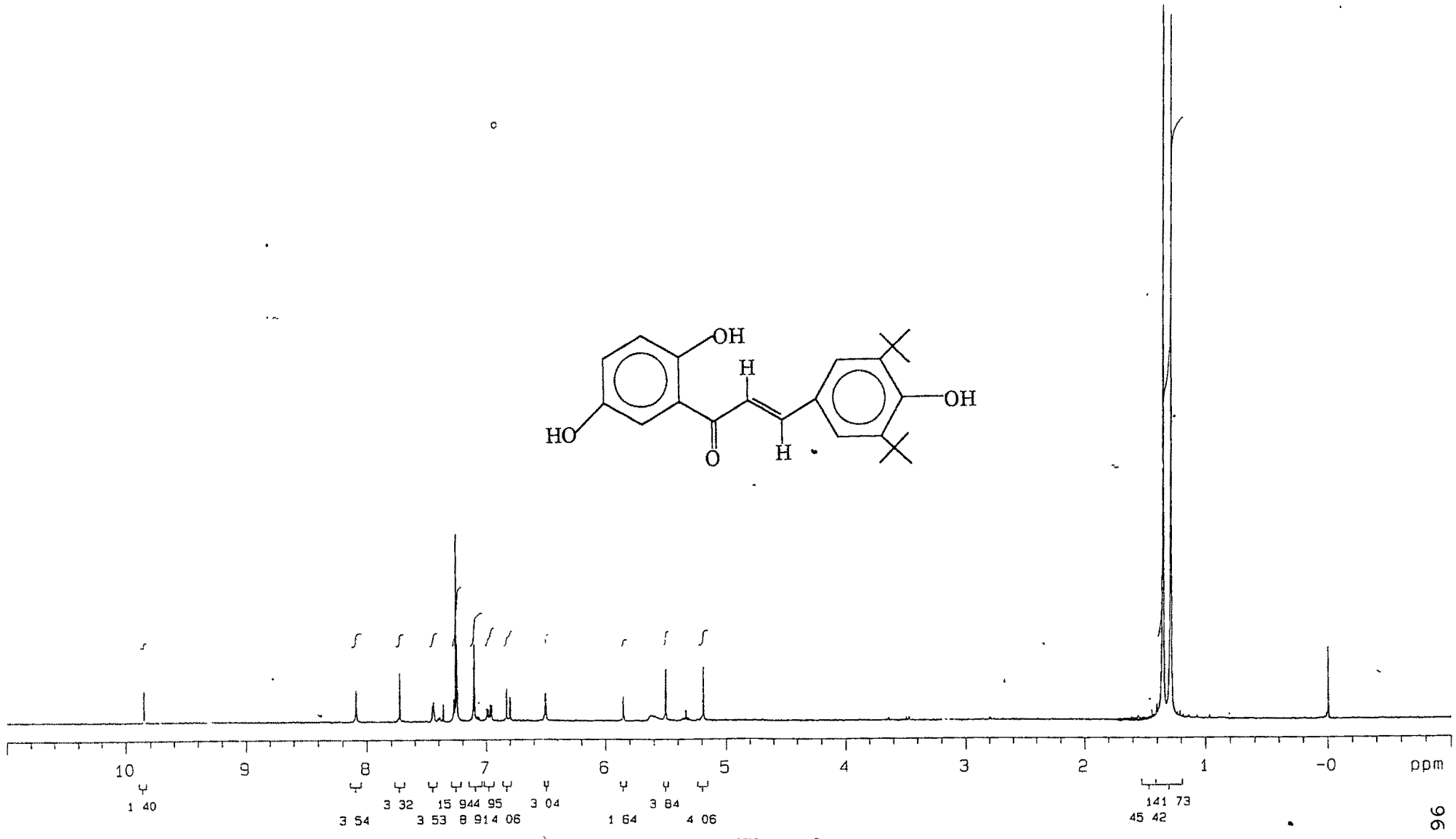
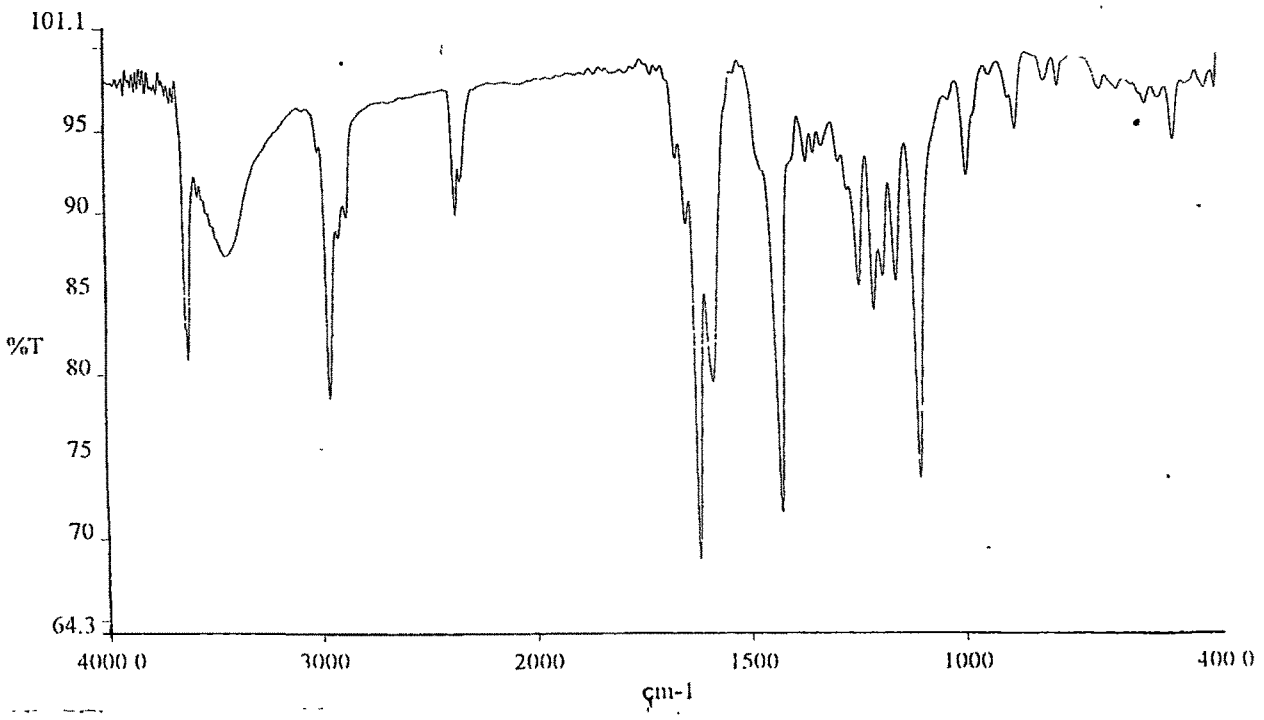
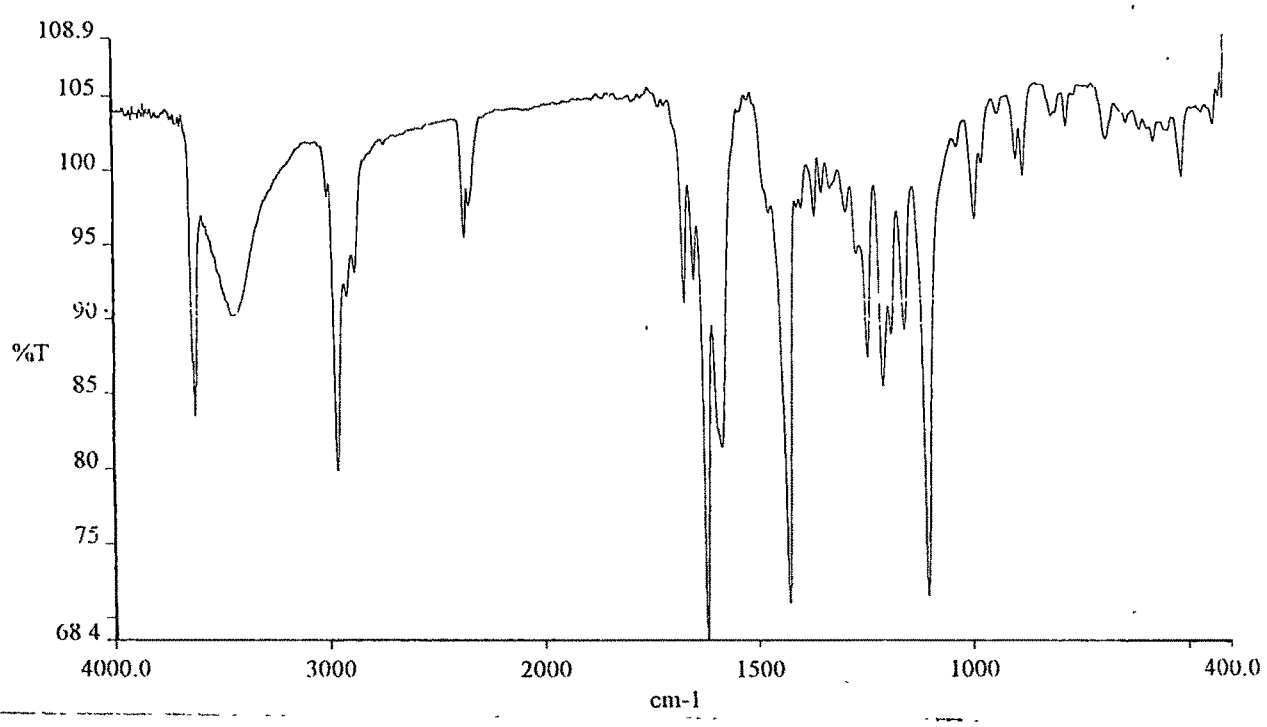

OH
H
OH
HO
O
H
10
9
8
7
6
5
4
3
2
1
-0
ppm
1 40
3 54
3 32
3 53
15 944 95
8 914 06
3 04
1 64
3 84
4 06
141 73
45 42
96

