CHAPTER 6

During the isolation procedure described in Chapter 5, a number of compounds had crystallised out of solution. After cleaning using various solvents these compounds were sent for spectroscopic analysis. Although some had not shown prior antibacterial activity, it was agreed to send them for analysis with the hope of discovering novel compounds.

6. STRUCTURE ELUCIDATION

The spectroscopic methods described in Chapter 4 (sections 4.9 and 4.10) were used to elucidate the structures of isolated compounds in this study. Seven compounds were isolated and identified as flavonoids, three of these being flavones and the remaining four flavonols.

The characterisation of flavonoids provides an ideal application for NMR spectra with the typical skeleton represented in Figure 6.1. The patterns of the aromatic protons reveal the nature of substitution of the various aromatic systems. The chemical shifts of the protons of rings A and B are independent of one another but are affected by ring C. Peaks arising from A occur upfield and are easily recognised, therefore examination of an unfamiliar spectrum usually starts with recognition of these peaks. Remaining peaks in the aromatic region reveal the pattern of oxygenation of ring B and confirm the nature of ring C. Comparison of the individual compounds often reveals the nature of the oxygen-borne groups [Batterham, 1963].

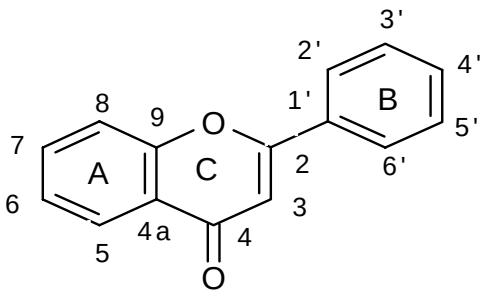
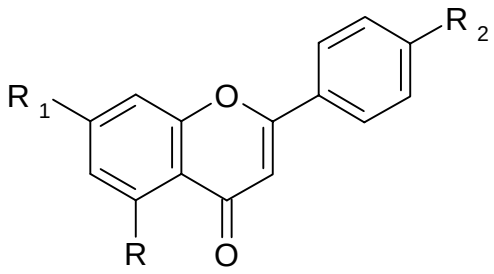


Figure 6.1: Skeleton structure of the flavonoids showing the numbering system

6.1 FLAVONES

Three flavones were isolated from *C. erythrophyllum* leaf extracts and identified as 5,7,4'-trihydroxyflavone (apigenin) (coded as CE144), 5,4'-dihydroxy-7-methoxy-flavone (genkwanin) (IIIa150) and 5-hydroxy-7,4'-dimethoxyflavone (CE36) (Fig. 6.2). These are reported in this family for the first time.



Compound	R	R_1	R_2
CE144	OH	OH	OH
IIIa150	OH	OCH_3	OH
CE36	OH	OCH_3	OCH_3

Figure 6.2: Flavones isolated from *C. erythrophyllum* leaf extracts

6.1.1 General characterization of flavones

Flavones are dehydrogenated in positions 2 and 3 (Fig. 6.2). Both the C-2 and C-3 resonances are olefinic, resonating between 157.4 – 165.8 and 102.3 – 113.7 ppm respectively. The presence of a 2,3-olefinic bond results in the quaternary behaviour of the C-2 resonance and methine behaviour of C-3 resonance. In unsubstituted flavones at C-2' and C-6' the chemical shift of the C-3 is usually between 102.0 and 108.6 ppm but when a hydroxyl or methoxyl group is attached to C-2' and/or C-6', a downfield shift occurs to between 110 and 115.5 ppm [Agrawal, 1989].

The 2,3-olefinic bond also leads to the upfield shift of the carbonyl group (C-4) that appears between 175.2-183.4 ppm, possibly due to conjugation. The presence of a hydroxyl at C-5 position causes a downfield shift of the C-4 resonance resulting in the appearance of C-4 resonance 182.5±0.7 ppm. This is as a result of intra-molecular hydrogen bond interactions between the ketone group (C-4) and C-5-attached hydroxyl. Another characteristic feature in 5-hydroxyflavones is the appearance of C-3 at 1.5±0.8 ppm upfield relative to the unsubstituted flavones at C-5 [Agrawal, 1989].

The carbon at 4a can also yield information about the substitution in ring A but is frequently obscured by larger peaks. With methoxyl substitution at C-5 and C-7, C-4a is shifted upfield from its usual position at 123 ppm by 10 and 6 ppm respectively. In simple benzene derivatives, carbons *ortho* to methoxyl are shielded by ca. 15 ppm and *para* carbons are shielded by ca. 9 ppm. Meta carbons are only slightly affected and therefore a change in the position of the methoxyl group around rings B and A will result in a sequential shielding of carbons which are in an *ortho* position to methoxyl. Methoxyl substitution in ring A at any given carbon will cause a large shift of the *ortho* carbon if the two carbons are joined by bonds of predominantly double-bond character, eg. C-5 and C-6. If, however, the bond is of single bond character, e.g. C-6 and C-7, methoxyl substitution seems to result in a small shift [Kingbury, 1974].

The splitting patterns for C-5 to C-8 are also informative as these carbons are split into either doublets or double doublets depending on whether or not the position *meta* to the carbon in question is substituted by methoxyl [Kingsbury, 1974].

The C-6 and C-8 resonances in flavones appear at between 92 - 100 ppm with C-6 downfield relative to C-8 resonance. This is important in establishing the site of C-alkylation at these sites [Agrawal, 1989].

The ^{1}H NMR spectrum shows that the presence of the double bond in ring C of flavones and flavonols causes a marked downfield shift of the 6,8 protons, producing a two-doublet pattern. In flavones, the presence of the C-ring double bond causes a shift of the 2',6'-protons and the spectrum shows the two complex multiplets, one centered at δ_H 8.0 (2',6') and the other at δ_H 7.6 (3',4',5').
With the introduction of a 4'-hydroxyl group the B ring protons appear effectively as a four-peak pattern. The hydroxyl group causes relative shielding on the adjacent 3',5'-protons and their peaks move substantially upfield. Introduction of the 2,3-double bond of flavones causes these protons to resonate at a much lower field (δ_H 8.0), while in flavonols the 3-hydroxy group causes a further slight downfield shift. In ring C the olefinic protons give rise to peaks in the general aromatic region. The singlets are easily identifiable and occur near δ = 6.8 [Batterham, 1963].

Table 6.1: ^{1}H NMR spectral data of isolated flavones from *C. erythrophyllum* leaf extracts

Position	CE144[a]	IIIa150[b]	CE36[b]
3	6.85 (s)	6.94 (s)	6.98 (s)
6	6.29 (d,2)	6.64 (d,2)	6.65 (d,2)
8	6.57 (d,2)	6.73 (d,2)	6.73 (d,2)
2'/6'	8.01 (d, 8.8)	7.97 (d, 8.8)	7.99 (m)
3'/5'	7.02 (d, 8.8)	7.28 (d, 8.8)	7.15 (m)

[a] Data obtained in DMSO-d_6 at 300MHz

[b] Data obtained in C_5D_5N at 400MHz

Coupling constant *J* in Hz is shown in parentheses

Table 6.2: ^{13}C NMR spectral data of isolated flavones from *C. erythrophyllum* leaf extracts

Position	CE144	IIIa150	CE36
2	164.5	164.4	-
3	103.2	103.4	104.8
4	182.1	182.3	-
4a	103.2	105.0	-
5	161.5	157.6	-
6	99.2	98.3	98.4
7	164.5	165.5	-
8	94.3	93.0	93.0
9	157.7	161.6	-
1'	121.5	121.4	-
2'/6'	128.8	128.9	128.4
3'/5'	116.3	116.3	114.9
4'	161.8	161.6	-

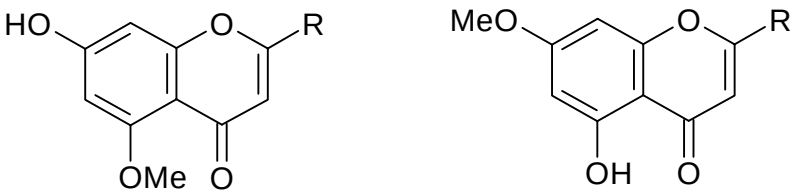
All data obtained in DMSO-d_6 except CE36 in $CDCl_3$

6.1.1.1 Characterisation of IIIa150

Compound IIIa150 was isolated as a yellow powder from column IIIa of the chloroform fraction. The Rf values were determined using three solvent systems and were calculated as follows: 0.34 (CEF), 0.70 (BEA) and 0.93 (2A:3MDC).

The ^{1}H NMR spectrum showed signals between 3.8 and 7.9 ppm indicating the presence of a methoxyl group δ_H 3.8, *meta*-coupling protons at δ_H 6.64 and δ_H 6.73 and aromatic protons at δ_H 6.94, 7.97 and 7.28. The *meta*-coupled protons (s, J = 2 Hz) are typical of the H-6 and H-8 in the A-ring of flavonoids. The singlet at δ_H 6.94 is the proton at H-3 while the signals at δ_H 7.97 and δ_H 7.28 (d, J = 8.8 Hz) are due to the *ortho*-coupling of protons 2'/6' and 3'/5' respectively [Spectrum 6.1]. ^{13}C NMR corresponded to the values obtained in Agrawal (1989) [Spectrum 6.2].

Two possible structures were suggested : 8-hydroxy-6-methoxyflavone (I) and 6-hydroxy-8-methoxyflavone (II). Agrawal [1989] suggests that C-4 resonates at a position that is dependant on the electron movements at C-5. Therefore in 5-hydroxylated flavones, C-4 is deshielded at δ_C 182.5±0.7, which correlates with ^{13}C NMR data (Table 6.2) and therefore Compound IIIa150 is likely to be II.



I II

Figure 6.3: Two possible substitution patterns in IIIa150

A Long Range COrrelation SpectroscopY (COSY-LR) experiment was also performed to provide evidence for the positioning of the methoxyl group on the A-ring of the flavone. Cross-peaks were visible between the methoxyl group and H-6 and H-8 confirming the positioning of the methoxyl group at C-7 [Spectrum 6.3].

Confirmation of structure was done by HREIMS (Spectrum 6.4), which showed the base peak and molecular ion M^+ to be at *m/z* 284 corresponding to $C_{16}H_{12}O_5$. Other prominent peaks were seen at *m/z* 255 $[M\text{-}CO]^+$ (26%), *m/z* 241 $[M\text{-}C_2H_3O]^+$ (10%), *m/z* 166 $[M\text{-}C_8H_6O]^+$ (8%) and *m/z* 118 $[M\text{-}C_8H_6O_4]^+$ (5.5%). The fragmentation is typical of the flavones and is illustrated in Fig. 6.4.

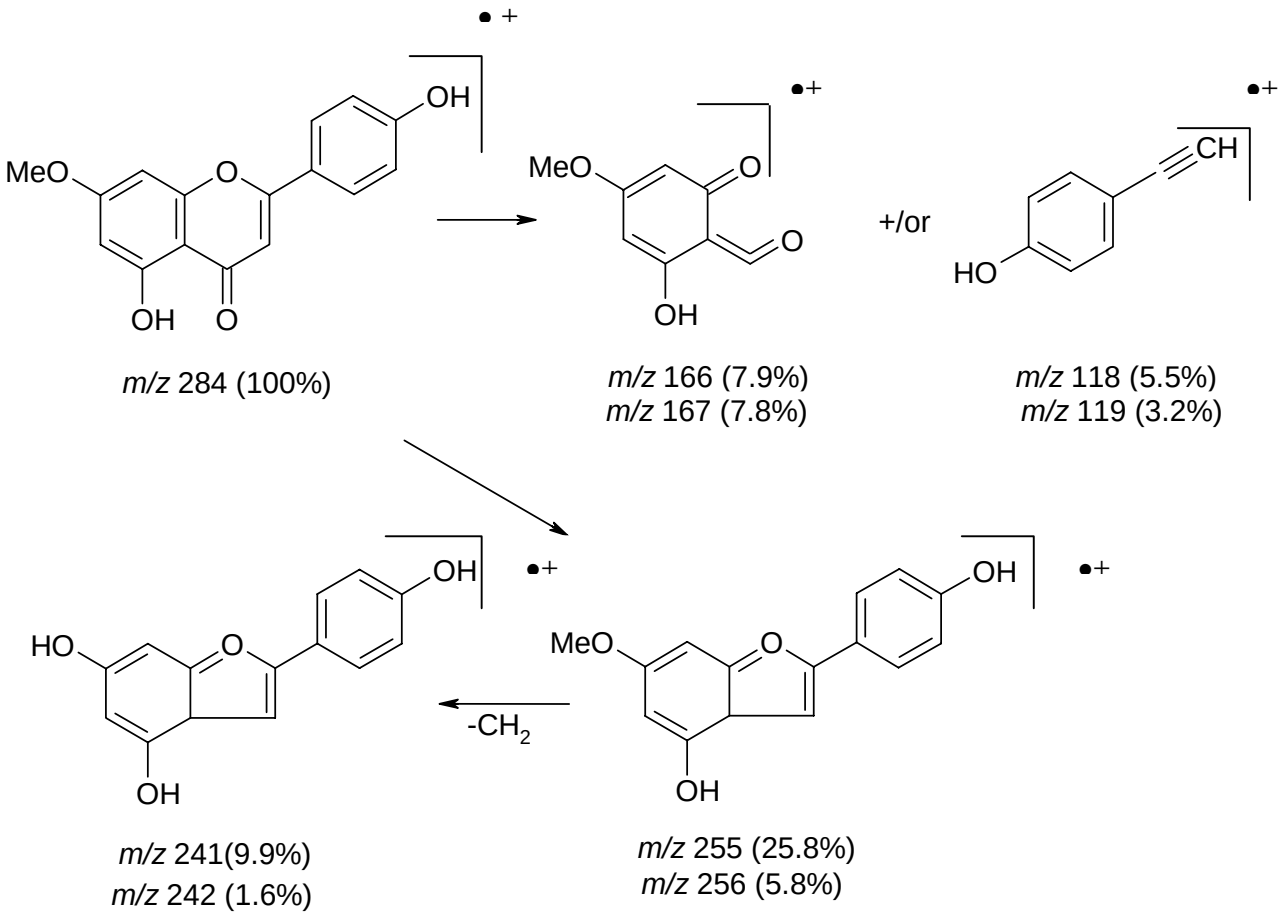


Figure 6.4: Suggested fragmentation pattern of IIIa150, typical of the flavones

IIIa150 was identified as genkwanin (5,4'-dihydroxy-7-methoxyflavone), which has previously been isolated from *Artemisia, Eupatorium, Alnus, Betula, Ostrya, Beyeria Rosmarinus, Salvia, Populus, Daphne, Larrea, Cheilanthes, Notholaena* species and *Pityrogramma tartarea* to name a few [Wollenweber, 1980]. This appears to be the first report of its isolation in Combretaceae.

6.1.1.2 Characterisation of CE144

CE144 was crystallised from fraction 144 and 145 of the first silica gel column using the chloroform fraction.

