CHAPTER 3

RESULTS AND DISCUSSION

As part of a research project aiming toward a search for chemotherapeutic agents from Thai marine invertebrates, we found that the preliminary screening of the methanolic extract from a Thai sponge, later identified as *Brachiaster* sp., exhibited potent antituberculosis activity (MIC 12.5 μg/mL). This result led to the initiation of a research project in search of active components responsible for such activity. The bioassay-monitored fractionation of the sponge yielded eight sesterterpenes, among which three were new naturally-occurring compounds. All the isolated sesterterpenes were found active in the antituberculsis assay with the MICs of 1.56-50 μg/mL (3-117 μM).

3.1 Isolation of the antituberculosis compounds from the sponge, *Brachiaster* sp.

The Thai sponge, *Brachiaster* sp., was collected at the depth of 18-20 m from Koh-Tao, Surat Thani, Thailand, in April 2001, and was recollected in April 2002 from the same location. The specimen from the first expedition was extracted with MeOH exhaustively (5×1.5 L) and then partitioned with organic solvents to yield hexane-, CH_2Cl_2- and *n*-BuOH-soluble materials (1.11, 1.64 and 1.32 g, respectively). The active CH_2Cl_2 fraction (MIC 6.25 μg/mL) was fractionated and the major active compound, heteronemin **(18)** was obtained (99

mg). The hexane fraction, which was also active (MIC 6.25 μg/mL), was subjected to the chromatographic isolation and two compounds, heteronemin acetate **(41)** and 12-epi-19-deoxyscalarin **(42)** were obtained (10 and 2 mg, respectively). These three compounds are known scalarane-type sesterterpenes.

The sponge was re-collected during the second excursion mentioned earlier. The lyophilized sponge was subsequently extracted with hexane (3×2 L), CH_2Cl_2 (3×2 L) and MeOH (3×2 L) to yield the extracts weighed 13.49, 1.36 and 26.90 g, respectively. The active hexane extract (MIC 3.125 μg/mL) was chosen for the further isolation of the bioactive compounds. An aliquot of the hexane fraction (4.3 g) was separated by means of chromatographic techniques to afford three new compounds, 12-deacetoxy-scalarin acetate **(40)** (3 mg), (*E*)-neomanoalide diacetate **(44)** (4 mg) and (*Z*)-neomanoalide diacetate **(45)** (7 mg), along with two known compounds, 12-deacetyl-12-epi-19-deoxyscalarin **(43)** (2 mg) and manoalide-25-acetate **(46)** (7 mg). Among these, **40** and **43** are also scalarane-type sesterterpenes, whereas **44**, **45** and **46** are the members of the manoalide family of sesterterpenes.

3.2 The structure elucidation of isolated compound

Due to the combining nature of the isolated compounds, i.e., five sesterterpenes of scalarane family and three sesterterpenes of manoalide family, the structure elucidation session of this report will therefore be addressed separately in two sessions. Here, the new compound(s) of either family are presented first, followed by the discussion regarding the identification of the known ones.

3.2.1 The scalarane-sesterterpenes

3.2.1.1 The structure elucidation of 40

Compound **40** was obtained as a white amorphous compound (3 mg) from the hexane-soluble material of the second-expedition specimen by successive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75), followed by reverse phase HPLC (ODS, isocratic 85% aqueous CH_3CN; UV detector, 220 nm).

Compound **40** has a molecular formula of $C_{27}H_{40}O_4$ as established by the ESI mass spectrum, which shows a molecular peak at *m/z* 429 ($[M+H]^+$) (Figure 25), and by its 27 carbon signals observed from the ^{13}C NMR spectrum (Figure 4). The proposed molecular formula was confirmed by the $[M]^+$ peak at *m/z* 428.2910 in the HR-EI mass spectrum (calc for $C_{27}H_{40}O_4$ 428.2927). The proposed molecular formula requires the unsaturation degrees of eight. The ^{13}C NMR spectrum (Figure 4) indicates the presence of two carbonyl carbons and one double bond; therefore, five ring systems are required for **40**. The infrared absorptions at ν 1770 and 1730 cm^{-1} (Figure 26) are consistent with the presence of lactone and carbonyl ester functionalities. The UV spectrum (Figure 27) shows the maximal absorption at λ 227 nm (log ε 3.78).

The ^1H NMR spectrum of **40** (Figure 5) in C_6D_6 (500 MHz) exhibits signals for five aliphatic methyl singlets (δ 0.35, 0.54, 0.70, 0.81 and 0.88), one acetate methyl (δ 1.59), one olefinic proton (δ 6.61) and an acetal proton (δ 6.60), along

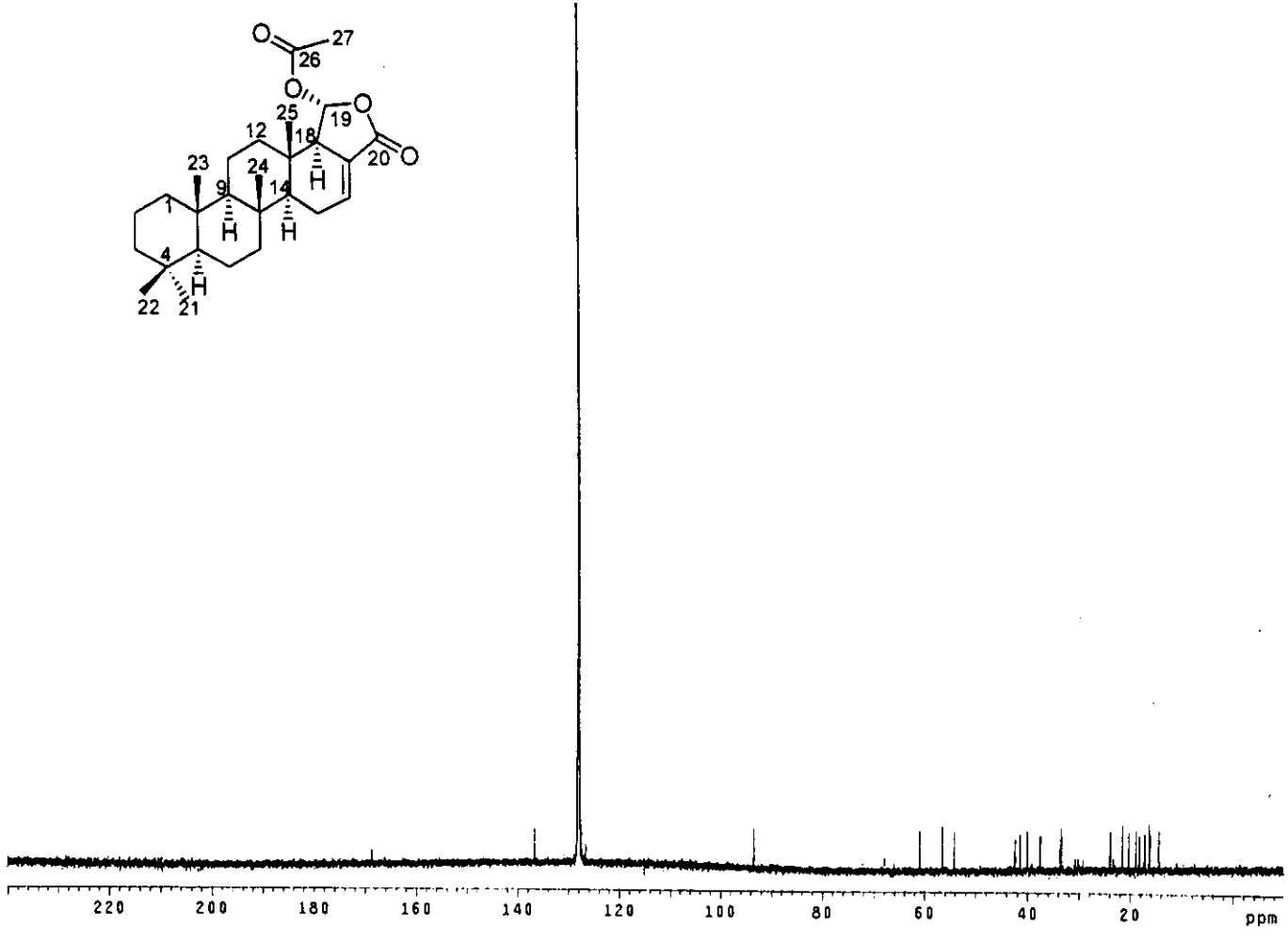


Figure 4 ^{13}C NMR spectrum of **40** (125 MHz; C_6D_6)

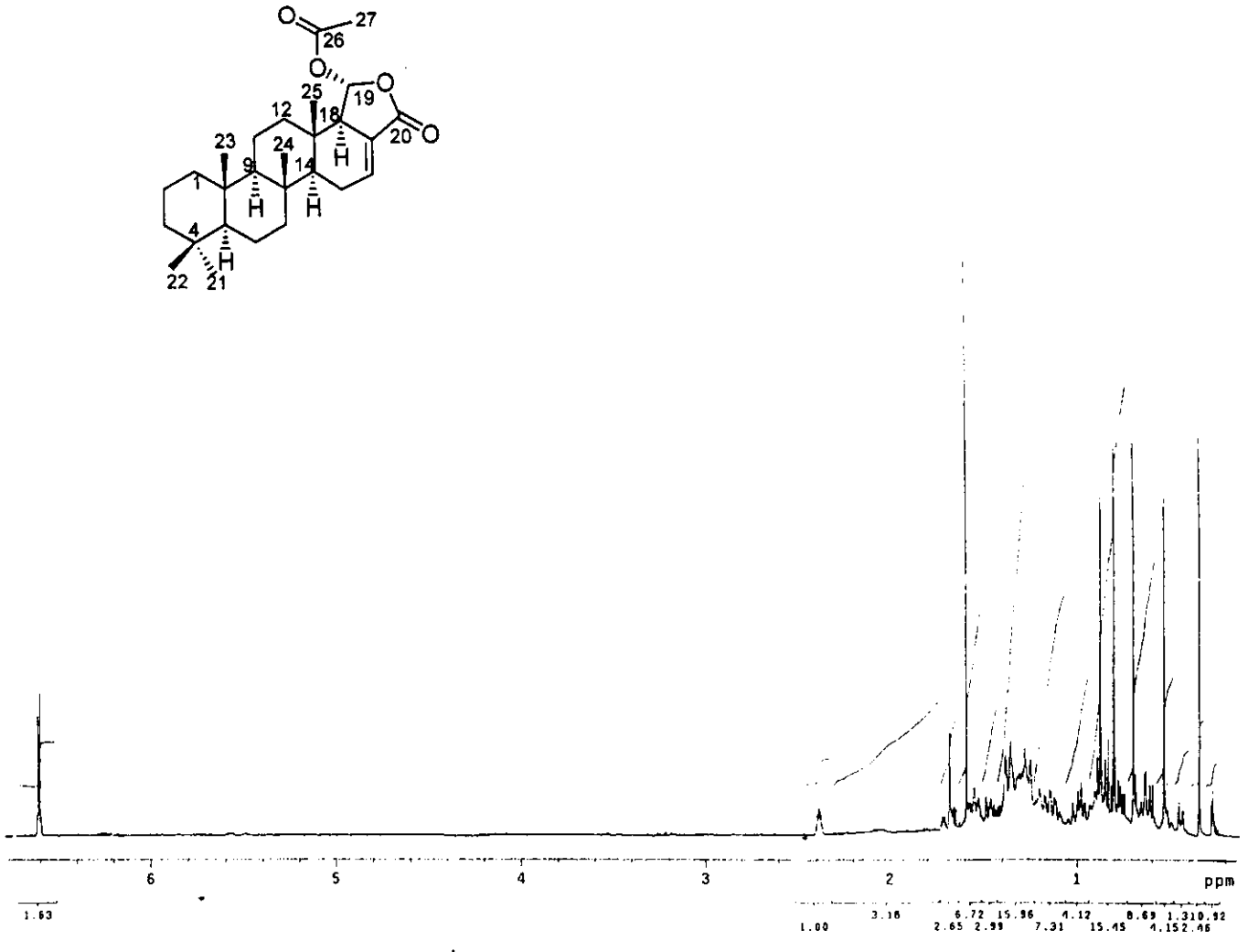


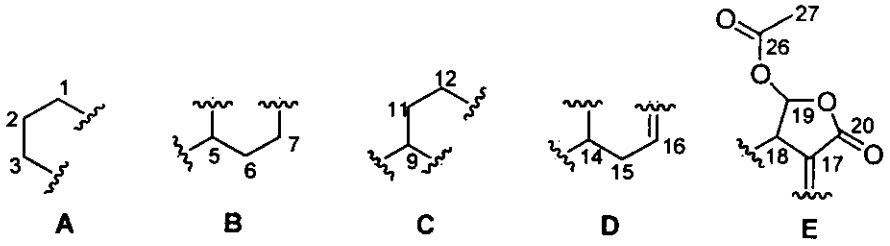
Figure 5 ^{1}H NMR spectrum of **40** (500 MHz; C_6D_6)

with multiplet methylene signals, integrated to belong to sixteen protons. The ^{13}C NMR spectrum (Figure 4) reveals 27 carbons including signals for an α,β-conjugated carbonyl (δ 136.6, 126.6 and 165.7), one ester carbonyl (δ168.6), four quarternary carbons, five methines, eight methylenes, and six methyls, thus accounted for 40 hydrogen atoms.

