CHAPTER IV

RESULTS AND DISCUSSION

The fresh bark (27 kg) of *Garcinia dulcis* (Roxb.) Kurz was extracted with 95 % ethanol (4 x 15 L) at room temperature. The ethanol extract was partitioned and then separated by repetitive chromatographic technique to afford seven isolates. The structural determinations of all the isolates were achieved through interpretation of their UV, IR, MS and NMR data and subsequently confirmed by comparison of these values with those reported in the literature.

1. Structure Determination of Isolate GD-1 [139 and 140]

Isolate GD-1, colorless needles, was obtained from fraction V-2. It was recrystallized from methanol (23 mg, 8.51 x 10^{-5} % based on fresh weight of the bark). It gave green to Libermann-Burchard's test, suggesting the presence of a steroidal skeleton.

The EI mass spectrum of isolate GD-1 (Figure 2) exhibited two molecular ions [M^+] at *m/z* 414 and 412, suggesting the molecular formulae $C_{29}H_{50}O$ and $C_{29}H_{48}O$, respectively. The IR spectrum (Figure 3) showed absorption bands at 3300 (O-H stretching), 2960 (C-H stretching), 1460 and 1380 (C-H bending,) and 1060 (C-O stretching) cm^{-1}.

Isolate GD-1 could be assigned as a mixture of sterol, β-sitosterol [139] and stigmasterol [140] by analysis of its ^1H and ^{13}C NMR spectral properties, including comparison of these data with those previously published for β-sitosterol and stigmasterol.

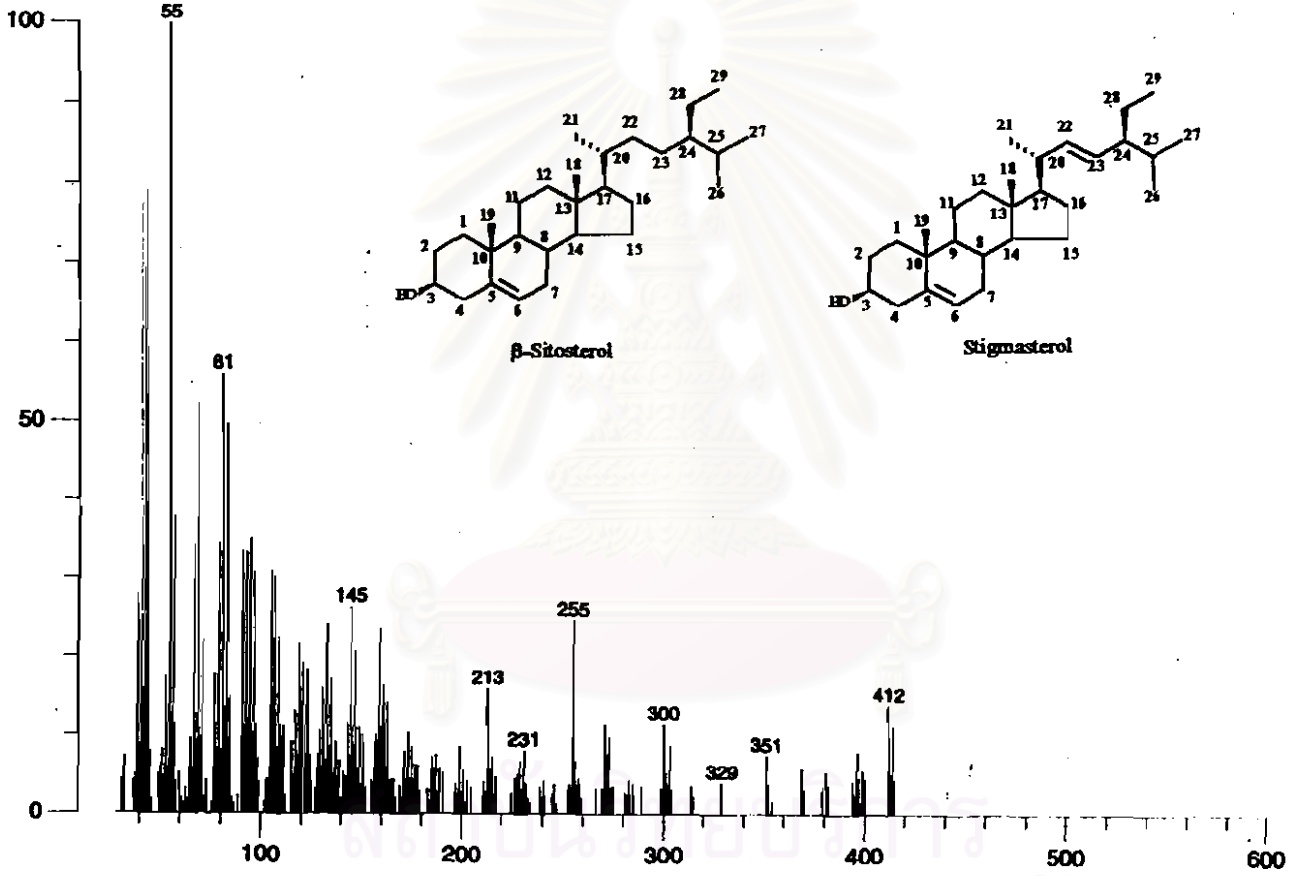


Figure 2 EI mass spectrum of isolate GD-1

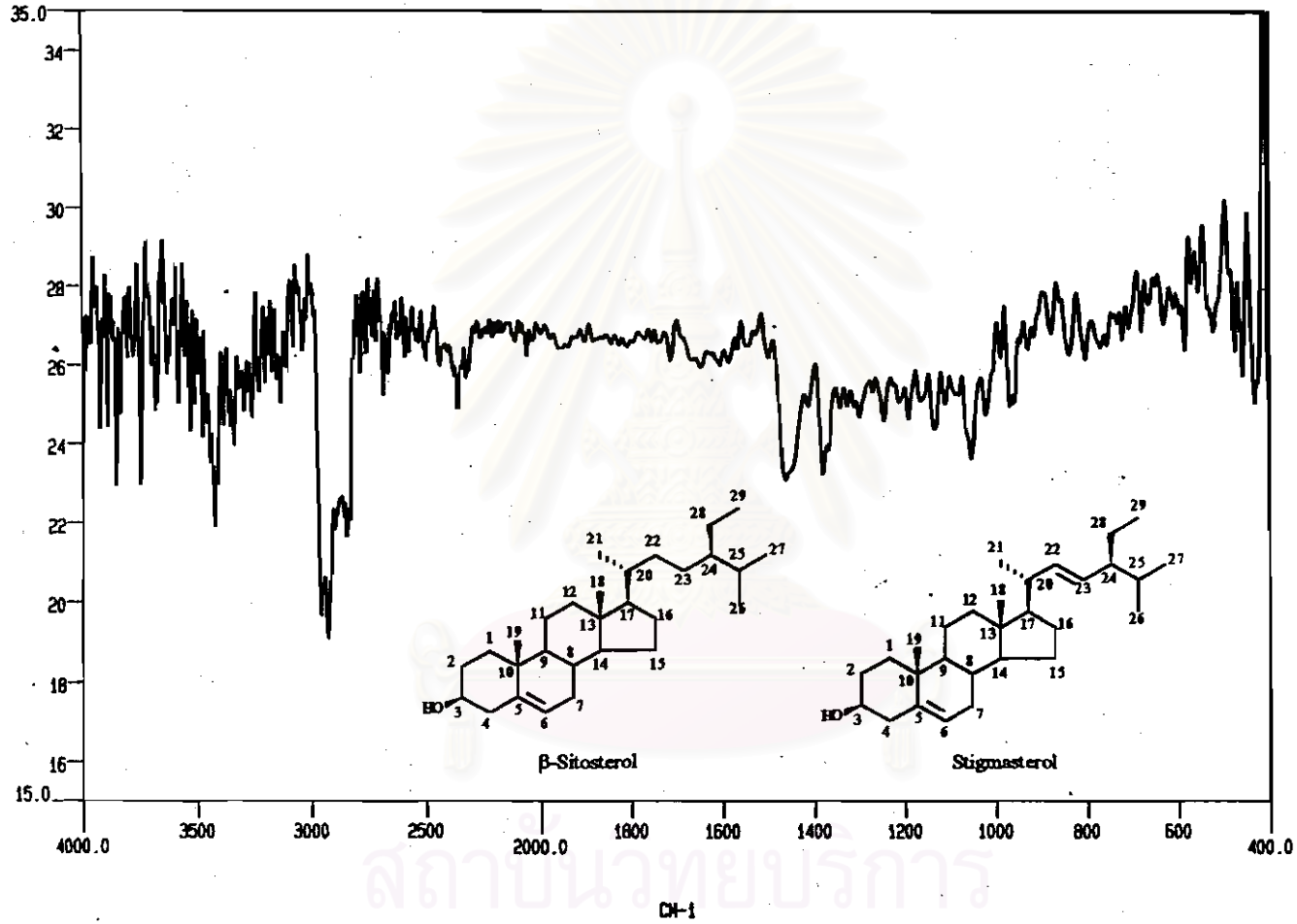


Figure 3 IR spectrum of isolate GD-1 (film)

The ^{1}H NMR spectrum of isolate GD-1 (Figures 4a-4c) showed methyl signals of the sterol skeleton at δ 0.66-1.01 ppm which could be assigned as shown in Table 8. The chemical shifts of the methyl protons of isolate GD-1 were in good agreement with of those of β-sitosterol and stigmasterol. The signals at δ 1.10-2.30 were the signals of methylene and methine protons. The signal at δ 3.50 (m) could be assigned to H-3 whereas the resonance at δ 5.33 (m) could be assigned to H-6. The olefinic signals at δ 5.00 (1H, dd, J = 15.2, 8.5 Hz) and δ 5.13 (1H, dd, J = 15.2, 8.5 Hz) were due to H-22 and H-23 of stigmasterol (Khalil and Idler, 1980, Iribarren and Pomilio, 1985 and Heupel *et al.*, 1986).

Table 8 ^{1}H NMR assignments of β-sitosterol, stigmasterol and isolate GD-1 (in $CDCl_3$)

Position	Chemical shift (ppm)		
	β-Sitosterol [a]	Stigmasterol [b]	Isolate GD-1
C-18	0.680 (s)	0.699 (s)	0.677 (s), 0.660 (s)
C-19	1.007 (s)	1.012 (s)	1.007 (s), 0.989 (s)
C-21	0.919 (d, J = 6.5 Hz)	1.021 (d, J = 6.5 Hz)	0.901 (d, J = 6.4 Hz), 1.00 (d, J = 6.7 Hz)
C-26	0.833 (d, J = 6.8 Hz)	0.846 (d, J = 7 Hz)	0.825 (d, J = 6.4 Hz), 0.793 (d, J = 6.7 Hz)
C-27	0.813 (d, J = 6.8 Hz)	0.797 (d, J = 7 Hz)	0.815 (d, J = 6.7 Hz), 0.775 (d, J = 6.4 Hz)
C-29	0.842 (t, J = 7.2 Hz)	0.805 (t, J = 7 Hz)	0.825 (d, J = 7.6 Hz), 0.784 (d, J = 7.6 Hz)

[a] From Rubinstein *et al.*, 1976

[b] From Matsumoto, Nakagawa and Itoh, 1984

From the ^{1}H NMR spectrum, the ratio of the integrals of either H-6 of β-sitosterol to H-22 of stigmasterol or H-6 of β-sitosterol to H-23 of stigmasterol was

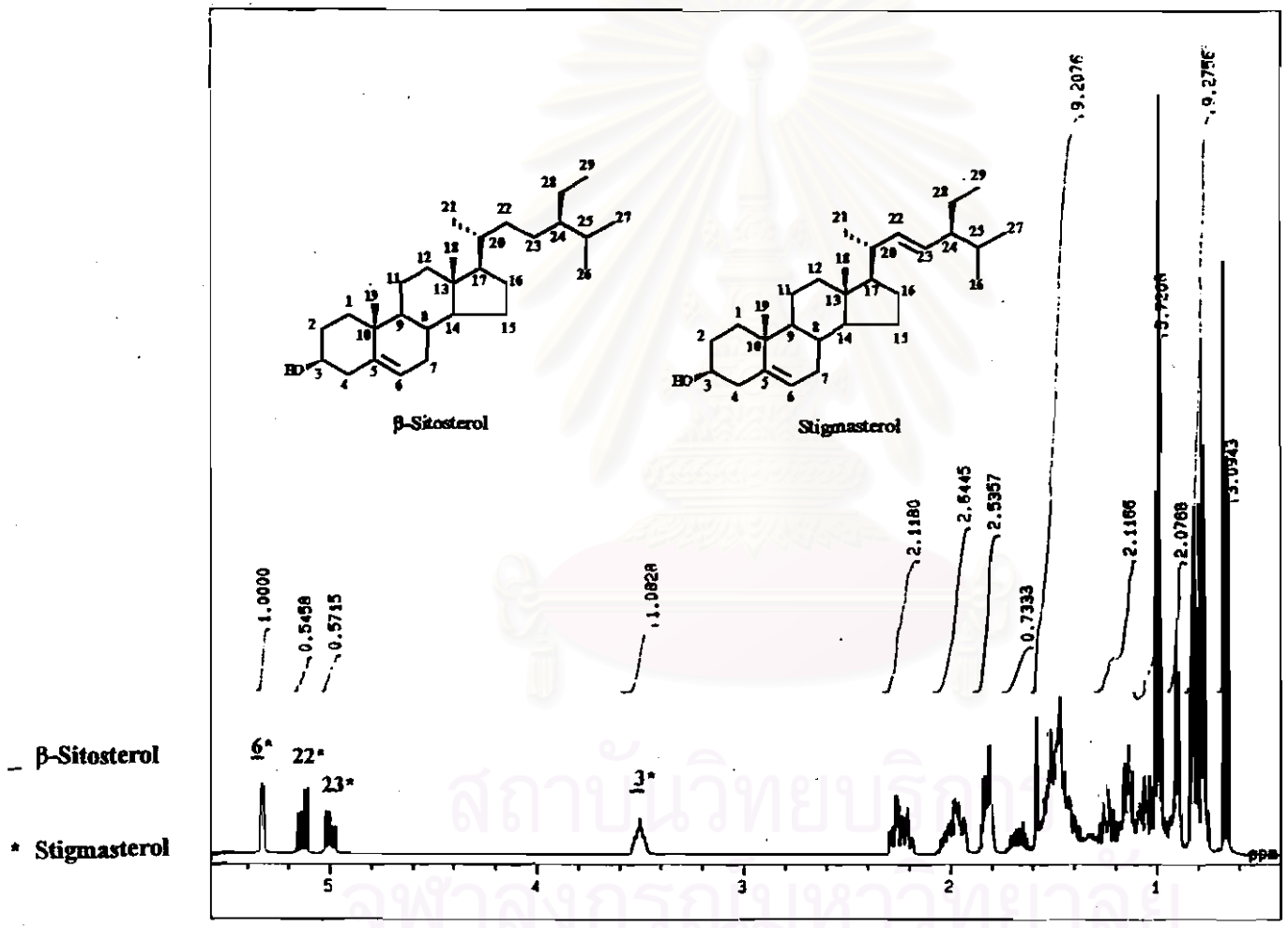


Figure 4a 500 MHz ^{1}H NMR spectrum of isolate GD-1 (in $CDCl_3$)

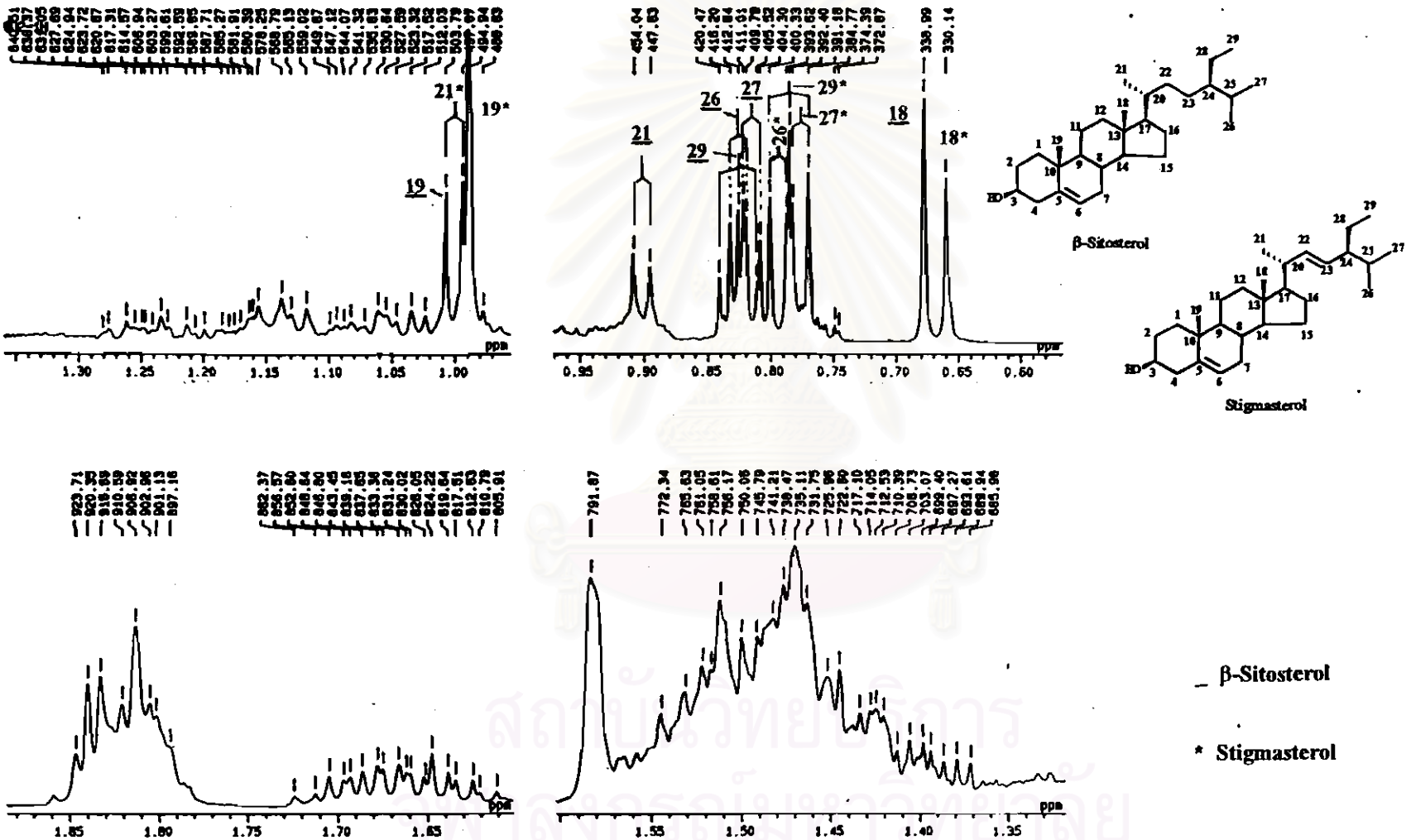


Figure 4b 500 MHz ^{1}H NMR spectrum of isolate GD-1 (in $CDCl_3$) (expanded from 0.60-1.85 ppm)

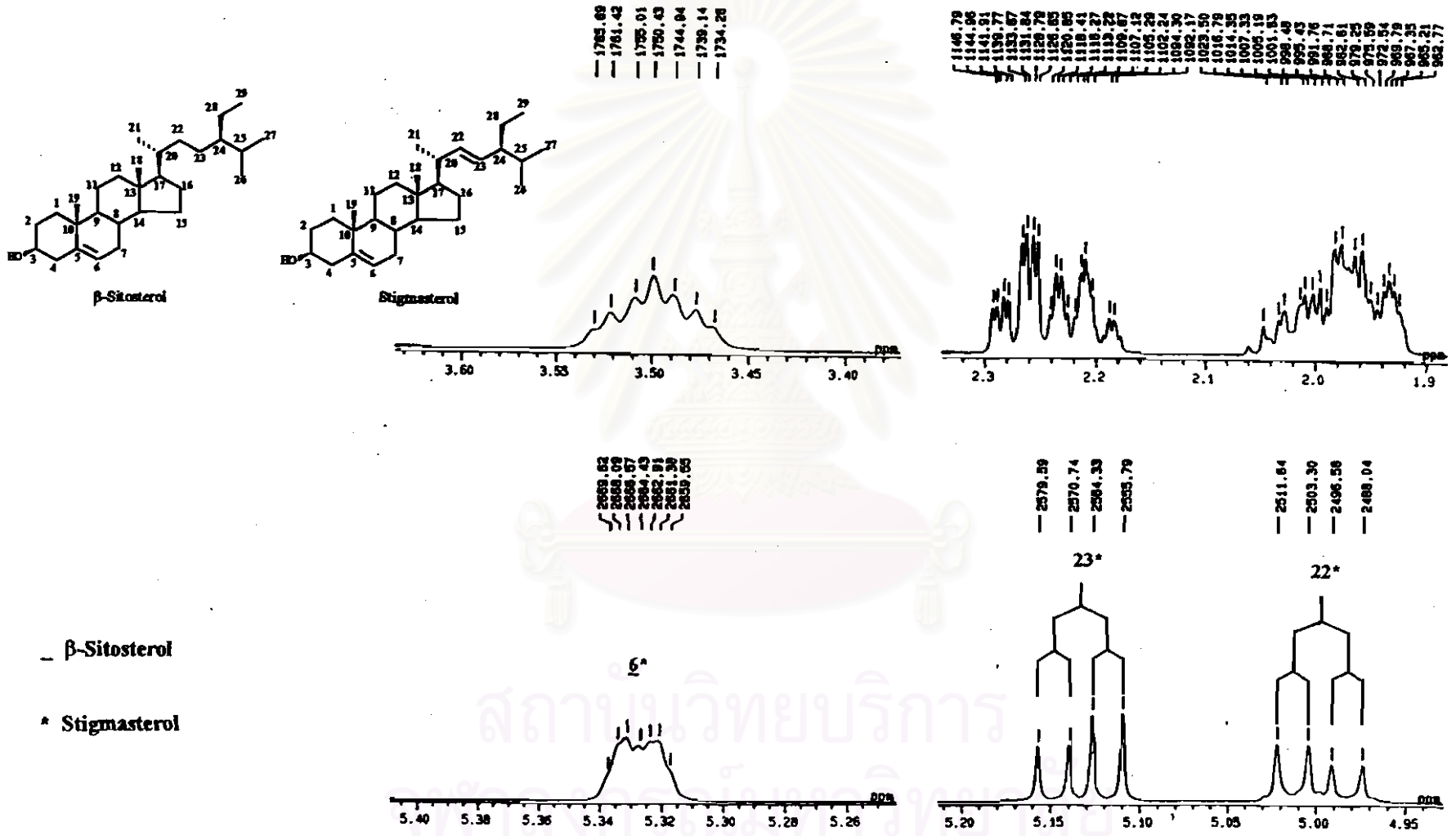


Figure 4c The 500 MHz ^{1}H NMR spectrum of isolate GD-1 (in $CDCl_3$) (expanded from 1.90-5.40 ppm)

found to be approximately 0.4 : 0.6 or 2 : 3. Therefore it was concluded that isolate GD-1 was a mixture of β-sitosterol and stigmasterol in a ratio of 2 : 3.

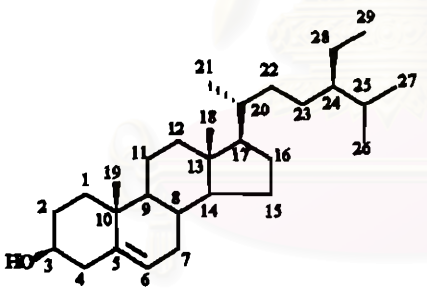
The ^{13}C NMR and DEPT spectra (Figures 5a-5b and 6) disclosed carbon resonances which are in good agreement with those of β-sitosterol and stigmasterol. (Wright *et al.*, 1978) Their carbon assignments are shown in Table 9.

Table 9 The ^{13}C NMR chemical shifts of β-sitosterol, stigmasterol and isolate GD-1 (in $CDCl_3$)

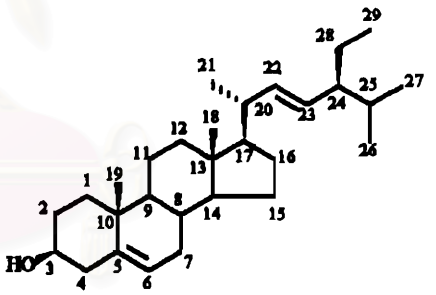
Carbon	Chemical shift (ppm)		
	β-Sitosterol	Stigmasterol	Isolate GD-1
1	37.31	37.31	37.25
2	31.57	31.69	31.66
3	71.69	71.81	71.80
4	42.25	42.35	42.21, 42.30
5	140.76	140.80	140.75
6	121.59	121.69	121.70
7	31.92	31.94	31.89
8	31.92	31.94	31.89
9	50.17	50.20	50.14
10	36.51	36.56	36.51
11	21.11	21.11	21.07
12	39.81	39.74	39.77, 39.67
13	42.33	42.35	42.30
14	56.79	56.91	56.77, 56.86
15	24.32	24.39	24.29, 24.36
16	28.26	28.96	28.24, 28.90
17	56.11	56.06	56.06, 55.96
18	11.87	12.07	11.85, 11.97
19	19.40	19.42	19.39

Table 9 (Continued)

Carbon	Chemical shift (ppm)		
	β-Sitosterol	Stigmasterol	Isolate GD-1
20	36.17	40.54	36.14, 40.46
21	18.82	21.11	18.78, 21.07
22	33.95	138.37	33.95, 138.30
23	26.13	129.32	26.08, 129.28
24	45.85	51.29	45.84, 51.24
25	29.18	31.94	29.16, 31.89
26	19.84	21.26	19.80, 21.20
27	19.07	19.02	19.03, 18.98
28	23.09	25.44	23.07, 25.39
29	12.32	12.27	12.23, 12.03



[139]



[140]

Figure 7 Structures of the compounds of isolate GD-1

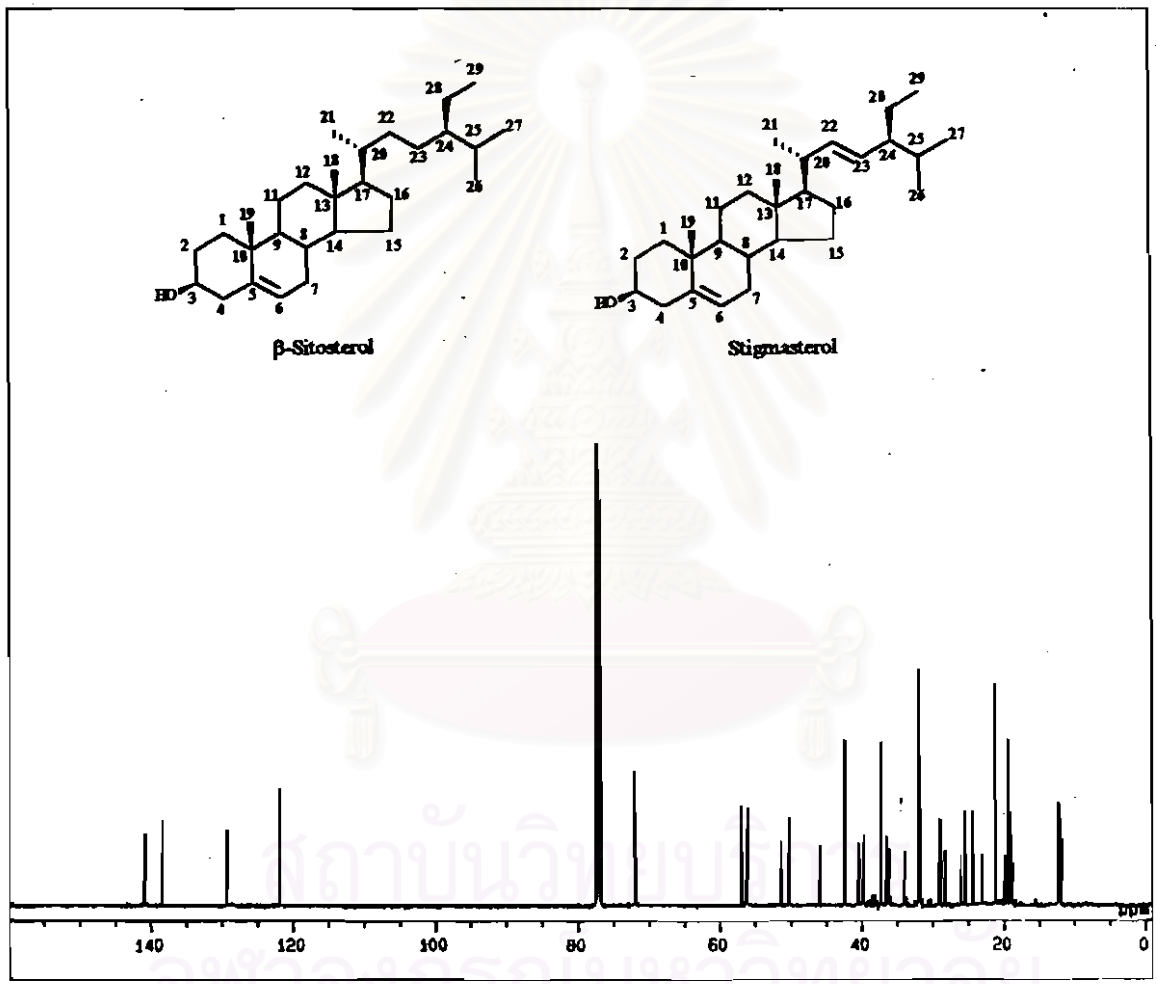


Figure 5a 125 MHz ^{13}C NMR spectrum of isolate GD-1 (in $CDCl_3$)

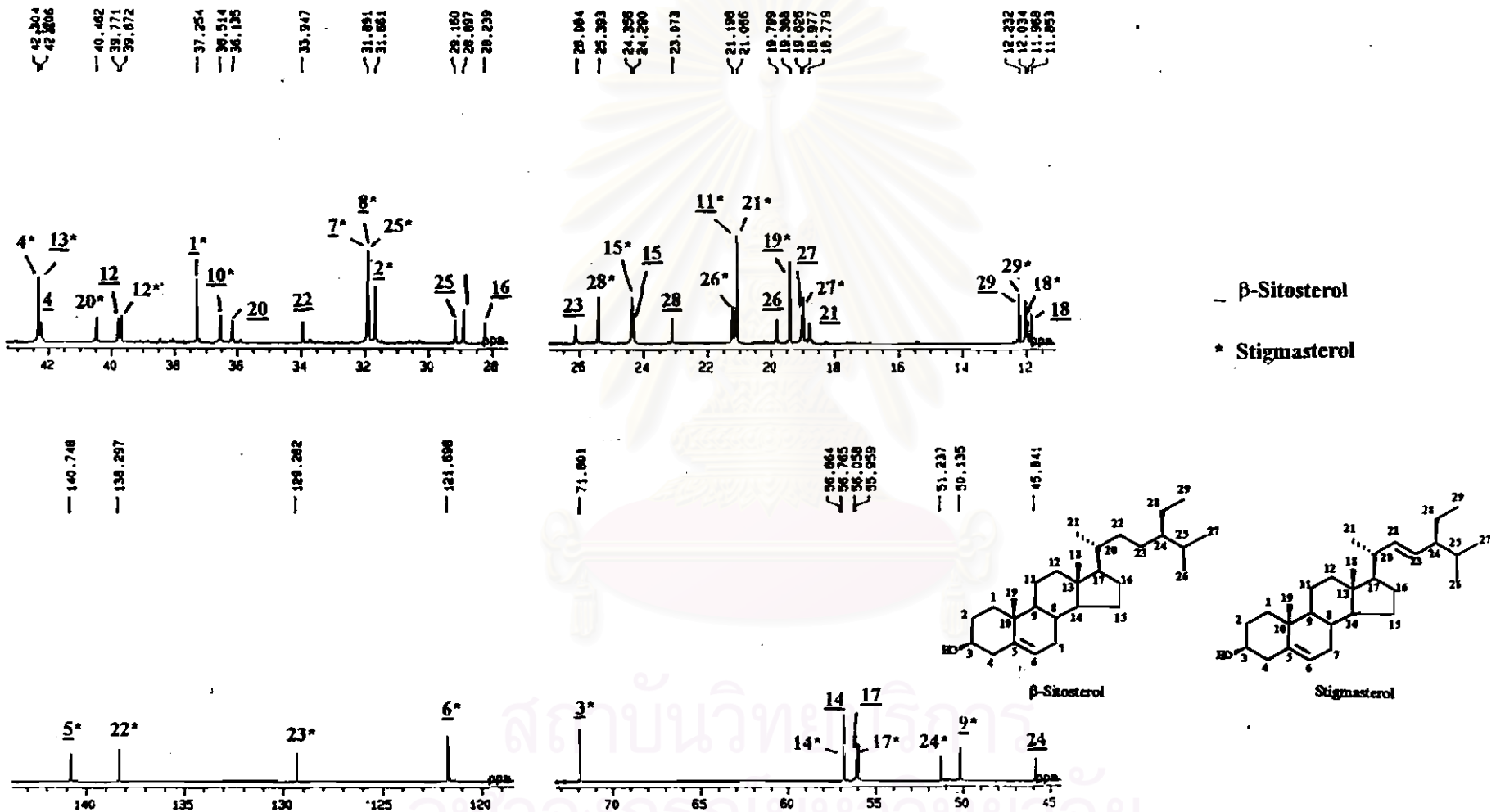
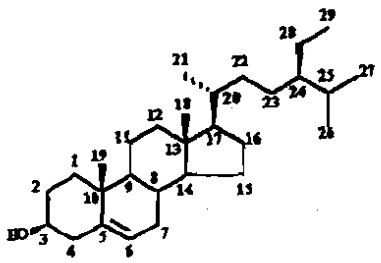
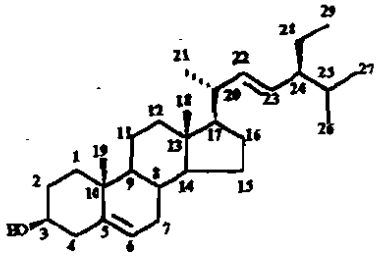


Figure 5b 125 MHz ^{13}C NMR spectrum of isolate GD-1 (in $CDCl_3$) (expanded from 12-140 ppm)



β-Sitosterol



Stigmasterol

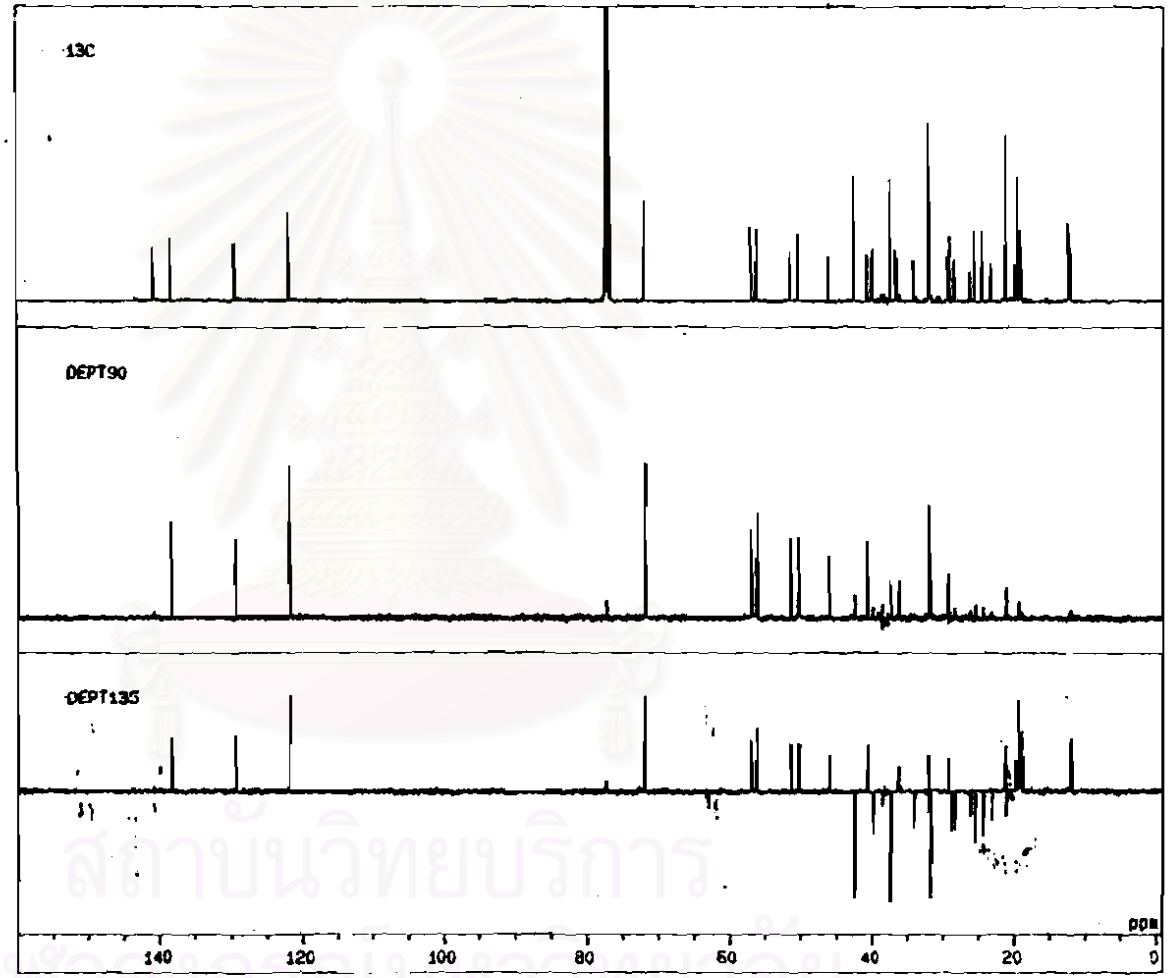


Figure 6 DEPT spectrum of isolate GD-1 (in $CDCl_3$)

2. Structure Determination of Compound GD-2 [10]

Compound GD-2, bright yellow needles, was obtained from fraction V-3 by repetitive chromatography (7 mg, 2.59 x 10^{-5} % based on fresh weight of the bark).

Compound GD-2 was identified as 1,7-dihydroxyxanthone or euxanthone [10] (Bandaranayake *et al.*, 1975) by analysis of its NMR spectral data.

Although euxanthone has been isolated from several plants of different genera (Sultanbawa, 1980 and Bennett and Lee, 1989), its complete proton assignments have never been reported. Gunatilaka and co-workers (1982) isolated euxanthone from the timber of *Hypericum mysorense* and reported only partial ^{1}H NMR spectral assignments.

The EIMS of compound GD-2 (Figure 8) displayed a molecular ion [M^+] at *m/z* 228, suggesting a molecular formula of $C_{13}H_8O_4$. The UV absorption bands in methanol at λ_{max} 385, 287, 259 and 234 nm (Figure 9) showed the typical xanthone chromophore (Scott, 1964). The IR spectrum (Figure 10) indicated the presence of a hydroxyl group at 3500 (broad), a carbonyl group at 1630, olefinic carbons at 1460 and ether linkage at 1220 cm^{-1}.

The ^{1}H NMR spectrum (Figures 11a-11b) showed the signals of 2 hydroxyl groups and 6 aromatic protons. The most downfield singlet signal at δ 12.62 was assigned to 1-OH because of its intramolecular hydrogen bonding to the carbonyl group C-9. The other hydroxy signal at δ 10.07 ppm was assigned to the hydroxyl substituent at C-7. Six aromatic proton signals appeared at δ 6.77 (1H, dd, J = 8.2, 0.6 Hz), 7.03 (1H, dd, J = 8.2, 0.6 Hz), 7.35 (1H, dd, J = 8.8, 3.0 Hz), 7.44 (1H, d, J = 3.0 Hz), 7.53 (1H, d, J = 8.8 Hz) and 7.69 (1H, dd, J = 8.2, 8.2 Hz) (Table 10). The assignments of these protons were based on the correlations shown in the ^{1}H-^{1}H COSY spectrum (Figures 12a-12b).

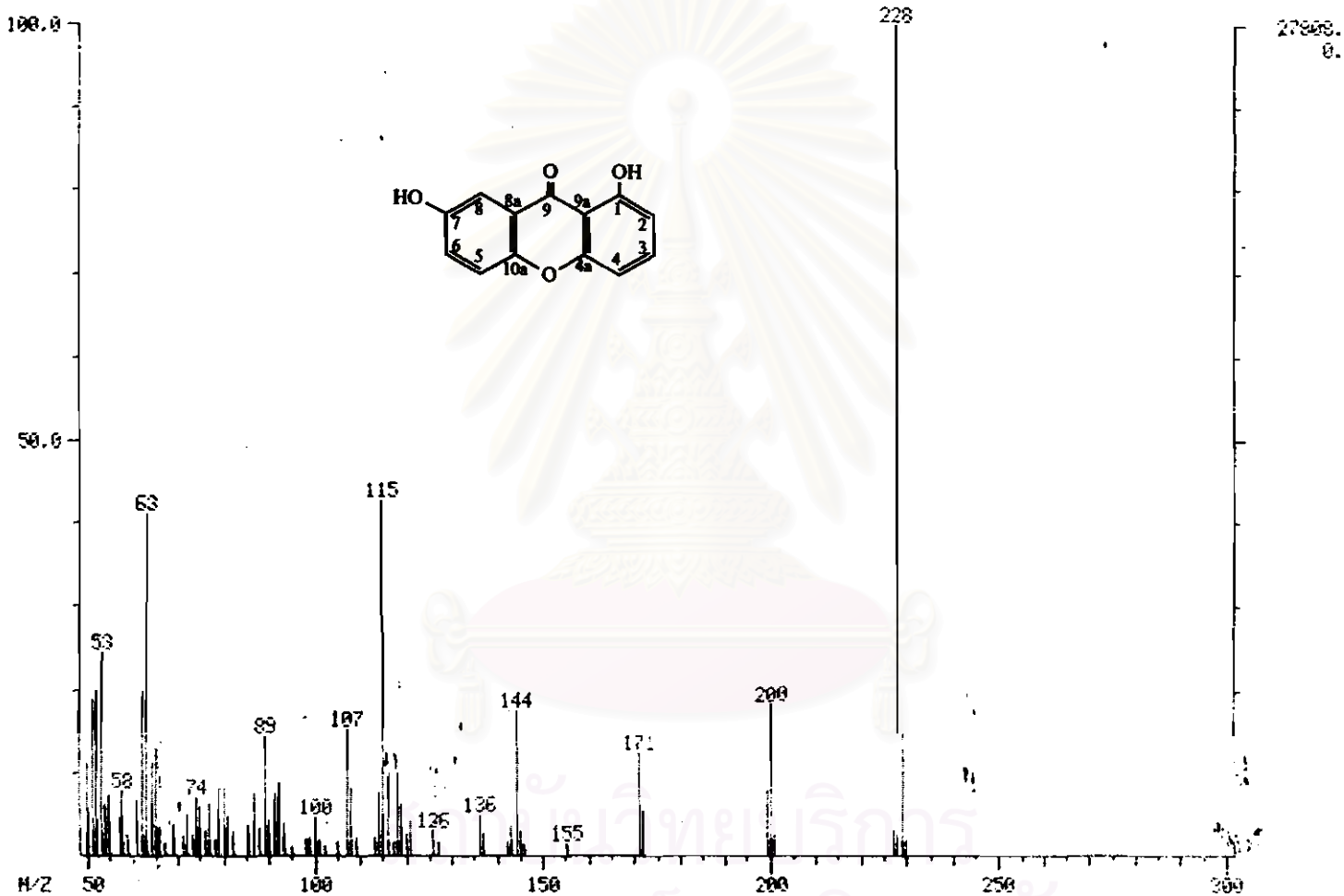


Figure 8 EI mass spectrum of compound GD-2

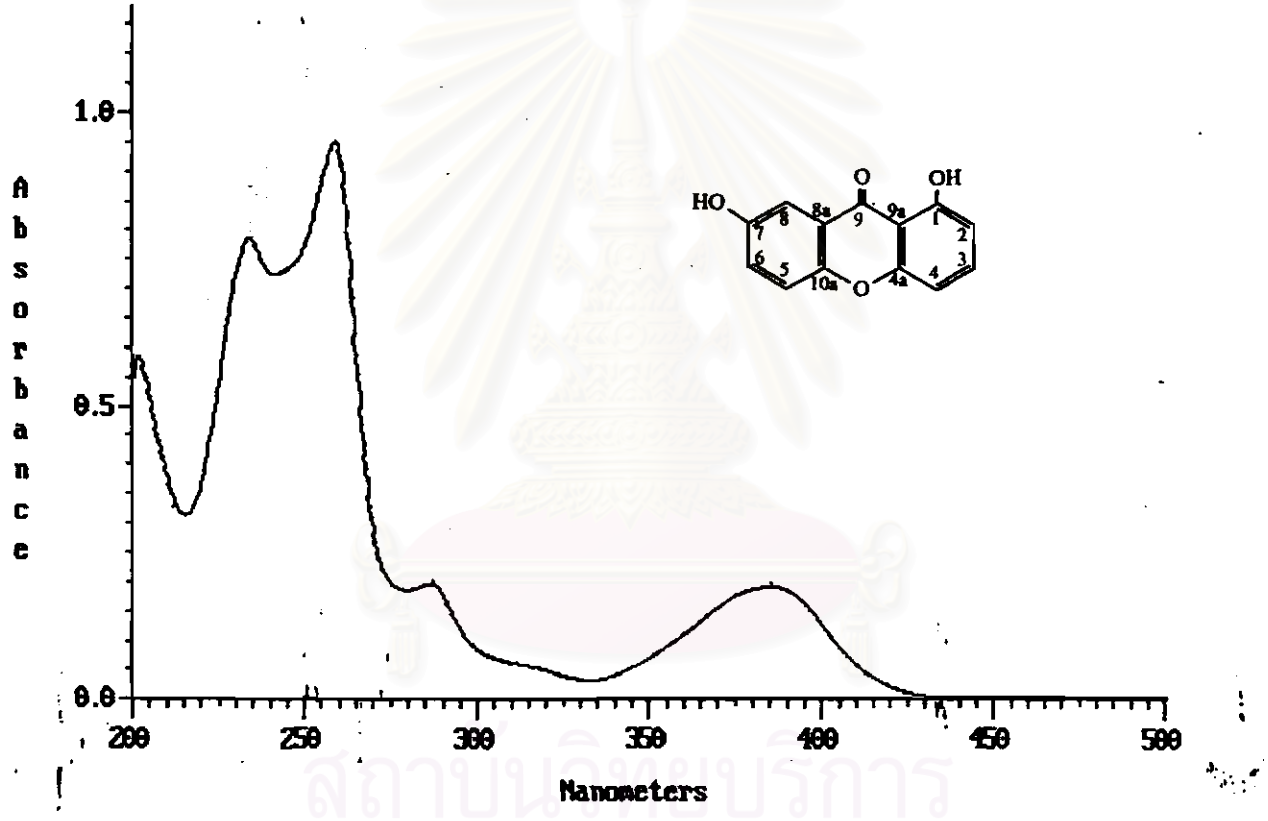


Figure 9 UV spectrum of compound GD-2 (in methanol)

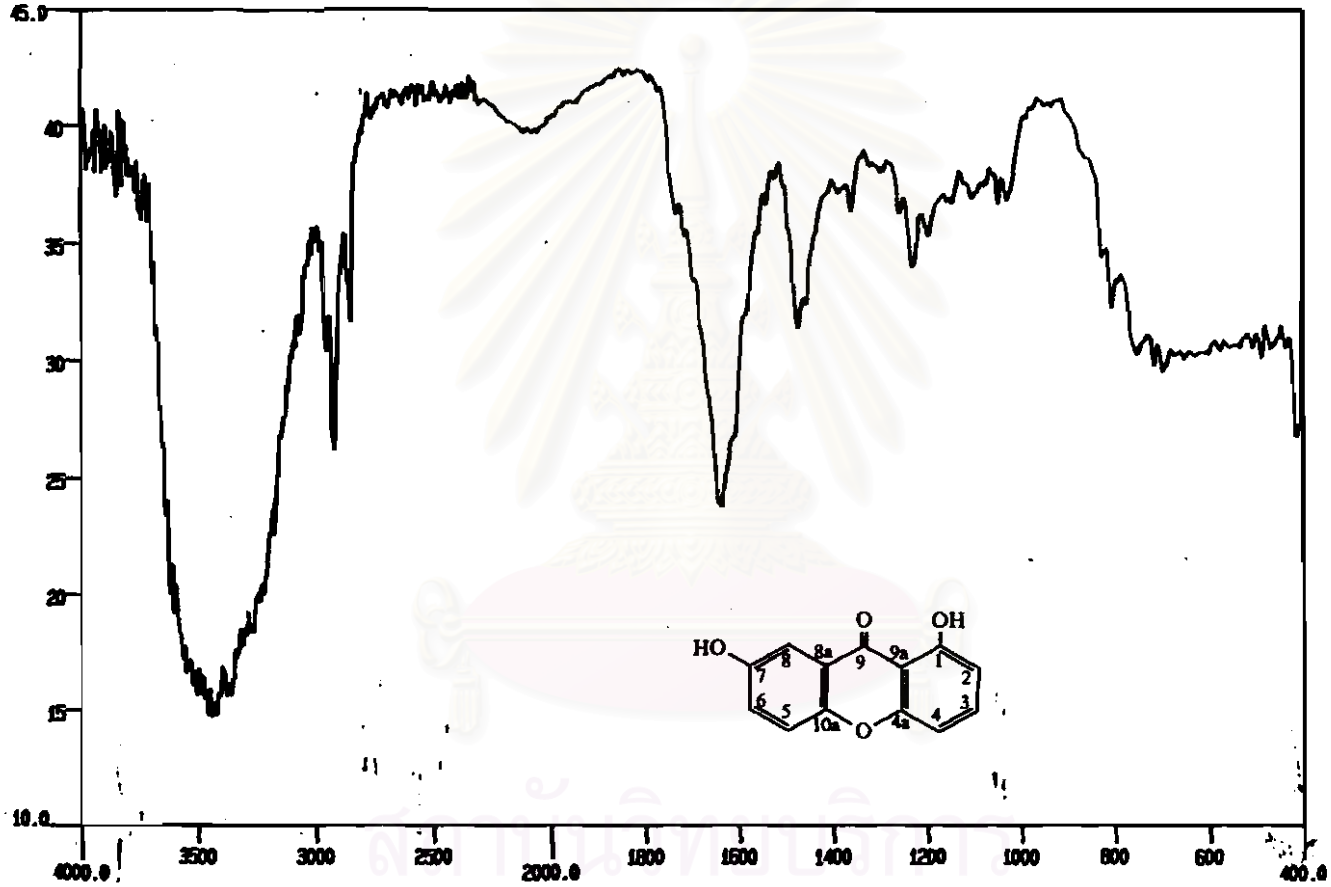


Figure 10 IR spectrum of compound GD-2 (film)

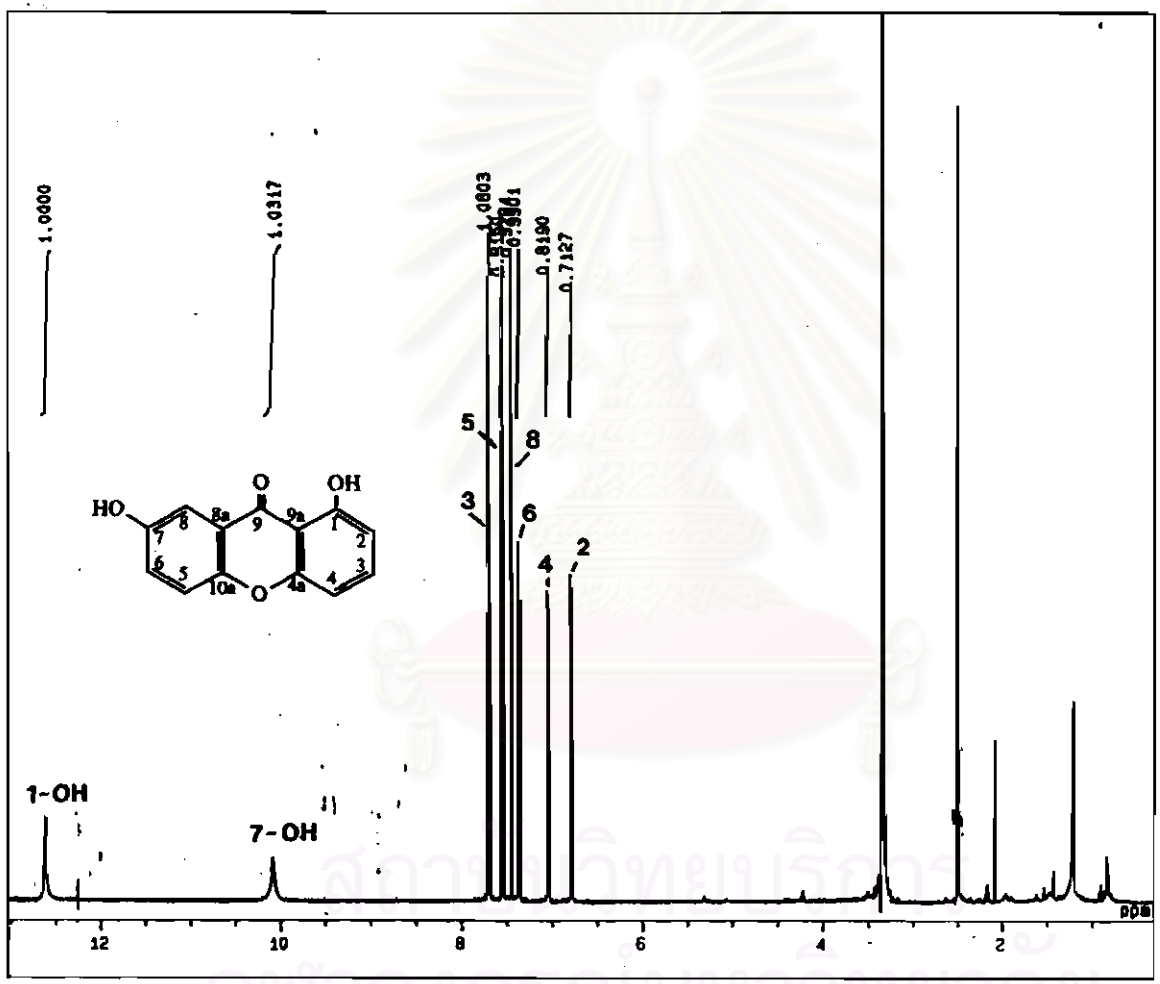


Figure 11a 500 MHz ^{1}H NMR spectrum of compound GD-2 (in DMSO-d_6)

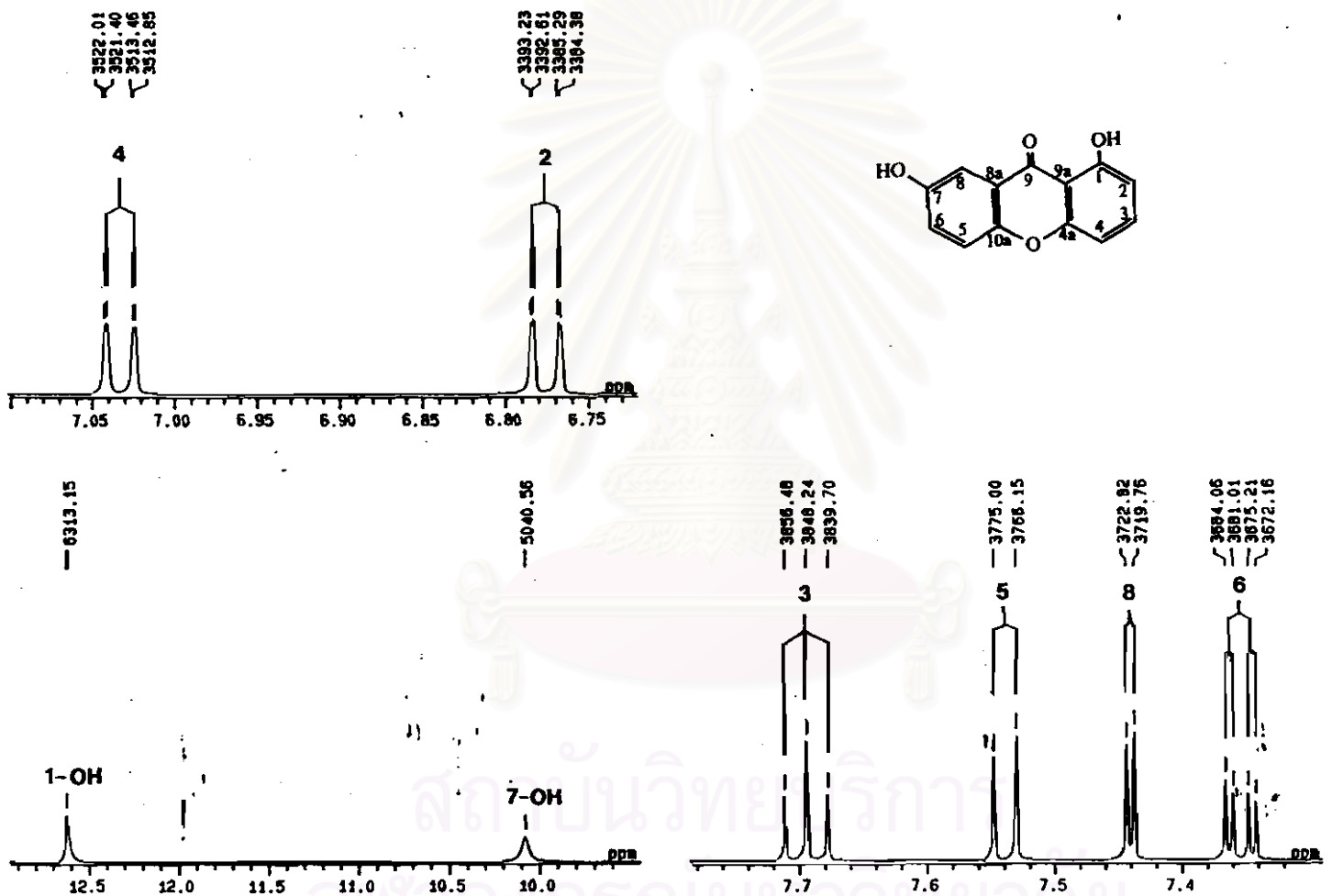


Figure 11b 500 MHz ^{1}H NMR spectrum of compound GD-2 (in DMSO-d_6) (expanded from 6.75-13.0 ppm)

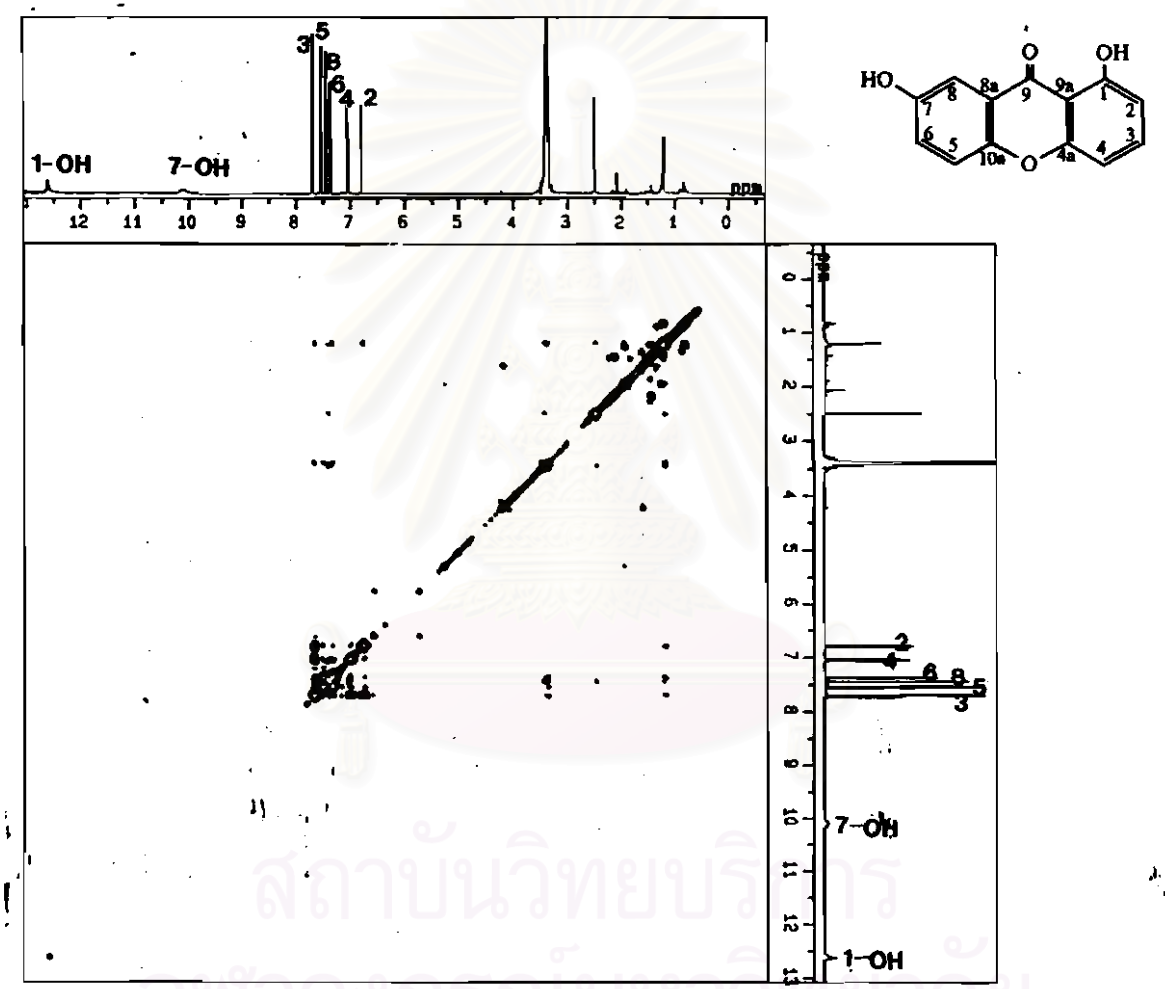


Figure 12a ^{1}H-^{1}H COSY spectrum of compound GD-2 (in DMSO-d_6)

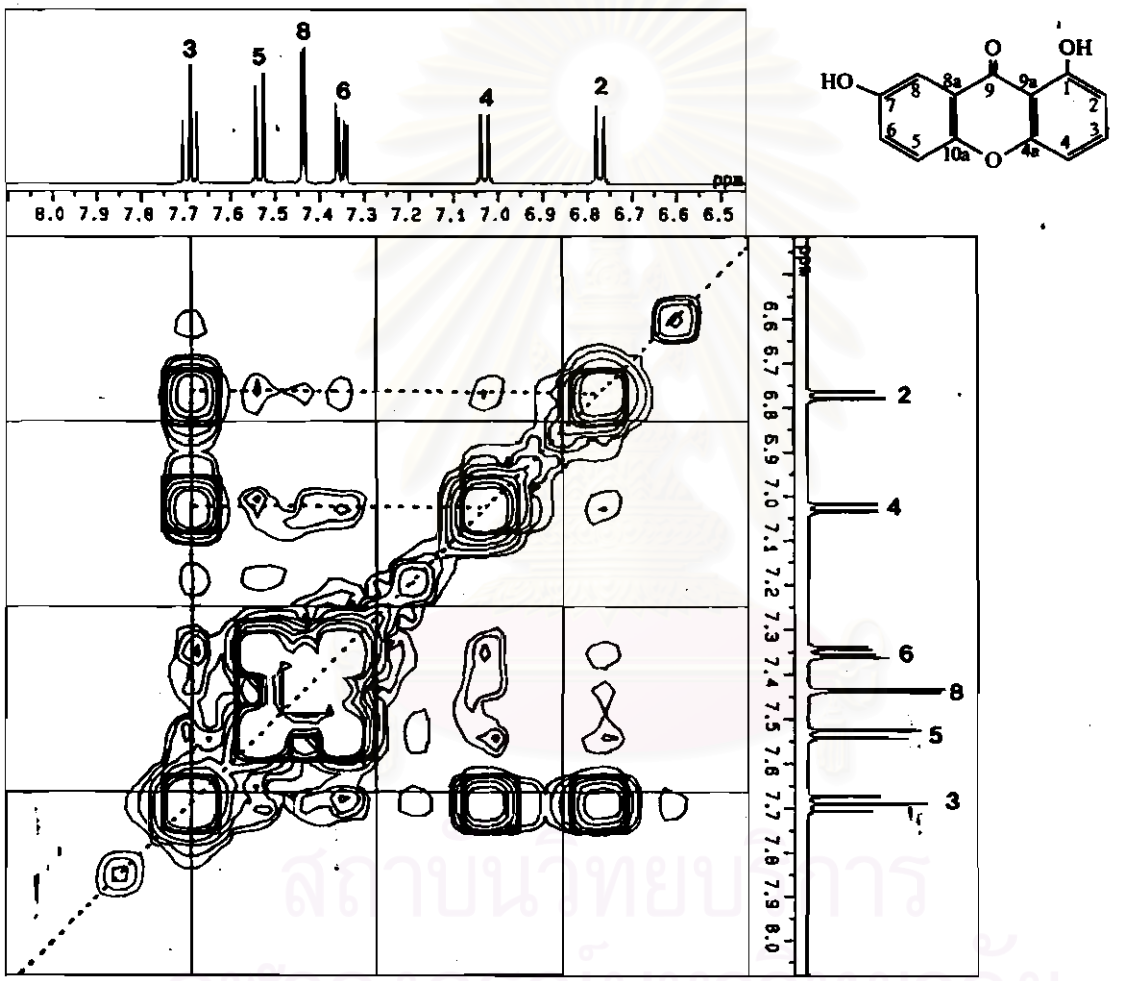


Figure 12b ^{1}H-^{1}H COSY spectrum of compound GD-2 (in DMSO-d_6) (expanded from 6.5-8.0 ppm)

In a 2-D NOESY experiment (Figures 13a-13b), the H-3 (δ 7.69) signal showed NOE interactions with the resonances of H-2 (δ 6.77) and H-4 (δ 7.03) as expected. The NOE between H-5 and H-6 was also observed. Results from the NOESY experiment are shown in Figure 14.