Figure 2



Product I



Product II

Figure 2a

Product I

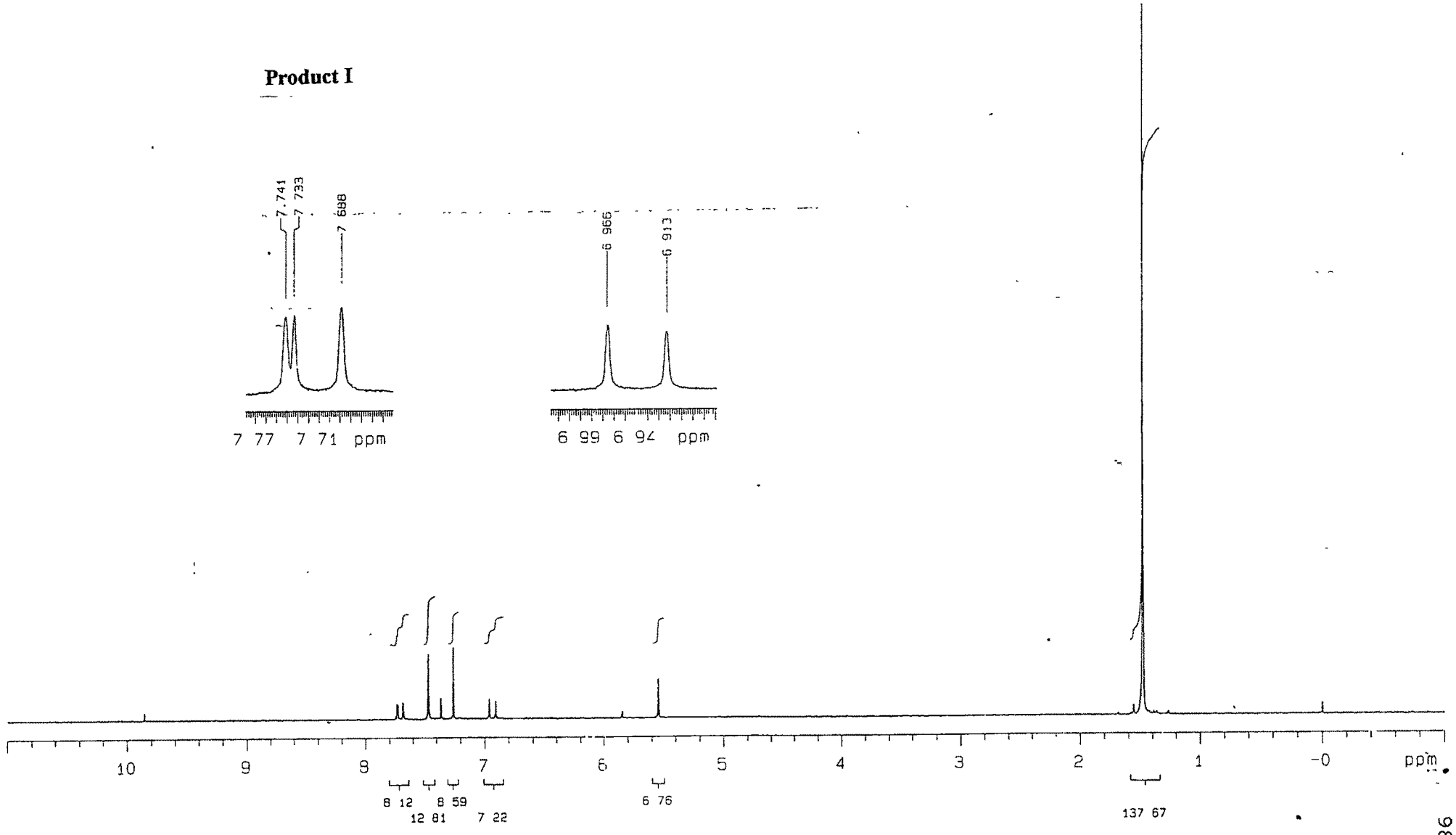


Figure 2b

Product II

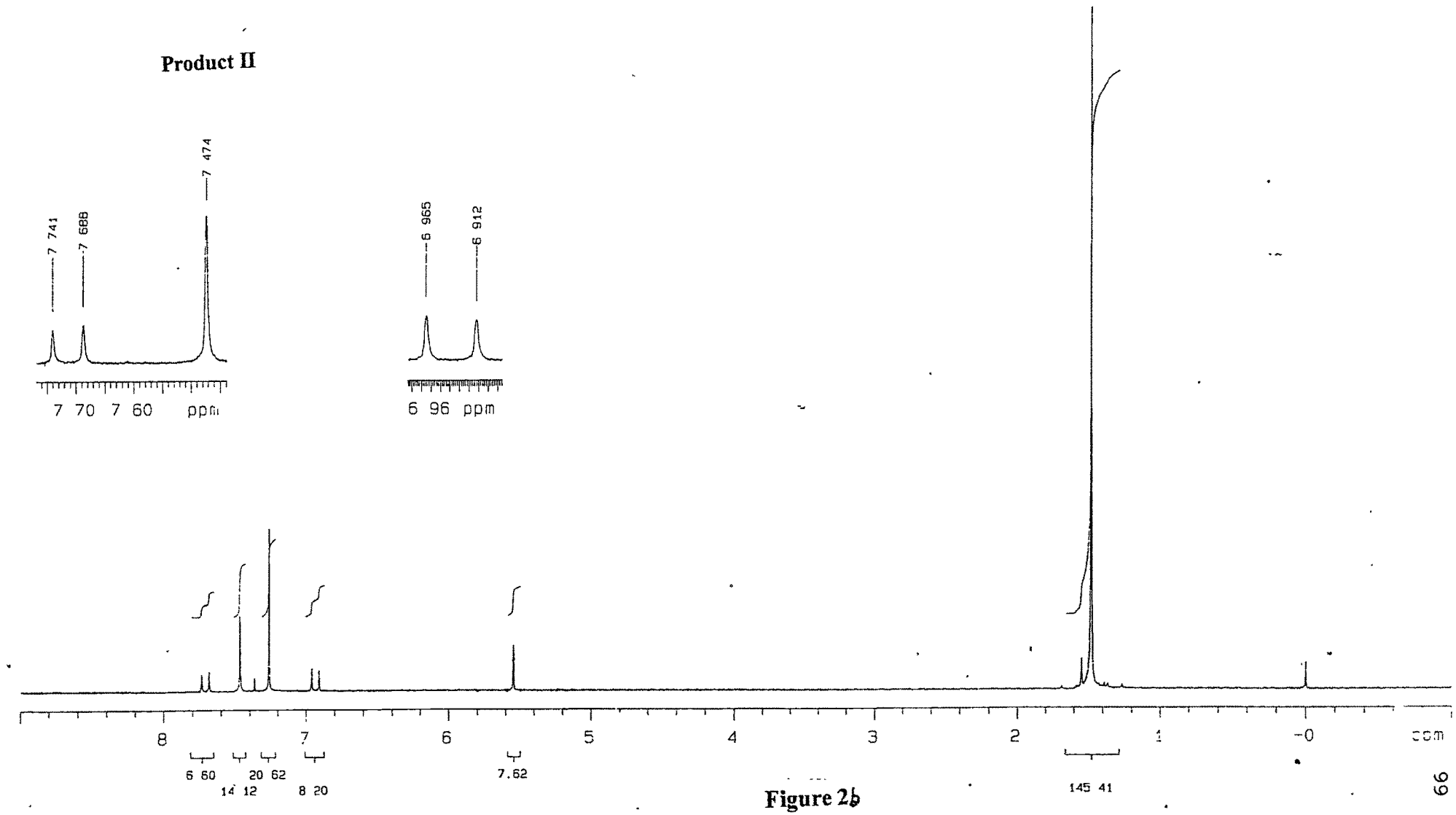
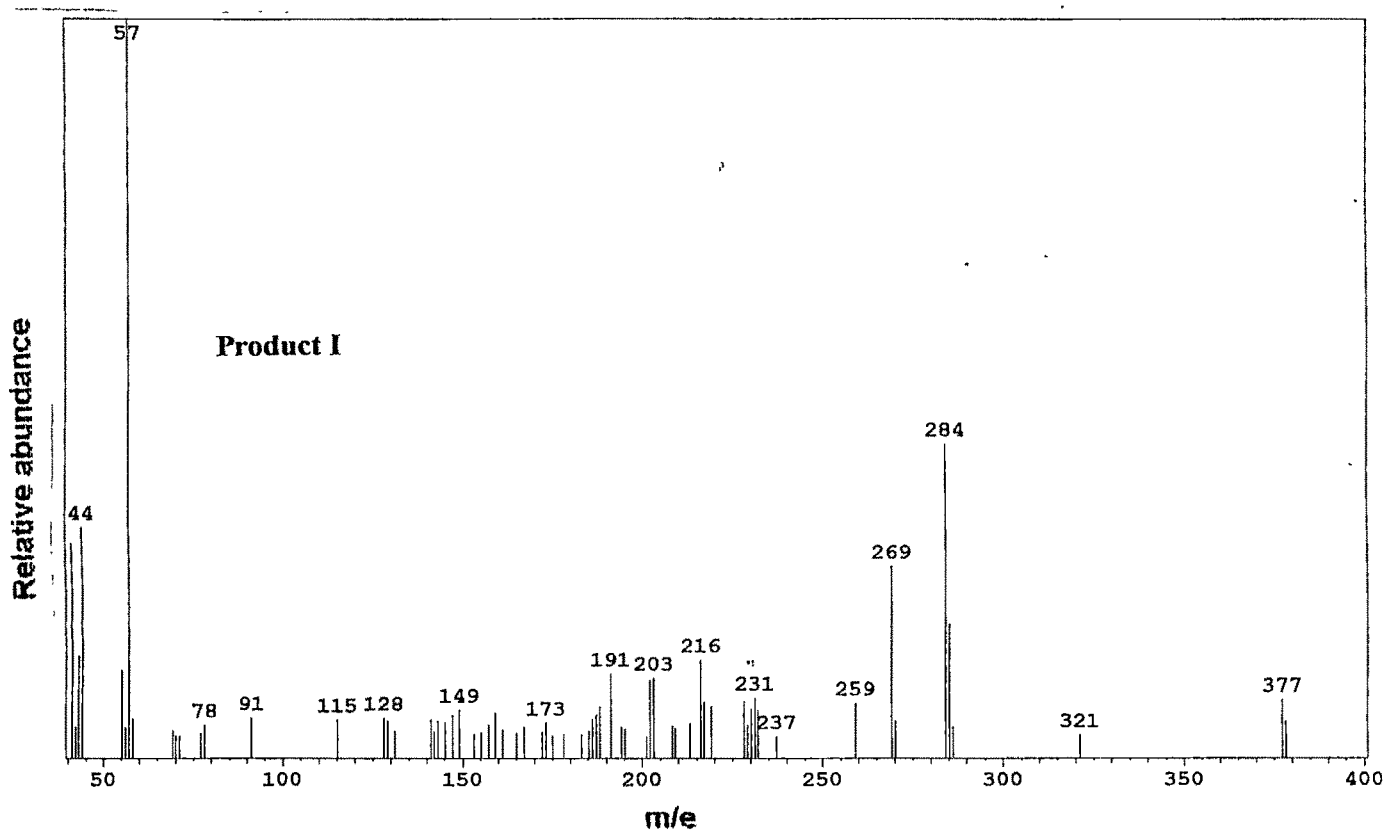


Figure 2b

δ 7.7 with same J value 15.9Hz. Singlet was observed at δ 7.21 and at δ 7.42 for two protons. Mass spectrum (Fig. 2c) of the compound **I** showed molecular ion peak at 378. Base peaks were observed at 57. Rests of the peaks were observed at 321, 293, 284, 269, 259, 235, 231, 216, 202, 191, 173, 159, 141, 129, 115, 91 and 78.

*Synthesis of 1-(7'-hydroxy benzopyran-2-[H]one)-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propenone **3d.***

3,5-Di-tert-butyl-4-hydroxybenzaldehyde **1** on reaction with 7-hydroxy-3-acetyl coumarin **2d** in ethanolic HCl afforded propenone **3d** (Scheme 1). Structure of propenone **3d** was established on the basis of IR, NMR and CMR spectral studies. IR spectrum (Fig. 3) recorded in Nujol showed band at 3610 cm^{-1} due to OH stretching frequency of hindered phenolic group. Band at 3300 cm^{-1} was observed due to hydroxy group at position $C_{7'}$. Carbonyl group of lactone appeared at 1740 cm^{-1} and that of chalcone observed at 1610 cm^{-1}. Unequal bands appeared at 1385 &1375 cm^{-1} are due to CH bending of tert-butyl groups. ^1H NMR spectrum (Fig. 4) of the compound recorded using DMSO as solvent and TMS as internal standard showed singlet at δ 1.42 for eighteen protons is due to two tert-butyl groups. Doublet at δ 6.70 with J = 2.1Hz for one proton is due to the presence of aromatic proton at $C_{8'}$. Double doublet at δ 6.8 with $J_{6'-8'}$ = 2.1 Hz and $J_{5'-6'}$ = 8.7 Hz for one proton is due to the presence of proton at $C_{6'}$. J value indicated that proton $H_{6'}$ is in the ortho coupling with $H_{5'}$ and in meta coupling with $H_{8'}$ proton. Singlet at δ 7.49 for two protons was observed due to two aromatic protons present in phenolic ring. Doublet observed at δ 7.59 with J=15.9 Hz for one proton is due to H_α proton present in chalcone linkage and doublet at δ 7.65 with same J value for one proton is due to the presence of H_β proton. Singlet observed at δ 7.81 corresponds to one proton with J value 8.7 Hz is for $H_{5'}$ proton and singlet at δ 8.60 corresponds to one proton is for $H_{4'}$ proton. ^{13}CMR spectrum (Fig. 5) taken


Product I
Relative abundance
m/e
57
44
78
91
115
128
149
173
191
203
216
231
237
259
269
284
321
377
50
100
150
200
250
300
350
400

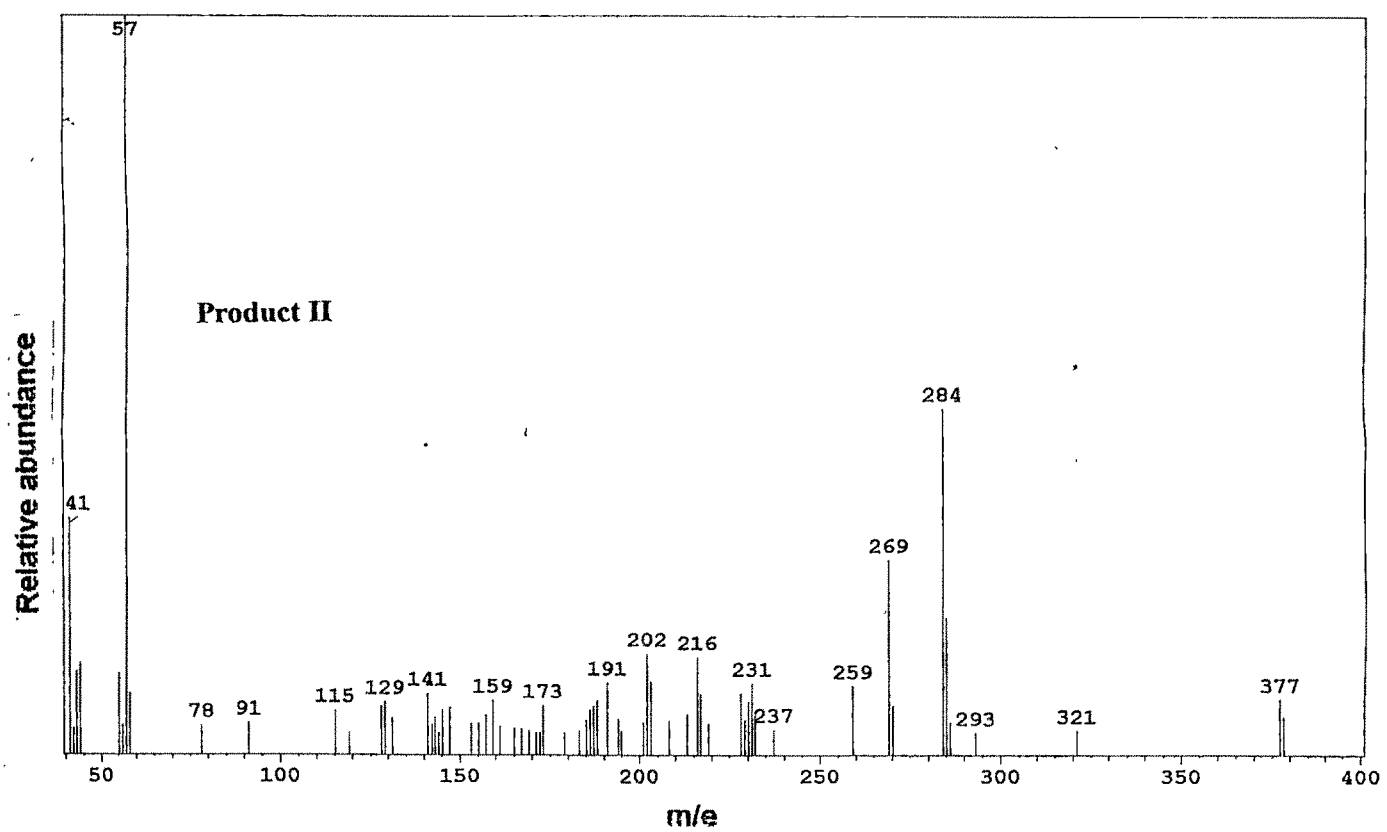

Product II
Relative abundance
m/e
57
41
78
91
115
129
141
159
173
191
202
216
231
237
259
269
284
293
321
377
50
100
150
200
250
300
350
400

Figure 2C

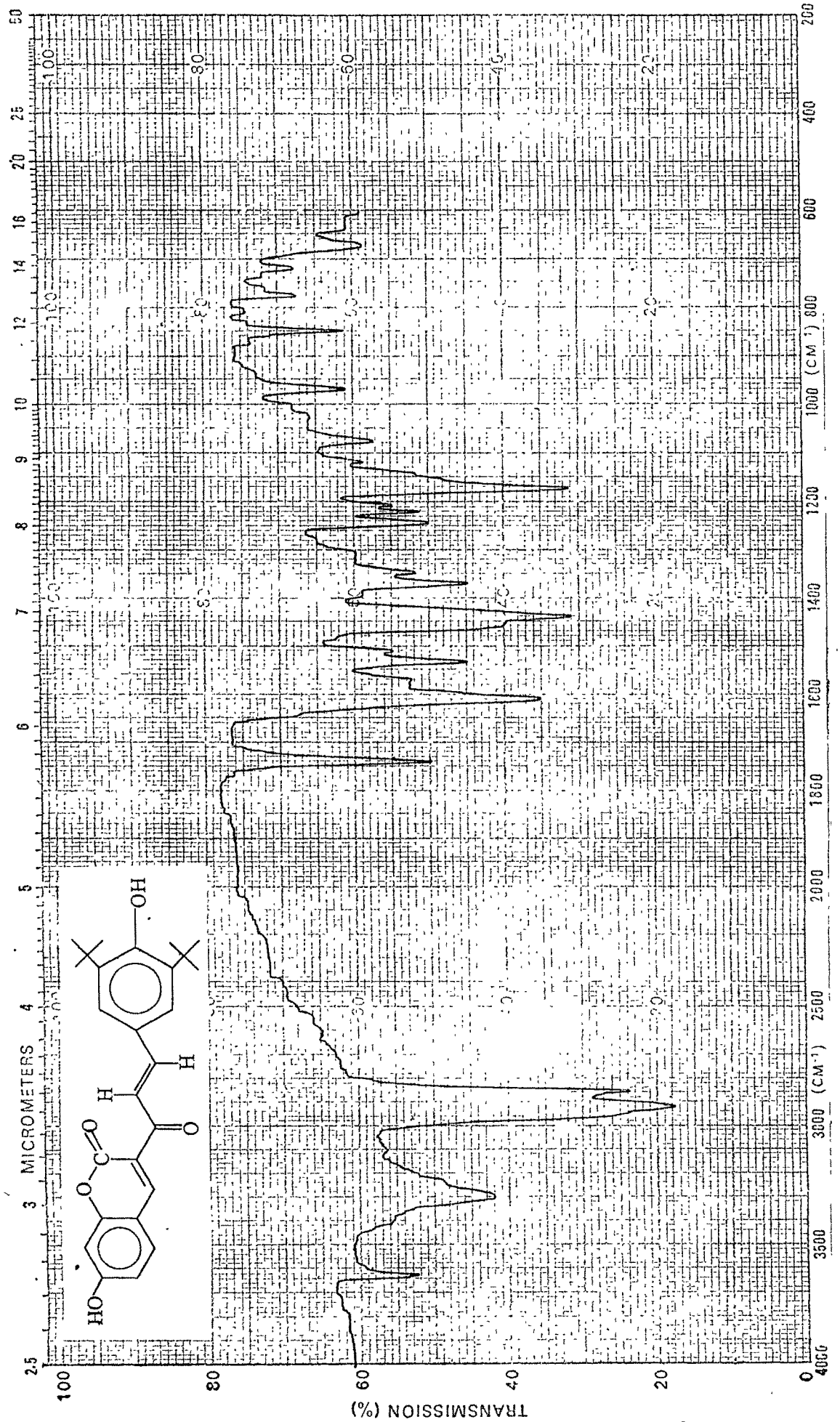
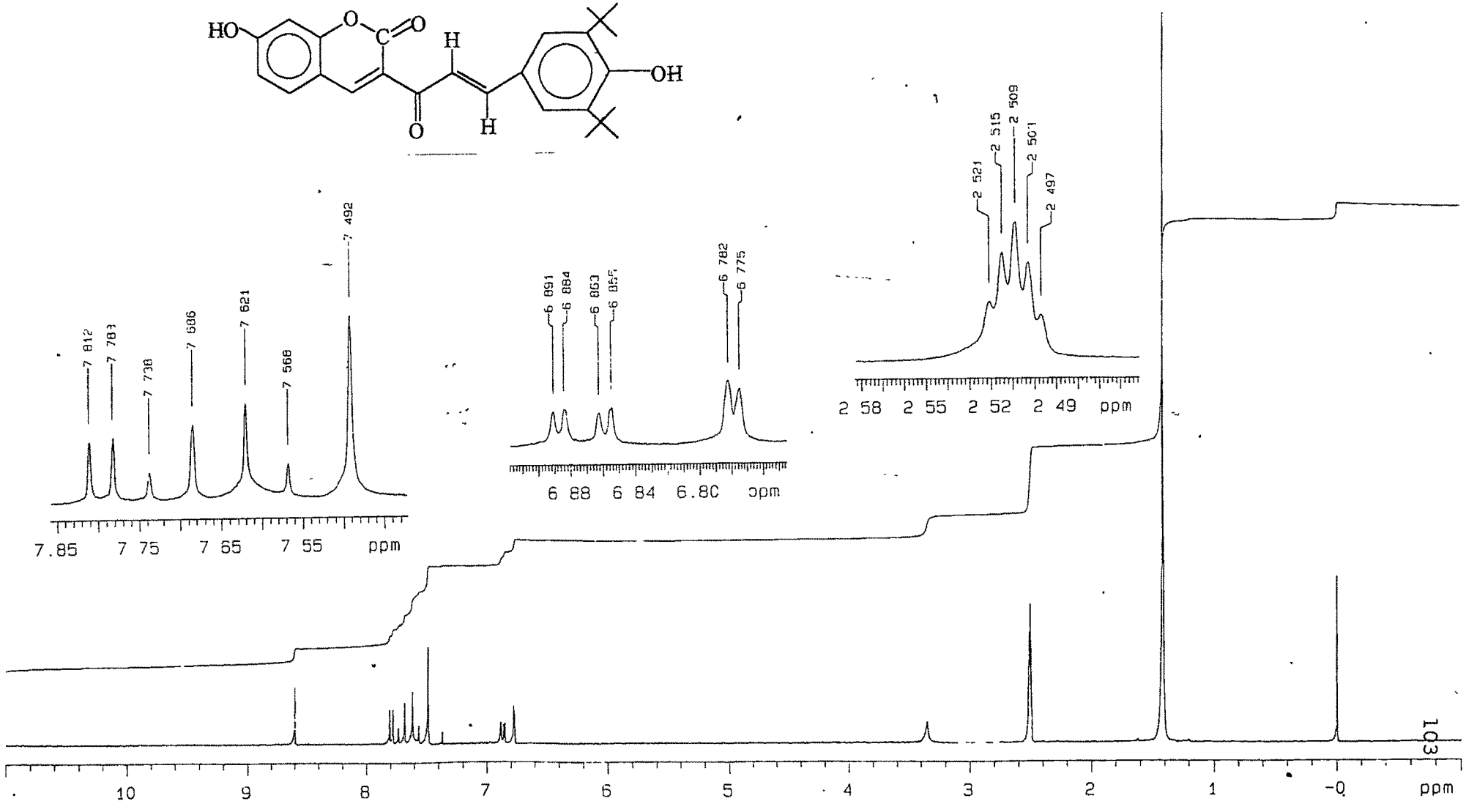