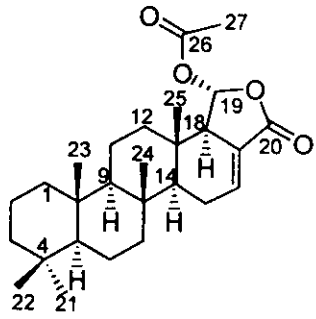
Interpretation of ^{1}H-^{1}H COSY cross peaks in the aliphatic methylene region (Figure 28) led to four partial structures, including fragment **A** [δ 0.64 (m, H-1ax), δ 1.53 (m, H-1eq), δ 1.36 (m, H-2ax), δ 1.55 (m, H-2eq), δ 1.14 (m, H-3ax) and δ 1.37 (m, H-3eq)]; fragment **B** [δ 0.63 (m, H-5), δ 1.15 (m, H-6ax), δ 1.39 (m, H-6eq), δ 0.52 (m, H-7ax) and δ 1.27 (m, H-7eq)]; fragment **C** [δ 0.45 (dd, J = 3.5, 9.6 Hz, H-9), δ 1.00 (m, H-11ax), δ 1.25 (m, H-11eq), δ 0.98 (m, H-12ax) and δ 1.47 (m, H-12eq)]; and fragment **D** [δ 0.78 (dd, J = 5.5, 10.7 Hz, H-14), δ 1.42 (m, H-15a), δ 1.71 (br.d, J = 17.5 Hz, H-15b) and δ 6.61 (ddd, J = 3.6, 4.0. 4.0 Hz, H-16)] as shown below. The assignment of axial and equatorial orientation as stated for each signal was carried out by mean of the coupling constants analysis, along with the observation for the chemical shift of each signal. The axial proton, shielded by 1,3-diaxial repulsion, is generally found at a comparatively higher-field chemical shift than its equatorial counterpart.

The remaining signals, which include those of the acetal proton at δ 6.60 (d, J = 5.5 Hz, H-19) coupling to the methine signal at δ 2.40 (br.ddd, J = 3.6, 3.6, 5.5 Hz, H-18), the signals of lactone carbonyl at δ 165.7 (C-20) and disubstituted

olefinic carbon at δ 126.6 (C-17), all construct the γ-oxygenated γ-lactol moiety. The remaining acetoxy group (δ 168.6, C-26; δ 1.59, s, 3H, H-27) was placed at C-19 on the basis of HMBC data. This structure part is shown as fragment **E**.



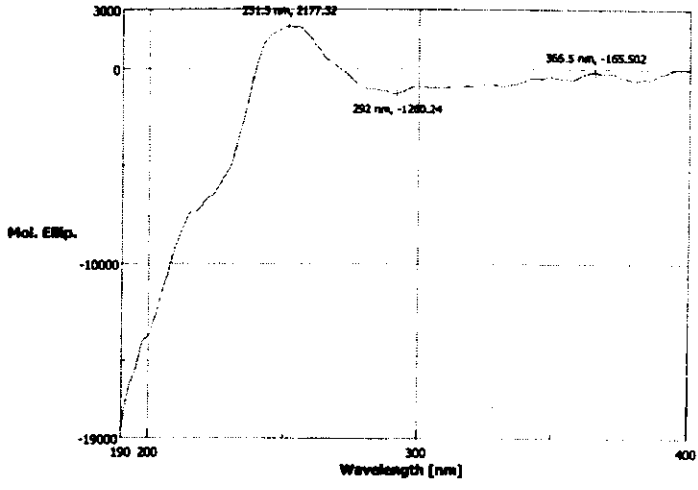
All the proposed structural units were interconnected over four quarternary carbons [δ 33.5 (C-4), 37.4 (C-8), 37.6 (C-10) and 33.7 (C-13)] and five singlet methyls [δ 0.35 (H-25), 0.54 (H-24), 0.70 (H-23), 0.81 (H-21) and 0.88 (H-22)] on the basis of HMBC data (see Table 4). The crucial HMBC correlations include those from C-3 to H-21 and H-22; from C-4 to H-5, H-21 and H-22; from C-5 to H-21, H-22 and H-23; from C-1 to H-9 and H-23; from C-10 to H-5, H-9 and H-23; from C-9 to H-23, H-24, H-5 and H-14; from C-8 to H-9, H-14 and H-24; from C-13 to H-11ax, H-11eq, H-14, H-18 and H-25; and from C-14 to H-24 and H-25. Thus, the tetracarbocyclic moiety (rings A-D) was constructed. The HMBC correlations from C-20 to H-16 and from C-18 to H-14, H-16 and H-25 indicate that the fragment **E** is was fused to ring D at C-17 and C-18. Therefore, the planar structure of **40** was determined as shown. The NMR spectral data are summarized in Table 4.



40

The conformation of tetracarbocyclic ring system of **40** was determined by a close observation of the ^{13}C chemical shifts of ring junction methyls, i.e., C-23, C-24 and C-25 (δ 16.5, 16.1 and 14.5, respectively.) On the basis of the observation of ^{13}C shifts in the models by Crews and Bescansa (1986), ring junction methyls on *trans*-fused ring in chair conformation are shielded (thus resonate at < 20 ppm) relative to those on *trans*-fused ring in boat conformation and *cis*-fused ring of both systems. Thus, the conformation of rings A, B and C is assigned as all *trans*. Accordingly, H-5, H-9 and H-14 are assigned as axial.

The stereochemistry of **40** at positions 18 and 19 was assigned on the basis of coupling constant and CD spectral analysis and was comfirmed by nOe observation. Allylic coupling between H-18 and H-16 (J = 3.6 Hz), and homoallylic coupling between H-18 and H-15a (J = 3.6 Hz), suggested that H-18 is pseudoaxial (Pretsch *et al.*, 1989). The CD spectrum of **40** (Figure 6) reveals the first positive Cotton effect ($[\theta]_{251.5}$ +2177), indicating that the configurations at C-13 and C-19 are *S* and *R*, respectively, according to the octant rule (Eliel and Wilen, 1994).



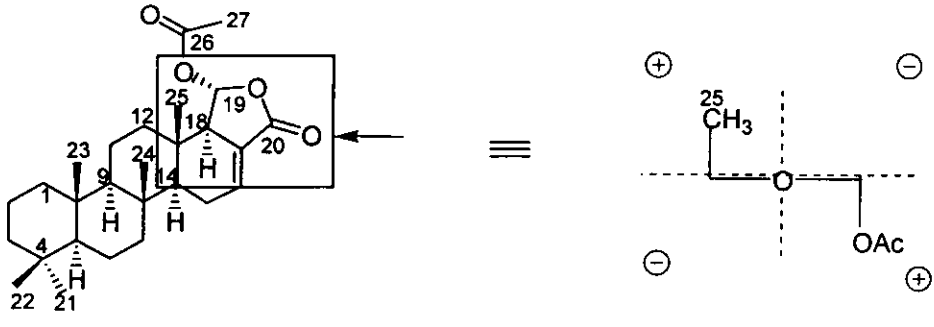


Figure 6 CD spectrum of compound **40**

This is comfirmed by comparison with the report data (Cimino *et al.*, 1977) of the positive cotton effect ($[\theta]_{244}$ +14980) and the $[\alpha]_D$ (-11.5°) of 12-epi-19-deoxyscalarin; i.e., both compounds thus share similar configuration. Furthermore, the dipolar couplings between H-18 and H-14, and between H-19 and H-25 observed from nOe difference spectra (Figures 31 and 32) indicate that the protons of each pair reside on the same plane of structure; thus strongly comfirm the proposed stereochemistry. Compound **40**, therefore, is proposed as 12-deacetoxy-scalarin acetate with the absolute of ring D and E portion configuration shown in figure 7.

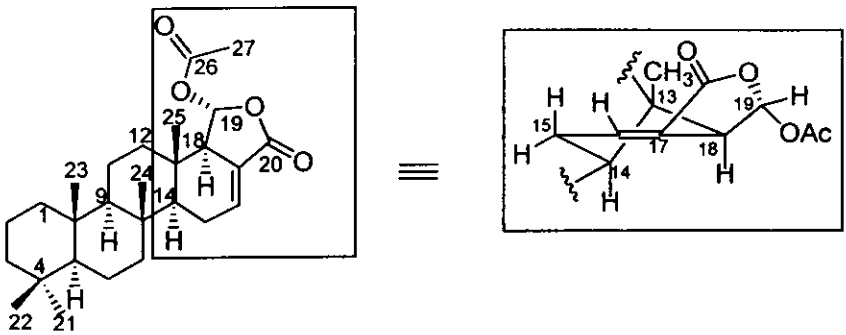


Figure 7 The stereochemistry of **40**

Table 4 NMR data (500 MHz for ^{1}H; in C_6D_6) of **40**

Position	δ_H (mult.; *J* in Hz)	δ_C (mult.)	HMBC correlation ($^{13}C \longrightarrow {}^{1}H$)
1	Hax, 0.64 (m)	40.0 (t)	H-2ax, H-2eq, H-9, H-23
	Heq, 1.53 (m)		
2	Hax, 1.36 (m)	18.9 (t)	H-1ax, H-3ax, H-3eq, H-21
	Heq, 1.55 (m)		
3	Hax, 1.14 (m)	42.4 (t)	H-21, H-22
	Heq, 1.37 (m)		
4	-	33.5 (s)	H-5, H-21, H-22
5	0.63 (m)	56.5 (d)	H-21, H-22, H-23
6	Hax, 1.15 (m)	18.2 (t)	H-5, H-7ax, H-7eq
	Heq, 1.39 (m)		
7	Hax, 0.52 (m)	41.4 (t)	H-5, H-24
	Heq, 1.27 (m)		
8	-	37.4 (s)	H-9, H-14, H-24
9	0.45 (dd, 3.5, 9.6)	61.0 (d)	H-5, H-14, H-23, H-24
10	-	37.6 (s)	H-5, H-9, H-24

Table 4 (cont.)

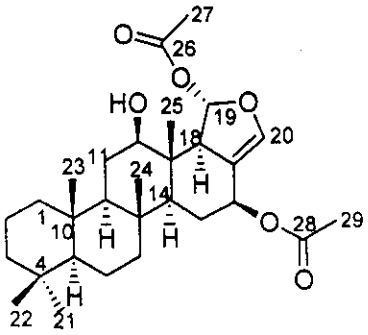
Position	δ_H (mult.; *J* in Hz)	δ_C (mult.)	HMBC correlation ($^{13}C \longrightarrow {}^{1}H$)
11	Hax, 1.00 (m)	17.1 (t)	H-9, H-12ax, H-12eq
	Heq, 1.25 (m)		
12	Hax, 0.98 (m)	40.0 (t)	H-9, H-25
	Heq, 1.47 (m)		
13	-	33.7 (s)	H-11ax, H-11eq, H-12eq, H-14, H-18, H-25
14	0.78 (dd, 5.5, 10.7)	54.2 (d)	H-24, H-25
15	Ha, 1.42 (m)	23.9 (t)	H-14
	Hb, 1.71 (br.d, 17.5)		
16	6.61 (br.ddd, 3.6, 4.0, 4.0)	136.6 (d)	-
17	-	126.6 (s)	-
18	2.40 (br.ddd, 3.6, 3.6, 5.5)	56.6 (d)	H-14, H-16, H-25
19	6.60 (d, 5.5)	93.4 (d)	H-27
20	-	165.7 (s)	H-16, H-19
21	0.81 (s, 3H)	21.4 (q)	H-22
22	0.88 (s, 3H)	33.3 (q)	H-5, H-21
23	0.70 (s, 3H)	16.5 (q)	H-5, H-9
24	0.54 (s, 3H)	16.1 (q)	H-9, H-14
25	0.35 (s, 3H)	14.5 (q)	H-14
26	-	168.6 (s)	H-19, H-27
27	1.59 (s, 3H)	20.2 (q)	-

3.2.1.2 The structure elucidation of 18

Compound **18**, which is the major component, is obtained as white needles (99 mg) from the CH_2Cl_2-soluble material of the first expedition using chromatographic technique with Sephadex LH-20 (methanol) then SiO_2 columns (3% EtOAc in CH_2Cl_2). Also, the hexane-soluble material of second expedition specimen was isolated by chromatographic technique using SiO_2 columns (20:5:75 of EtOAc:acetone: hexane) and re-crystallized in CH_2Cl_2-methanol mixture (1:3) to afford **18** (390 mg).