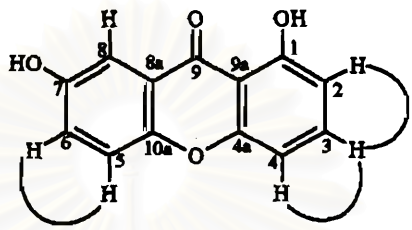


Figure 14 Results from the NOESY experiment of compound GD-2

The ^{13}C NMR spectrum (Figure 15) showed 13 carbons which could be classified by examination of the DEPT spectrum (Figure 16). These spectral data suggested the presence of one carbonyl group, 6 methine carbons and 6 quaternary carbons. The unambiguous carbon assignment of compound GD-2 (Table 10) was accomplished through analysis of the HMQC (Figures 17a-17c) and HMBC (Figures 18a-18e) spectra.

Table 10 ^{1}H and ^{13}C NMR spectral data of compound GD-2 (in DMSO-d_6) and ^{13}C NMR spectral data of 1,7-dihydroxyxanthone* [10]

Position	1,7-Dihydroxyxanthone	Compound GD-2	
	δ_C (ppm)	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)
1	160.9	160.8	12.62 (1-OH, s)
2	109.5	109.6	6.77 (dd, J = 8.2, 0.6)
3	137.0	137.1	7.69 (dd, J = 8.2, 8.2)
4	107.0	107.1	7.03 (dd, J = 8.2, 0.6)
4a	155.7	155.8	-
5	119.2	119.3	7.53 (d, J = 8.8)
6	125.5	125.5	7.35 (dd, J = 8.8, 3.0)

Table 10 (Continued)

Position	1,7-Dihydroxyxanthone	Compound GD-2	
	δ_C (ppm)	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)
7	154.0	154.1	10.07 (7-OH, s)
8	107.8	107.7	7.44 (d, J = 3.0)
8a	120.4	120.4	-
9	181.4	181.5	-
9a	107.8	107.8	-
10a	149.2	149.3	-

* From Westerman *et al.*, 1977

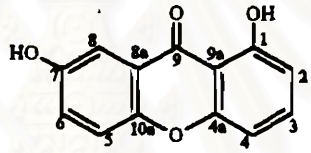


Figure 19 Structure of compound GD-2

The HMQC spectrum (Figures 17a-17c) revealed correlation between the directly coupled ^1H and ^{13}C nuclei. According to the HMQC spectrum, all protons and protonated carbons of compound GD-2 could be assigned. The directly coupled ^1H and ^{13}C are summarized in Table 11.

Table 11 The carbon-proton correlations of compound GD-2 observed in the HMQC spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-2	109.6	6.77
C-3	137.1	7.69
C-4	107.1	7.03
C-5	119.3	7.53
C-6	125.5	7.35
C-8	107.7	7.44

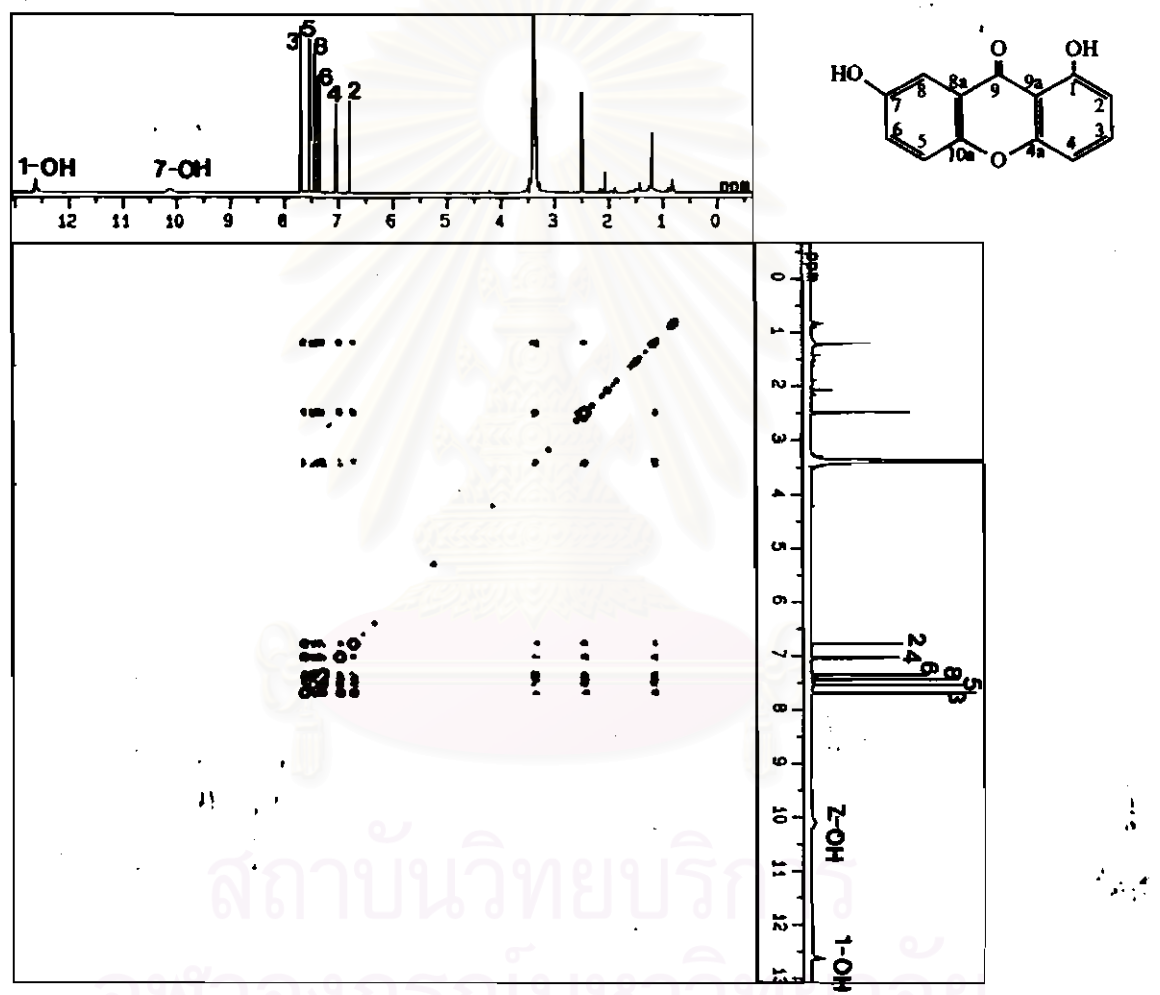


Figure 13a NOESY spectrum of compound GD-2 (in DMSO-d_6)

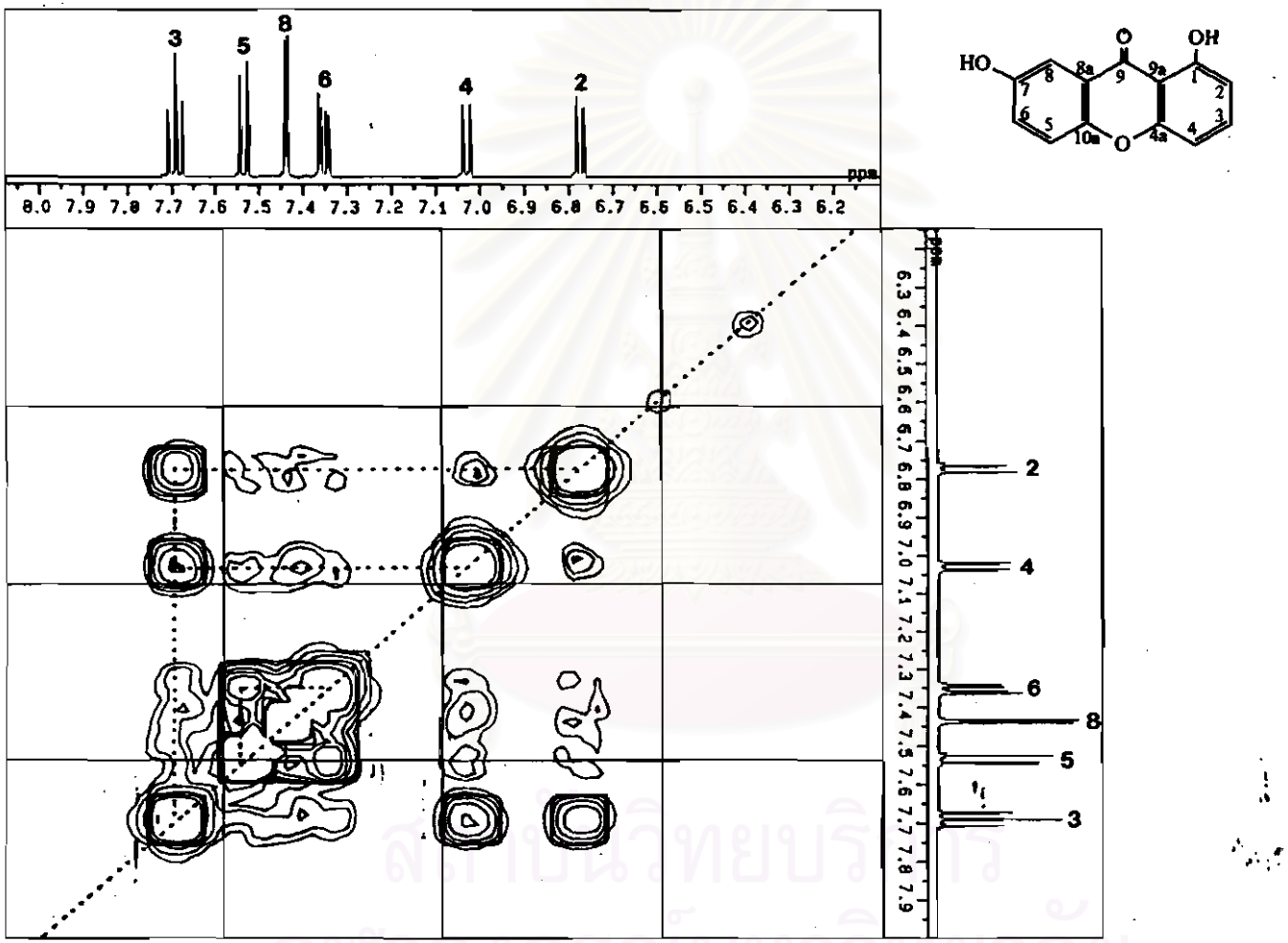


Figure 13b NOESY spectrum of compound GD-2 (in DMSO-d_6) (expanded from 6.2-8.0 ppm)

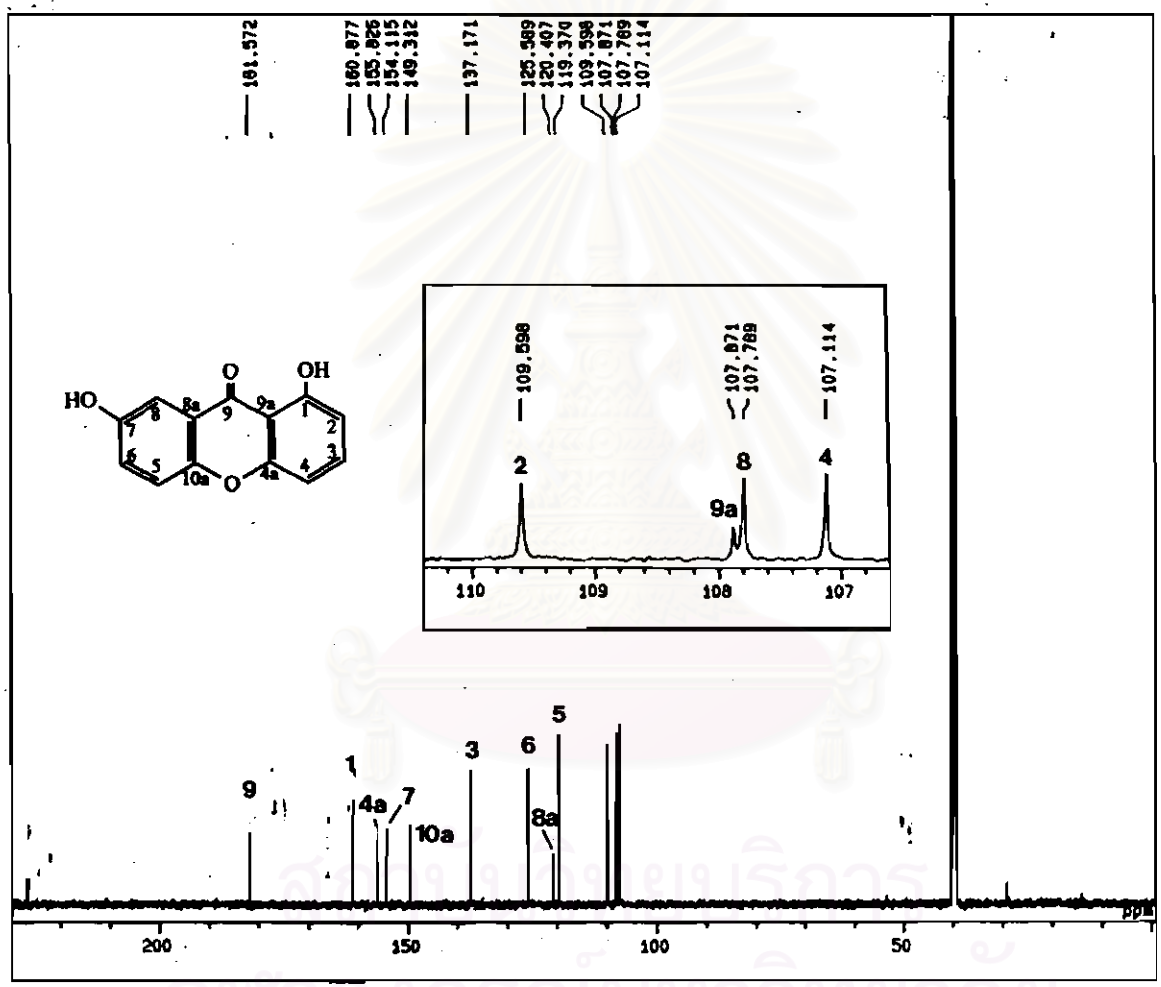


Figure 15 125 MHz ^{13}C NMR spectrum of compound GD-2 (in DMSO-d_6)

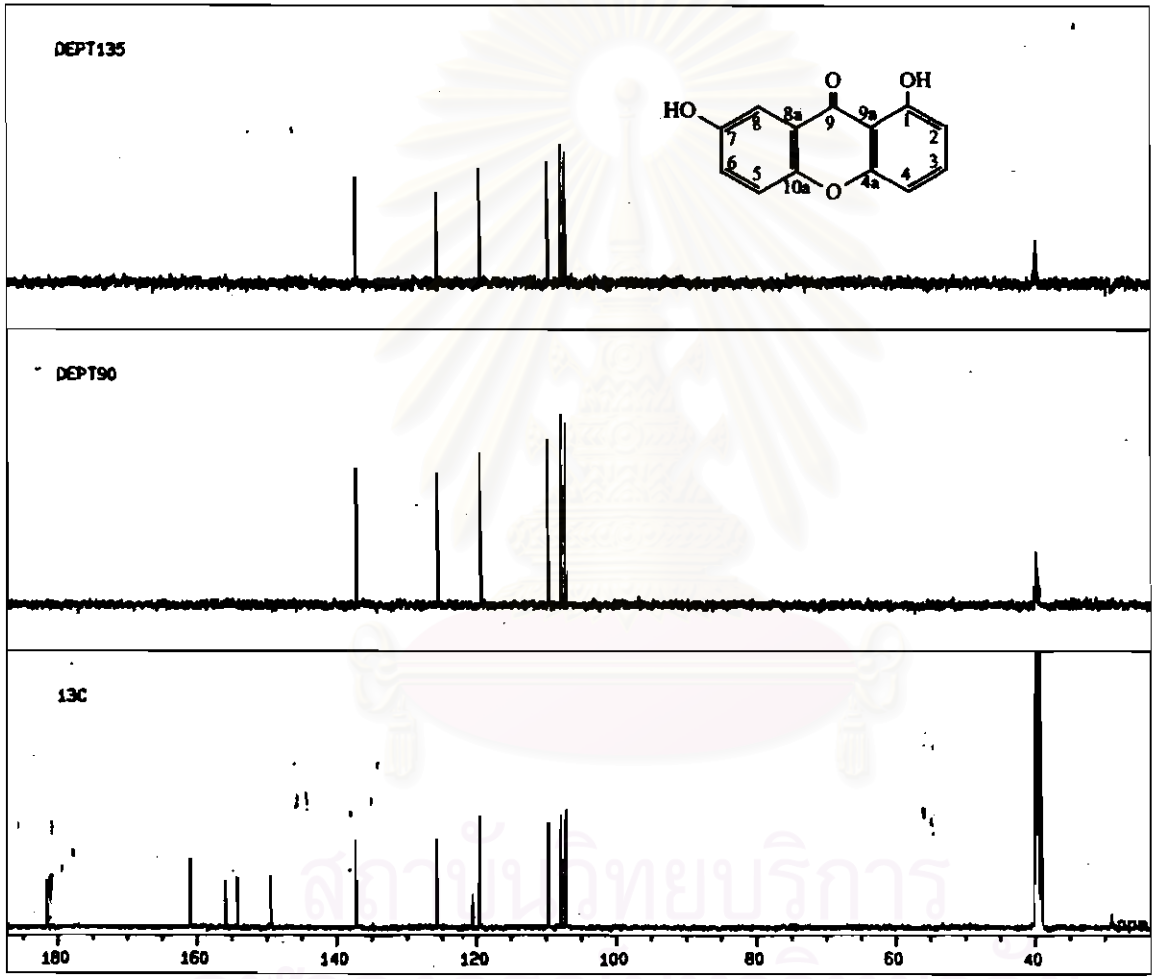


Figure 16 DEPT spectrum of compound GD-2 (in DMSO-d_6)

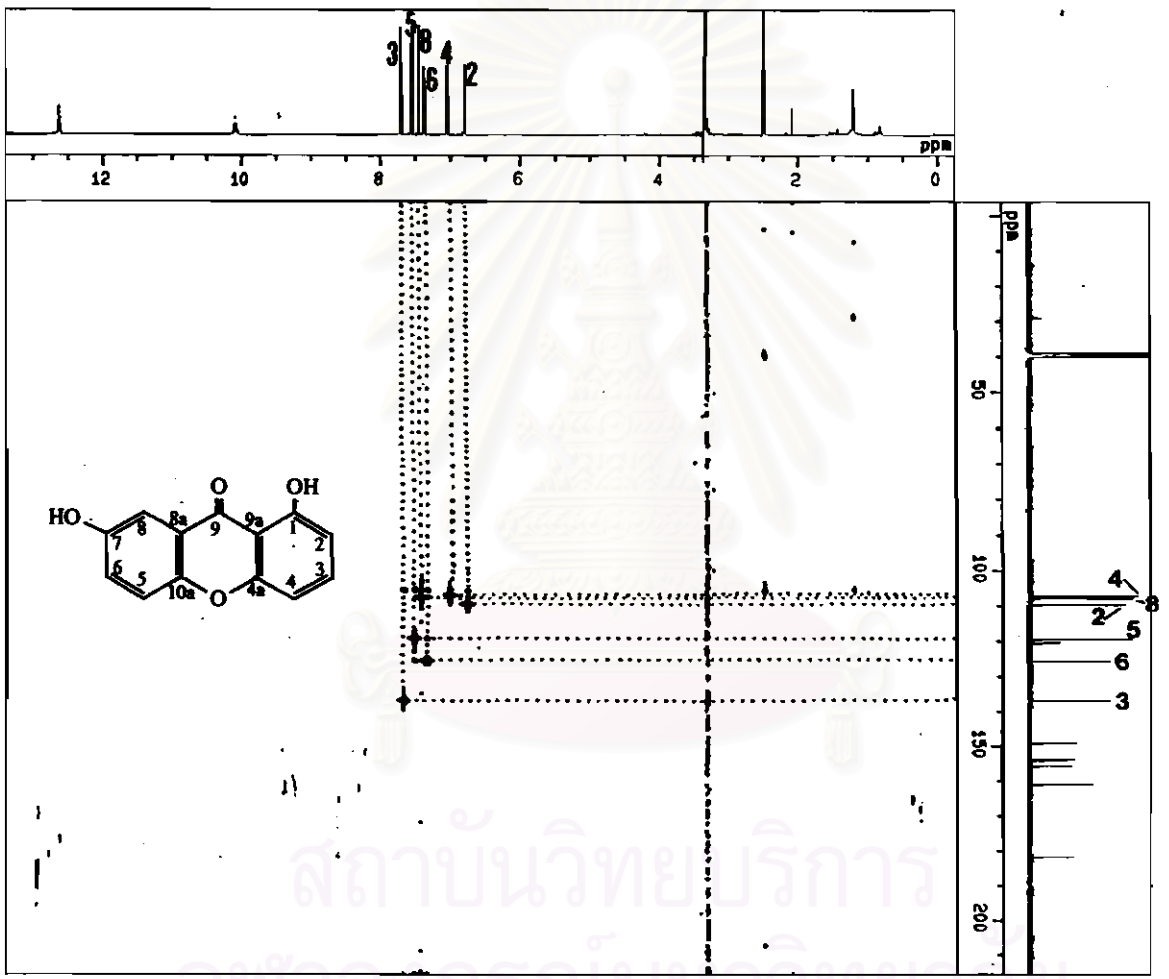


Figure 17a HMQC spectrum of compound GD-2 (in DMSO-d_6), [δ_H 0.00-12.75 ppm, δ_C 0-200 ppm]

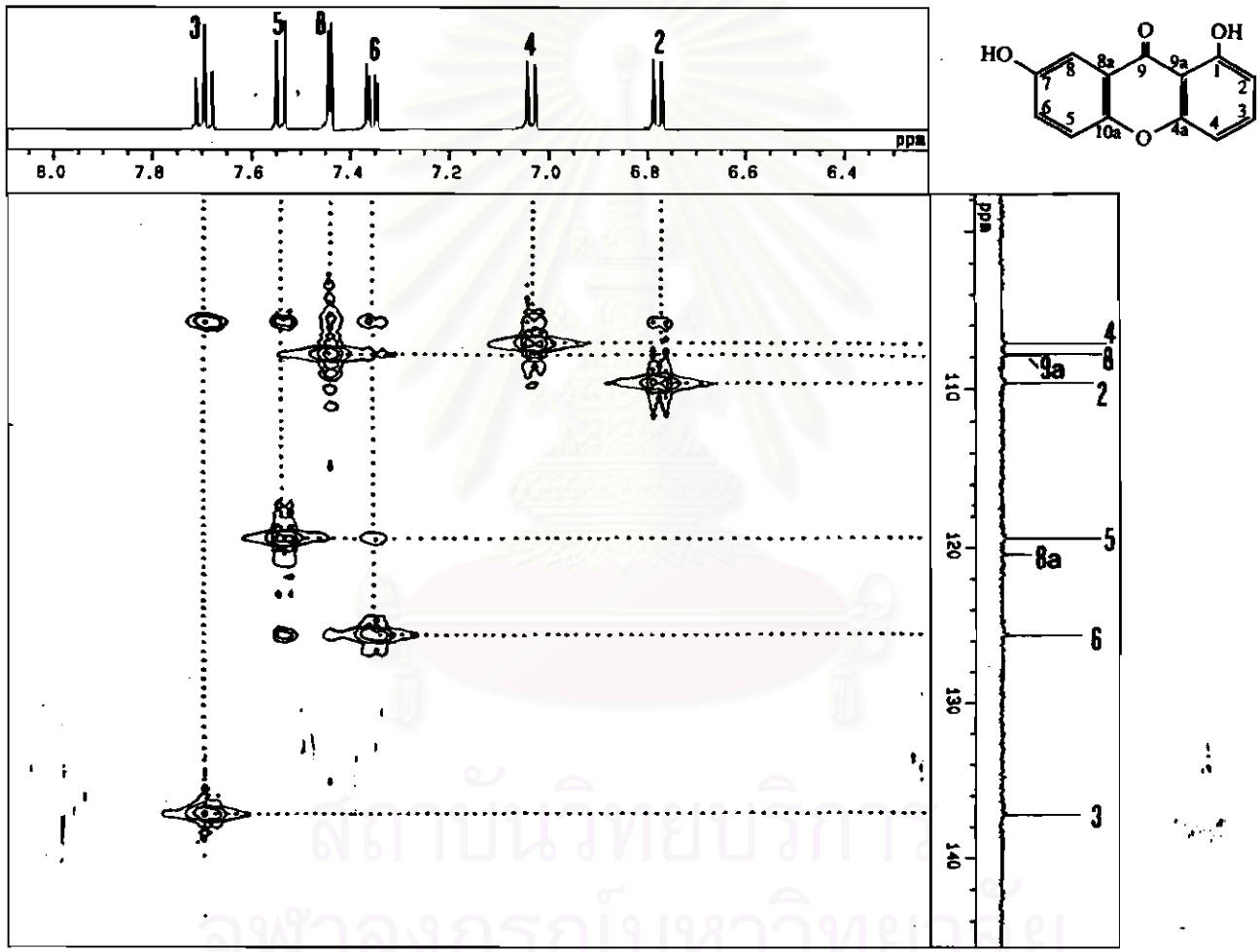


Figure 17b HMQC spectrum of compound GD-2 (in DMSO-d_6), [δ_H 6.40-8.00 ppm, δ_C 100-146 ppm]

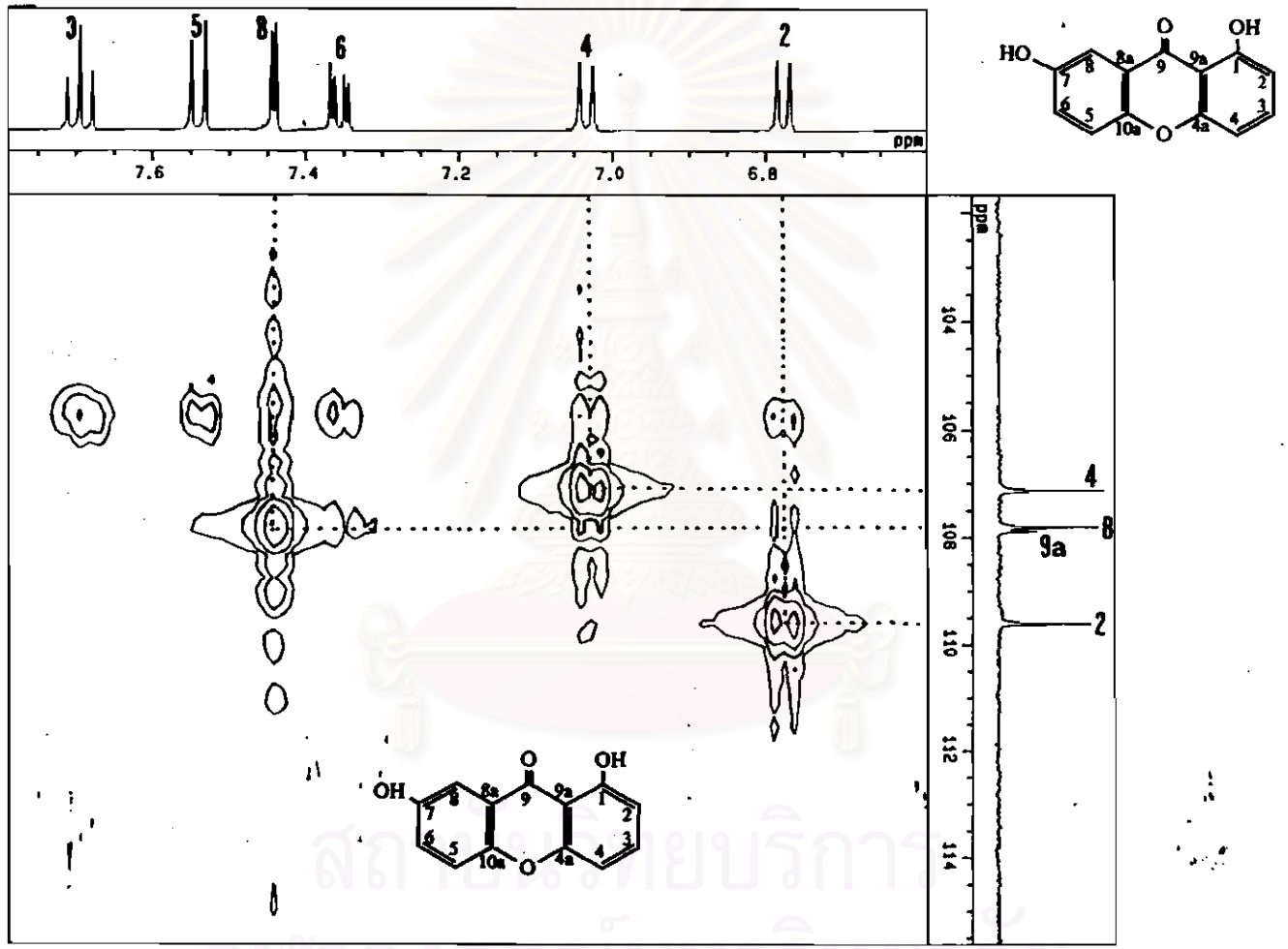


Figure 17c HMQC spectrum of compound GD-2 (in DMSO-d_6), [δ_H 6.6-7.8 ppm, δ_C 102-114 ppm]

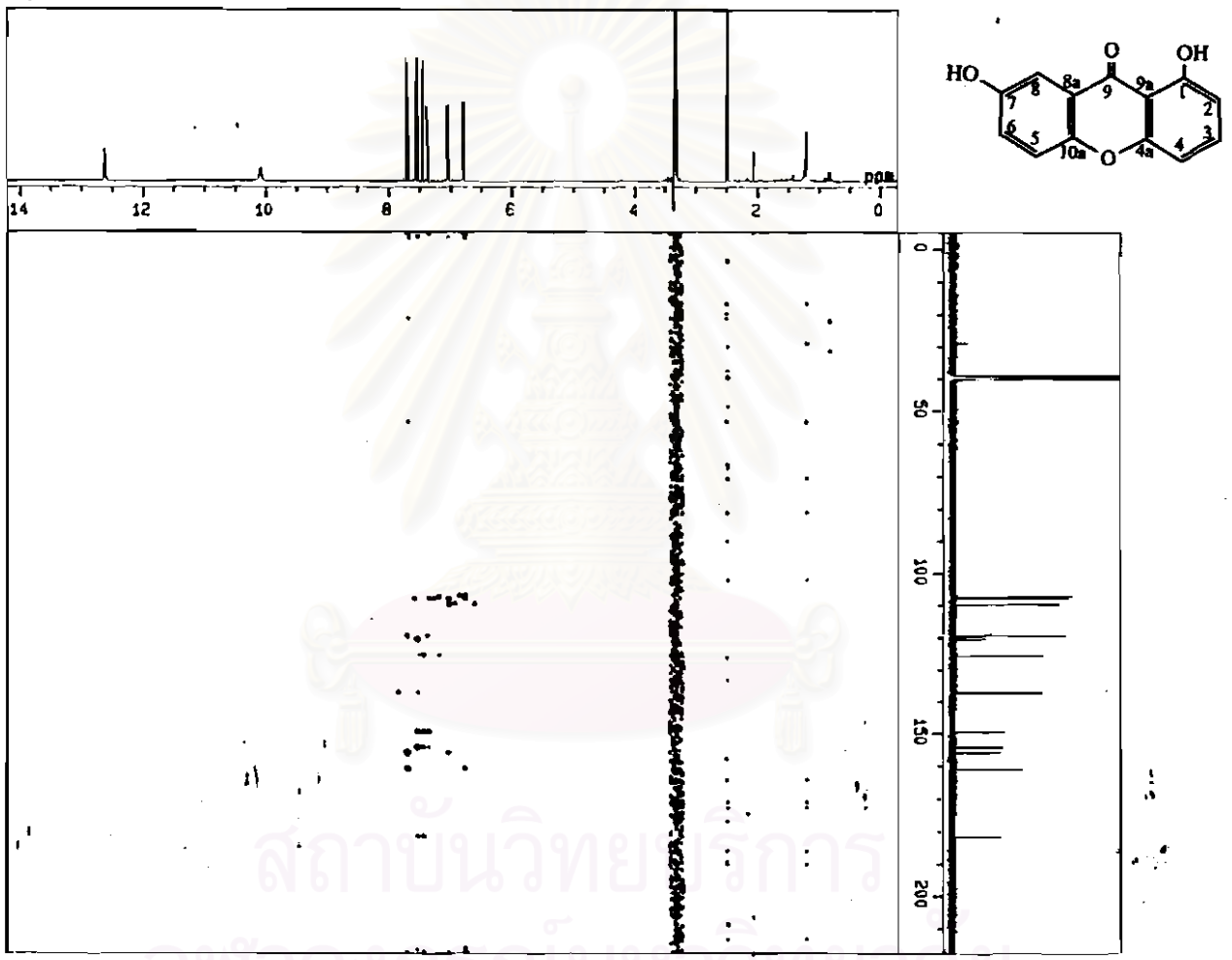


Figure 18a HMBC spectrum of compound GD-2 (in DMSO-d_6), [δ_H 0.00-14.00 ppm, δ_C 0-200 ppm]

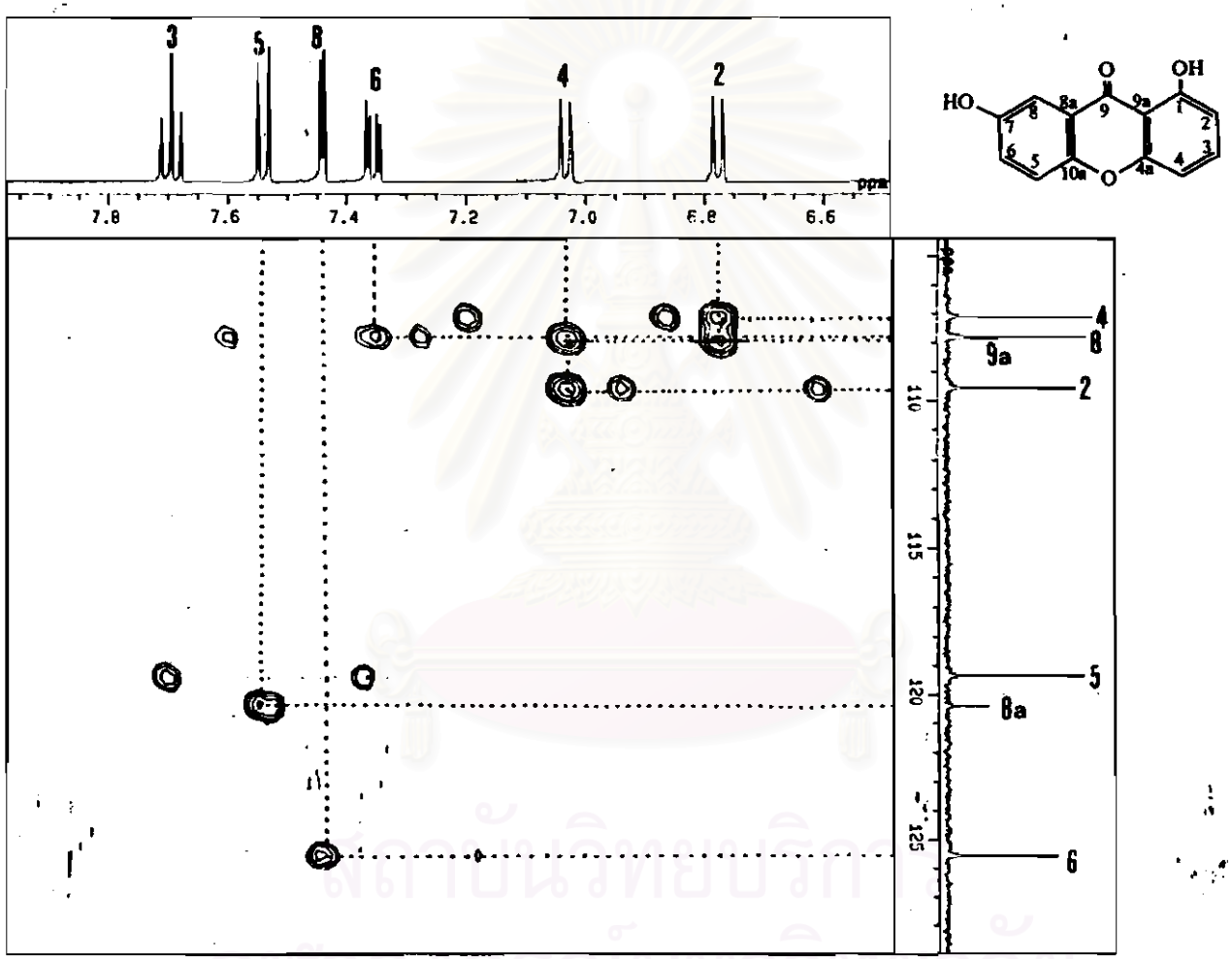


Figure 18b HMBC spectrum of compound GD-2 (in DMSO-d_6), [δ_H 6.70-8.00 ppm, δ_C 105-129 ppm]

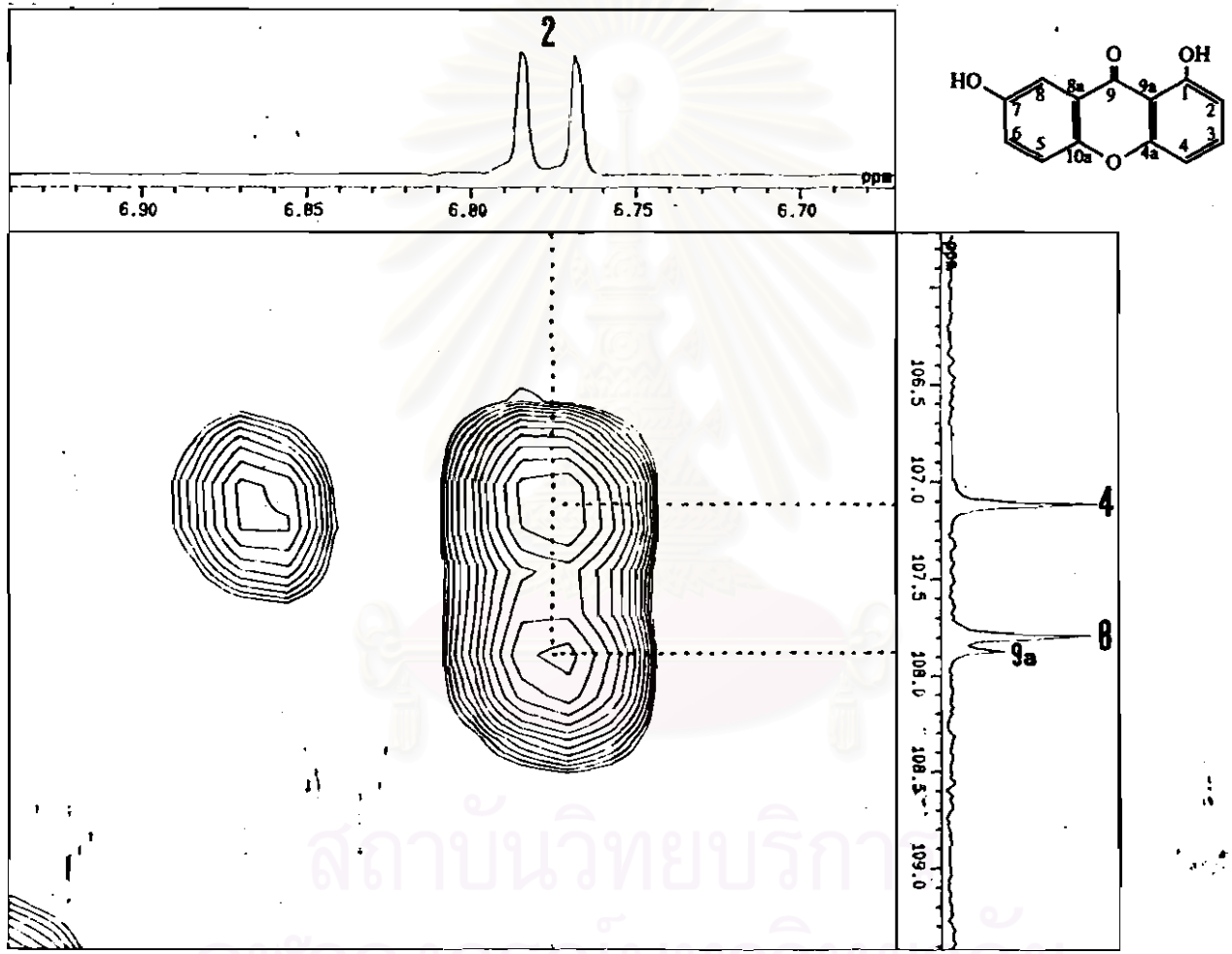


Figure 18c HMBC spectrum of compound GD-2 (in DMSO-d_6), [δ_H 6.70-6.90 ppm, δ_C 106-109 ppm]

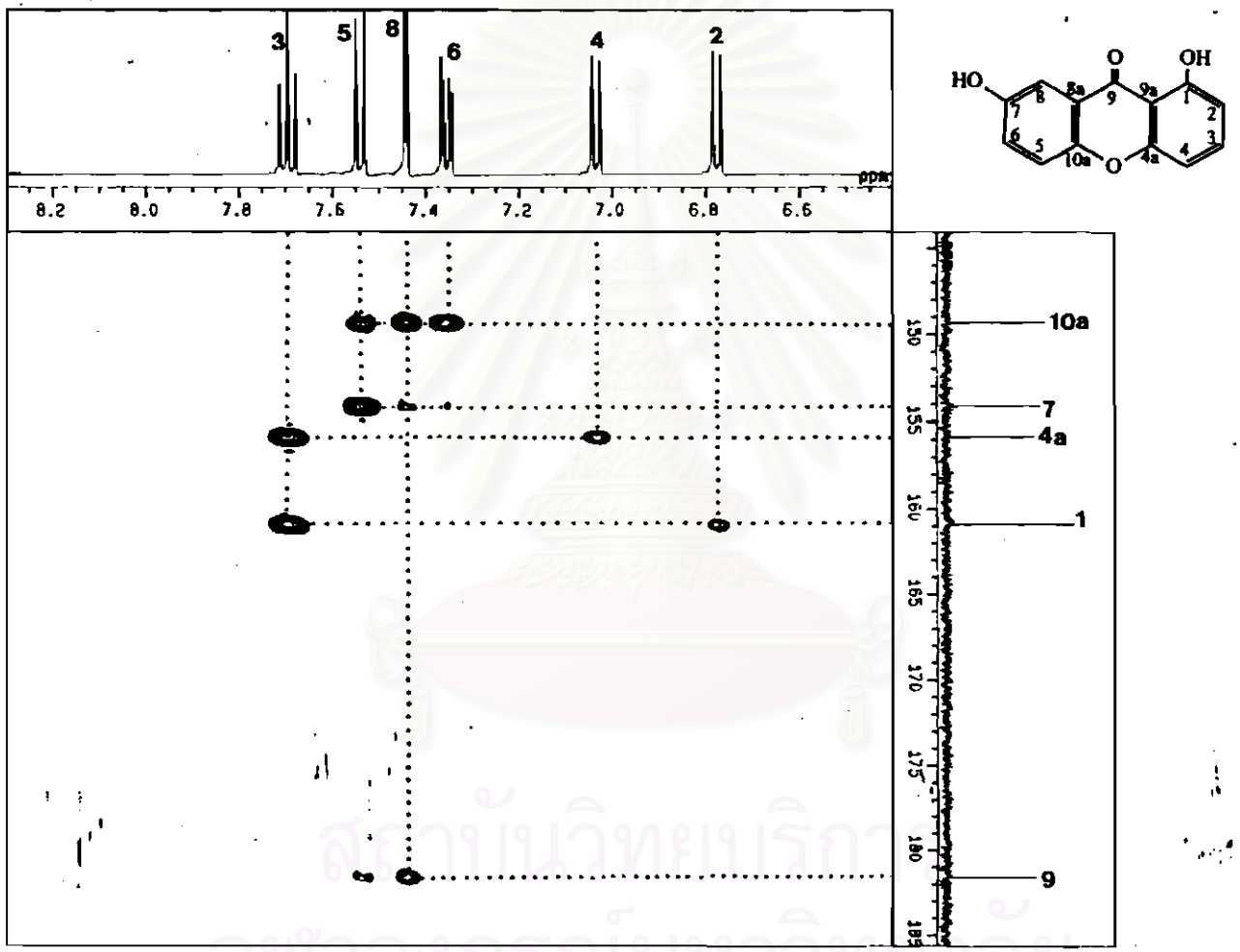


Figure 18d HMBC spectrum of compound GD-2 (in DMSO-d_6), [δ_H 6.40-8.20 ppm, δ_C 145-185 ppm]

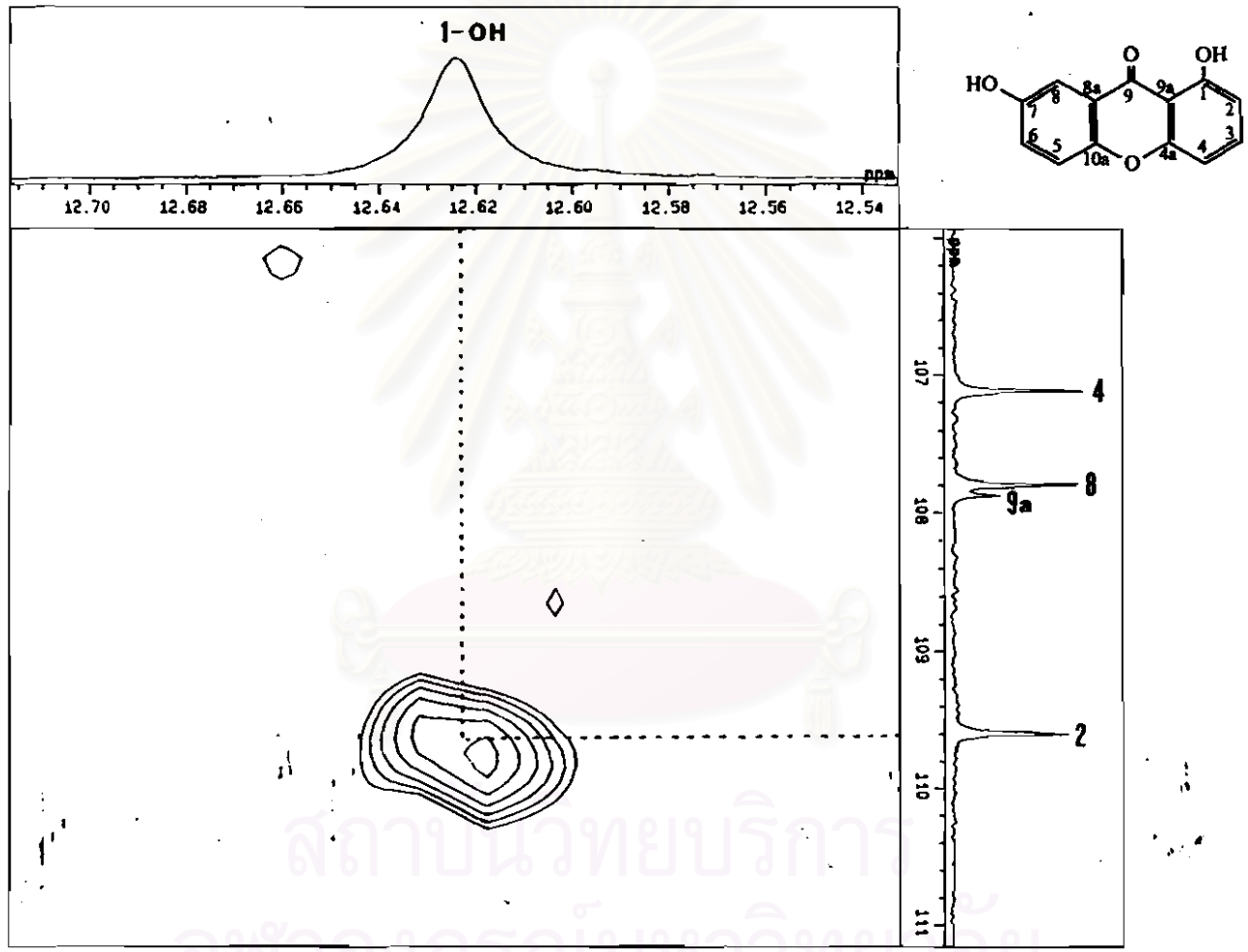


Figure 18e HMBC spectrum of compound GD-2 (in DMSO-d_6), [δ_H 12.54-12.70 ppm, δ_C 106-111 ppm]

The HMBC spectrum (Figures 18a-18c) showed correlations of the long-range coupled ^{1}H and ^{13}C nuclei, providing information for the assignment of the quaternary carbons, as shown in Figure 20.

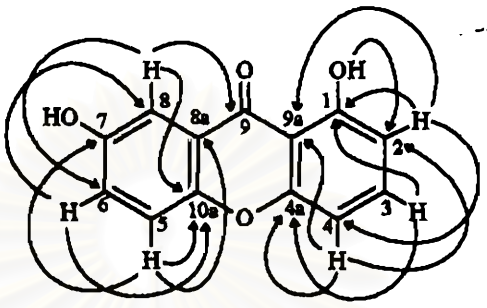
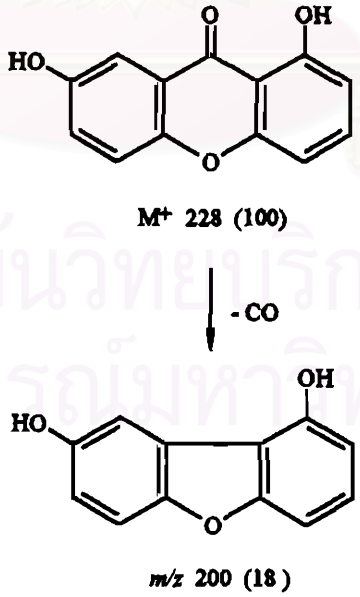


Figure 20 Long-range C-H correlations of compound GD-2 observed in HMBC spectrum

The mass spectrum of compound GD-2 exhibited a characteristic of the xanthone nucleus by the loss of carbonmonoxide giving an ion peak at *m/z* 200 (Budzikiewicz, Djerassi and Williams, 1964).



Scheme 2 Mass fragmentation of compound GD-2

3. Structure Determination of Compound GD-3 [19]

Compound GD-3 was obtained as golden yellow needles from fraction V-4 of the chloroform extract by vacuum liquid chromatographic technique.

The EI mass spectrum of compound GD-3 (Figure 21) exhibited a molecular ion at *m/z* 312, consistent with a molecular formula of $C_{18}H_{16}O_5$. The UV (Figure 22) and IR (Figure 23) spectra of compound GD-3 were similar to those of compound GD-2, suggesting a xanthone basic skeleton. The UV spectrum demonstrated absorptions in methanol at λ_{max} 406, 316, 265 and 248 nm. The IR spectrum exhibited maximum absorption bands at 3300 (O-H stretching), 2960 (C-H stretching), 1585 (C=O stretching), 1460 (olefinic carbon) and 1290 (ether linkage) cm^{-1}.

In the ^1H NMR spectrum (Figures 24a-24b), the presence of the following features was indicated: a chelated hydroxyl group [δ 12.75 (1H, s)], a 1,1-dimethylallyl moiety [δ 1.46 (6H, s), 4.99 (1H, dd, J = 18.0, 1.5 Hz), 5.00 (1H, dd, J = 10.3, 1.5 Hz) and 6.23 (1H, dd, J =18.0, 10.3 Hz)] and four aromatic protons [δ 7.27 (1H, s), 7.28 (1H, t, J = 7.9 Hz), 7.34 (1H, dd, J = 7.9, 1.5 Hz) and 7.59 (1H, dd, J = 7.9, 1.5 Hz)]. The ^{13}C NMR (Figure 25) and DEPT spectra (Figure 26) showed one carbonyl group, five methine carbons, one methylene carbon, two methyl carbons and nine quaternary carbons.

Comparison of the ^1H and ^{13}C NMR spectral data of compound GD-3 with the literature data established that compound GD-3 was identical with 12b-hydroxy-des-D-garcigerrin A [19]. The xanthone 12b-hydroxy-des-D-garcigerrin A was first isolated from the root bark of *G. gerrardii* (Sordat-Diserens *et al.*, 1989). The ^1H and ^{13}C NMR spectral properties of this compound have been extensively studied (Sordat-Diserens *et al.*, 1989 and Iinuma *et al.*, 1995c).

In the present investigation, several 2-D NMR experiments, including ^1H-^1H COSY, NOESY, HMQC and HMBC, were performed. The results from these

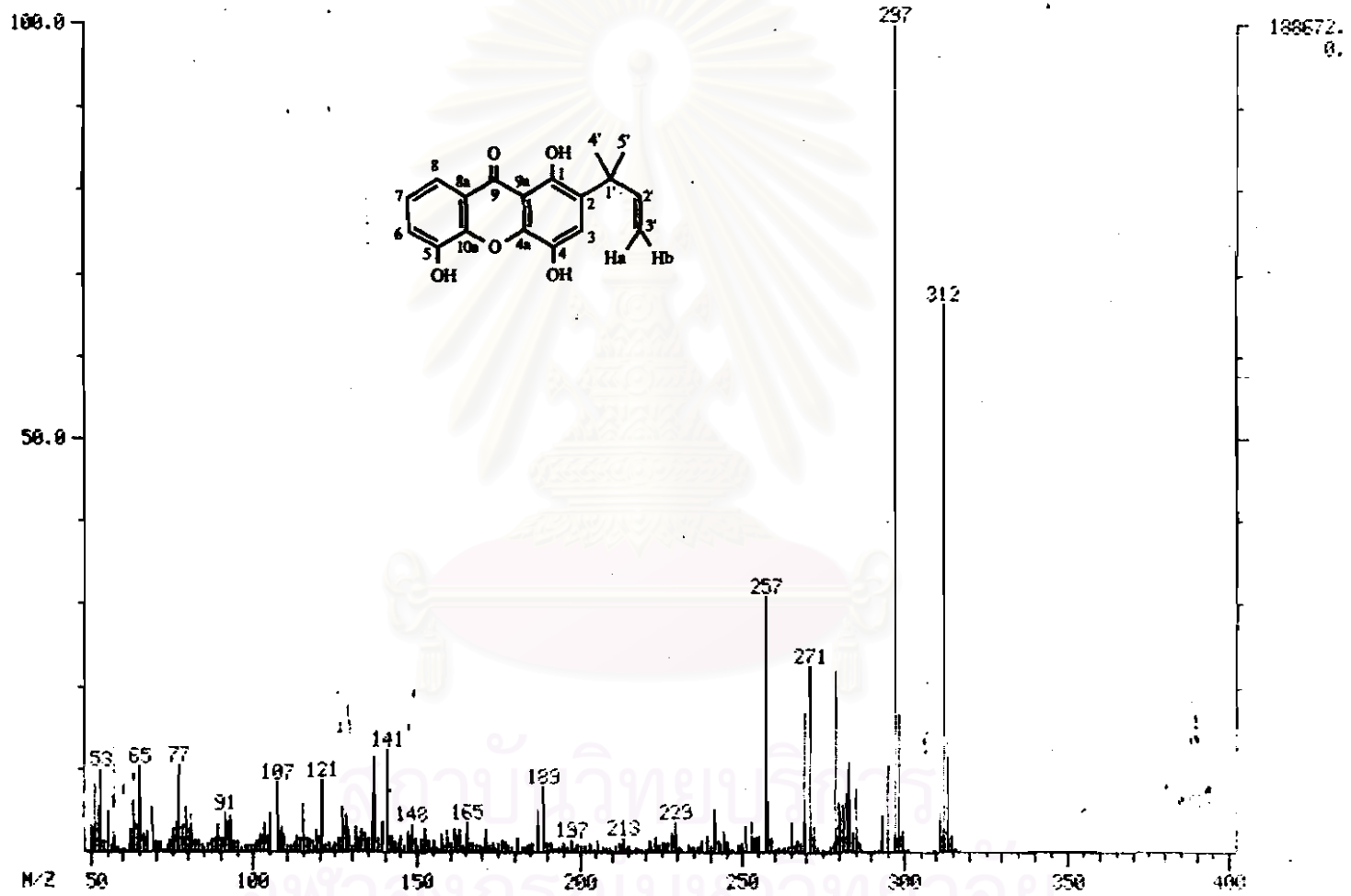


Figure 21 EI mass spectrum of compound GD-3

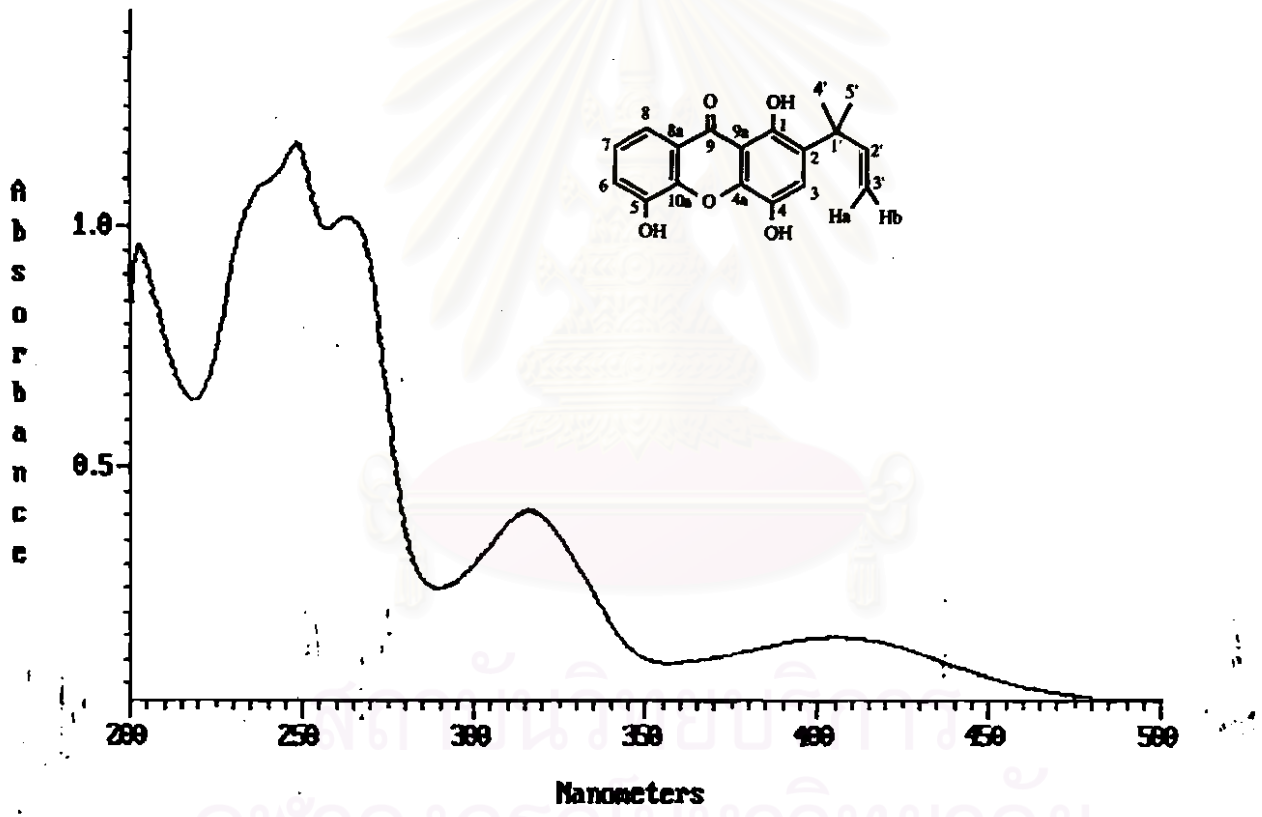


Figure 22 UV spectrum of compound GD-3 (in methanol)

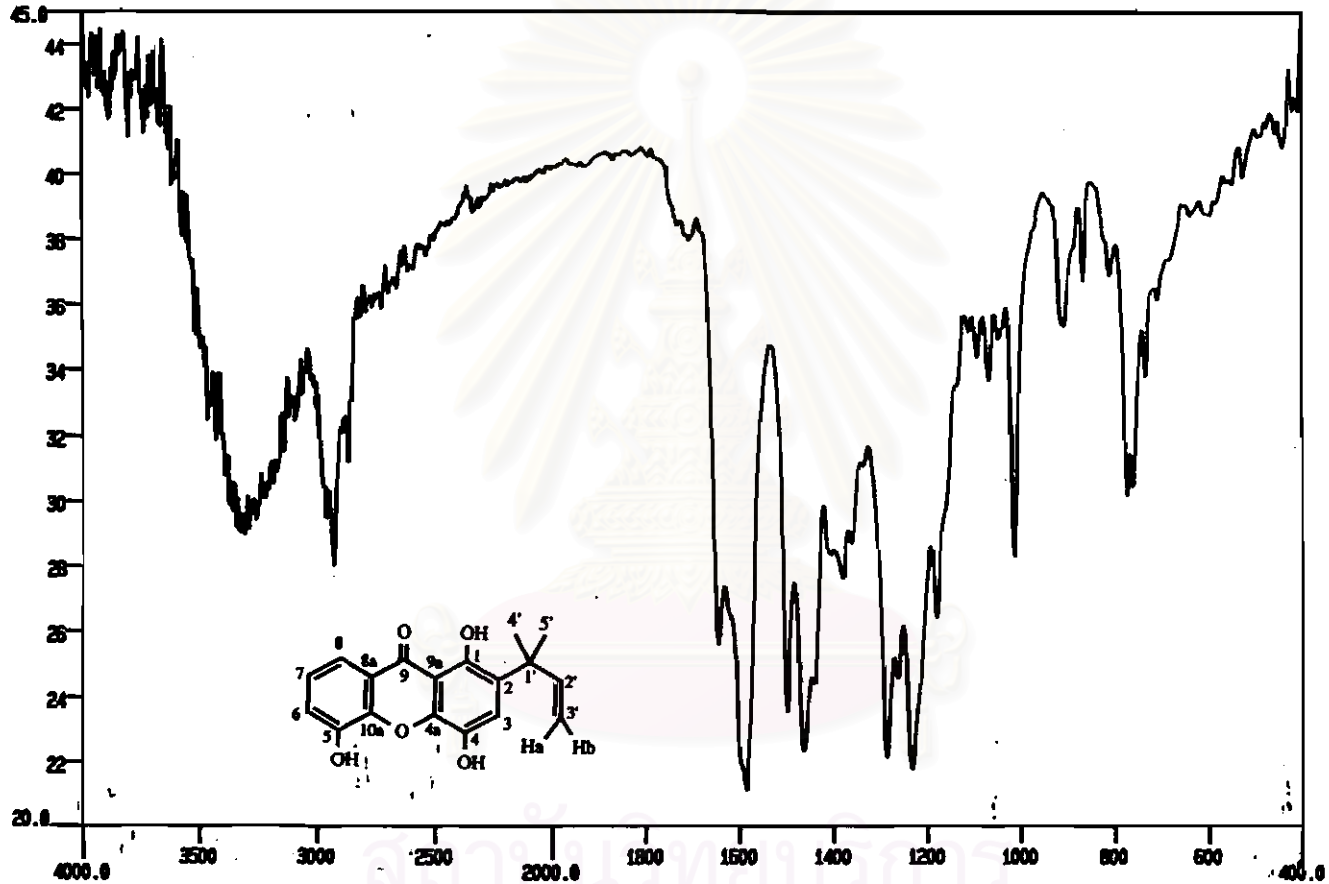


Figure 23 IR spectrum of compound GD-3 (film)