Figure 3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O
O
C
O
H
H
O
OH
7 812
7 783
7 738
7 686
7 621
7 568
7 492
6 891
6 884
6 863
6 855
6 782
6 775
2 521
2 515
2 509
2 501
2 497
7.85 7 75 7 65 7 55 ppm
6 88 6 84 6.80 ppm
2 58 2 55 2 52 2 49 ppm
10 9 8 7 6 5 4 3 2 1 -0 ppm
103

Figure 4

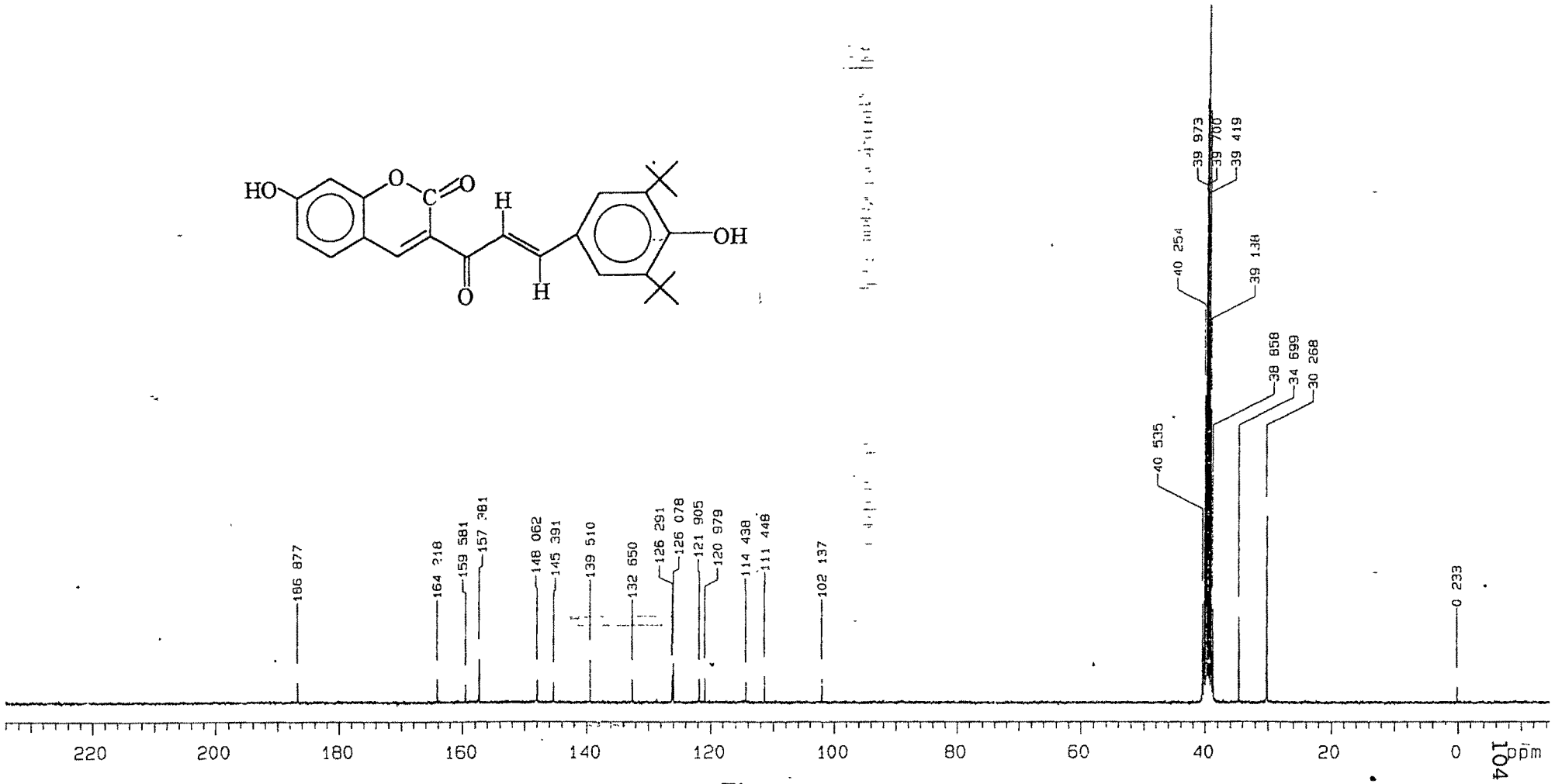

HO
O
O
C
O
H
H
O
OH
186 877
164 218
159 581
157 381
148 062
145 391
139 510
132 650
126 291
126 078
121 905
120 979
114 438
111 448
102 137
40 535
40 254
39 973
39 700
39 419
39 138
38 858
34 699
30 268
0 233
220
200
180
160
140
120
100
80
60
40
20
0
ppm

Figure 5

using DMSO as solvent showed methyl carbon at δ 30.26, tert butyl carbon appeared at δ 34.69. Signal observed at δ 121.90 is for aromatic carbon present in the phenolic ring at $C_{2'}$ and $C_{6'}$. C_α carbon appeared at δ 126.08 and that of C_β carbon appeared at δ 145.39. Carbonyl carbon of chalcone appeared at δ 186.87.

Synthesis of 1-(7'-hydroxy-2'-methyl benzopyran-4-[H]one)-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propenone ***3e.***

Aldehyde **1** on condensation with ketone **2e** in ethanolic HCl gave chalcone **3e** and compound **II** (Scheme 1). Structure of chalcone **3e** has been established on the basis of IR, NMR and Mass spectral studies. IR spectrum (Fig. 6) recorded in Nujol showed band at 3620 cm^{-1} due to OH stretching frequency of hindered phenolic OH group. Band at 3250 cm^{-1} was observed due to presence of hydroxyl group at $C_{7'}$. Carbonyl group of chalcone appeared at 1660 cm^{-1} and that of chromone appeared at 1620 cm^{-1}. ^1H NMR (Fig. 7) taken in $CDCl_3$ using TMS as internal standard showed two singlets at δ 1.41 & 1.44 corresponding to eighteen protons for two tert-butyl groups. Two singlets observed at δ 2.40 & 2.70 for three protons are due to methyl group. From these it has been concluded that compound existed in two isomeric forms E and Z. From the integral calculation amount of one of the isomers in the reaction mixture was found to be 43% and the other 57%. R_f value of both these isomers was same on TLC (benzene : alcohol :: 9:1). Due to the presence of these isomers the aromatic region was showing large number of peaks merging in each other. Mass spectrum (Fig. 8) showed molecular ion peak at m/z 434 and base peak at 203. Rest of the peaks obtained were at 419, 405, 377. 319, 335, 321, 303, 203, 175.

Elemental analysis and spectral characterisation of product **II** were found to be identical with that of product **I.**

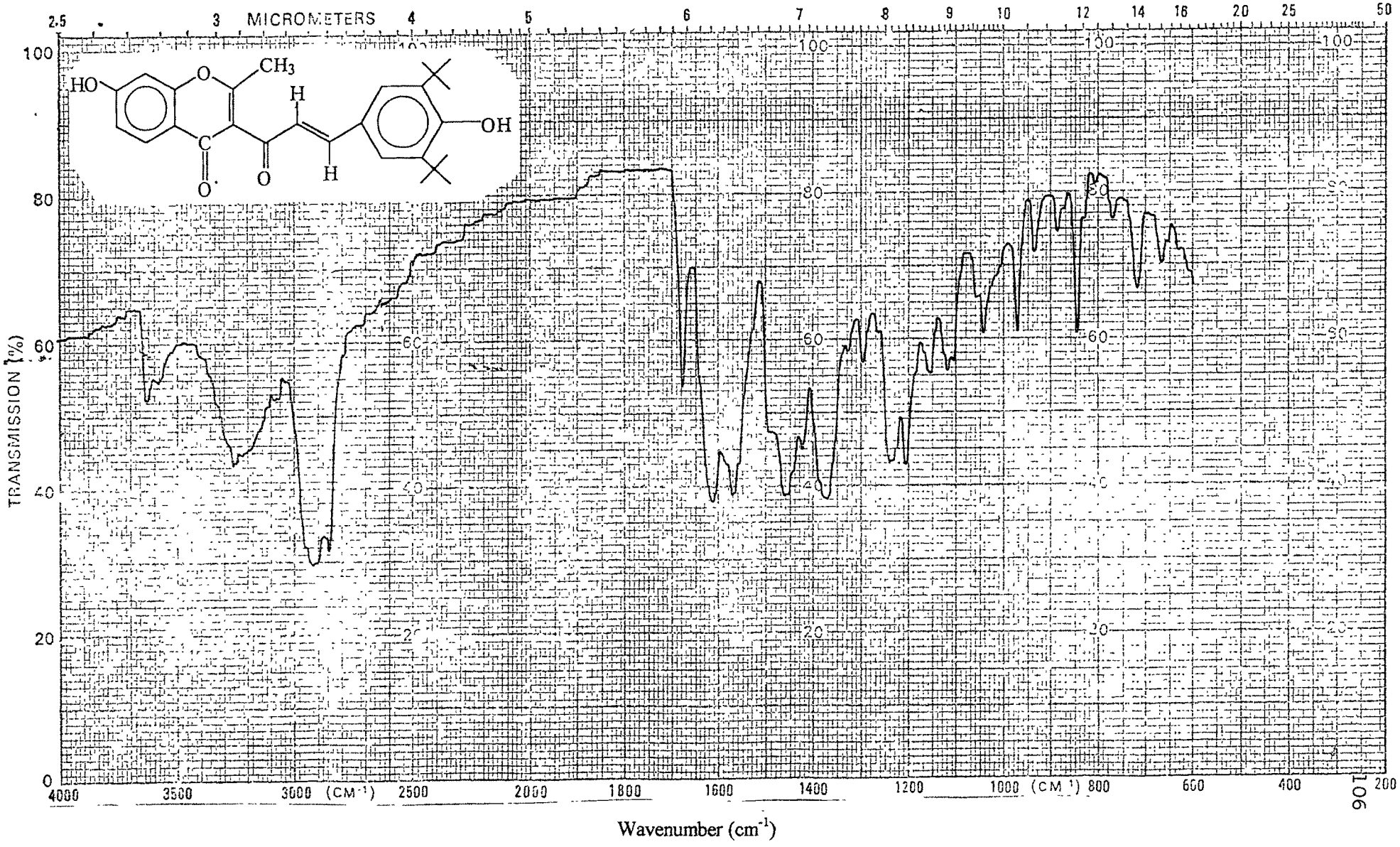

2.5
3
MICROMETERS
4
5
6
7
8
9
10
12
14
16
20
25
50
100
80
60
40
20
0
TRANSMISSION (%)
HO
O
CH3
H
OH
C
O
O
H
4000
3500
3000 (CM-1)
2500
2000
1800
1600
1400
1200
1000 (CM-1)
800
600
400
200
Wavenumber (cm-1)

Figure 6

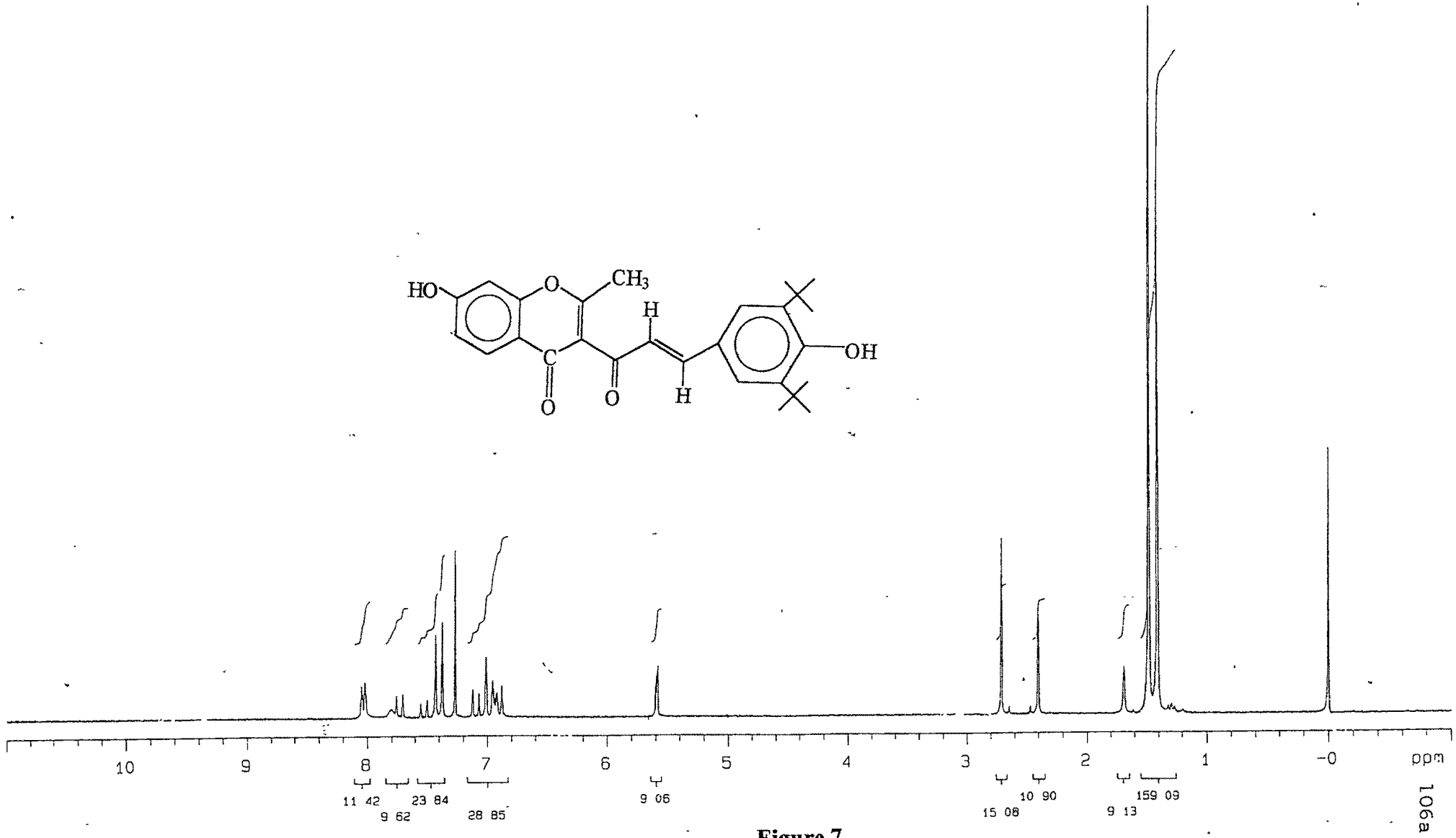

HO
O
CH3
H
C
O
O
H
OH
10
9
8
7
6
5
4
3
2
1
-0
ppm
11 42
9 62
23 84
28 85
9 06
15 08
10 90
9 13
159 09
106a