The ESI mass spectrum of **18** exhibits a molecular ion peak at *m/z* 511 ($[M+Na]^+$) (Figure 33). Along with 29 carbon signals observed from its ^{13}C NMR spectrum (Figure 8), this corresponds with the molecular formula of $C_{29}H_{44}O_6$. Accordingly, the eight degrees of unsaturation are determined as two carbonyl carbons, two sp^2 carbons and five ring systems. The major absorption band at ν 3500 cm^{-1} was assigned to the hydroxyl group, whereas those at 1740 and 1235 cm^{-1} were assigned to the ester functionality in the IR spectrum (Figure 34). The UV spectrum (Figure 35) shows the absorption maximum at λ 229 nm (log ε 2.34).

The ^1H NMR spectrum of **18** (Figure 9) displays signals of five singlet aliphatic methyls, two acetate methyls, seven methylenes, seven methines (which include one acetal proton and two oxygenated methine protons), and one olefinic proton. The 29 carbon signals in the ^{13}C NMR spectrum (Figure 8) indicate the presence of seven methyls, seven methylenes, one acetal methine, two carbonol



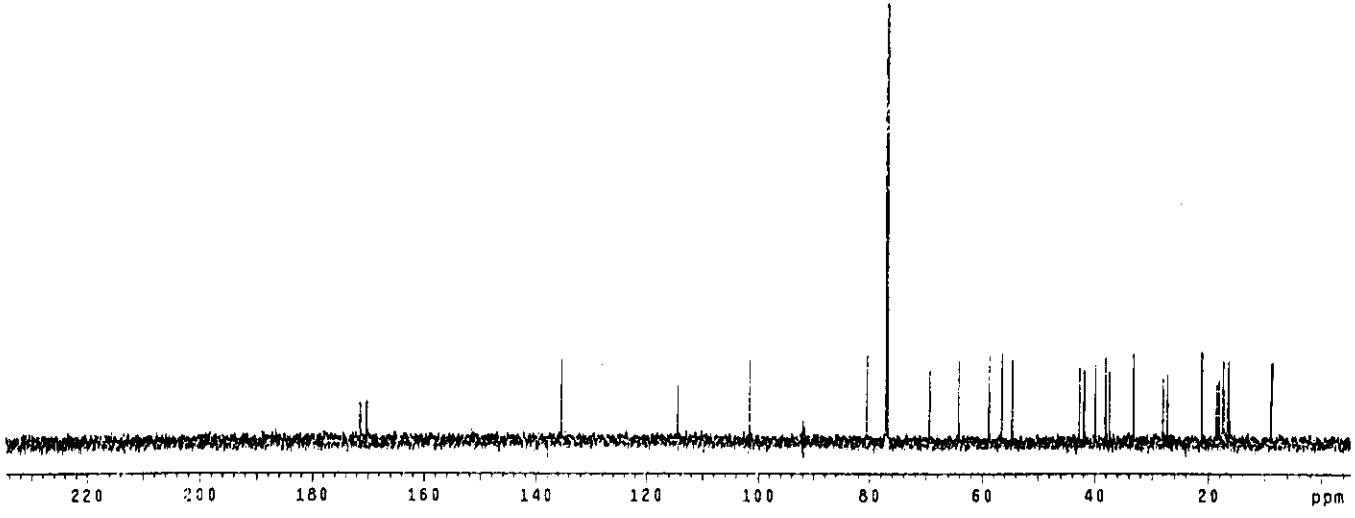


Figure 8 ^{13}C NMR spectrum of **18** (125 MHz; $CDCl_3$)

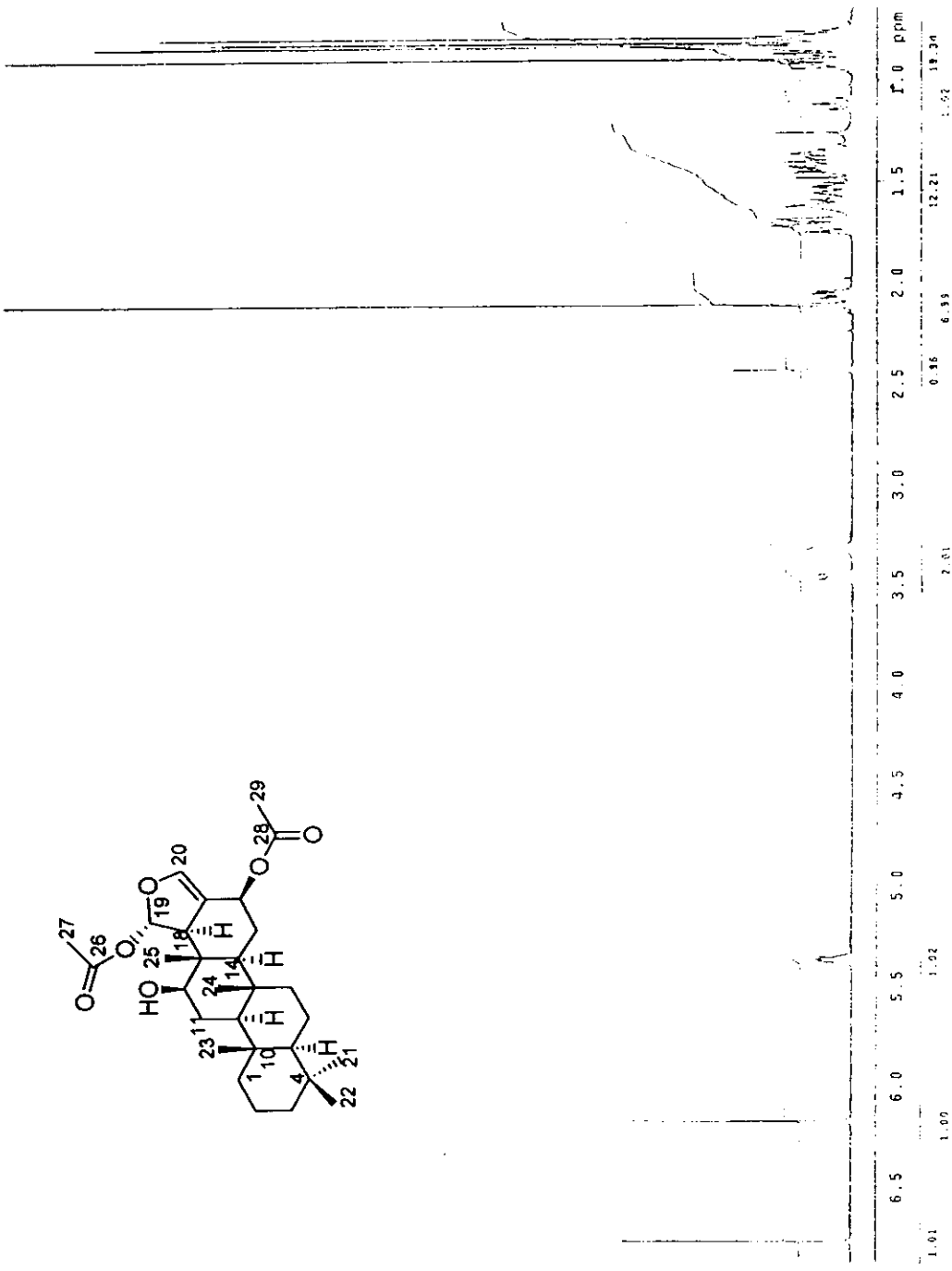


Figure 9 ^{1}H NMR spectrum of **18** (500 MHz; $CDCl_3$)

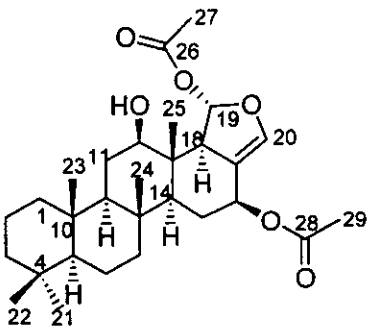
methines, four aliphatic methines, four sp^3 quarternary carbons, two olefinic carbons and two carbonyls. Interpretation of the ^1H-^1H COSY and HMQC spectra (Figures 36 and 37) led to the assembly of the partial structures of C-1 to C-3 and C-5 to C-7 similar to those seen in **40**. The subunit of C-9 to C-12 and C-14 to C-16 were established in the same fashion, however, with some slight differences in oxygenating patterns on C-12 and C-16. Here, a hydroxy group was proposed to attach on C-12 due to the chemical shift at 3.45 ppm (br.d, J = 11.4 Hz, H-12), whereas an acetoxy group (δ_C 170.1, C-28; δ_H 2.11, s, 3H, H-29) was linked to C-16 as a signal at δ 5.37 (dddd, J = 1.8, 1.8, 6.1, 10.4 Hz, H-16) was observed.

The remaining acetal proton at δ 6.78 (d, J = 1.4 Hz, H-19) is coupled to a methine proton at δ 2.43 (br.s, H-18), which shows further allylic coupling to an olefinic proton at δ 6.17 (t, J = 1.8 Hz, H-20). Also, by the C-H long range correlations, the acetoxy moiety with the carbonyl at δ 171.3 (C-26) and the methyl at δ 2.11 (s, 3H, H-27) was placed onto C-19. This information suggests the presence of a dihydrofuranol acetate moiety, which fuses to the C-17-C-18 bond.

Connectivities of all the proposed partial structures were deduced by the HMBC analysis (Figure 38) and led to the proposed structure of **18** as shown. The all *trans* fused ring system was demonstrated based on the ^{13}C NMR chemical shifts of the axial methyl groups including signals of C-23 (δ 16.3), C-24 (δ 17.3) and C-25 (δ 8.7). Here, all the methyl groups, except for C-22, are resonating at high field region with the chemical shifts less than 25 ppm, characteristic to the axial methyl shielded by the 1,3-diaxial effect.

The stereochemistry at positions 12, 16, 18 and 19 of **18** was determined by the observations of chemical shifts and coupling constants. H-12 and H-16 are axial, as established from their large coupling constants (J = 11.4 and 10.4 Hz, respectively). Extended to the nearby position 18, C-25 shift was used as an indirect determination of the relative stereochemistry at C-12 and C-18 in the scalarane series. (Cimino *et al.*, 1977; Crew and Bescansa, 1986). It is the additive γ-effect that induces the up-field shift of C-25 signals in those of with 12β and 18β skeleton. The chemical shift of C-25 in **18** (δ 8.7), similar to that found in 12β,18β-substituted scalaranes, indicates that H-12 and H-18 are axial.

Similar to **40**, the relative stereochemisty between H-18 and H-19 is *anti*, as determined by the small coupling constant (J = 1.4 Hz) (Crews and Bescansa, 1986). The similar sign in the specific rotation ($[\alpha]_D^{25}$ –71.4°) to those previously report (Bourquet-Kondracki *et al.*,1994) indicates the same configuration; thus the structure of **18** is shown below.



18

By comparison of the ^1H and ^{13}C spectral data of **18** with the previously reported data (Kazlauskas *et al.*, 1976; Crews and Bescansa, 1986), compound **18**

was identical with heteronemin, which was first isolated from the sponge *Heteronema erecta* (Kazlauskas *et al.*, 1976). The NMR spectral data of **18** are shown in Table 5.

3.2.1.3 The structure elucidation of 41

Compound **41** was isolated as white amorphous solid (10 mg). It was purified from hexane-soluble material of the first expedition specimen using schromatographic techniques with SiO_2 column (5% EtOAc in CH_2Cl_2) then reverse phase HPLC (ODS, isocratic 87% aqueous CH_3CN; UV detector, 220 nm).

The molecular formula of compound **41** was deduced to be $C_{31}H_{46}O_7$ by means of the analyses of its ESI mass spectrum (Figure 39), which shows molecular ion peak at *m/z* 553 ($[M+Na]^+$), as well as its and ^{13}C NMR spectrum (Figure 10). The nine-degree of unsaturation is designated as three carbonyl functionalities, one carbon-carbon double bond and five ring systems. The IR spectrum of **41** (Figure 40) shows the bands at ν 1740 and 1235 cm^{-1}, which were assigned to the ester carbonyl functionality, while the UV spectrum (Figure 41) shows the absorption maximum at λ 241 nm (log ε 2.52).