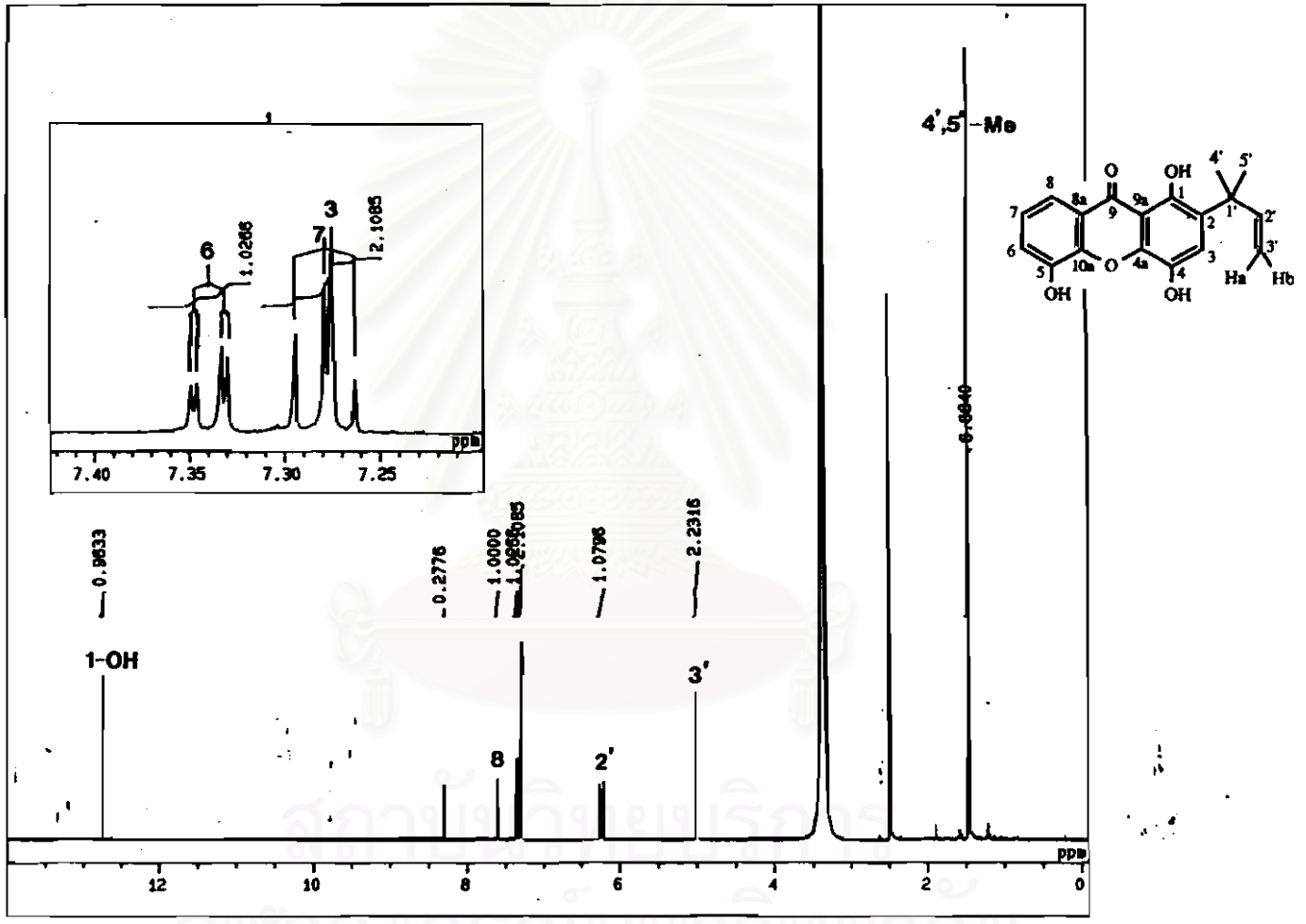


Figure 24a 500 MHz ^{1}H NMR spectrum of compound GD-3 (in DMSO-d_6)

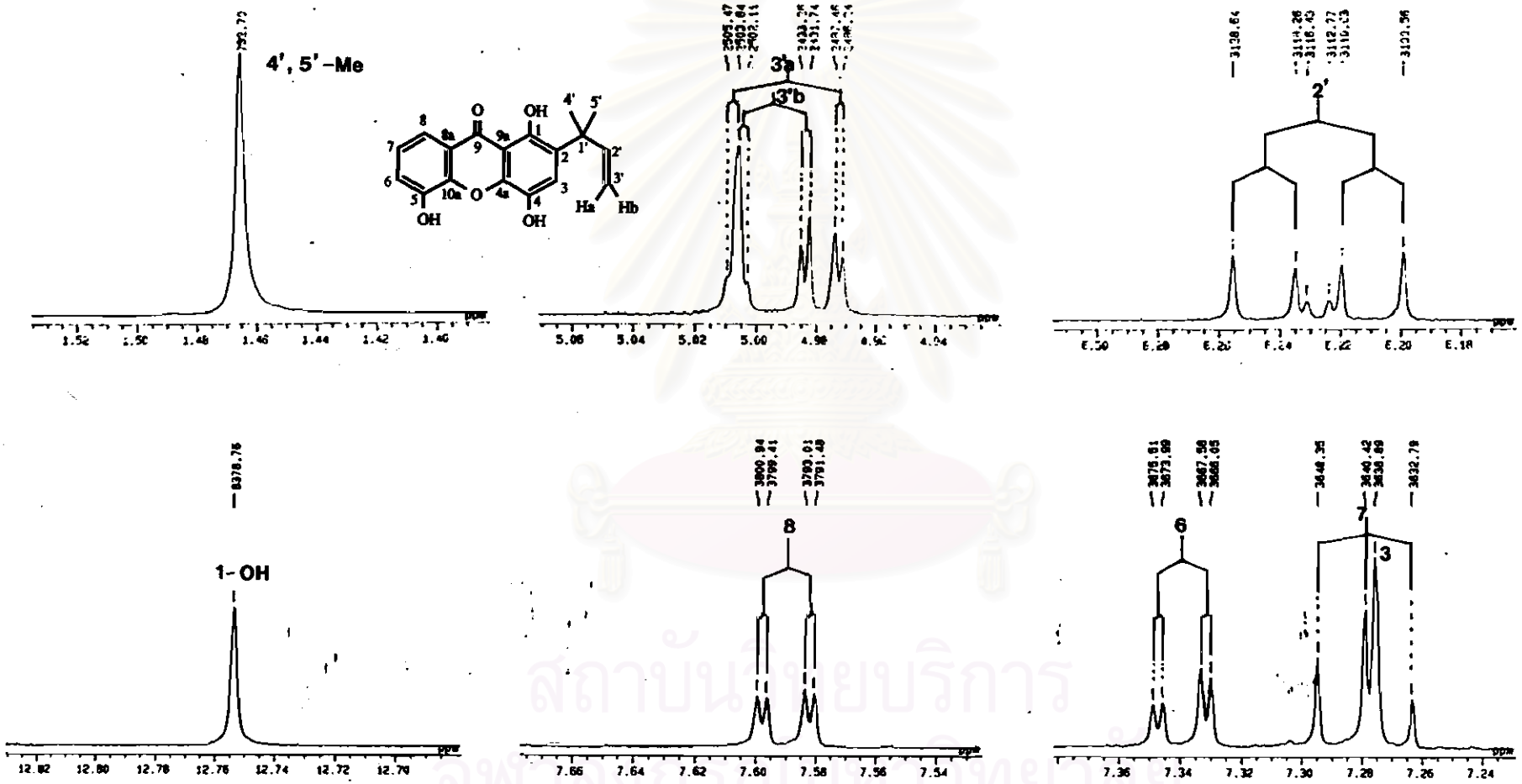


Figure 24b 500 MHz ^{1}H NMR spectrum of compound GD-3 (in DMSO-d_6) (expanded from 1.40-12.82 ppm)

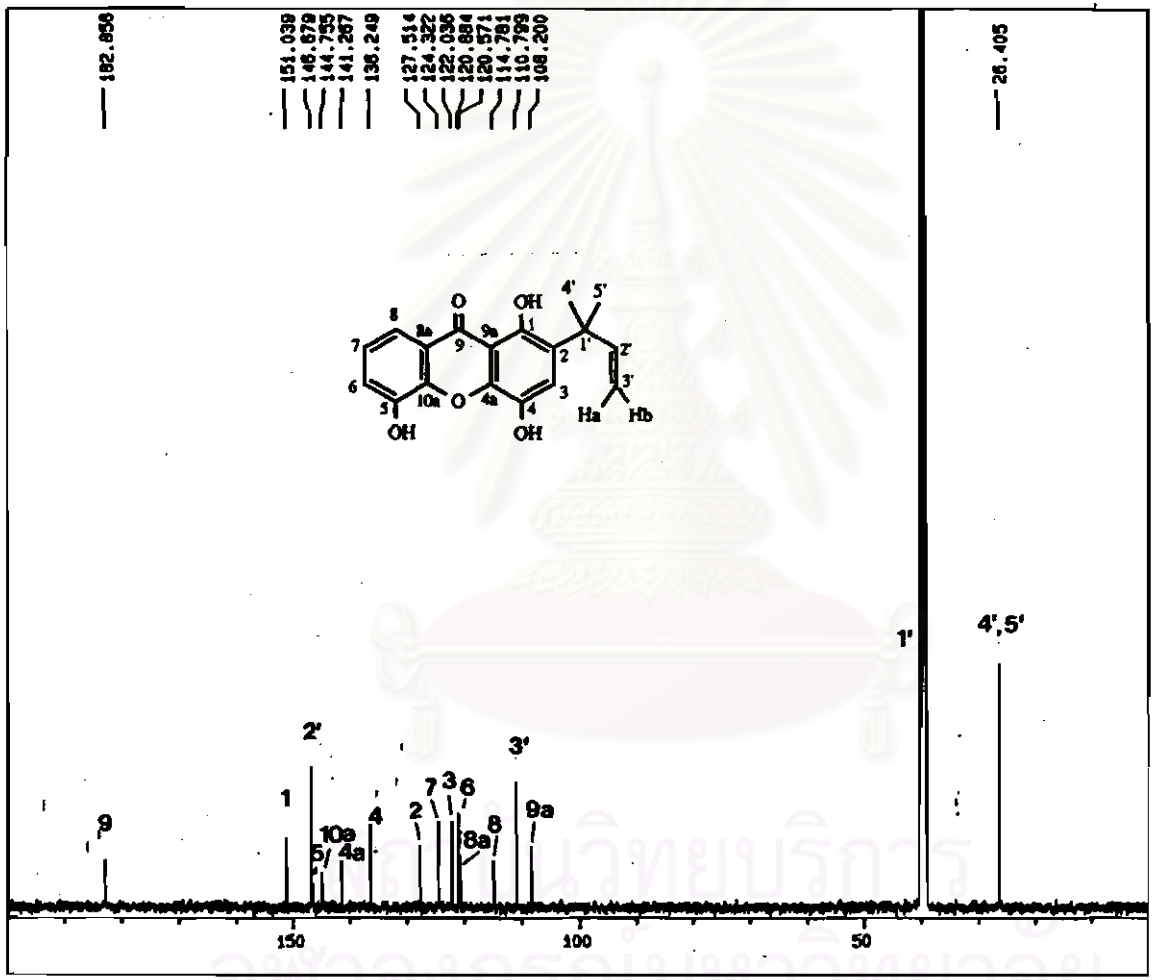


Figure 25 125 MHz ^{13}C NMR spectrum of compound GD-3 (in DMSO-d_6)

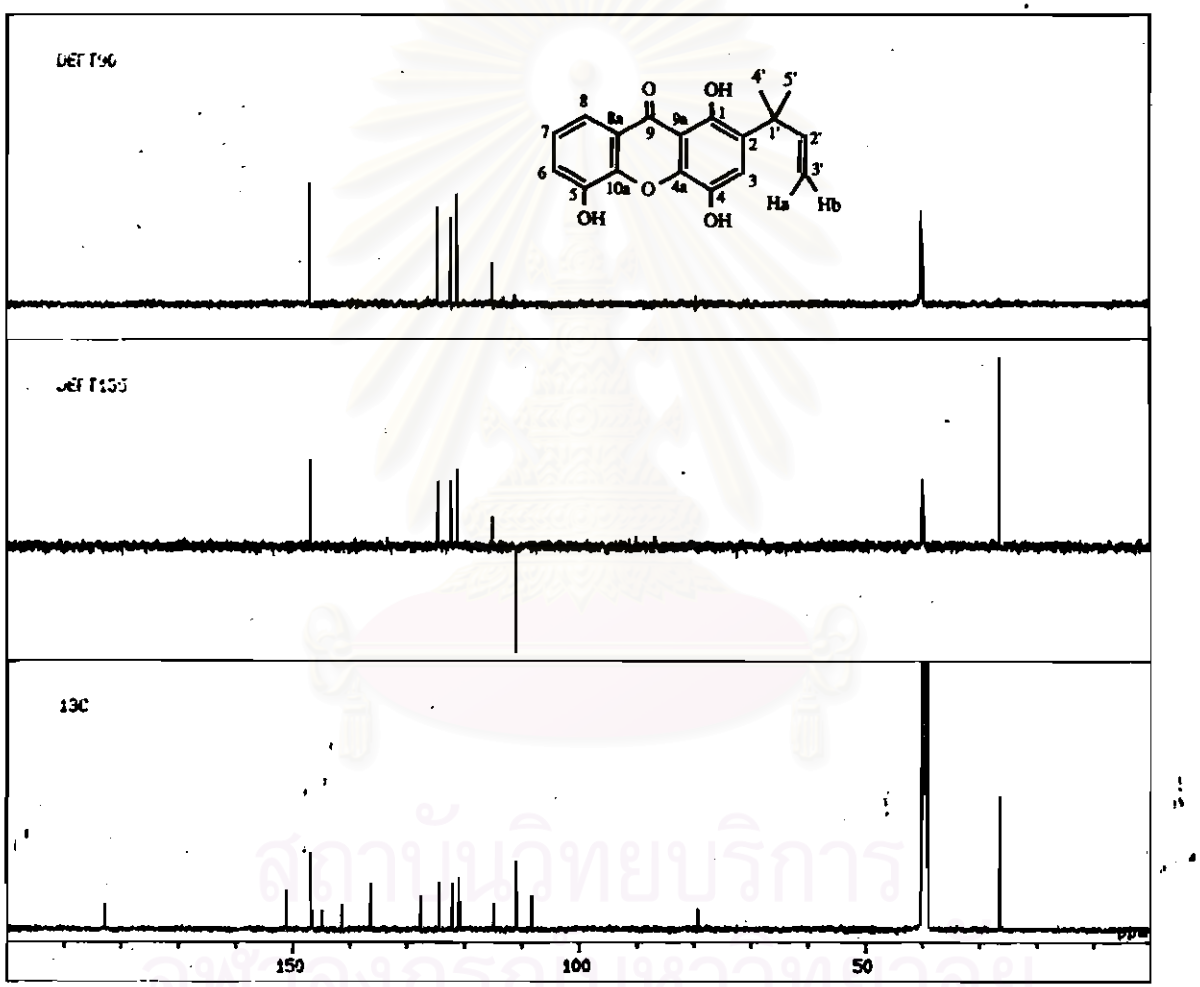


Figure 26 DEPT spectrum of compound GD-3 (in DMSO-d_6)

experiments confirmed ealier assignments (Sordat-Diserens *et al.*, 1989 and Iinuma *et al.*, 1995c).

The ^{1}H-^{1}H COSY spectrum (Figures 27a-27b) showed aromatic coupling between H-6 and H-7, H-7 and H-8, and H-6 and H-8. Olefinic proton coupling was also observed between H-2′ and H-3′a, H-2′ and H-3′b, and H-3′a and H-3′b.

A NOESY experiment (Figures 28a-28c) revealed an NOE interaction between the methyl signals of 1,1-dimethylallyl moiety at δ 1.46 and a phenolic proton at δ 12.75 (1-OH). In addition, an NOE between the two methyl signals and H-3 was also displayed. The NOE effects confirmed the location of 1,1-dimethylallyl moiety and the substitution pattern of the aromatic rings. The NOE interactions observed in the NOESY spectrum of compound GD-3 are shown in Figure 29.

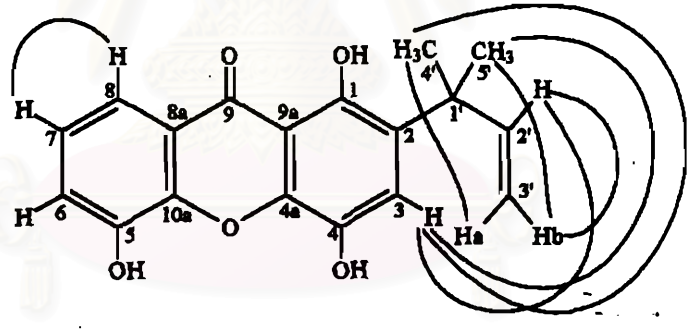


Figure 29 Results from the NOESY experiment of compound GD-3

According to the HMQC spectrum of compound GD-3 (Figures 30a-30d), all protonated carbons could be assigned (Table 12).

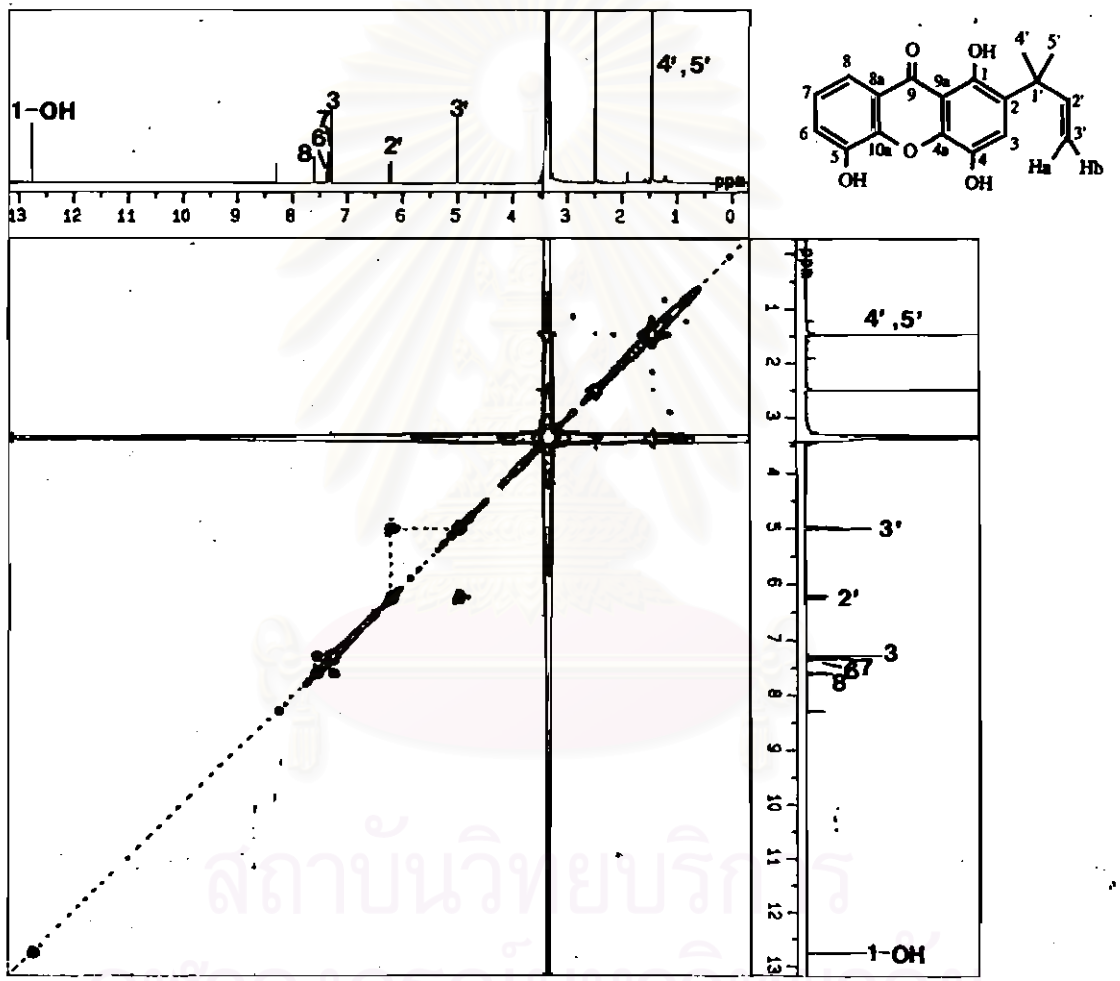


Figure 27a ^{1}H-^{1}H COSY spectrum of compound GD-3 (in DMSO-d_6)

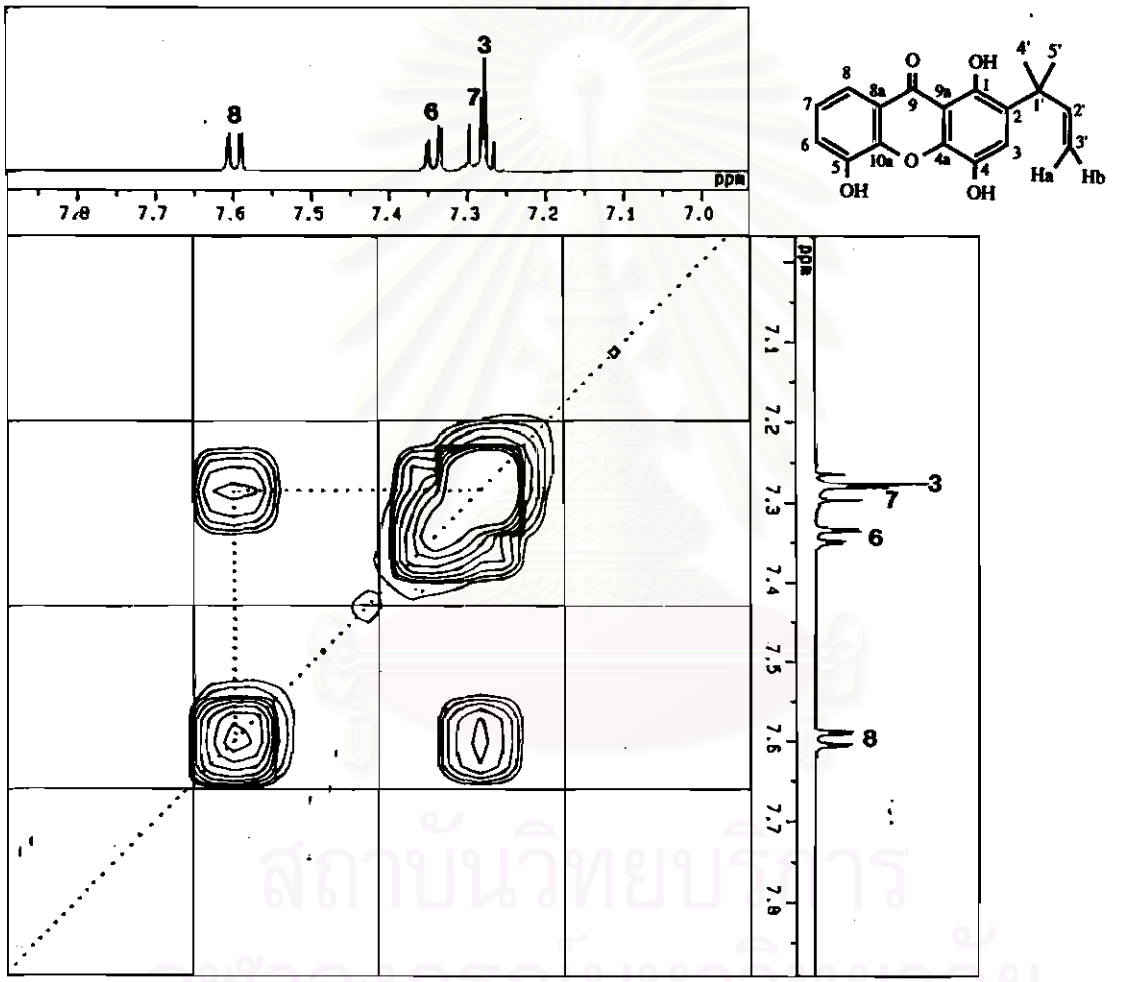


Figure 27b ^{1}H-^{1}H COSY spectrum of compound GD-3 (in DMSO-d_6) (expanded from 7.0-7.8 ppm)

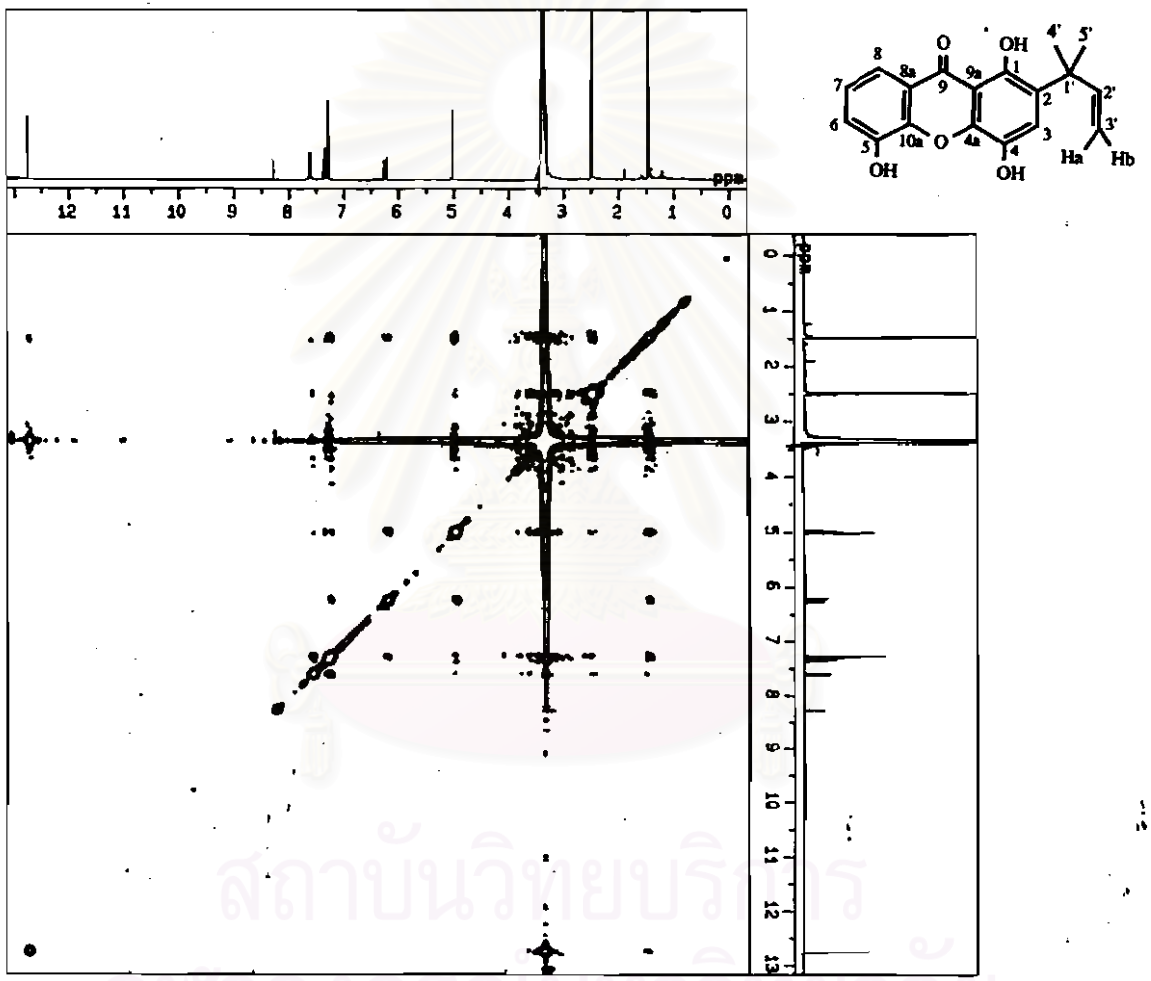


Figure 28a NOESY spectrum of compound GD-3 (in DMSO-d_6)

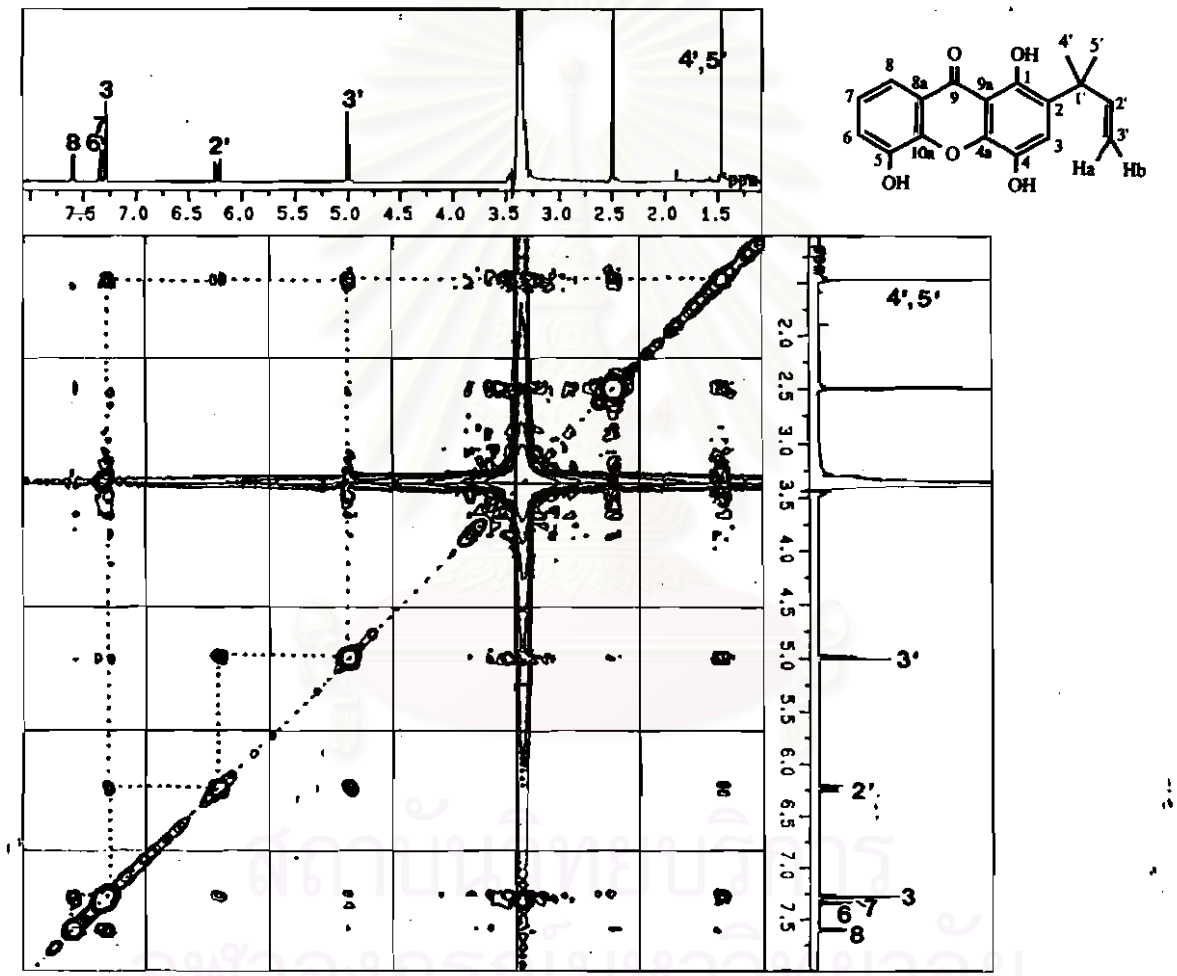


Figure 28b NOESY spectrum of compound GD-3 (in DMSO-d_6) (expanded from 1.5-7.5 ppm)

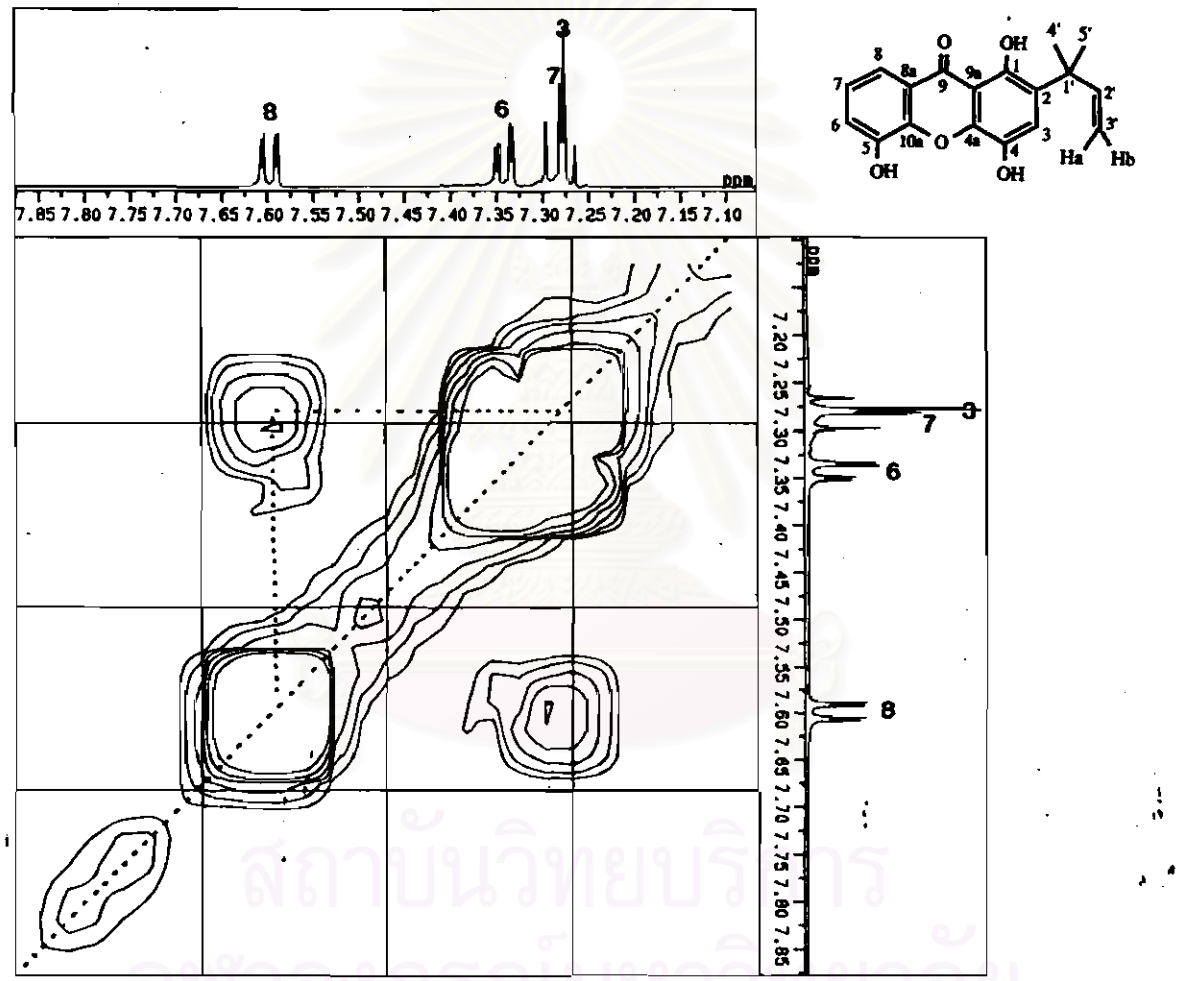


Figure 28c NOESY spectrum of compound GD-3 (in DMSO-d_6) (expanded from 7.10-7.85 ppm)

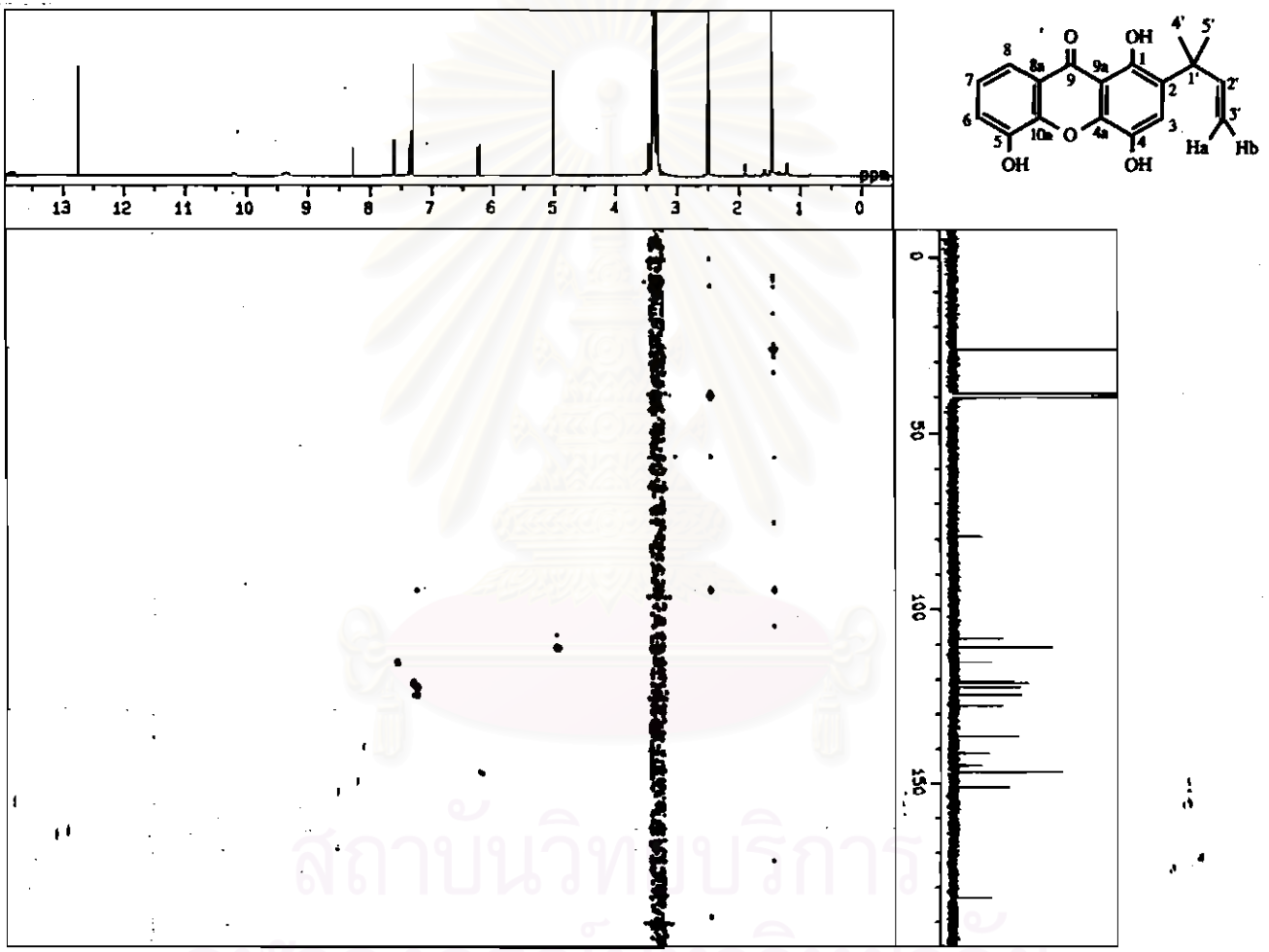


Figure 30a HMQC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 0.00-14.00 ppm, δ_C 0-200 ppm]

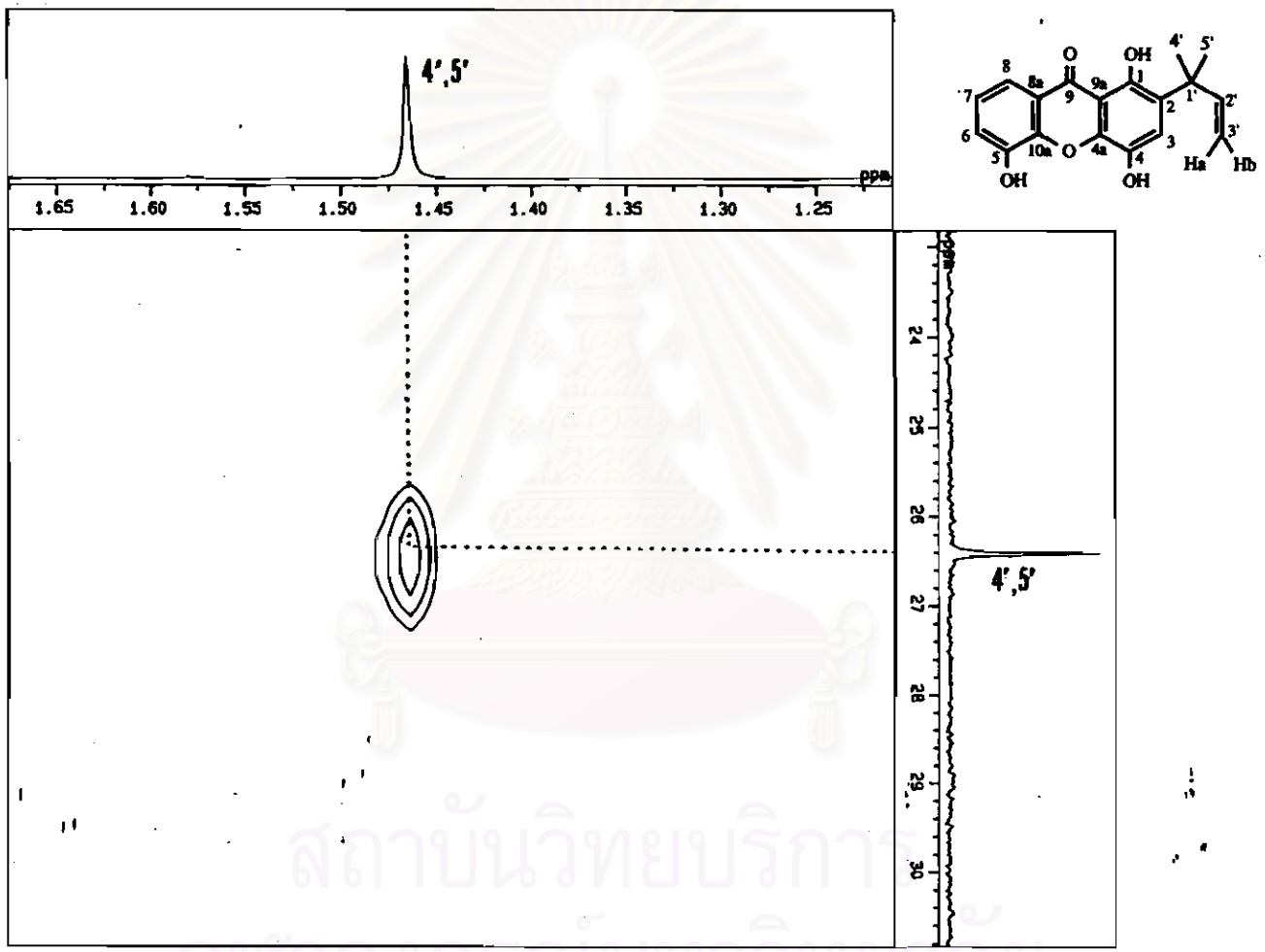


Figure 30b HMQC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 1.25-1.65 ppm, δ_C 23-30 ppm]

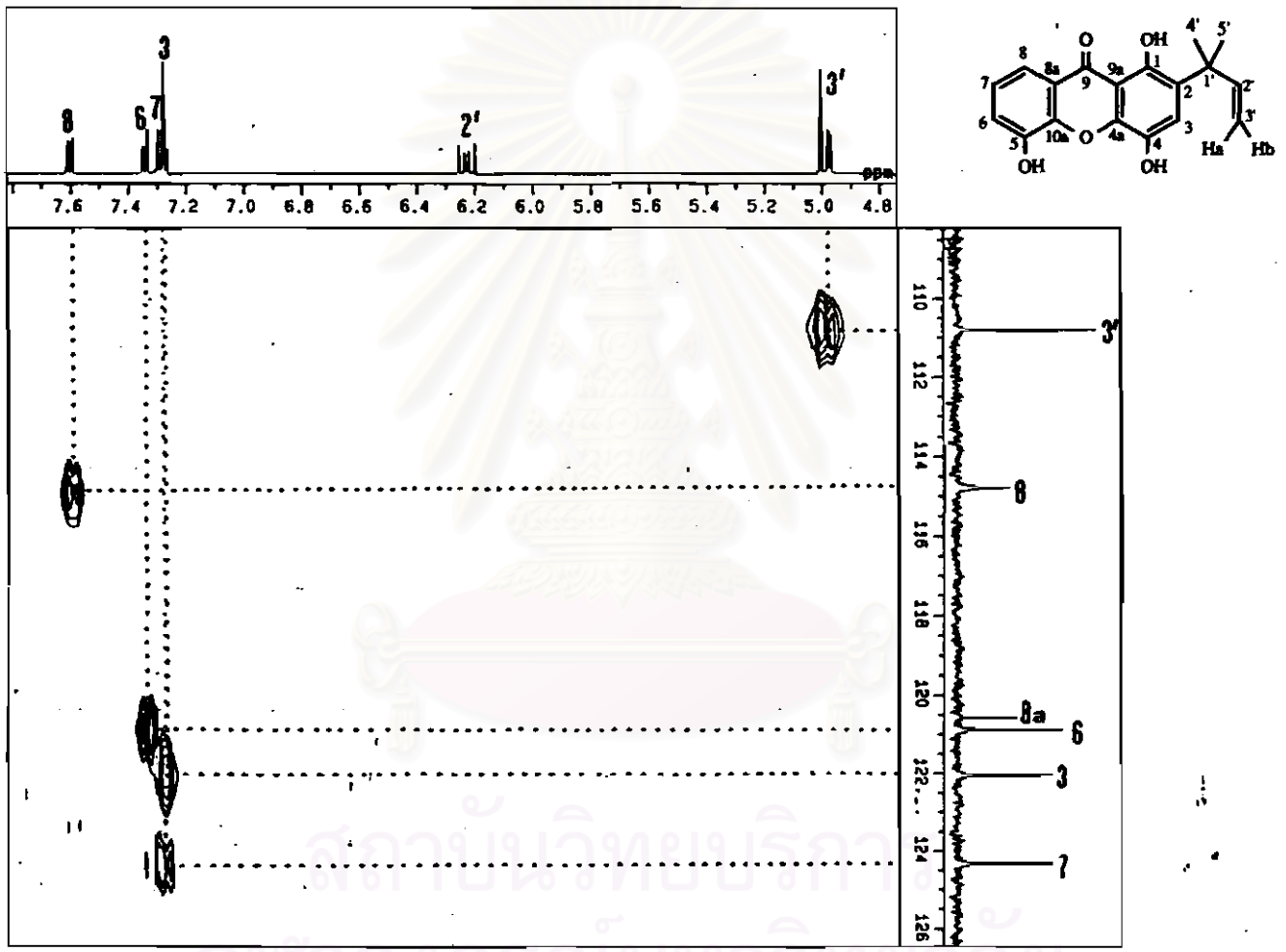


Figure 30c HMQC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 4.8-7.8 ppm, δ_C 108-126 ppm]

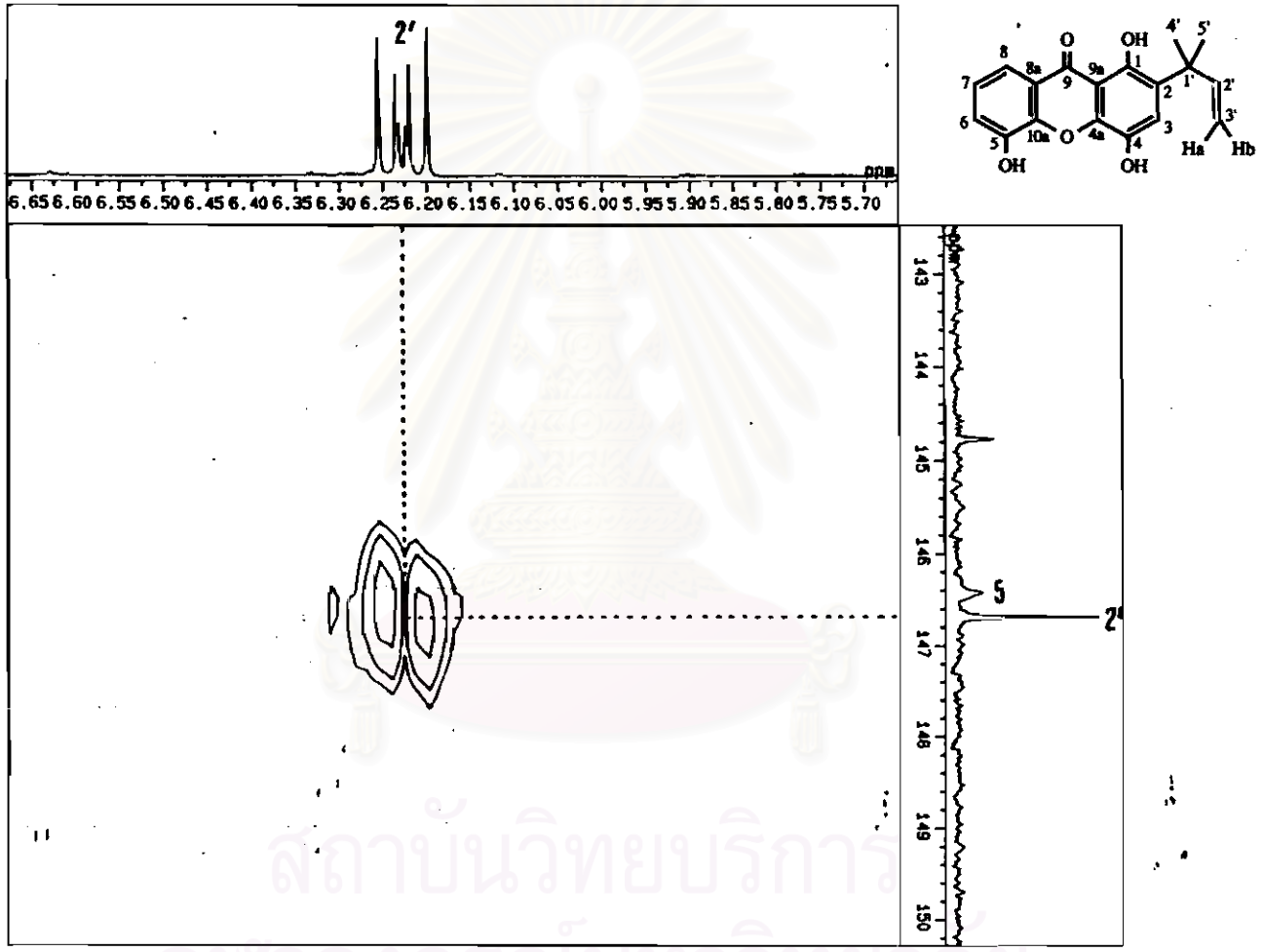


Figure 30d HMQC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 5.70-6.65 ppm, δ_C 143-150 ppm]

Table 12 The carbon-proton correlations of compound GD-3 observed in the HMQC spectrum

Carbon	δ_C (ppm)	Correlation with proton(s) at δ_H (ppm)
C-3	122.0	7.27
C-6	120.8	7.34
C-7	124.3	7.28
C-8	114.7	7.59
C-2′	146.6	6.23
C-3′	110.7	4.98, 5.00
C-4′, 5′	26.4	1.46

The long-range C-H correlations of compound GD-3 could be observed from HMBC spectrum (Figures 31a-31g). The results from the HMBC experiment confirmed the structure of compound GD-3 and led to the unequivocal assignments of all quaternary carbons. The long-range ^{1}H and ^{13}C couplings are shown in Figure 32.

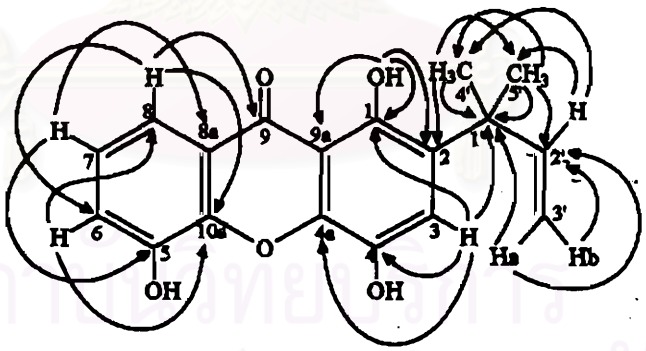


Figure 32 Long-range C-H correlations of compound GD-3 observed in HMBC spectrum

The complete proton and carbon assignments of compound GD-3 were compared to those of 12b-hydroxy-des-D-garcigerrin A (Sordat-Diserens *et al.*, 1989) as summarized in Table 13.

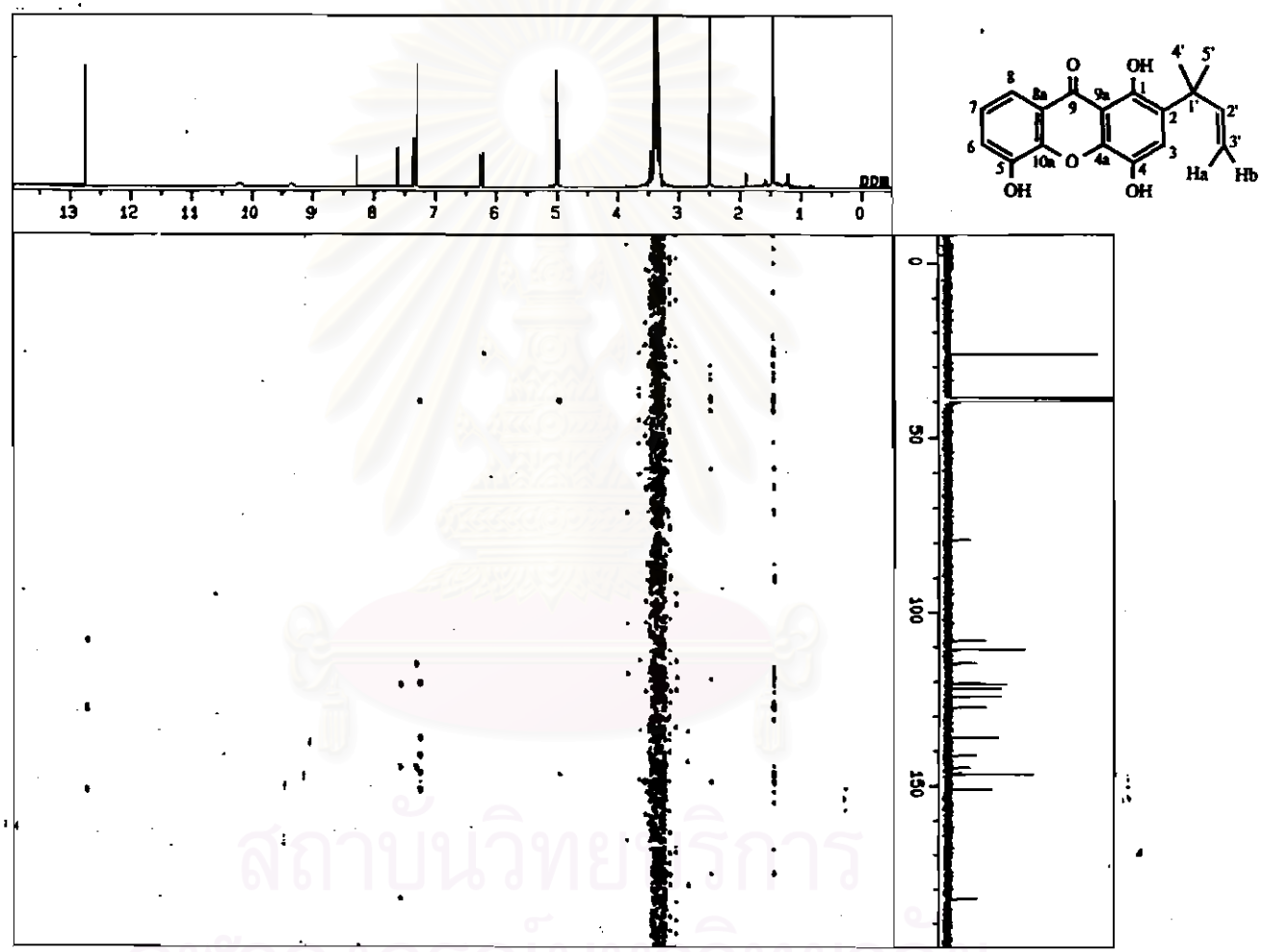


Figure 31a HMBC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 0.00-14.00 ppm, δ_C 0-200 ppm]

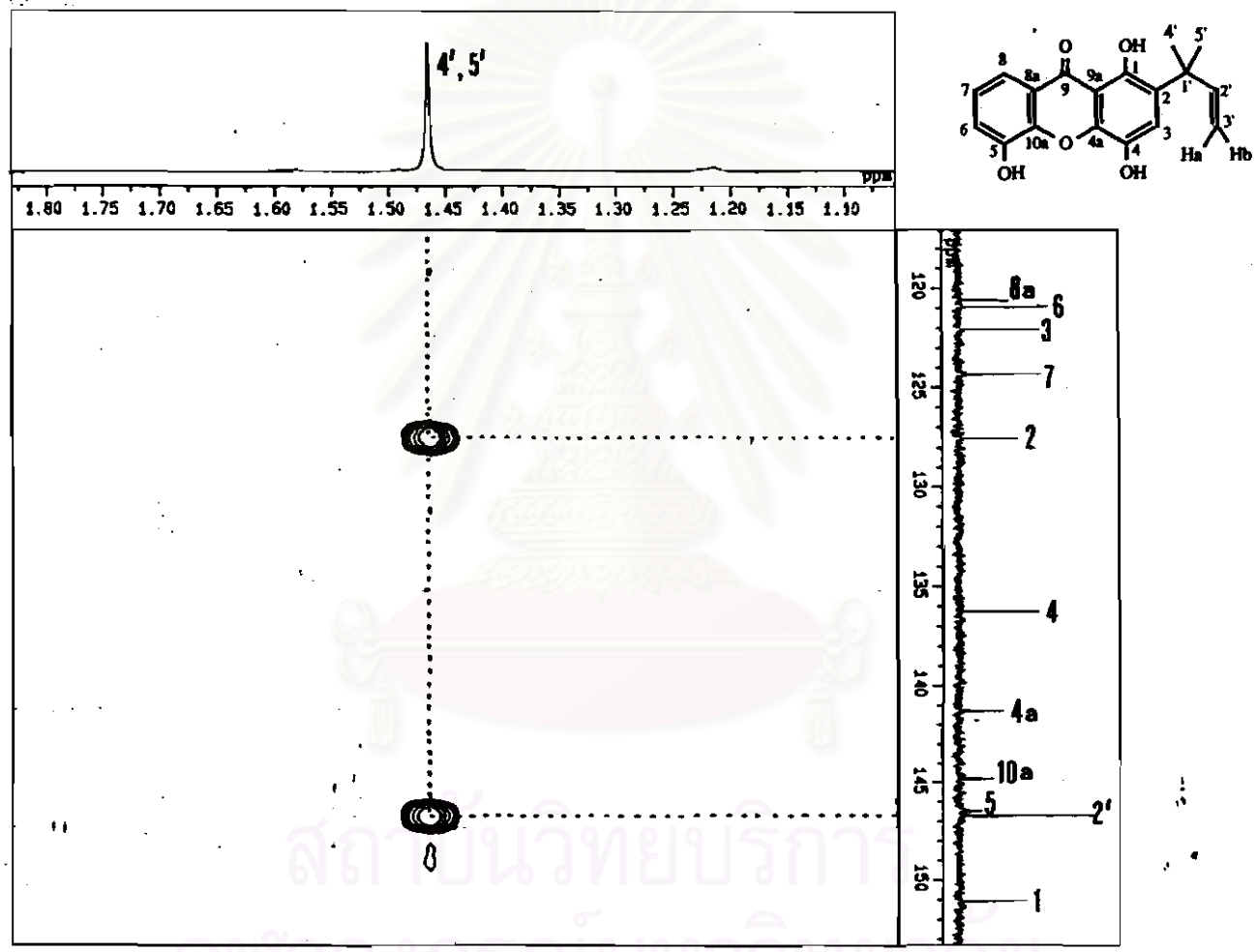


Figure 31b HMBC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 1.05-1.80 ppm, δ_C 118-153 ppm]

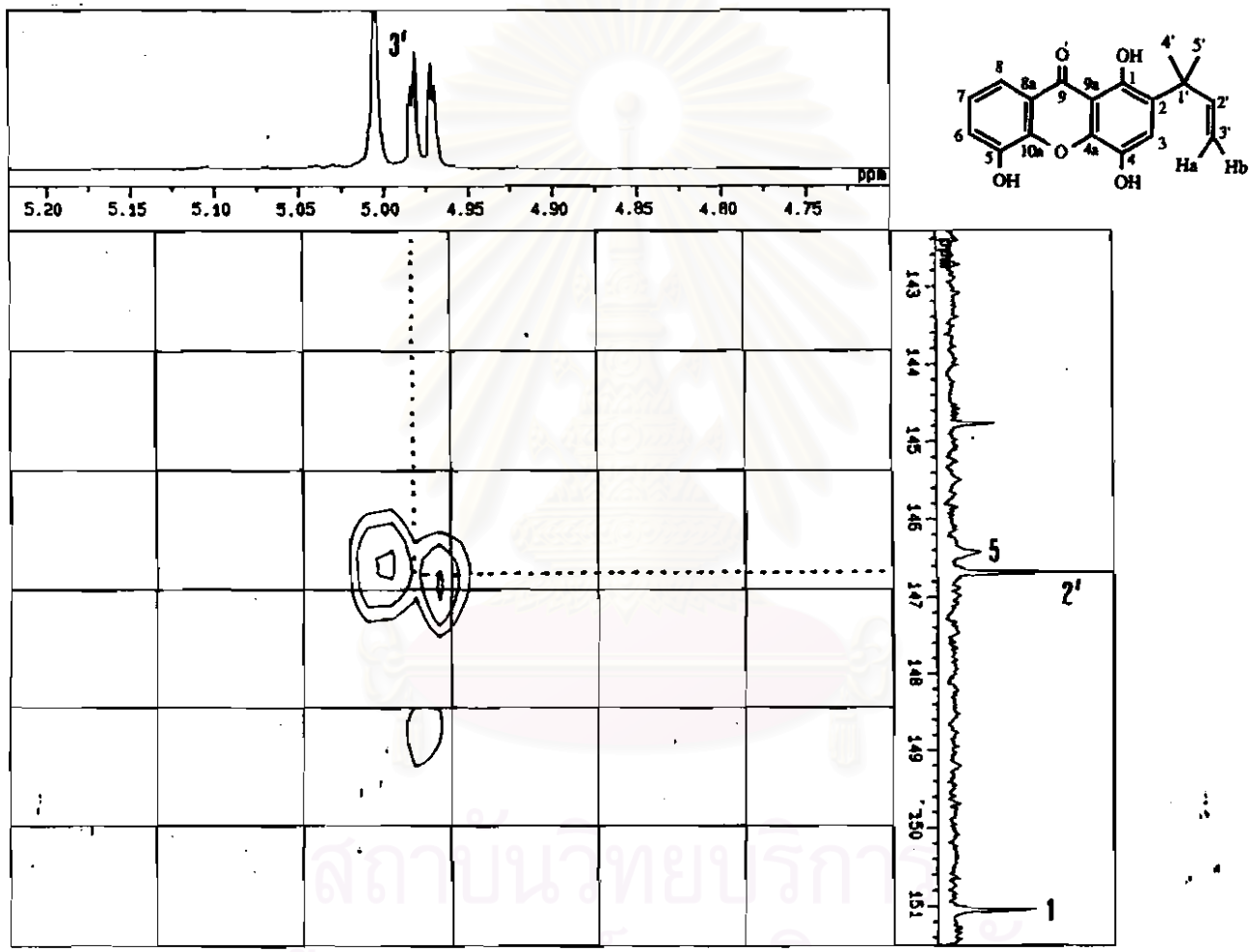


Figure 31c HMBC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 4.70-5.20 ppm, δ_C 143-151 ppm]