^{13}C NMR data revealed identical values for the C-atoms in the B and C-rings compared to that of IIIa150 [Spectrum 6.6]. Since hydroxylation in positions C-5 and C-7 corresponds to δ_C 99.2 and 94.4 of C-6 and C-8 respectively [Agrawal, 1989], it was concluded that this compound differed from IIIa150 only in the C-7-attached group. In comparison to hydroxy-substitution, methoxyl substitution at C-7 causes a downfield shift of the carbons in the *ortho*-position (C-6 and C-8) and an upfield shift of the C-4a carbon which lies in the *para*-position. ^{1}H NMR [Spectrum 6.5] was not compared to that of IIIa150 as different solvents were used but appeared similar to IIIa150 except for absence of a methoxyl group.

HREIMS gave the molecular ion M^+ at *m/z* 270, which corresponds to $C_{15}H_{10}O_5$ as the molecular formula. Other prominent fragments were *m/z* 242 $[M\text{-}CO]^+$, *m/z* 152 $[M\text{-}C_8H_6O]^+$ (13%), *m/z* 138 $[M\text{-}C_8H_4O_2]^+$ (4%) and *m/z* 119 $[M\text{-}C_7H_3O_4]^+$ (5%) [Spectrum 6.7]. The fragmentation pattern is typical of flavones as illustrated earlier (Figure 6.4).

By comparison of the ^{13}C NMR data with that of the literature, CE144 was identified as 5,7,4'-trihydroxyflavone, commonly known as apigenin [Agrawal, 1989].

Apigenin is a very commonly occuring flavone and has been isolated in many plant species including *Barleria christata, Chrysanthemium cinereriaefolium, Alnus* spp., *Betula* spp., *Garcinia multiflora, Coleus amboinicus,Salvia glutinosa, Acacia ixiophylla, Crudia amazonia, Prosopis* spp., *Elaegia utilis, Populus* spp., *Antirhinum* spp., *Larrea* spp., *Cheilanthes* spp. and *Notholaena* spp [Wollenweber, 1980]. In *Combretum* spp. it has been recently isolated in *C. apiculatum* [Katerere, unpublished 2001].

6.1.1.3 Characterisation of CE36

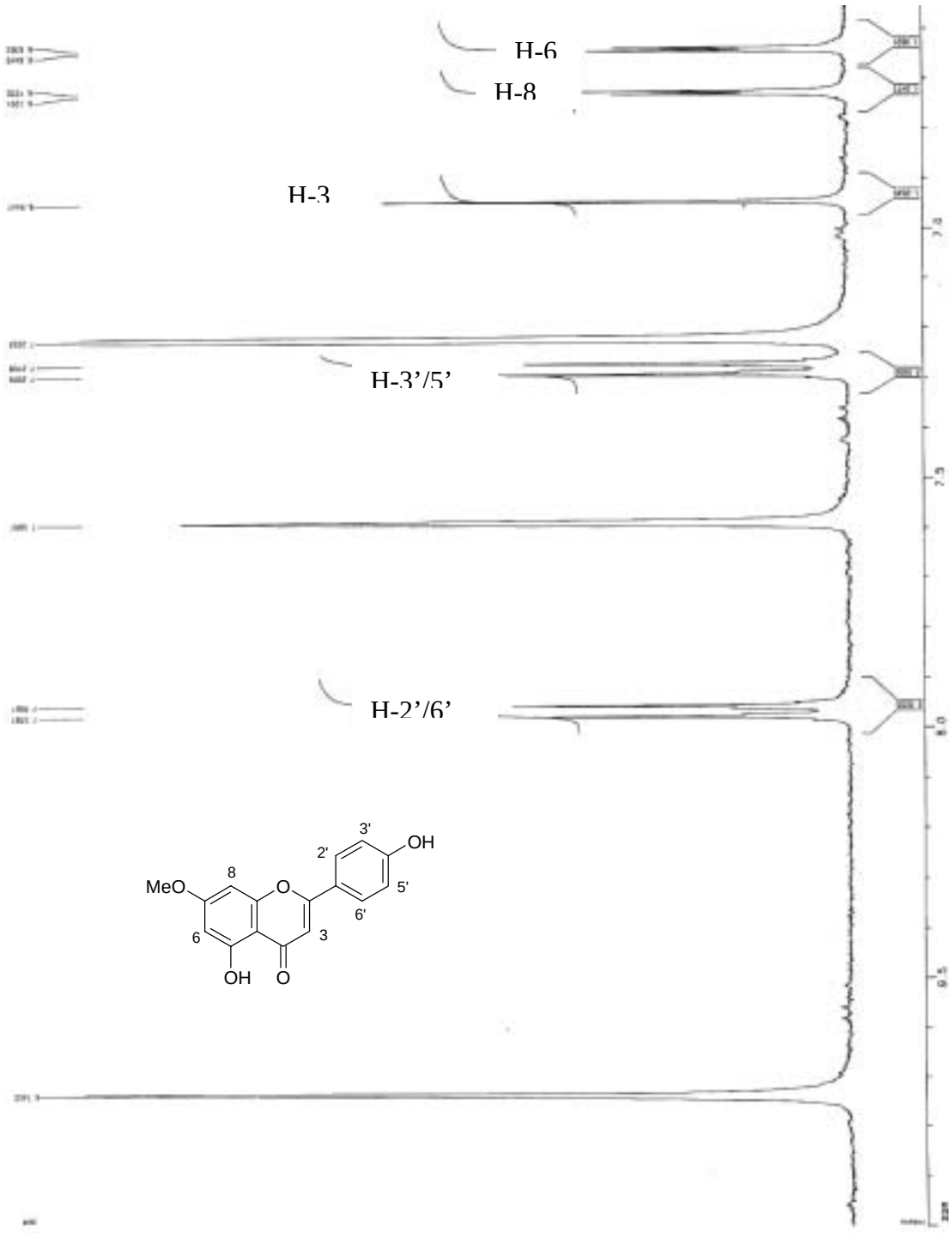
CE36 was one of the first compounds to come off column I of the chloroform fraction. It formed yellow needle-like crystals in solution and exhibited the following Rf values in the three solvent systems: 0.70 (CEF), 0.78 (BEA) and 0.95 (2A:3MDC).

^{1}H NMR revealed resonances at δ_H 3.78 (s, 3H) and δ_H 3.81 (s, 3H), representing 2 methoxyl groups. H-6 (δ_H 6.65) and H-8 (δ_H 6.73) were superimposable on spectra obtained for IIIa150, signifying a possible methoxyl-substitution at H-7 [Spectrum 6.8]. ^{13}C NMR verified the C-7 methoxyl substitution since C-6 and C-8 were identical to spectra obtained with IIIa150 (i.e. δ_C 98.3 and δ_C 93.0 respectively) [Spectrum 6.9]. These values also suggested the hydroxyl group to be at position C-5. Values obtained for C-3' and C-5' were further upfield (δ_C 114.9) than that obtained with a hydroxyl group (δ_C 116.3) and it was therefore suggested that the methoxyl groups were at positions C-7 and C-4'.

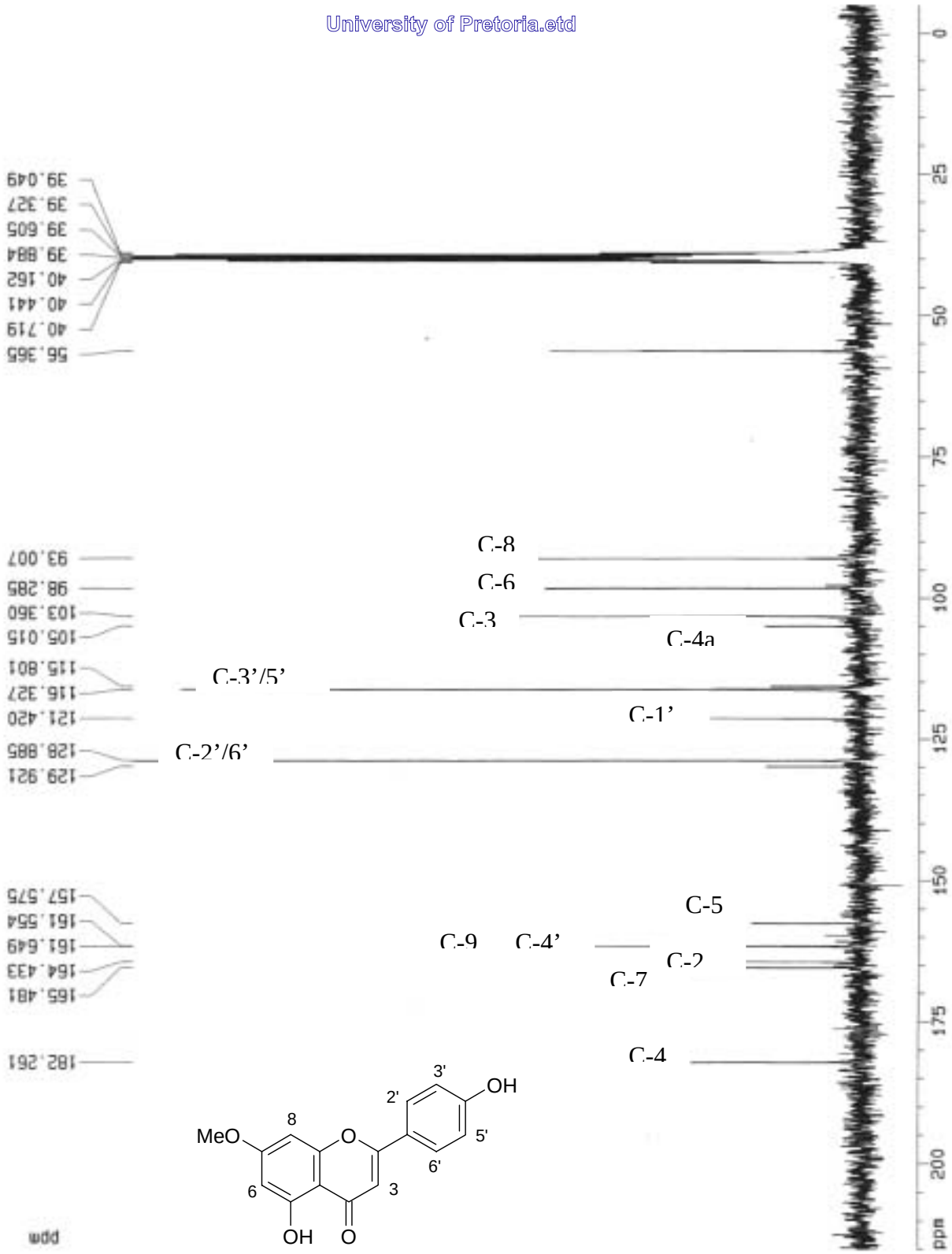
COSY-LR was performed to verify the structure [Spectrum 6.10]. Cross-peaks were visible between H-6 (δ_H 6.65) and H8 (δ_H 6.73) and the methoxyl group confirming the presence of the methoxyl group on C-7. Another cross-peak was also seen between the protons attached to C-3' and C-5' and the methoxyl group (C-5), which further confirms the positioning of the methoxyl group on the B-ring at position C-4'.

HREIMS data gave a molecular formula of $C_{17}H_{14}O_5$ (*m/z* 298) with other prominent peaks at *m/z* 297 $[M-H]^+$ (29%), *m/z* 281 $[M-OH]^+$ (23%), *m/z* 256 $[M-C_2H_2)\}^+$ (17%), *m/z* 219 $[M-C_2H_7)_3]^+$(33%), *m/z* 167 $[M-C_9H_7)]^+$ (10%) and *m/z* 193 $[M-C_7H_50]^+$ (34%) [Spectrum 6.11]. The fragmentation is typical of flavones (Figure 6.4). CE36 was identified as 5-hydroxy-7,4'-dimethoxyflavone.

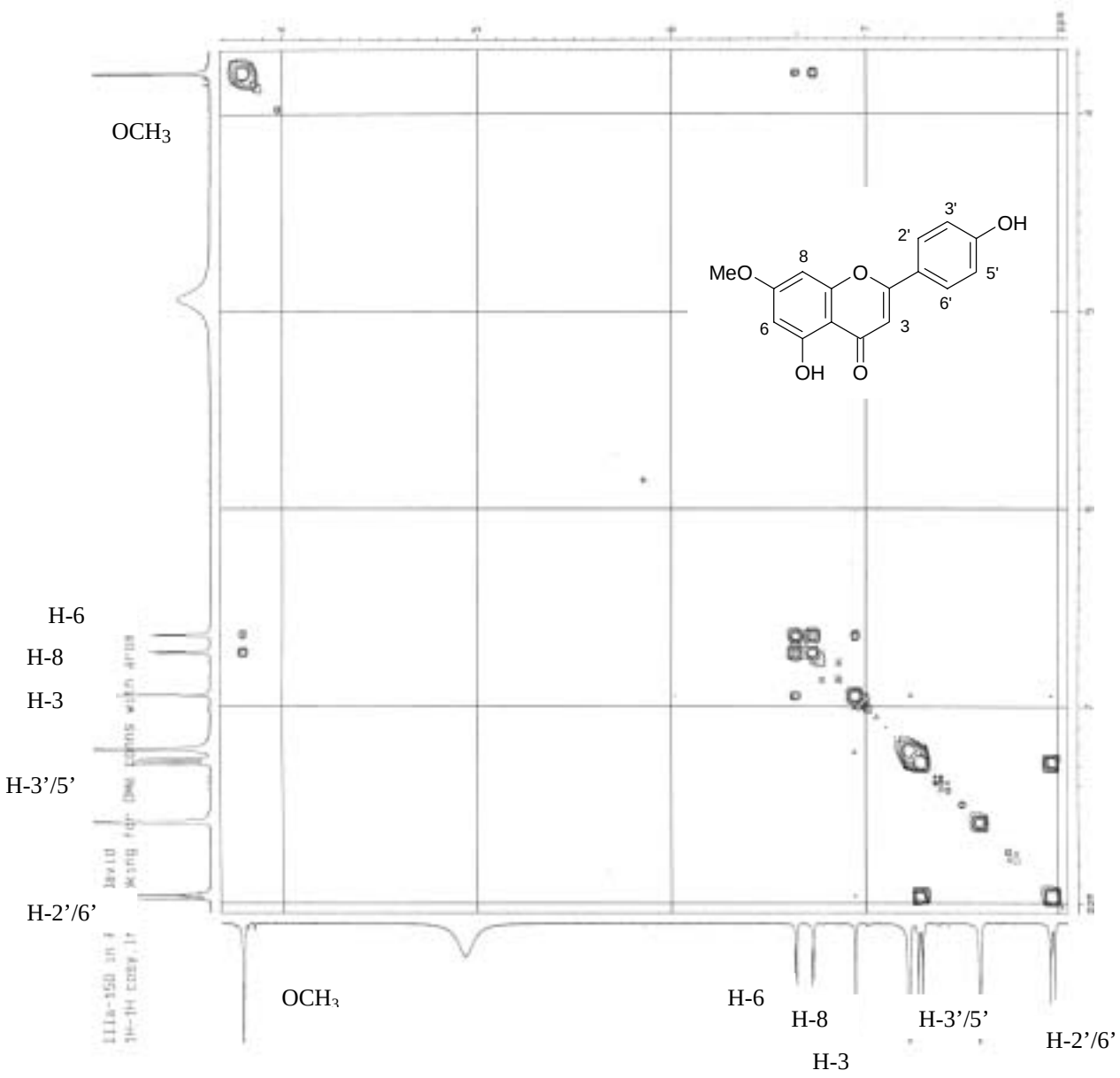
This compound has been found in *Biota orientalis* [Yang, 1995] and in the leaves of *Rosmarinus officinalis* [Brieskorn, 1967]. No known reports in Combretaceae have been documented. This is the first report of its isolation from *C. erythrophyllum* and family Combretaceae.