Despite the difference operating NMR solvents, the ^{13}C NMR spectrum of **41** (in C_6D_6) and **18** (in $CDCl_3$) were almost identical, suggesting that both shared a common skeleton. However, the ^1H NMR spectrum of **41** (Figure 11) obtained from C_6D_6 exhibits several protons that resonates at higher field than those of **18** ($CDCl_3$). The up-field shifts, particularly of the protons residing in the proximity

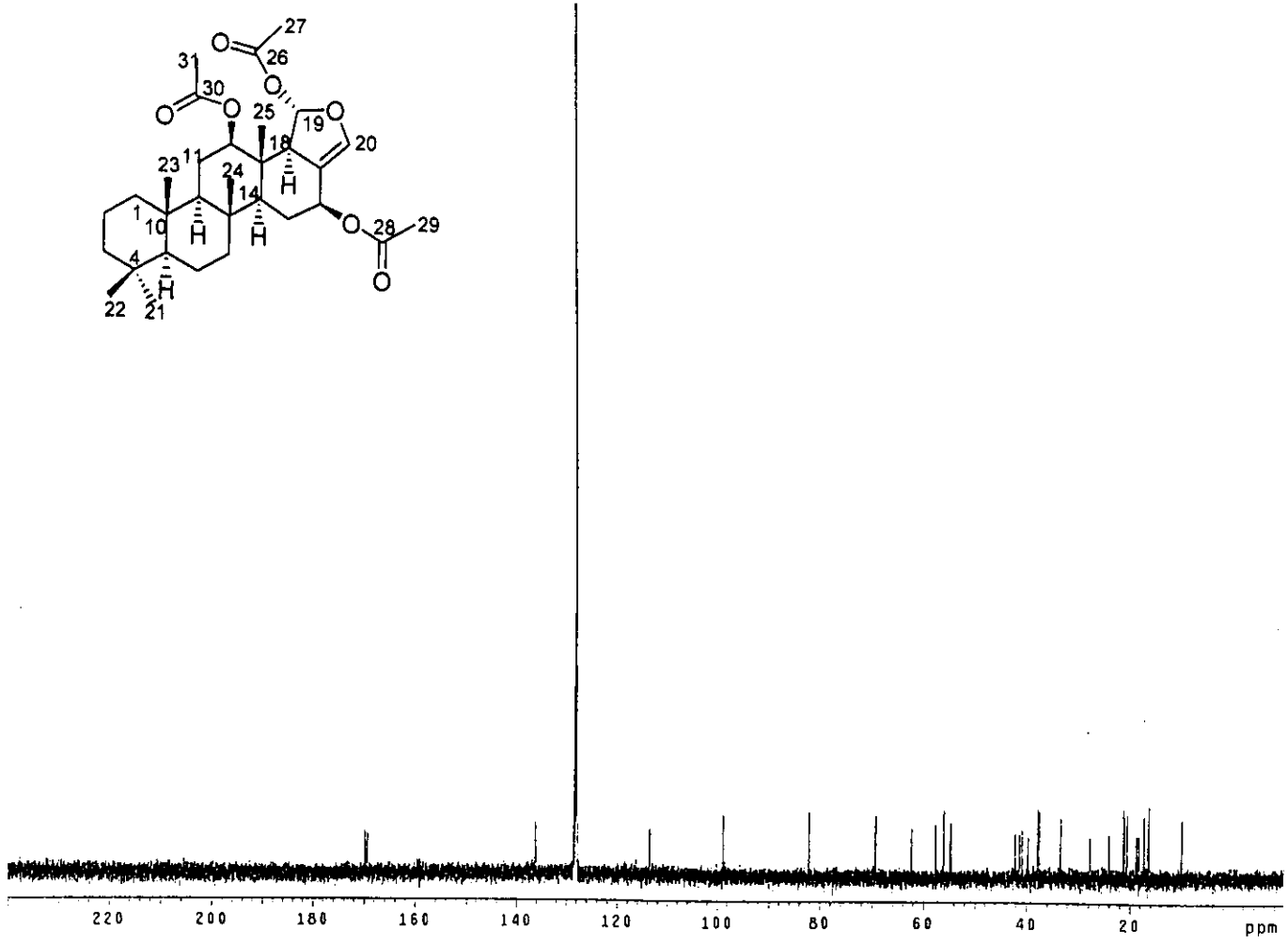


Figure 10 ^{13}C NMR spectrum of **41** (125 MHz; C_6D_6)

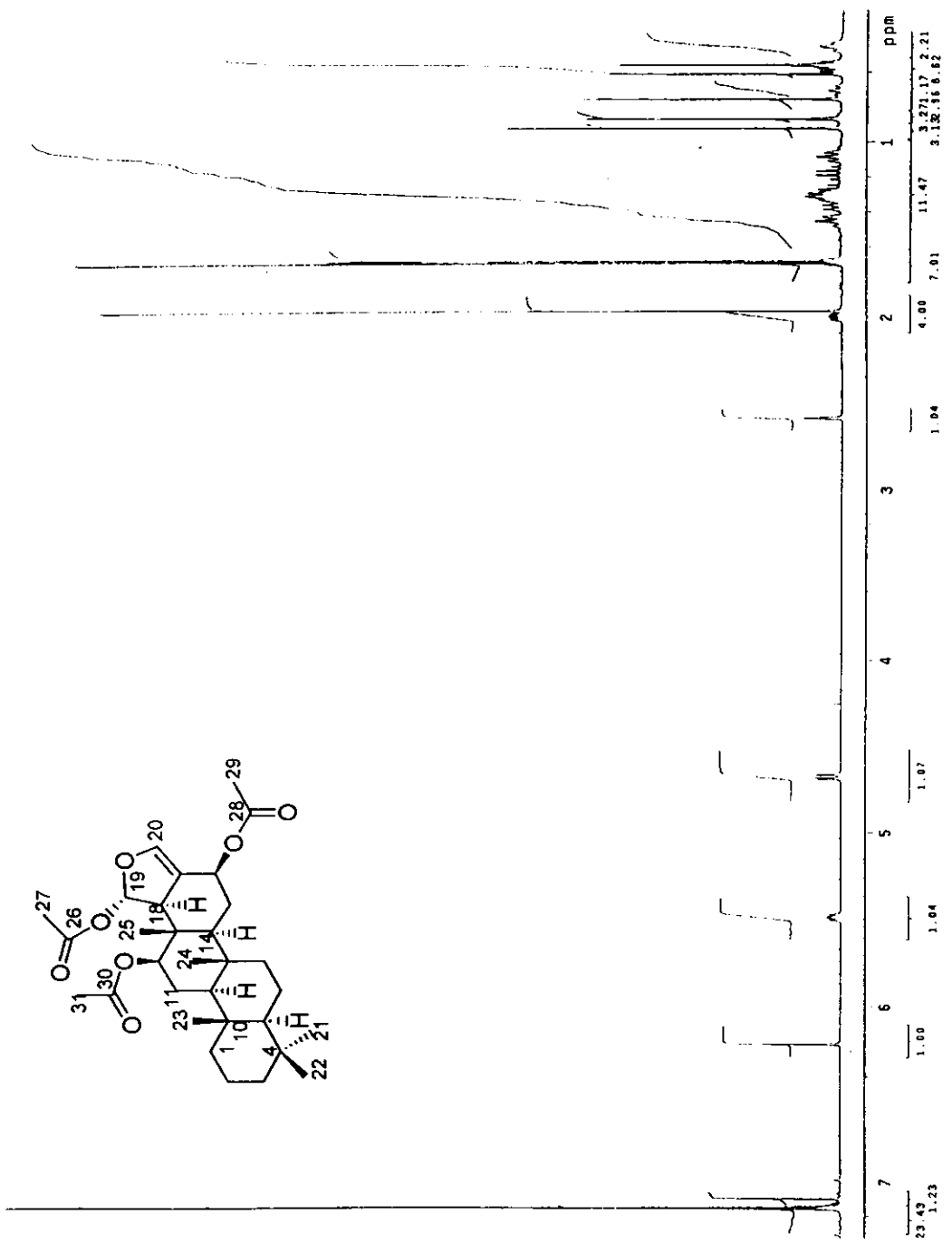
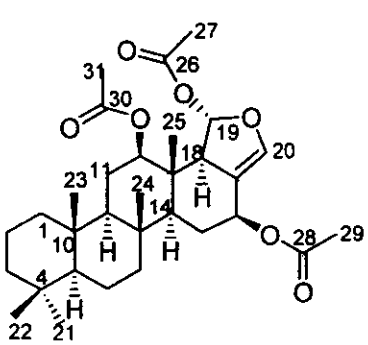


Figure 11 ^{1}H NMR spectrum of **41** (500 MHz; C_6D_6)

of the carbonyls, are resulted from the diamagnetic anisotropy caused by the complex between the carbonyl groups and solvent benzene (Williams and Bhacca, 1965).

Apart form the up-field shifts caused by the solvent effect stated above, the major differences between the ^{1}H spectra of **41** and **18** are the otherwise down-field signal at δ 4.68 (dd, J = 4.2, 11.4 Hz, H-12), along with the additional acetoxy signals at δ 169.9 (C-30), 21.1 (C-31) and 1.97 (s, 3H, H-31). This clearly indicates that **41** in fact is an acetate analog of **18**, of which the acetate group was added onto 12-OH group. The large coupling constant (J = 11.4 Hz) observed for H-12 of **41** indicates the axial orientation similar to that of **18**.

Therefore, compound **41** was identified as heteronemin acetate. The NMR spectral data of **41** are consistent with the data for previously reported from the sponge *Hyrtios erecta*, furthermore, the stereochemistry of **41** was confirmed by the specific rotation ($[\alpha]_D^{25}$ –48.6°), which is in the same sign as those report ($[\alpha]_D$ –30°) (Crews and Bescansa, 1986). The NMR spectral data of **41** are summarized in Table 5.



41

Table 5 NMR data (500 MHz for ^1H) of **18** (in $CDCl_3$) and **41** (in C_6D_6)

Position	δ_H (mult.; *J* in Hz)		δ_C (mult.)	
	18	**41**	**18**	**41**
1	Hax, 0.78 (m)	Hax, 0.69 (m)	39.9 (t)	39.7 (t)
	Heq, 1.69 (m)	Heq, 1.45 (m)		
2	Hax, 1.34 (m)	Hax, 1.48 (m)	18.2 (t)	18.3 (t)
	Heq, 1.54 (m)	Heq, 1.54 (m)		
3	Hax, 1.12 (m)	Hax, 1.09 (m)	42.0 (t)	41.4 (t)
	Heq, 1.37 (m)	Heq, 1.32 (m)		
4	-	-	33.2 (s)	33.4 (s)
5	0.77 (m)	0.58 (m)	56.5 (d)	56.1 (d)
6	Hax, 1.42 (m)	Hax, 1.06 (m)	18.6 (t)	18.7 (t)
	Heq, 1.62 (m)	Heq, 1.31 (m)		
7	Hax, 0.91 (m)	Hax, 0.47 (m)	41.8 (t)	40.8 (t)
	Heq, 1.74 (m)	Heq, 1.39 (ddd, 3.2, 3.2 ,12.7)		
8	-	-	37.4 (s)	37.5 (s)
9	0.86 (m)	0.56 (m)	58.7 (d)	57.6 (d)
10	-	-	38.0 (s)	37.8 (s)
11	Hax, 1.46 (m)	Hax, 1.18 (m)	27.2 (t)	24.1 (t)
	Heq, 1.71 (m)	Heq, 1.70 (m)		
12	3.45 (br.d, 11.4)	4.68 (dd, 4.2, 11.4)	80.5 (d)	82.1 (d)
13	-	-	42.7 (s)	42.2 (s)
14	0.94 (m)	0.59 (m)	54.6 (d)	54.7 (d)
15	Hax, 1.41 (m)	Hax, 1.29 (m)	28.0 (t)	28.0 (t)
	Heq, 2.06 (ddd, 2.4, 6.1, 12.1)	Heq, 2.01 (ddd, 2.2, 6.0, 12.0)		
16	5.37 (dddd, 1.8, 1.8, 6.1, 10.4)	5.47 (dddd, 1.9, 1.9, 6.0, 10.4)	69.3 (d)	69.3 (d)
17	-	-	114.4 (s)	113.6 (s)
18	2.43 (br.s)	2.58 (br.s)	64.2 (d)	62.3 (d)

Table 5 (cont.)