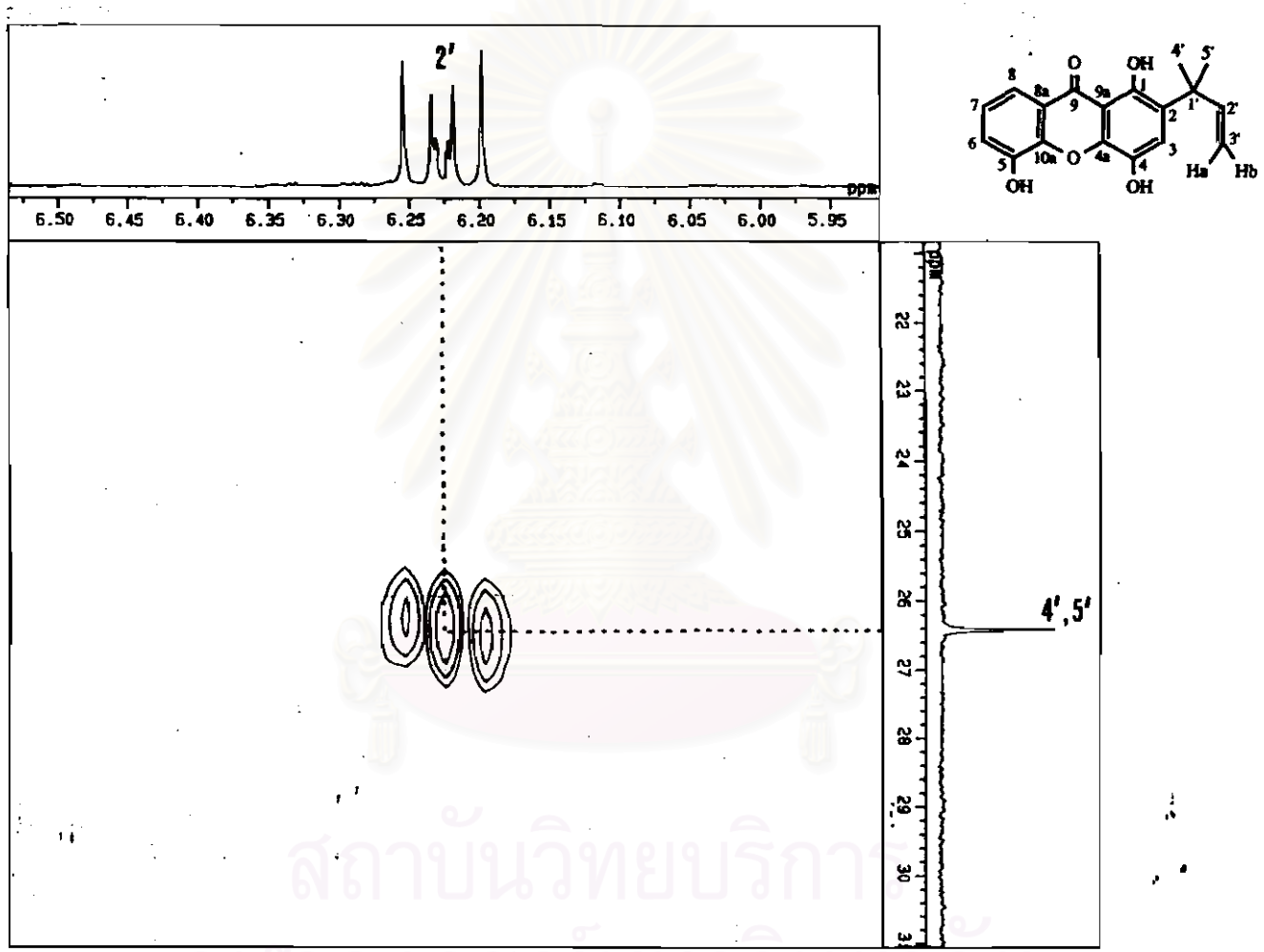


Figure 31d HMBC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 5.95-6.50 ppm, δ_C 21-31 ppm]

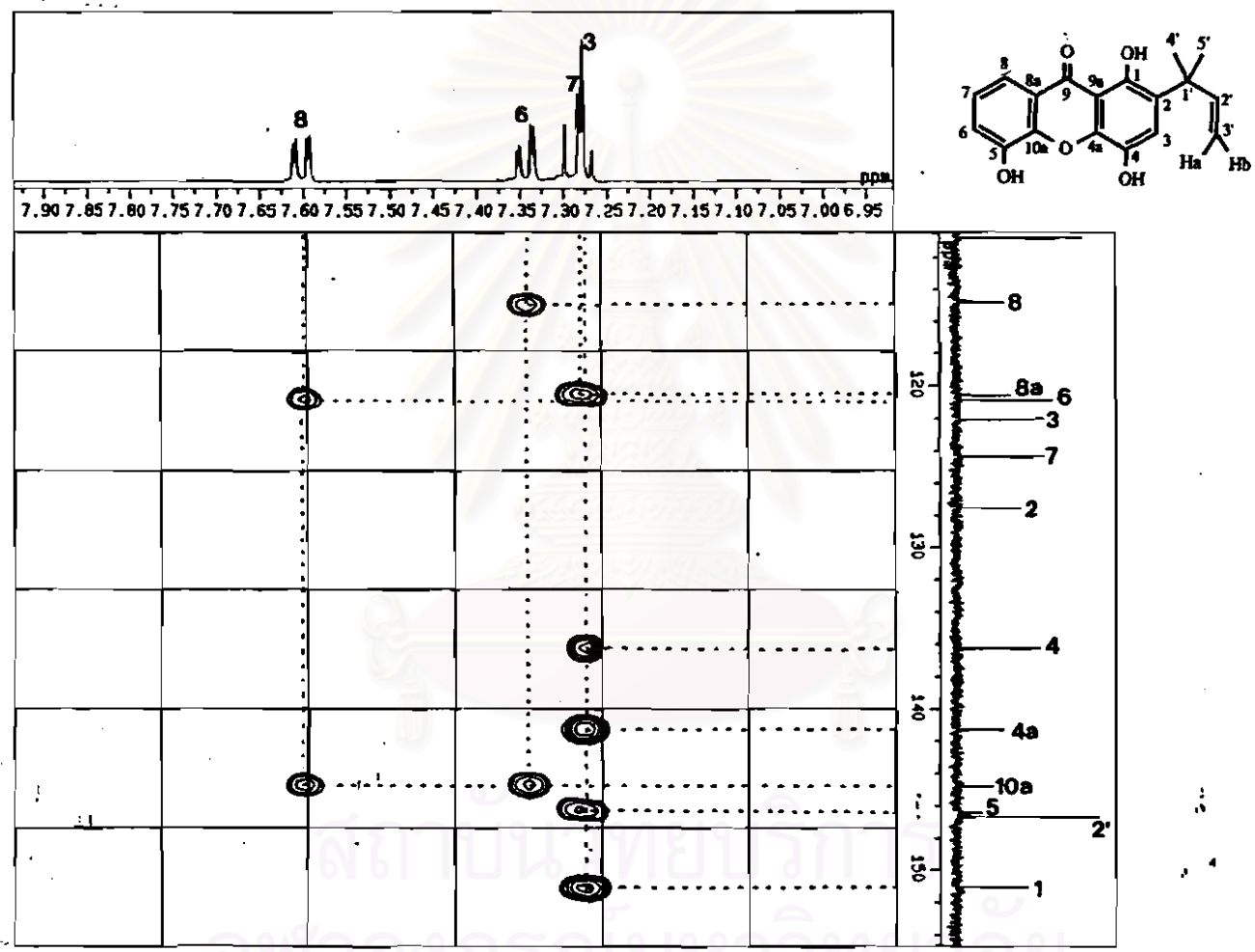


Figure 31e HMBC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 6.95-7.90 ppm, δ_C 110-154 ppm]

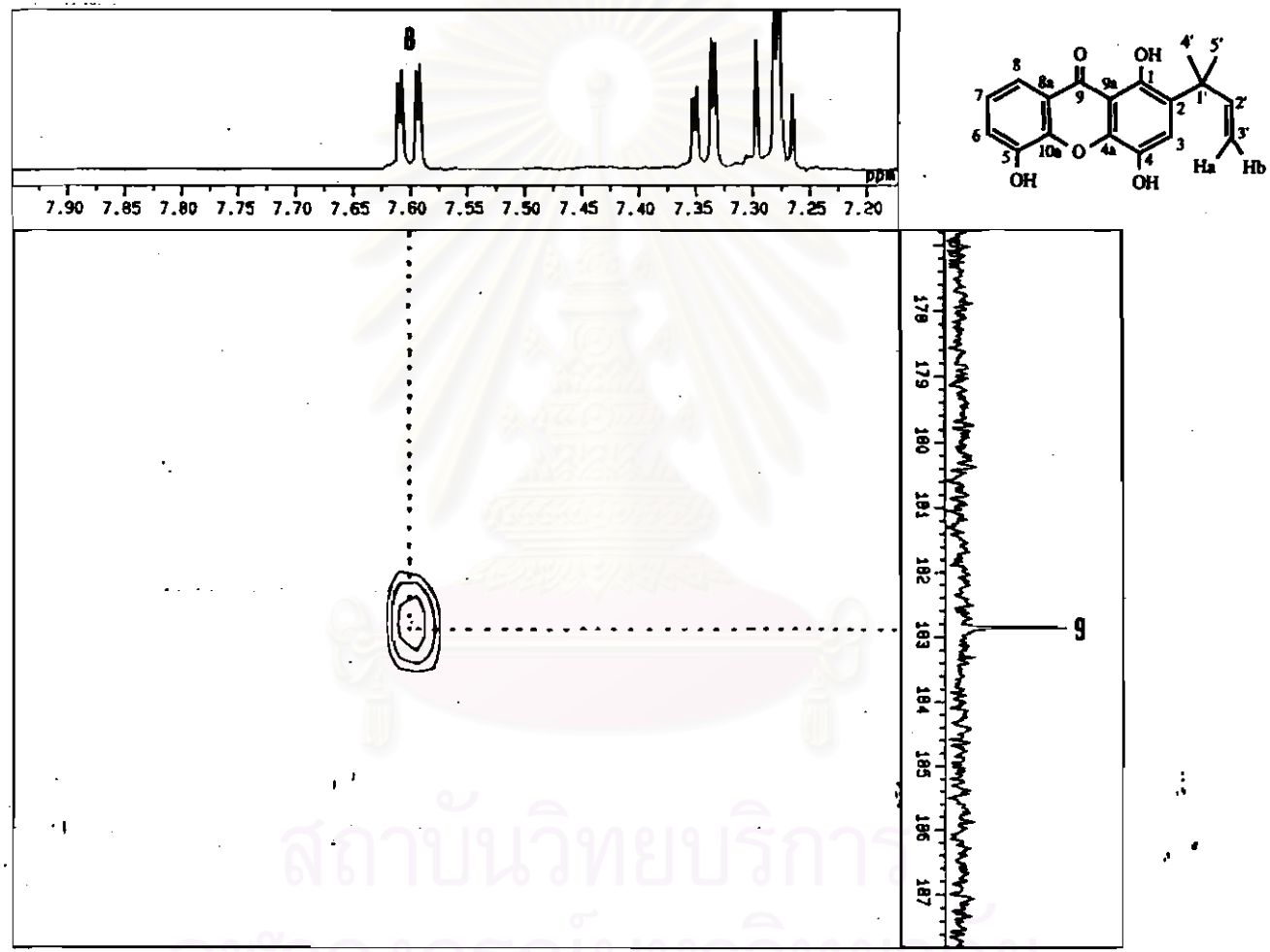


Figure 31f HMBC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 7.20-8.00 ppm, δ_C 177-187 ppm]

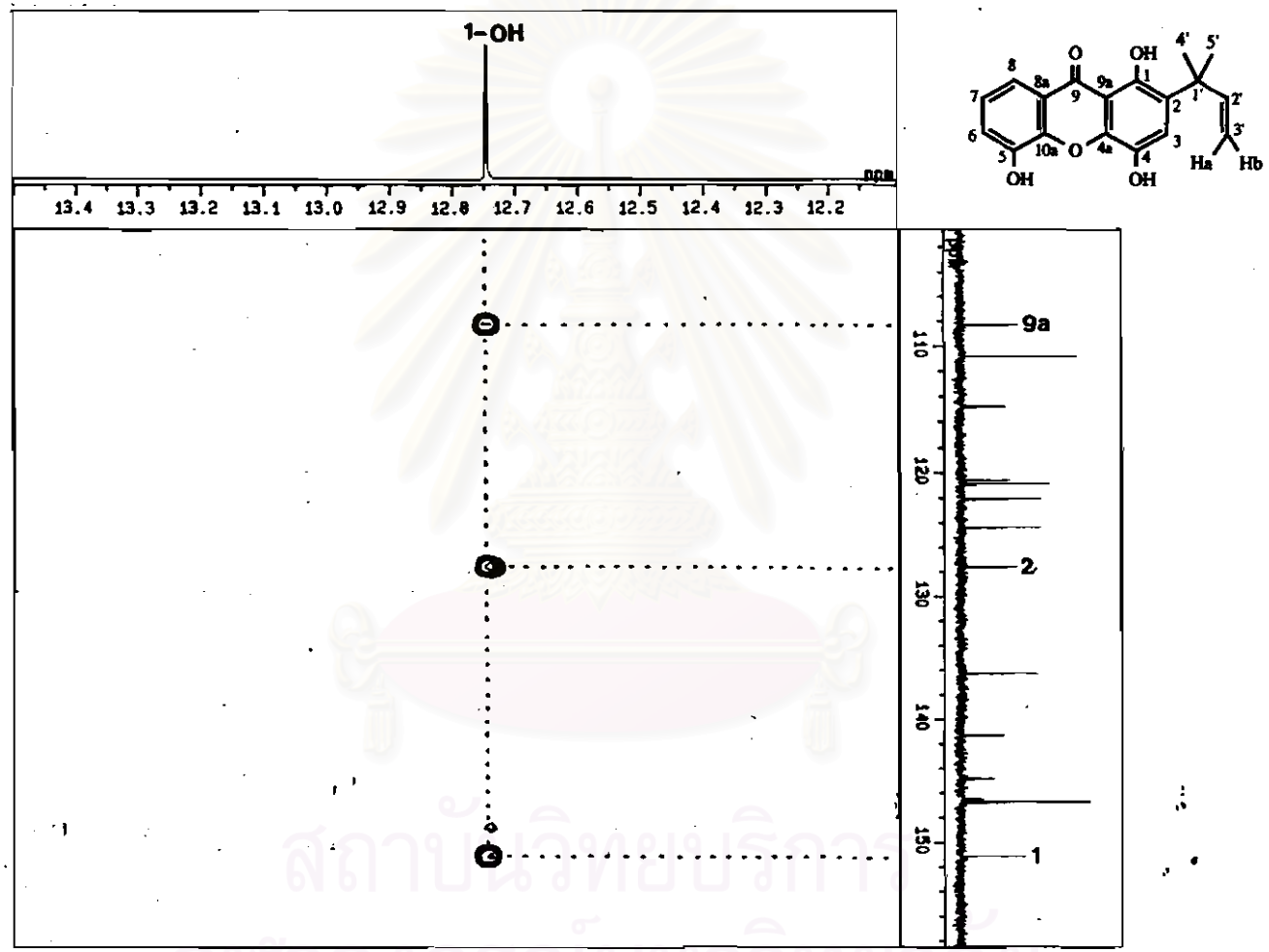


Figure 31g HMBC spectrum of compound GD-3 (in DMSO-d_6), [δ_H 12.1-13.5 ppm, δ_C 105-154 ppm]

Table 13 ^{1}H and ^{13}C spectral data of compound GD-3 and 12b-hydroxy-des-D-garcigerrin A [19] (in DMSO-d_6)

Position	Compound GD-3		12b-Hydroxy-des-D-garcigerrin A	
	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)
1	151.0	12.75 (1-OH, s)	151.0	12.77 (1-OH, s)
2	127.5	-	127.5	-
3	122.0	7.27 (s)	121.9	7.29 (s)
4	136.2	-	136.2	-
4a	141.2	-	141.2	-
5	146.4	-	146.5	-
6	120.8	7.34 (dd, J = 7.9, 1.5)	120.7	7.36 (dd, J =7.9, 2.1)
7	124.3	7.28 (t, J = 7.9)	124.2	7.29 (dd, J = 7.9, 7.5)
8	114.7	7.59 (dd, J = 7.9, 1.5)	114.6	7.60 (dd, J = 7.5, 2.1)
8a	120.5	-	120.5	-
9	182.8	-	182.8	-
9a	108.2	-	108.1	-
10a	144.7	-	144.7	-
1′	40.0	-	40.0	-
2′	146.6	6.23 (dd, J = 18.0, 10.3)	146.6	6.25 (dd, J = 17.8, 10.4)
3′a	110.7	4.99 (dd, J = 18.0, 1.5)	110.7	5.01 (dd, J = 17.8, 1.3)
3′b	-	5.00 (t, J = 10.3, 1.5)	-	5.01 (dd, J = 10.5, 1.3)
4′	26.4	1.46 s	26.4	1.49 s
5′	26.4	1.46 s	26.4	1.49 s

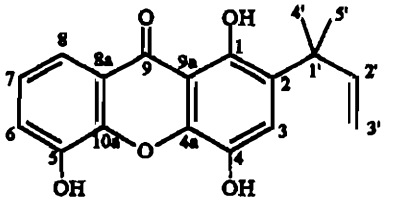


Figure 33 Structure of compound GD-3

According to Iinuma and co-workers (1995c), 12b-hydroxy-des-D-garcigerrin A was found in the root bark of *G. subelliptica* and its structure was determined by NMR spectral analysis (in acetone-d_6). The proton and carbon assignments of compound GD-3 in comparison with those of 12b-hydroxy-des-D-garcigerrin A (in acetone-d_6) are shown in Table 14 and Figures 34a; 34b and 35.

Table 14 ^{1}H and ^{13}C spectral data of compound GD-3 and 12b-Hydroxy-des-D-garcigerrin A [19] (in acetone-d_6)

Position	Compound GD-3		12b-Hydroxy-des-D-garcigerrin A	
	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)
1	153.2	12.87 (1-OH, s)	153.5	12.85 (1-OH, s)
2	129.5	-	129.8	-
3	123.3	7.34 (s)	123.4	7.34 (s)
4	136.9	-	137.0	8.70 (OH, br s)
4a	142.1	-	142.2	-
5	146.9	-	147.0	-
6	121.9	7.37 (dd, J = 7.9, 1.8)	122.0	7.35 (dd, J = 8, 2)
7	125.1	7.32 (t, J = 7.9)	125.2	7.30 (t, J = 8)
8	116.5	7.72 (dd, J = 7.9, 1.8)	116.8	7.71 (dd, J = 8, 2)
8a	121.5	-	121.9	-
9	183.8	-	183.9	-
9a	109.2	-	109.4	-
10a	145.6	-	145.8	-
1′	40.9	6.30 (dd, J = 17.0, 10.6)	41.1	6.31 (dd, J = 18, 11)
2′	147.8	5.03 (dd, J = 17.0, 1.5)	148.1	5.01 (dd, J = 18, 1)
3′	111.0	5.00 (dd, J = 10.6, 1.5)	111.5	5.01 (dd, J = 11, 1)
4′	26.9	1.53 (s)	29.3	1.54 (s)
5′	26.9	1.53 (s)	29.3	1.54 (s)

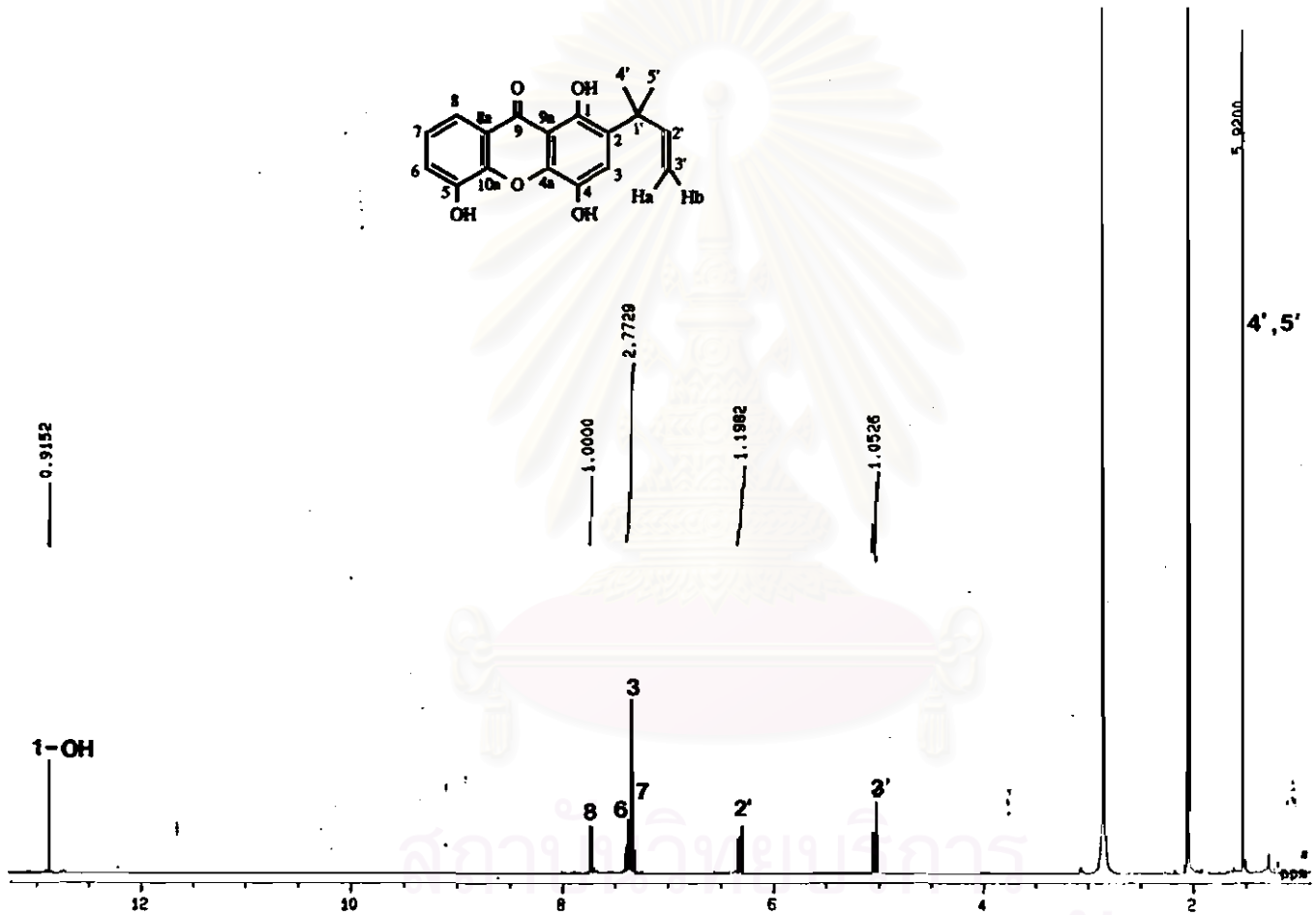


Figure 34a 500 MHz ^{1}H NMR spectrum of compound GD-3 (in acetone-d_6)

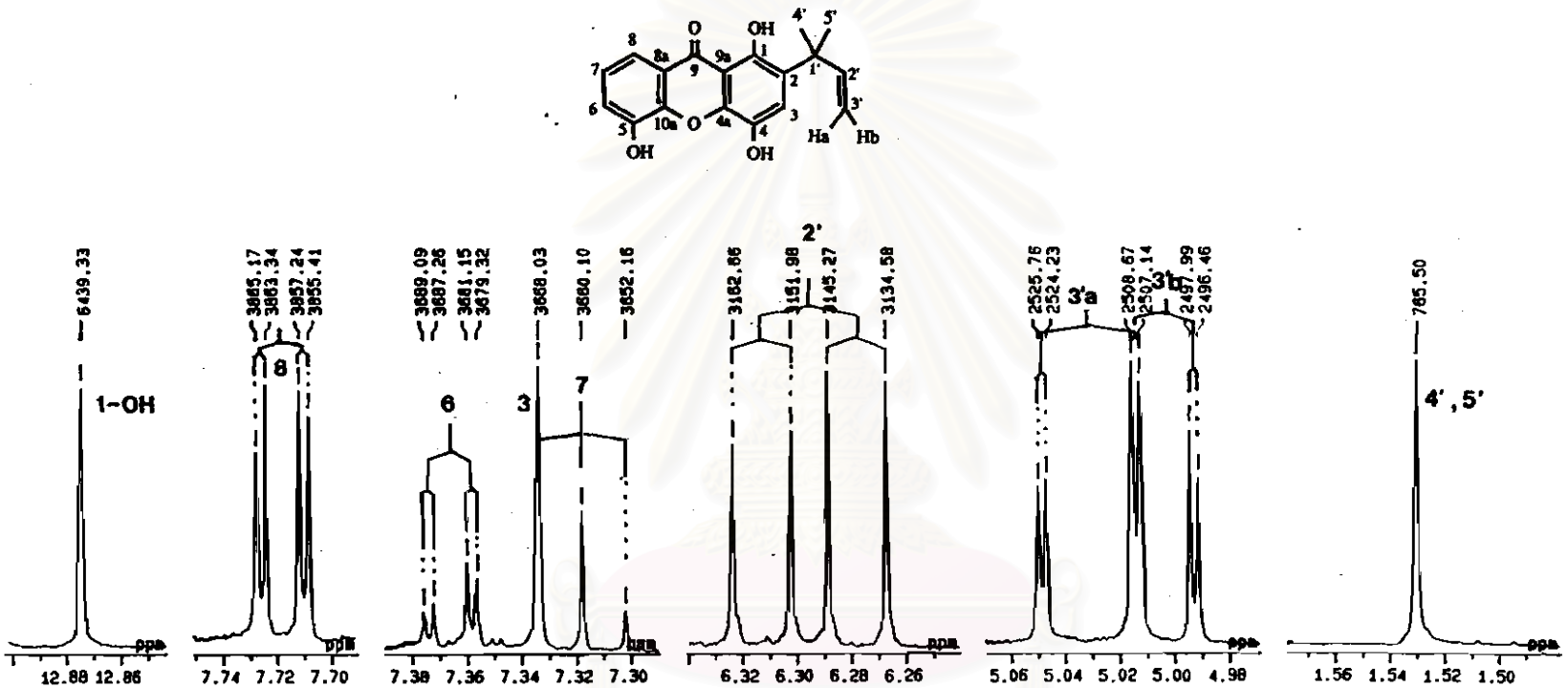


Figure 34b 500 MHz ^{1}H NMR spectrum of compound GD-3 (in acetone-d_6) (expanded from 1.50-12.90 ppm)

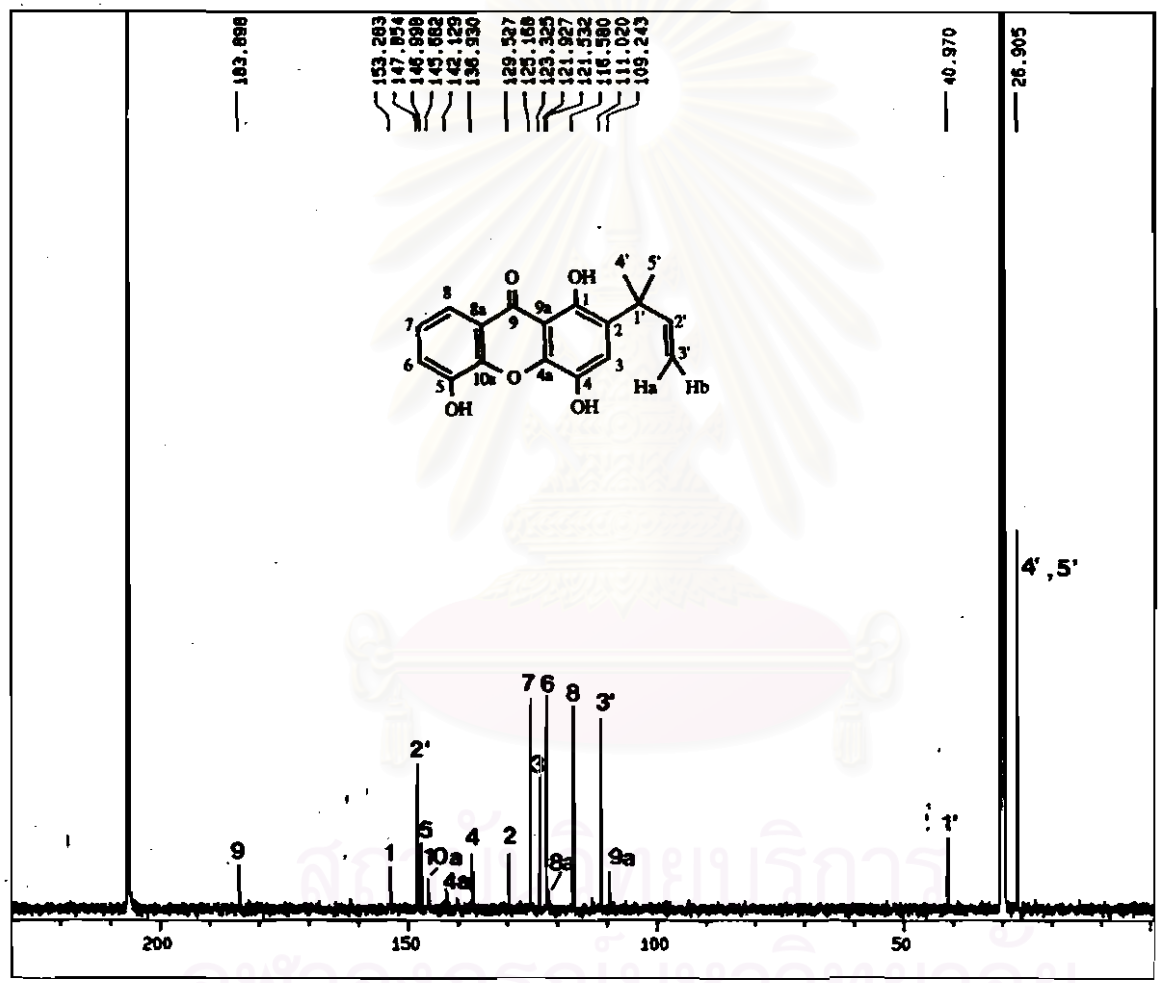


Figure 35 125 MHz ^{13}C NMR spectrum of compound GD-3 (in acetone-d_6)

In addition, the ^1H NMR properties of compound GD-3 in $CDCl_3$ were studied. (Figures 36a-36b). The proton signals between δ 7.2-7.4 (2H) were under the solvent signal and consequently could not be assigned. The proton spectral data are shown in Table 15.

Table 15 ^1H spectral data of compound GD-3 (in $CDCl_3$)

Position	δ_H (ppm) (multiplicity), *J* (Hz)
1	12.75 (1-OH, s)
3	7.12 (s)
6	7.2-7.4 (overlapping)
7	7.2-7.4 (overlapping)
8	7.79 (dd, *J* = 7.8, 1.2)
2′	6.25 (dd, *J* = 17.4, 10.7)
3′a	5.02 (dd, *J*= 17.4, 1.2)
3′b	5.03 (dd, *J* = 10.7, 1.2)
4′	1.53 (s)
5′	1.53 (s)

It should be noted that the ^1H and ^{13}C NMR spectra of compound GD-3 taken in acetone-d_6 gave the best resolution, with clear splitting patterns for H-3′a and H-3′b, and without interference from the solvent signals on the C-1′ resonance of the 1,1-dimethylallyl moiety.

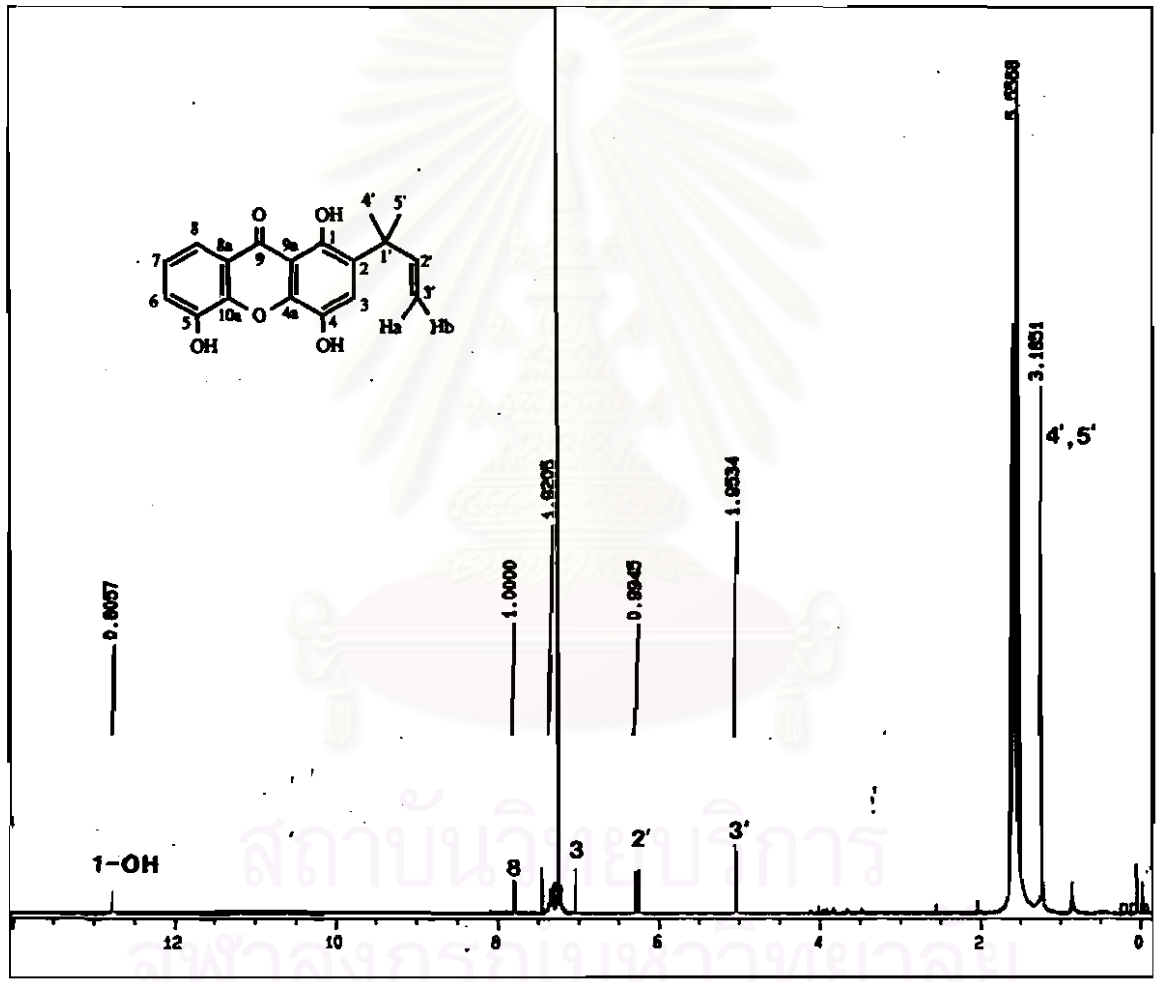


Figure 36a 500 MHz ^{1}H NMR spectrum of compound GD-3 (in $CDCl_3$)

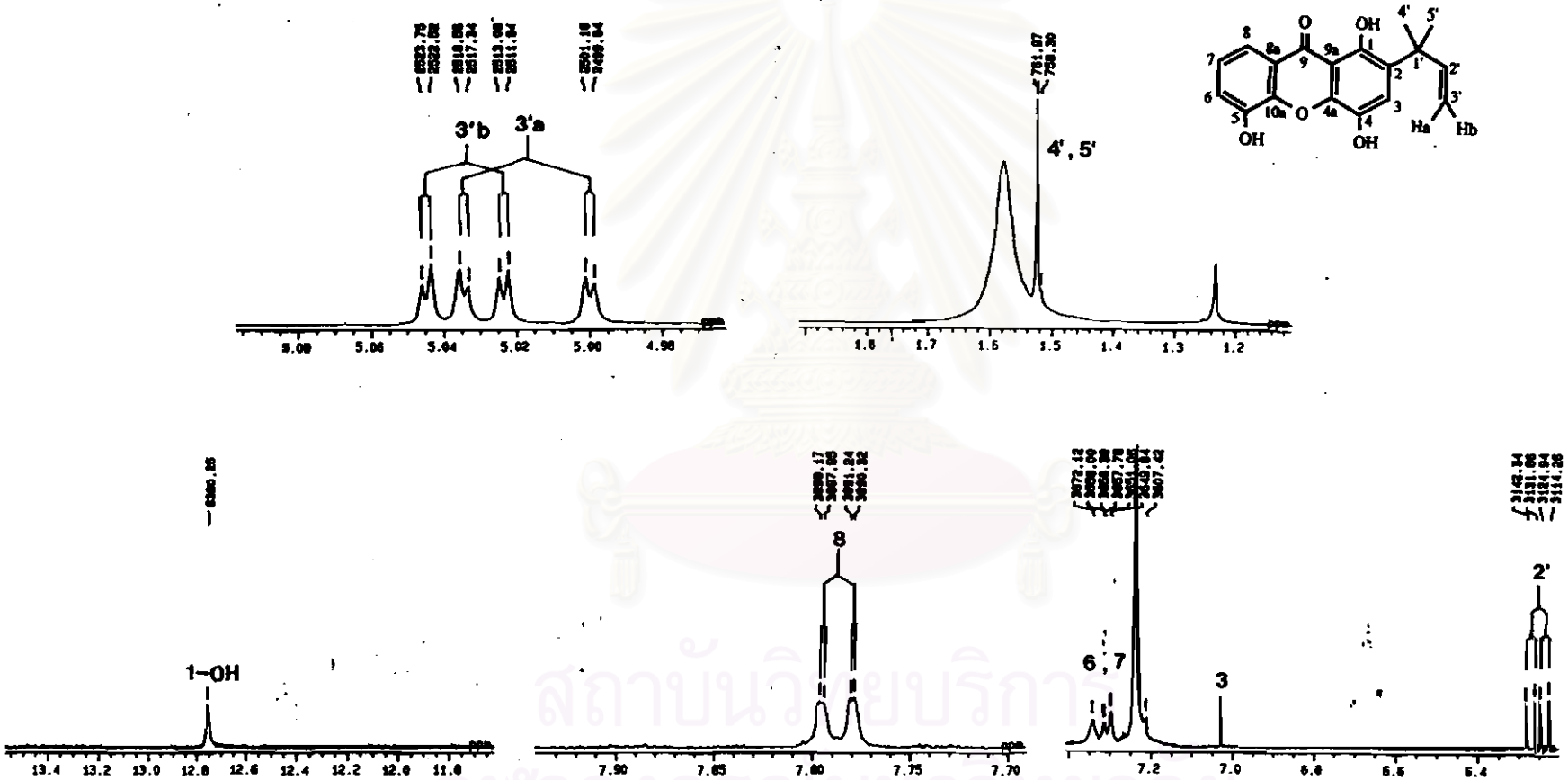


Figure 36b 500 MHz ^{1}H NMR spectrum of compound GD-3 (in $CDCl_3$) (expanded from 1.3-13.4 ppm)

4. Structure Determination of Compound GD-4 [179]

Compound GD-4 was obtained as colorless needles from fraction V-5 by repeated chromatographic technique. It produced pink with Libermann-Burchard's test which suggested that it was a triterpenoid compound.

Although the molecular ion of compound GD-4 was not observed in the EIMS (Figure 37), important ions at *m/z* 248 and 208 were found (see below). The IR spectrum of compound GD-4 (Figure 38) showed absorption bands at 3400 (O-H stretching), 2900 (C-H stretching of CH_3, CH_2), 1700 (C=O stretching of carboxyl group), 1490 (C-H bending of CH_3), 1060 (C-O stretching) and 790 (C-H bending of trisubstituted alkene) cm^{-1}.

The ^1H NMR spectrum (Figures 39a-39c) showed seven methyl singlet signals between δ 0.73-1.11. The olefinic signal at δ 5.26 ppm (t, J = 3.6 Hz) was typical of H-12 of the Δ^{12} oleanene skeleton (Karliner and Djerassi, 1966). The signal of H-18 appeared at δ 2.80 (1H, dd, J = 13.7, 4.5 Hz). The signal at δ 3.20 ppm (dd, J = 11.2, 4.5 Hz) suggested the location of hydroxyl group at C-3 (Maillard, Adewunmi and Hostettman, 1992).

Careful examination of the ^{13}C NMR (Figure 40) and DEPT spectra (Figure 41) revealed the presence of 7 methyl, 10 methylene, 5 methine, 7 quaternary carbons and one carbonyl carbon. Comparison of the NMR spectral properties of compound GD-4 with the literature data showed that compound GD-4 was identical with oleanolic acid [179]. The carbon assignments of compound GD-4 are shown in Table 16.

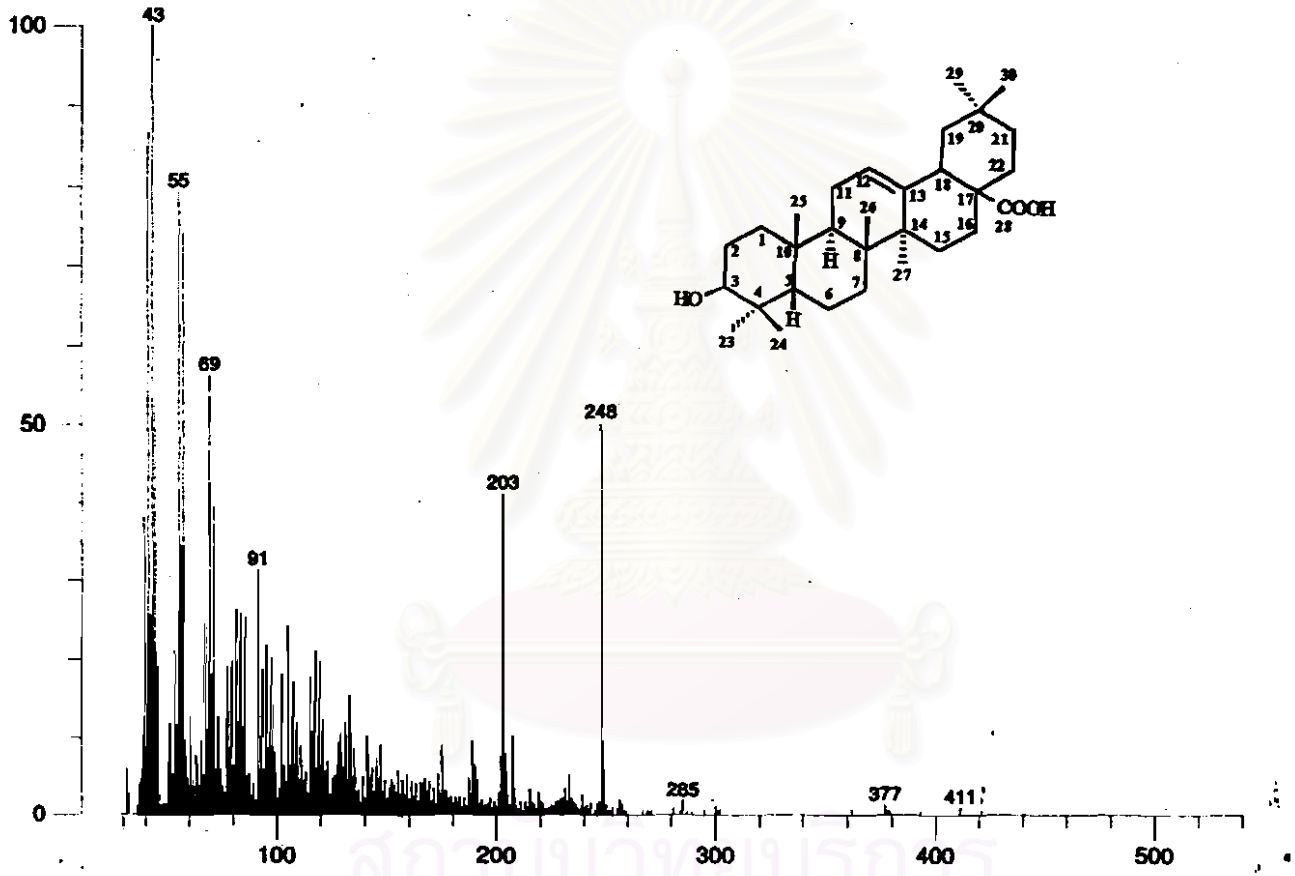


Figure 37 EI mass spectrum of compound GD-4

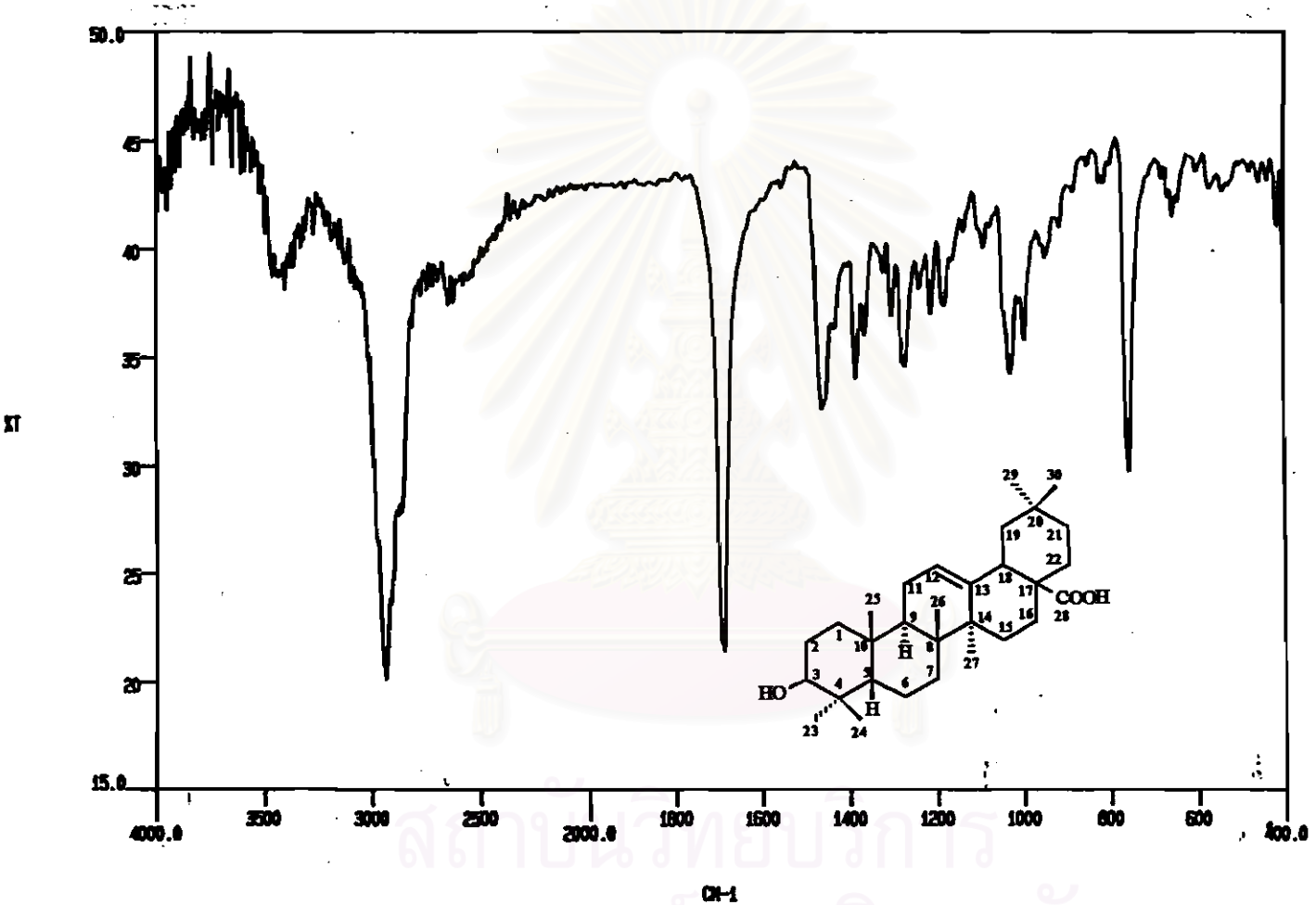


Figure 38 IR spectrum of compound GD-4 (film)

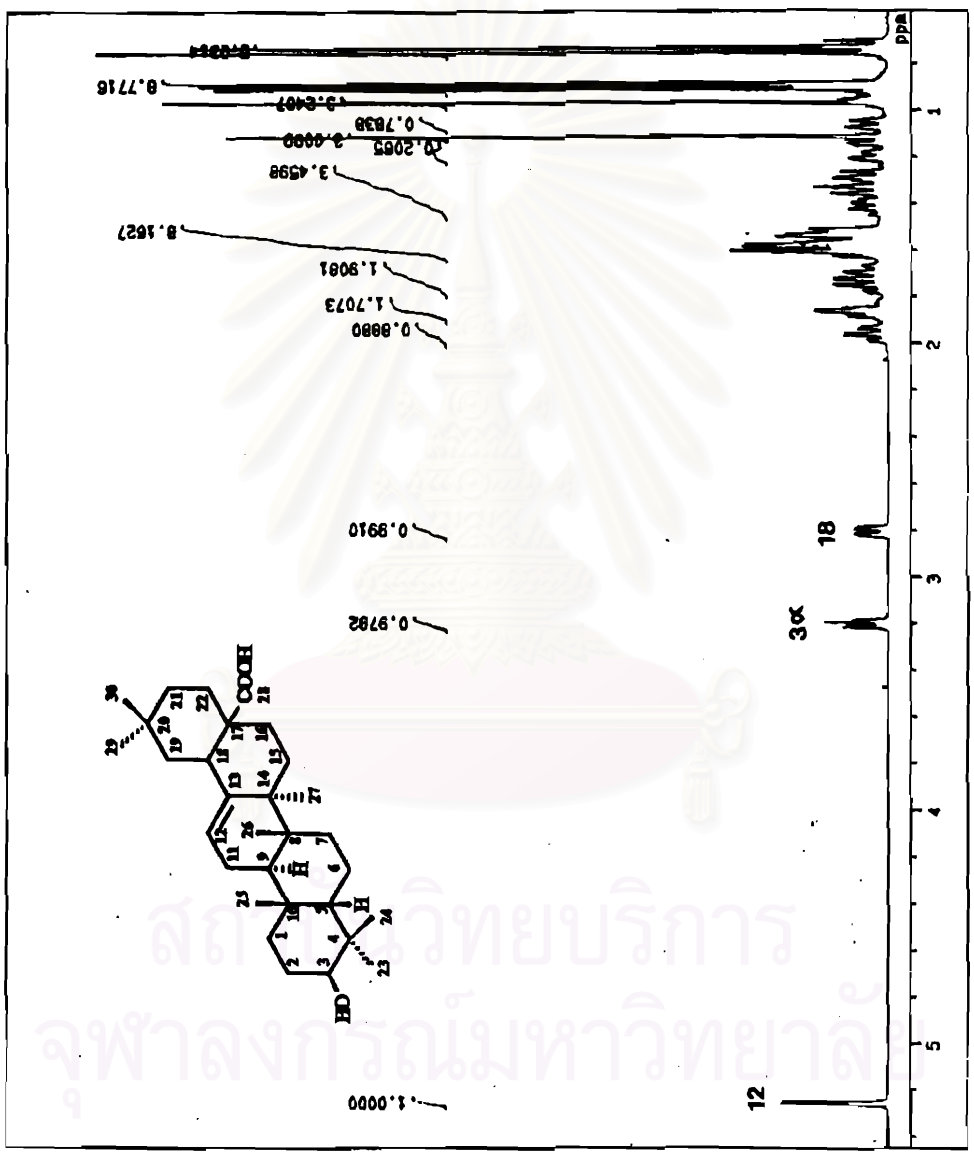


Figure 39a 500 MHz ^{1}H NMR spectrum of compound GD-4 (in $CDCl_3$)

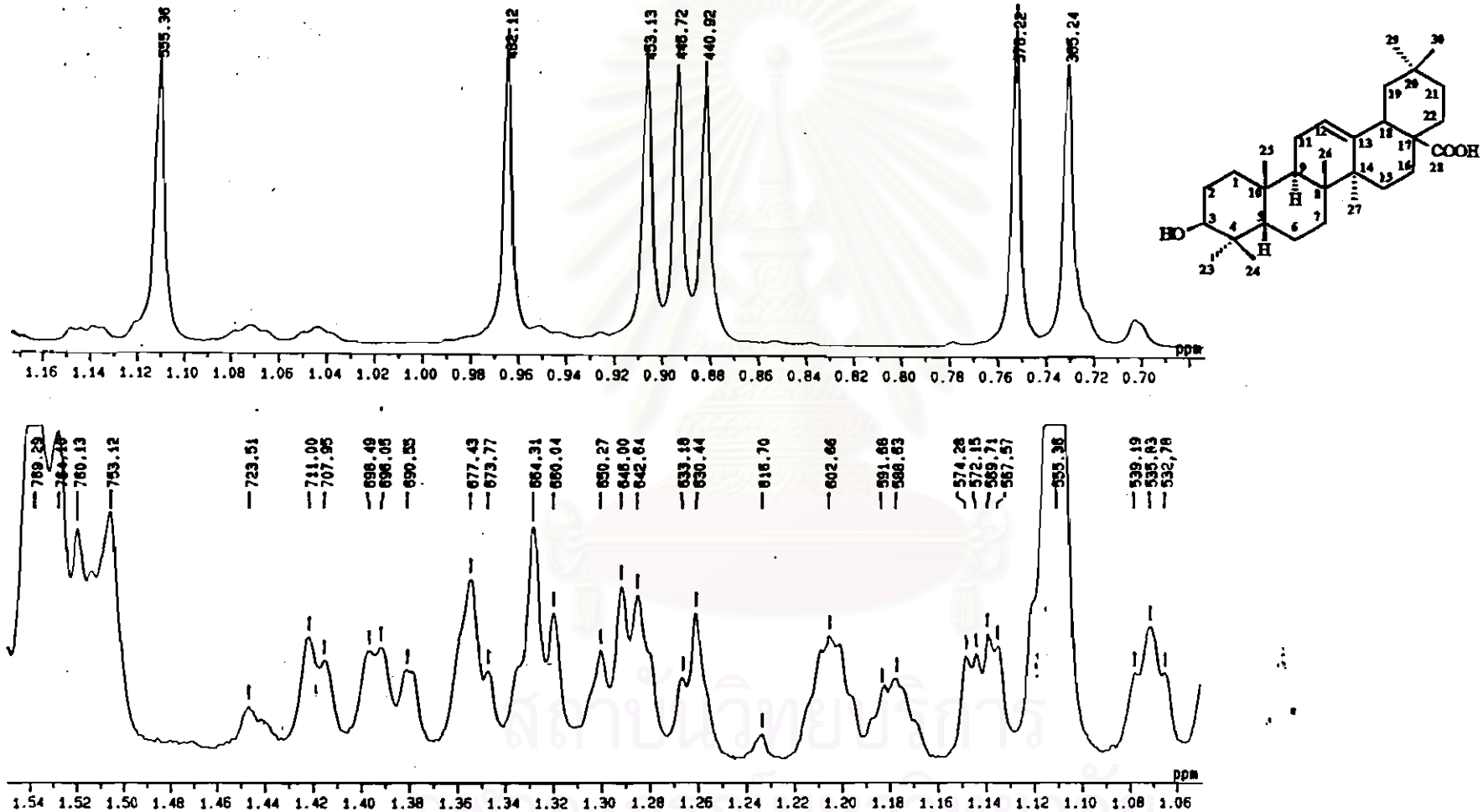


Figure 39b 500 MHz ^{1}H NMR spectrum of compound GD-4 (in $CDCl_3$) (expanded from 0.70-1.54 ppm)

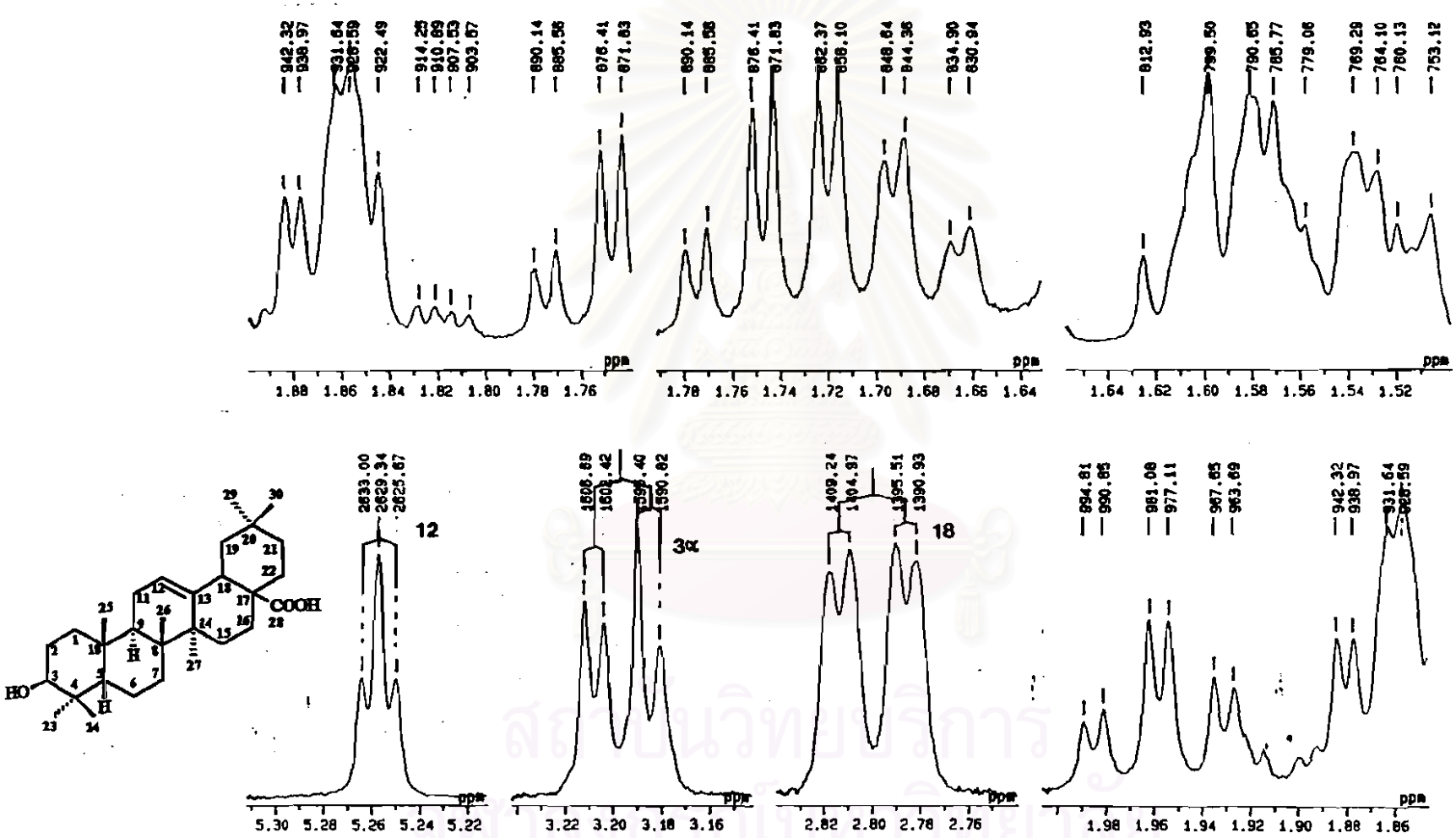


Figure 39c 500 MHz ^{1}H NMR spectrum of compound GD-4 (in $CDCl_3$) (expanded from 1.52-5.30 ppm)

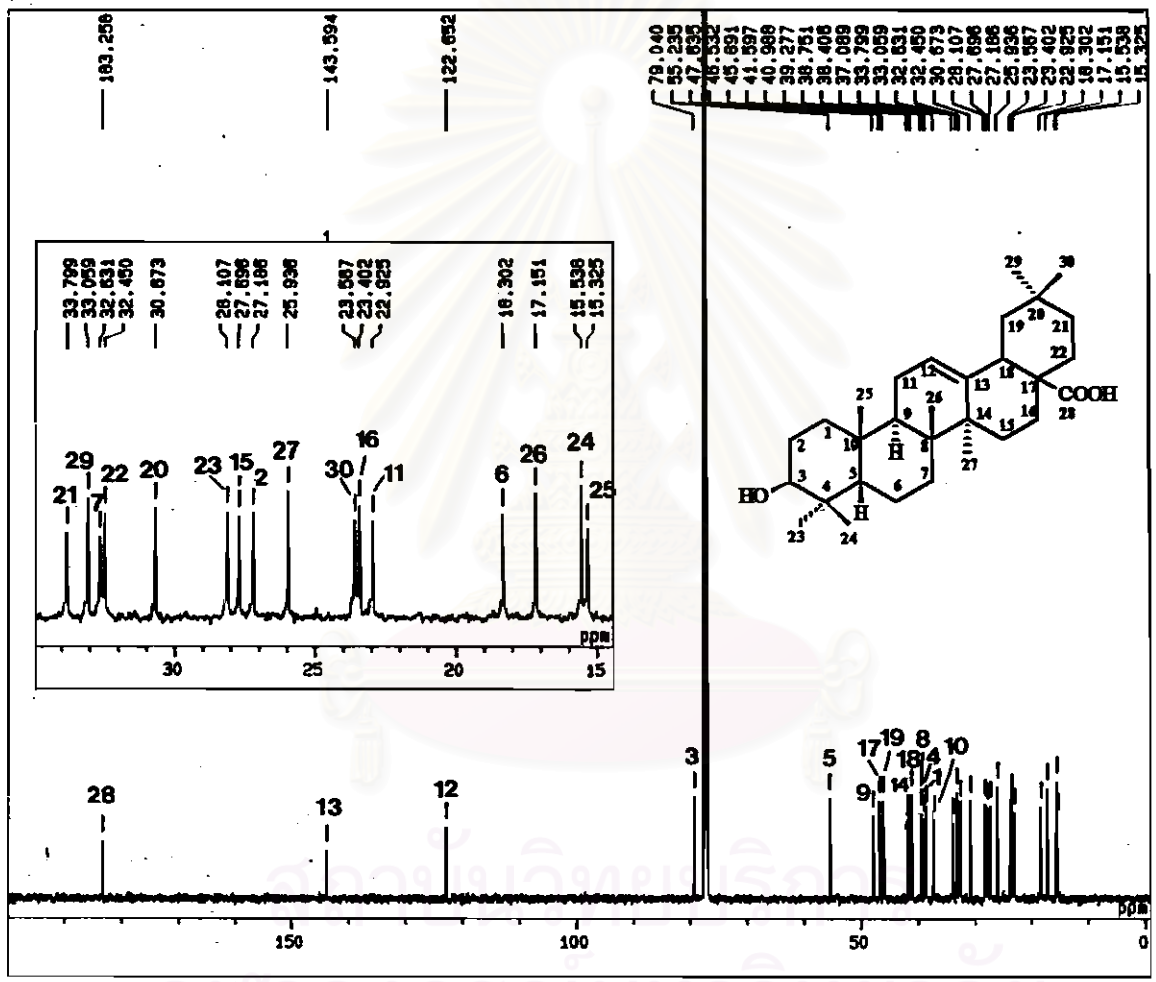


Figure 40 125 MHz ^{13}C NMR spectrum of compound GD-4 (in $CDCl_3$)

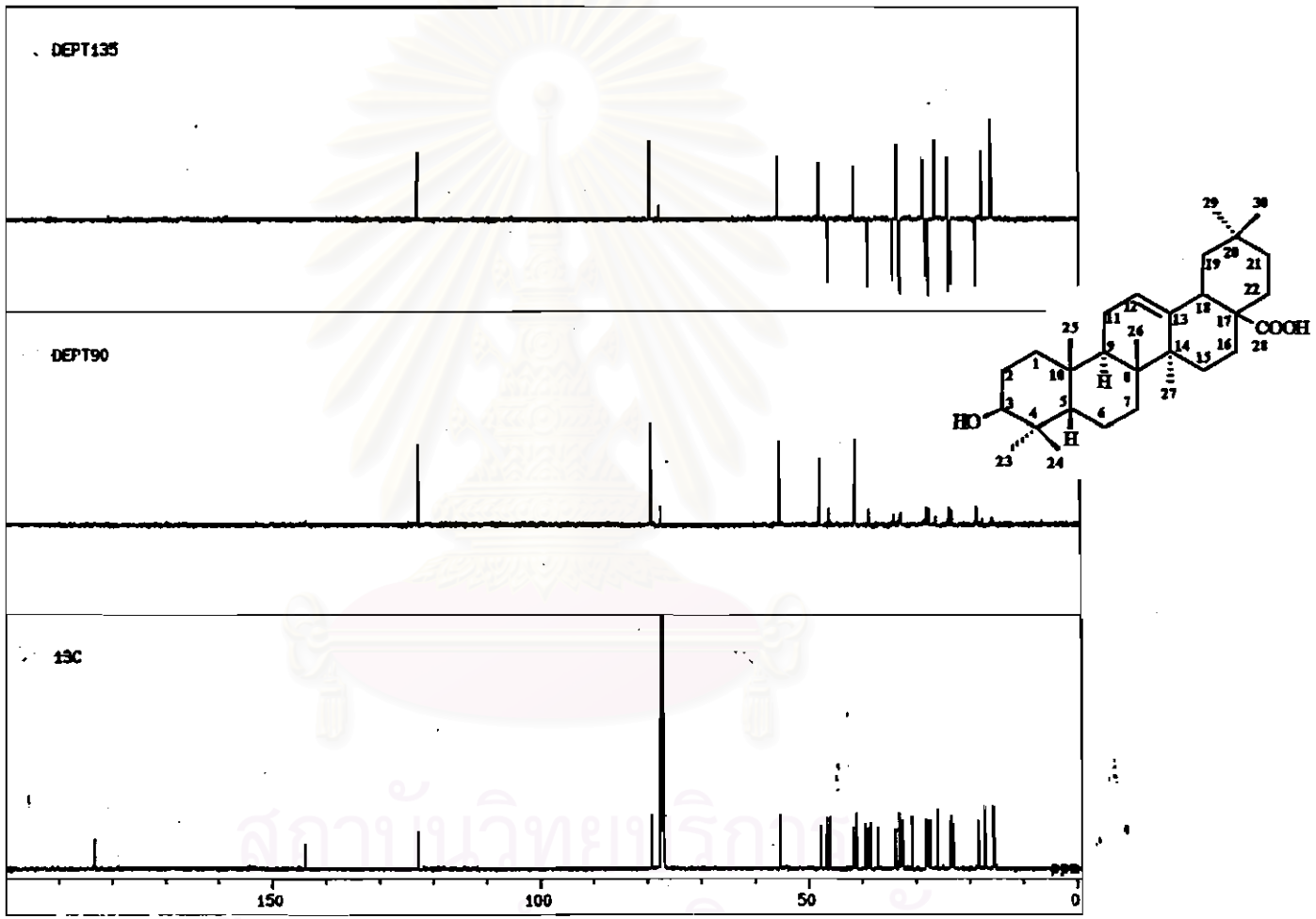
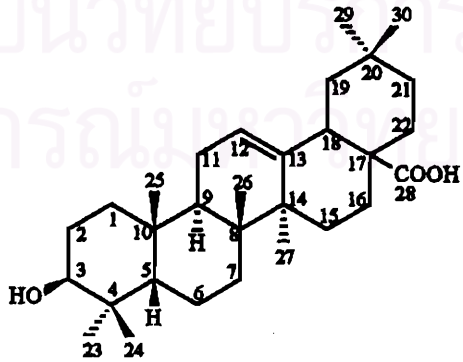


Figure 41 DEPT spectrum of compound GD-4 (in $CDCl_3$)

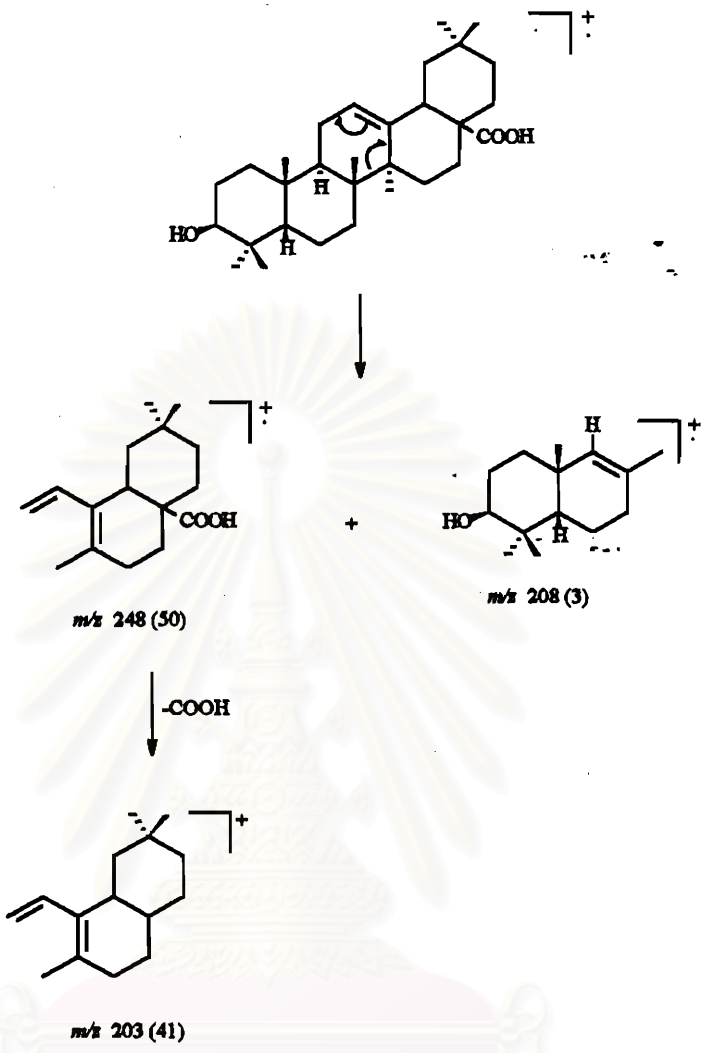
Table 16 The carbon assignments of oleanolic acid [179] and compound GD-4

Carbon	Chemical shift (ppm)		Carbon	Chemical shift (ppm)	
	Oleanolic acid	Compound GD-4		Oleanolic acid	Compound GD-4
1	38.5	38.4	16	23.4	23.4
2	27.4	27.1	17	46.6	46.5
3	78.7	79.0	18	41.3	40.9
4	38.7	38.7	19	45.8	45.9
5	55.2	55.2	20	30.6	30.6
6	18.3	18.3	21	33.8	33.8
7	32.6	32.6	22	32.3	32.4
8	39.3	39.2	23	28.1	28.1
9	47.6	47.6	24	15.6	15.5
10	37.0	37.0	25	15.3	15.3
11	23.1	22.9	26	16.8	17.1
12	122.1	122.6	27	26.0	25.9
13	143.4	143.6	28	181.0	183.2
14	41.6	41.6	29	33.1	33.0
15	27.7	27.6	30	23.6	23.5



[179]

Figure 42 Structure of compound GD-4



Scheme 3 Mass fragmentation of compound GD-4

The presence of a Δ^{12} double bond, a hydroxyl and a carboxyl group in compound GD-4 was reflected in its mass spectrum by the major fragmentation of this compound with the retro-Diels-Alder cleavage of ring C to give peaks at *m/z* 248 (50), and *m/z* 208 (3) (Scheme 3) (Budzikiewicz, Wilson and Djerassi, 1963). Loss of the C-28 carboxyl group gave another major peak at *m/z* 203 (41).