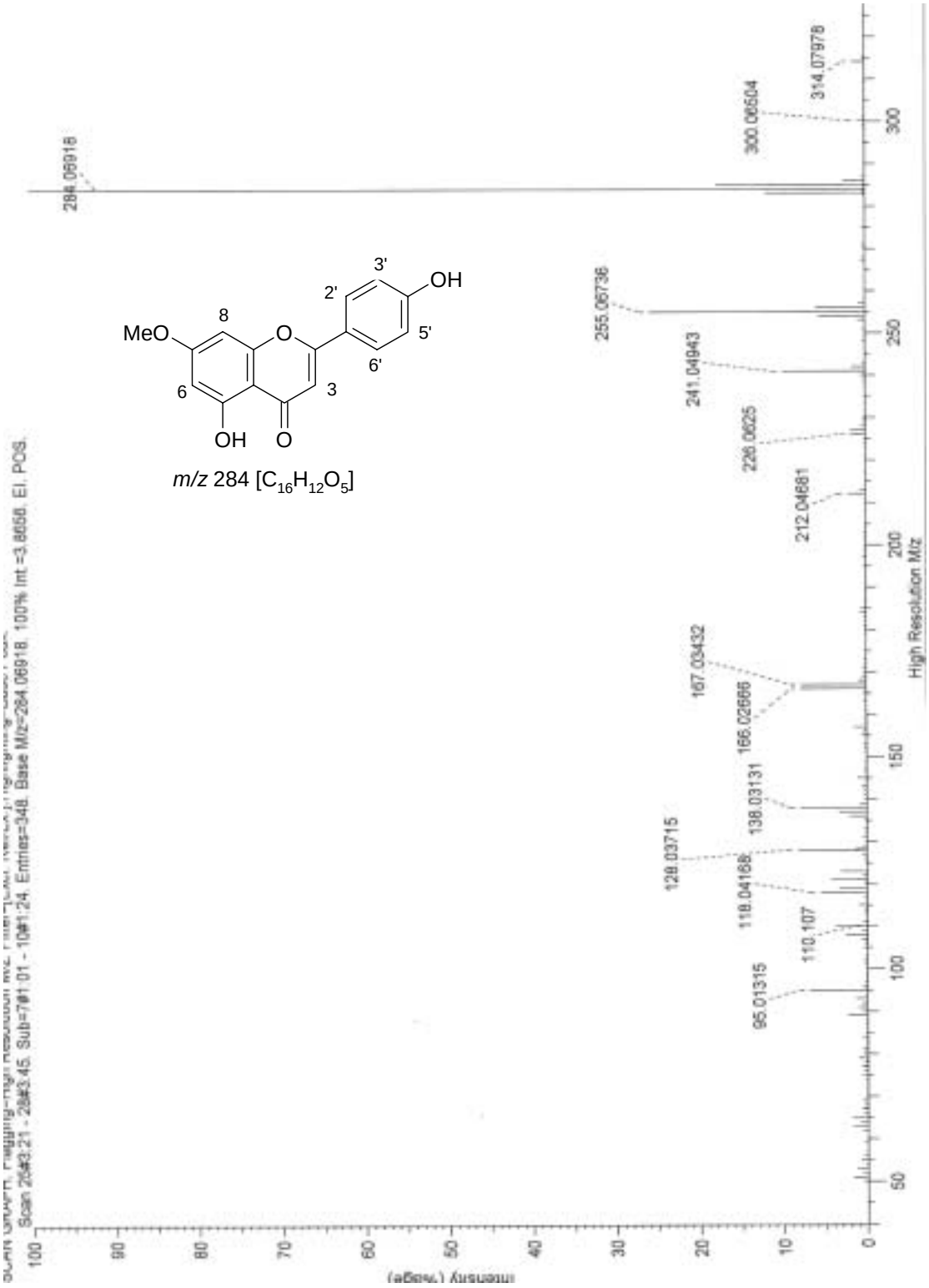
Spectrum 6.1: ^{1}H NMR (400 MHz, C_5D_5N) of IIIa150



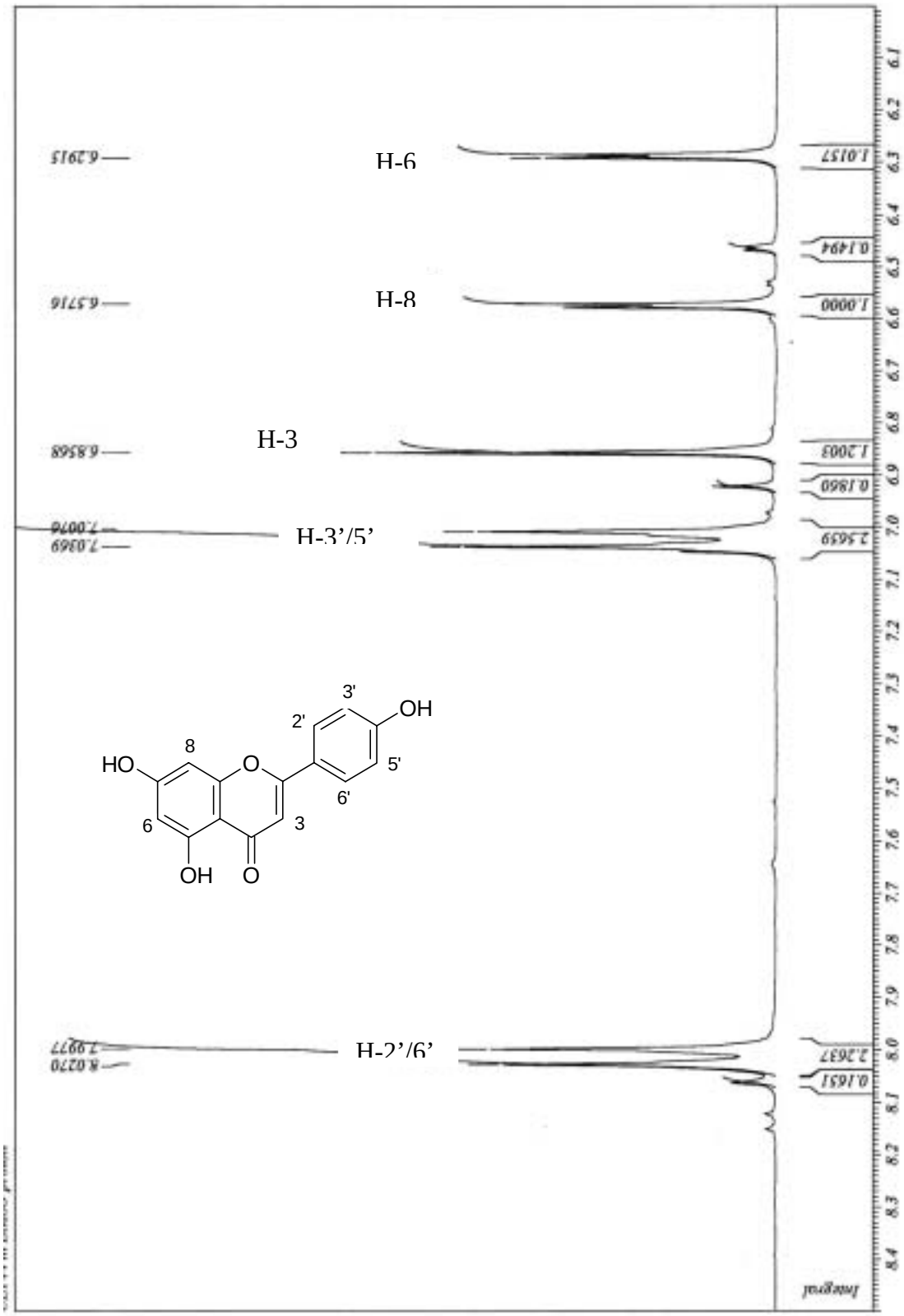
Spectrum 6.2: ^{13}C NMR (75 MHz, DMSO-d_6) of IIIa150



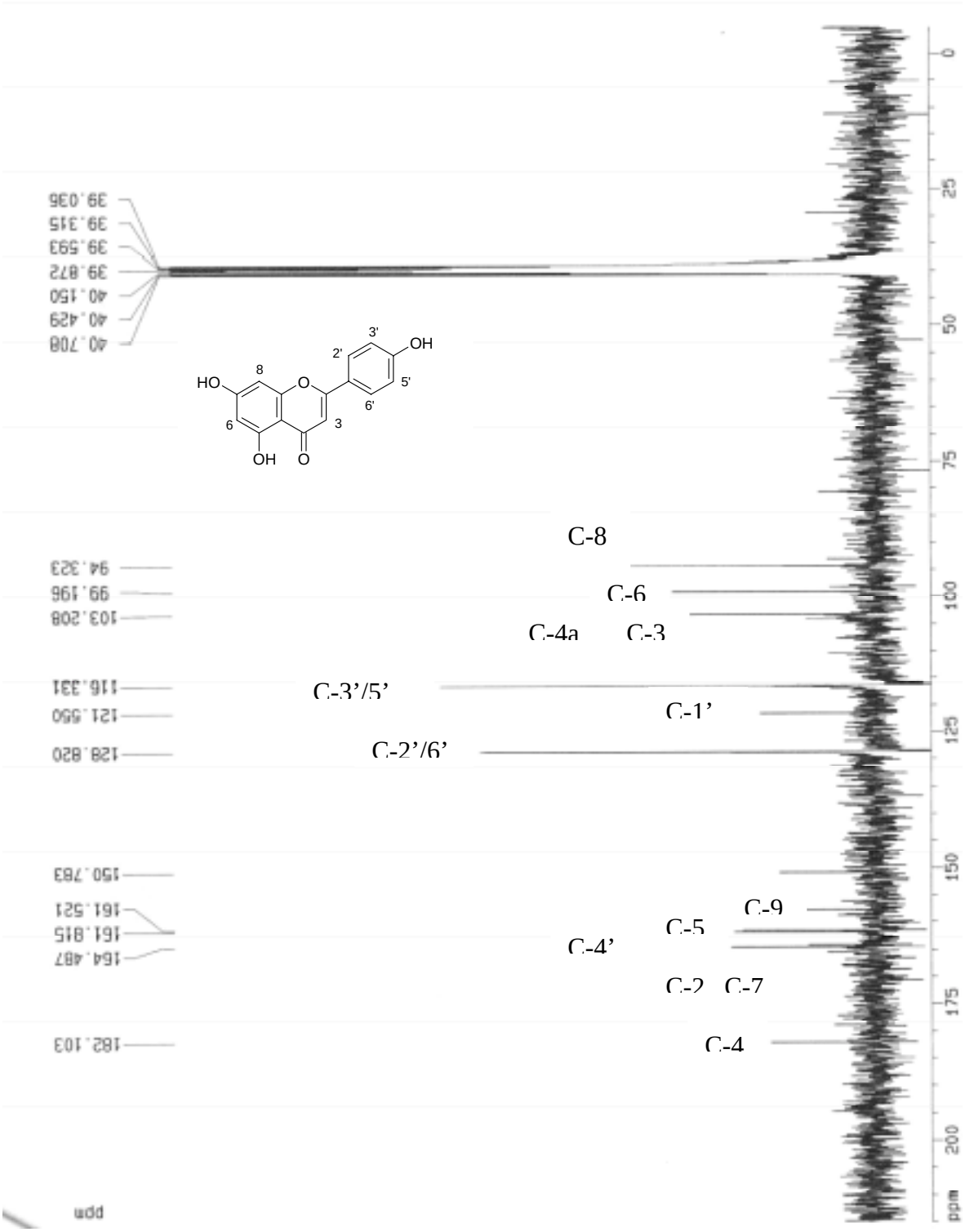
Spectrum 6.3: ^{1}H-^{1}H COSY-LR (400 MHz, C_5D_5N) of IIIa150