Position	δ_H (mult.; *J* in Hz)		δ_C (mult.)	
	18	**41**	**18**	**41**
19	6.78 (d, 1.4)	7.10 (d, 2.2)	101.7 (d)	98.8 (d)
20	6.17 (t, 1.8)	6.21 (t, 1.9)	135.3 (d)	136.2 (s)
21	0.79 (s, 3H)	0.75 (s, 3H)	21.4 (q)	21.3 (q)
22	0.83 (s, 3H)	0.87 (s, 3H)	33.2 (q)	33.3 (q)
23	0.82 (s, 3H)	0.61 (s, 3H)	16.3 (q)	16.4 (q)
24	0.84 (s, 3H)	0.56 (s, 3H)	17.3 (q)	17.3 (q)
25	0.90 (s, 3H)	0.92 (s, 3H)	8.7 (q)	10.0 (q)
26	-	-	171.3 (s)	169.7 (s)
27	2.11 (s, 3H)	1.69 (s, 3H)	21.2 (q)	20.5 (q)
28	-	-	170.1 (s)	169.3 (s)
29	2.11 (s, 3H)	1.67 (s, 3H)	21.1 (q)	20.5 (q)
30	-	-	-	169.9 (s)
31	-	1.97 (s, 3H)	-	21.3 (q)
12-OH	3.32 (d, 4.7)	-	-	-

3.2.1.4 The structure elucidation of 42

As white amorphous solid (2 mg), compound **42** was obtained from hexane-soluble material of the first expedition specimen within the same step as that for **41**, i.e., SiO_2 column (5% EtOAc in CH_2Cl_2) then reverse phase HPLC (ODS, isocratic 87% aqueous CH_3CN; UV detector, 220 nm).

Similar to that of **40**, compound **42** has the molecular formula of $C_{27}H_{40}O_4$, as deduced from its molecular ion peak at *m/z* 429 ($[M+H]^+$) in the ESI mass

spectrum (Figure 45), and from the numbers of carbons and protons observed from the ^{13}C and ^{1}H spectra (Figures 12 and 13). Also similar to **40**, the nine-degree of unsaturation is assigned as two carbonyls, one carbon-carbon double bonds and five ring systems. The lactone and ester functionalities was determined from the major absorption bands at ν 1765, 1735 and 1240 cm^{-1} in its IR spectrum (Figure 46). The UV maximal absorption of **42** (Figure 47) was observed at λ 223 nm (log ε 3.77).

Despite similar NMR spectral pattern, thus suggesting **18**, **40** and **42** share the same skeleton, close inspection indicates that the NMR signals associated with the acetal moiety as seen in **18** and **40** are absent here. On the other hand, compound **42** shows two characteristic lactone methylene signals at δ 3.82 (dd, J = 9.2, 9.3 Hz, H-19a) and 3.92 (dd, J = 9.1, 9.2 Hz, H-19b), corresponding to the carbon at δ 67.2 (C-19). These indicate the presence of a saturated γ-lactone, replacing a lactol moiety seen previously in **18** and **40**. Additionally, an acetoxy group (δ_C 169.3, C-26; δ_H 1.54, s, 3H, H-27) was proposed to attached on C-12 due to the chemical shift at δ 4.46 (dd, J = 4.3, 11.5 Hz, H-12). Here, **42** is proposed to be a 12-acetoxy analog of the scalarin family with an extended saturated lactone ring E. This proposed structure is identical to the known sesterterpene, 12-epi-19-deoxyscalarin, as shown.

The coupling constant of 11.5 Hz observed for H-12 indicates the axial orientation similar to that of **18**. Also, the orientation of H-18 of **42** is pseudoaxial, similar to that of **18** and **40**, due to the allylic coupling constant (3.6 Hz) between

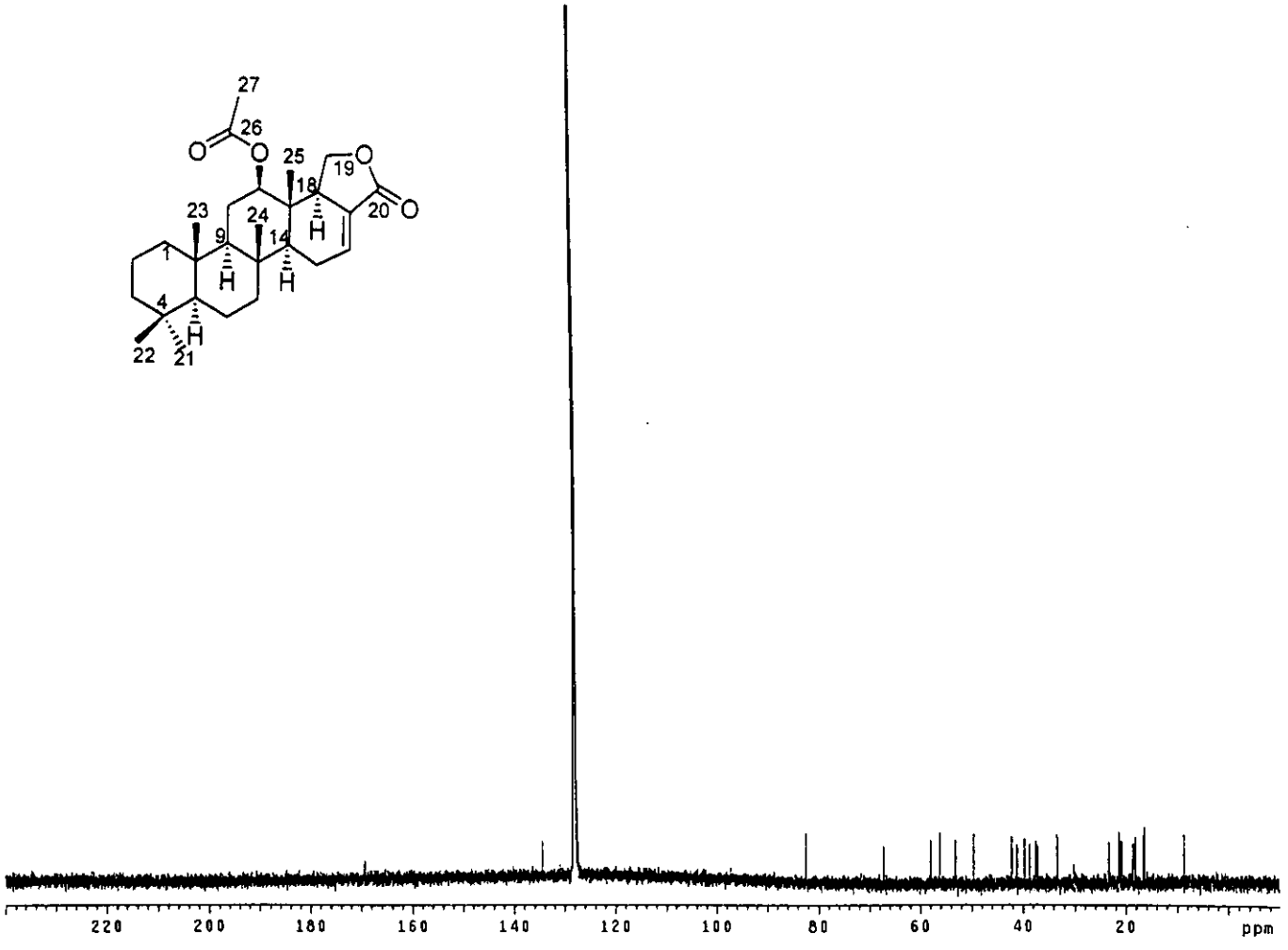


Figure 12 ^{13}C NMR spectrum of **42** (125 MHz; C_6D_6)

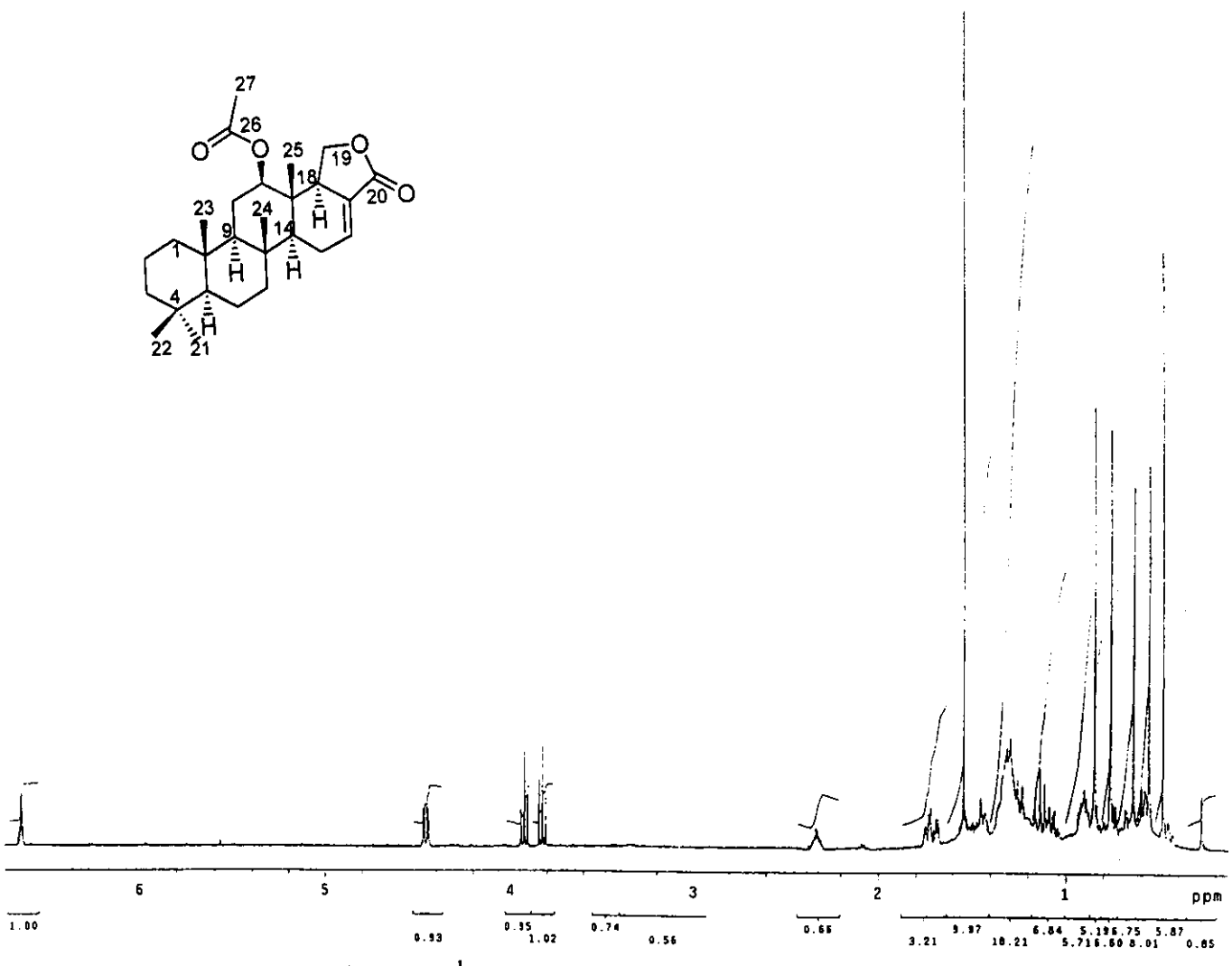
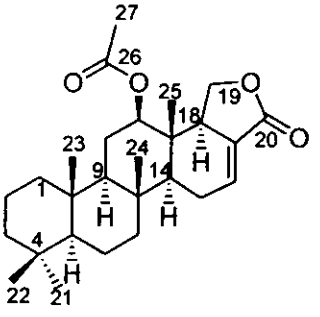


Figure 13 ^{1}H NMR spectrum of **42** (500 MHz; C_6D_6)

H-18 and H-16, and the homoallylic coupling constant (3.9 Hz) between H-18 and H-15a (Pretsch *et al.*, 1989).

Comparison between ^{1}H and ^{13}C NMR data and specific rotation ($[\alpha]_D^{25}$ –33.0°) of **42** with those previously reported strongly supports the proposed structure (Cimino *et al.*, 1977). Therefore, compound **42** was identified as 12-epi-19-deoxyscalarin. The NMR spectral data of **42** are summarized in Table 6.



42

3.1.2.5 The structure elucidation of 43

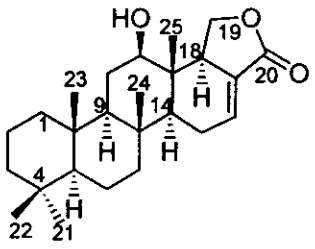
Compound **43** was isolated as white needles (2 mg) from hexane-soluble material of the second-expedition specimen by successive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75) followed by semi-preparative normal phase HPLC (SiO_2, isocratic 5% isopropanol in hexane; UV detector, 220 nm) and re-crystallized in acetonitrile.

The molecular formula of $C_{25}H_{38}O_3$ was determined by means of the ESI mass spectral analysis (Figure 51), which show molecular ion peak at *m/z* 387 ($[M+H]^+$). The ^{13}C NMR spectrum (Figure 14) indicates the presence of one

carbonyl carbons and one double bond, thus, five ring systems are required to fit the unsaturation degree of seven. The IR spectrum (Figure 52) shows absorption bands at ν 3450 and 1745 cm^{-1}, which were assigned to hydroxyl and lactone functionalities, respectively. The UV spectrum (Figure 53) shows the absorption maximum at λ 224 nm (log ε 3.72).