Figure 7

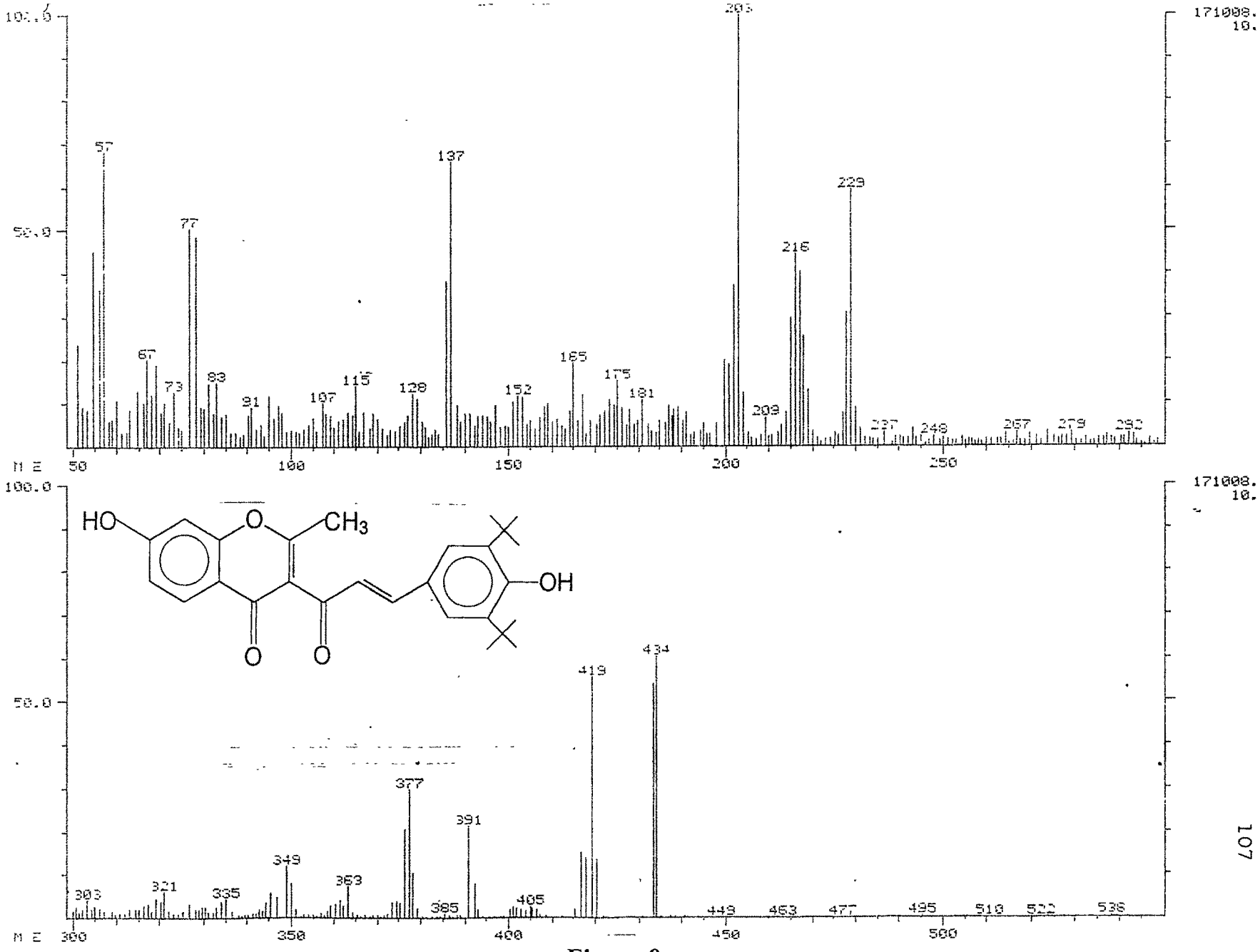

171008.
10.
100.0
50.0
M E
50
100
150
200
250
57
77
67
73
83
91
107
115
120
137
152
165
175
181
203
209
216
229
237
248
257
279
292
HO
O
CH3
OH
O
O
171008.
10.
100.0
50.0
M E
300
350
400
450
500
303
321
335
349
363
377
385
391
405
419
434
449
463
477
495
510
522
536

Figure 8

Products **I** and **II** were formed from the reactions where aldehyde used was same but the ketones used were different. From the elemental and spectral characteristics a conclusion can be drawn that the products **I** and **II** might be generated from the reaction between aldehyde **1**, ethanol and HCl. In order to support this assumption, a reaction was performed between 3,5-di-tert-butyl-4-hydroxybenzaldehyde, ethanol and HCl.

3,5-Di-tert-butyl-4-hydroxybenzaldehyde on stirring with ethanolic HCl gave product **III**. The ^{1}H NMR spectrum (Fig. 2d) of **III** recorded in $CDCl_3$ using TMS as internal standard showed singlet at δ 1.45 for eighteen protons. Singlet was observed at δ 5.51 for one proton. Two doublets were observed at δ 6.93 and at δ 7.7 with same J value 15.9 Hz. Two singlets were observed at δ 7.21 and at δ 7.42 for two protons.

From the above results it can be concluded that products **I** and **II** are same as that of **III**, generated through the reaction between aldehyde **1**, ethanol and HCl. However the structure of this compound is yet to be established.

Synthesis of 1-(2',4'-dichloro-5'-fluorophenyl)-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propenone 3g

Condensation of 3,5-di-tert-butyl-4-hydroxybenzaldehyde **1** with 2,4-di-chloro-5-fluoro acetophenone **2g** in ethanolic HCl gave chalcone **3g** (Scheme 1). Structure of the compound was established on the basis of IR, NMR and CMR spectral studies. IR spectrum (Fig. 9) recorded in Nujol showed band at 3622 cm^{-1} for hindered phenolic OH stretching frequency. Band appeared at 1660 cm^{-1} is due to CO stretching frequency of carbonyl group in chalcone. ^{1}H NMR (Fig. 10) data taken in $CDCl_3$ as solvent and TMS as internal standard showed singlet at δ 1.40 for eighteen protons is due to two tert-butyl groups. Singlet at δ 5.65 correspond to

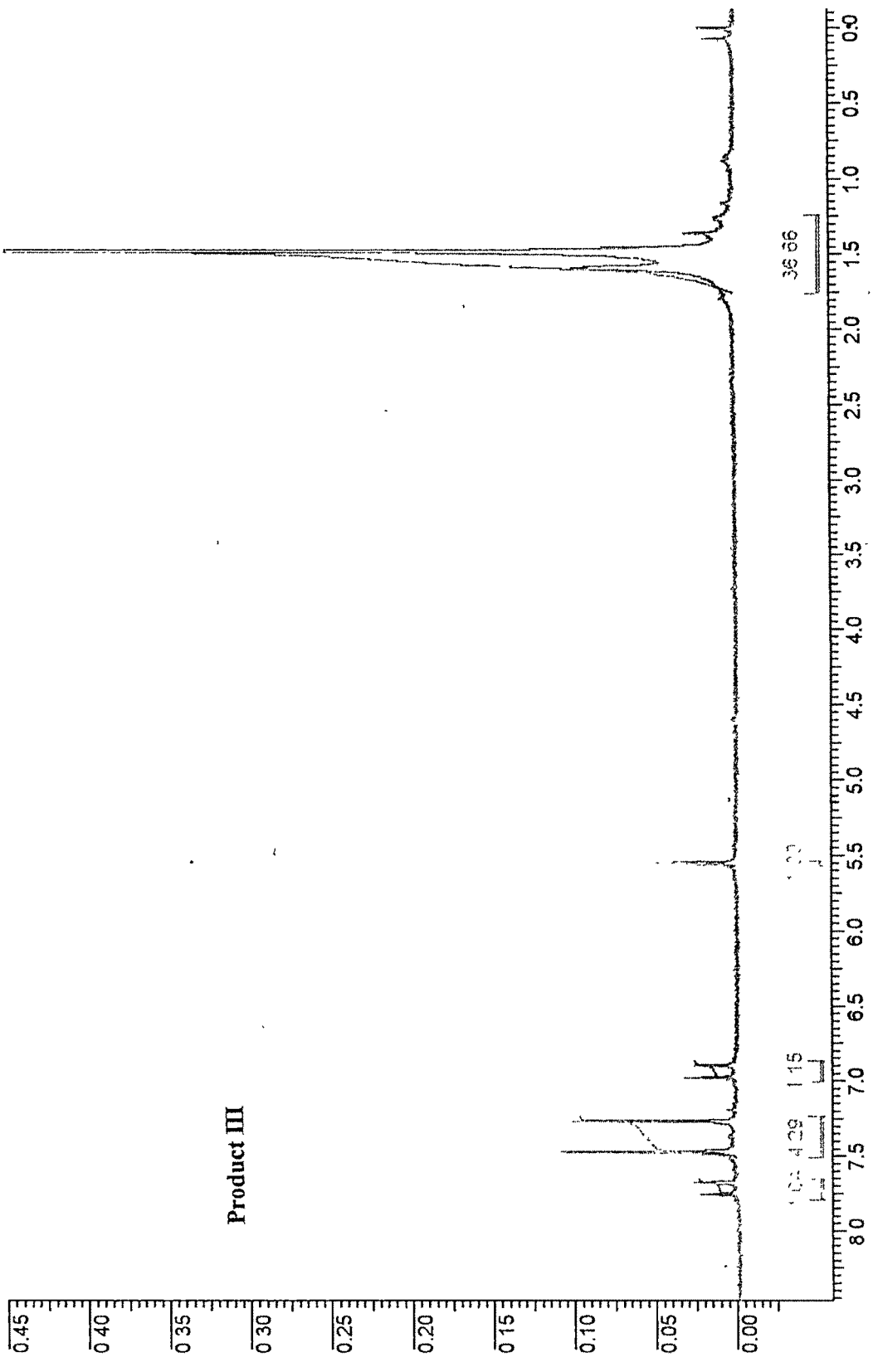

Product III
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0

Figure 2d

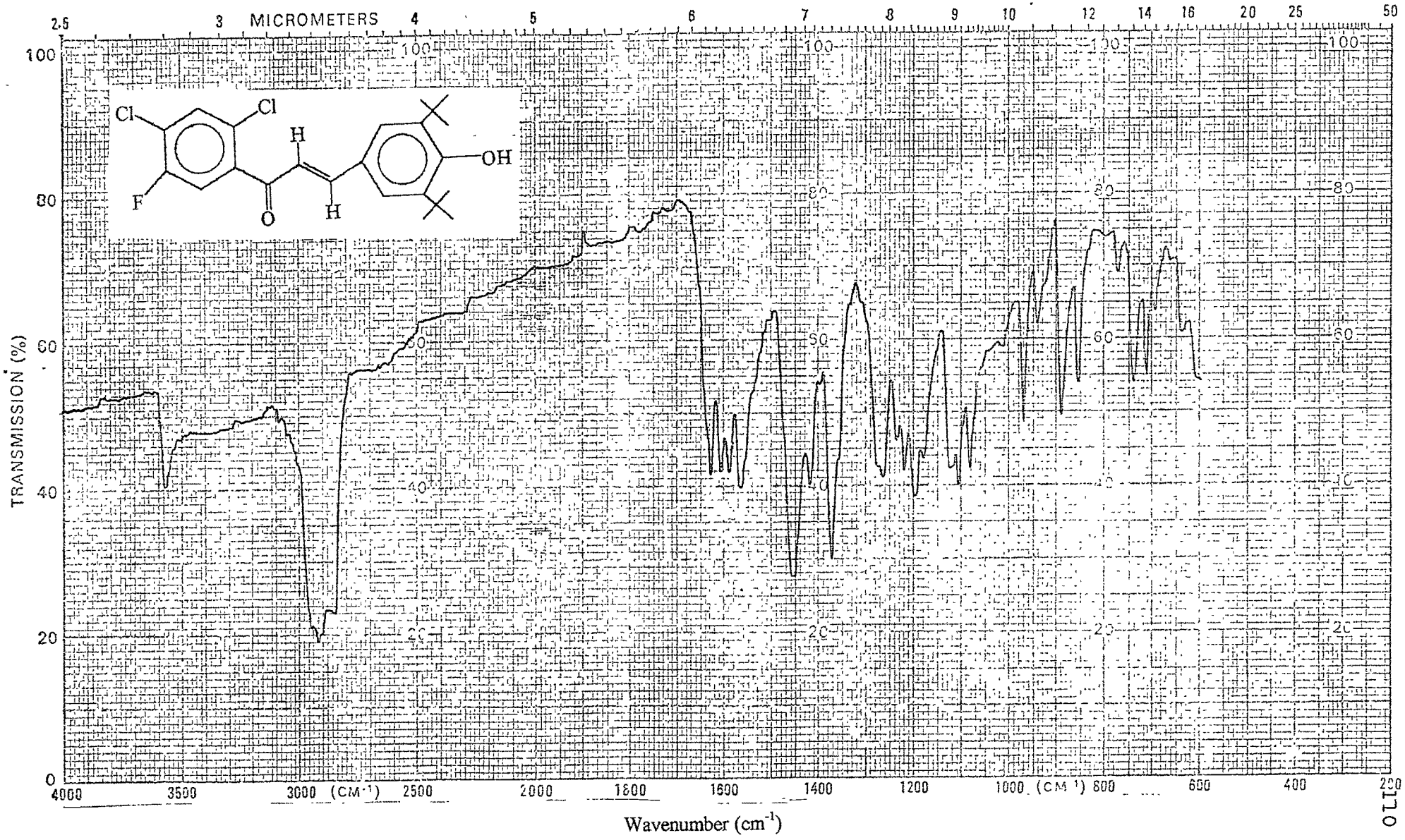


Figure 9

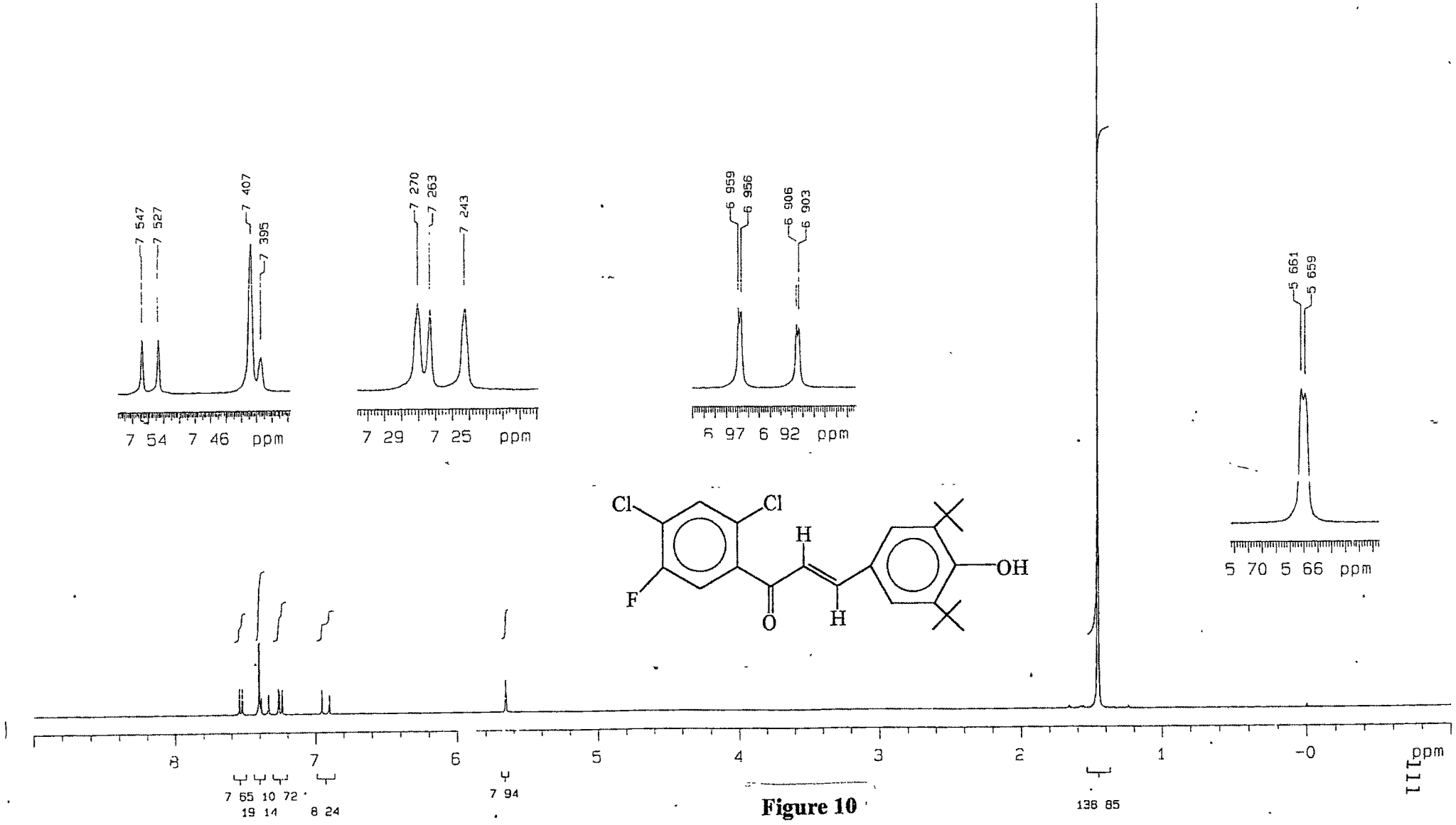

7 547
7 527
7 407
7 395
7 54 7 46 ppm
7 270
7 263
7 243
7 29 7 25 ppm
6 959
6 956
6 906
6 903
6 97 6 92 ppm
5 661
5 659
5 70 5 66 ppm
Cl
Cl
H
OH
F
O
H
8
7
6
5
4
3
2
1
-0
ppm
7 65 10 72
19 14 8 24
7 94
138 85