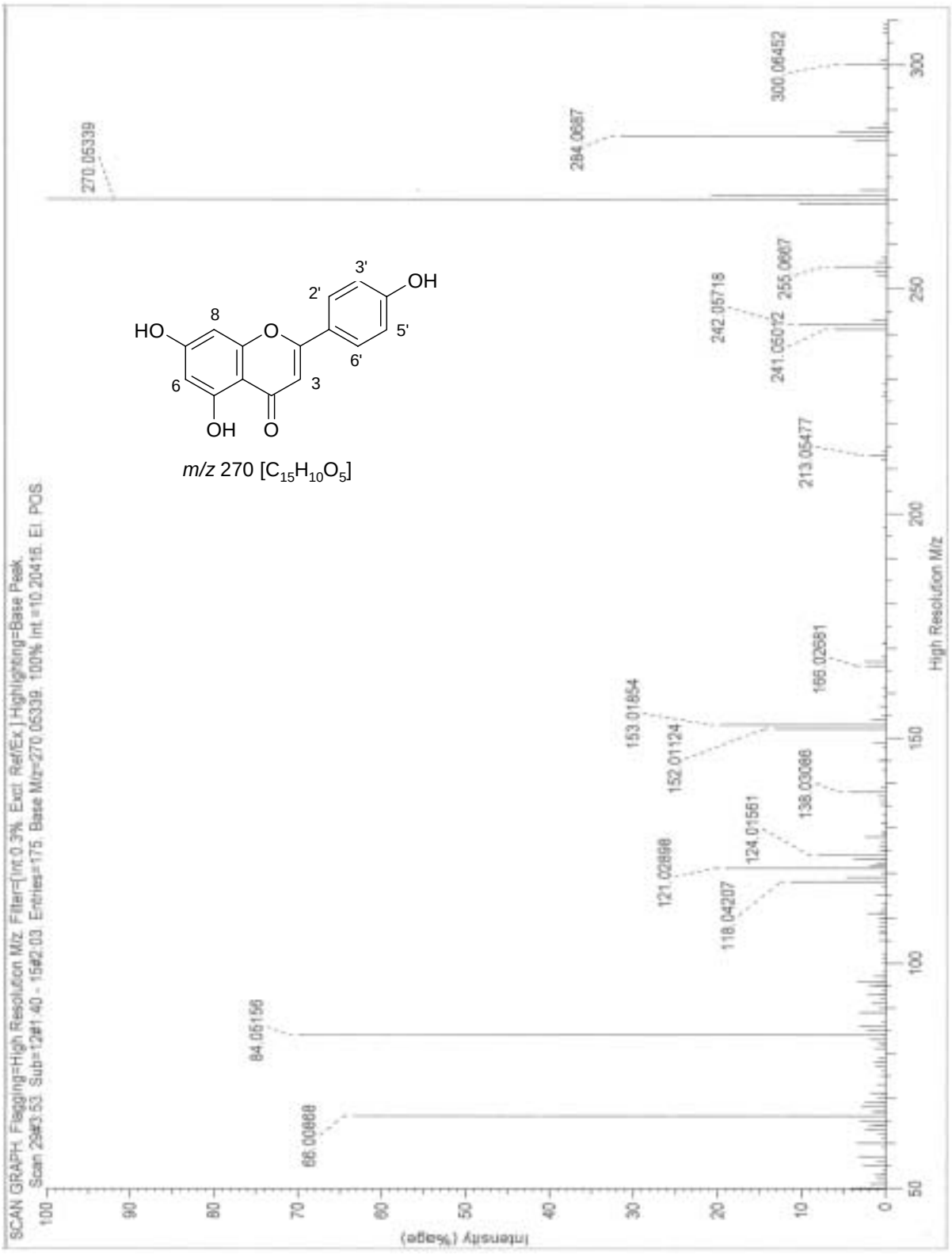
Spectrum 6.4: HREIMS of IIIa150



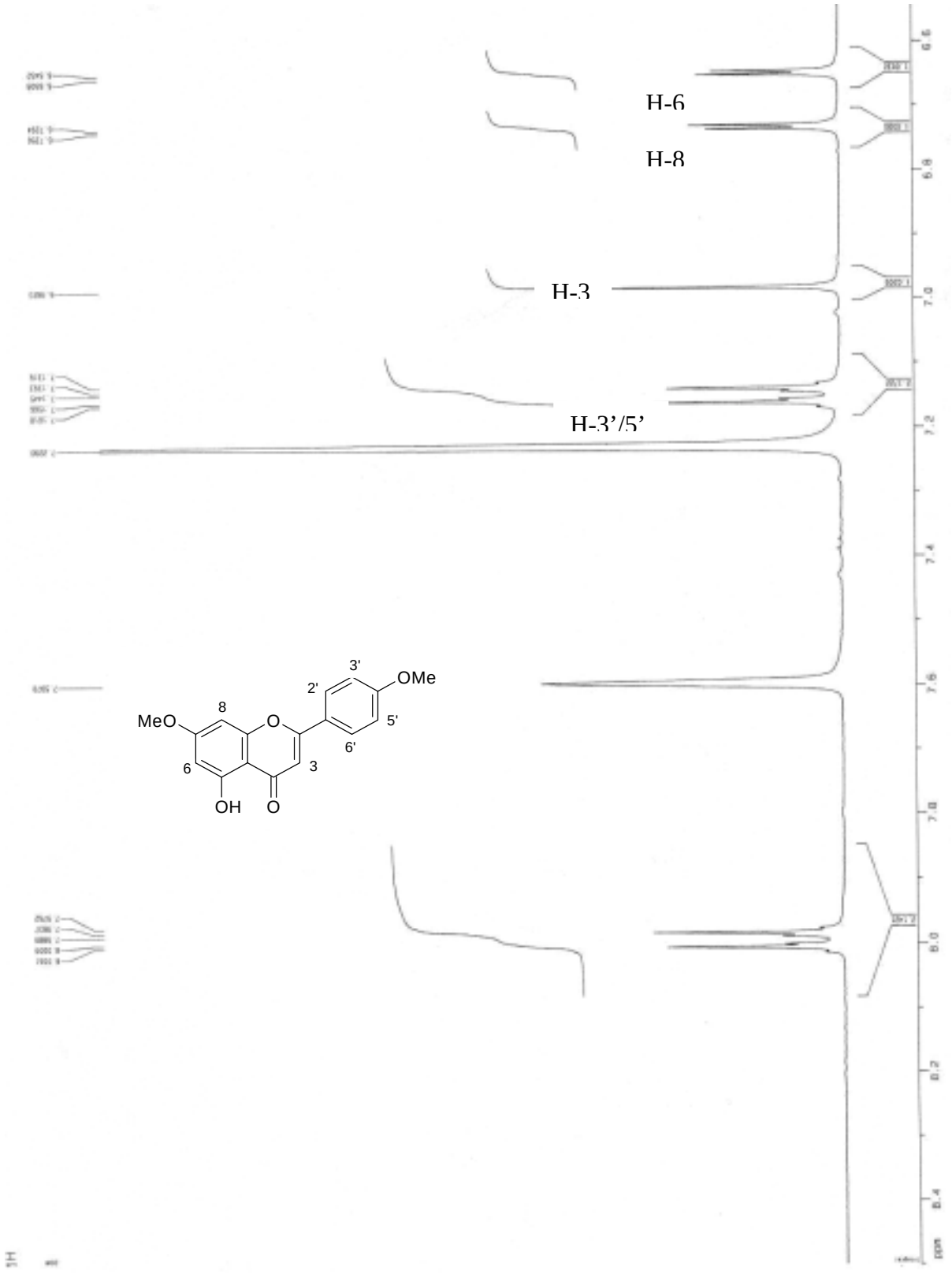
Spectrum 6.5: ^{1}H NMR (300 MHz, DMSO-d_6) of CE144



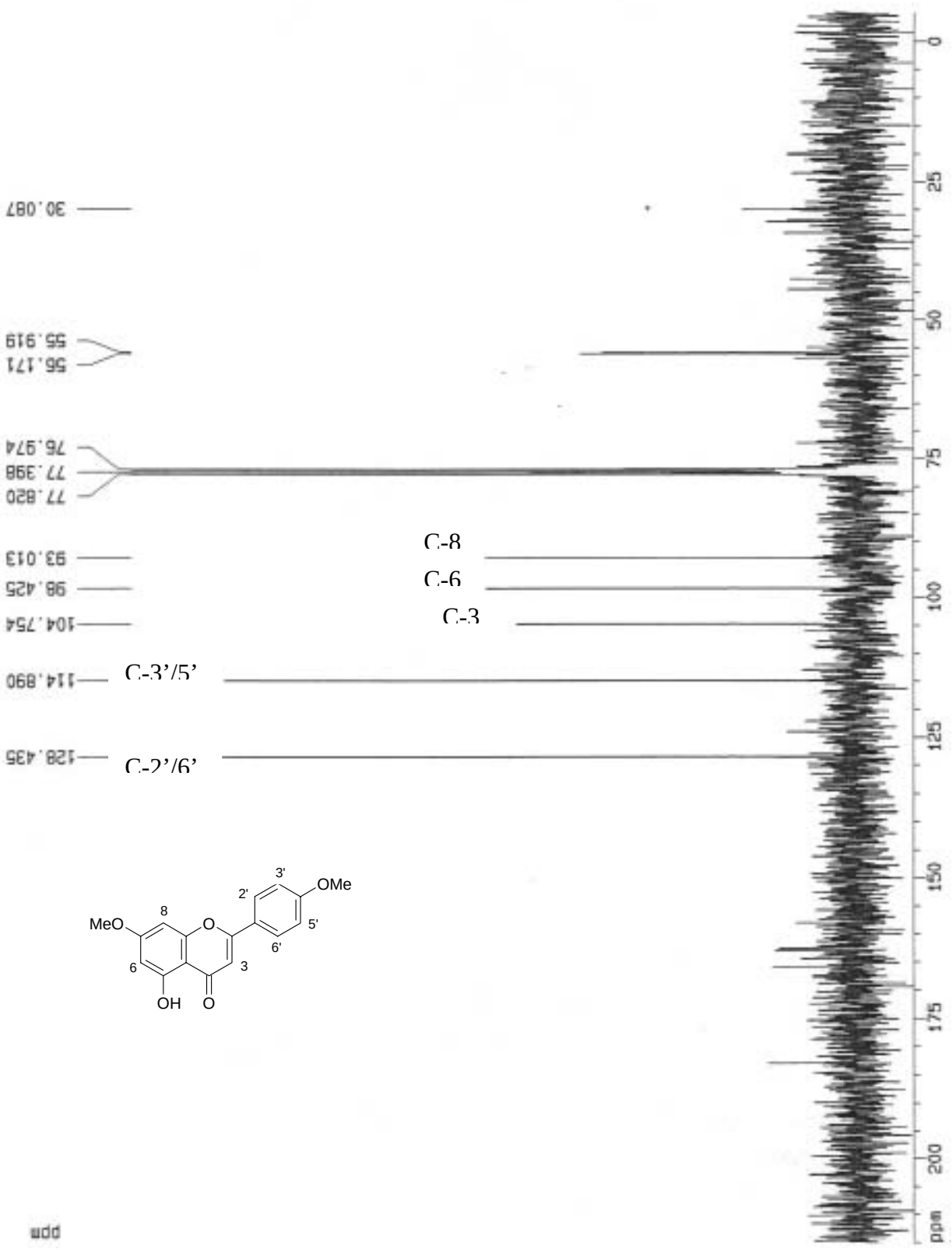
Spectrum 6.6: ^{13}C NMR (75 MHz, DMSO-d_6) of CE144



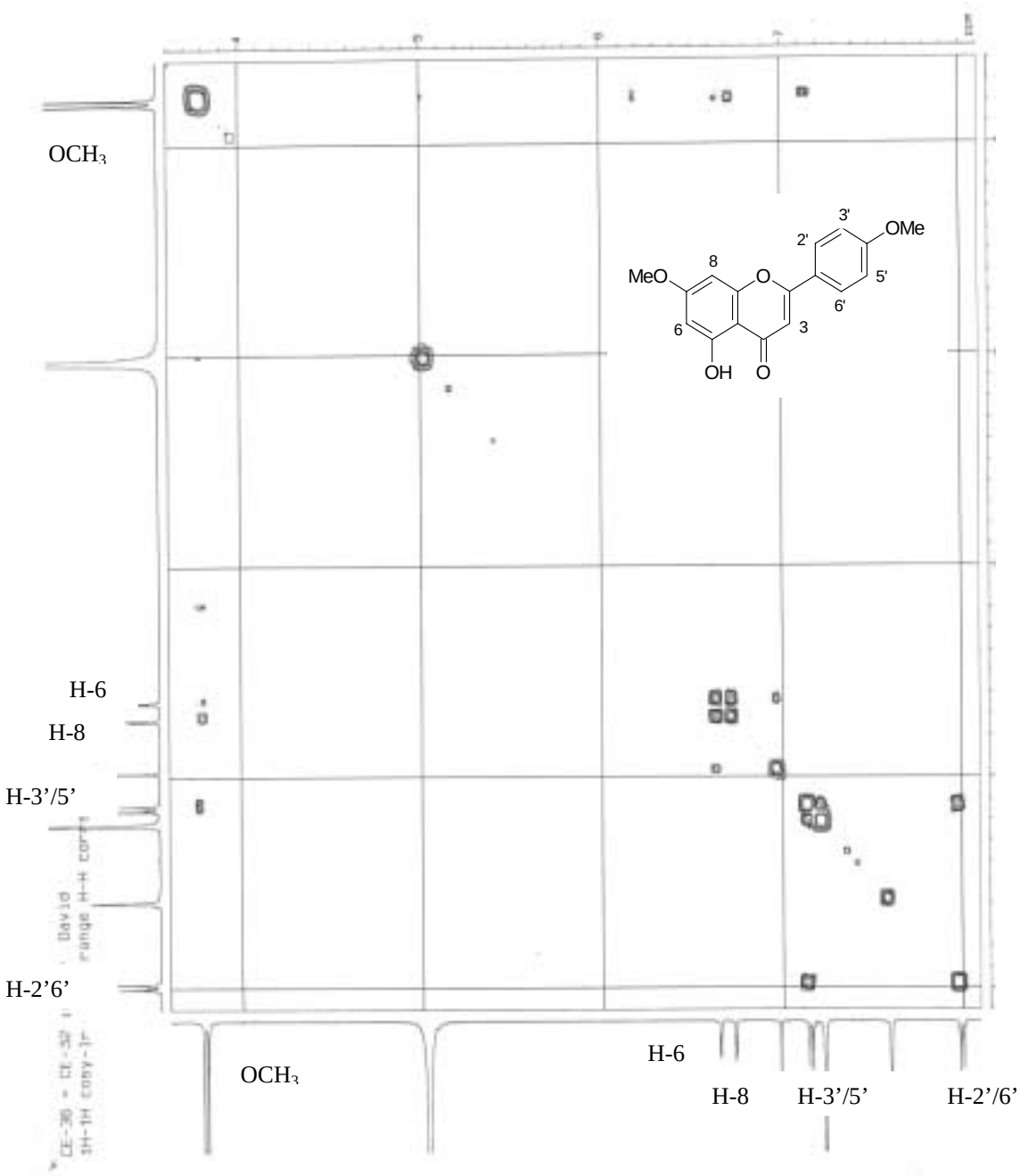
Spectrum 6.7: HREIMS of CE144



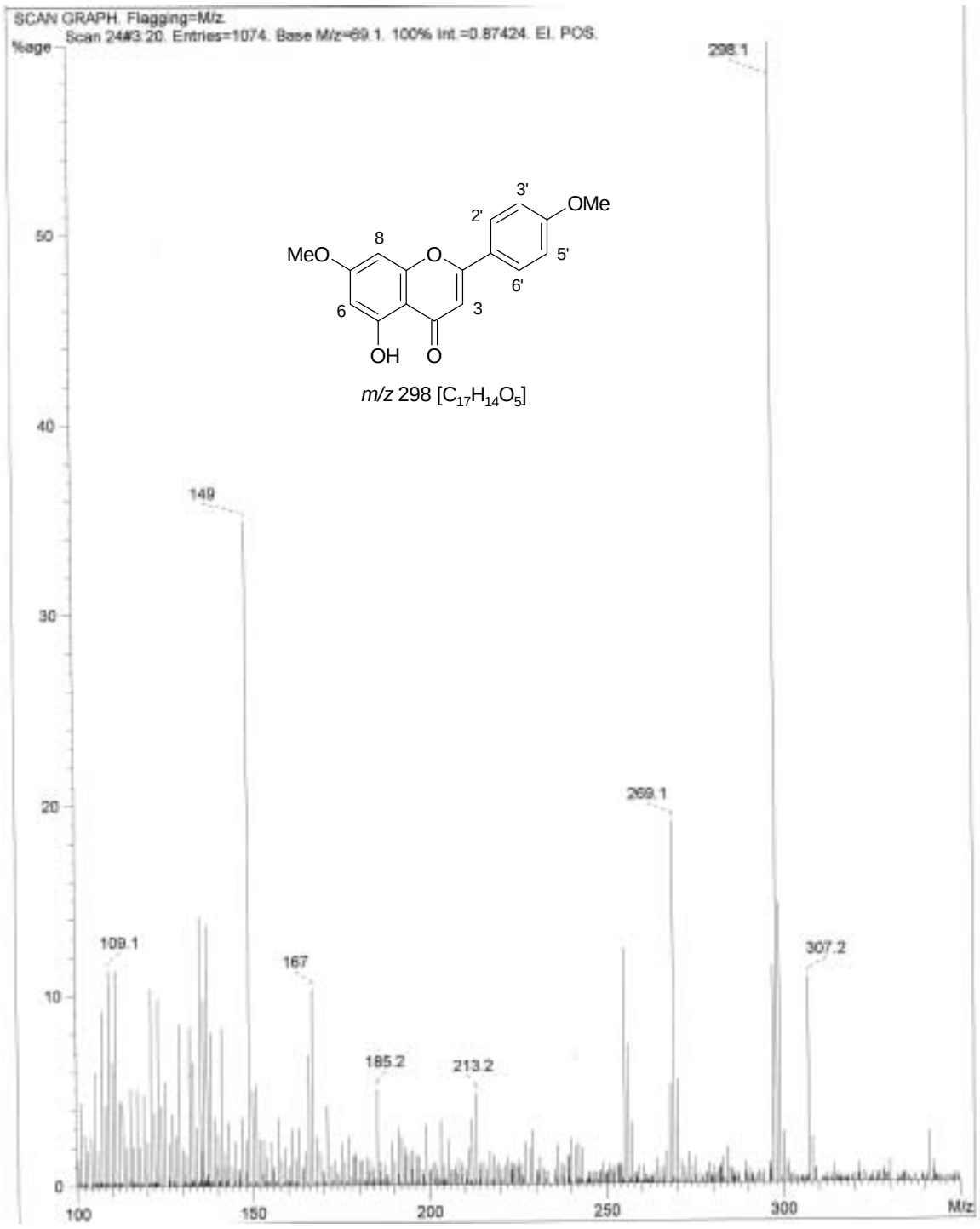
Spectrum 6.8: ^{1}H NMR (400 MHz, C_5D_5N) of CE36