The ^{13}C and ^{1}H NMR spectra of **43** (Figures 14 and 15) were almost identical to those of **42**. The only difference is the absence of the acetyl group at C-12. The up-field shift of H-12 (δ 2.72, ddd, J = 4.5, 5.0, 11.4 Hz) indicates that **43** is a deacetyl analog of **42**. The orientation of H-12 is assigned as axial, similar to **18**, **41** and **42**, as determined by the coupling constant of 11.4 Hz. Interestingly, the chemical shift of H-12 at 2.72 ppm is uncharacteristically high-fielded, presumably due to diamagnetic anisotropy from solvent effect (Williams and Bhacca, 1965).

Compound **43** was, therefore, identified as 12-deacetyl-12-epi-19-deoxyscalarin. The substitution pattern of **43** was confirmed by comparison with the spectral data of those previously reported (Cimino *et al.*, 1977). Although its specific rotation has never been reported, the similar sign to that of **42**, suggests the two compounds share the same configuration. The NMR spectral data of **43** are summarized in Table 6.



43

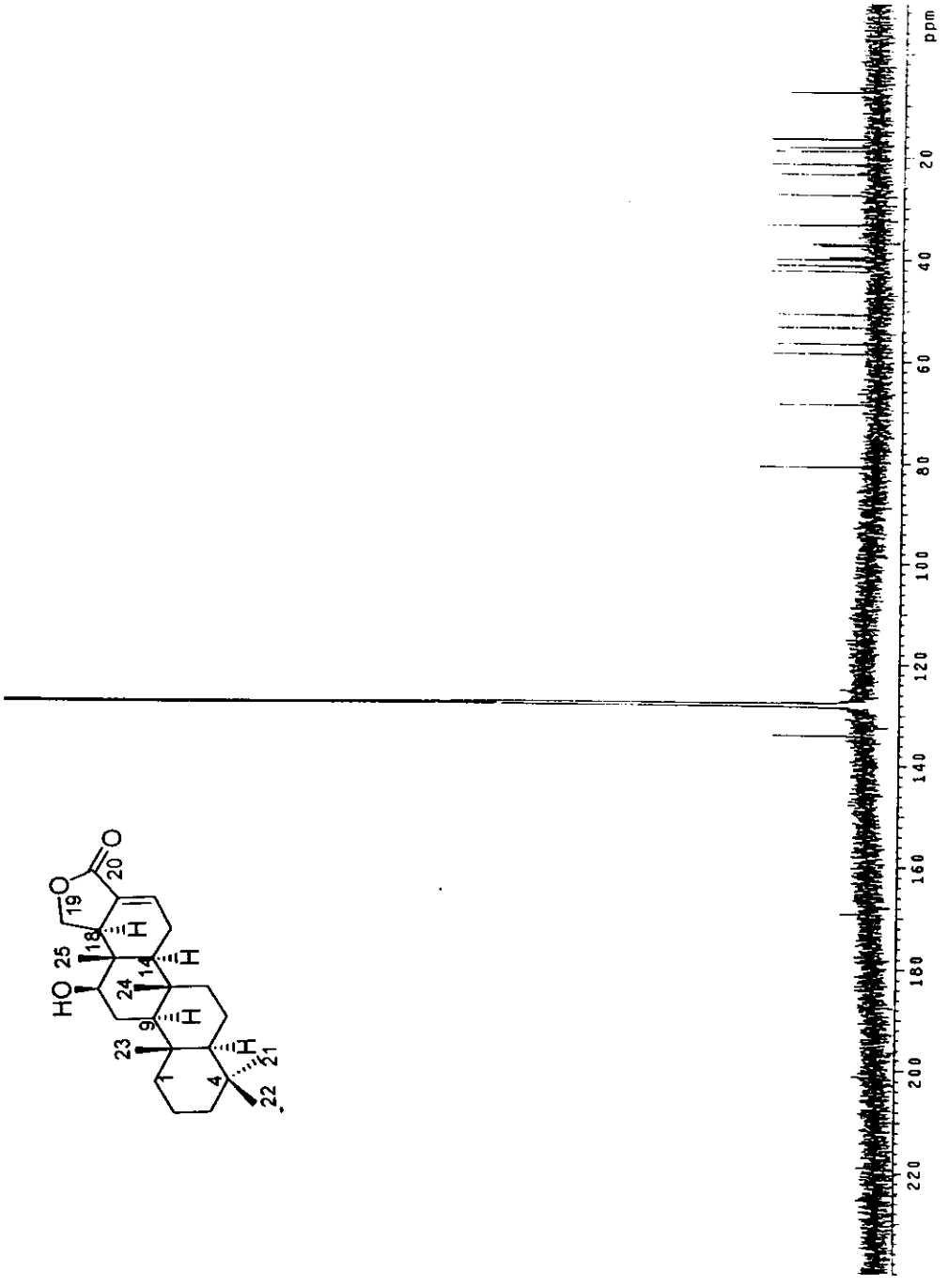


Figure 14 ^{13}C NMR spectrum of **43** (125 MHz; C_6D_6)

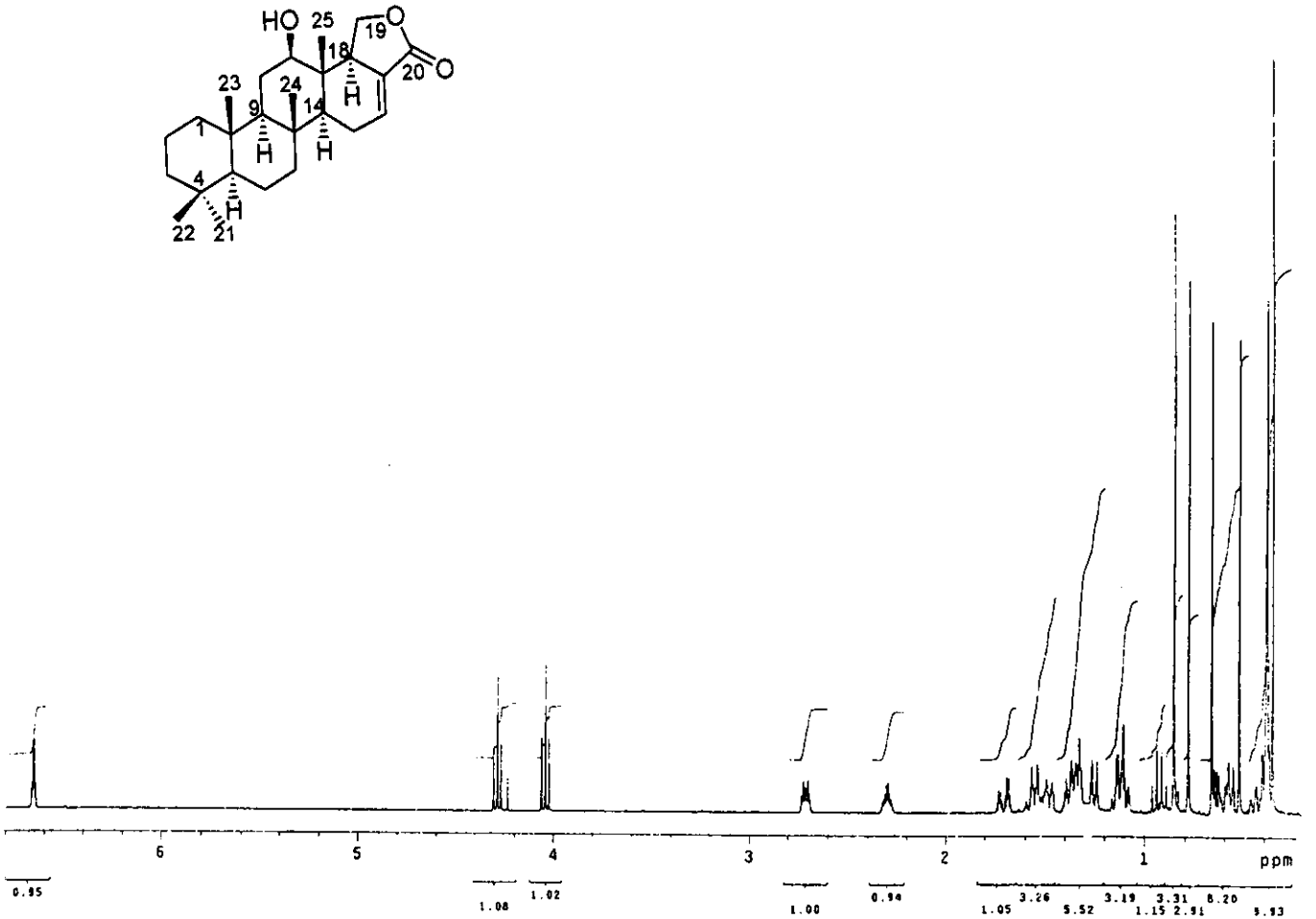


Figure 15 ^{1}H NMR spectrum of **43** (500 MHz; C_6D_6)

Table 6 NMR data (500 MHz for ^1H; in C_6D_6) of **42** and **43**

Position	δ_H (mult.; *J* in Hz)		δ_C (mult.)	
	42	**43**	**42**	**43**
1	Hax, 0.68 (m)	Hax, 0.60 (m)	39.7 (t)	40.1 (t)
	Heq, 1.45 (m)	He, 1.48 (m)		
2	Hax, 1.28 (m)	Hax, 1.39 (m)	18.1 (t)	18.9 (t)
	Heq, 1.48 (m)	Heq, 1.56 (m)		
3	Hax, 1.07 (m)	Hax, 1.11 (m)	42.1 (t)	42.3 (t)
	Heq, 1.30 (m)	Heq, 1.36 (m)		
4	-	-	33.4 (s)	33.4 (s)
5	0.58 (m)	0.57 (dd, 2.3, 12.4)	56.2 (d)	56.4 (d)
6	Hax, 1.13 (m)	Hax, 1.13 (m)	18.6 (t)	18.2 (t)
	Heq, 1.32 (m)	Heq, 1.33 (m)		
7	Hax, 0.46 (m)	Hax, 0.45 (m)	41.1 (t)	41.2 (t)
	Heq, 1.25 (m)	Heq, 1.26 (ddd, 3.2, 3.2, 12.8)		
8	-	-	37.2 (s)	37.1 (s)
9	0.59 (m)	0.40 (m)	58.0 (d)	58.4 (d)
10	-	-	37.4 (s)	37.5 (s)
11	Hax, 1.13 (m)	Hax, 0.94 (m)	23.3 (t)	27.5 (t)
	Heq, 1.73 (ddd, 1.8, 4.3, 12.1)	Heq, 1.14 (m)		
12	4.46 (dd, 4.3, 11.5)	2.72 (ddd, 4.5, 5.0, 11.4)	82.4 (d)	80.6 (d)
13	-	-	38.7 (s)	39.8 (s)
14	0.74 (m)	0.66 (m)	53.2 (d)	53.1 (d)
15	Ha, 1.70 (m)	Ha, 1.51 (m)	23.4 (t)	23.5 (t)
	Hb, 1.71 (m)	Hb, 1.71 (dddd, 3.6, 4.1, 5.5, 20.1)		
16	6.64 (ddd, 3.4, 3.6, 3.7)	6.67 (ddd, 3.6, 3.6, 3.6)	134.4 (d)	134.0 (d)
17	-	-	127.0 (s)	127.6 (s)

Table 6 (cont.)

Position	δ_H (mult.; *J* in Hz)		δ_C (mult.)	
	42	**43**	**42**	**43**
18	2.33 (dddd, 3.6, 3.9, 9.1, 9.2)	2.33 (dddd, 3.6, 4.1, 9.6, 9.6)	49.6 (d)	50.7 (d)
19	Ha, 3.82 (dd, 9.2, 9.3) Hb, 3.92 (dd, 9.1, 9.2)	Ha, 4.05 (dd, 9.6, 9.6) Hb, 4.30 (dd, 9.6, 9.6)	67.2 (t)	68.5 (t)
20	-	-	169.3 (s)	169.2 (s)
21	0.76 (s, 3H)	0.79 (s, 3H)	21.4 (q)	21.4 (q)
22	0.85 (s, 3H)	0.87 (s, 3H)	33.3 (q)	33.3 (q)
23	0.64 (s, 3H)	0.68 (s, 3H)	16.5 (q)	16.7 (q)
24	0.56 (s, 3H)	0.54 (s, 3H)	16.6 (q)	16.5 (q)
25	0.48 (s, 3H)	0.38 (s, 3H)	8.7 (q)	7.6 (q)
26	-	-	169.3 (s)	-
27	1.54 (s, 3H)	-	20.8 (q)	-

3.2.2 The manoalide-sesterterpenes

3.2.2.1 The structure elucidation of 44

Compound **44** was obtained as viscous colorless liquid (4 mg) from the hexane-soluble material of the second-expedition specimen by consecutive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75), semi-preparative normal phase HPLC (SiO_2, isocratic 5% isopropanol in hexane; UV detector, 220 nm) and reverse phase HPLC (ODS, isocratic 75% aqueous CH_3CN; UV detector, 220 nm).