5. Structure Determination of Compound GD-5 [92]

The molecular formula of compound GD-5 was deduced as $C_{19}H_{18}O_6$ from its [M^+] at *m/z* 342 in the EIMS (Figure 43). Its UV spectrum (Figure 44) showed characteristic absorptions of a xanthone structure at λ_{max} 257 and 320 nm, whereas the IR spectrum (Figure 45) revealed the presence of a hydroxyl group (3300 cm^{-1}, br), a conjugated carbonyl group (1600 cm^{-1}), aromatic structures (1490 cm^{-1}) and ether linkage (1200 cm^{-1}).

Comparison of its ^1H and ^{13}C NMR spectra with reported data (Minami *et al.*, 1996) suggested that compound GD-5 was identical with 1-*O*-methylsymphoxanthone [92] previously obtained from the wood of *G. subelliptica.*

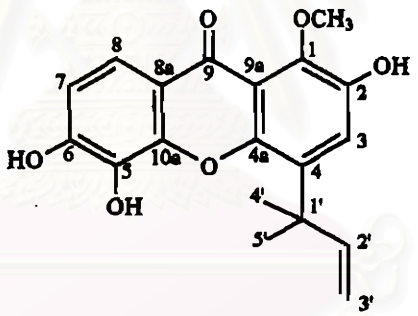


Figure 46 Structure of compound GD-5

The results from several 1-D and 2-D NMR experiments in the present study, including ^1H-^1H COSY, NOESY, HMQC and HMBC, suggested that the reported assignments of C-2, C-4, C-5, C-6, C-8, C-8a, C-9a and C-10a of 1-*O*-methylsymphoxanthone (Minami *et al.*, 1996) should be revised.

The ^1H NMR spectrum (Figures 47a-47b) showed signals for one methoxy group δ 3.77 (3H, s), one 1,1-dimethylallyl moiety [δ 1.59 (6H, s), 5.01 (1H, dd, *J* = 10.6, 1.2 Hz), 5.09 (1H, dd, *J* = 17.7, 1.2 Hz) and 6.39 (1H, dd, *J* = 17.7, 10.6

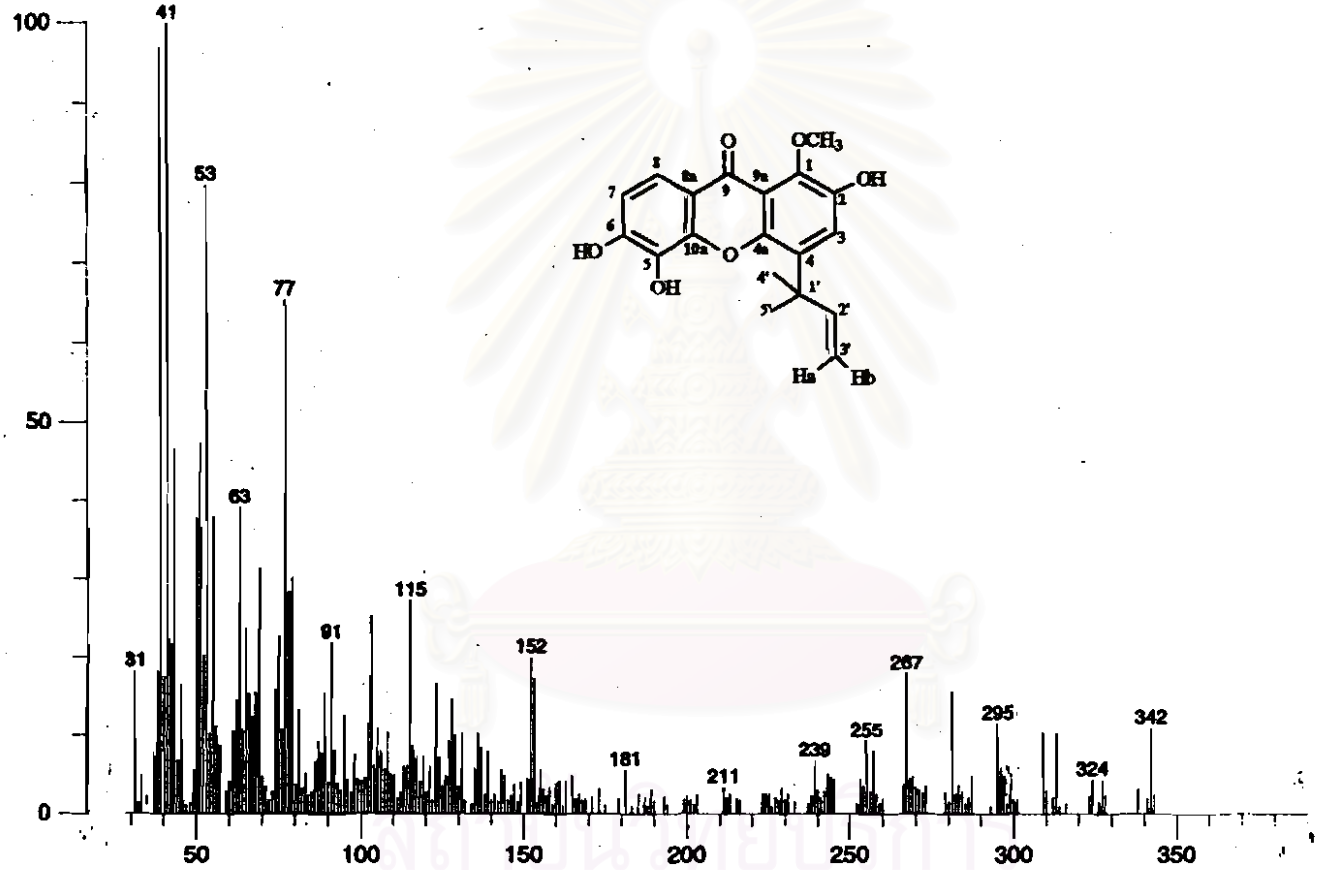


Figure 43 EI mass spectrum of compound GD-5

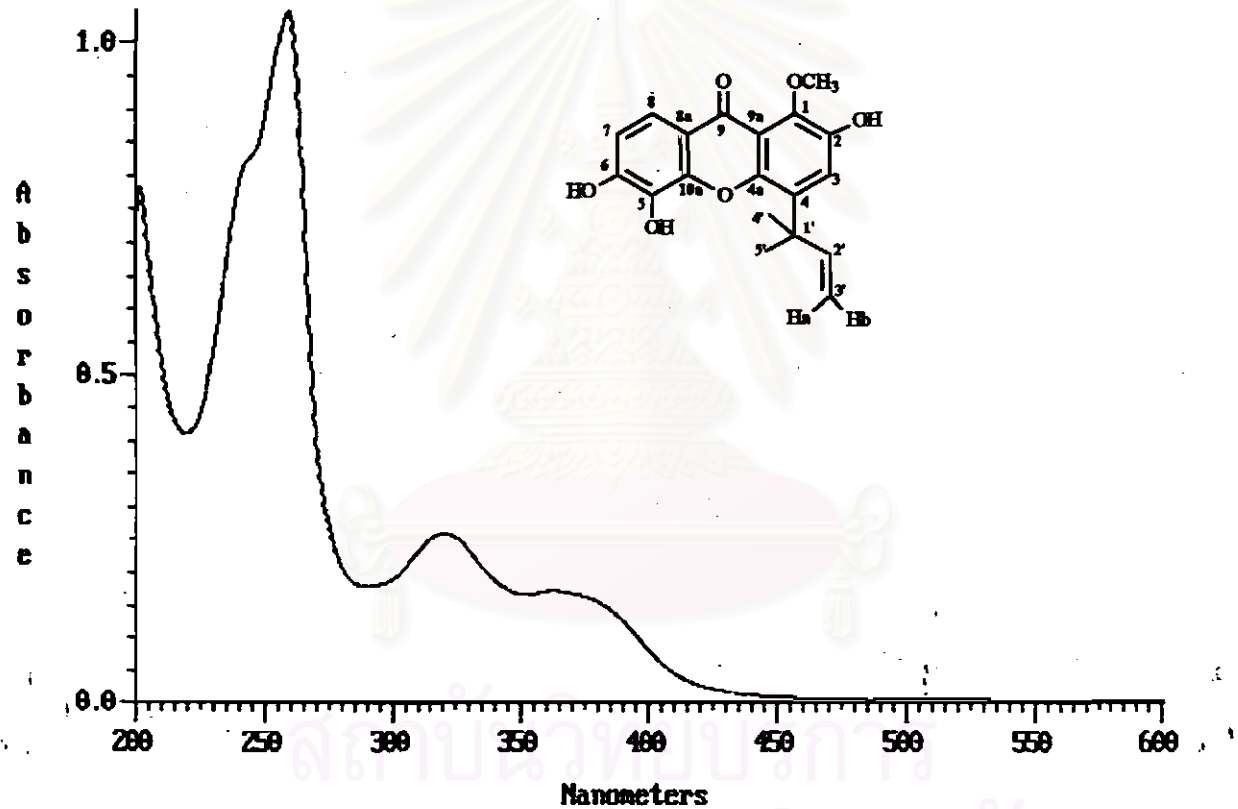


Figure 44 UV spectrum of compound GD-5 (in methanol)

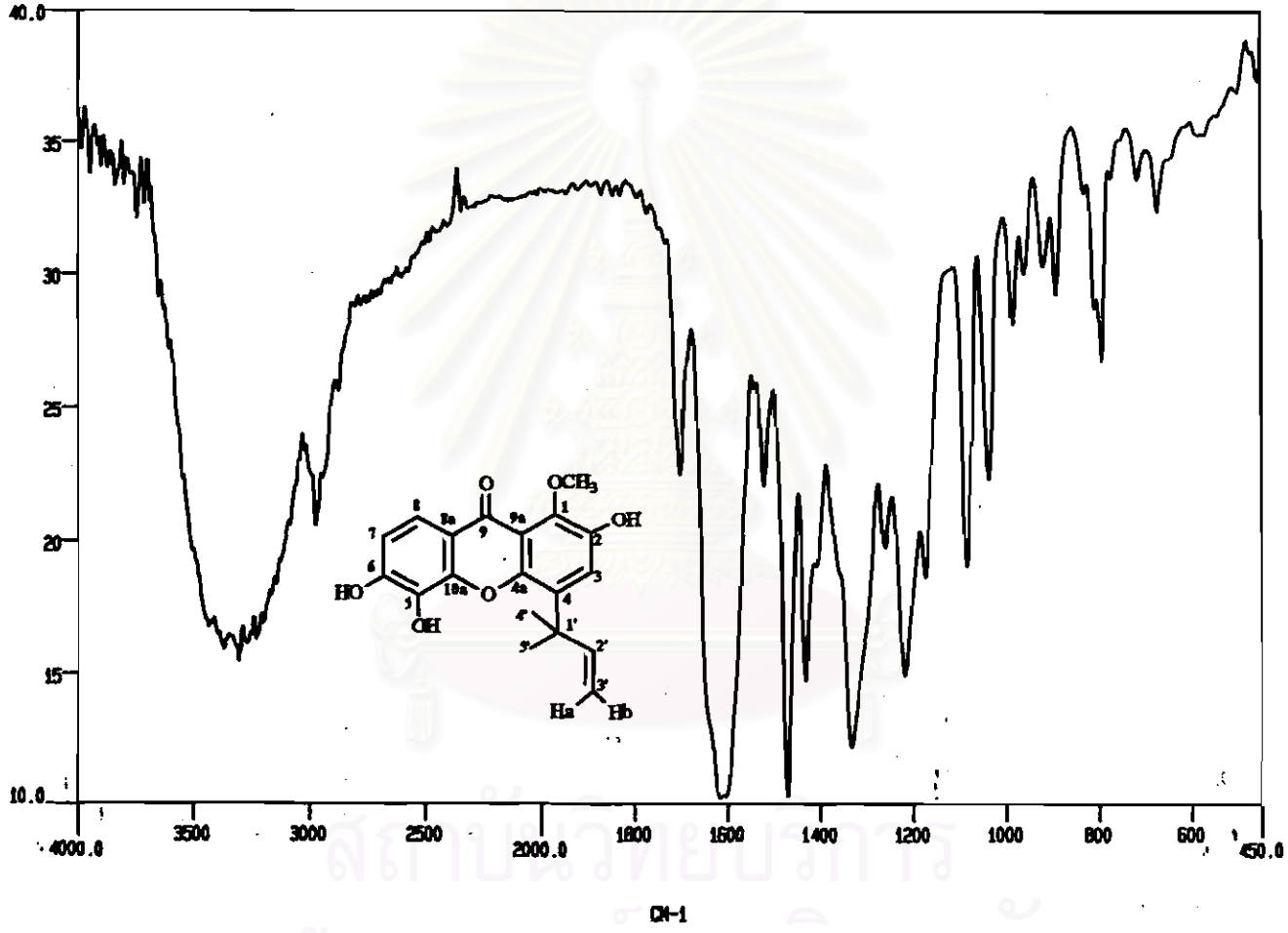


Figure 45 IR spectrum of compound GD-5 (film)

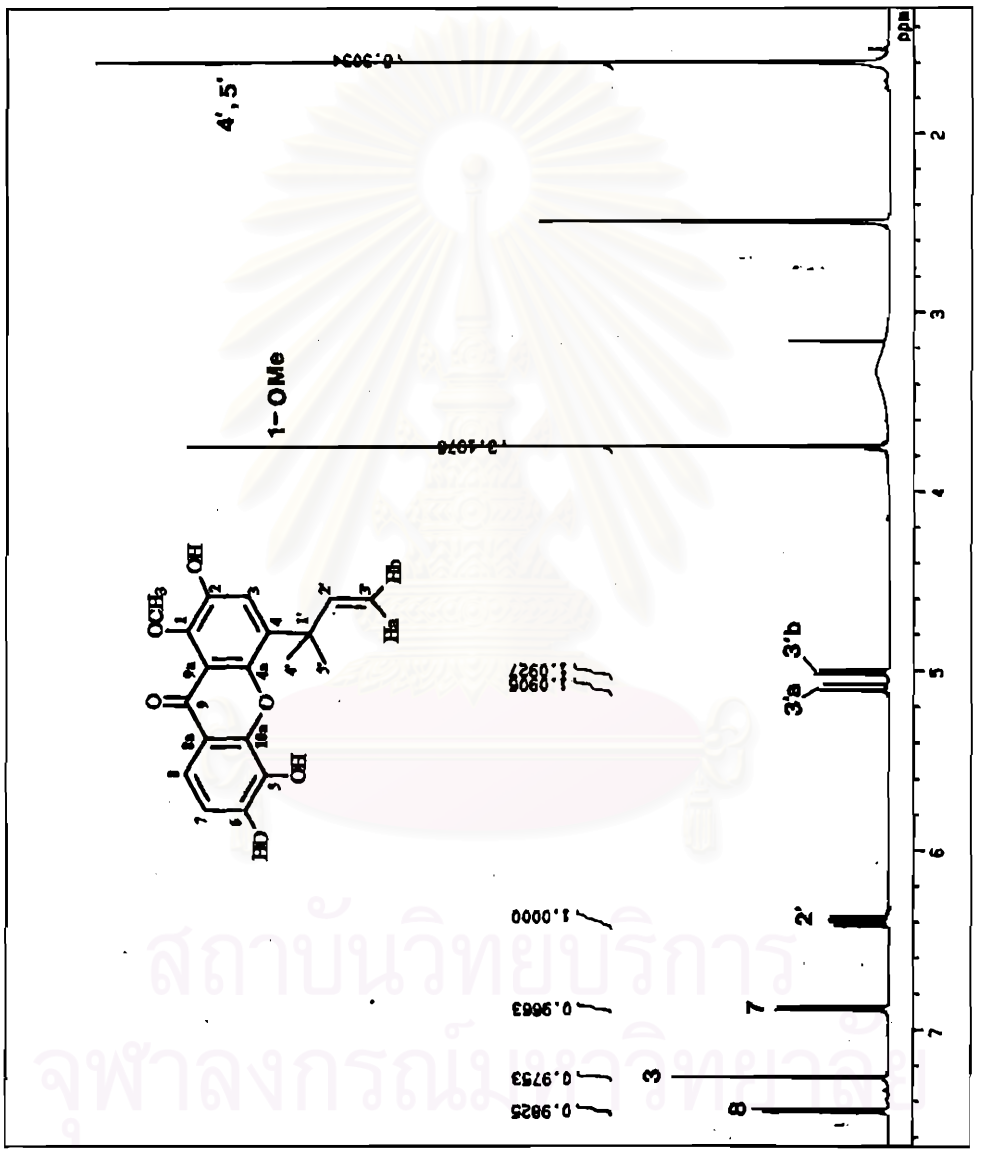


Figure 47a 500 MHz ^{1}H NMR spectrum of compound GD-5 (in DMSO-d_6)

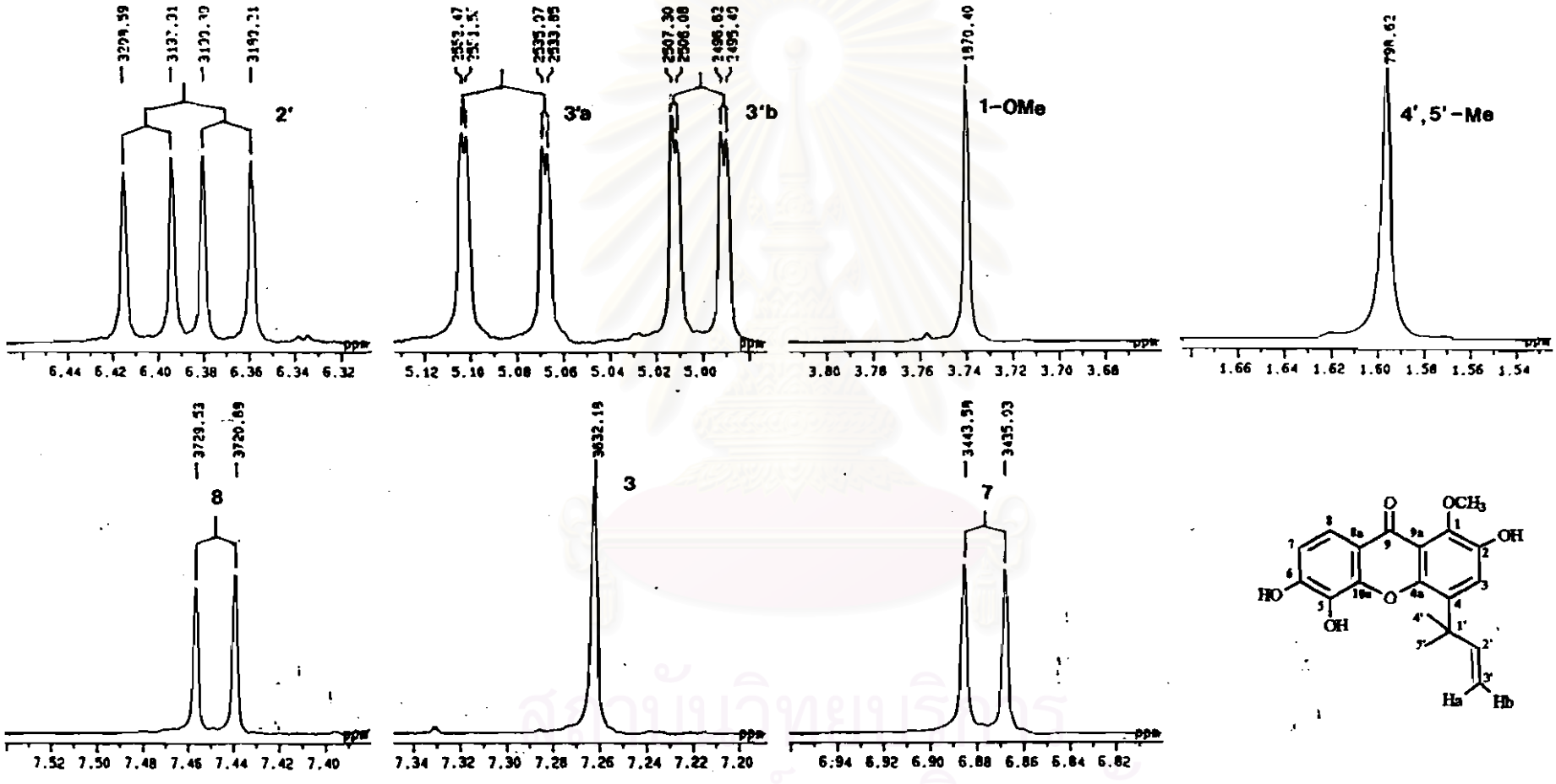


Figure 47b 500 MHz ^{1}H NMR spectrum of compound GD-5 (in DMSO-d_6) (expanded from 1.54-7.52 ppm)

Hz)] and three aromatic protons [δ 6.88 (1H, d, J = 8.8 Hz), 7.26 (1H, s) and 7.45 (1H, d, J = 8.8 Hz)].

The ^{1}H-^{1}H COSY spectrum (Figure 48) showed aromatic coupling between H-7 and H-8, and olefinic proton couplings were also observed between H-2′ and H-3′a, and H-2′ and H-3′b.

In a NOESY experiment (Figure 49), the NOE interactions confirmed the location of 1,1-dimethylallyl moiety and the substitution pattern of the aromatic rings. The results from the NOESY experiment of compound GD-5 are shown in Figure 50.

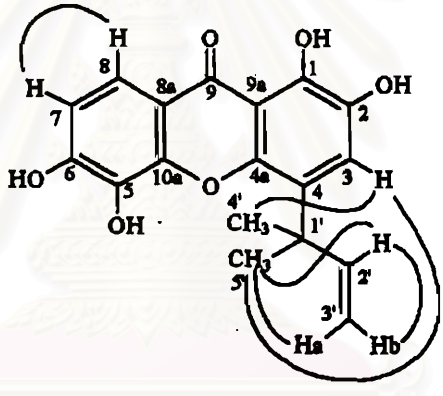


Figure 50 Results from NOESY experiment of compound GD-5

The ^{13}C NMR spectrum (Figure 51) and DEPT spectrum (Figure 52) provided signals for one carbonyl group, one methoxy carbon, two methyl carbons, one methylene carbon, four methine carbons and ten quaternary carbons.

Based on the information obtained from the HMQC spectrum (Figures 53a-53e), all protonated carbons of compound GD-5 were assigned, as shown in Table 17.

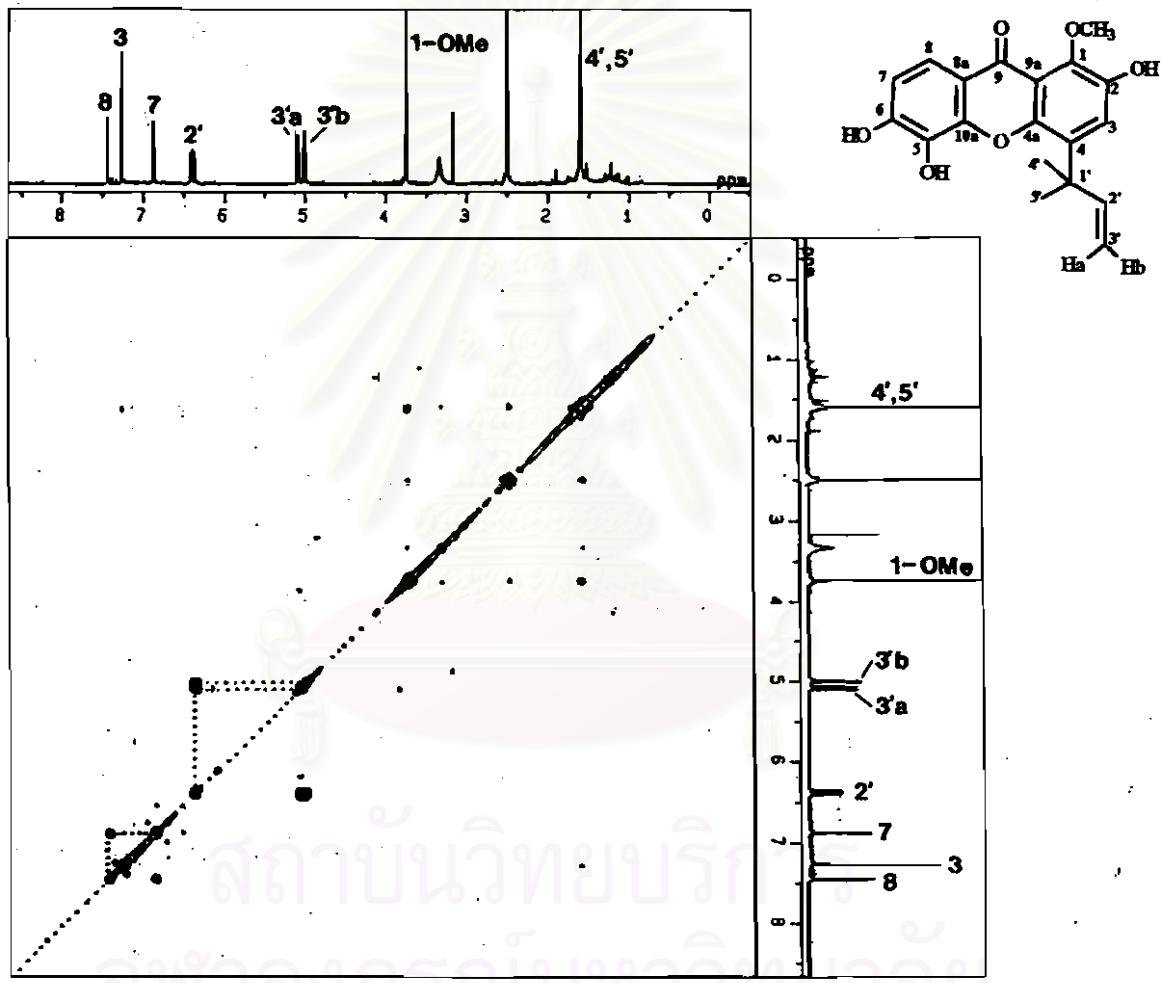


Figure 48 ^{1}H-^{1}H COSY spectrum of compound GD-5 (in DMSO-d_6)

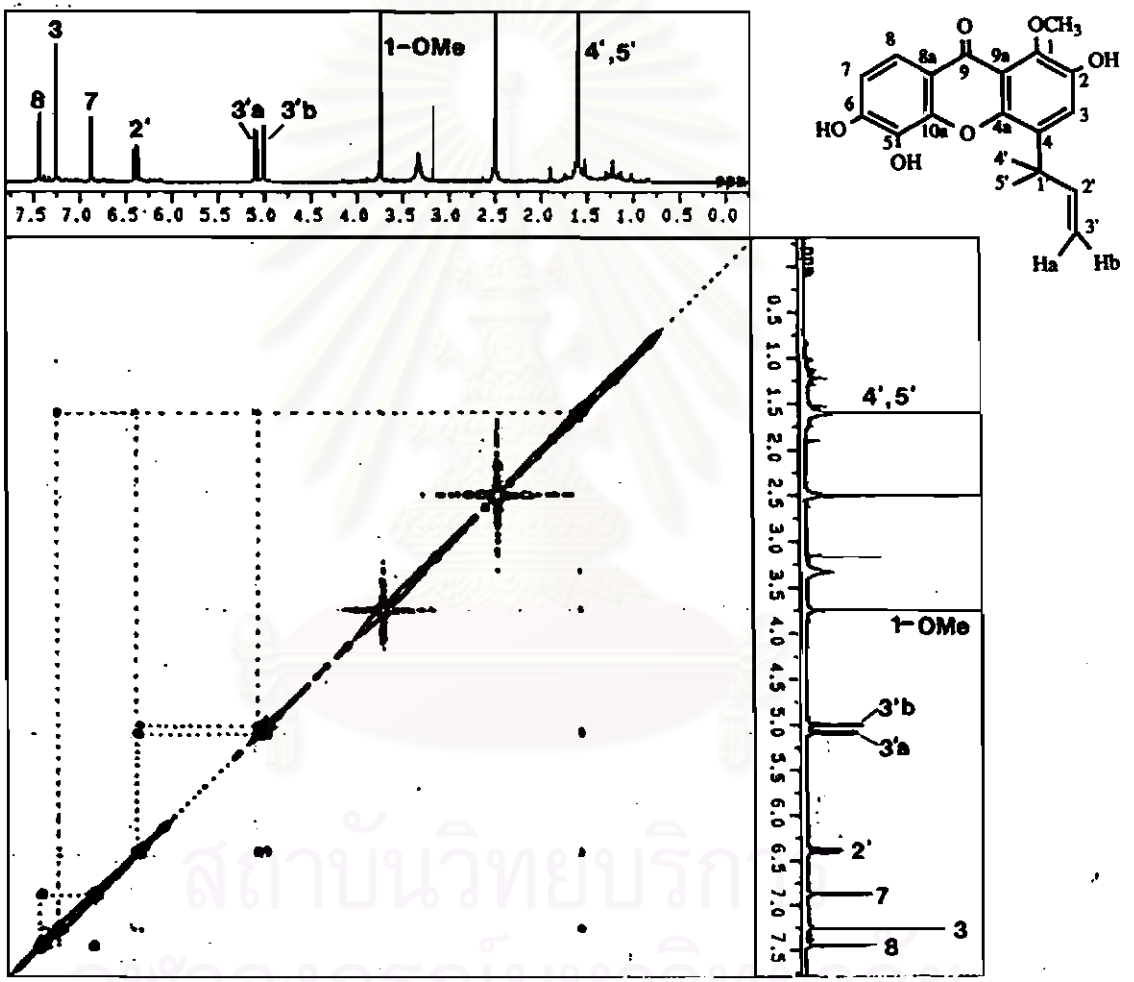


Figure 49 NOESY spectrum of compound GD-5 (in DMSO-d_6)

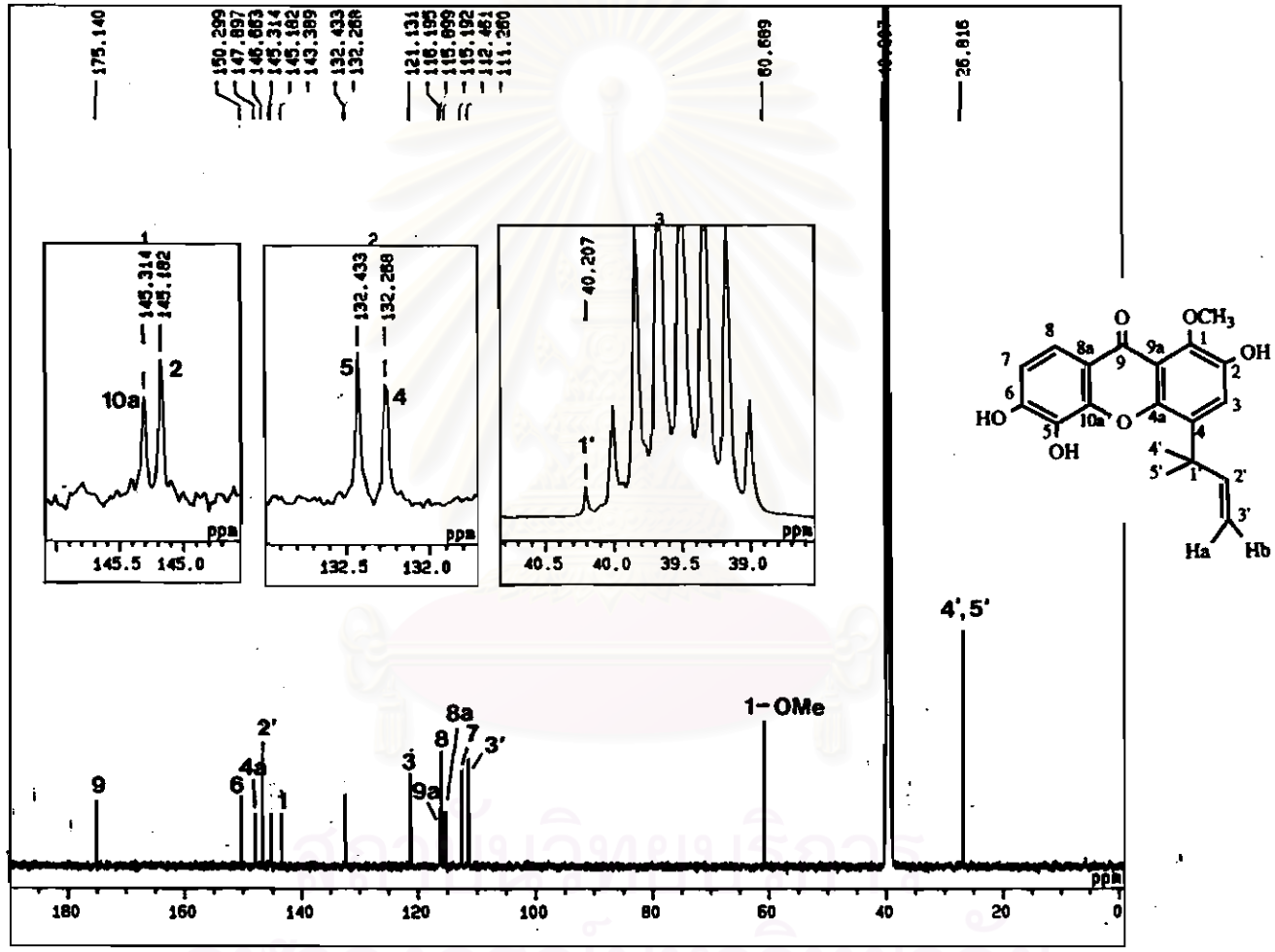


Figure 51 125 MHz ^{13}C NMR spectrum of compound GD-5 (in DMSO-d_6)

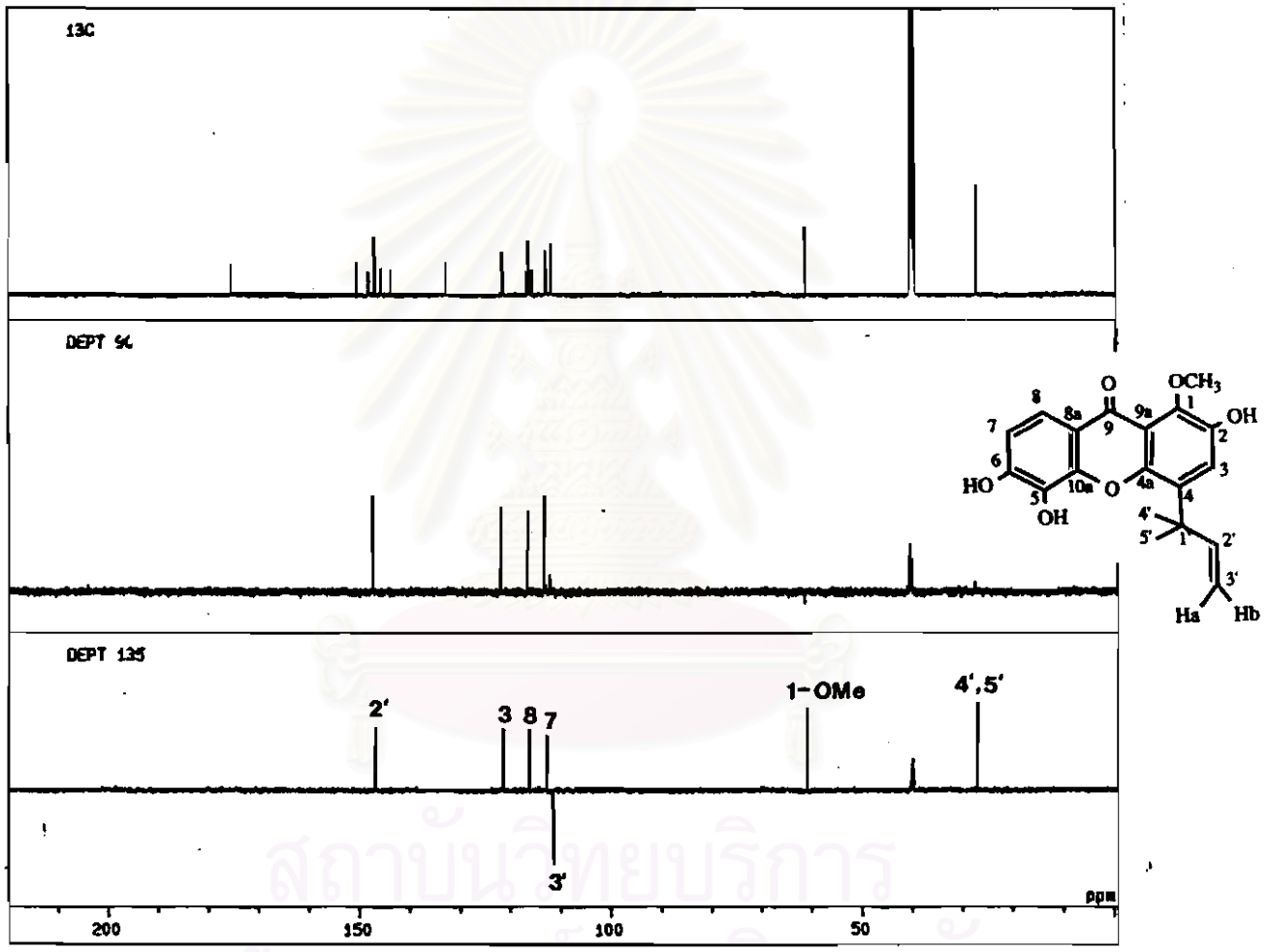


Figure 52 DEPT spectrum of compound GD-5 (in DMSO-d_6)

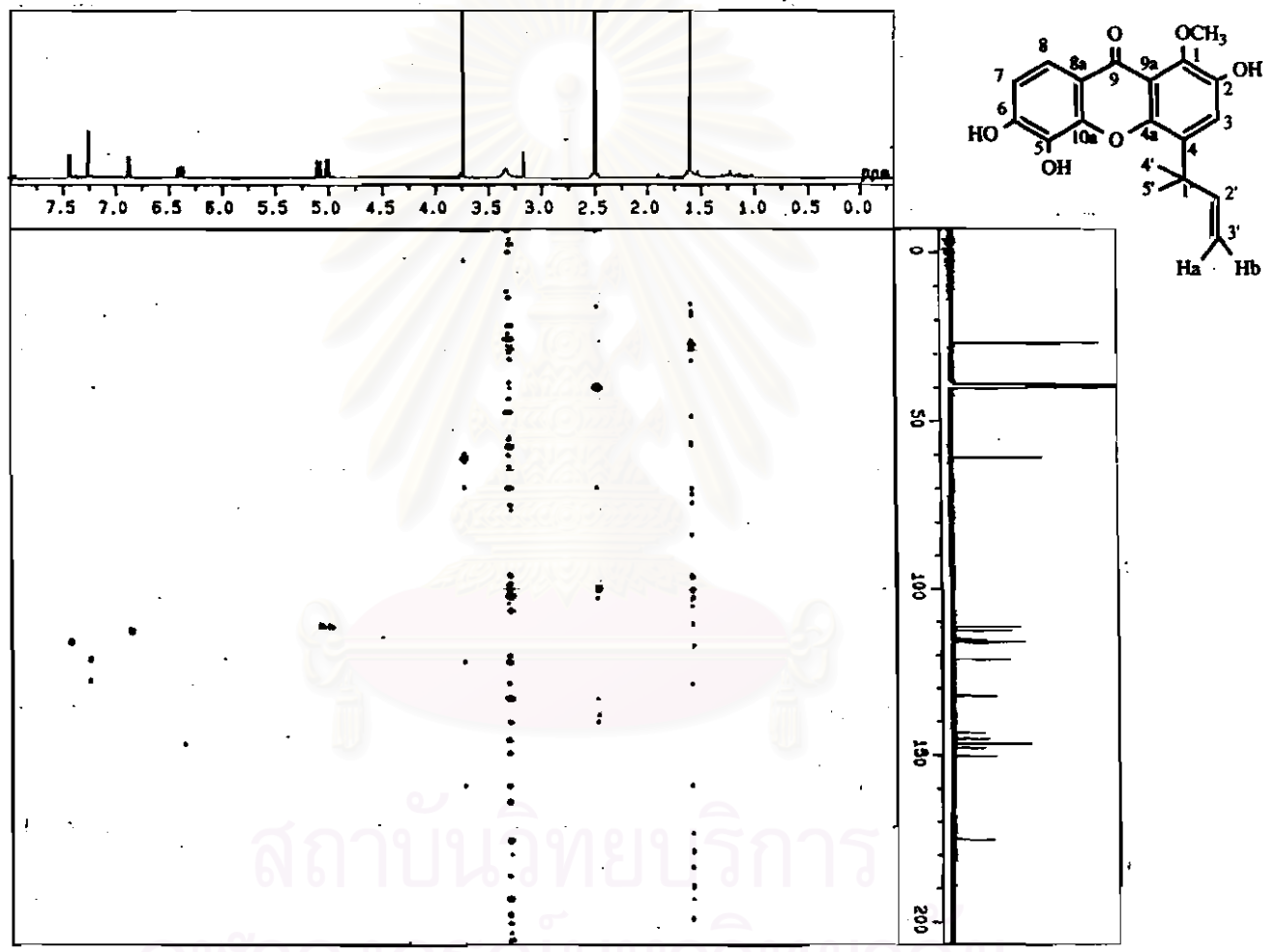


Figure 53a HMQC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 0.00-7.5 ppm, δ_C 0-200 ppm]

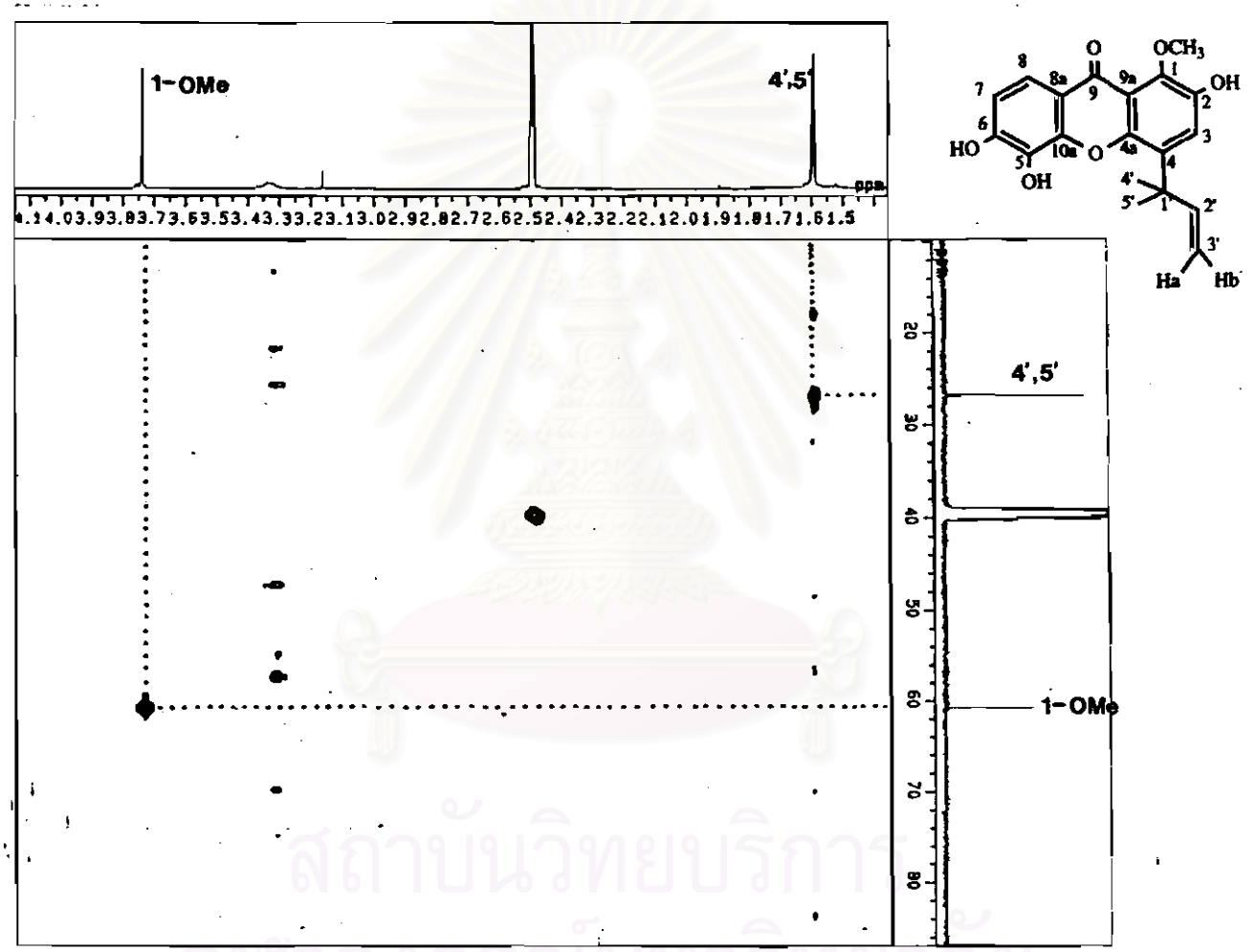


Figure 53b HMQC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 1.5-4.1 ppm, δ_C 20-80 ppm]

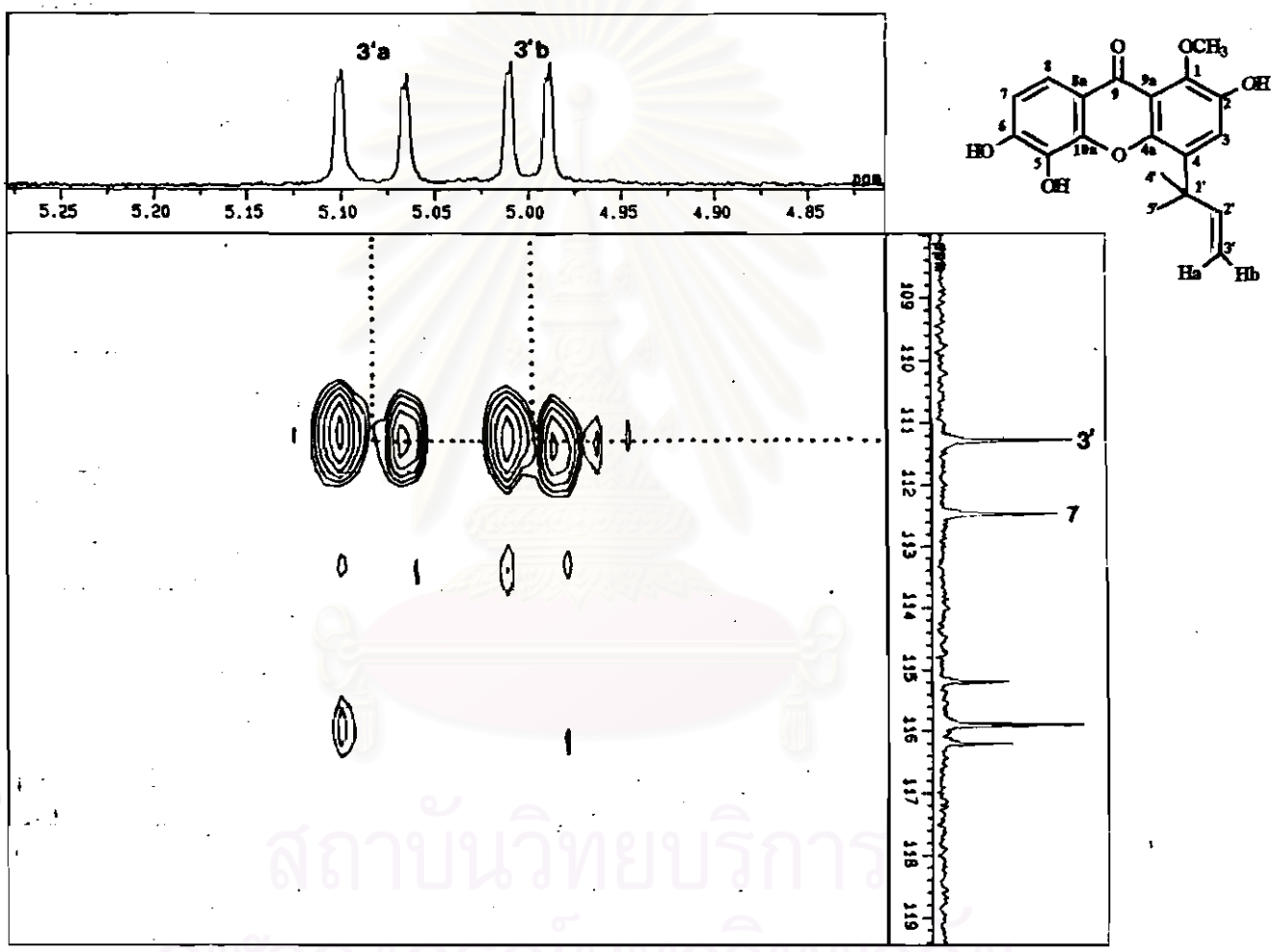


Figure 53c HMQC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 4.85-5.25 ppm, δ_C 109-119 ppm]

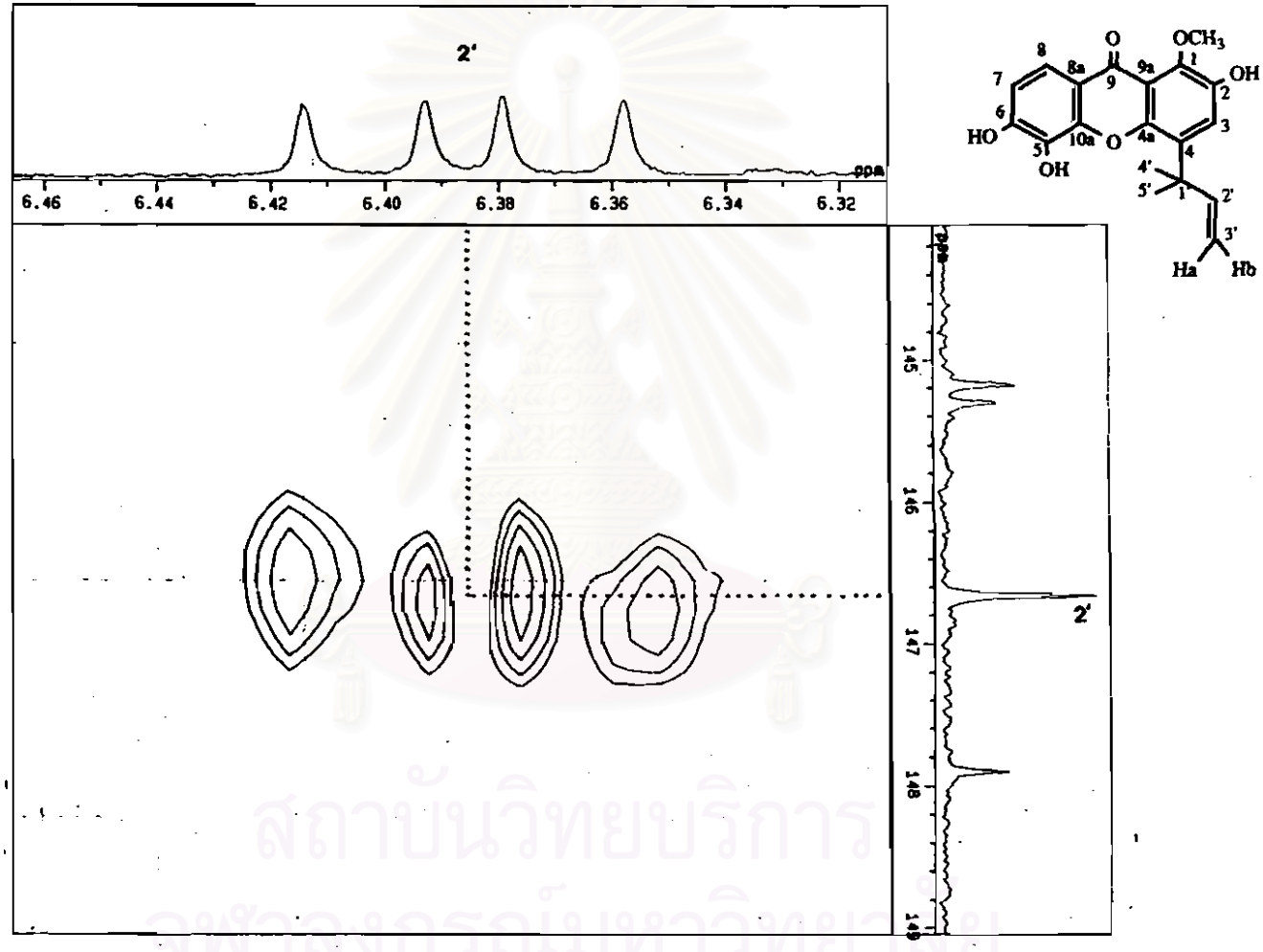


Figure 53d HMQC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 6.32-6.46 ppm, δ_C 145-149 ppm]

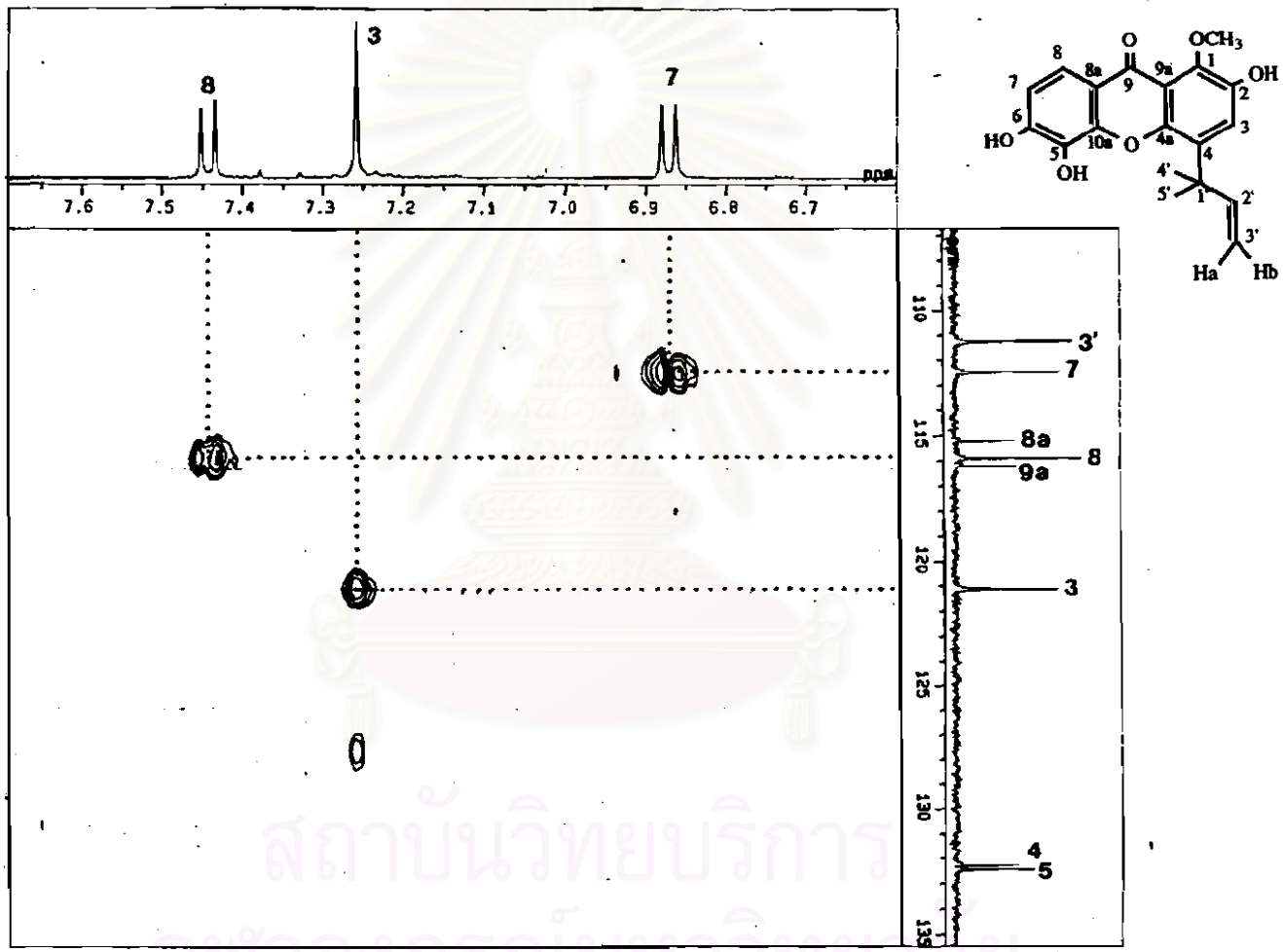


Figure 53e HMQC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 6.7-7.6 ppm, δ_C 110-135 ppm]

Table 17 The carbon-proton correlations of compound GD-5 observed in the HMQC spectrum

Carbon	δ_C (ppm)	Correaltion with proton at δ_H (ppm)
C-3	121.1	7.26
C-7	112.4	6.88
C-8	115.9	7.45
C-2′	146.6	6.39
C-3′a	111.2	5.09
C-3′b	111.2	5.01
C-4′,5′	26.8	1.59
C_1-OMe	60.6	3.74

According to the DEPT and HMQC spectra, the signal at δ 115.9 was assigned to C-8. The signals of C-8a and C-9a were assigned at δ 115.2 and 116.2, respectively from the HMBC correlations.