Spectrum 6.9: ^{13}C NMR (75 MHz, $CDCl_3$) of CE36



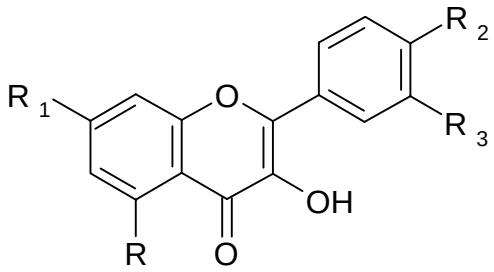
Spectrum 6.10: ^{1}H-^{1}H COSY LR (400MHz, C_5D_5N) of CE36



Spectrum 6.11: HREIMS of CE36

6.2 FLAVONOLS

Four flavonols were isolated from the *C. erythrophyllum* leaf extracts and identified as 5,6,4'-trihydroxyflavonol (kaempferol) (coded Seph 51), 5,4'-dihydroxy-7-methoxyflavonol (rhamnocitrin) (IIIa90), 5,4'-dihydroxy-7,5'-dimethoxyflavonol (rhamnazin) (CE51) and 7,4'-dihydroxy-5,5'-dimethoxyflavonol (quercetin-5,3'-dimethylether) (CE46). These are reported in Combretaceae for the first time.



Compound	R	R_1	R_2	R_3
IIIa90	OH	OCH_3	OH	H
Seph51	OH	OH	OH	H
CE46	OCH_3	OH	OH	OCH_3
CE51	OH	OCH_3	OH	OCH_3

Figure 6.5: Flavonols isolated from *C. erythrophyllum* leaf extracts

6.2.1 General characterisation of flavonols

Flavonols are 3-hydroxy derivatives of flavones. The chemical shifts of C-2 and C-3 are dramatically affected by the introduction of the 3-OH substituent on the C-ring of the flavone skeleton. The C-3 shifts downfield by ca. 32 ppm and C-2 and C-4 move upfield by ca. 17 and 5 ppm respectively. C-2 usually resonates at 5.0 – 13.0 ppm lower than C-3 resonance. In this case C-2 and C-3 are oxygenated quaternary carbons resonating around δ_C 133.5 and 151.2 respectively. Generally the resonance appearing at 140.0 – 151.2 ppm corresponds to C-2 and 133.5 – 140.0 ppm to C-3 [Agrawal, 1989].

In 5,7-dihydroxylated flavonols, C-6 resonates at about 5 ppm lower field relative to C-8 [Agrawal, 1989]. The presence of the double bond in ring C of the flavonols, as in the flavones, causes a marked shift of H-6 and H-8 in ^{1}H NMR, producing a two-doublet pattern. Introduction of a 3-hydroxy group causes a further downfield shift of the 2'/6'-protons and 3'/5'-protons than the flavones [Batterman, 1963].

Table 6.6: ^{1}H NMR (300MHz) and ^{13}C NMR (75MHz) spectral data of isolated flavonols

C	^{13}C	^{1}H	C	^{13}C	^{1}H
CE46[a]			**Seph 51[b]**		
6	98.8	6.63 (d,2)	6	99.6	6.31 (d,2)
8	93.2	6.80 (d,2)	8	94.9	6.57 (d,2)
2’		8.27(dd,2,8.4)	2’	130.7	8.19 (m,8.8)
3’	116.5	7.43 (d,8.4)	3’	116.8	7.06(m, 8.8)
5’	116.5	-	5’	116.8	7.06(m,8.8)
6’		8.33 (d,2)	6’	130.7	8.19(m,8.8)
CE51[a]			**IIIa90[a]**		
6	NOT	6.63 (d,2)	6	98.8	6.63 (d,2)
8	DONE	6.80 (d,2)	8	93.1	6.73 (d,2)
2’		8.27 (dd	2’	130.8	8.57 (m)
3’		7.43 (d,8.4)	3’	116.8	7.37 (m)
5’		-	5’	116.8	7.37 (m)
6’		8.32 (d,2)	6’	130.8	8.57 (m)

[a] Data obtained in C_5D_5N

[b] Data obtained in Acetone-d_6

Coupling constant *J* is shown in parentheses

6.2.1.1 Characterisation of CE51

Analysis of the ^{1}H-NMR spectra of CE51 showed the presence of two methoxyl groups δ_H 3.78 and δ_H 3.91[Spectrum 6.12]. The resonances of two *meta*-coupled doublets at δ_H 6.63 and δ_H 6.80 (1H, d, *J*=2 Hz), are typical of the 6- and 8-protons of the A-ring. The doublet at δ_H 7.43 1H, d, *J*=8.4) was attributed to H-3' which is *ortho*-coupled to H-2' at δ_H 8.27 (1H, dd, *J*=8.4). It is also further split by *meta*-coupling to the proton at δ_H 8.32.

To confirm the positioning of the methoxyl groups, a COSY-LR was done and this showed cross-peaks between H-6 and H-8 with a methoxyl group (δH 3.78), placing the methoxyl at position H-7. Another cross-peak was seen between H-6' and the methoxyl group (δH 3.9) placing it at position H-5'. From these results it was concluded that the methoxyl groups were situated at H-7 and H-5' and the hydroxyl groups at H-5 and H-4' [Spectrum 6.14].

This suggested structure was confirmed by HREIMS which exhibited a molecular peak at *m/z* 330 $[M]^+$ (100%) and fragmentation peaks at *m/z* 329 $[M-H]^+$ (20%), *m/z* 298 $[M-O_2]^+$ (11.6%), *m/z* 287 $[M-C_2H_30]^+$ (9%); *m/z* 167 $[M-C_9H_7O_3]^+$ (12.5%) and *m/z* 149 $[M-C_9H_9O_4]^+$(39.8%) [Spectrum 6.15]. This fragmentation pattern is closely related to that of flavones, illustrated in Figure 6.6.

CE51 was identified as 5,4'-dihydroxy-7,5'-dimethoxyflavonol, commonly called rhamnazin and is found in *Artemisia pygmaea, Alnus* spp., *Betula* spp., *Aesculus* 4 spp., *Polygonum hydropiper, Rhamnus* spp., *Populus* spp., *Larrea* spp., *Cheilanthes* spp and *Notholaena* spp. to name a few [Wollenweber, 1980]. It has not previously been reported in Combretaceae.

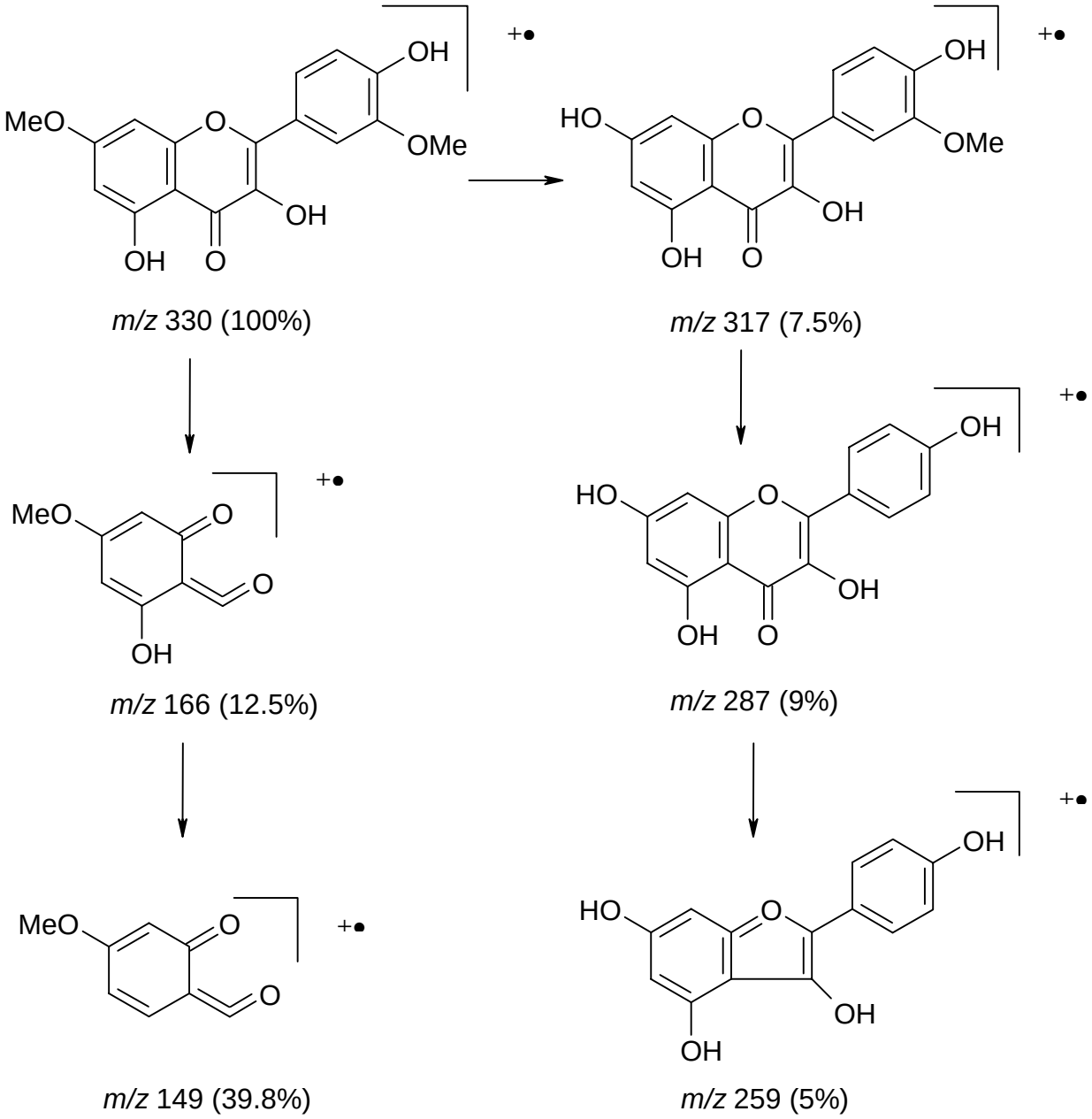


Figure 6.6: Suggested fragmentation pattern for CE51, which is typical of the flavonols

6.2.1.2 Characterisation of CE46

This compound was isolated as a yellow amorphous powder from Column I and exhibited Rf values of 0.38 with CEF, 0.74 with BEA and 0.93 with 2A:3MDC.
The ^{1}H-NMR spectra of CE46 matched that of CE51 showing the presence of two methoxyl groups δ_H 3.78 and δ_H 3.91 and the two *meta*-coupled protons at δ_H 6.63 and δ_H 6.80 (1H, d, *J*=2 Hz) characteristic of H-6 and H-8. The doublet at δ_H 7.43 (*J*=8.4) was attributed to H-3' while the double-doublets at δH 8.27 and doublet δH 8.32 were attributed to the *meta*-coupled H-2' and H-6' [Spectrum 6.8].

Initially it was thought that the A-ring methoxyl group was at C-5 and therefore a COSY-LR was done [Spectrum 6.14]. This showed cross-peaks between H-6 and H-8 with stronger absorption at H-8 placing the methoxyl at position H-7. Another cross-peak was seen between H-6' and the methoxyl group (δH 3.9) placing it at position H-5'. From these results it was concluded that the methoxyl groups were situated at H-7 and H-5' and the hydroxyl groups at H-5 and H-4' making this compound possibly identical to CE51. HMBQ data would be able to deliver more accurate results but was unfortunately not available in time for inclusion.

6.2.1.3 Characterisation of IIIa90

IIIa90 was isolated from column IIIa as a yellow powder and exhibited the following Rf values in the three solvent systems: 0.28 (CEF), 0.75 (BEA) and 0.94 (2A:3MDC).

^{1}H NMR spectra showed the presence of one methoxyl group at δ_H 3.81. Two protons with *meta*-coupling in ring A appeared at δ_H 6.63 and δ_H 6.73 (d, J = 2) and also two double doublets at δ_H 7.37 and δ_H 8.57 representing H-3'/5' and H-2'/6' respectively. ^{13}C NMR [Spectrum 6.17] of C-2'/6' and C-3'/5' (δ_C 130.8 and δ_C 116.8 respectively) indicated the presence of a hydroxyl group at C-4' [Spectrum 6.16].

Confirmation of structure was by COSY-LR [Spectrum 6.18] and HREIMS [Spectrum 6.19]. COSY-LR showed cross-peaks between H-6 and H-8 and the methoxyl group, confirming the position of the methoxyl at H-7.

HREIMS gave the molecular formula of $C_{16}H_{12}O_6$ *m/z* 300 $[M]^+$. Other fragmentation peaks were as follows *m/z* 299 $[M-H]^+$ (14.6%); *m/z* 284 $[M-OH]^+$ (16.6%); *m/z* 271 $[M-CHO]^+$ (9.2%); *m/z* 257 $[M-C_2H_30]^+$ (13.6%); *m/z* 167 $[M-C_9H_7O_3]^+$ (11.2%); *m/z* 149 $[M-C_9H_9O_4]^+$ (20%) and *m/z* 121 $[M-C_9H_7O_4]^+$ (37.7%).