Figure 10

one proton is due to hydroxyl group present in the molecule. Doublet observed at δ 6.90 for one proton is due to proton at position $H_{6'}$ and doublet at δ 6.95 for one proton is due to proton at position $H_{3'}$. H_α proton of chalcone appeared at δ 7.25 as doublet with J = 15.8 Hz and that of H_β proton appeared at δ 7.53 as doublet with same J value. Singlet at δ 7.40 for two protons was observed due to two aromatic protons present in phenol ring. ^{13}C NMR spectrum (Fig. 11) taken in $CDCl_3$ exhibited peak at δ 30.10 for the methyl group and peak appeared at δ 34.37 is for tert-carbon present in tert-butyl group. C_α carbon of chalcone appeared at δ126.88 and C_β carbon appeared at δ 155.26. C_2 and C_6 carbon atoms present in phenol ring were identical and appeared at δ 122.8. Carbons on which tert-butyl groups attached were same and appeared at δ 149.46.

*Synthesis of 2-pyrazoline **4 a-g**.*

Propenone **3 a-g** on cyclocondensation with hydrazine hydrate yielded pyrazoline **4 a-g** (Scheme 1). Structures of the synthesised compounds were established on the basis of IR, NMR and Mass spectral studies. Spectral data of representative compound **4g** is discussed here. IR spectrum (Fig. 12) taken in Nujol showed a band at 3620 cm^{-1} due to hindered phenolic group. Band at 3200 cm^{-1} appeared due to NH stretching frequency of NH group present in pyrazoline ring. ^1H NMR spectrum (Fig. 13) recorded in $CDCl_3$ using TMS as internal standard showed singlet at δ 1.47 for eighteen protons is due to presence of two tert- butyl groups. Two double doublets at δ 3.20 and at δ 3.55 for one proton each with same J value indicated the diasterotropic nature of methylene protons present in pyrazoline ring. Triplet observed at δ 4.88 showed the presence of one methine proton in pyrazoline ring. Hydroxyl hydrogen appeared at δ 5.4 as singlet. Two aromatic protons of phenol ring resonated at δ 7.40. Multiplet observed in the region of δ 7.48-7.57 is due to aromatic protons present in the ring containing halogen. Mass

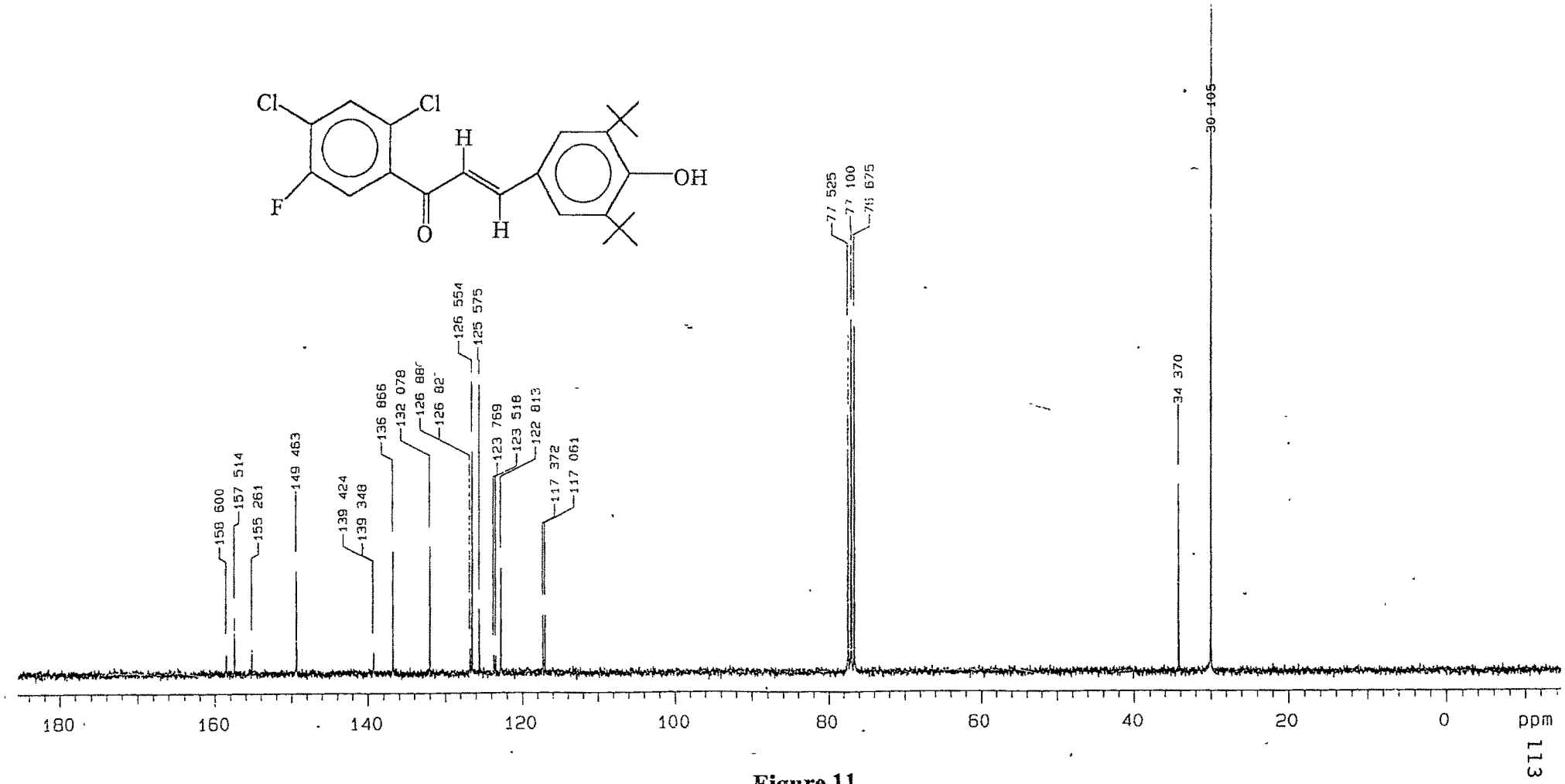

Cl
Cl
H
OH
F
O
H
158 600
157 514
155 261
149 463
139 424
139 348
136 866
132 078
126 88
126 82
126 554
125 575
123 769
123 518
122 813
117 372
117 061
77 525
77 100
76 675
34 370
30 405
180
160
140
120
100
80
60
40
20
0
ppm

Figure 11

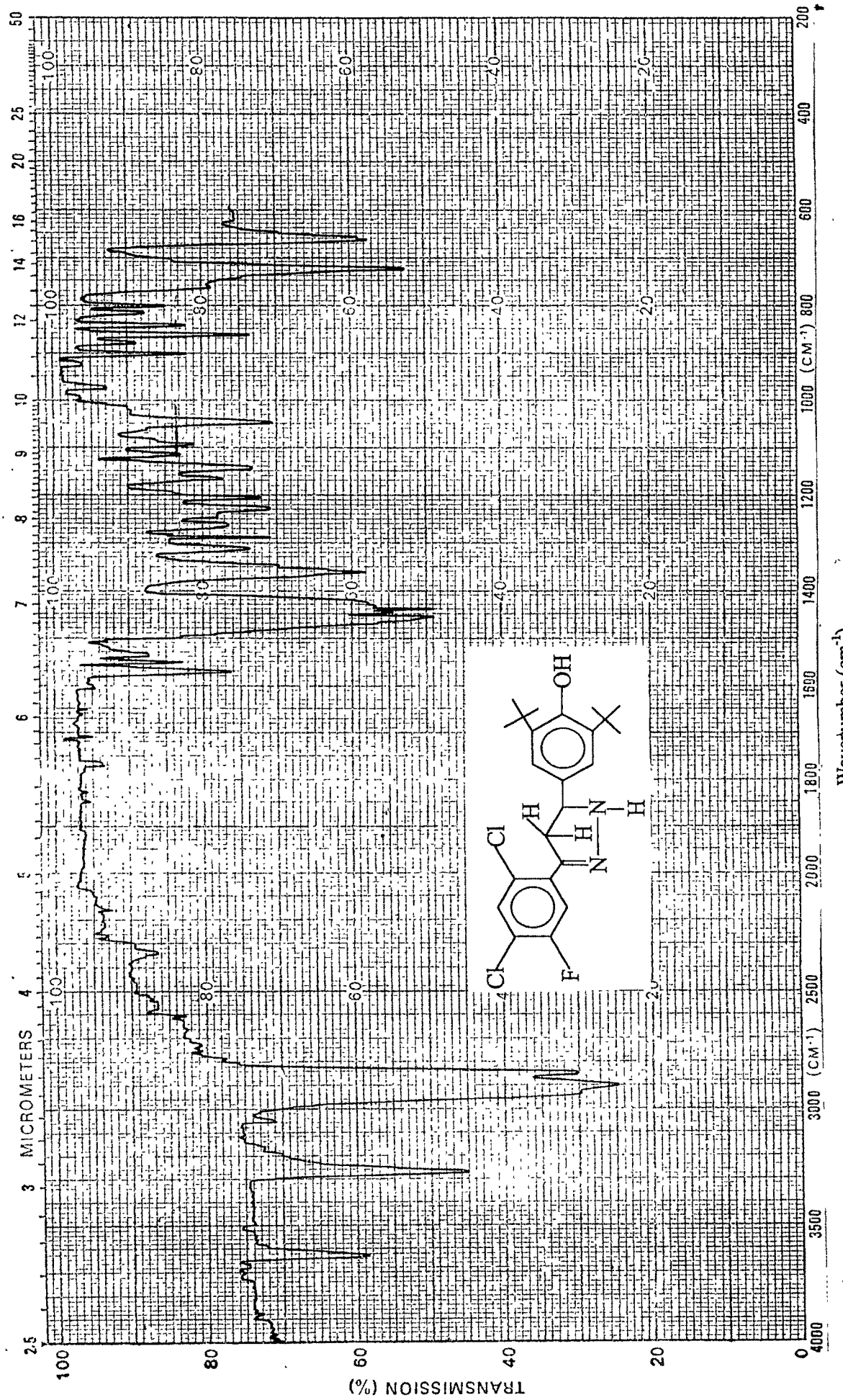


Figure 12

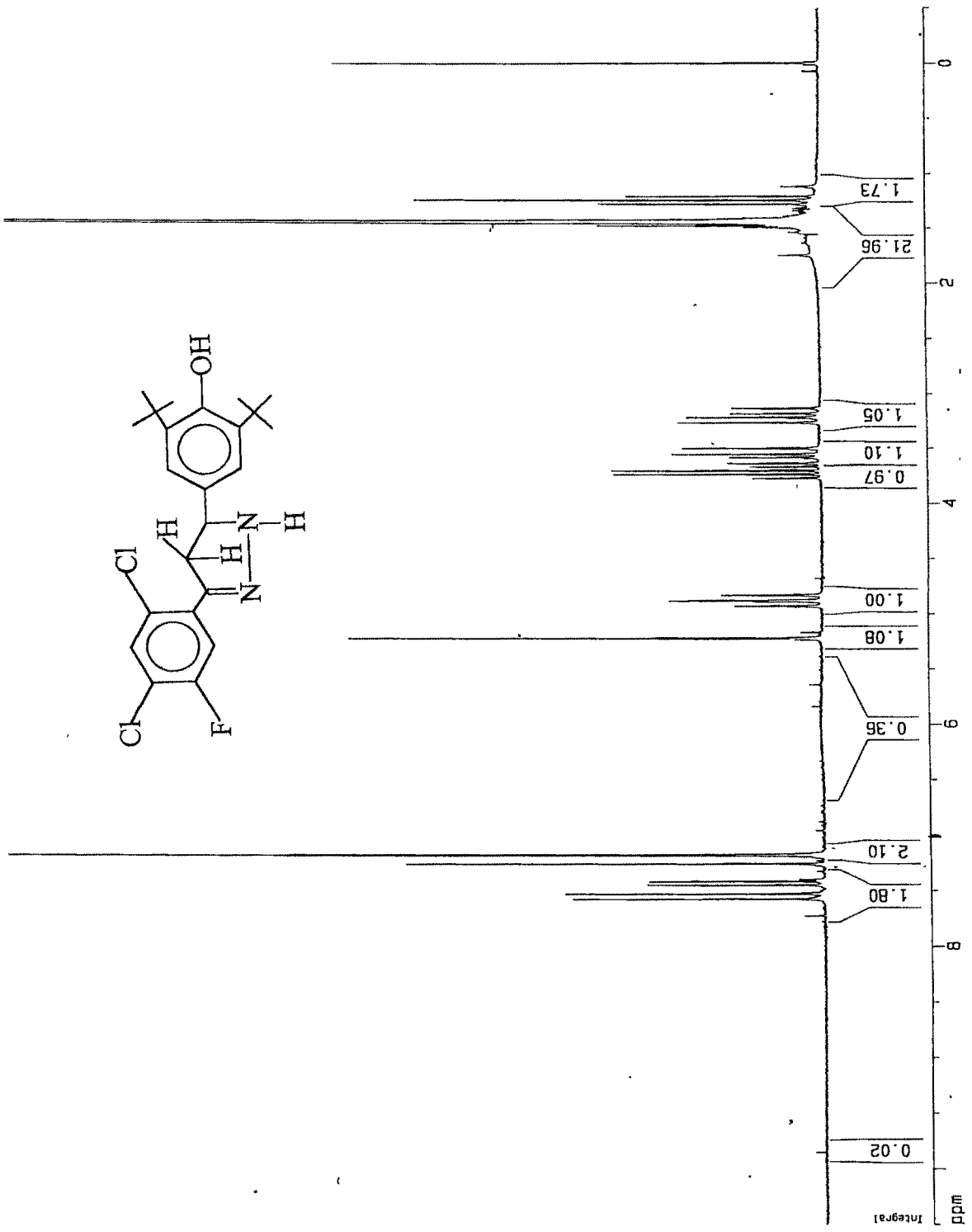

OH
H
N
H
H
N
N
Cl
Cl
F
Integral
ppm
0.02
1.80
2.10
0.36
1.08
1.00
0.97
1.10
1.05
21.96
1.73
0
2
4
6
8

Figure 13

spectrum (Fig. 14) of this compound showed molecular ion peak at 437 which indicated molecular weight of the compound. Base peak was observed at 436. Peaks were also observed at 421, 393, 379, 365, 351, 323, 231, 205.