The assignments of quaternary carbons of compound GD-5 and the position of 1,1-dimethylallyl group on the aromatic ring were obtained from examination of the HMBC spectra (Figures 54a-54h). The spectral data revealed that the 1,1-dimethylallyl group was on C-4.

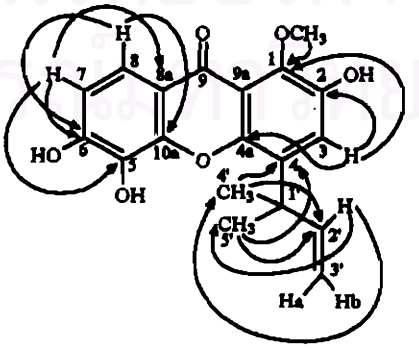


Figure 55 Long-range C-H correlations of compound GD-5 observed in HMBC spectrum

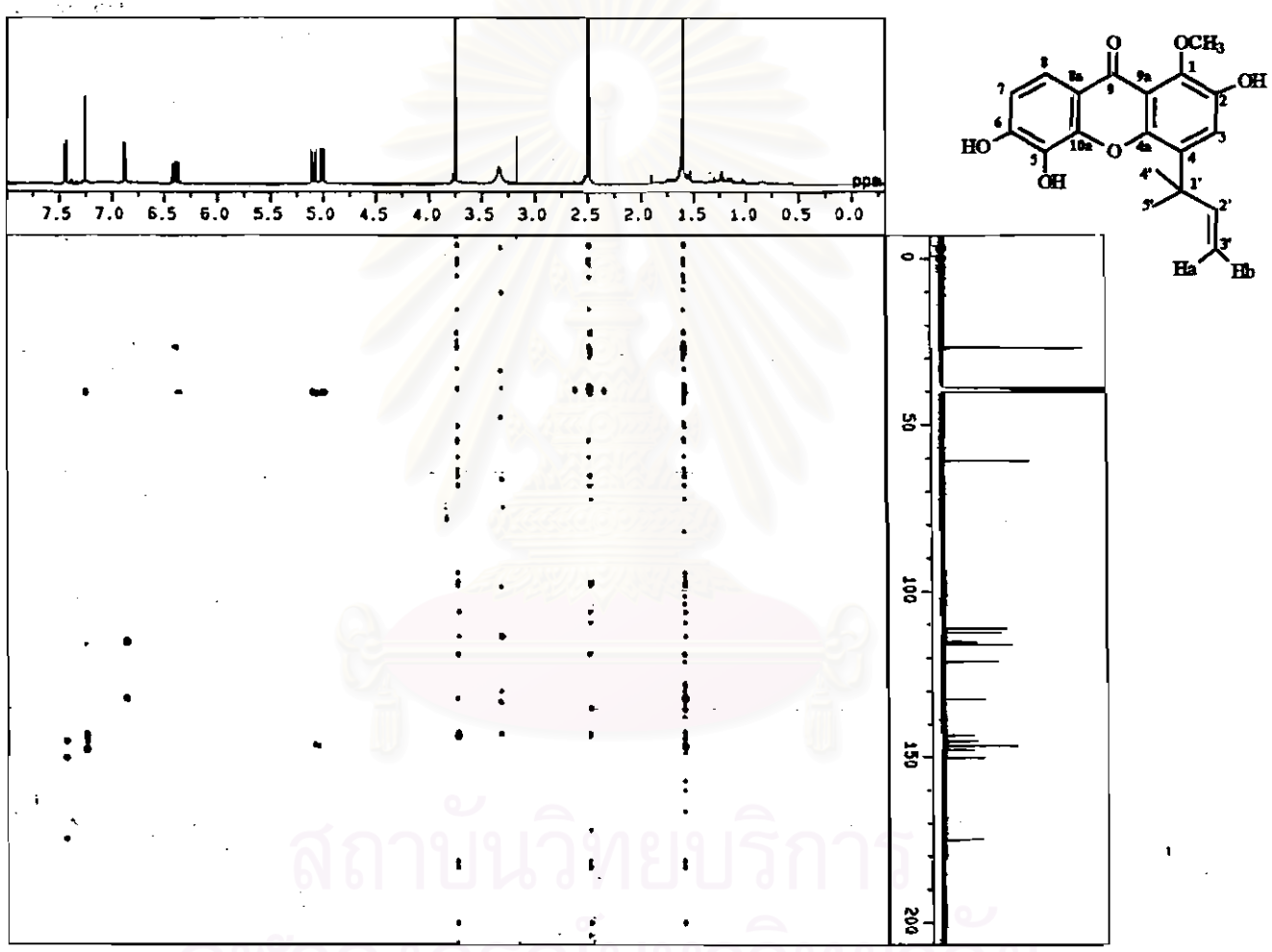


Figure 54a HMBC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 0.00-7.5 ppm, δ_C 0-200 ppm]

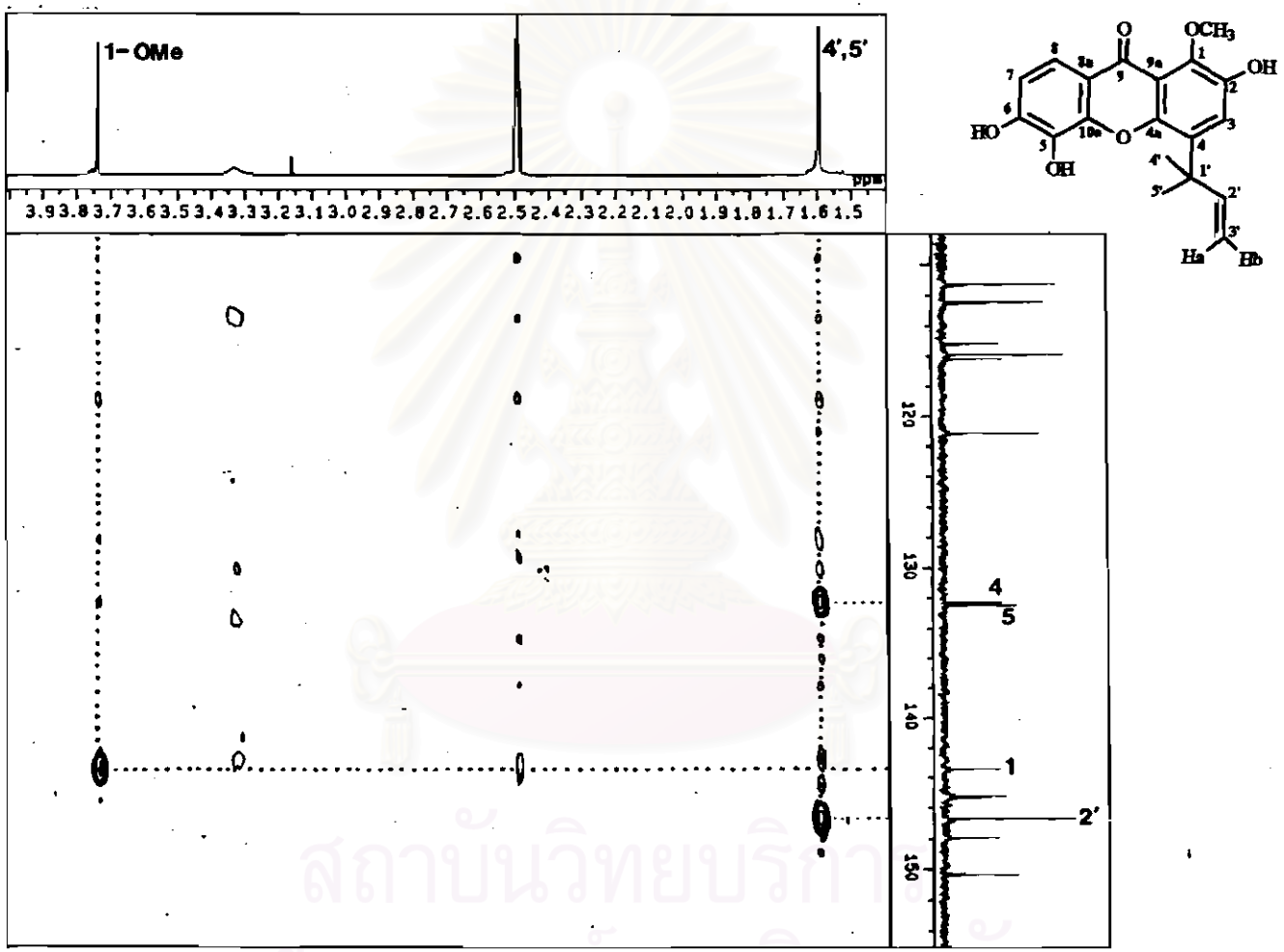


Figure 54b HMBC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 1.5-3.9 ppm, δ_C 110-152 ppm]

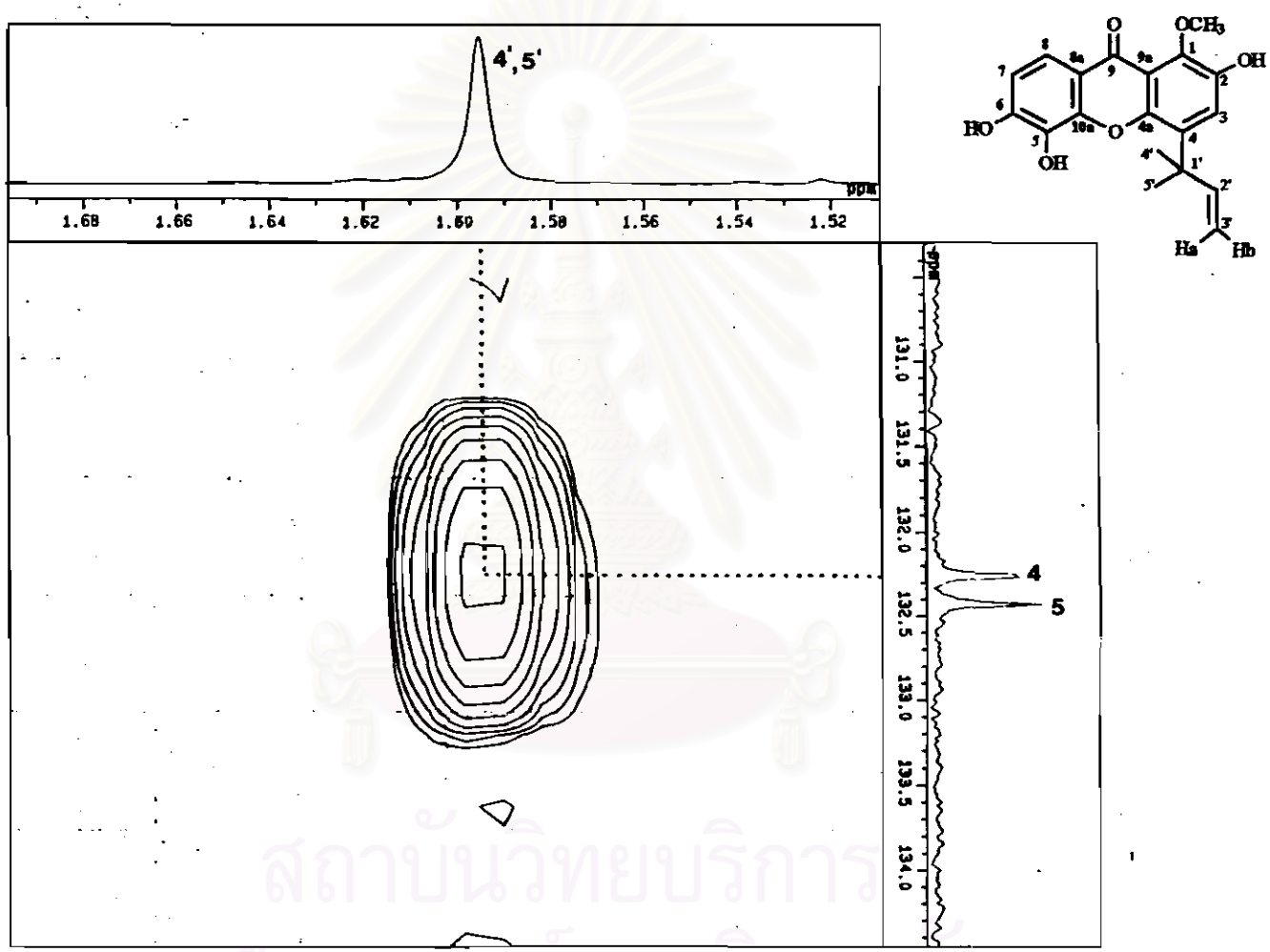


Figure 54c HMBC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 1.52-1.68 ppm, δ_C 131-134 ppm]

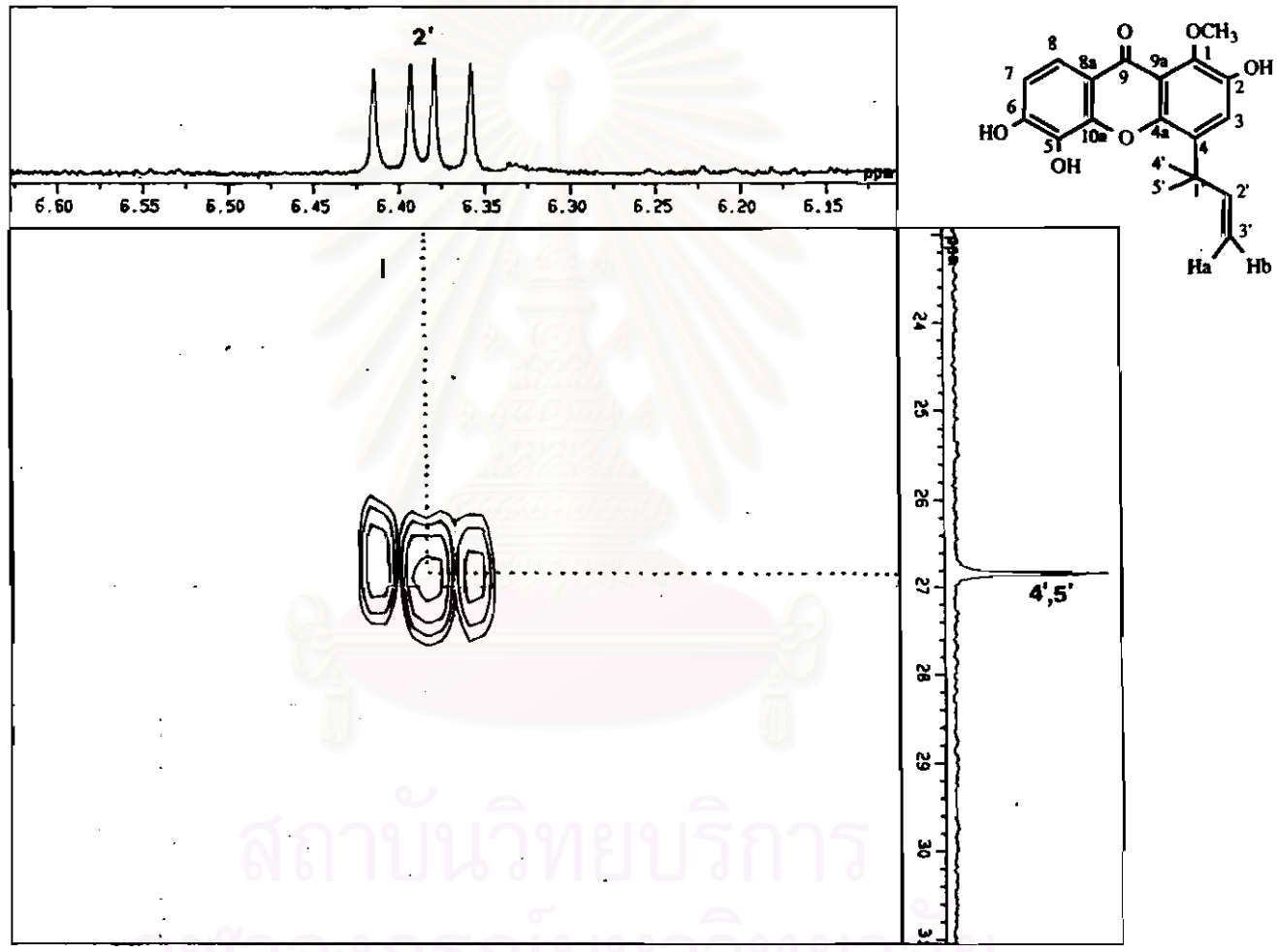


Figure 54d HMBC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 6.15-6.60 ppm, δ_C 24-31 ppm]

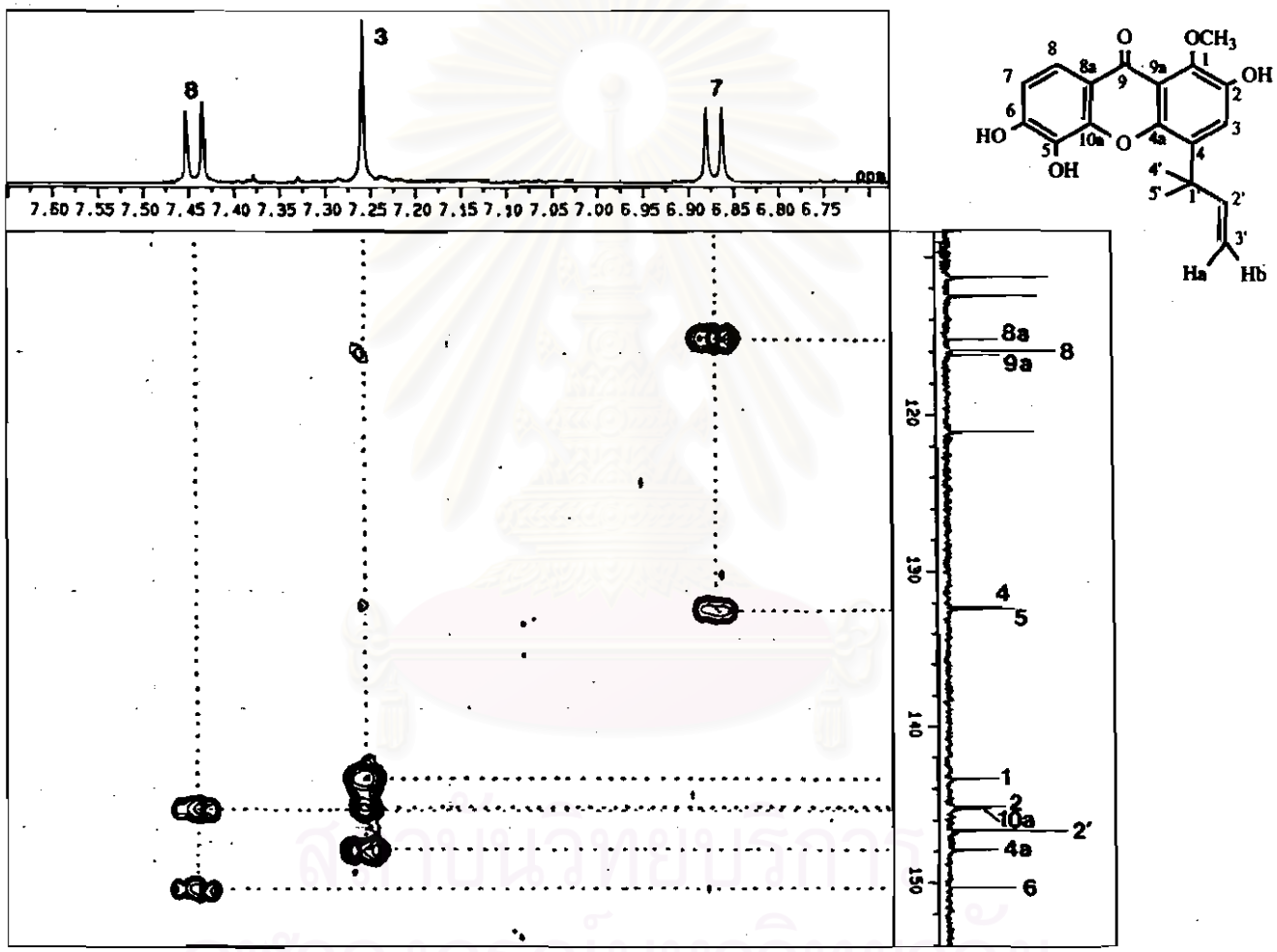


Figure 54e HMBC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 6.75-7.60 ppm, δ_C 110-152 ppm]

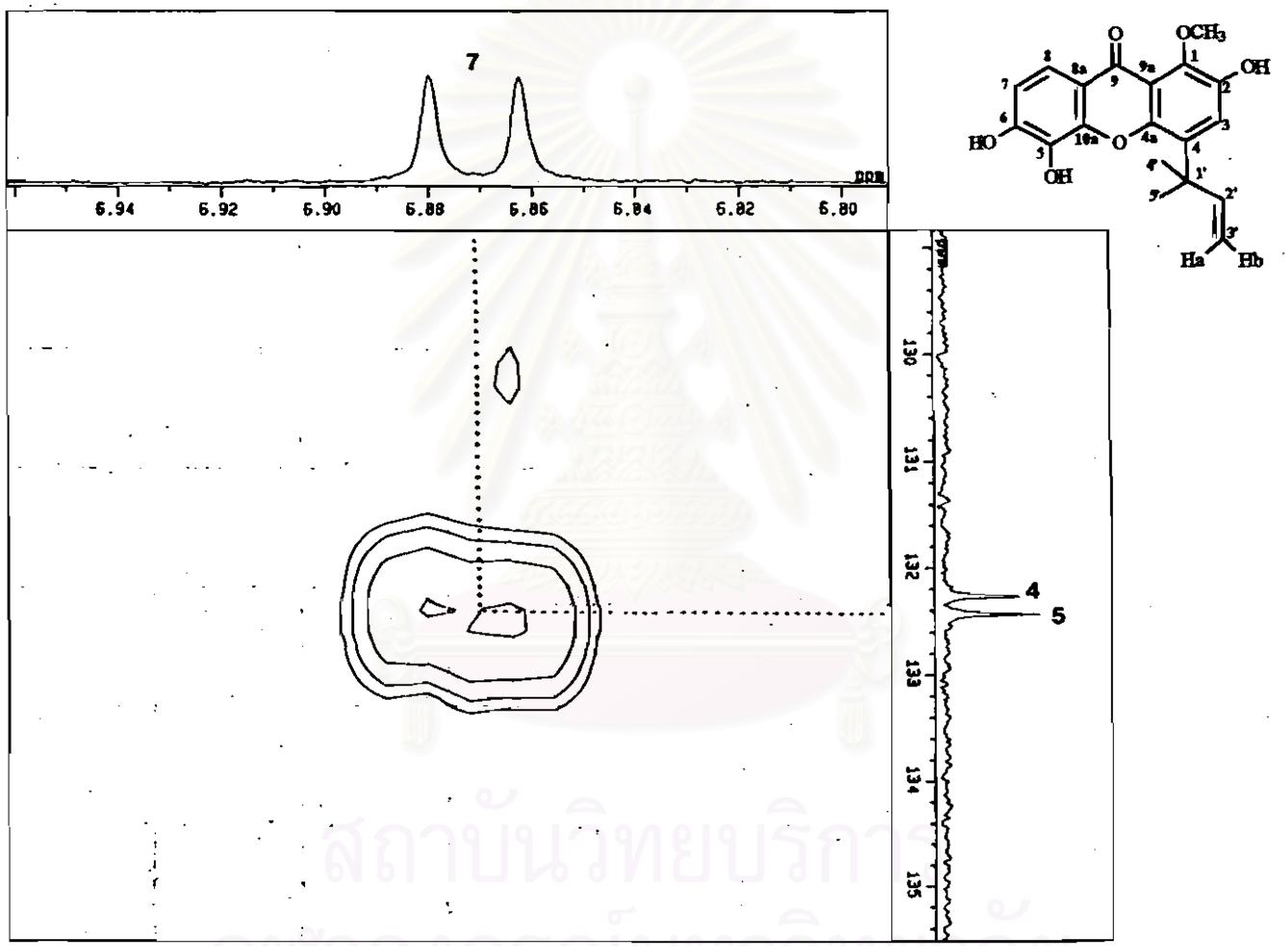


Figure 54f HMBC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 6.80-6.94 ppm, δ_C 130-135 ppm]

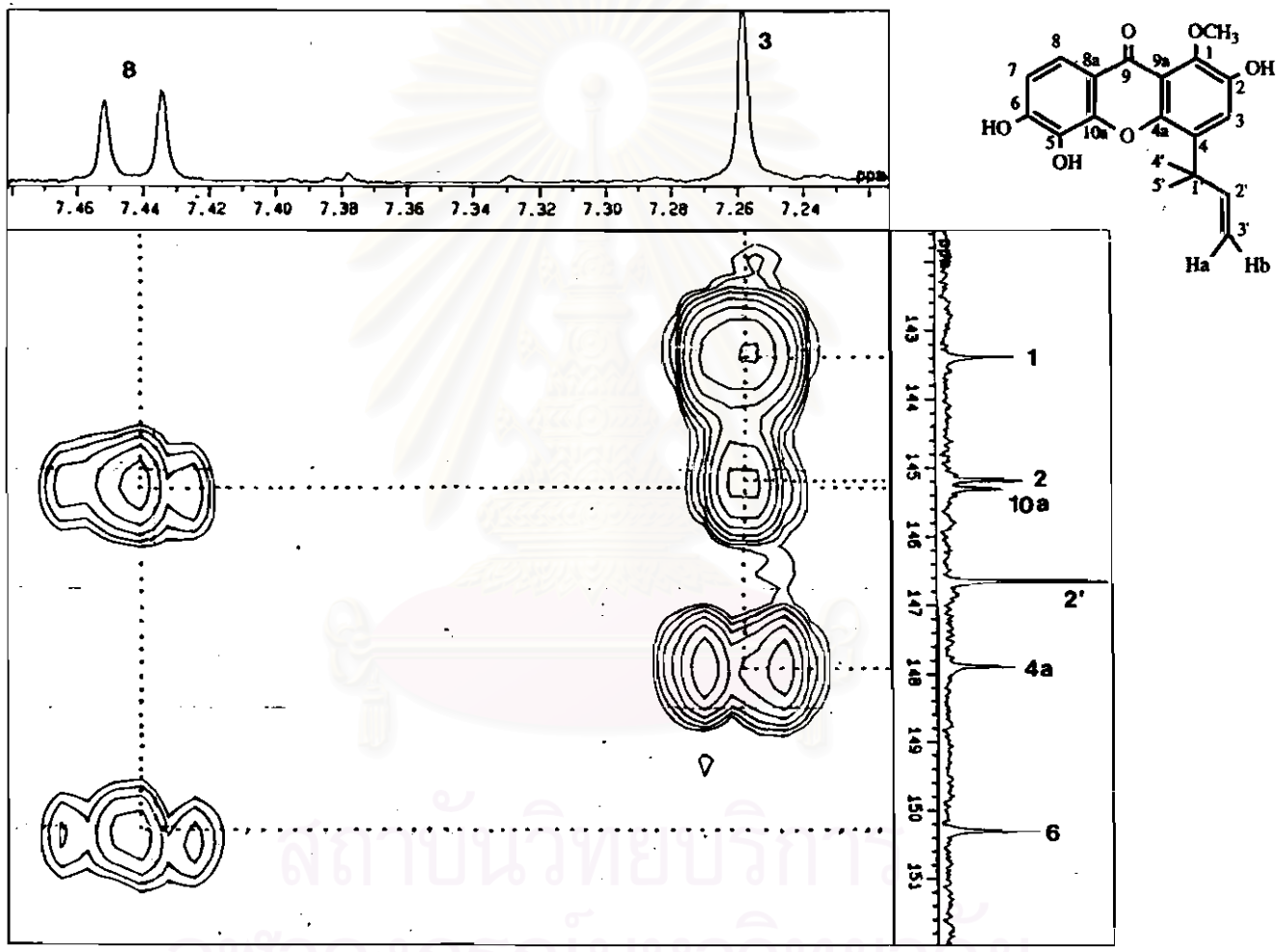


Figure 54g HMBC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 7.22-7.48 ppm, δ_C 143-151 ppm]

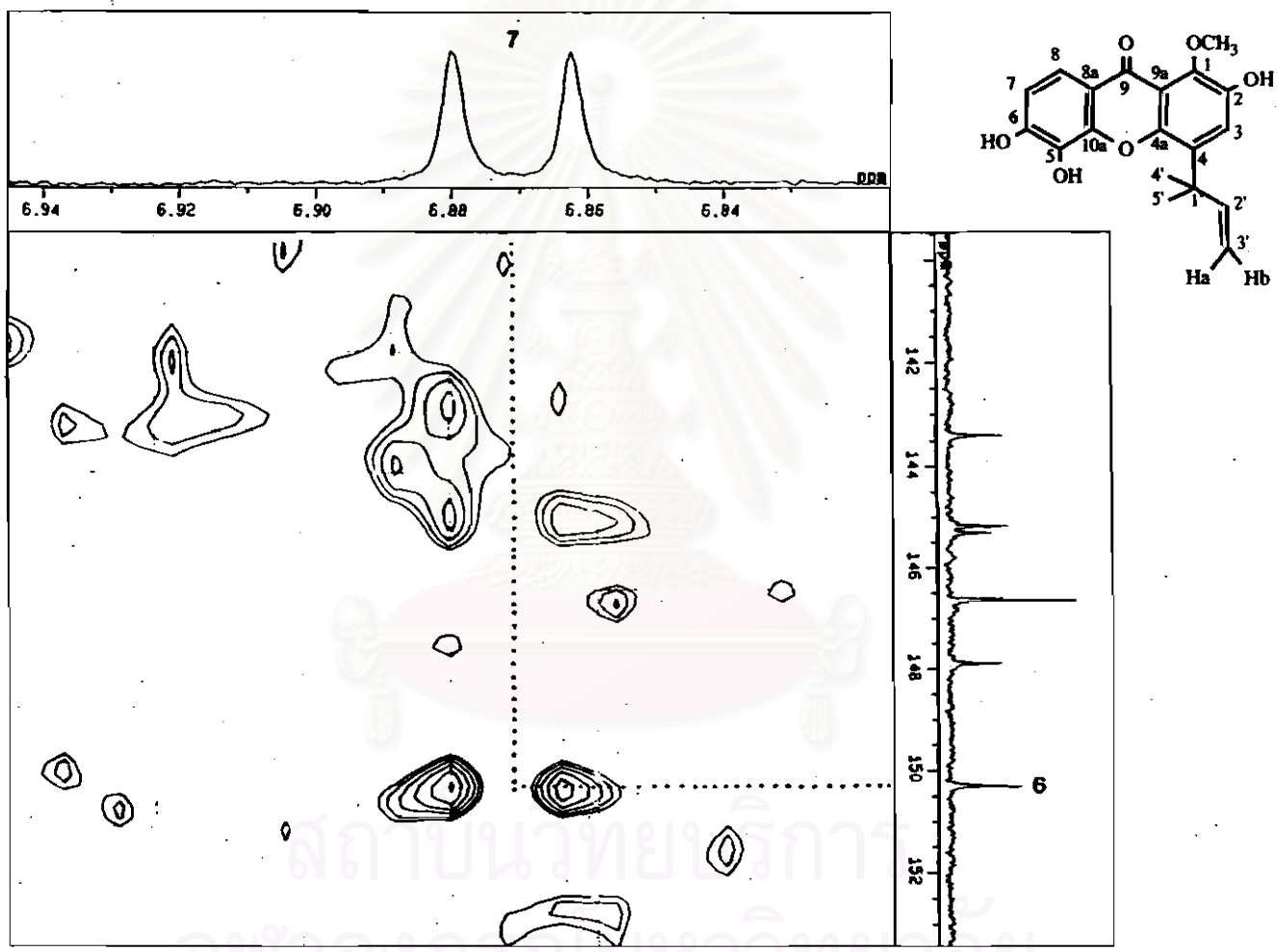


Figure 54h HMBC spectrum of compound GD-5 (in DMSO-d_6), [δ_H 6.84-6.94 ppm, δ_C 142-152 ppm]

From the HMBC spectrum, the signals at δ 132.2, 132.4, 145.1 and 145.3 were assigned to C-4, C-5, C-2 and C-10a, respectively. In addition, the assignments of C-6 and C-10a in the previous report (Minami *et al.*, 1996) were reversed.

The complete proton and carbon assignments of compound GD-5 and those of 1-*O*-methylsymphoxanthone are shown in Table 18.

Table 18 ^{1}H and ^{13}C NMR spectral data of compound GD-5 and 1-*O*-methylsymphoxanthone [92] (in DMSO-d_6)

Carbon	Compound GD-5		1-*O*-Methylsymphoxanthone*	
	δ_C (ppm)	δ_H Multiplicity, *J* (Hz)	δ_C (ppm)	δ_H Multiplicity, *J* (Hz)
1	143.3	-	143.4	-
2	145.1	-	145.3	-
3	121.1	7.26 s	121.2	7.27 s
4	132.2	-	132.3	-
4a	147.9	-	147.9	-
5	132.4	-	132.2	-
6	150.2	-	145.3	-
7	112.4	6.88 (d, *J* = 8.8)	112.5	6.89 (d, *J* = 8.8)
8	115.9	7.45 (d, *J* = 8.8)	115.2	7.45 (d, *J* = 8.8)
8a	115.2	-	116.2	-
9	175.1	-	175.3	-
9a	116.2	-	115.9	-
10a	145.3	-	150.3	-
1′	40.2	-	40.3	-
2′	146.6	6.39 (dd, *J* = 17.7, 10.6)	146.7	6.39 (dd, *J* = 17.6, 11.0)
3′a	111.2	5.09 (dd, *J* = 17.7, 1.2)	111.4	5.09 (d, *J* = 17.6)
3′b	-	5.01 (dd, *J* = 10.6, 1.2)	-	5.09 (d, *J* = 11.0)
4′	26.8	1.59 s	26.8	1.60 s

Table 18 (Continued)

Carbon	Compound GD-5		1-*O*-Methylsymphoxanthone*	
	δ_C (ppm)	δ_H Multiplicity, *J* (Hz)	δ_C (ppm)	δ_H Multiplicity, *J* (Hz)
5′	26.8	1.59 s	26.8	1.60 s
C_1-OMe	60.6	3.74 s	60.7	3.77 s

* From Minami *et al.*, 1996

6. Structure Determination of Compound GD-6 [102]

Compound GD-6 was obtained as yellow needles from fraction S-333 by centrifugal and preparative TLC techniques.

Compound GD-6 gave a molecular ion [M^+] at *m/z* 328 in the EIMS (Figure 56), suggesting a tentative molecular formula of $C_{18}H_{16}O_6$. The UV (Figure 57) and IR (Figure 58) spectral data closely resembled those of compound GD-5, suggesting a xanthone with a similar substitution pattern.

The structure of compound GD-6 was determined to be symphoxanthone [102] by analysis of its 1-D and 2-D NMR (HMBC, HMQC, ^{1}H-^{1}H COSY and NOESY) spectra. Symphoxanthone has been isolated from the wood of *Symphonia globulifera* (Locksley, Moore and Scheinmann, 1966) and the wood of *G. subelliptica* (Minami *et al.*, 1996). Regarding its NMR spectral data, only ^{1}H NMR assignments have been reported.

From the ^{1}H NMR spectrum (Figures 59a-59b), the structure of compound GD-6 contained one 1,1-dimethylallyl group and three aromatic protons. The ^{13}C NMR (Figure 60) and DEPT (Figure 61) spectra disclosed the presence of one carbonyl group, four methine carbons, one methylene carbon, two methyl carbons and ten quaternary carbons. The ^{1}H NMR data were similar to those of compound GD-5 except for the signals attributed to the hydroxyl moiety (δ 12.96) which was replaced by a methoxy functionality (δ 3.77).

The coupling between H-7 and H-8 and the couplings of the olefinic protons H-2′, H-3′a and H-3′b were observed in the ^{1}H-^{1}H COSY spectrum (Figures 62). All protonated carbons were assigned by analysis of the HMQC spectrum (Figures 63a-63b). The information from the HMQC spectrum of compound GD-6 is summarized in Table 19.

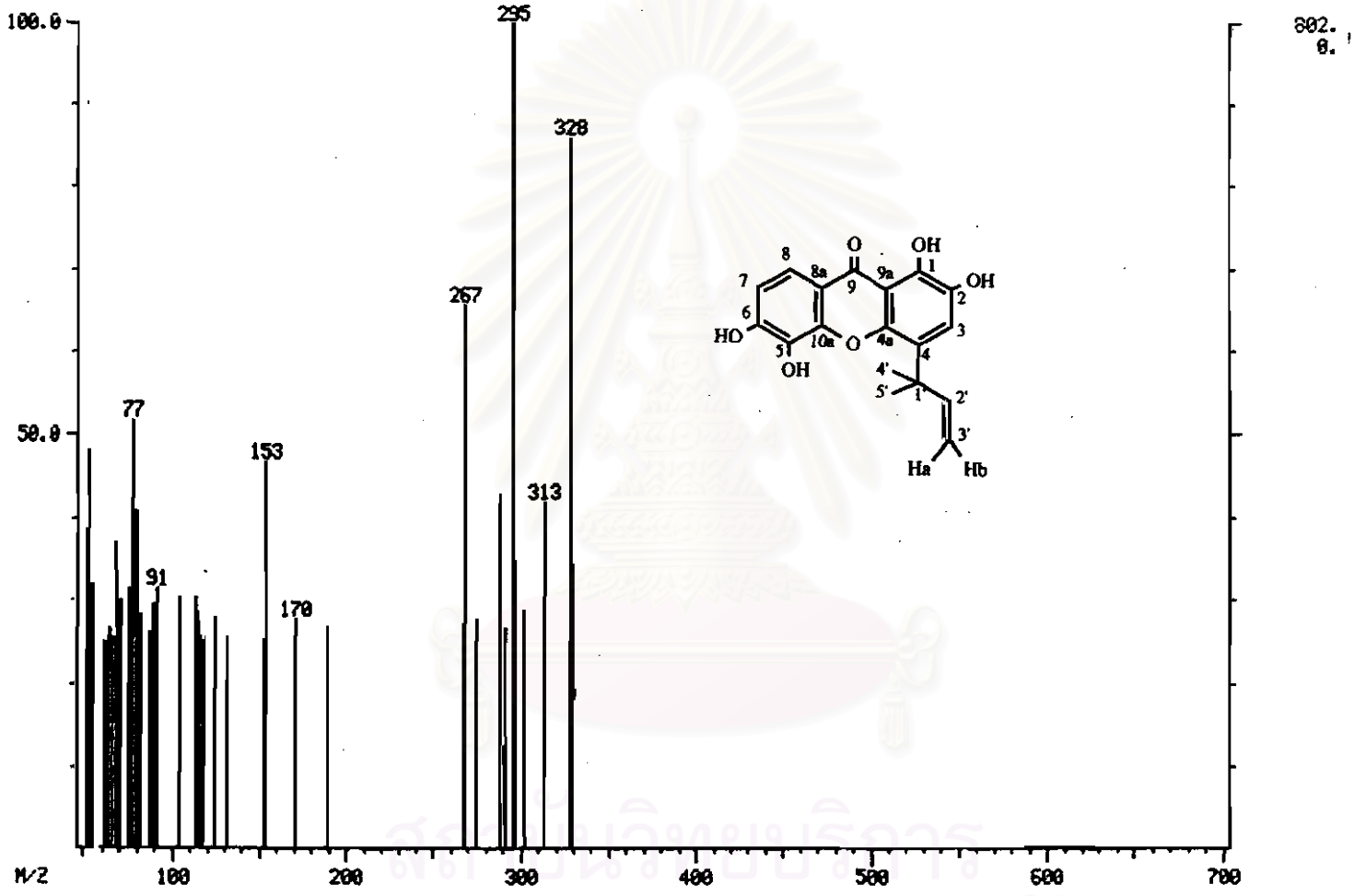


Figure 56 EI mass spectrum of compound GD-6

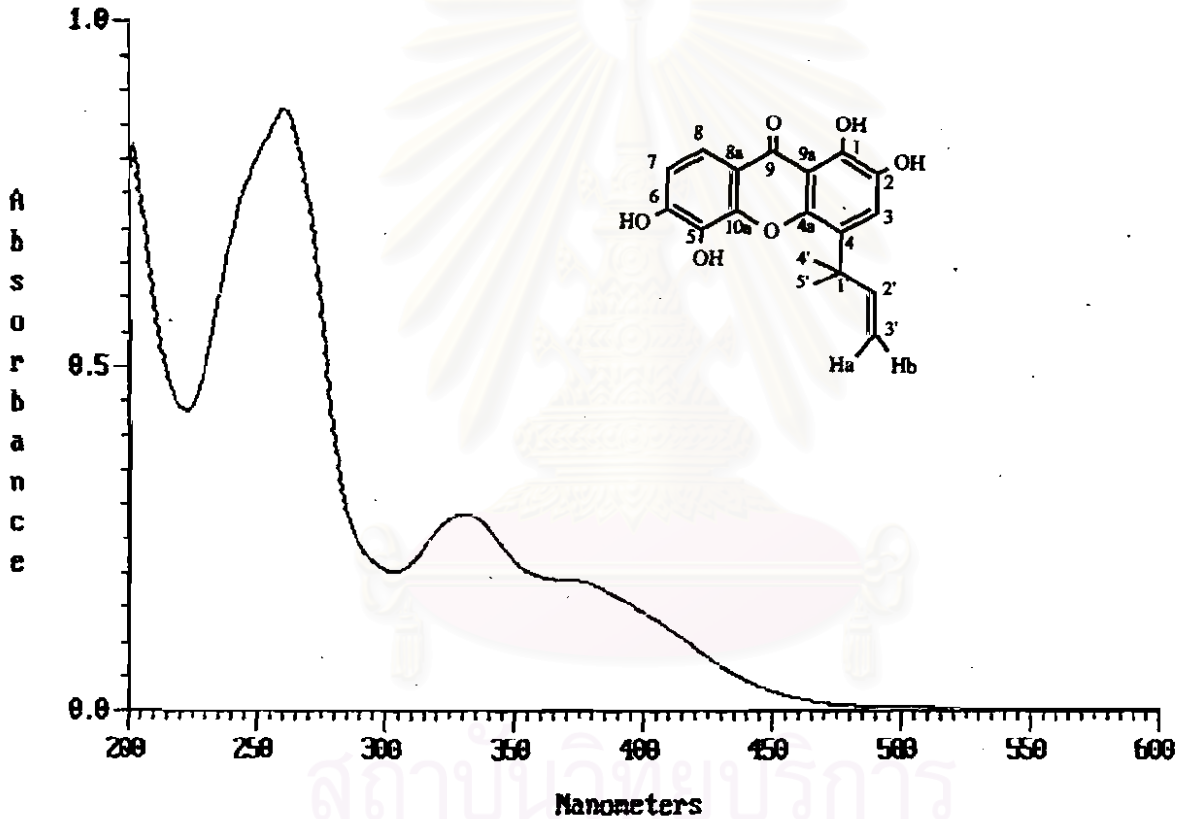


Figure 57 UV spectrum of compound GD-6 (in methanol)

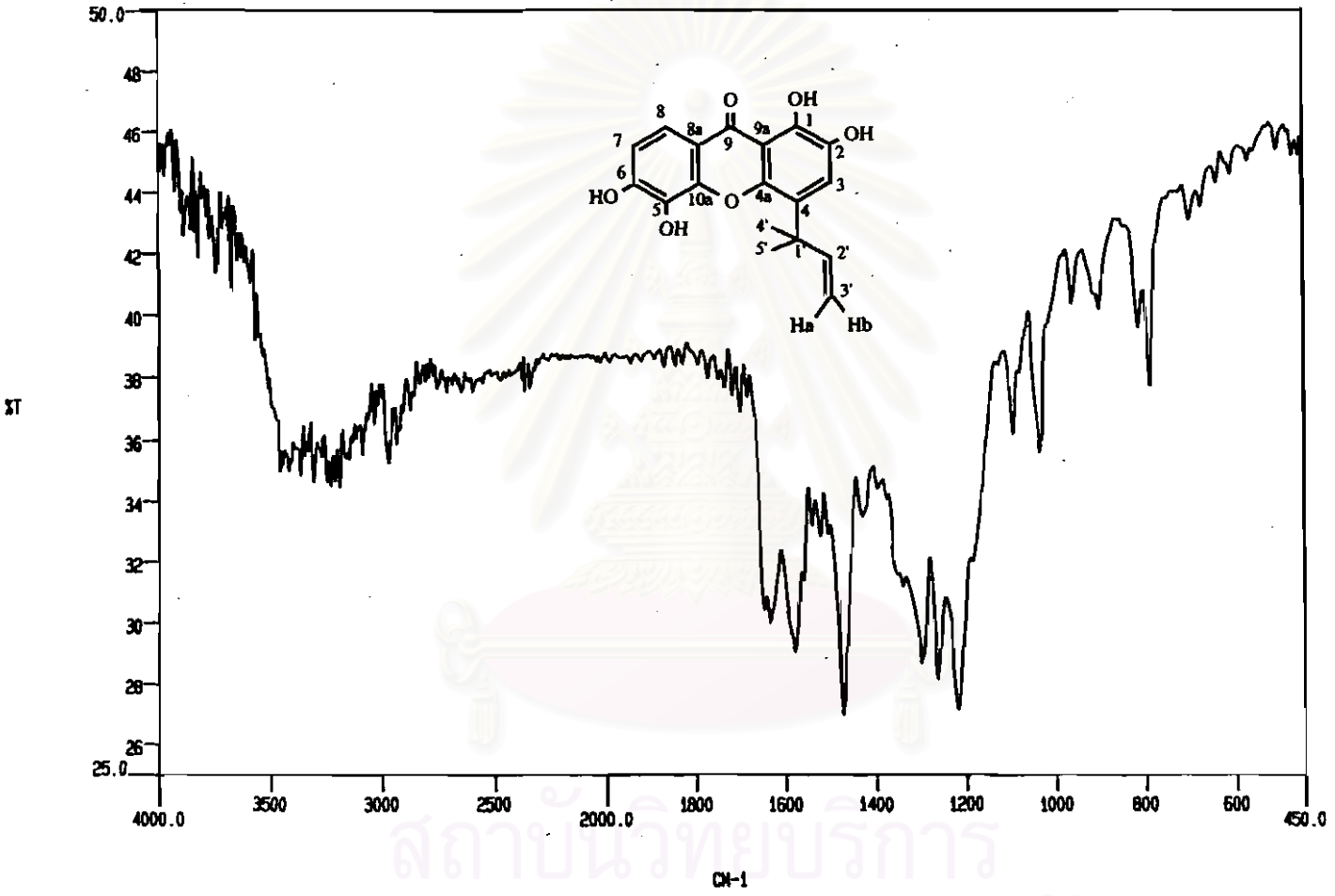


Figure 58 IR spectrum of compound GD-6 (film)

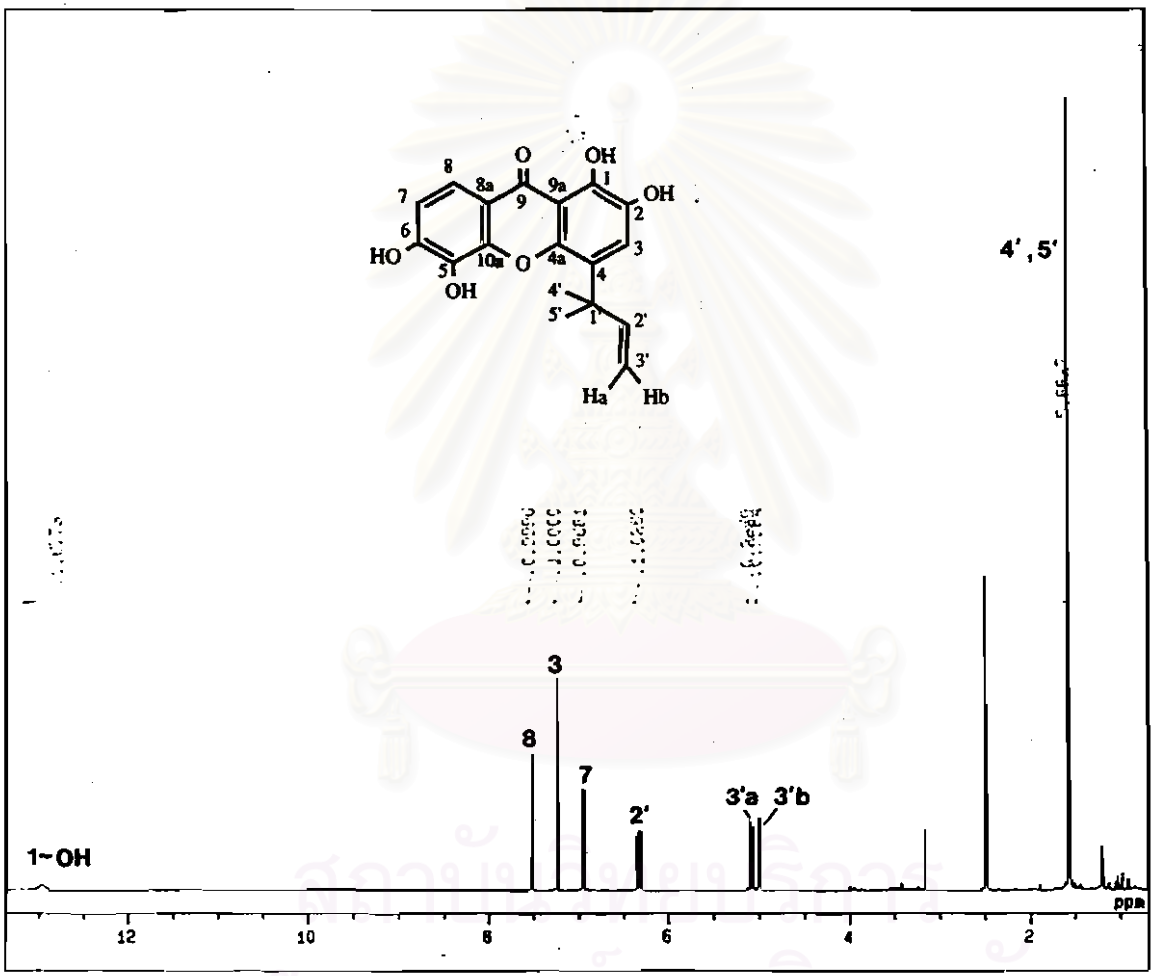


Figure 59a 500 MHz ^{1}H NMR spectrum of compound GD-6 (in DMSO-d_6)

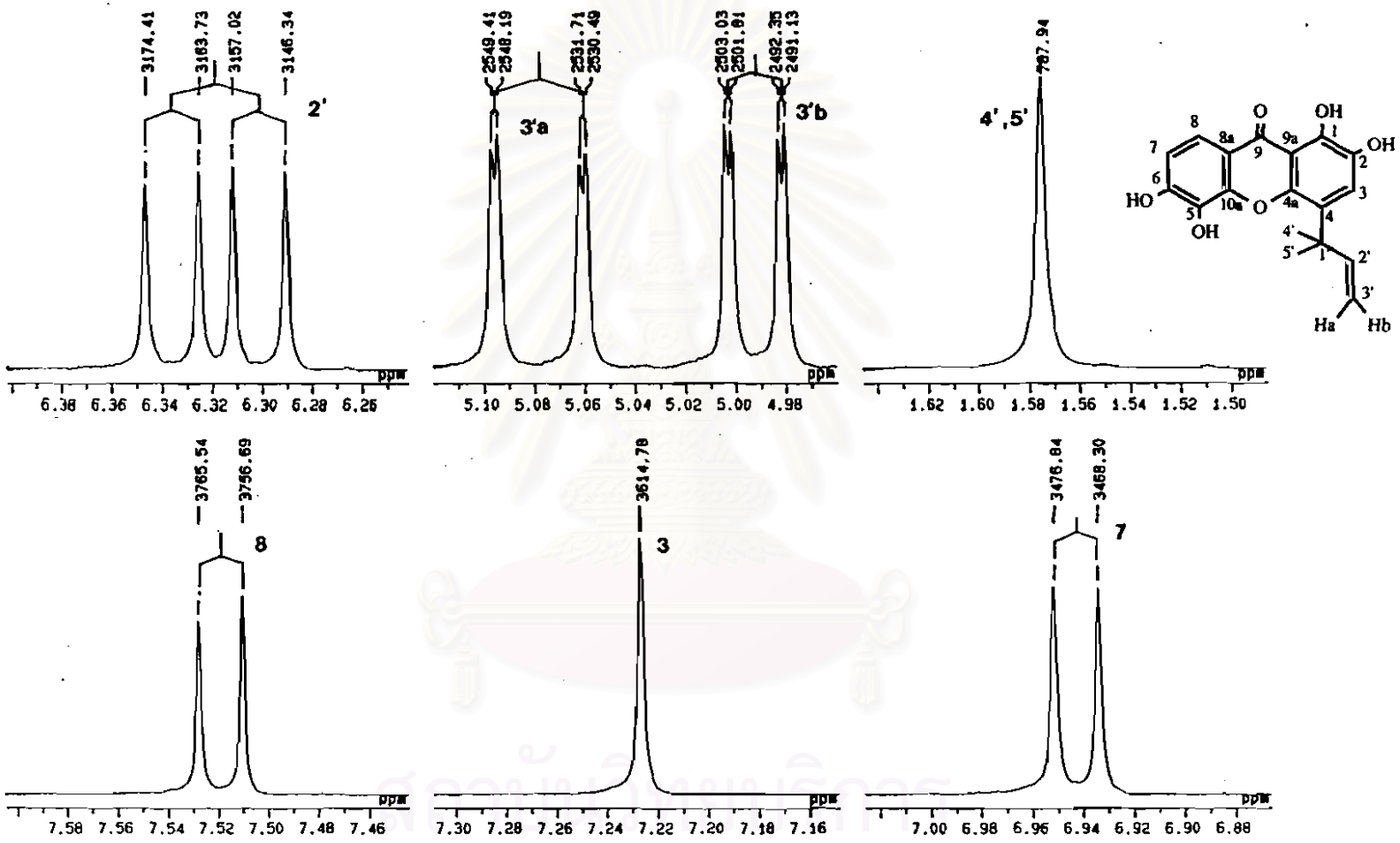


Figure 59b 500 MHz ^{1}H NMR spectrum of compound GD-6 (in DMSO-d_6) (expanded from 1.50-7.58 ppm)

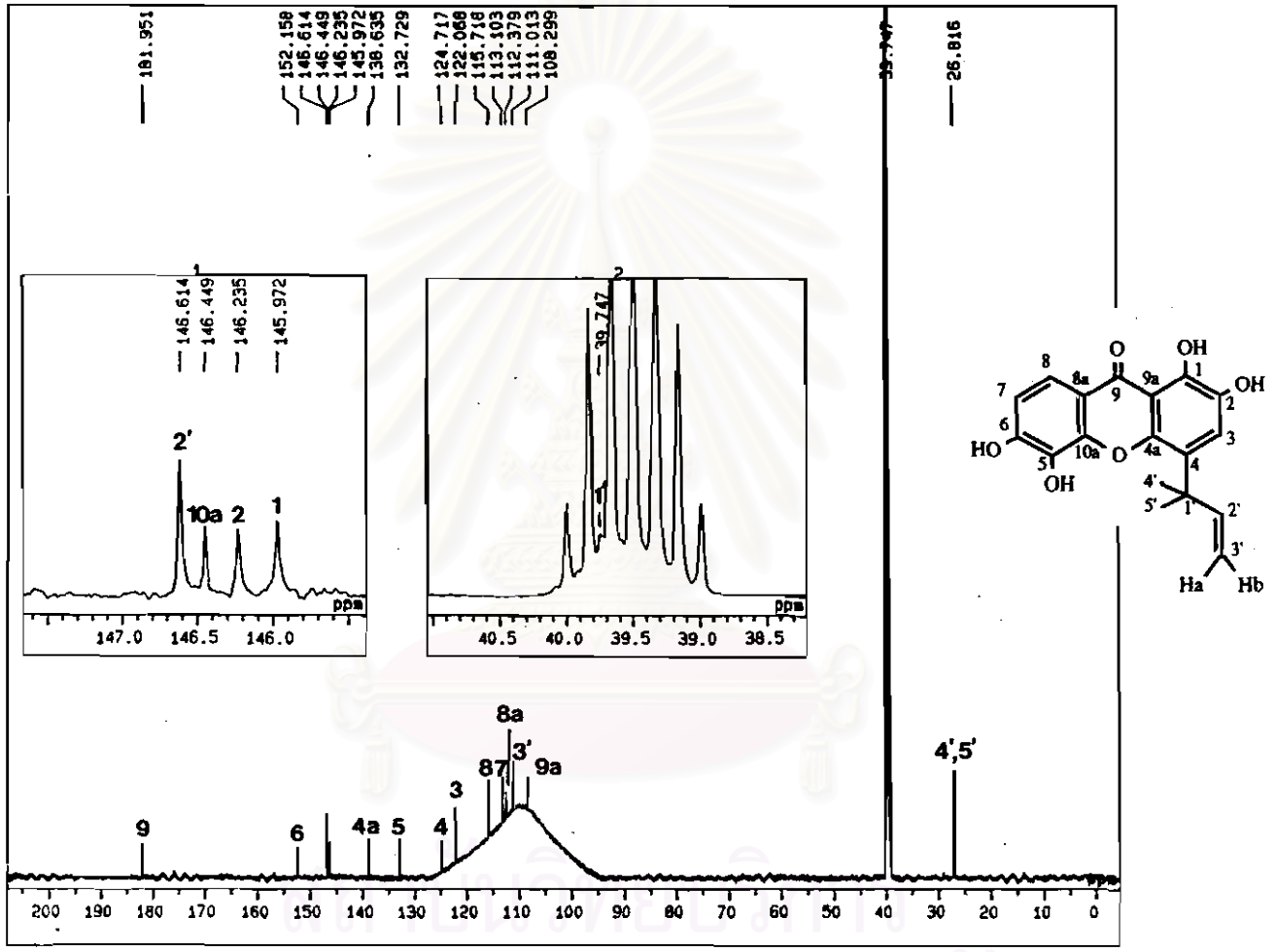


Figure 60 125 MHz ^{13}C NMR spectrum of compound GD-6 (in DMSO-d_6)

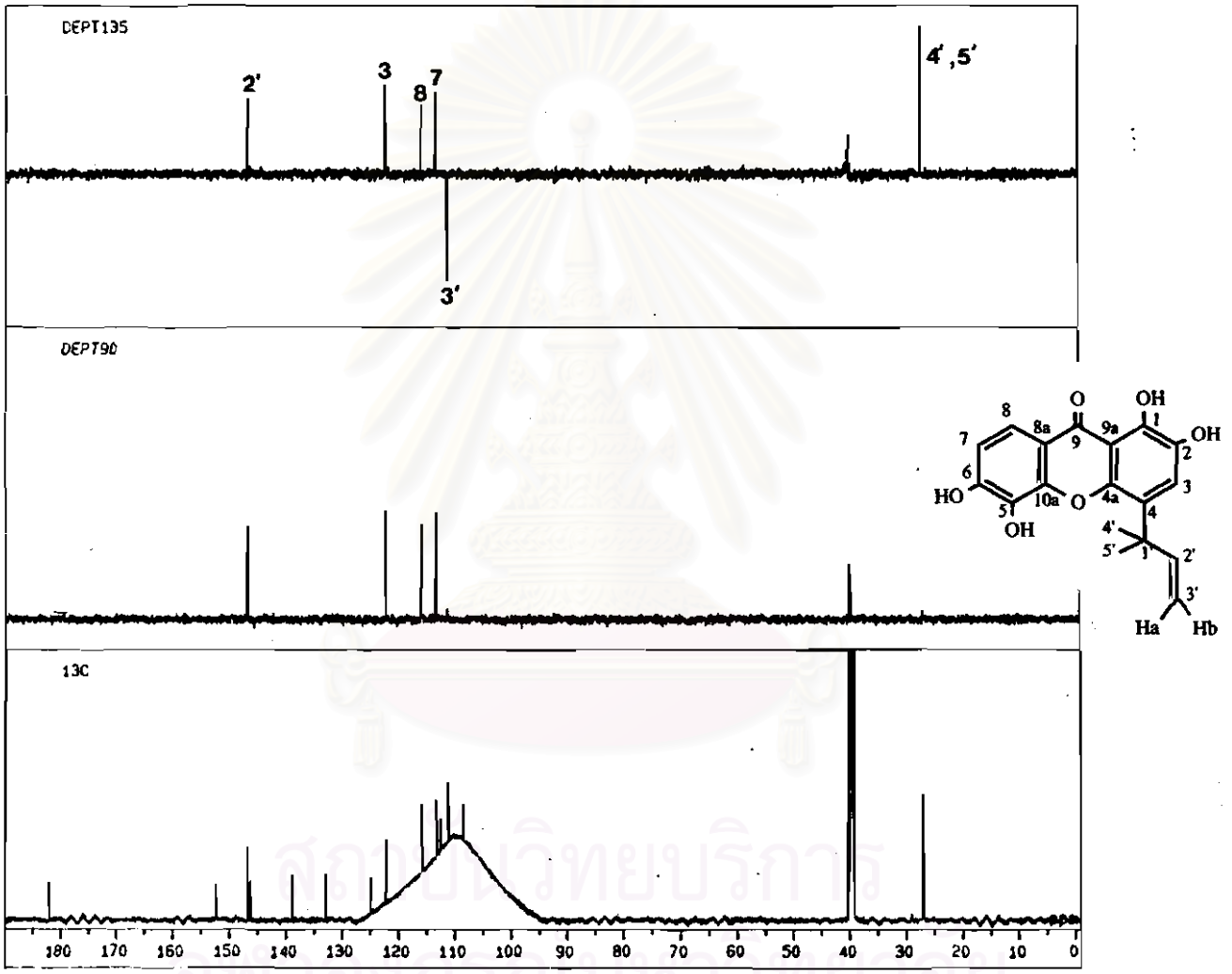


Figure 61 DEPT spectrum of compound GD-6 (in DMSO-d_6)