This compound was identified as 5,4-dihydroxy-7-methoxyflavonol, commonly known as rhamnocitrin and has been found in *Alnus* spp., *Betula* spp., *Ostrya* spp., *Aesculus* spp., *Rhamnus* spp., *Populus* spp., *Alpinia japonica, Alpinia kumatake, Larrea* spp., *Cheilanthes* spp., *Notholeana* spp. and *Pityrogramma tartarea* [Wollenweber, 1980]. This appears to be the first report of its isolation in Combretaceae.

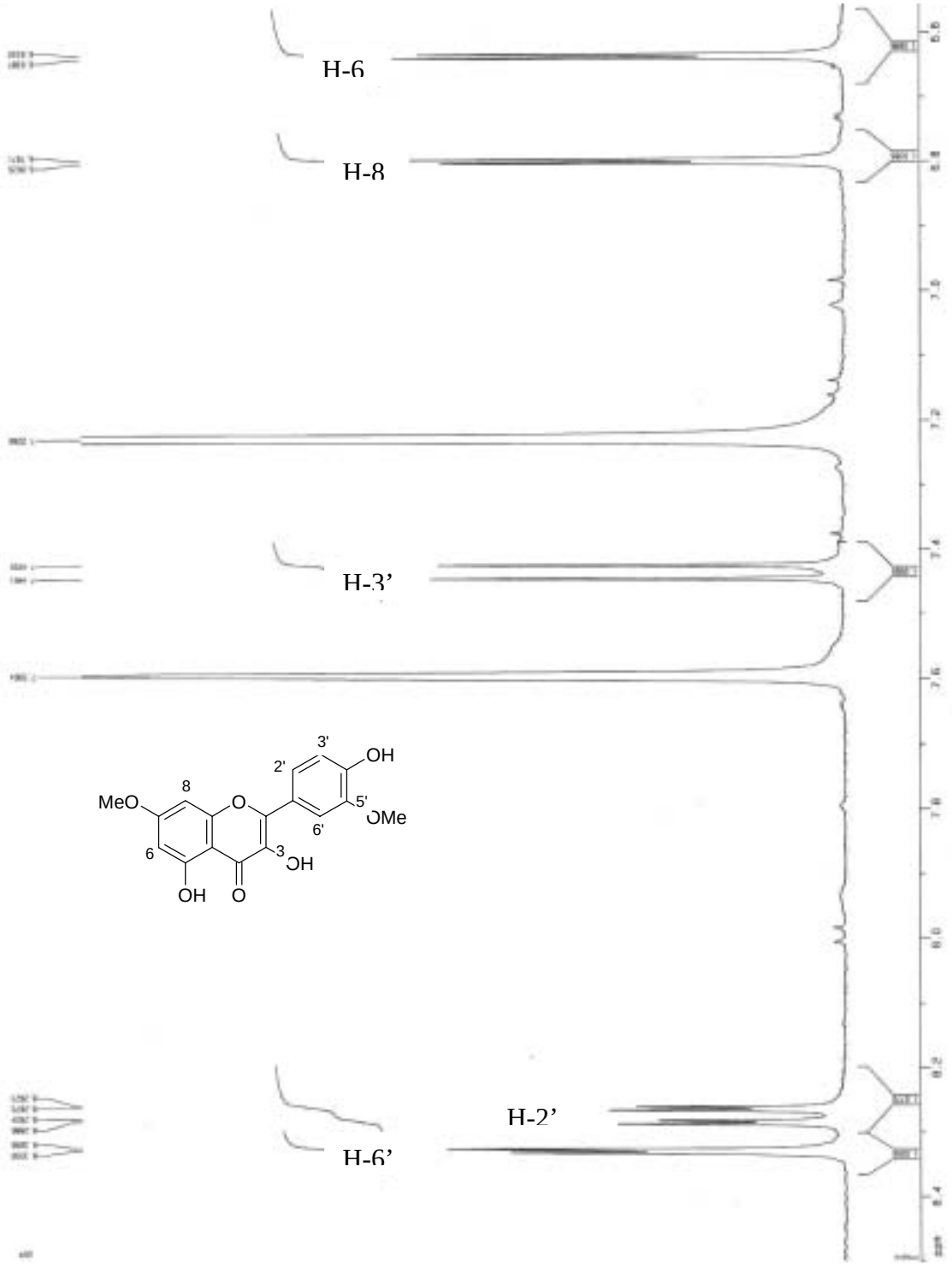
6.2.1.4 Characterisation of Seph 51

This compound was isolated from Column II and afforded a yellow powder. ^1H NMR [Spectrum 6.20] exhibited doublets at δ_H 6.31 (1H, *J* = 2) and δ_H 6.57 (1H, *J* =2), which corresponded to *meta*-coupled protons H-6 and H-8 respectively. These protons are attached to C-6 (δ_C 99.6) and C-8 (δ_C 94.9) carbons with ^{13}C NMR [Spectrum 6.21].

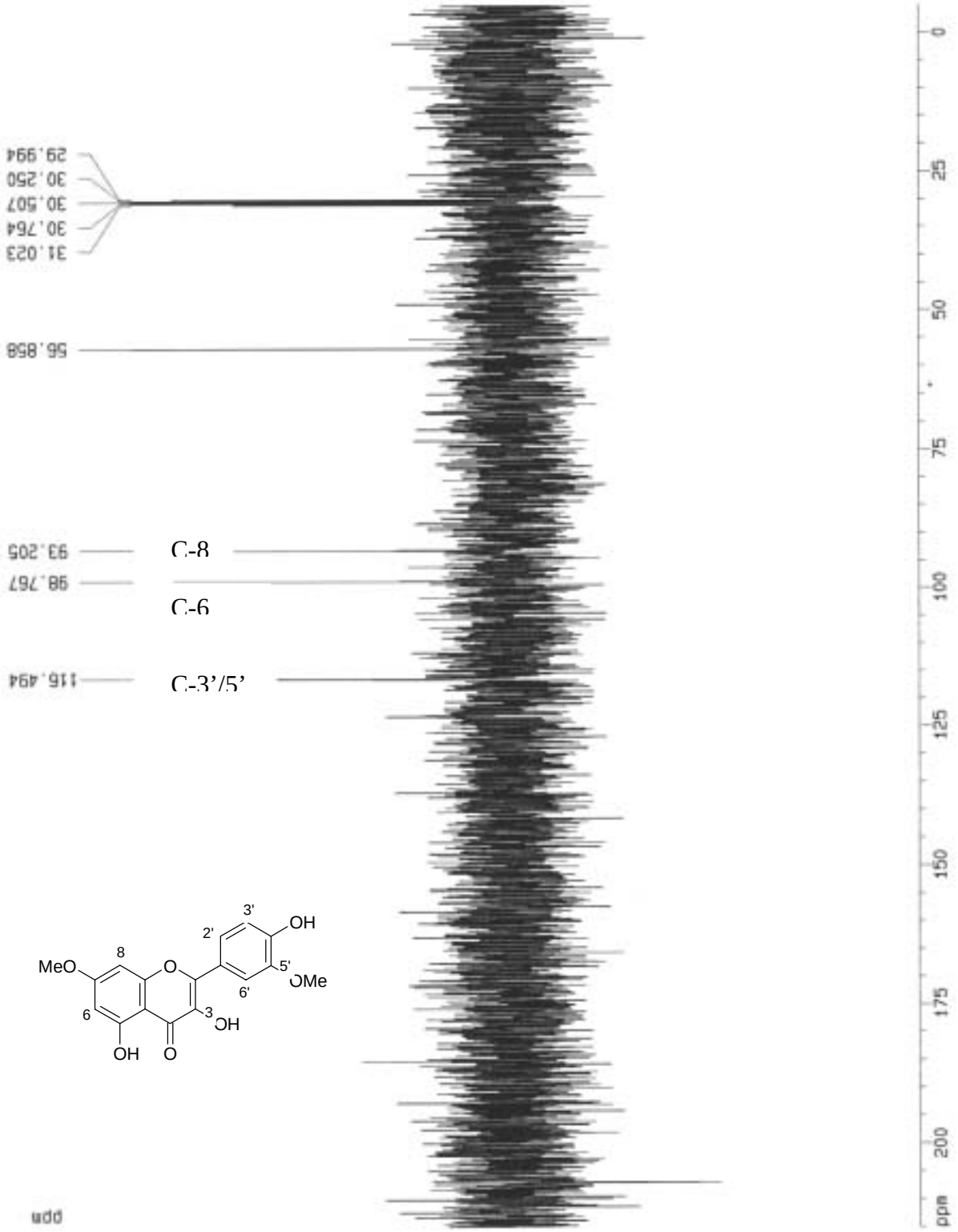
^{13}C NMR of the C-ring carbons was superimposable on the IIIa90 carbon-13 spectrum with resonances at δ_C 130.7 (C-2'/6') and δ_C 116.8 (C-3'/5'), suggesting therefore that a hydroxyl group was present on C-4'. C-6 and C-8 resonated at δ_C 99.6 and δ_C 94.9 respectively suggesting a hydroxyl group on position C-7 [Spectrum 6.17].

This structure was confirmed by HREIMS, which gave a molecular ion peak at *m/z* 286 and corresponds to $C_{15}H_{10}O_6$. Other fragments were seen at *m/z* 258 $[M\text{-}CO]^+$ (10%), *m/z* 153 $[M\text{-}C_8H_6O_2]^+$ (6.4%), *m/z* 134 $[M\text{-}C_7H_4O_4]^+$ (3%), *m/z* 229 $[M\text{-}C_2O_2H]$+ (7.8%) [Spectrum 6.22].

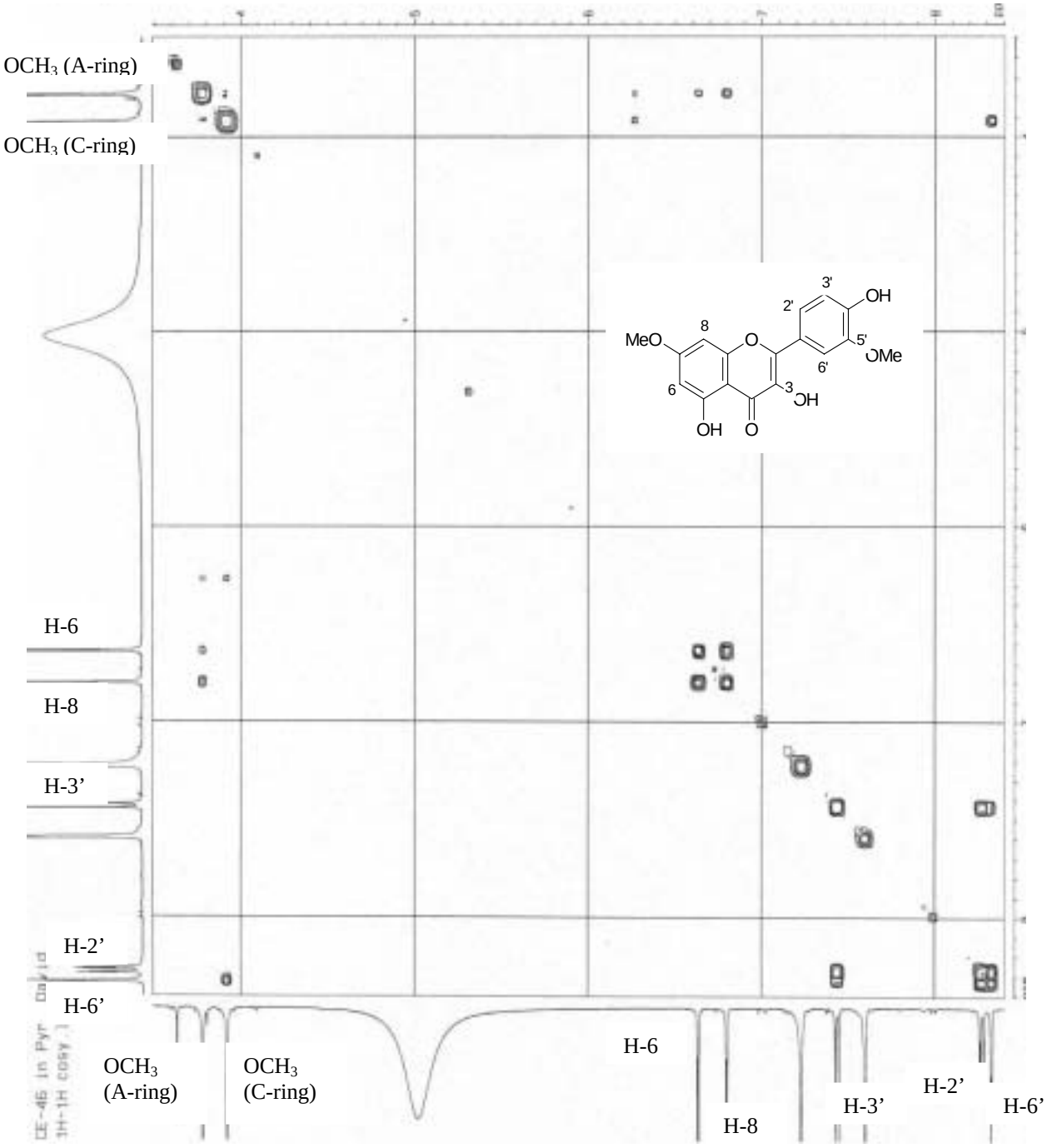
This compound was characterised as 5,6,4'-trihydroxyflavonol. It is also known as kaempferol. This is a very common compound and is found in many species including *Betula* spp., *Alluaudia ascendens, Acrotema uniflora, Aesculus* spp., *Prunus* spp., *Populus* spp., *Pterospermum acerifolium, Alpinia officinarum, Larrea* spp., *Cheilanthes* spp., *Notholaena* spp. and *Pityrogramma* spp [Wollenweber, 1980]. As far as it can be ascertained, this appears to be the first report of its isolation in Combretaceae.