Synthesis of 1-phenyl-2-pyrazoline ***5 a-g.***

Cyclocondensation of propenone **3a-g** with phenyl hydrazine in the presence of acetic acid and ethanol afforded 1-phenyl-2-pyrazoline **5 a-g** (Scheme 1). Structures of these 1-phenyl-2-pyrazolines were established on the basis of IR and NMR spectral data. Spectral data of the representative compound **5g** is discussed here. IR spectrum (Fig. 15) taken in Nujol showed band at 3610 cm^{-1} due to hindered phenolic group. ^1H NMR spectrum (Fig. 16) recorded in $CDCl_3$ using TMS as internal standard showed singlet at δ 1.47 for two tert-butyl groups. Double doublet at δ 3.29 for one proton with J value 3.26 and double doublet at δ 5.22 for one proton with same J value suggest that both protons of methylene group present in pyrazoline ring are diasterotropic in nature. Triplet observed at δ 4.0 is due to the presence of methine proton. Hydroxy proton appeared at δ 5.15 and all the aromatic protons appeared as multiplet in the region of δ 6.8 to 7.70.

Synthesis of flavanone ***6 a-c.***

Propenone **3a-c** on treatment with ethanolic HCl undergoes cyclization to yield flavanone **6a-c** (Scheme 1). An IR spectrum of the representative compound **6a** is given (Fig. 17). Presence of band at 3400 cm^{-1} due to hydroxyl group at position 2' in compound **3a** and absence of same band in compound **6a** confirmed cyclization.

Synthesis of 4-(3',5'-di-tert-butyl-4'-hydroxyphenyl)-5-carbethoxy-6-methyl dihydropyrimidine-2-one **7.**

Condensation of 3,5-di-tert-butyl-4-hydroxybenzaldehyde **1** with ethylacetoacetate and urea in the presence of mineral acid afforded dihydropyrimidine-2-one **7**

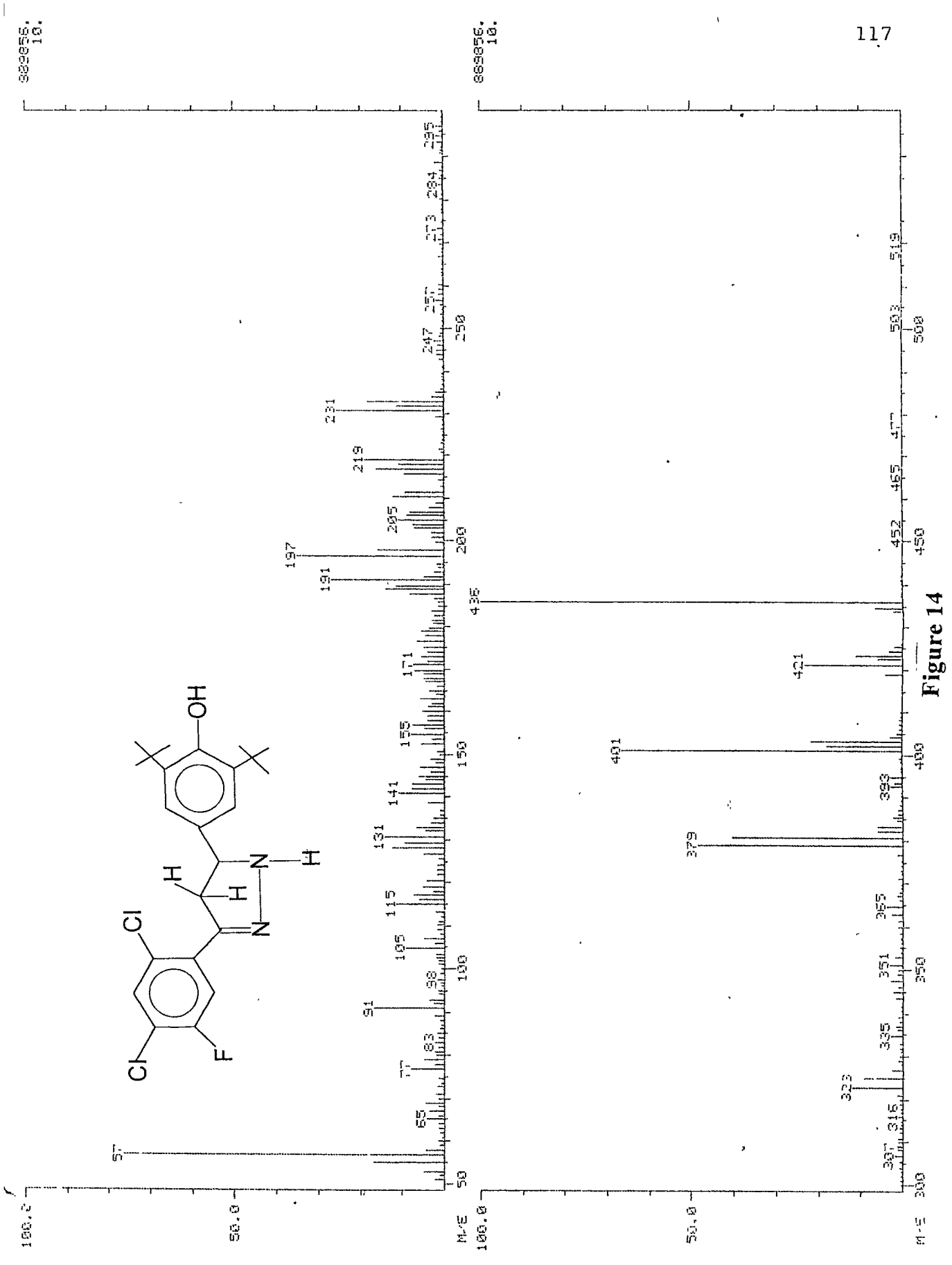

OH
Cl
Cl
F
H
H
N
N
H
M/E
100.0
50.0
57
91
197
191
231
219
205
421
401
379
436

Figure 14

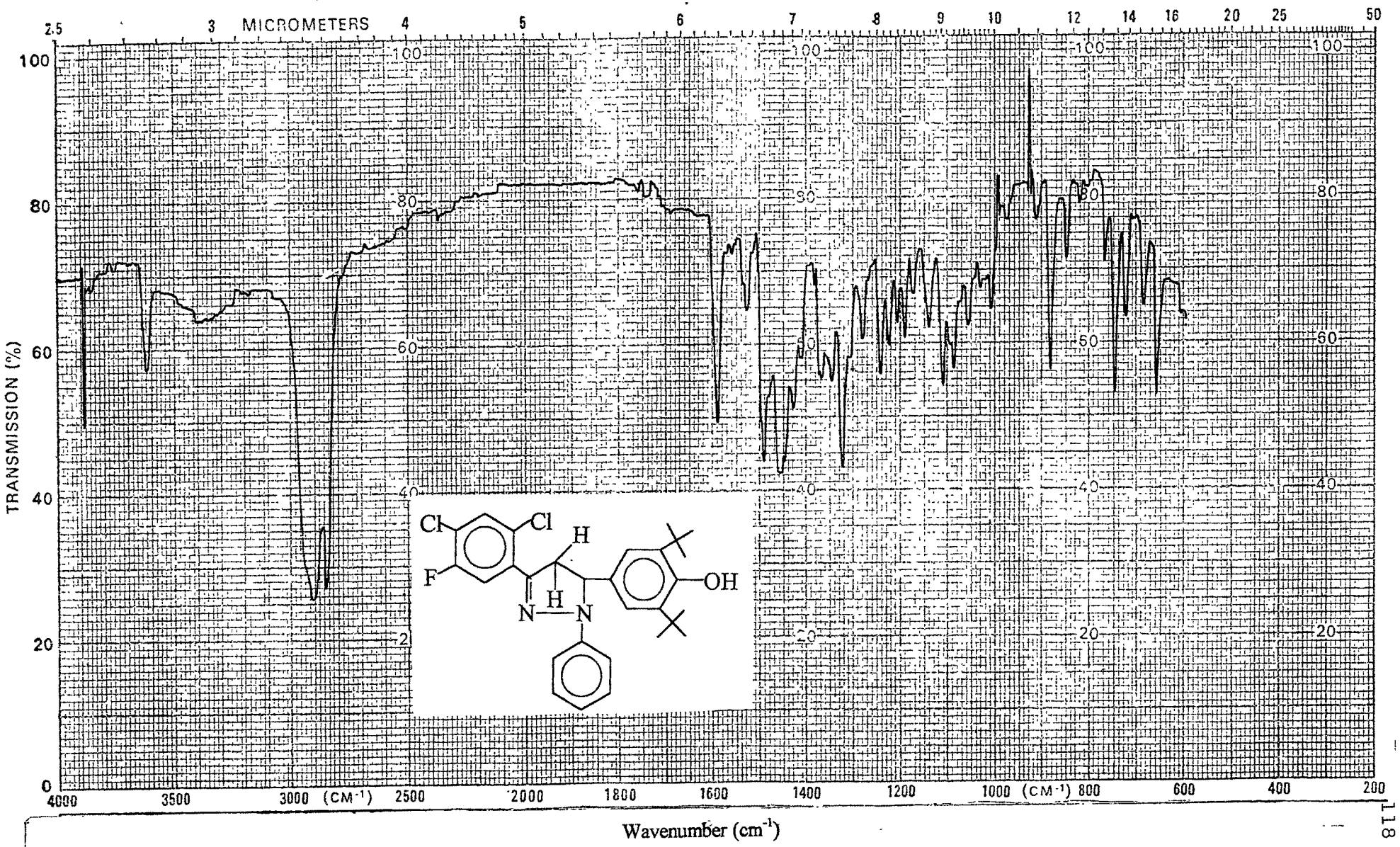

2.5
3
MICROMETERS
4
5
6
7
8
9
10
12
14
16
20
25
50
100
80
60
40
20
0
TRANSMISSION (%)
4000
3500
3000 (CM-1)
2500
2000
1800
1600
1400
1200
1000 (CM-1)
800
600
400
200
Wavenumber (cm-1)
Cl
Cl
H
F
OH
N
H
N

Figure 15

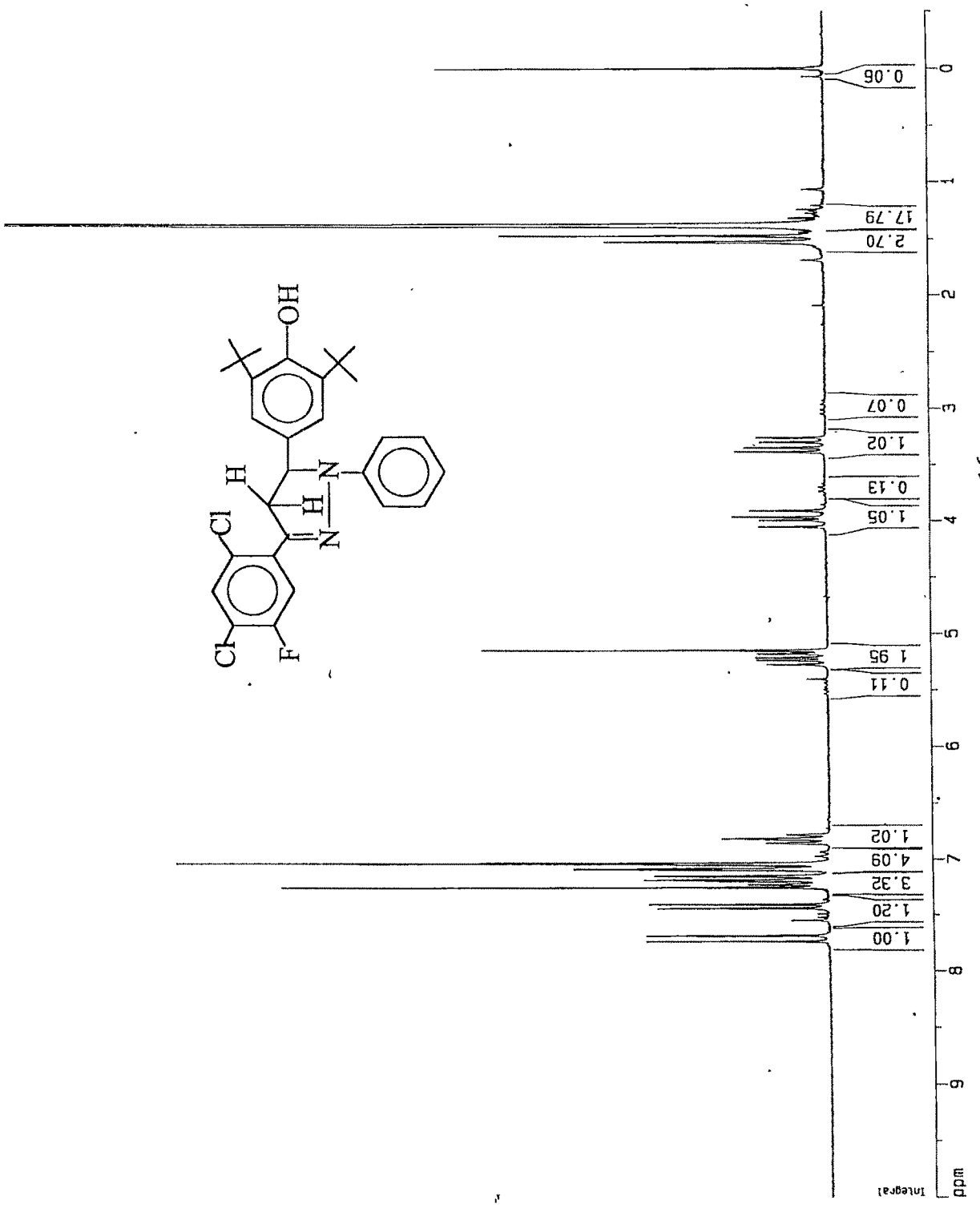

OH
H
N
H
N
Cl
Cl
F
0.06
17.79
2.70
0.07
1.02
0.13
1.05
1.95
0.11
1.02
4.09
3.32
1.20
1.00
Integral
ppm
0
1
2
3
4
5
6
7
8
9