The ESI mass spectrum of **44** (Figure 57) shows a molecular peak at *m/z* 509 ($[M+Na]^+$). Thus, along with 29 carbon signals observed from its ^{13}C NMR spectrum, the molecular formula was suggested as $C_{29}H_{42}O_6$. The proposed molecular formula was comfirmed by the $[M+Na]^+$ peak at *m/z* 509.2898 in the HR-ESI mass spectrum (calc for $C_{29}H_{42}O_6Na$ 509.2868). The proposed molecular formula requires the unsaturation degree of nine. The ^{13}C NMR spectrum (Figure 16) indicates the presence of three carbonyl carbons and eight sp^2 carbons, leaving two sites of unsaturation unassigned. Therefore, two ring systems are required for **44**. The IR spectrum (Figure 58) shows the major absorption bands at ν 1755 and 1225 cm^{-1}, suggesting the presence of lactone functionality. The UV spectrum (Figure 59) shows the maximal absorption at λ 224 nm (log ε 3.72).

The ^1H NMR spectrum of **44** (Figure 17) shows the signals of three olefinic protons, one oxygenated methine proton, two oxygenated methylene groups and six methyl groups; thus, three spin systems were deduced. The first spin system is an α,β-unsaturated γ-lactone moiety with an acetoxy methylene substituent. This ring is composed of the signals at δ 5.62 (ddd, J = 1.6, 1.6, 3.4 Hz, H-2), coupling to the signal at δ 4.26 (m, H-4). The quarternary carbons of the lactone ring, i.e., the carbonyl carbon and the β carbon, were found resonating at δ 170.6 (C-1) and 164.6 (C-3), respectively, as determined by the analysis of the HMBC spectrum (see Table 8). The acetoxy methylene group is composed of the methylene protons at δ 4.15 (dd, J = 1.6, 16.2 Hz, H-25a) and 4.25 (dd, 1.6, 16.2 Hz, H-25b), one carbonyl carbon at δ 169.1 (C-28) and one methyl group at δ 1.53 (s, 3H, H-29).

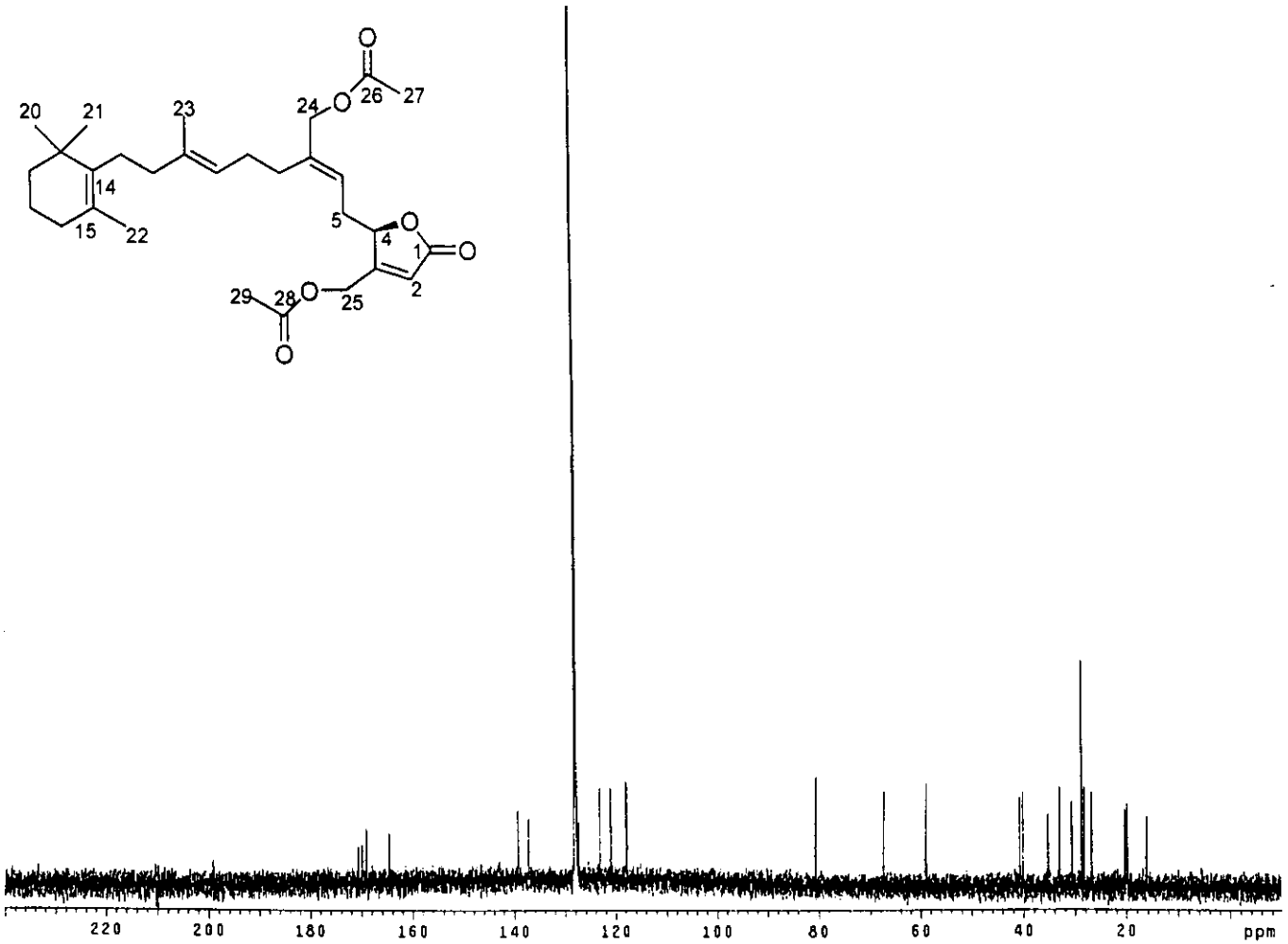


Figure 16 ^{13}C NMR spectrum of **44** (125 MHz; C_6D_6)

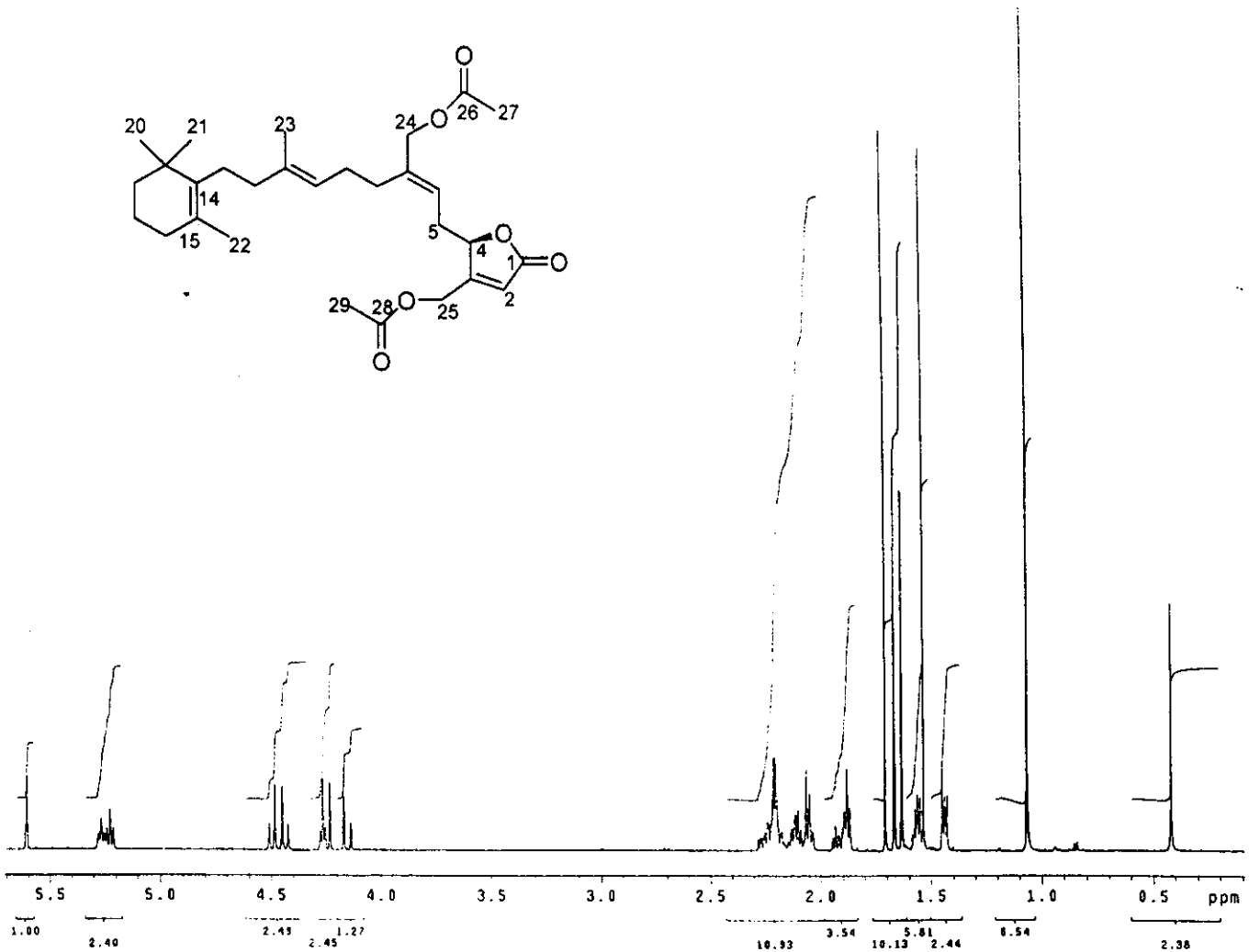
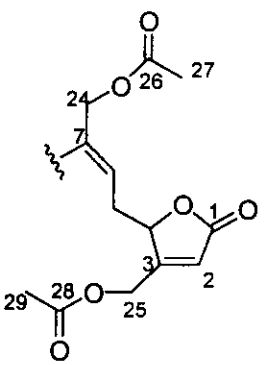


Figure 17 ^{1}H NMR spectrum of **44** (500 MHz; C_6D_6)

The HMBC correlations from C-2 (δ 117.2) and C-3 (δ 164.6) to H-25a and H-25b helped the connection of this acetoxy methylene onto C-3 (Table 8).

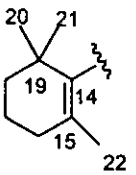
In addition, the γ-carbon of the α,β-unsaturated γ-lactone moiety also linked to one methylene, which was then extended to an olefin and the other acetoxy methylene. By means of the ^{1}H-^{1}H COSY spectral analysis, the extension from H-4 through the methylene (δ 1.93, ddd, J = 6.5, 7.3, 15.3 Hz, H-5a and δ 2.26, ddd, J = 5.0, 7.3, 15.3 Hz, H-5b) to the olefin (δ 5.23, t, J = 7.3 Hz, H-6) was achieved. The chemical shifts of the olefin carbons were assigned to be 121.1 (C-6) and 139.2 (C-7) ppm, on the basis of HMBC analysis. Also by the HMBC analysis, the acetoxy methylene moiety (δ 4.43, d, J = 12.4 Hz, H-24a; δ 4.49, d, J = 12.4 Hz, H-24b; δ 169.8, C-26; δ1.71, s, H-27) were placed onto C-7, thus furnished fragment **A** as shown.



A

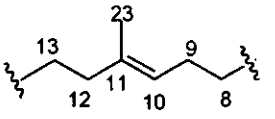
The second spin system is a tetrasubstituted cyclohexenyl moiety. Six methylene protons at δ 1.88 (dd, 2H, J = 6.2, 6.4 Hz, H-16), 1.56 (m, 2H, H-17) and 1.44 (m, 2H, H-18), were connected by means of ^{1}H-^{1}H COSY analysis.

These were further linked to an sp^3 carbon at δ 35.2 (C-19) and two olefinic carbons at δ137.2 (C-14) and δ 127.3 (C-15). The crucial HMBC correlation within this cyclohexeneyl moiety include those from C-19 to all the three methylene groups, from C-14 to H-16 and from C-15 to H-16. The placements of three methyl groups on C-19 and C-15 were assigned according to the C-H correlations observed in the HMBC spectrum. The two methyl groups resonating at δ 1.07 (s, 6H, H-20, H-21) correlate with C-19, whereas another at δ 1.63 (s, 3H, H-22) does with C-15. This structural part is shown as fragment **B**.