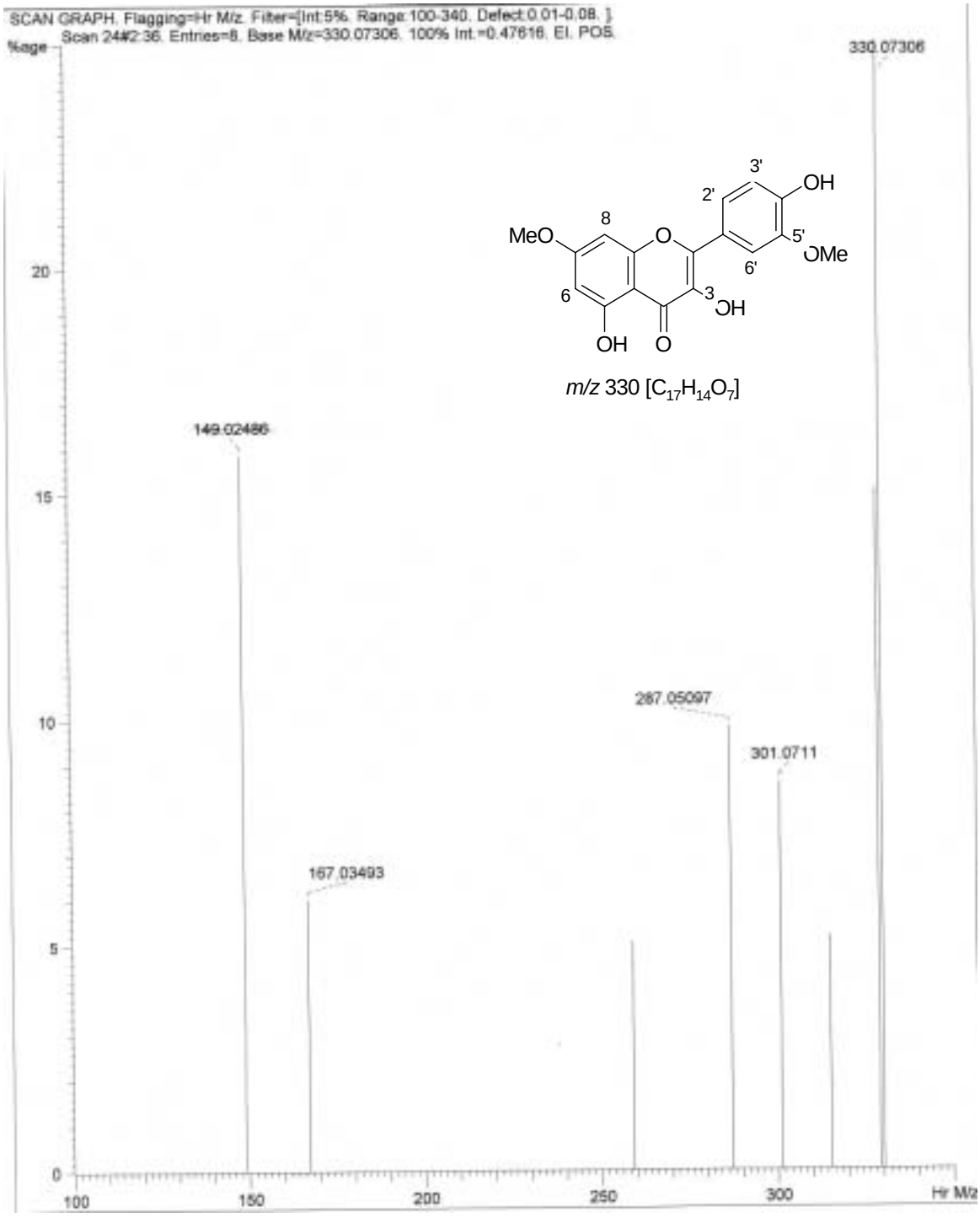
Spectrum 6.12: ^{1}H NMR (400MHz, C_5D_5N) of CE46 / CE51



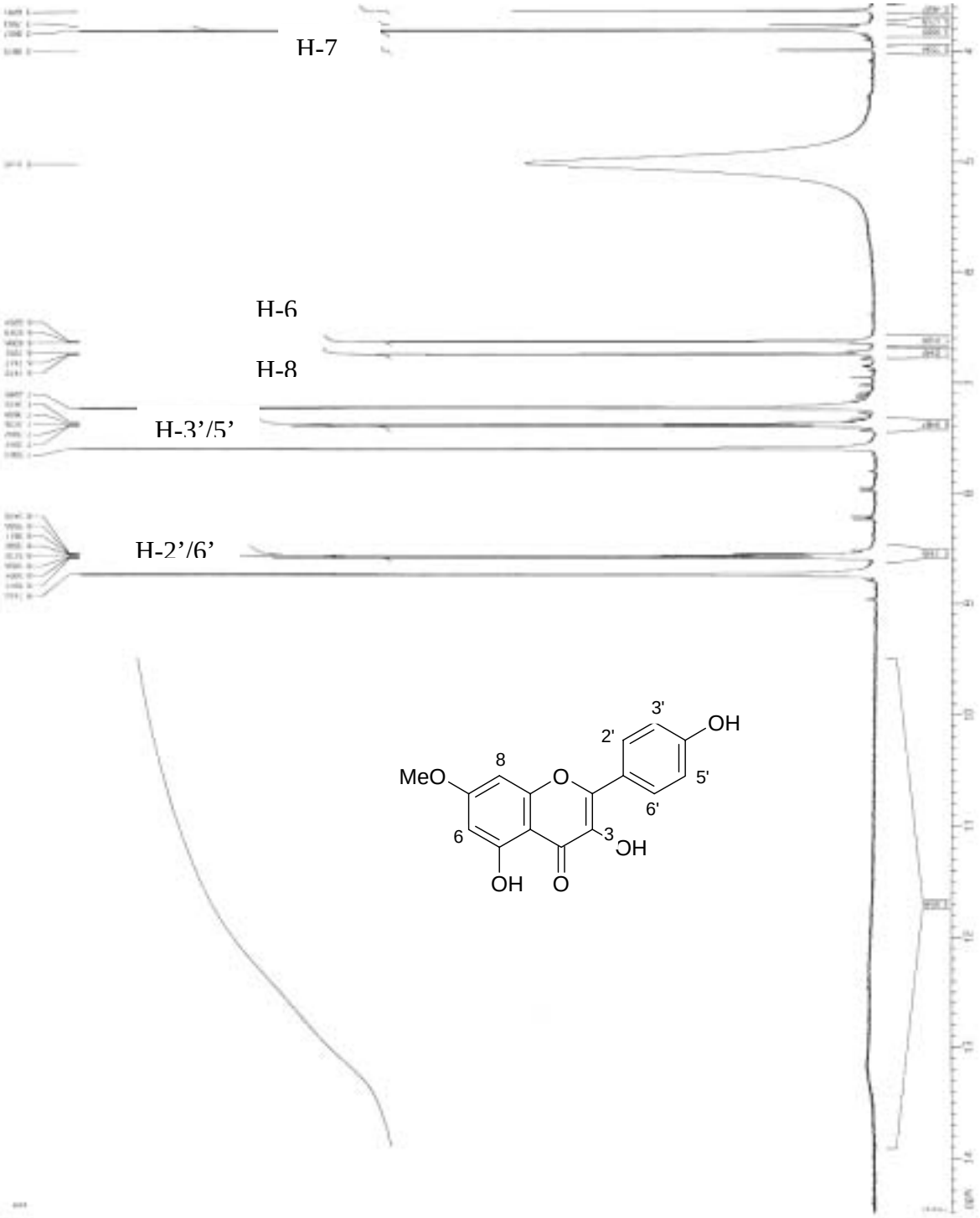
Spectrum 6.13: ^{13}C NMR (75 MHz, Acetone-d_6) of CE46



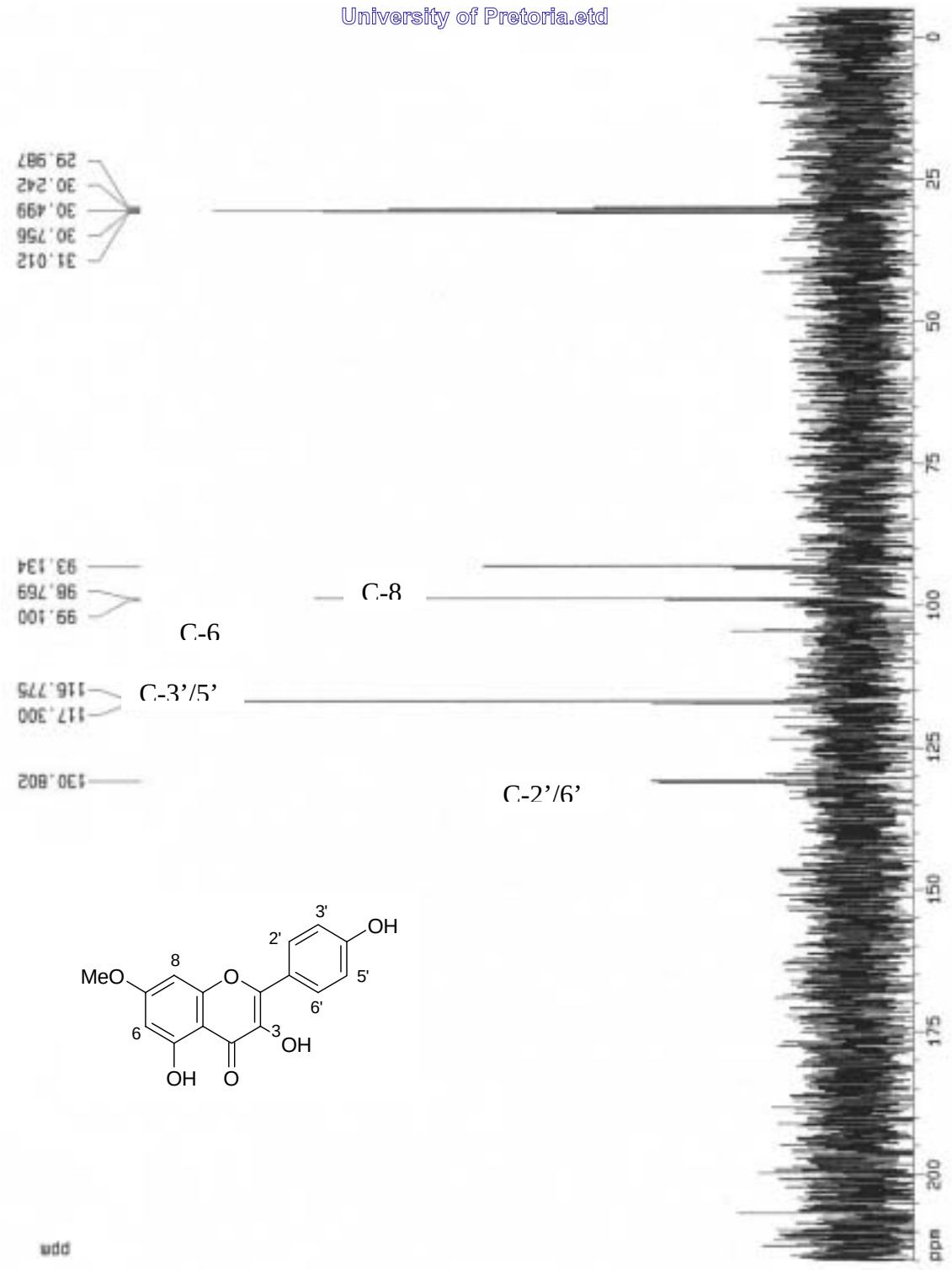
Spectrum 6.14: ^{1}H-^{1}H COSY LR (400MHz, C_5D_5N) of CE46