Figure 16

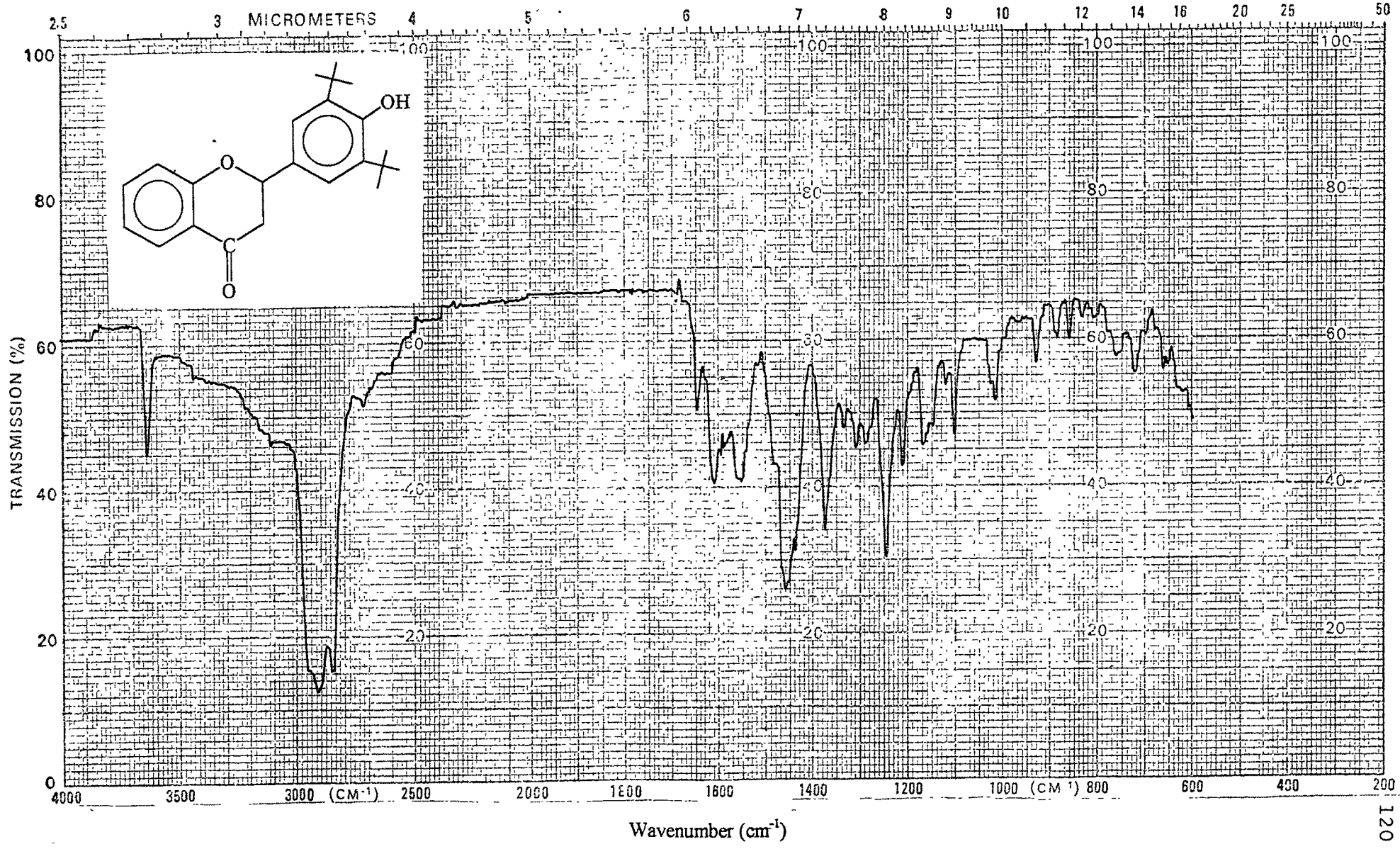
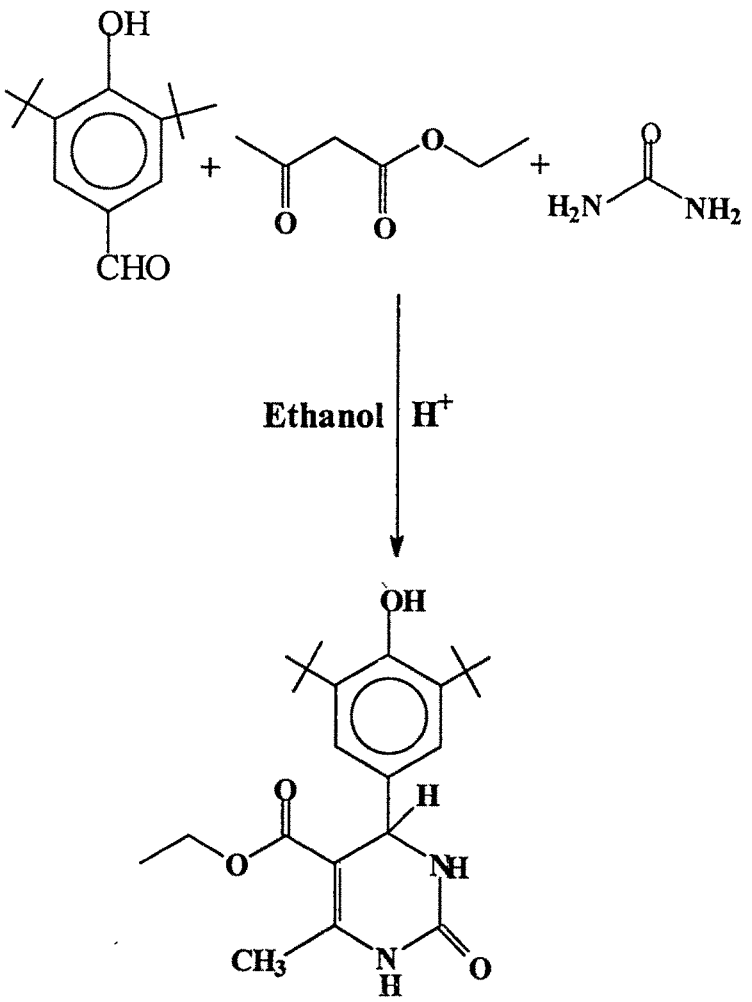


Figure 17

(Scheme 2). Structure of synthesised compound has been established on the basis of elemental analysis, IR and NMR spectral studies. IR spectrum (Fig. 18) of the compound showed band at 3610 cm^{-1} due to OH stretching frequency of hindered phenolic group. Bands observed at 3250 cm^{-1} and at 3100 cm^{-1} are due to NH stretching frequency of amide group. Carbonyl stretching frequency of an ester appeared at 1720 cm^{-1} and that of amide appeared at 1660 cm^{-1}. ^{1}NMR spectrum (Fig. 19) recorded in $CDCl_3$ using TMS as internal standard showed triplet at δ 1.20 for three protons and quartet at δ 4.12 for two protons are due to presence of ethyl group. Singlet observed at δ 1.42 for eighteen protons is due to the presence of tert-butyl group. Three protons of methyl group appeared as singlet at δ 2.25. Phenolic proton resonated at δ 5.2 as singlet. Two NH protons present in the dihydropyrimidine ring were observed at δ 5.26 and at δ 5.45 as singlets. Singlet observed at δ 7.15 for two protons is due to the presence of two aromatic protons. Methine protons appeared as a singlet at δ 8.05.

Experimental

All the melting points were determined in open capillaries using paraffin bath and are uncorrected. Elemental analyses were performed using Perkin-Elmer-2400 (Norwalk, CT) C, H, N and S analyser. IR spectra recorded on Perkin Elmer-781 spectrophotometer in Nujol. NMR spectra were recorded on Brukers-200mHz (Wissenbourg-France) spectrophotometer or on Varian-Gemini 300mHz spectrophotometer using $CDCl_3$ or DMSO as solvent and TMS as internal standard. Signal positions (δ values) were measured relative to the TMS signal (δ 0). Coupling constant (J) values are given in Hz. ^{13}CMR spectra were recorded on Varian-Gemini 75mHz spectrophotometer. Mass spectra were recorded on Fillisinnigan MAT-1020B instrument. Acmes silica gel used for column chromatography with mesh size 60-120. Analytical TLC was performed on precoated E.Merck silica gel 60 F_{254} aluminium plates.



[7]

Biginelli Synthesis

Scheme 2

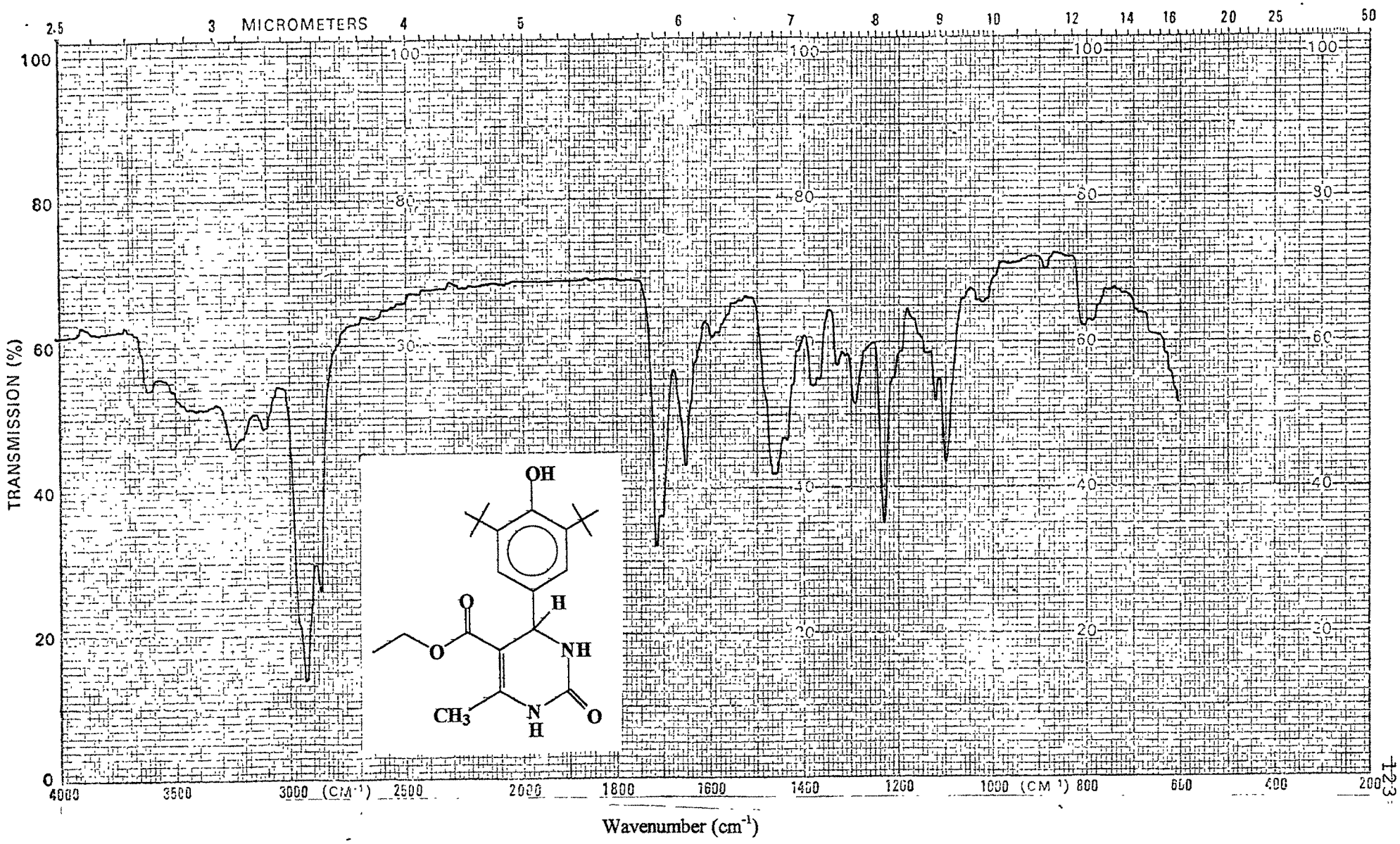

2.5
3
MICROMETERS
4
5
6
7
8
9
10
12
14
16
20
25
50
100
80
60
40
20
0
TRANSMISSION (%)
4000
3500
3000 (CM⁻¹)
2500
2000
1800
1600
1400
1200
1000 (CM⁻¹)
800
600
400
200
Wavenumber (cm⁻¹)
OH
H
O
O
NH
CH3
N
H
O

Figure 18

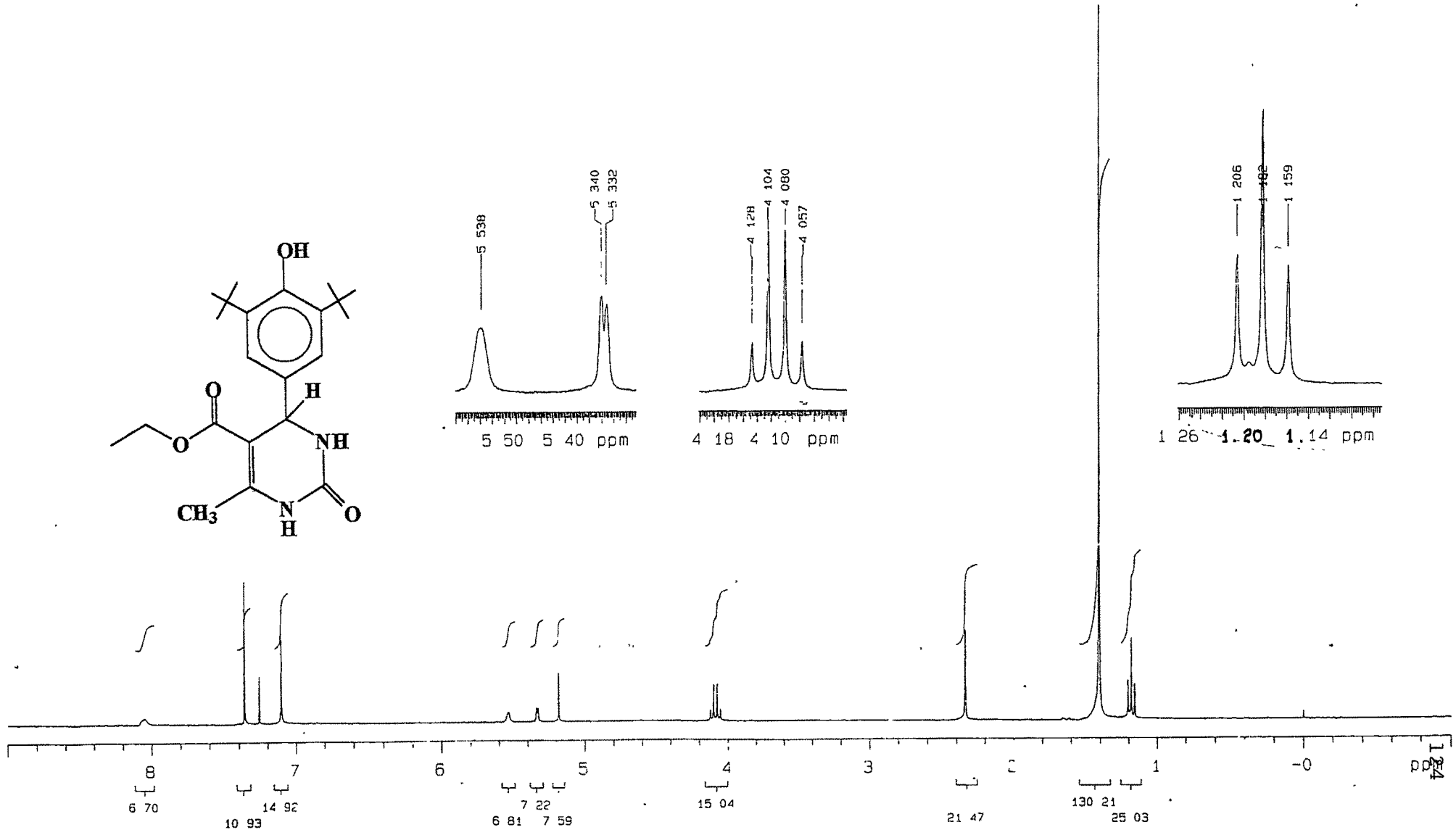

OH
O
H
O
NH
CH3
N
H
O
5 538
5 340
5 332
5 50 5 40 ppm
4 128
4 104
4 080
4 057
4 18 4 10 ppm
1 206
1 159
1 26 1.20 1.14 ppm
8
7
6
5
4
3
2
1
-0
6 70
10 93
14 92
6 81
7 59
7 22
15 04
21 47
130 21
25 03

Figure 19

3,5-Di-tert-butyl-4-hydroxybenzaldehyde 1 was synthesized from 2,6-di-tert-butyl–4-methyl phenol (BHT) according to the method reported by *Coppinger* and *Campbell*[70].

Synthesis of 1-(2',5'-dihydroxyphenyl)-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propenone 3b

3,5-Di-tert-butyl-4-hydroxybenzaldehyde (0.01mol) in ethanol (10ml) was added to the saturated solution of ethanolic HCl (100ml). To this, a solution of 2,5-dihydroxyacetophenone **2b** (0.01mol) in ethanol (10ml) was added in small portions. The solution was stirred for 24 hr at room temperature. The reaction mixture was poured in to crushed ice and the products obtained were filtered, dried and separated by column chromatography using benzene as eluent. Compound **3b** was crystallised from benzene-ethanol. m.p. 280^{0}C, yield 52%.

Elemental Analysis	Found :	C, 74.69	H, 8.07
$C_{23}H_{28}O_4$/368.24	Calcd :	C, 75.00	H, 7.60%

Product **I*** was crystallised from benzene-ethanol. m.p. 238 –240^{0}C, yield 8%.

Elemental analysis	Found:	C, 79.00;	H, 9.09 %.

Synthesis of 1-(7'-hydroxy benzopyran-2[H]one)-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propenone 3d.

To a saturated solution of ethanolic HCl (100ml), aldehyde **1** (0.01mol) was added followed by solution of ketone **2d** (0.01mol) in ethanol (10ml) in small portions. The mixture was stirred for 24 hr at room temperature. The reaction mixture was decomposed by pouring into ice cold water and the product obtained was filtered, dried and purified through column chromatography using benzene as eluent. Propenone **3d** was crystallised from benzene. m.p. 282^{0}C, yield 68%.

Elemental Analysis	Found:	C, 74.25	H, 6.43
$C_{26}H_{28}O_5$/420.50	Calcd.:	C,74.28	H, 6.66%

Synthesis of 1-(7'-hydroxy-2'-methyl benzopyran-4-[H]one]-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propenone 3e.

3,5-Di-tert-butyl-4-hydroxybenzaldehyde **1** (0.01mol) was added to a saturated solution of ethanolic HCl (100ml) followed by ketone **2e** in ethanol (10ml) in small portions. The solution was stirred for 24 hr at room temperature. Reaction mixture was decomposed by pouring it into ice cold water. Products obtained were filtered, dried and separated through column chromatography using benzene-petroleum ether (9:1) as eluent. Propenone **3e** was crystallised from benzene-ethanol. m.p. 260^{0}C, yield 46%.