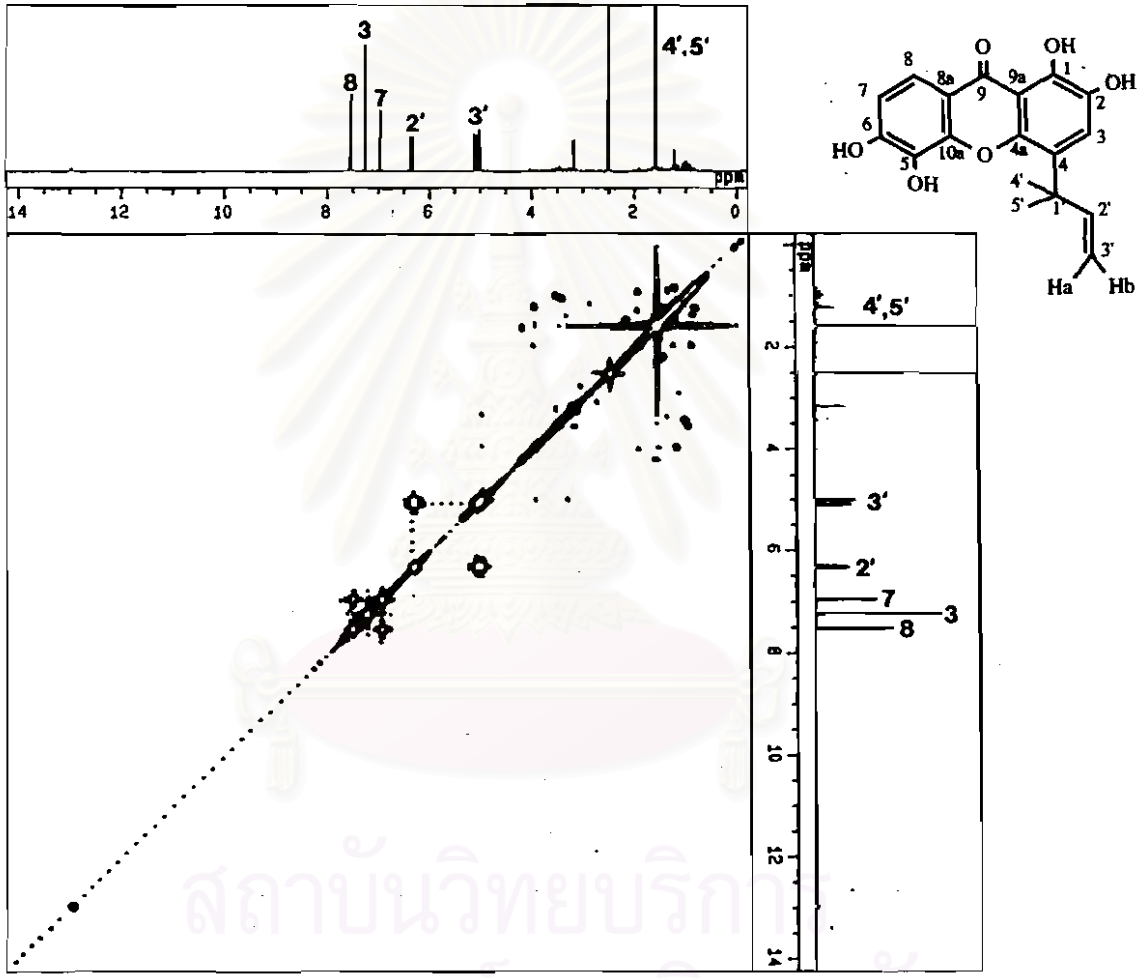


Figure 62 ^{1}H-^{1}H COSY spectrum of compound GD-6 (in DMSO-d_6)

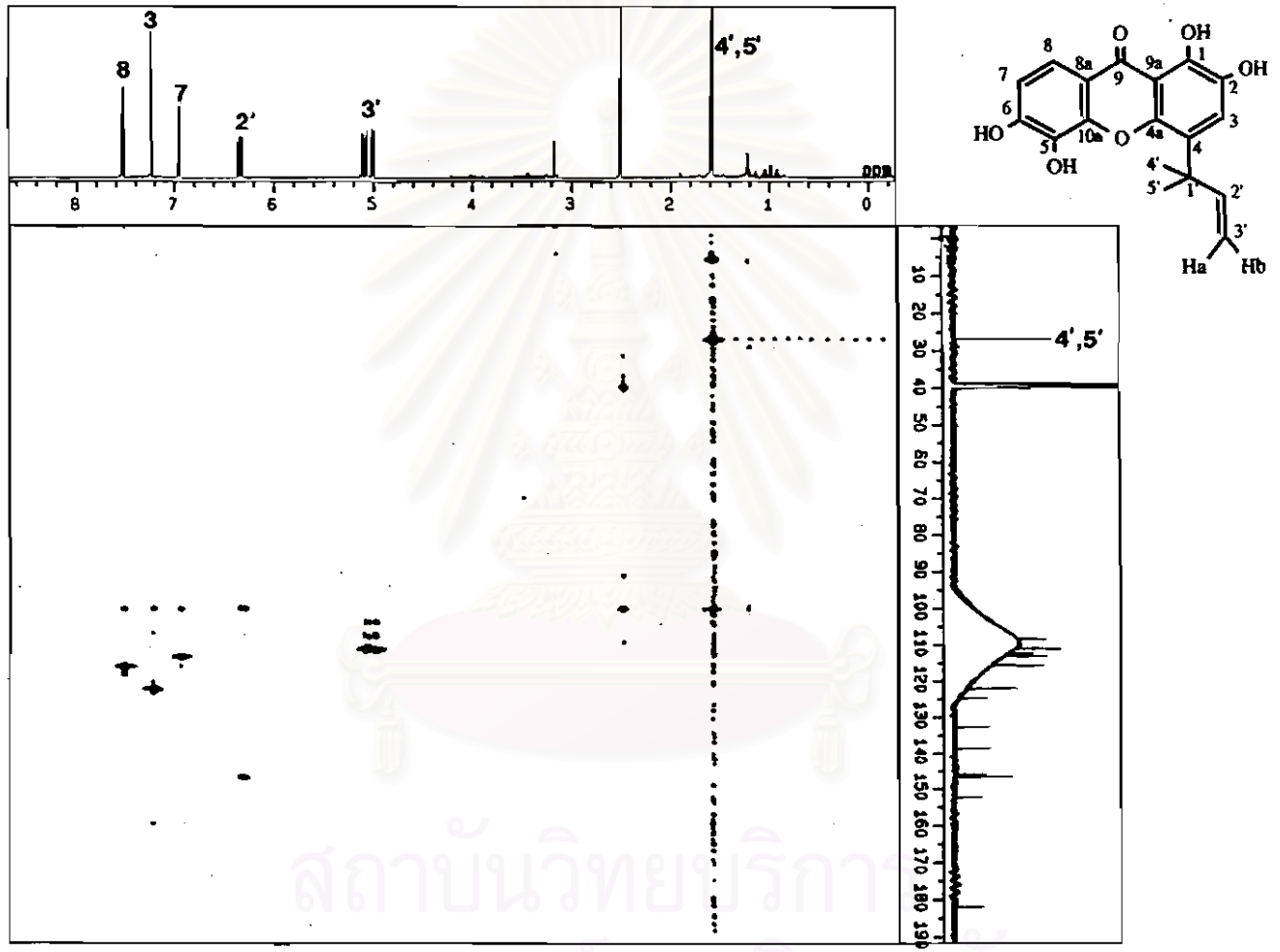


Figure 63a HMQC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 0.0-8.0 ppm, δ_C 10-190 ppm]

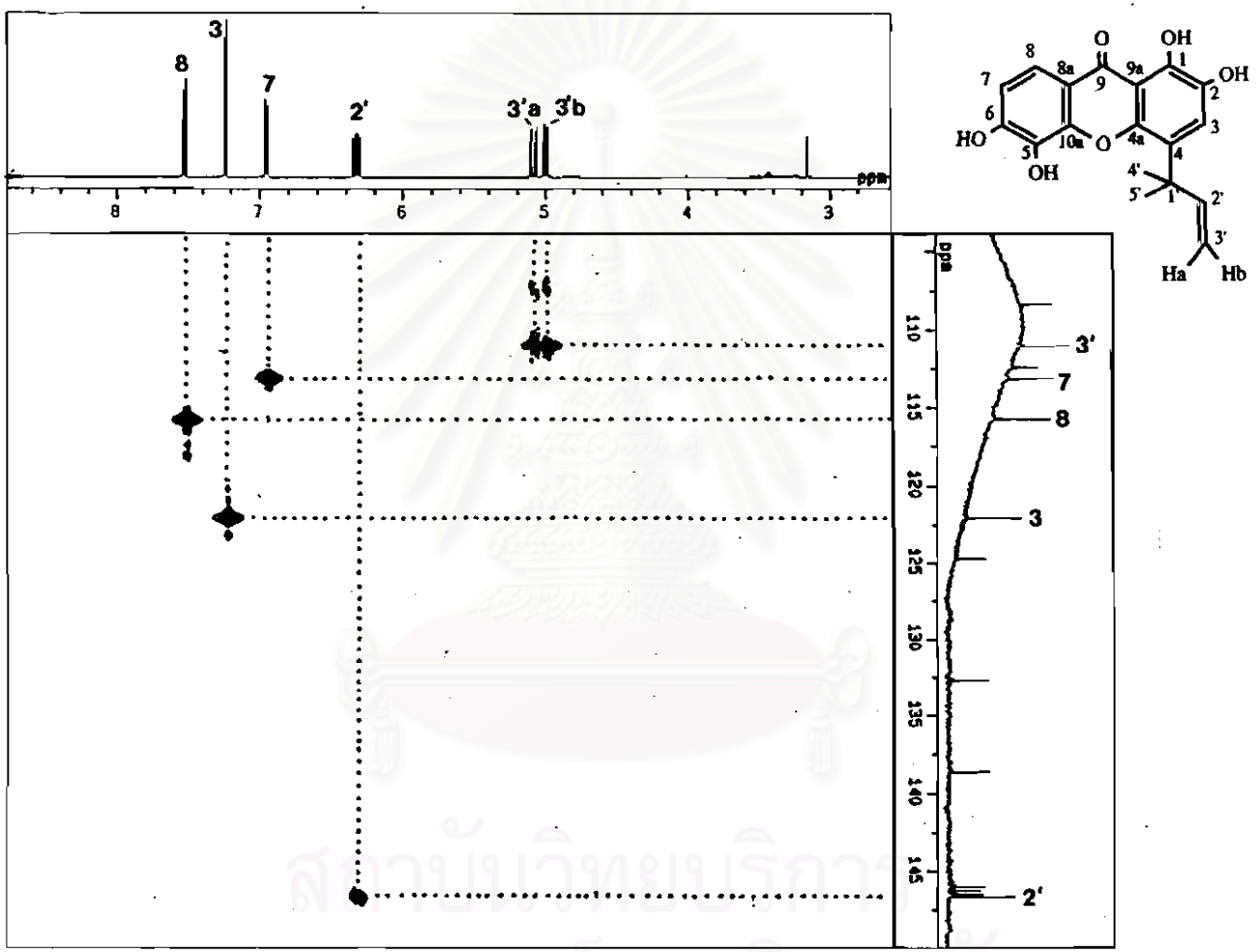


Figure 63b HMQC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 3.0-8.0 ppm, δ_C 105-150 ppm]

Table 19 The carbon-proton correlations of compound GD-6 observed in the HMQC spectrum

Position	δ_C (ppm)	Correlation with proton at δ_H (ppm)
3	122.0	7.23
7	113.1	6.94
8	115.7	7.52
2′	146.6	6.32
3′a	111.0	5.08
3′b	-	4.99
4′, 5′	26.8	1.57

A NOESY experiment (Figure 64) showed the 2-D NOE correlations between H-7 and H-8, H-3 and H-4′, H-3 and H-5′, and H-3 and H-2′, supporting the presence of 1,1-dimethylallyl group on C-4. The results are summarized in Figure 65.

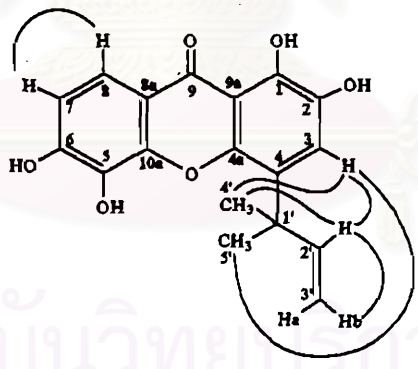


Figure 65 Results from NOESY experiment of compound GD-6

Finally, the quaternary carbons could be assigned from the ^{1}H-^{13}C long-range couplings displayed in the HMBC spectrum (Figures 66a-66i). The correlation between H-3 and C-1′ confirmed the location of 1,1-dimethylallyl moiety on C-4, whereas the correlation between H-8 and C-9 was evidence for the presence of two hydroxyl groups at C-5 and C-6.

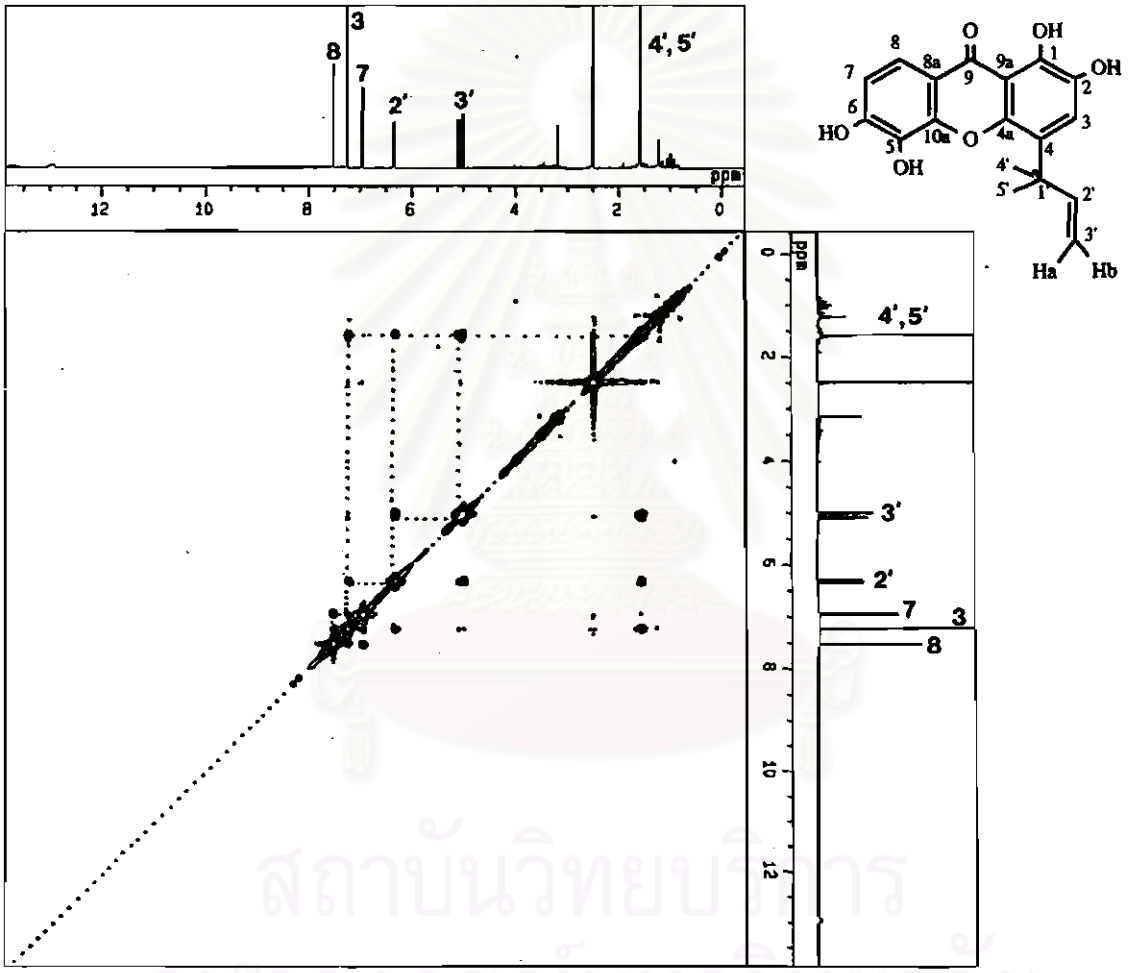


Figure 64 NOESY spectrum of compound GD-6 (in DMSO-d_6)

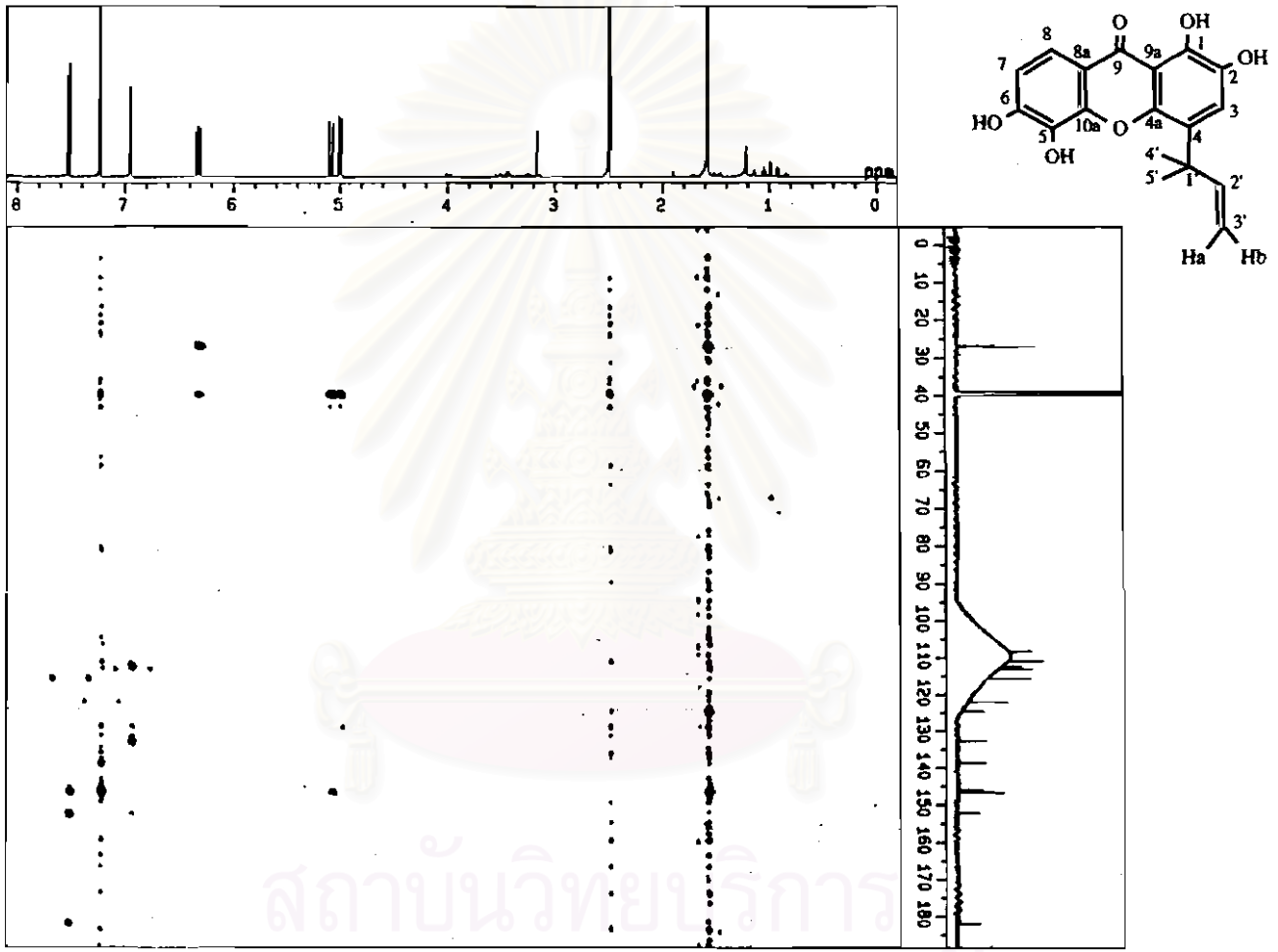


Figure 66a HMBC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 0.0-8.0 ppm, δ_C 0-190 ppm]

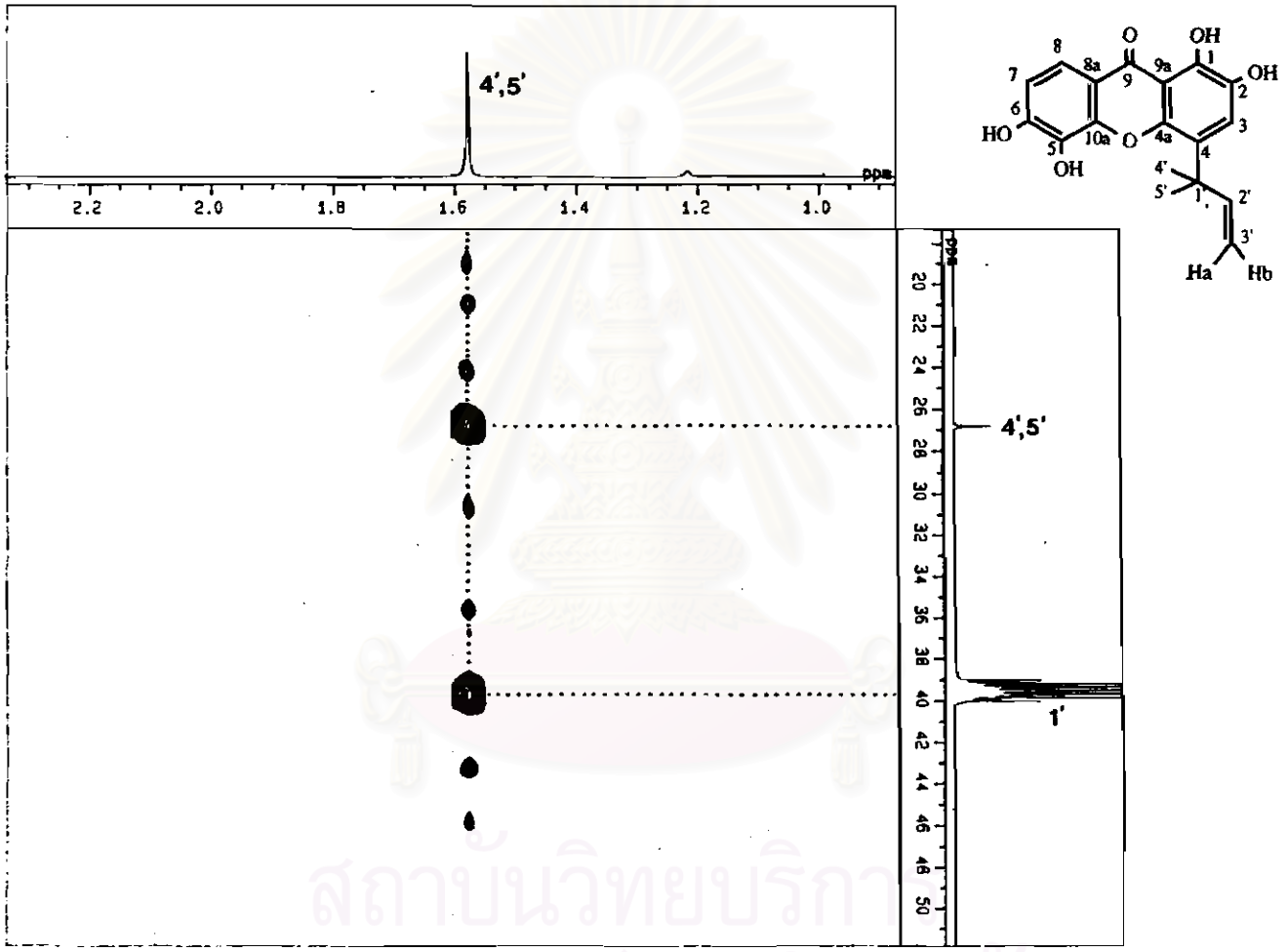


Figure 66b HMBC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 1.0-2.2 ppm, δ_C 20-50 ppm]

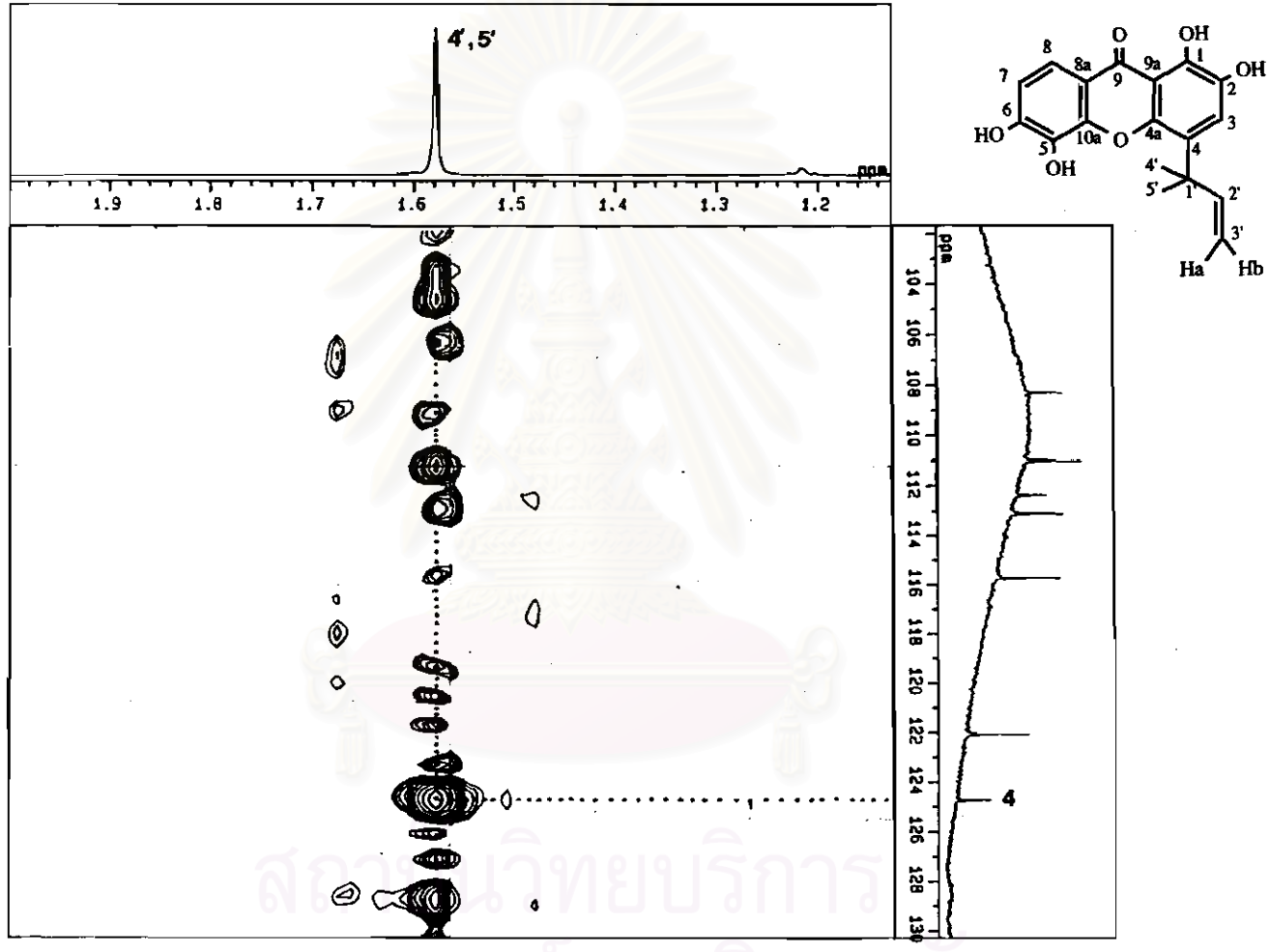


Figure 66c HMBC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 1.2-1.9 ppm, δ_C 104-130 ppm]

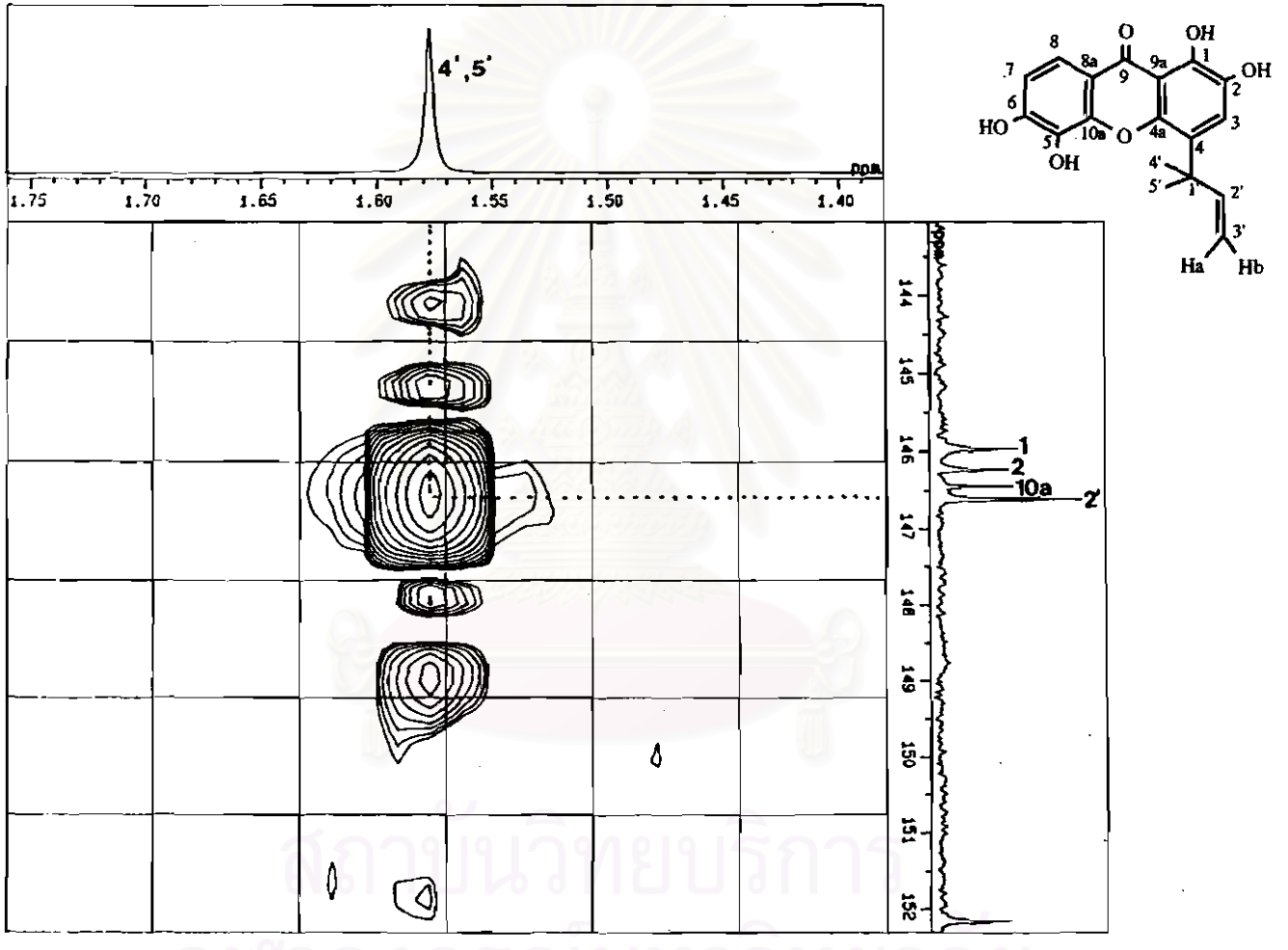


Figure 66d HMBC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 1.40-1.75 ppm, δ_C 144-152 ppm]

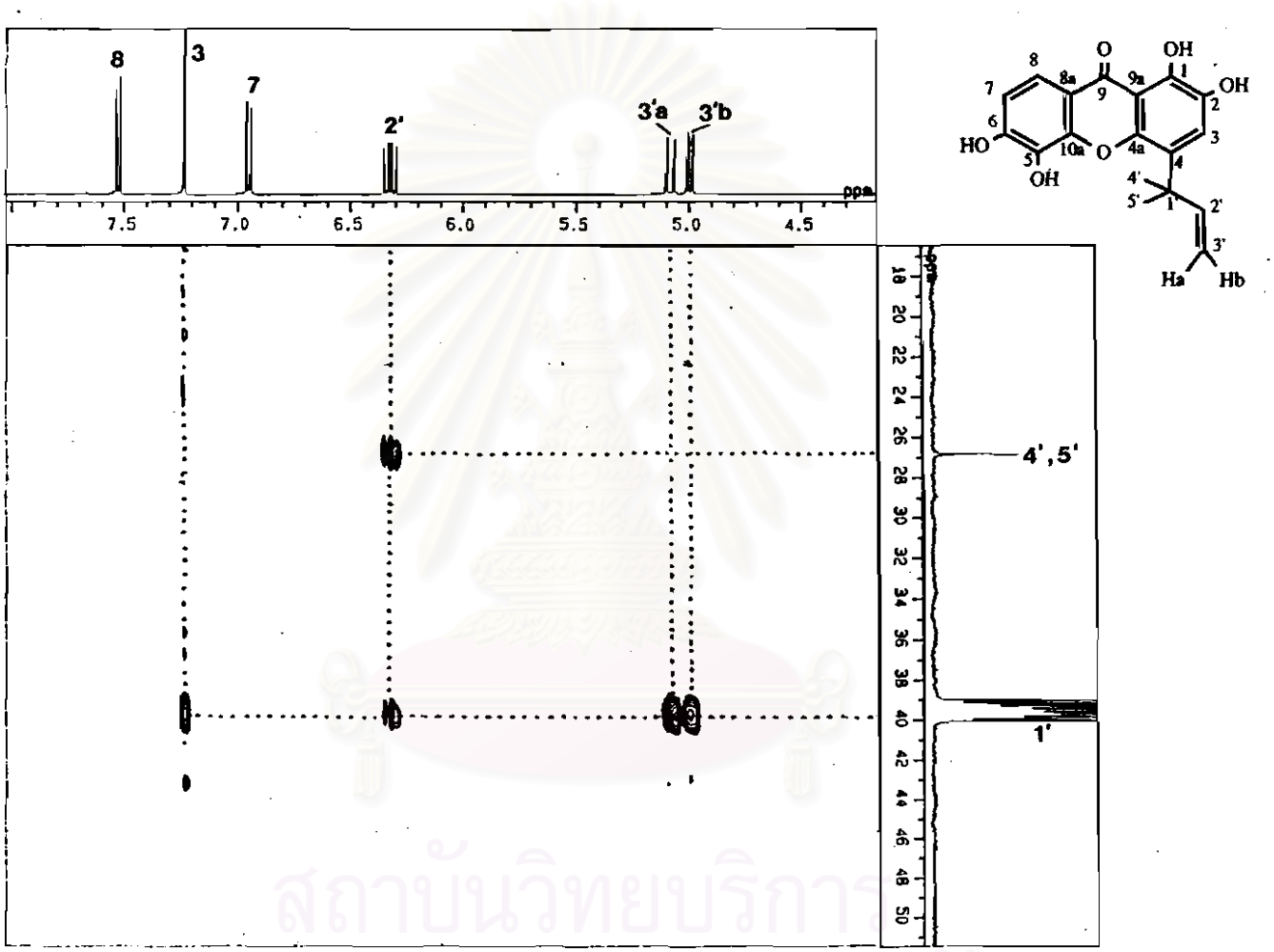


Figure 66e HMBC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 4.5-8.0 ppm, δ_C 18-50 ppm]

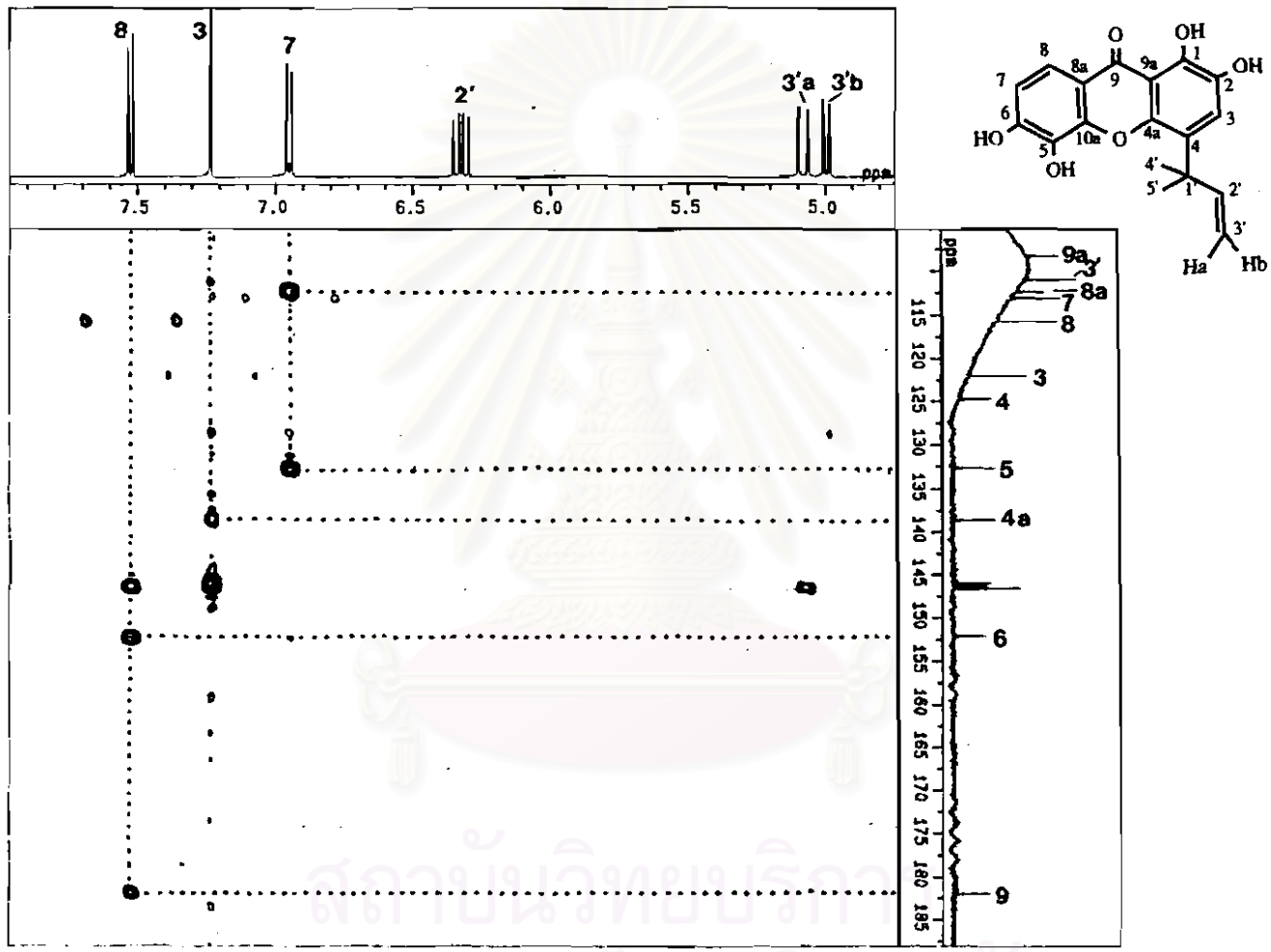


Figure 66f HMBC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 5.3-8.0 ppm, δ_C 105-185 ppm]

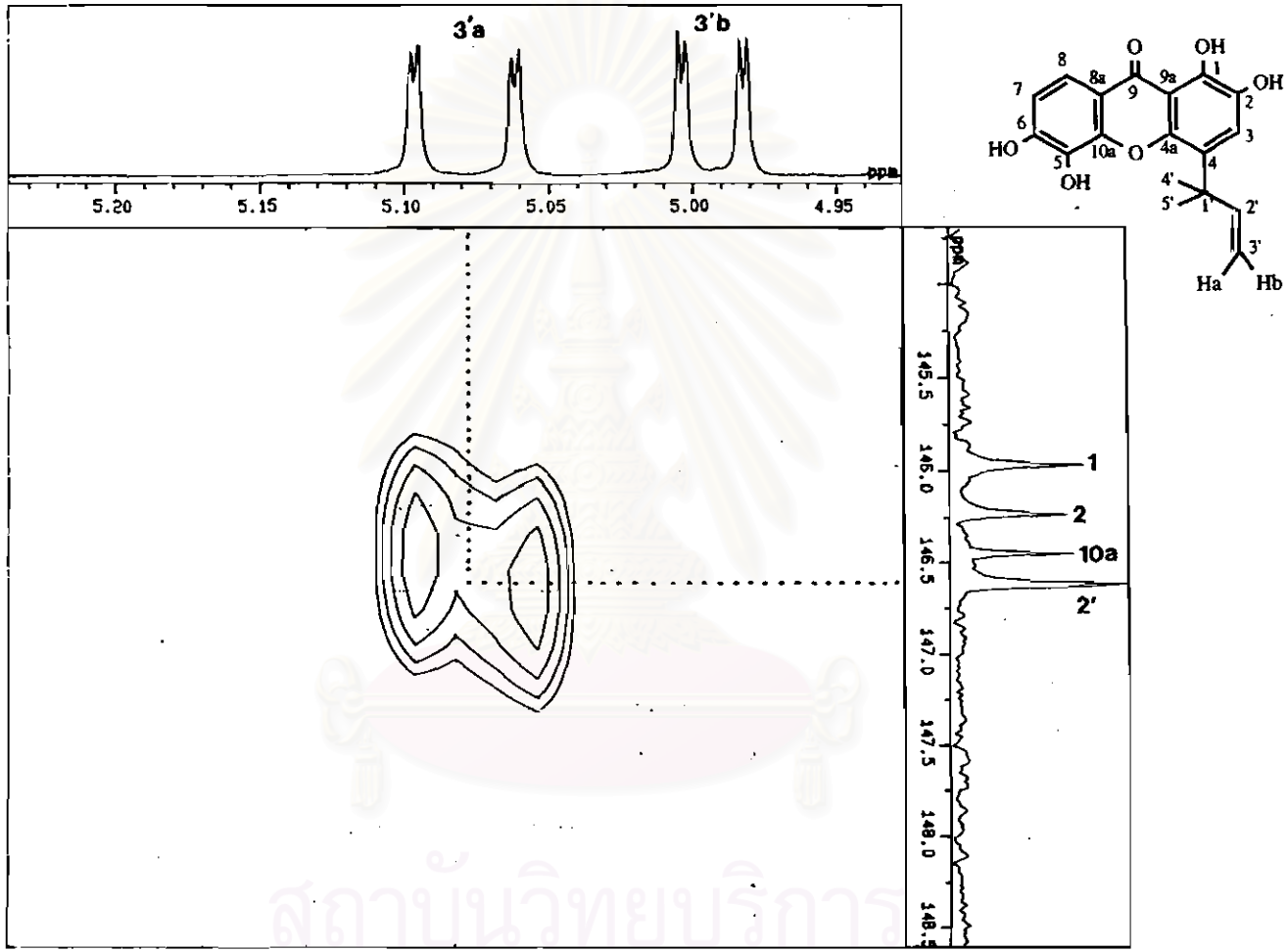


Figure 66g HMBC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 4.95-5.20 ppm, δ_C 145-148 ppm]

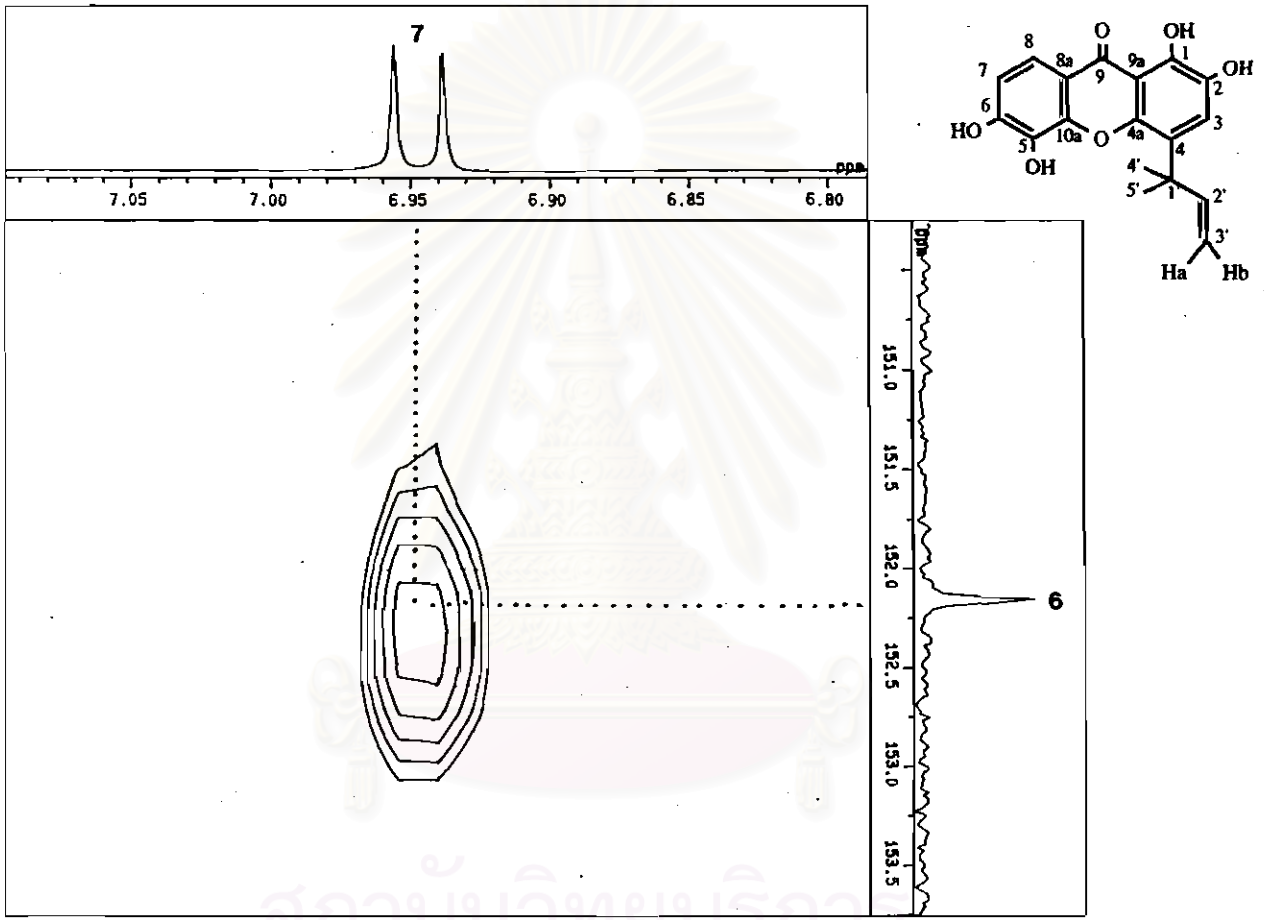


Figure 66h HMBC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 6.80-7.05 ppm, δ_C 151.0-153.5 ppm]

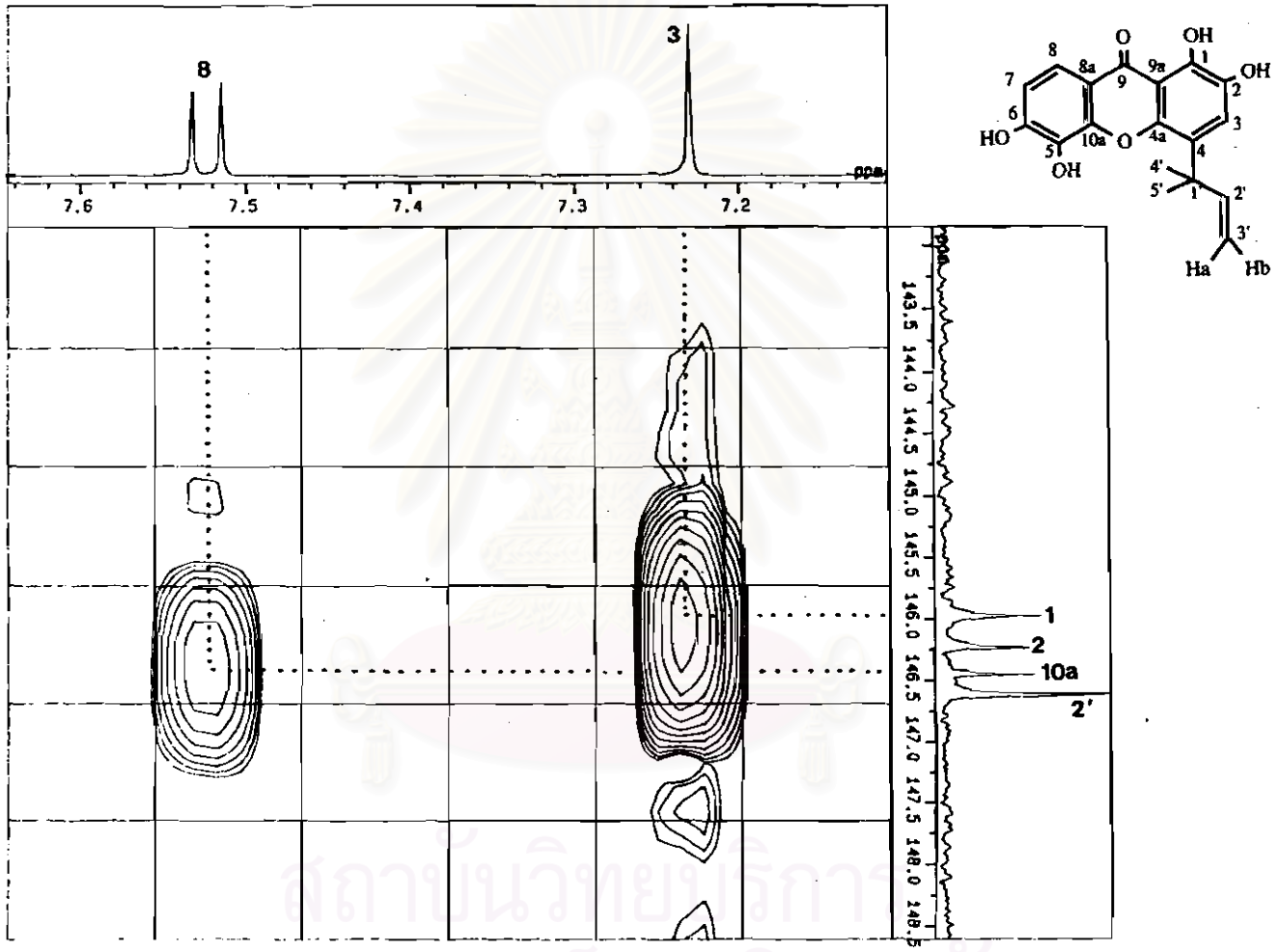


Figure 66i HMBC spectrum of compound GD-6 (in DMSO-d_6), [δ_H 7.2-7.6 ppm, δ_C 143.5-148.5 ppm]

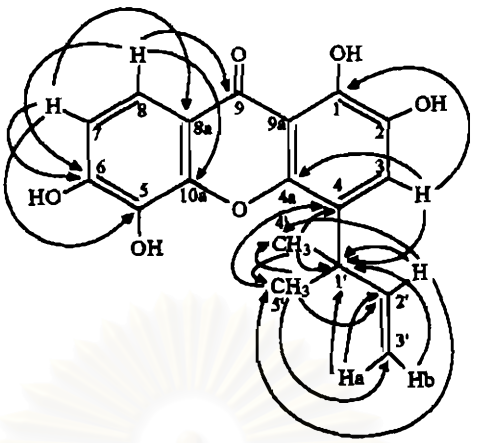


Figure 67 Long-range C-H correlations of compound GD-6 observed in HMBC spectrum

The complete proton and carbon assignments of compound GD-6 (in DMSO-d_6) and proton assignments of symphoxanthone (in acetone-d_6) are summarized in Table 20.

Table 20 ^{1}H and ^{13}C NMR spectral data of compound GD-6 (in DMSO-d_6) and symphoxanthone (in acetone-d_6)

Position	Compound GD-6		Symphoxanthone
	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)	δ_H (ppm) (multiplicity), J (Hz)
1	145.9	12.96 (1-OH, s)	13.00 (1-OH, s)
2	146.2	-	-
3	122.0	7.23 (s)	7.37 (s)
4	124.7	-	-
4a	138.6	-	-
5	132.7	-	-
6	152.1	-	-
7	113.1	6.94 (d, J = 8.8)	7.05 (d, J = 9 Hz)
8	115.7	7.52 (d, J = 8.8)	7.71 (d, J = 9 Hz)

Table 20 (Continued)

Position	Compound GD-6		Symphoxanthone
	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)	δ_H (ppm) (multiplicity), *J* (Hz)
8a	112.3	-	-
9	181.3	-	-
9a	108.2	-	-
10a	146.4	-	-
1′	39.7	-	-
2′	146.6	6.32 (dd, *J* = 17.7, 10.6)	6.47 (q)
3′a	111.0	5.08 (dd, *J* = 17.7, 1.2)	5.11 (t)
3′b	-	4.99 (dd, *J* = 10.6, 1.2)	5.11 (t)
4′	26.8	1.57 (s)	1.70 (s)
5′	26.8	1.57 (s)	1.70 (s)

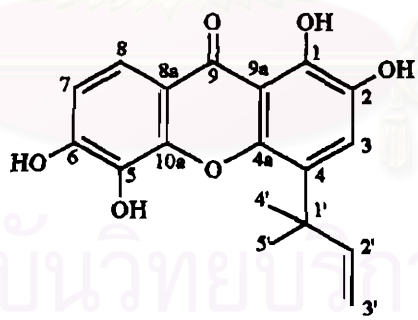


Figure 68 Structure of compound GD-6

7. Structure Determination of Compound GD-7

Compound GD-7, a yellow powder, was obtained from fraction S-334 by repetitive chromatography using a combination of centrifugal TLC and gel filtration techniques. The EIMS of compound GD-7 (Figure 69) showed the molecular ion [M^+] at *m/z* 464, corresponding to $C_{28}H_{32}O_6$. This compound showed typical xanthone UV absorptions (Figure 70) in methanol at λ_{max} 255 and 325 nm. The IR spectrum of compound GD-7 (Figure 71) exhibited maximum absorption bands at 3802-3024 (O-H stretching), 1650, 1599 (carbonyl of α, β unsaturated ketone), 1273 (C-O stretching of ether linkage) and 1542-1375 (conjugated carbon moieties) cm^{-1}.

Compound GD-7 was identified as 7-geranyl-1,3,5,6-tetrahydroxy-8-(3-methyl-2-butenyl)xanthone or garciniaxanthone E [90] (Minami *et al.*, 1996), by analysis of the 1-D and 2-D NMR data, including the HMQC and HMBC spectra. Its NMR properties are in excellent agreement with the literature values (Minami *et al.*, 1996).

Minami and co-workers (1996) isolated garciniaxanthone E from the wood of *G. subelliptica* and determined its structure by NMR analysis, using DQFCOSY, HMQC and HMBC experiments. The information from DQFCOSY and HMQC indicated the presence of 3-methyl-2-butenyl and geranyl groups, and the locations of these moieties were confirmed by examination of HMBC correlations.

The ^1H NMR spectrum of compound GD-7 (Figures 72a-72c) disclosed the presence of one chelated hydroxy group, 5 methyl groups, 2 aromatic protons, one 3-methyl-2-butenyl group and one geranyl group. The ^{13}C NMR (Figure 73) and the DEPT (Figure 74) spectra provided the signals for 5 methyl, 4 methylene, 5 methine and 13 quaternary carbons, as well as and one carbonyl carbon.