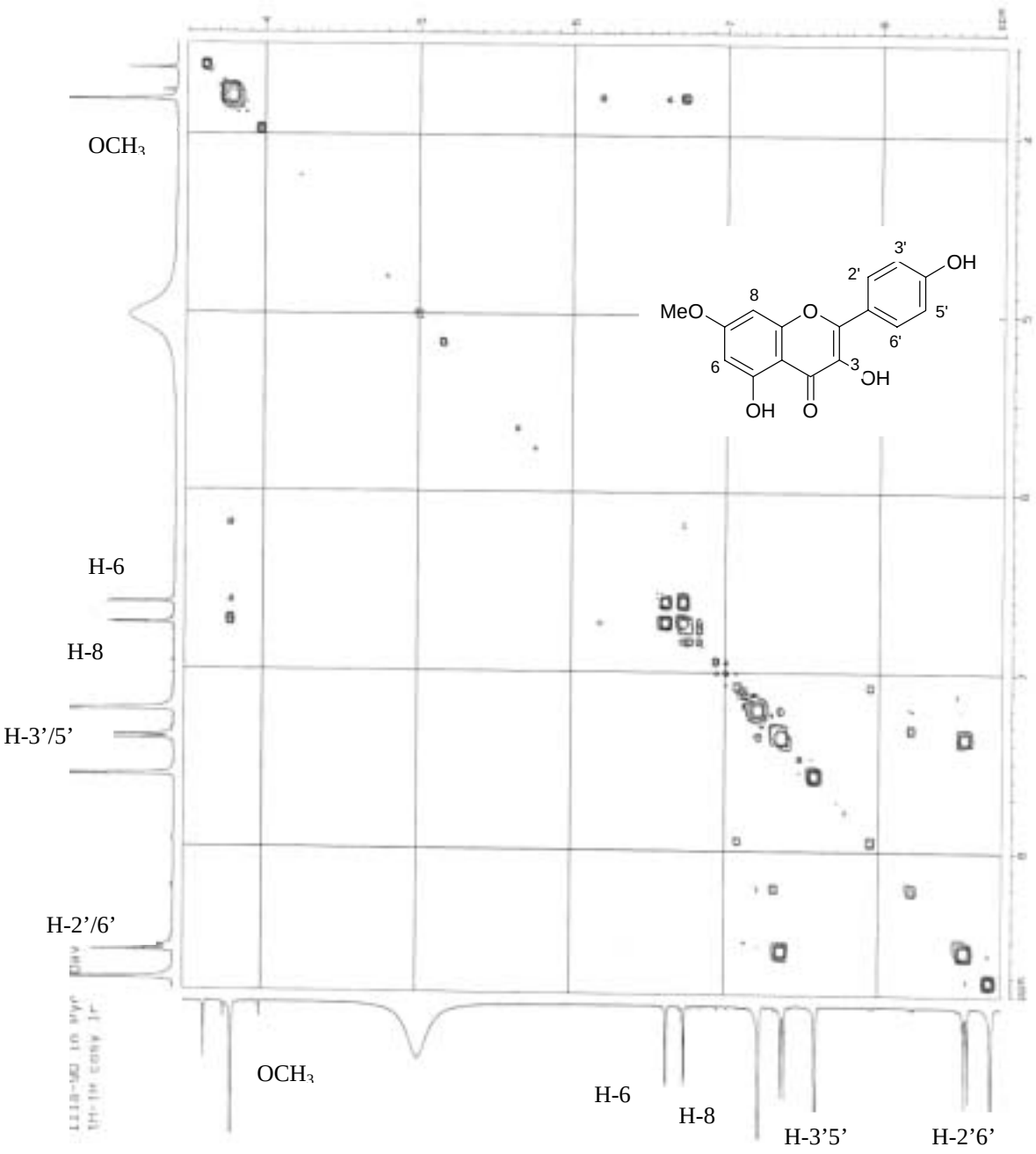
Spectrum 6.15: HREIMS of CE46



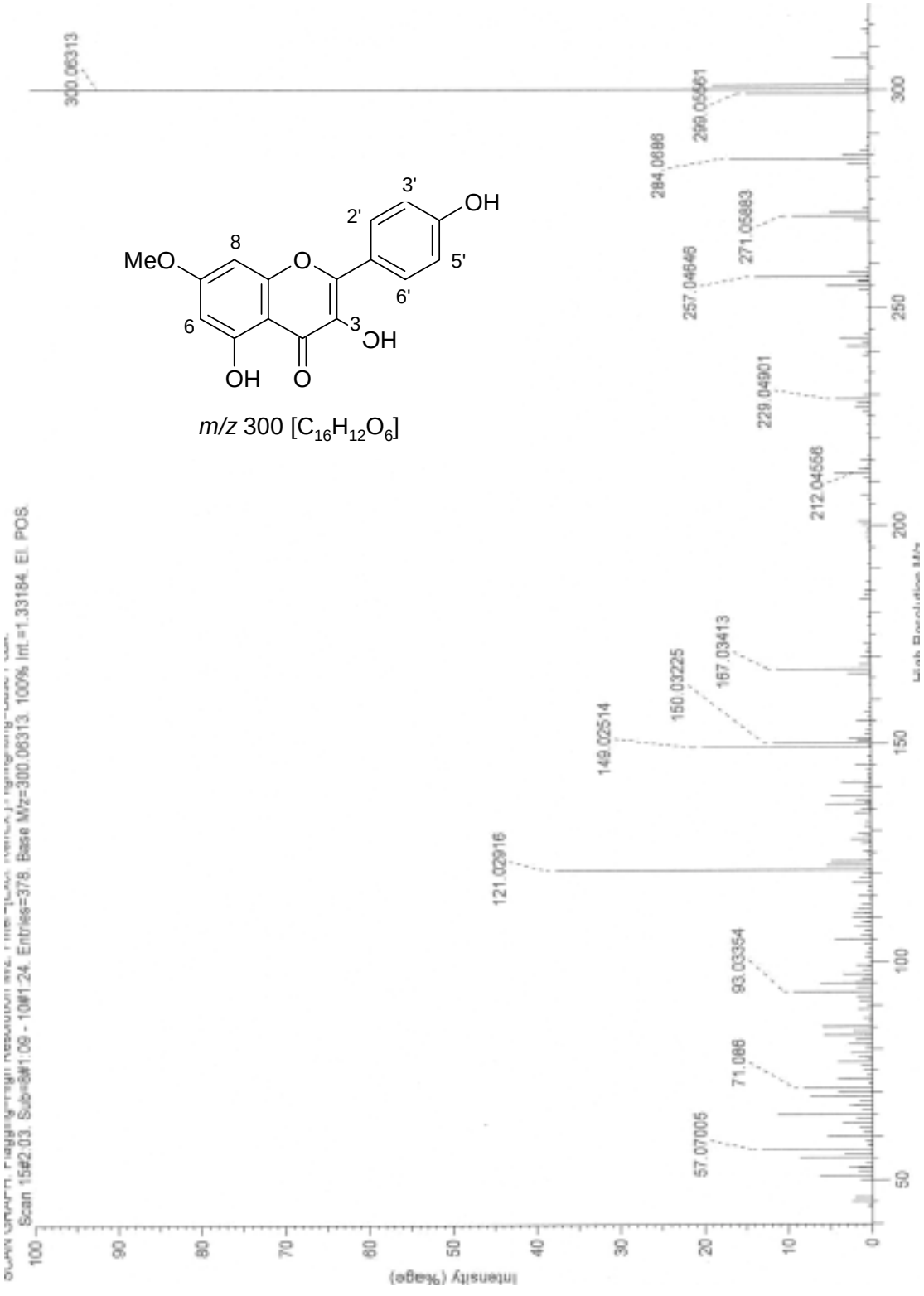
Spectrum 6.16: ^{1}H NMR (400MHz, C_5D_5N) of IIIa90



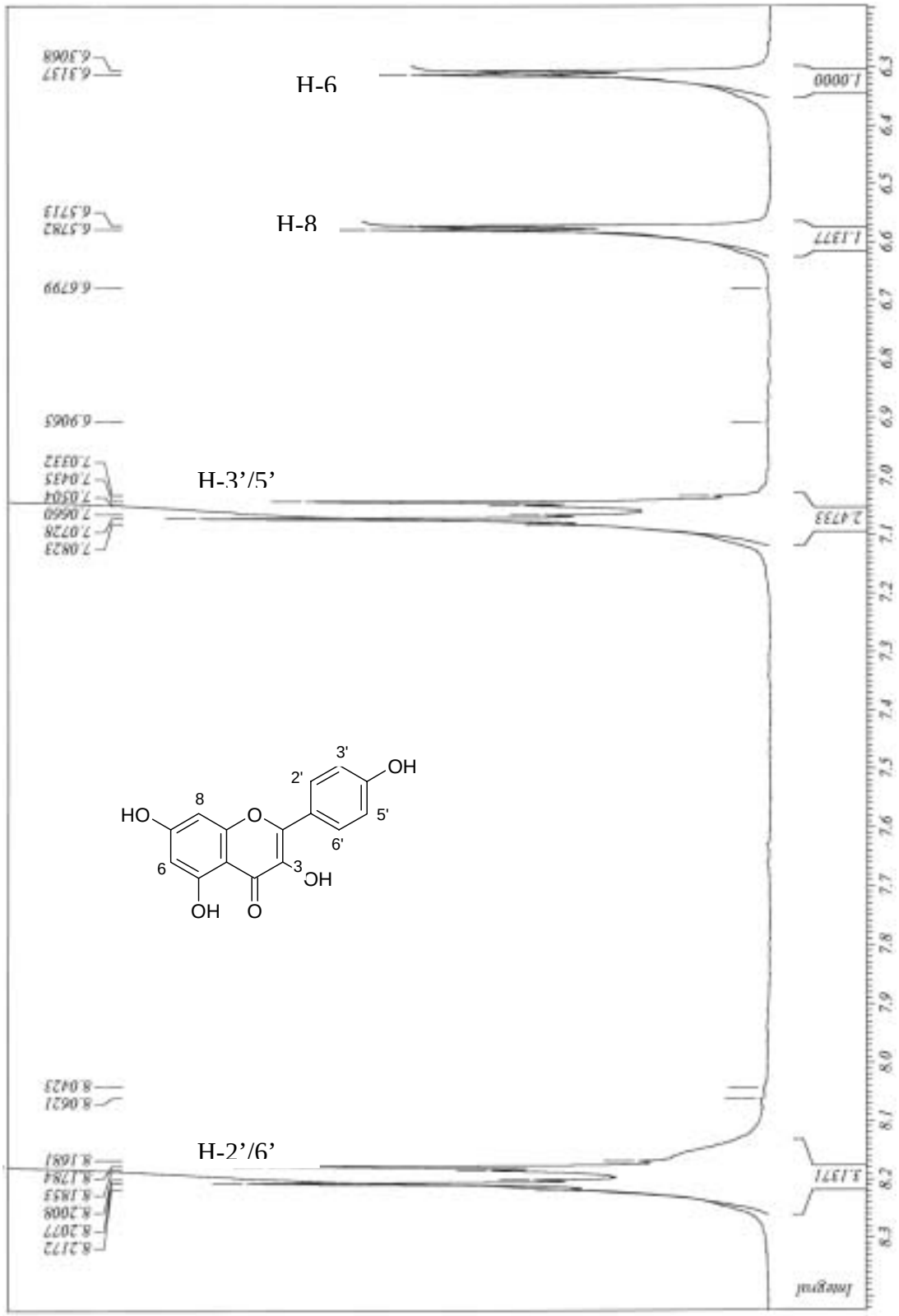
Spectrum 6.17: ^{13}C NMR (75MHz, Acetone-d_6) of IIIa90



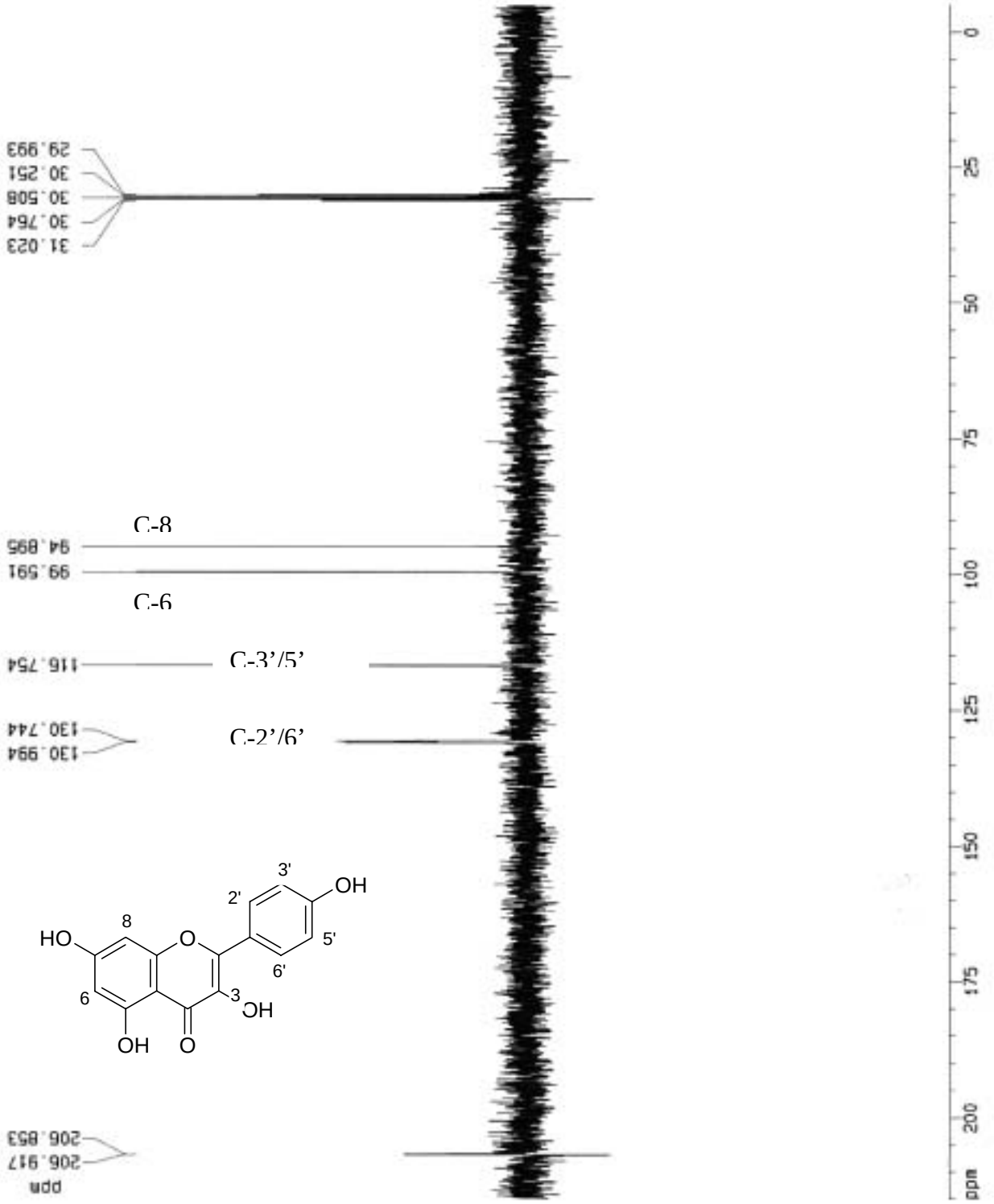
Spectrum 6.18: ^{1}H-^{1}H COSY LR (400MHz, C_5D_5N) of IIIa90



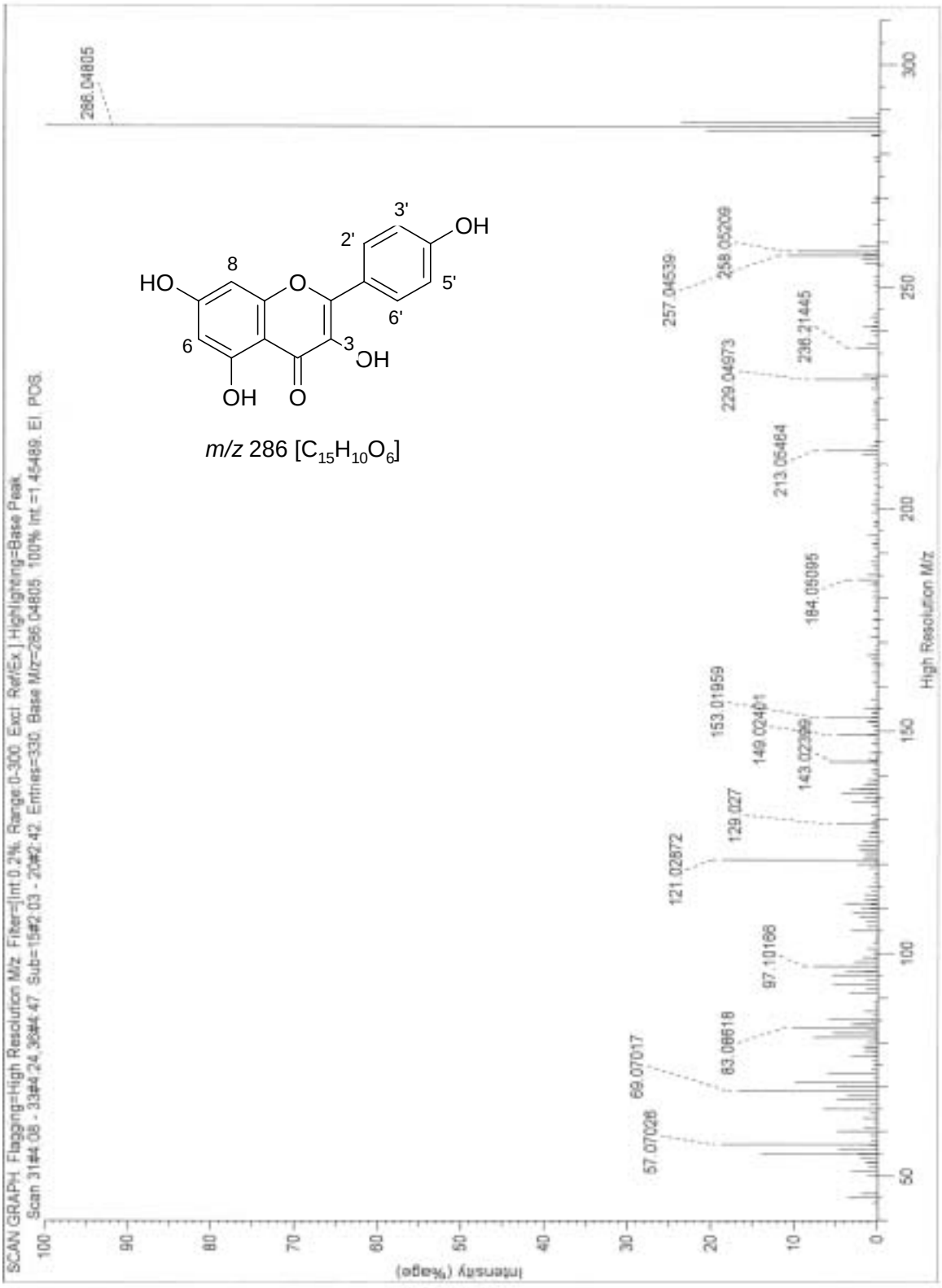
Spectrum 6.19: HREIMS of IIIa90



Spectrum 6.20: ^{1}H NMR (300MHz, Acetone-d_6) of Seph 51



Spectrum 6.21: ^{13}C NMR (300MHz, Acetone-d_6) of Seph 51



Spectrum 6.22: HREIMS of Seph 51