Elemental Analysis	Found:	C, 74.53	H, 6.74
$C_{27}H_{30}O_5$\434.52	Calc.:	C, 74.65	H, 6.91%

Product **II*** was crystallised from benzene-ethanol. m.p. 238-240^{0}C, yield 7%.

Elemental analysis Found: C, 79.02; H, 9.05 %.

*Synthesis of compound III**

3,5-Di-tert-butyl-4-hydroxybenzaldehyde (0.01mol) was added to the saturated solution of ethanolic HCl (100ml). The mixture was stirred for 24 hr at room temperature and was decomposed by pouring in water. The solid separated was filtered, dried, purified through column chromatography using benzene as eluent and crystallised from benzene - ethanol. m.p. 239^{0}C, yield 10%.

Elemental analysis Found: C, 79.03; H, 9.01%

**Spectra of the products I, II and III are identical and the structure is yet to be established.*

Synthesis of 1-(2',4'-dichloro-5'-fluorophenyl)-3-(3,5-di-tert-butyl-4-hydroxyphenyl) propenone ***3g.***

2,4-Dichloro-5-fluoro acetophenone (0.01mol) was added to homogeneously stirred mixture of aldehyde **1** (0.01mol) in saturated ethanolic HCl (100ml) in portions. The stirring was continued for 24 hr. Product obtained after pouring it in water was dried and crystallised from petroleum ether. m.p. 146^{0}C, yield 76%

Elemental Analysis	Found:	C, 64.81	H, 5.47
$C_{23}H_{25}Cl_2FO$/423.12	Calc.:	C, 65.24	H, 5.91%

Melting point, yield, molecular formula, molecular weight and elemental analysis results of all the synthesised chalcones are listed in Table 1.

Synthesis of 2-pyrazoline ***4a-g.***

Propenone **3 a-g** (0.1mol) was dissolved in distilled ethanol and to this hydrazine hydrate 98-100% (0.1mol) was added followed by few drops of acetic acid. The mixture was refluxed for 5 hr and was kept overnight. The solid obtained was filtered, dried and recrystallised from appropriate solvents.

Melting point, yield, molecular formula, molecular weight and elemental analysis results of all the synthesised 2- pyrazolines are listed in Table 2.

Synthesis of 1-phenyl-2-pyrazoline ***5 a-g.***

Propenone **3 a-g** (0.1mol) was dissolved in ethanol (25ml). To this phenyl hydrazine (0.1mol) was added followed by few drops of acetic acid. The mixture was refluxed for 6 hr and kept overnight. The product obtained after pouring it into water was filtered, dried and recrystallised from appropriate solvents.

Melting point, yield, molecular formula, molecular weight and elemental analysis results of all the synthesised phenyl pyrazolines are listed in Table 3.

Synthesis of flavanone. ***6 a-c***

Chalcone **3 a-c** (0.01mol) was refluxed in ethanol in the presence of HCl (0.5ml) for about 6 hr. Excess of ethanol was removed by distillation. Product obtained was crystallised from appropriate solvents.

Melting point, yield, molecular formula, molecular weight and elemental analysis results of all the synthesised flavanone are listed in Table 4.

Synthesis of 4-(3',5'-di-tert-butyl-4'-hydroxyphenyl)-5-carbethoxy-6-methyl dihydropyrimidine-2-one. **7**

3,5-Di-tert-butyl-4-hydroxybenzaldehyde **1** (0.01mol) was dissolved in ethanol (50ml). To this, ethylacetoacetate (0.01mol) and urea (0.01mol) were added followed by conc. HCl (0.1ml). Reaction mixture was refluxed for about 6 hr and excess of ethanol was removed by distillation. Product obtained after pouring the reaction mixture in cold water was filtered, dried and recrystallised from ethanol. m.p. 170 ^{0}C, yield 80%.

Elemental Analysis	Found :	C, 68.61	H, 8.45	N, 7.10
$C_{22}H_{32}O_4N_2$/ 388	Calc.:	C, 68.04	H, 8.24	N, 7.21%

Code*	M.P. (^{0}C)	Yield%	Mol. formula (Mol.Wt.)	Calcd./Found (%) % C	%H
3a^{B+P}	194	80	$C_{23}H_{28}O_3$ (352.24)	78.40 78.68	7.95 8.29
3b^{A+B}	280	52	$C_{23}H_{28}O_4$ (368.24)	75.00 74.69	7.60 8.07
3c^{A+B}	210	54	$C_{23}H_{28}O_4$ (368.24)	75.00 74.96	7.60 7.98
3d^{B}	282	68	$C_{26}H_{28}O_5$ (420.50)	74.28 74.25	6.66 6.43
3e^{A+B}	260	46	$C_{27}H_{30}O_5$ (434.52)	74.65 74.53	6.91 6.74
3f^{A+B}	225	50	$C_{27}H_{30}O_5$ (434.52)	74.65 74.92	6.91 6.92
3g^{B+P}	146	76	$C_{23}H_{25}Cl_2FO_2$ (423.12)	65.24 64.81	5.91 5.47

* Solvent used for crystallisation
A= Alcohol, B= Benzene, P= Petroleum ether

Table 1

Code*	M.P (°C)	Yield%	Mol formula (Mol.wt)	Calcd./Found (%)		
				C	H	N
4a^{B+P}	185	67	$C_{23}H_{30}N_2O_2$	75.40	8.91	7.65
			(366.26)	75.10	7.92	7.35
4b^{A+B}	207	52	$C_{23}H_{30}N_2O_2$	72.25	7.65	7.32
			(382.26)	72.02	7.65	7.29
4c^{A+B}	170	59	$C_{23}H_{30}N_2O_2$	72.25	7.65	7.32
			(382.26)	72.46	7.90	7.37
4d^{A+B}	205	65	$C_{26}H_{30}N_2O_4$	71.88	6.91	6.45
			(434.52)	71.64	6.77	6.36
4e^{A+B}	240	47	$C_{27}H_{32}N_2O_4$	72.32	7.14	6.29
			(448.55)	72.67	7.29	6.35
4f^{A+B}	235	54	$C_{27}H_{32}N_2O_4$	72.32	7.14	6.29
			(448.55)	72.95	7.36	6.41
4g^{B+P}	132	70	$C_{23}H_{27}Cl_2FN_2O$	63.15	6.17	6.40
			(437.58)	62.97	5.92	6.32

* Solvent used for crystallisation
A= Alcohol, B= Benzene, P= Petroleum ether

Table 2

Code*	M.P (^{0}C)	Yield%	Mol. formula (Mol.Wt.)	Calcd./Found(%) C	H	N
5a^{A+B}	201	66	$C_{29}H_{34}N_2O_4$	78.73	7.69	6.33
			(442.59)	78.65	7.59	6.20
5b^{A}	>300	60	$C_{29}H_{34}N_2O_3$	75.98	7.42	6.11
			(458.67)	76.01	7.57	6.34
5c^{A+B}	230	62	$C_{29}H_{34}N_2O_3$	75.98	7.42	6.11
			(458.67)	75.65	7.24	6.00
5d^{A+B}	250	52	$C_{32}H_{34}N_2O_4$	75.29	6.66	5.49
			(510.62)	75.37	6.63	5.50
5e^{A}	>300	59	$C_{33}H_{36}N_2O_4$	75.57	6.87	5.34
			(524.65)	75.77	6.85	5.47
5f^{A}	>300	45	$C_{33}H_{36}N_2O_4$	75.57	6.87	5.34
			(524.65)	75.63	6.89	5.27
5g^{B+P}	162	68	$C_{29}H_{29}Cl_2FN_2O$	68.10	5.67	4.69
			(511.65)	67.73	5.45	4.83

* Solvent used for crystallisation
A= Alcohol, B= Benzene, P= Petroleum ether

Table 3

Code	M.P (^{0}C)	Yield(%)	Mol. Formula (Mol.Wt.)	Calcd./Found(%)	
				C	H
6a[B]	160	80	$C_{23}H_{28}O_3$ (352.47)	78.40 78.82	7.95 7.75
6b[A]	237	84	$C_{23}H_{28}O_4$ (368.24)	75.00 75.50	7.60 7.53
6c[A]	190	78	$C_{23}H_{28}O_4$ (368.24)	75.00 75.47	7.60 7.20

* Solvent used for crystallisation
A= Alcohol, B= Benzene, P= Petroleum ether

Table 4

Screening for Anti-bacterial activity

INTRODUCTION[74-77]

In 1884, Danish physician Gram discovered the stain known as Gram stains. Staining reaction has widest applications, which divided all the bacteria in to two categories, namely, Gram +ve and Gram –ve. The Gram +ve bacteria resist discoloration and remain stained as dark purple colour while gram –ve bacteria are decolourised.

The micro-organisms capable of producing disease in animal or human being are known as "pathogenic". Most of the micro-organisms present on the skin and mucous membrane are non-pathogenic.

Paul Ehrlich, the father of chemotherapy used the term chemotherapy to describe the cure of an infectious disease without injury to the host. These agents are known as chemotherpaeutic agents and are classified according to disease and the infections, such as anti-bacterial, anti-protozoal, anti-viral, anti-neoplastic, anti-tubercular and anti-fungal agents.

This part describes methods used for "invitro" assessment of anti-bacterial agents. Anti-bacterial substances and preparations are classified as disinfectants, antiseptics and chemotherapeutic agents. The term disinfectant is used to eliminate or destroy infection and should be capable of killing a wide range of bacteria. An antiseptic is used to control or eliminate bacterial infection. A chemotherapeutic agent is an anti-bacterial substance administrated systematically for the treatment of infection, may be either bacteriostatic or bactericidal in its action, its main function is to prevent the multiplication of infective organism.

Anti-bacterial agents

Anti-bacterial agents are types of chemotherapeutic agents used against the bacterial disease and divided in to two types according to their action on bacteria namely bacteriostatic and bactericidal agents. An agent is considered bacteriostatic when it inhibits further growth or multiplication of bacteria and classified bactericidal when it kills the bacteria. Anti-microbial agents are the chemotherapeutic substances that destroy or inhibit the growth of micro-organism in the living tissue. Antibiotics are substances produced by living organism and are sufficiently non-toxic to be used as anti-microbial agents.

Evaluation of anti-bacterial activity

Varieties of "invivo" screening methods have been used to evaluate the anti microbial activity. Testing in mice has become standard, the sensitivity of bacteria to anti-microbial agents is tested by the same method as in other form of microbiological assay.

In vitro testing is useful for anti-bacterial spectrum determination of a compound and comparing it with other agent. Several types of procedure are in use for assaying the potency of antibiotic preparation for therapeutic purpose. These methods have been modified and used for sensitivity test of unknown organisms.

Invitro testing

Bacteriostatic activity can be determined on solid or liquid media, each depends on assessing the extent of inhibition of growth. We have adopted the disk or cup plate method for sensitivity testing.

The Disk method

After the report of the "International Collaborative Study"[78] involved with investigating "the disk test", method recommended has been adopted in Sweden.

In U.S.A. "Food and Drug Administration" have adopted the modified *Kirby-Bauer*[79] technique as an official method. The main stimulus for standardization in U.K. has come from recommendation of use of the controlled single disk method.[80] In this method, nutrient agar of appropriate composition is heavily inoculated with desired organism all over the surface of the solidified agar or mixed with agar, while still fluid, before pouring the plate. If an antibiotic solution of known potency is being assayed, the organism used is stoke strain of known sensitivity to standard dosage of antibiotic. Measured strengths of the antibiotic solution are applied to the inoculated agar in disks of uniform thickness, or sterile filter paper, and are placed on the surface of the agar plate before incubating. The width of the zone indicates the sensitivity of the organism being tested through the presence or absence of a zone is of greater significance.

2,6-Di-tert-butyl –4-methyl phenol acts as potential anti-viral agent against AIDS virus.[81] It possesses anti-inflammatory, anti-arthritic and anti-allergic activities.[82] *Branen et al.* [83] reported that BHT showed complete inhibition against the bacteria staphylococcus aureus at 0.7μmol/ml.

The compounds described in chapter 3 have been screened for anti-bacterial activity against Gram +ve and Gram –ve bacteria using bore-well method and the results are tabulated in Table 5, Table 6 and in Table 7.

Procedure used for anti-bacterial activity screening

Anti-microbial activity of the products was tested by the bore-well method. Agar plates were surface inoculated uniformly with standard cultures of Gram negative (Gram –ve) bacteria (*Escherichia coli, Salmonella typhi, Proteus vulgari* and *Shigella dysenteriae*) and Gram positive (Gram+ve) bacteria (*Staphylococcus aureus*). BHT was used as reference in this investigation. The synthesised

compounds 0.7μmol/ml were dissolved in solvent (DMF). The bacteria were grown on Luria- Bertani agar medium in petridish and 1cm bore size wells were created in the agar. The test compounds were pipetted out in the wells and were allowed to diffuse in the bacterial area at 37°C for 24 hr. Positive activity was indicated by clearance zone surrounding the wells due to inhibition of growth by the compound. Solvent did not show any activity towards all the microbes tested.

Results and Discussions

To the best of our knowledge, for the first time the anti-microbial activity of BHT and oxygen heterocyclics bridged by chalcone, pyrazoline and 1-phenyl-2-pyrazoline is reported against the bacteria *S. dysenteriae, S.typhi, and P.vulgaris* responsible for the bacterial diarrhoea, typhoid and urinary tract infections respectively. Almost all the synthesised compounds exhibited significant activity against *Shigella dysenteriae* bacteria. Most of the compounds exhibited moderate to good activity against bacteria *E.coli* and *S.aureus*. Only one compound **3c** exhibited activity against the bacteria *Proteus vulgaris*.

From the Tables 5,6 and 7 it has been observed that compounds **3(b-f), 4(b-g)** and **5(b-g)** are found to be active against the bacteria *Shigella dysenteriae*. The zone of inhibition for BHT was found to be 2.0cm. Activity of the compounds **3c, 3d, 3f, 4b, 4g, 5b, 5d, 5g** is comparable with that of BHT.

Compounds **3(c,d,f), 4(b,c,d,g)** ,**5**(b,d,e,f,g) are found to be active against the bacteria *E.coli.* Out of these, the activity of compounds **3c, 3d, 3f, 4b, 4g, 5b, 5d, 5g** is higher than that of BHT.

Compounds **3(b,c,d,f), 4(b,c,d,e,f), 5(c,d,e)** are found to be active against the bacteria *S.aureus* , where as the parent molecule BHT is inactive against *S.aureus* bacteria.

In conclusion, BHT served as potential biologically active molecule. By incorporating nitrogen or oxygen heterocyclics at the position para to hydroxy group, the biological activity is enhanced in most of the cases. Although numereous phytochemicals already have been isolated as anti-microbial agents, their individual activity usually is not potent enough to be considered for practical use.[85]

In the current scenario of drug design especially for hypertension, aging, cancer and heart diseases the first choice is in the development of molecule having biological as well as antioxidant property.

Comp.	Inhibition zone in cm against Micro-organisms			
	E.coli	*S.aureus*	*S.dysenteriae*	*P.vulgaris*
3a	--	--	--	--
3b	--	1.3	1.4	--
3c	1.8	1.7	1.2	2.1
3d	1.8	1.7	2.0	--
3e	--	--	1.8	--
3f	1.7	2.0	1.8	--
3g	--	--	--	--

Table 5 : Anti-microbial activity of the chalcone

Comp.	Inhibition zone in cm against Micro-organisms			
	E.coli	*S.aureus*	*S.dysenteriae*	*P.vulgaris*
4a	--	--	--	--
4b	2.0	1.5	1.7	--
4c	1.2	1.4	1.2	--
4d	1.9	1.6	1.8	--
4e	--	1.5	1.9	--
4f	--	2.1	1.6	--
4g	1.6	--	1.8	--

Table 6 : Anti-microbial activity of 2-Pyrazoline

Comp.	Inhibition zone in cm against Micro-organisms			
	E.coli	*S.aureus*	*S.dysenteriae*	*P.vulgaris*
5a	--	--	--	--
5b	1.9	--	1.3	--
5c	--	1.2	1.9	--
5d	1.7	1.6	2.1	--
5e	1.3	1.5	1.2	--
5f	1.6	--	1.3	--
5g	1.7	--	2.0	--
BHT	1.3	--	2.0	--

Table 7 : Anti-microbial activity of 1-phenyl-2-Pyrazoline

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