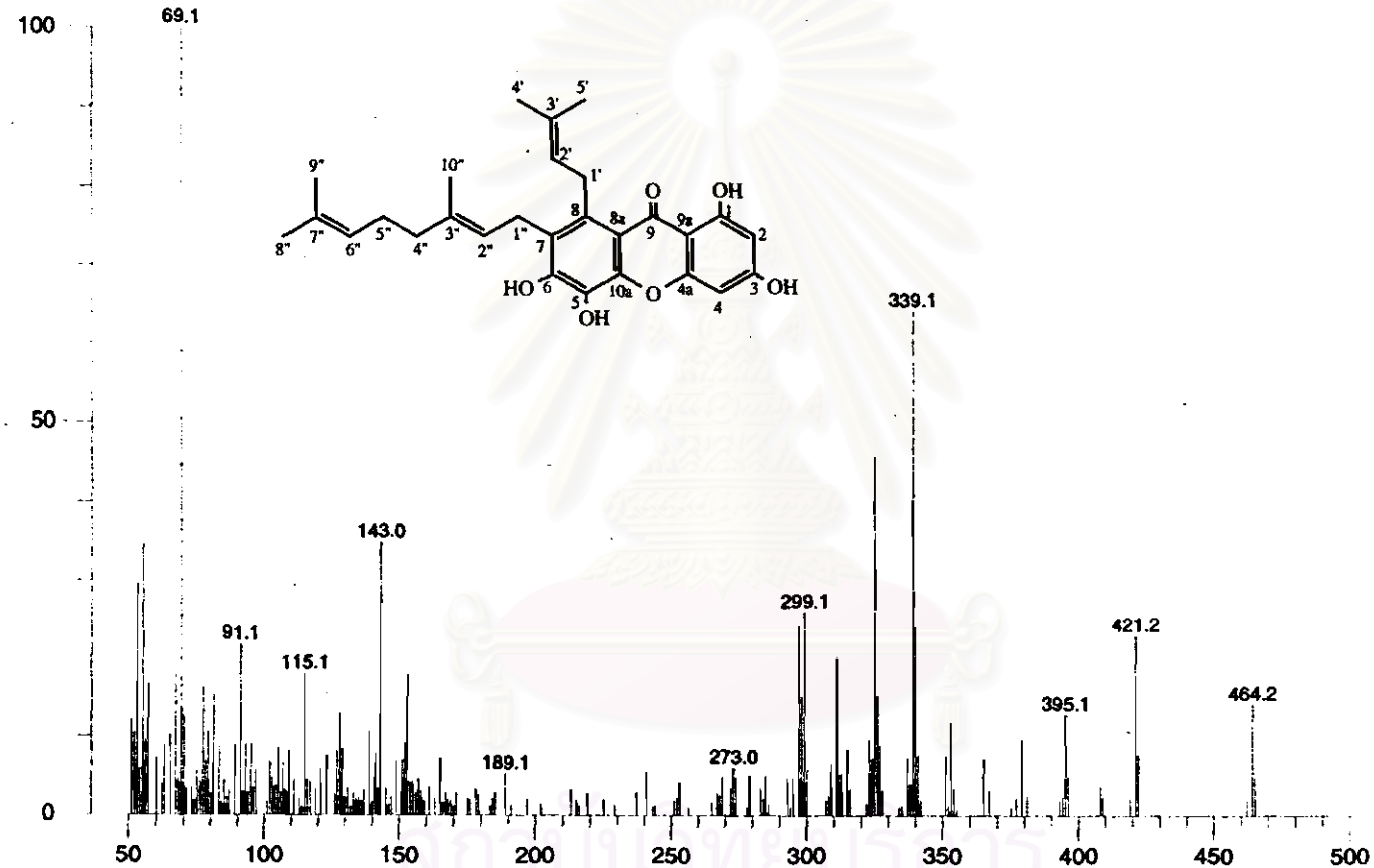


Figure 69 EI mass spectrum of compound GD-7

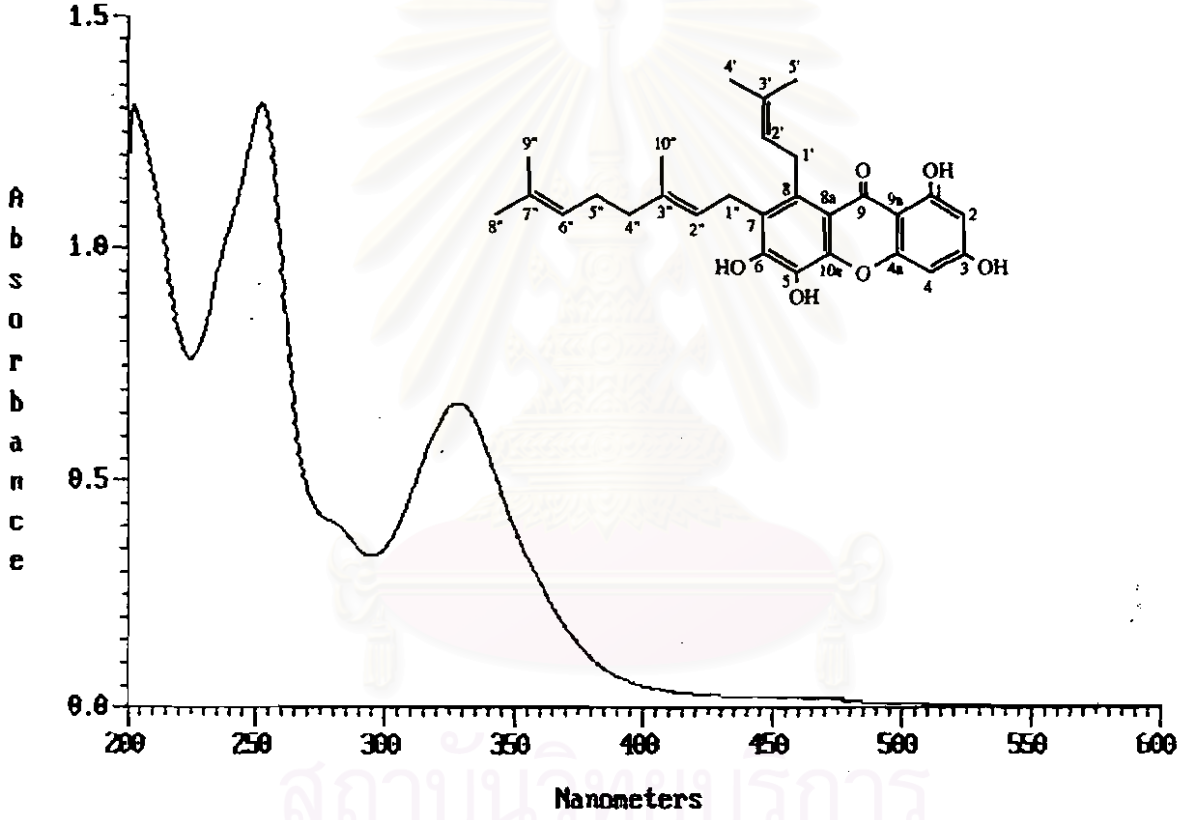


Figure 70 UV spectrum of compound GD-7 (in methanol)

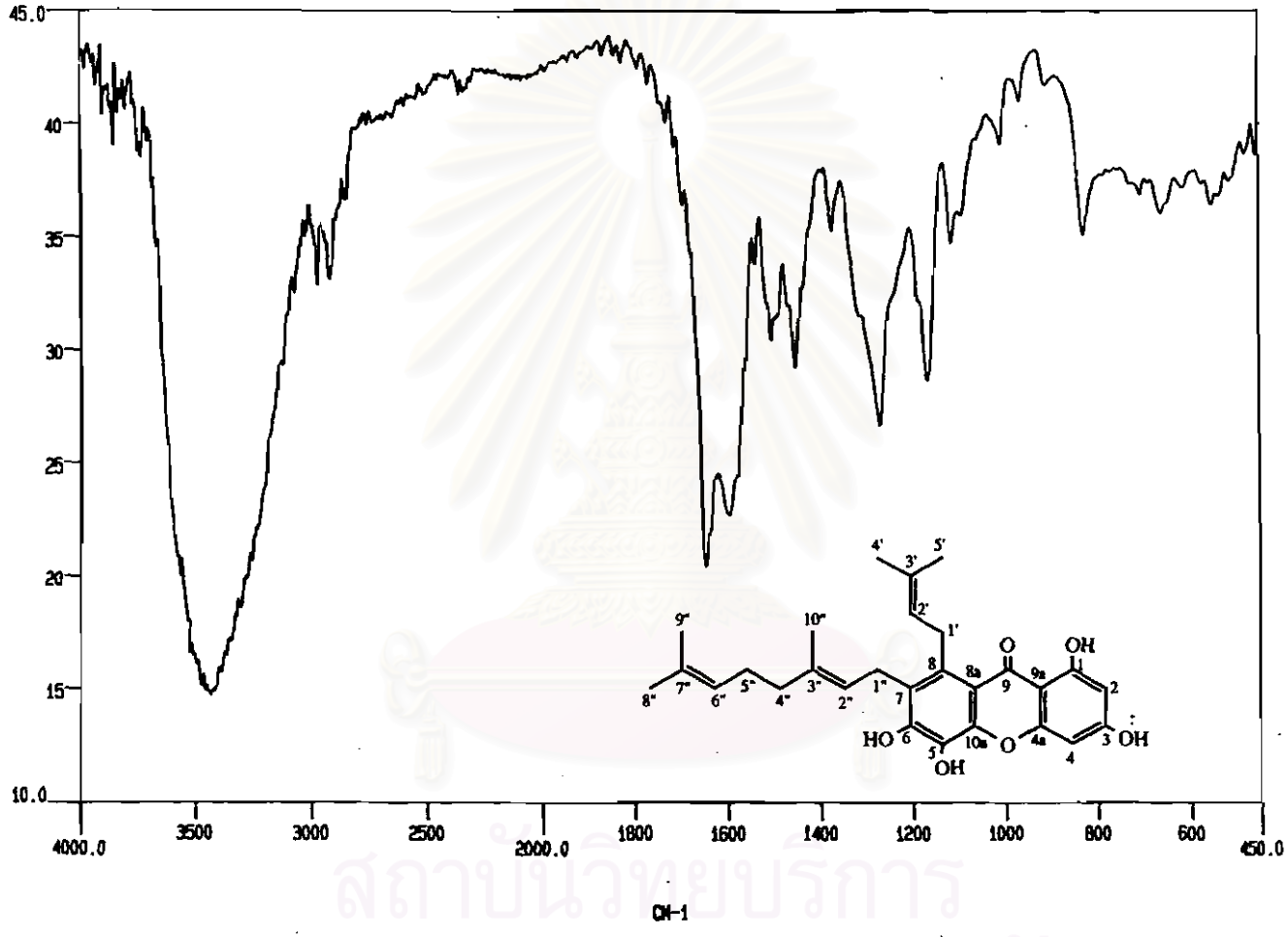


Figure 71 IR spectrum of compound GD-7 (film)

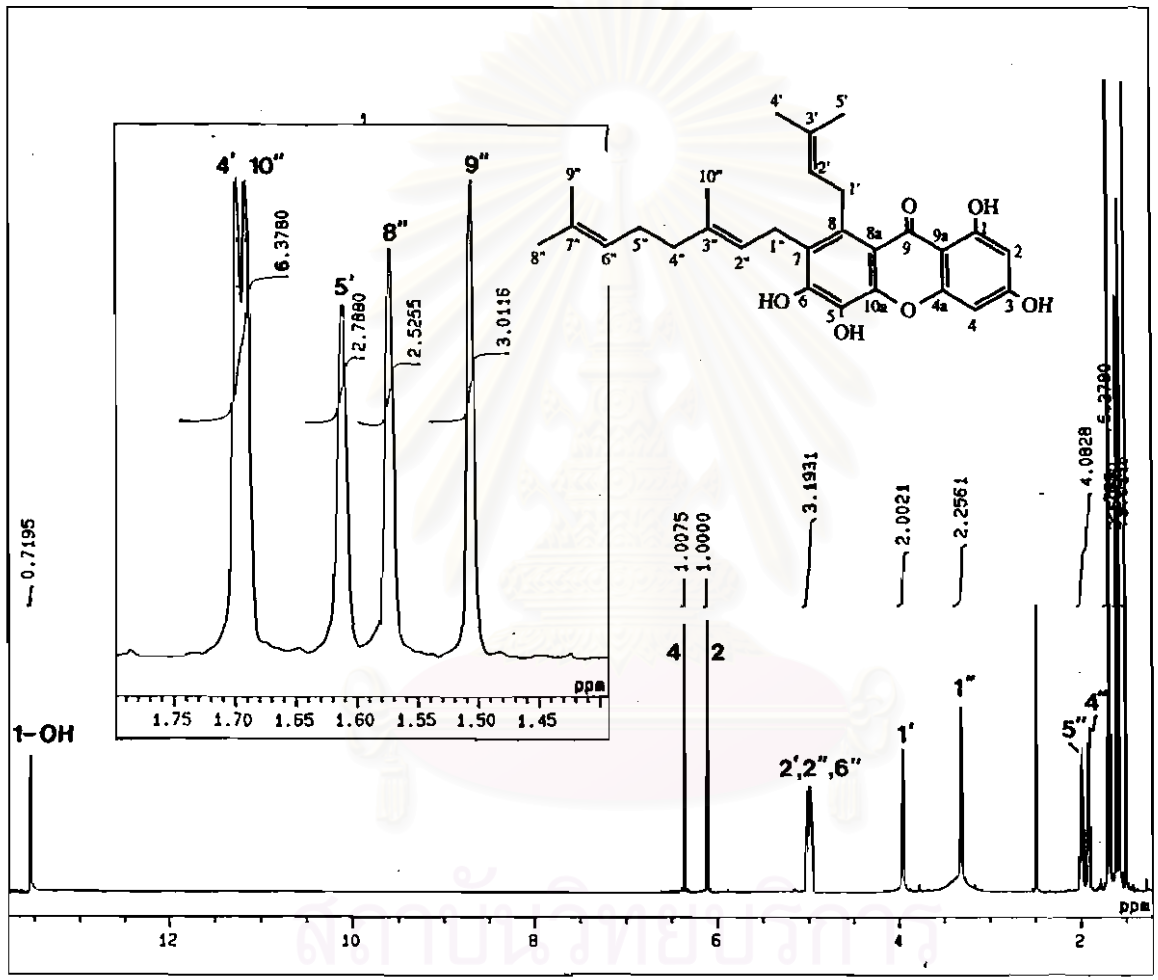


Figure 72a 500 MHz ^{1}H NMR spectrum of compound GD-7 (in DMSO-d_6)

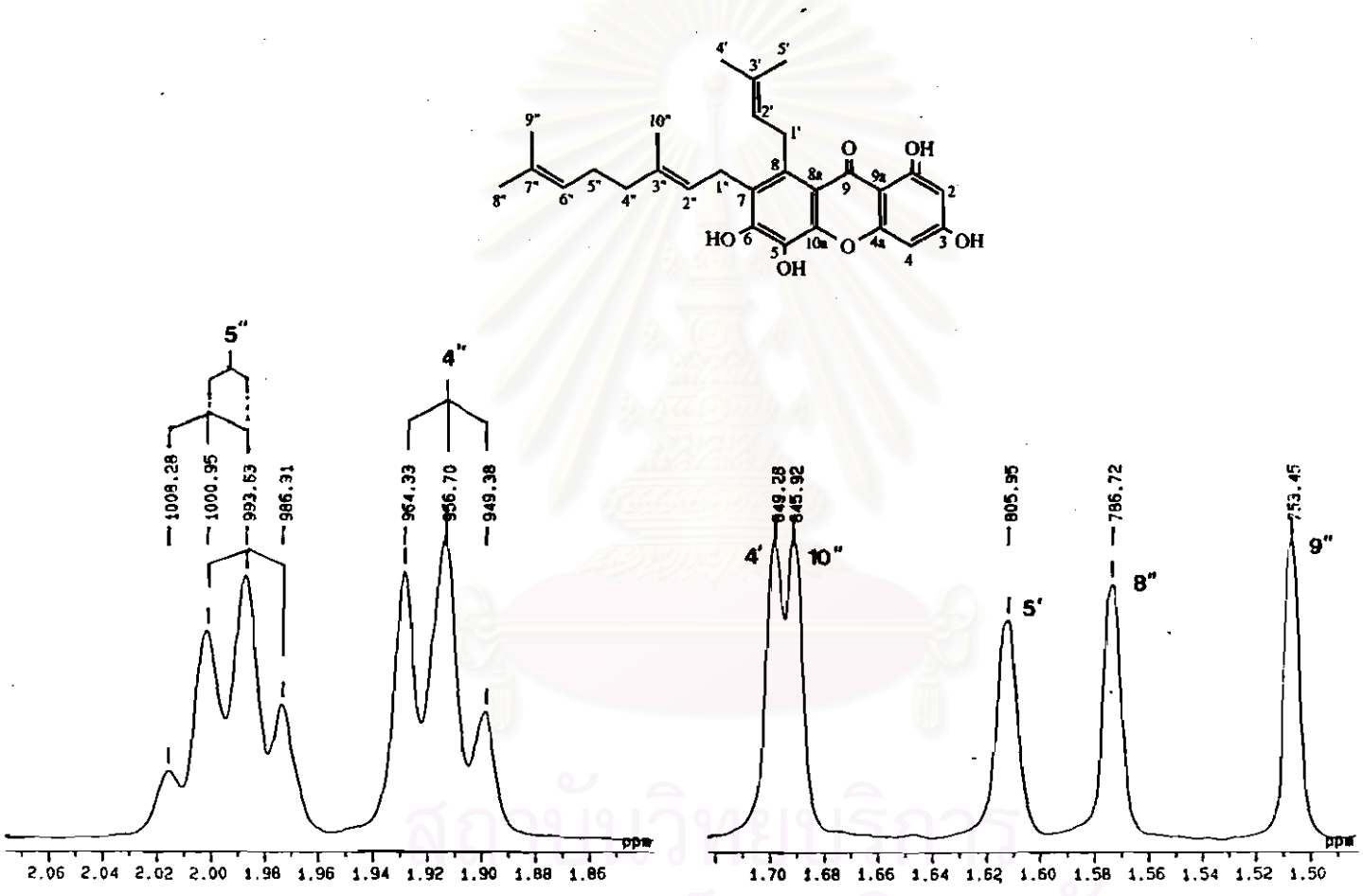


Figure 72b 500 MHz ^{1}H NMR spectrum of compound GD-7 (*in* DMSO-d_6) (expanded from 1.50-2.06 ppm)

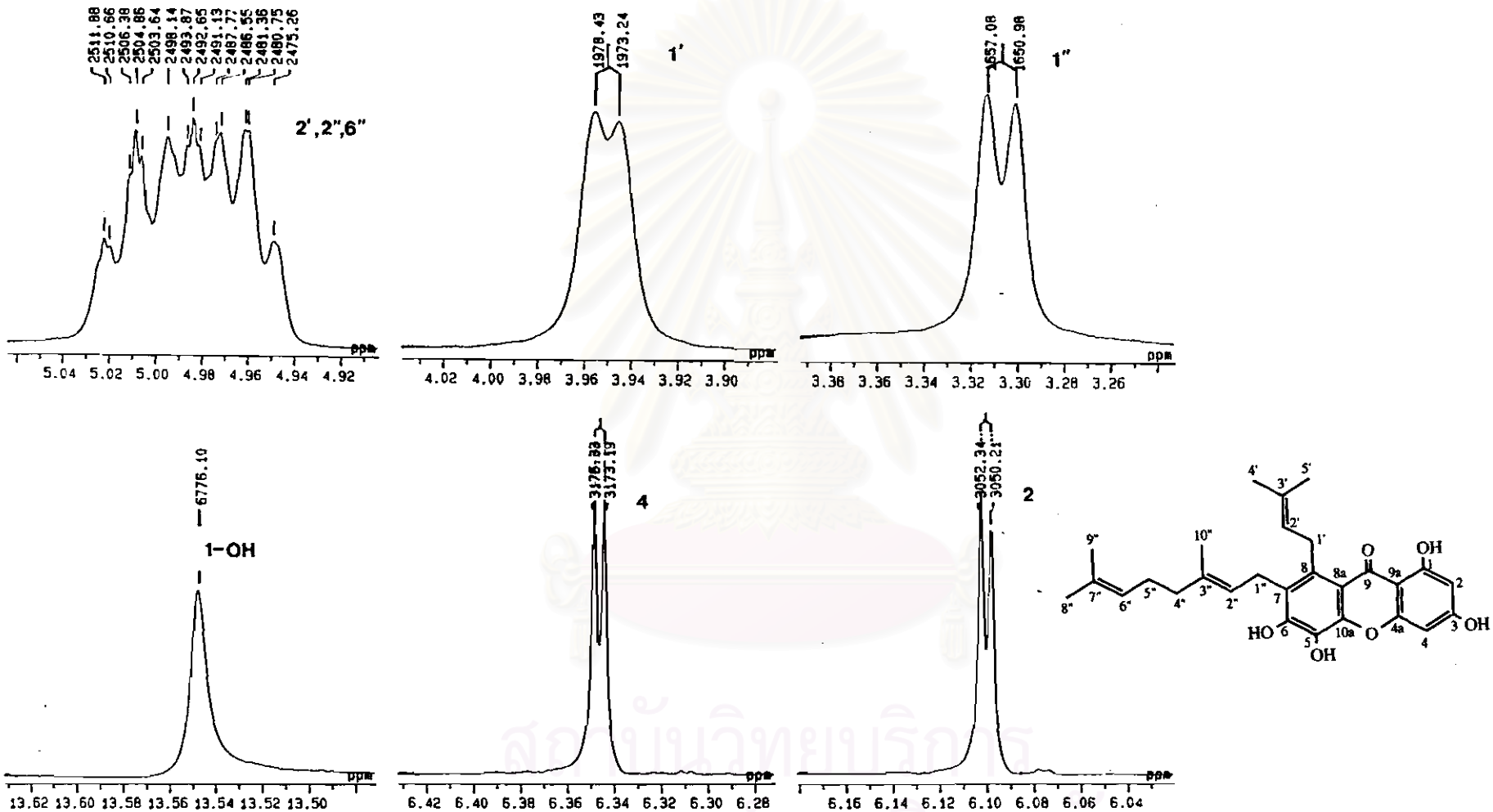


Figure 72c 500 MHz ^{1}H NMR spectrum of compound GD-7 (in DMSO-d_6) (expanded from 3.26-13.62 ppm)

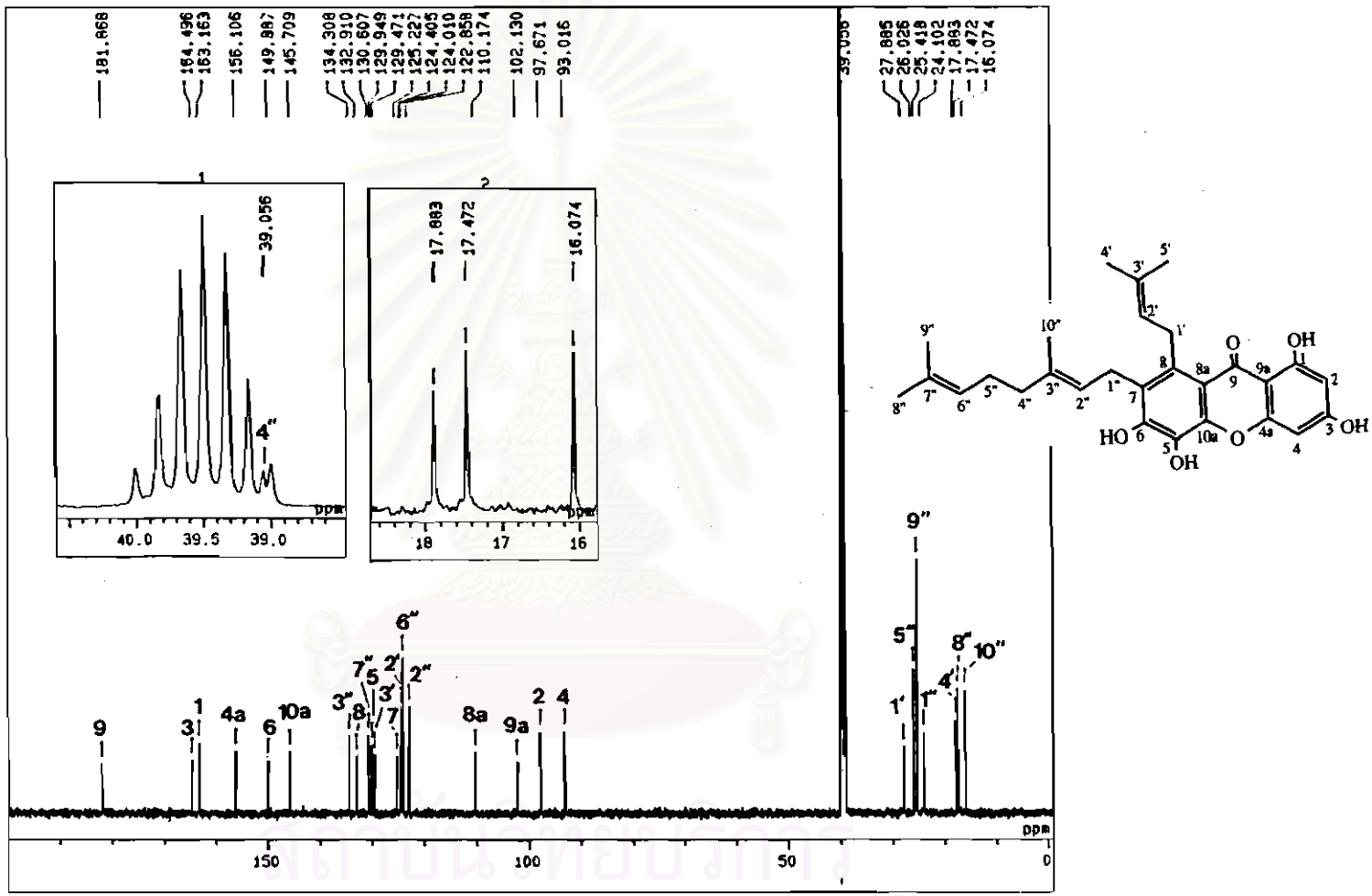


Figure 73 125 MHz ^{13}C NMR spectrum of compound GD-7 (in DMSO-d_6)

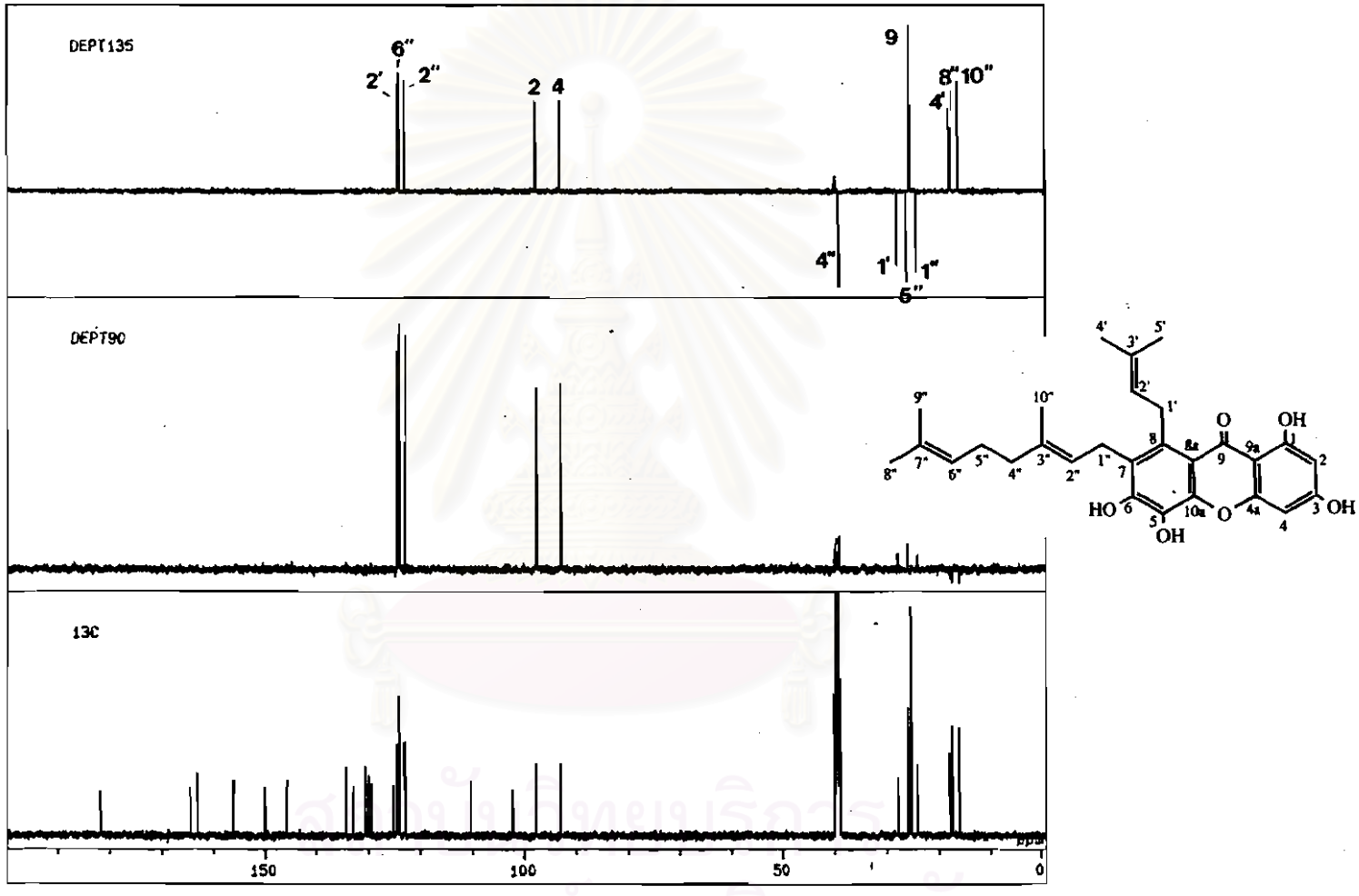


Figure 74 DEPT spectrum of compound GD-7 (in DMSO-d_6)

From HMQC experiment (Figures 75a-75d), 2 aromatic protonated carbons (C-2 and C-4) , some carbons of geranyl and 3-methyl-2-butenyl group could be assigned. The directly coupled ^{1}H and ^{13}C are summarized in Table 21.

Table 21 The carbon-proton correlations of compound GD-7 observed in HMQC spectrum

Carbon	δ_C (ppm)	Correlation with proton at δ_H (ppm)
C-2	97.6	6.10
C-4	93.0	6.34
C-1′	27.8	3.95
C-2′	124.4	4.98
C-1″	24.1	3.31
C-2″	122.8	4.98
C-4″	39.0	1.91
C-5″	26.0	2.00
C-6″	124.0	4.98

The positions of the 3-methyl-2-butenyl and geranyl groups were confirmed by the HMBC correlations (Figure 76a-76f). The spectral data indicated that the 3-methyl-2-butenyl and geranyl groups were on C-7 and C-8, respectively. The proton and carbon assignments of compound GD-7 and garciniaxanthone E are summarized in Table 22.

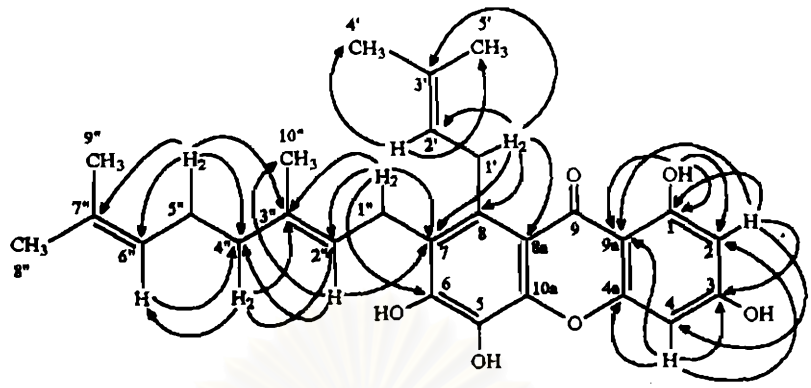


Figure 77 Long-range C-H correlations of compound GD-7 observed in HMBC spectrum

Table 22 ^{1}H and ^{13}C NMR spectral data of compound GD-7 and garciniaxanthone E [90] (in DMSO-d_6).

Position	Compound GD-7		Garciniaxanthone E	
	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), J (Hz)
1	163.1	13.54 (1-OH, s)	163.2	13.56 (1-OH, s)
2	97.6	6.10 (d, J = 2.1)	97.7	6.11 (d, J = 1.5)
3	164.4	-	164.5	-
4	93.0	6.34 (d, J = 2.1)	93.0	6.36 (d, J = 1.5)
4a	156.1	-	156.1	-
5	129.9	-	129.9	-
6	149.8	-	149.9	-
7	125.2	-	125.2	-
8	132.9	-	132.9	-
8a	110.1	-	110.1	-
9	181.8	-	181.9	-
9a	102.1	-	102.1	-
10a	145.7	-	145.7	-
1′	27.8	3.95	27.9	3.96 (m)

Table 22 (Continued)

Position	Compound GD-7		Garciniaxanthone E	
	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)	δ_C (ppm)	δ_H (ppm) (multiplicity), *J* (Hz)
2′	124.4	4.98	124.4	4.99 (m)
3′	129.4	-	129.5	-
4′	17.8	1.70 (s)	17.9	1.70 (s)
5′	25.4	1.61 (s)	25.4	1.62 (s)
1″	24.1	3.31 (d, *J* = 6.1)	24.1	3.31 (d, *J* = 5.9)
2″	122.8	4.98 (m)	122.8	4.97 (t, *J* = 5.9)
3″	134.3	-	134.3	-
4″	39.0	1.91 (t, *J* = 7.3)	39.3	1.92 (t, *J* = 7.3)
5″	26.0	2.00 (td, *J* = 7.3, 6.7)	26.0	2.01 (td, *J* = 7.3, 6.8)
6″	124.0	4.98 (m)	124.0	5.03 (t, *J* = 6.8)
7″	130.6	-	130.7	-
8″	17.4	1.57 (s)	17.5	1.58 (s)
9″	25.4	1.50 (s)	25.4	1.52 (s)
10″	16.0	1.69 (s)	16.1	1.70 (s)

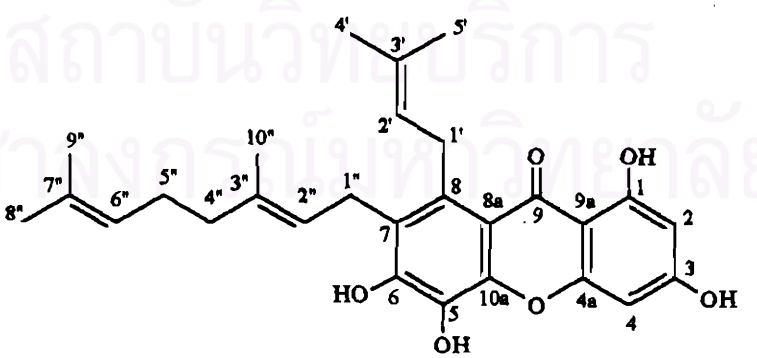


Figure 78 Structure of compound GD-7

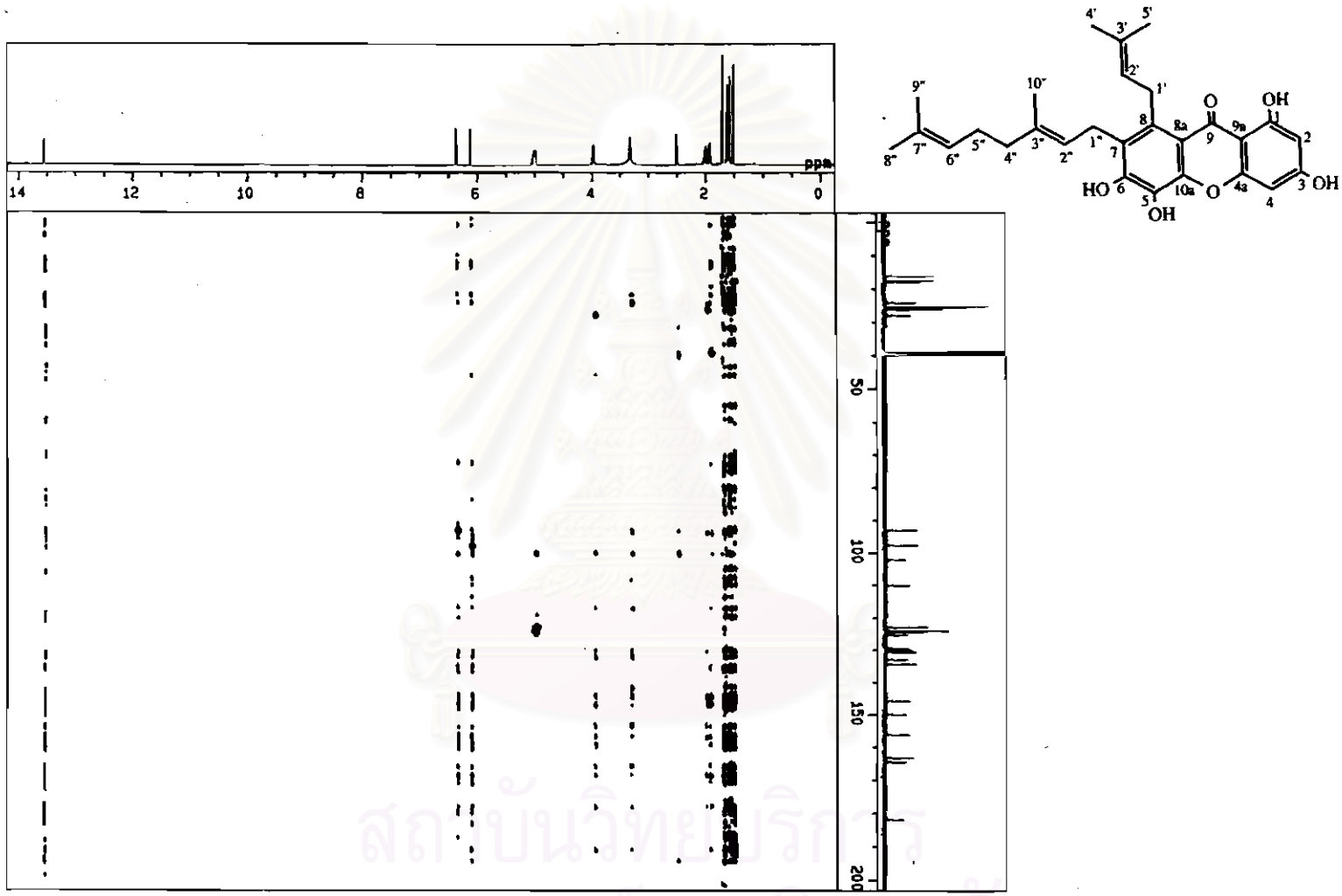


Figure 75a HMQC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 0.0-14.0 ppm, δ_C 0-200 ppm]

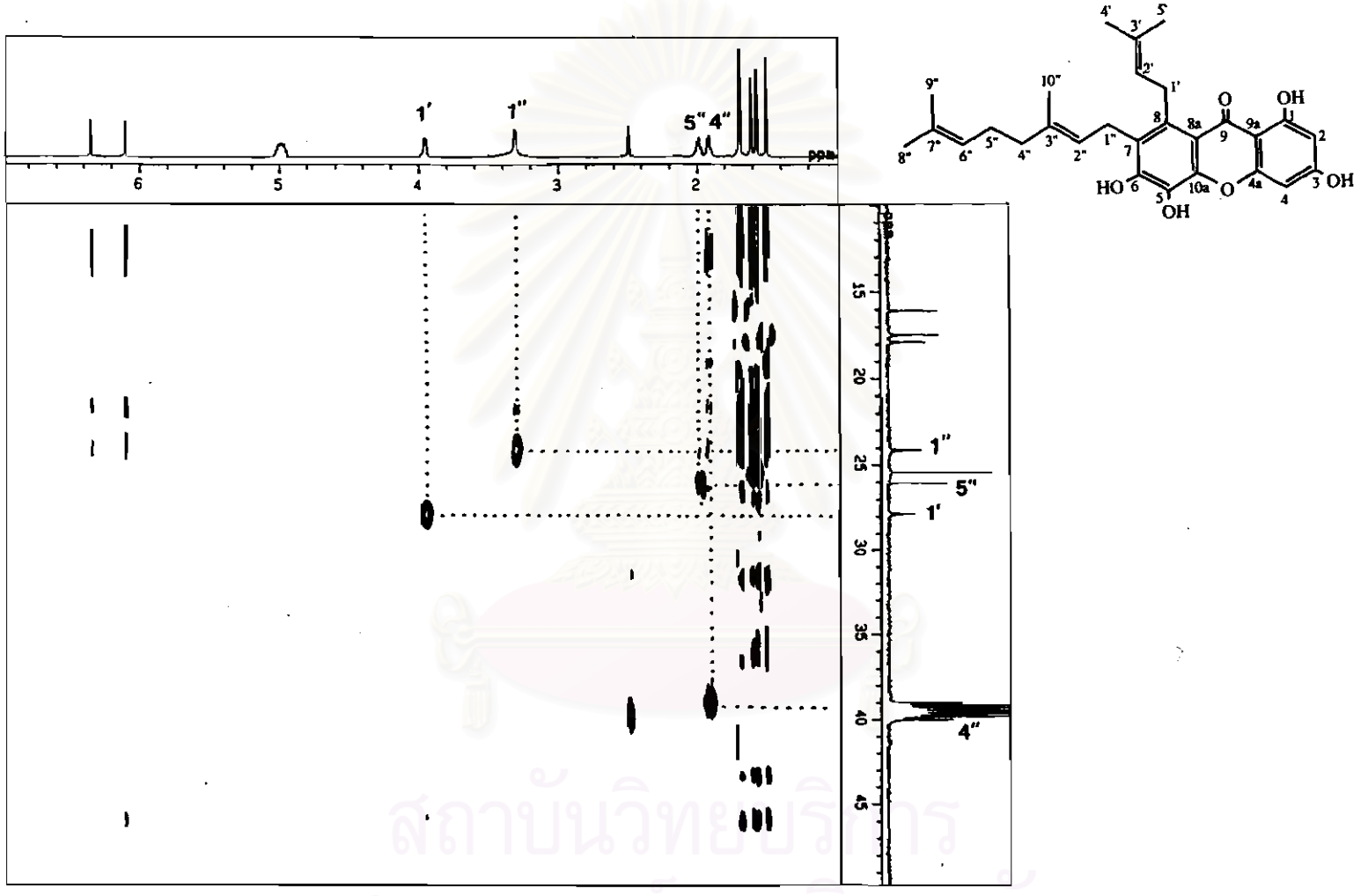


Figure 75b HMQC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 1.0-7.0 ppm, δ_C 10-45 ppm]

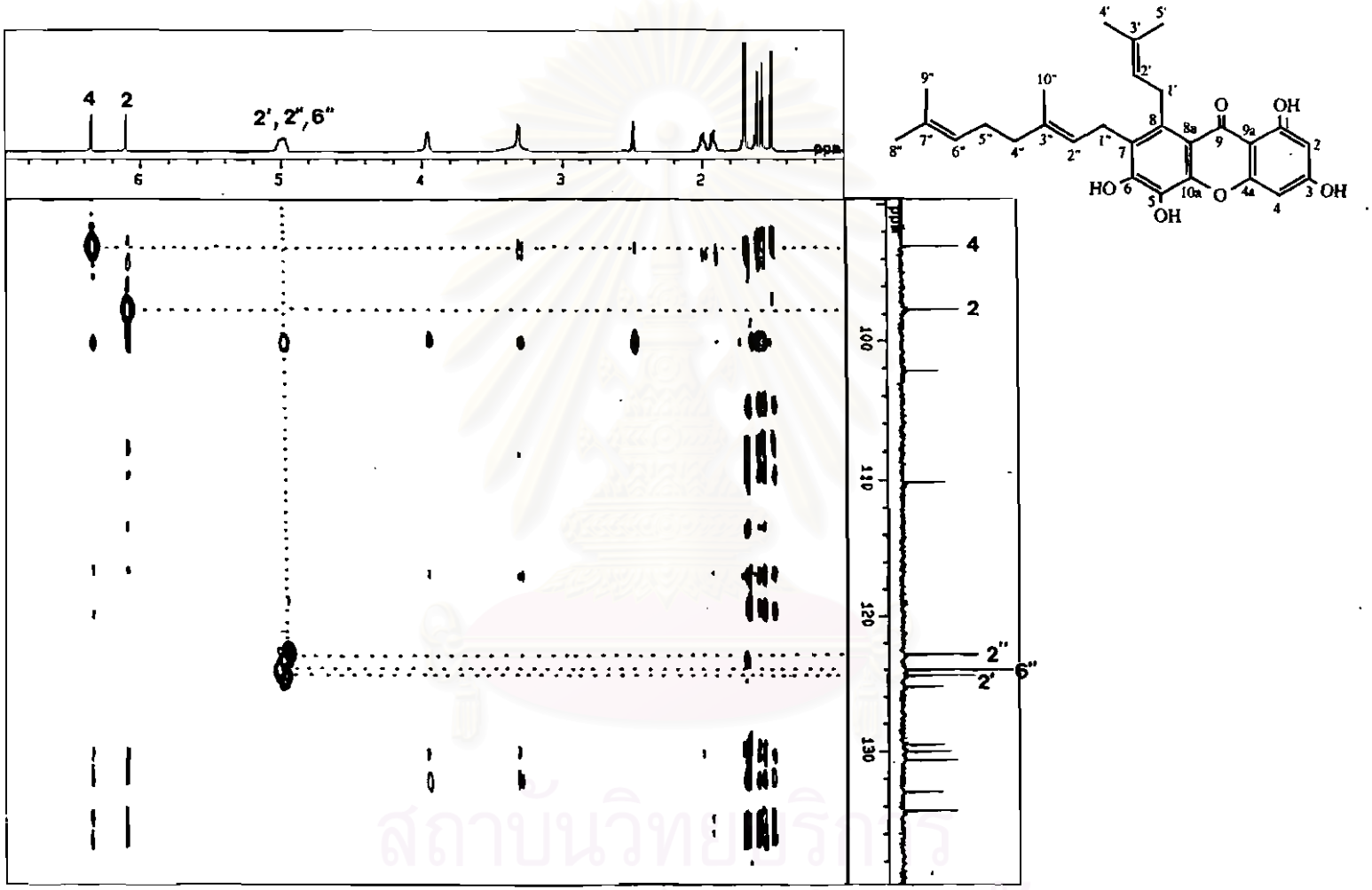


Figure 75c HMQC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 1.0-7.0 ppm, δ_C 90-140 ppm]

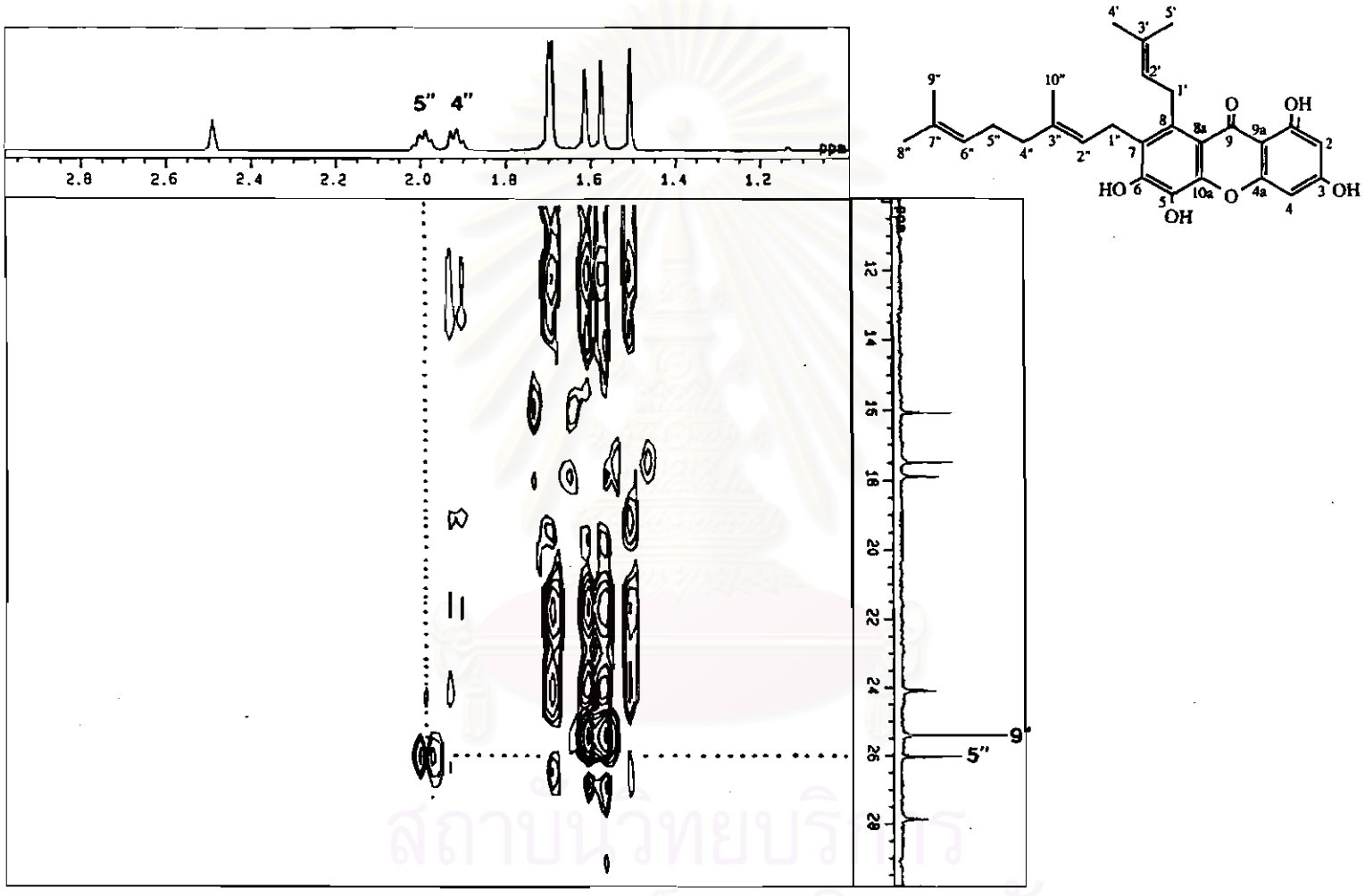


Figure 75d HMQC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 1.2-2.8 ppm, δ_C 10-30 ppm]

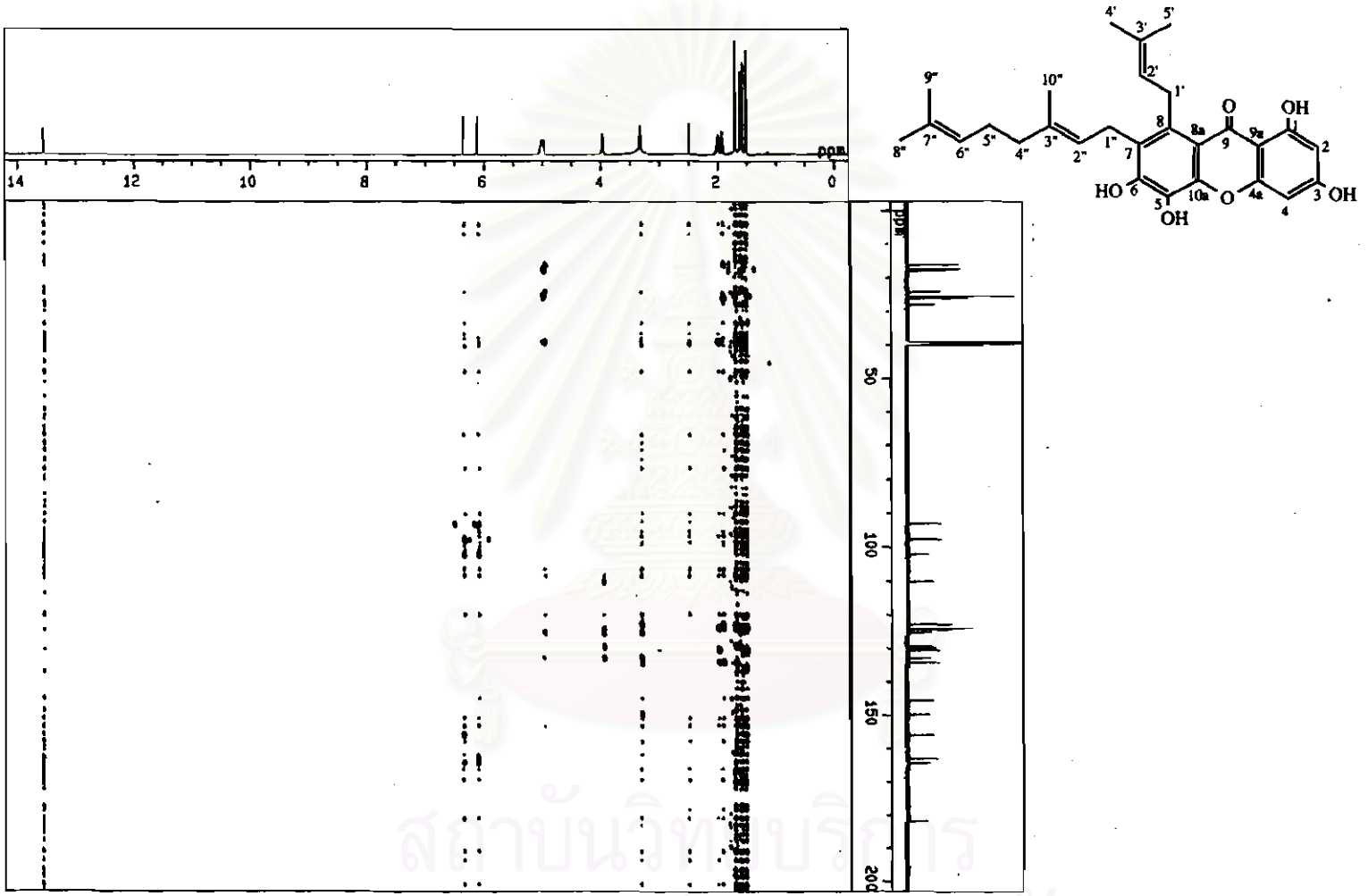


Figure 76a HMBC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 0.0-14.0 ppm, δ_C 0-200 ppm]

Figure 76b HMBC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 1.0-7.0 ppm, δ_C 10-50 ppm]

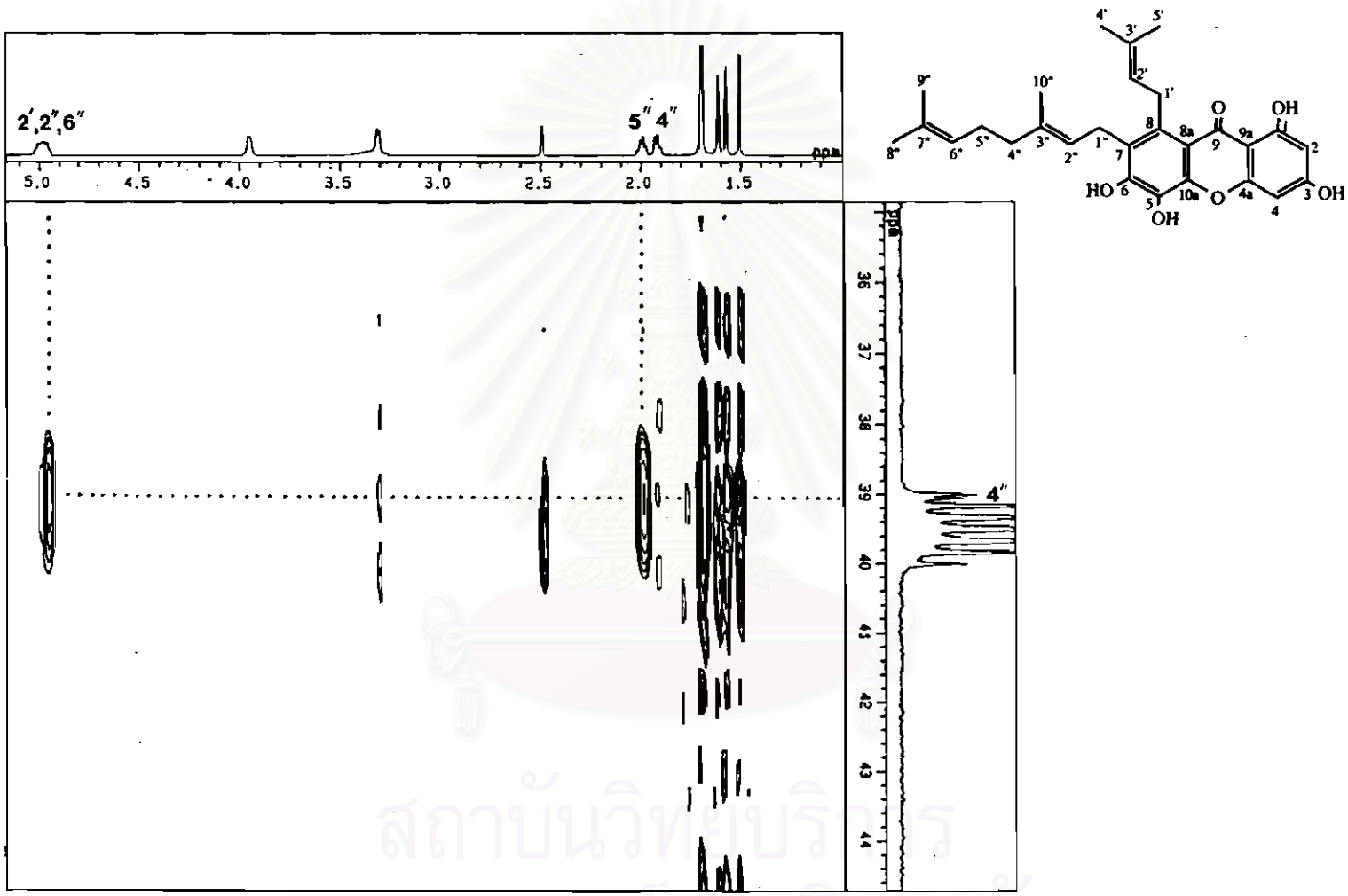


Figure 76c HMBC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 1.0-5.2 ppm, δ_C 35-44 ppm]

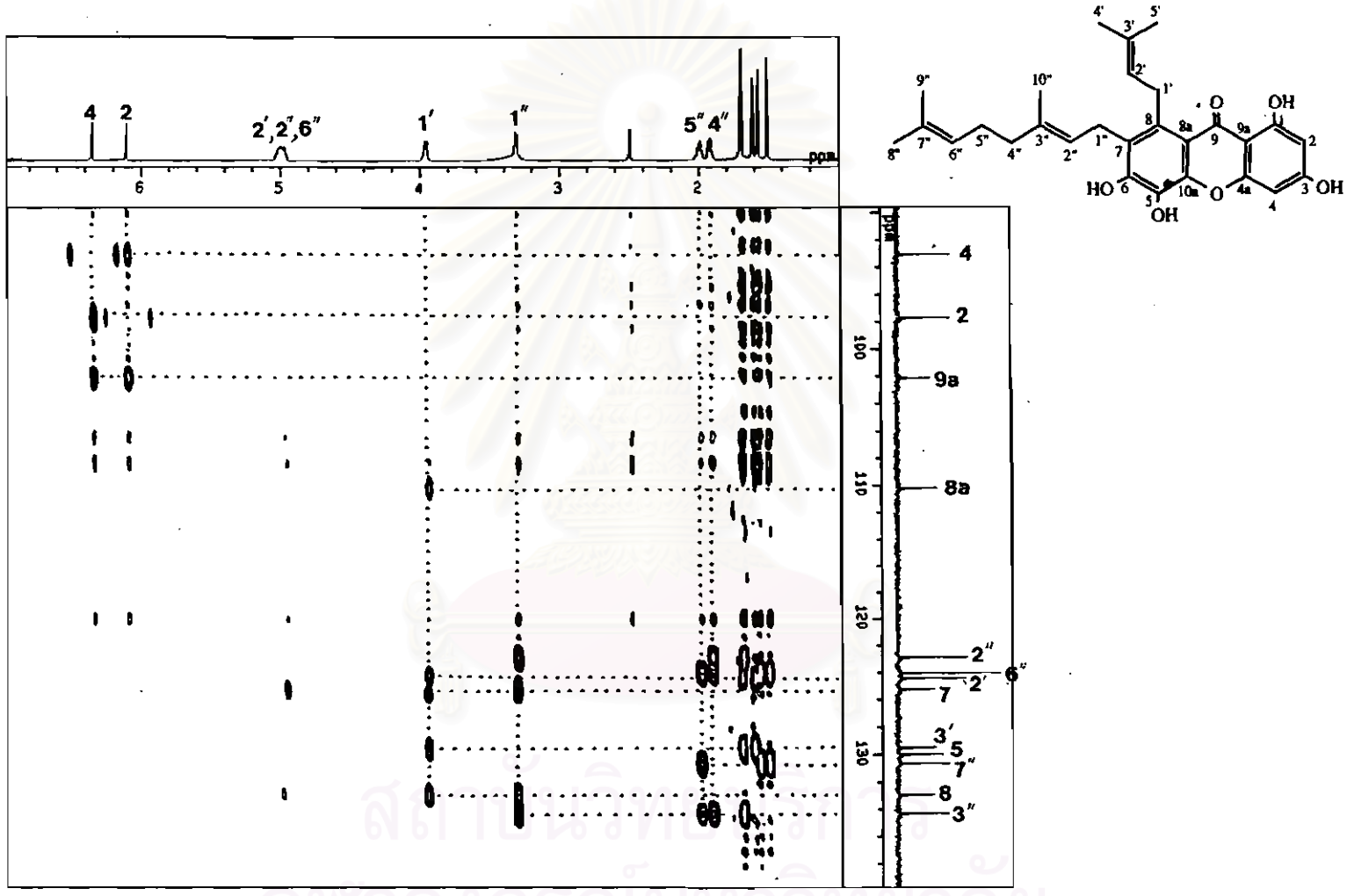


Figure 76d HMBC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 1.0-7.0 ppm, δ_C 90-140 ppm]

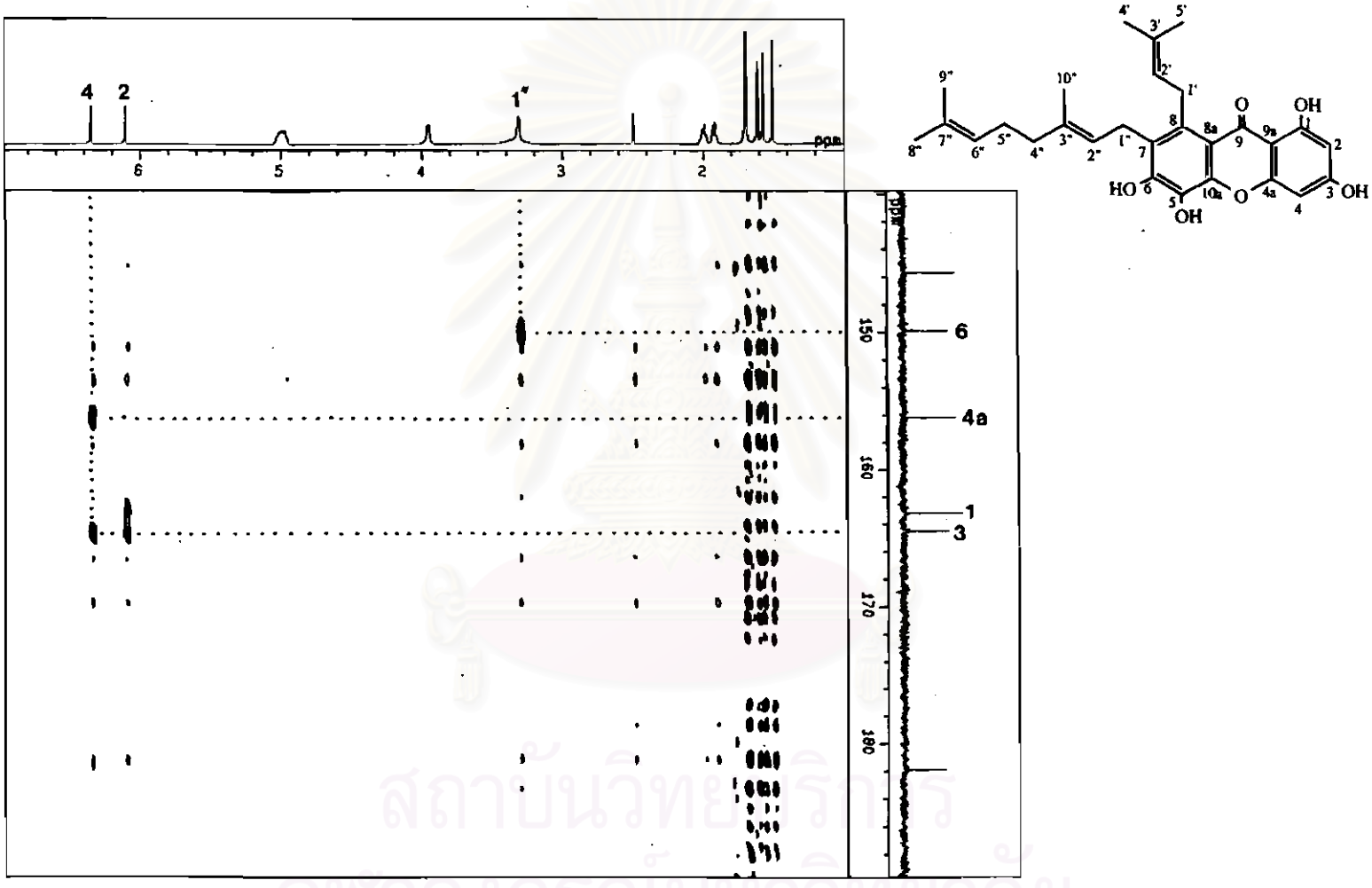


Figure 76e HMBC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 1.0-7.0 ppm, δ_C 140-190 ppm]

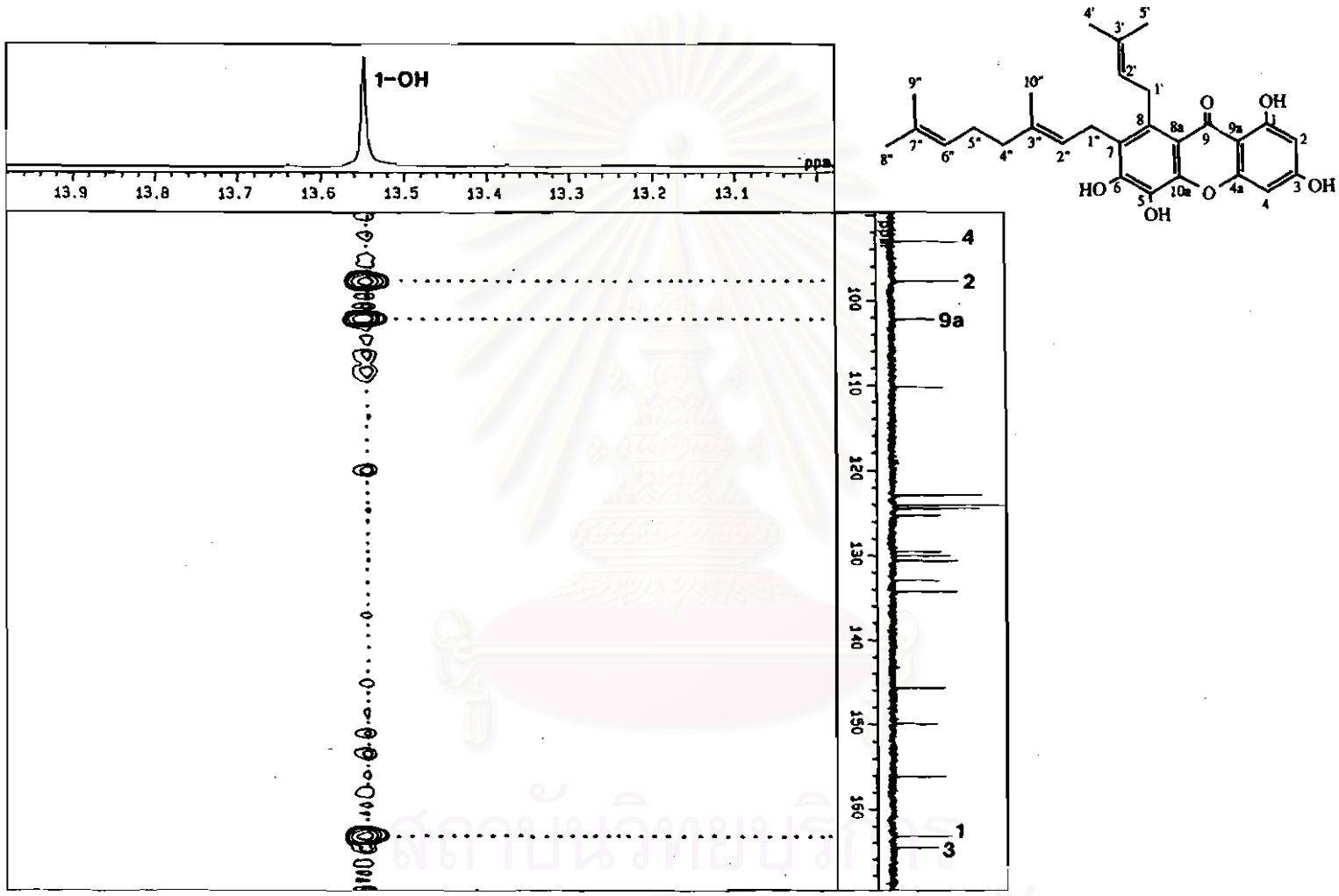


Figure 76f HMBC spectrum of compound GD-7 (in DMSO-d_6), [δ_H 13.1-13.9 ppm, δ_C 90-170 ppm]

The ^{1}H and ^{13}C NMR spectral data of all isolated xanthones from the bark of *G. dulcis* (compound GD-2, GD-3, GD-5, GD-6 and GD-7) are summarized in Tables 23 and 24.

Table 23 ^{1}H NMR spectral data* of five isolated xanthones (compound GD-2, GD-3, GD-5, GD-6 and GD-7) (500 MHz, DMSO-d_6)

Position	δ_H (ppm)				
	Compound GD-2	Compound GD-3	Compound GD-5	Compound GD-6	Compound GD-7
1	12.62 (OH)	12.75 (OH)	-	12.96 (OH)	13.54 (OH)
2	6.77	-	-	-	6.10
3	7.69	7.27	7.26	7.23	-
4	7.03	-	-	-	6.34
5	7.53	-	-	-	-
6	7.35	7.34	-	-	-
7	10.07 (OH)	7.28	6.88	6.94	-
8	7.44	7.59	7.45	7.52	-

* only position 1-8 of the xanthone nucleus

From Table 23, all xanthones were oxygenated at C-1 with a hydroxyl or methoxy group. The proton signal of 1-OH appeared in the range of δ 12.60-13.60. The H-8 aromatic proton was found between δ 7.40 and δ 7.60.

Table 24 ^{13}C NMR spectral data of five isolated xanthones (compound GD-2, GD-3, GD-5, GD-6 and GD-7) (125 MHz, DMSO-d_6)

Position	δ_C (ppm)				
	Compound GD-2	Compound GD-3	Compound GD-5	Compound GD-6	Compound GD-7
1	160.8	151.0	143.3	145.9	163.1
2	109.6	127.5	145.1	146.2	97.6
3	137.1	122.0	121.1	122.0	164.4
4	107.1	136.2	132.2	124.7	93.0
4a	155.8	141.2	147.9	138.6	156.1
5	119.3	146.4	132.4	132.7	129.9
6	125.5	120.8	150.2	152.1	149.8
7	154.1	124.3	112.4	113.1	125.2
8	107.7	114.7	115.9	115.7	132.9
8a	120.4	120.5	115.2	112.3	110.1
9	181.5	182.8	175.1	181.9	181.8
9a	107.8	108.2	116.2	108.2	102.1
10a	149.3	144.7	145.3	146.4	145.7
1′	-	40.0	40.2	39.7	27.8
2′	-	146.6	146.6	146.6	124.4
3′	-	110.7	111.2	111.0	129.4
4′	-	26.4	26.8	26.8	25.4
5′	-	26.4	26.8	26.8	17.8
1″	-	-	-	-	24.1
2″	-	-	-	-	122.8
3″	-	-	-	-	134.3
4″	-	-	-	-	39.0
5″	-	-	-	-	26.0
6″	-	-	-	-	124.0
7″	-	-	-	-	130.6

Table 24 (Continued)

Position	δ_C (ppm)				
	Compound GD-2	Compound GD-3	Compound GD-5	Compound GD-6	Compound GD-7
8″	-	-	-	-	25.4
9″	-	-	-	-	17.4
10″	-	-	-	-	16.0
C_1-OMe	-	-	60.6	-	-