B

The last spin system is a hexenyl bridge, composed of the olefinic signals at δ 123.2 (C-10) and 137.2 (C-11). This olefin is linked to two ethylenes and one methyl groups. The first ethylene moiety (δ 2.06, m, H-8a; 2.17, m, H-8b; 2.23, m, 2H, H-9) is placed on C-10, whereas the second one (δ 2.19, m, 2H, H-12; 2.05, m, 2H, H-13) is on C-11, as determined from the HMBC spectrum. Due to the C-H long-range correlation from C-9 (δ 28.3), C-10 (δ 123.2) and C-11 (δ 137.2) to the methyl group at δ 1.67 (s, 3H, H-23), this methyl group (H-23) is placed on C-11. This spin system is shown as fragment **C**.



C

All the three fragments are linked on the basis of the analysis of C-H long-range correlations. The fragment **C** is used as a bridge, linking fragments **A** and **B**, due to the observation of the long-range correlations from C-14 and C-15 to H-12; from C-19 to H-13; from C-7 to H-8a and H-8b; from C-8 to H-6; and from C-8 to H-24a and H-24b (Figure 62). Therefore, the structure of **44** was proposed as shown below. It was designated as neomanoalide diacetate. The NMR spectral data are summarized in Tables 7 and 8.

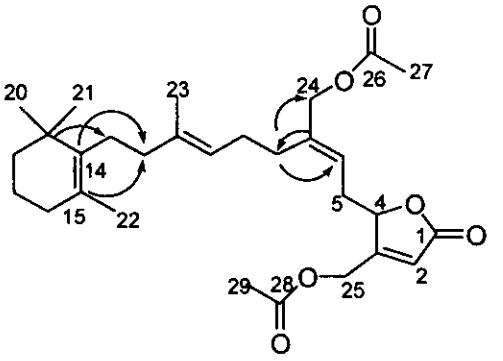


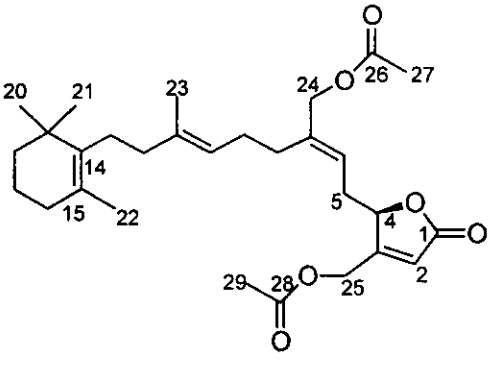
Figure 18 Crucial HMBC correlations from ^{13}C to ^{1}H of **44**

The stereochemistry of **44** was determined by means of a close observation of chemical shifts and CD spectral analysis. The up-field shift of C-8 (δ 28.8) and down-field shift of C-24 (δ 67.4), as compared to those reported by de Silva and Scheuer (1981), suggest the electronic repulsion on C-8, thus indicating the *E*-

configuration of the $\Delta^{6,7}$ olefin. Furthermore, the dipolar couplings observed among H-24a, H-24b and H-6 support the proposed configuration.

Similarly, the configuration of $\Delta^{10,11}$ double bond is also *E*, inferred from the up-field signal at 16.2 ppm of C-23. Such methyl group would resonate at lower field in *Z*-isomer. For example, the chemical shift of the methyl signal in (*E*)-3 methyl-3-hexene is at 15.7 ppm, whereas that of *Z* one is at 22.9 ppm (Yunker and Scheuer, 1978).

The absolute configuration at C-4 of **44** was determined by means of the CD spectral analysis. The CD spectrum of **44** (Figure 19) shows the first negative Cotton effect with $[\theta]_{218}$ of –5504, indicating that the configuration at C-4 is *R*, according to the octant rule (Eliel and Wilen, 1994). Furthermore, the negative specific rotation of **44** ($[\alpha]_D^{25}$ –33°) corresponds well with those previously reported for the neomanoalides (Kobayashi *et al.*, 1994), thus confirming the proposed configuration.



44

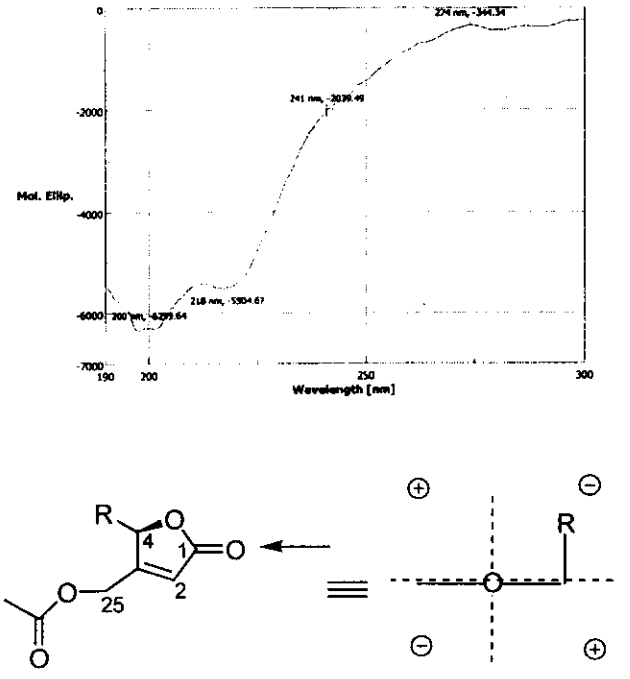


Figure 19 CD spectrum of compound **44**

Compound **44** is identified as (*E*)-neomanoalide diacetate. This compound was originally derived as part of the structure elucidation of (*E*)-neomanoalide (de Silva and Scheuer, 1981). However, all the spectral data, physical properties, and biological activities have never been reported. Therefore, here is the first report of naturally occuring 4*R*-(*E*)-neomanoalide diacetate.

3.2.2.2 The structure elucidation of 45

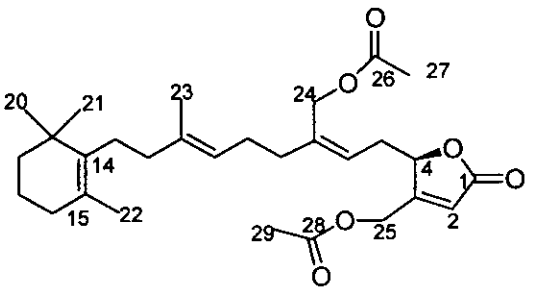
Also viscous colorless liquid similar to **44**, compound **45** (7 mg) was obtained along with **44** by successive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75), semi-preparative normal phase HPLC (SiO_2, isocratic 5% isopropanol in hexane; UV detector, 220 nm) and reverse phase HPLC (ODS, isocratic 75% aqueous CH_3CN; UV detector, 220 nm), respectively.

The molecular formula of **45** was proposed to be $C_{29}H_{42}O_6$ as deduced from its molecular peak at *m/z* 509 ($[M+Na]^+$) in the ESI mass spectrum (Figure 65), along with 29 carbon signals observed from its ^{13}C NMR spectrum. The proposed molecular formula was comfirmed by the $[M+Na]^+$ peak at *m/z* 509.2794 in the HR-ESI mass spectrum (calc 509.2868). Similar to **44**, the nine-degree of unsaturation are assigned as three carbonyl carbons, four carbon-carbon double bonds and two ring systems. The lactone functionality was also observed as the major absorption bands at ν 1755 and 1225 cm^{-1} in its IR spectrum (Figure 66). The UV spectrum (Figure 67) shows the absorption maximum at λ 224 (log ε 3.72).

Except some slight chemical shift differences, the ^{13}C and ^{1}H NMR spectra (Figures 20 and 21, respectively) of **45** were almost identical to those of **44**. Here, the two methylene protons previously assigned to H-24a and H-24b in **44**, were found to collide into a singlet resonating at δ 4.52. Also the signal at δ 35.6 that

was assigned to C-8 of **45** was found to shift toward lower field than that of **44**, whereas the signal at δ 61.4, assigned to C-24, otherwise moved toward higher field. These evidences suggest that **44** and **45** do in fact share the similar empirical structure, but differ only at the $\Delta^{6,7}$ configuration, which was assigned as *Z* for **45**. This assignment is supported by the comparison with that previously reported by de Silva and Scheuer (1981).

Also similar to **44**, the first negative Cotton effect ($[\theta]_{214.5}$ −10485), in its CD spectrum (Figure 71) was observed, thus indicating an *R* configuration at C-4. Compound **45** thus was proposed as 4*R*-(*Z*)-neomanoalide diacetate as shown below. The compound is first reported here as a new naturally-occurring sesterterpene; however, also similar to **44,** it was reported non-informatively by de Silva and Scheuer (1981). The NMR spectral data of **45** are summarized in Tables 7 and 8.



45

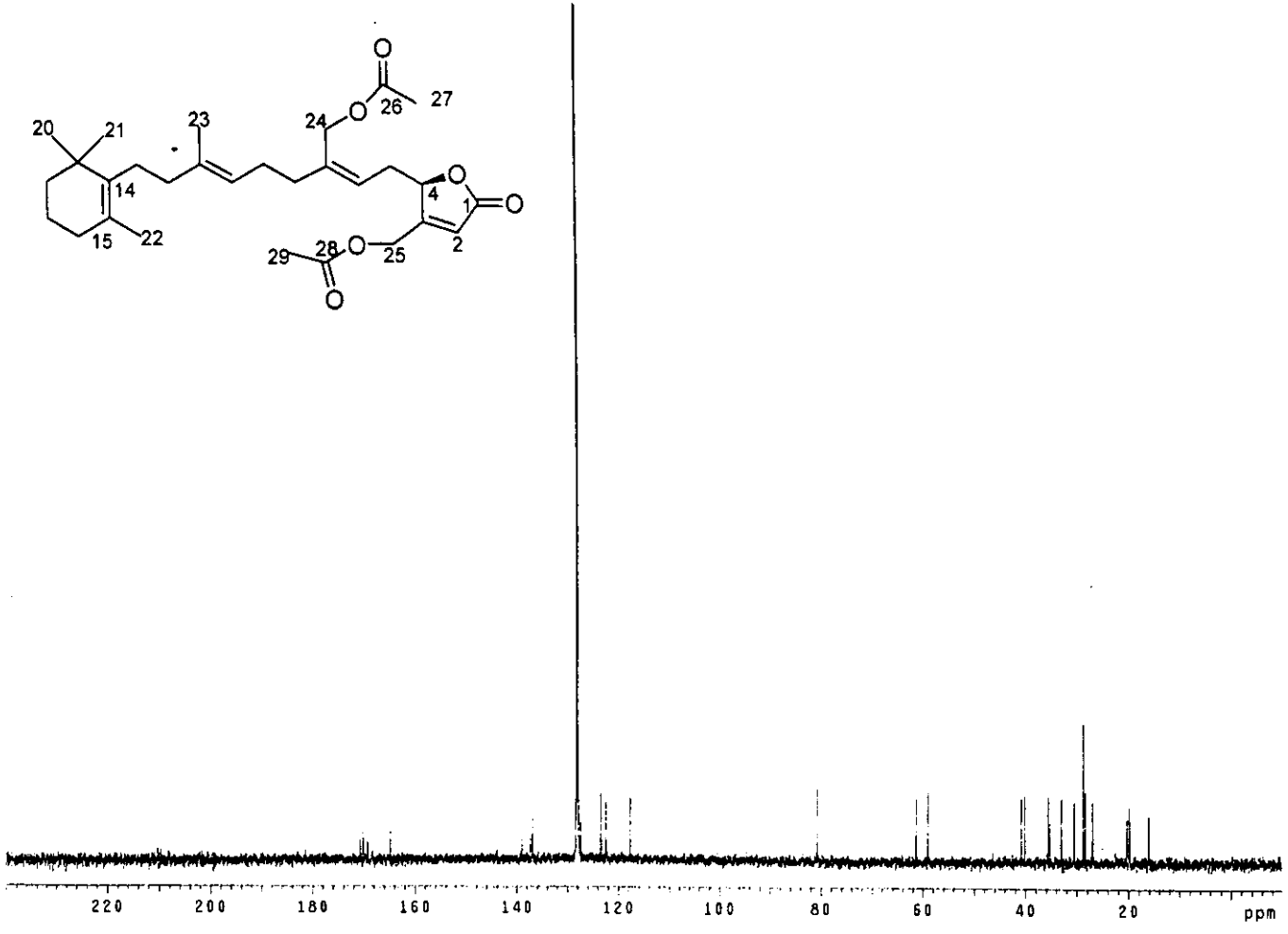


Figure 20 ^{13}C NMR spectrum of **45** (125 MHz; C_6D_6)

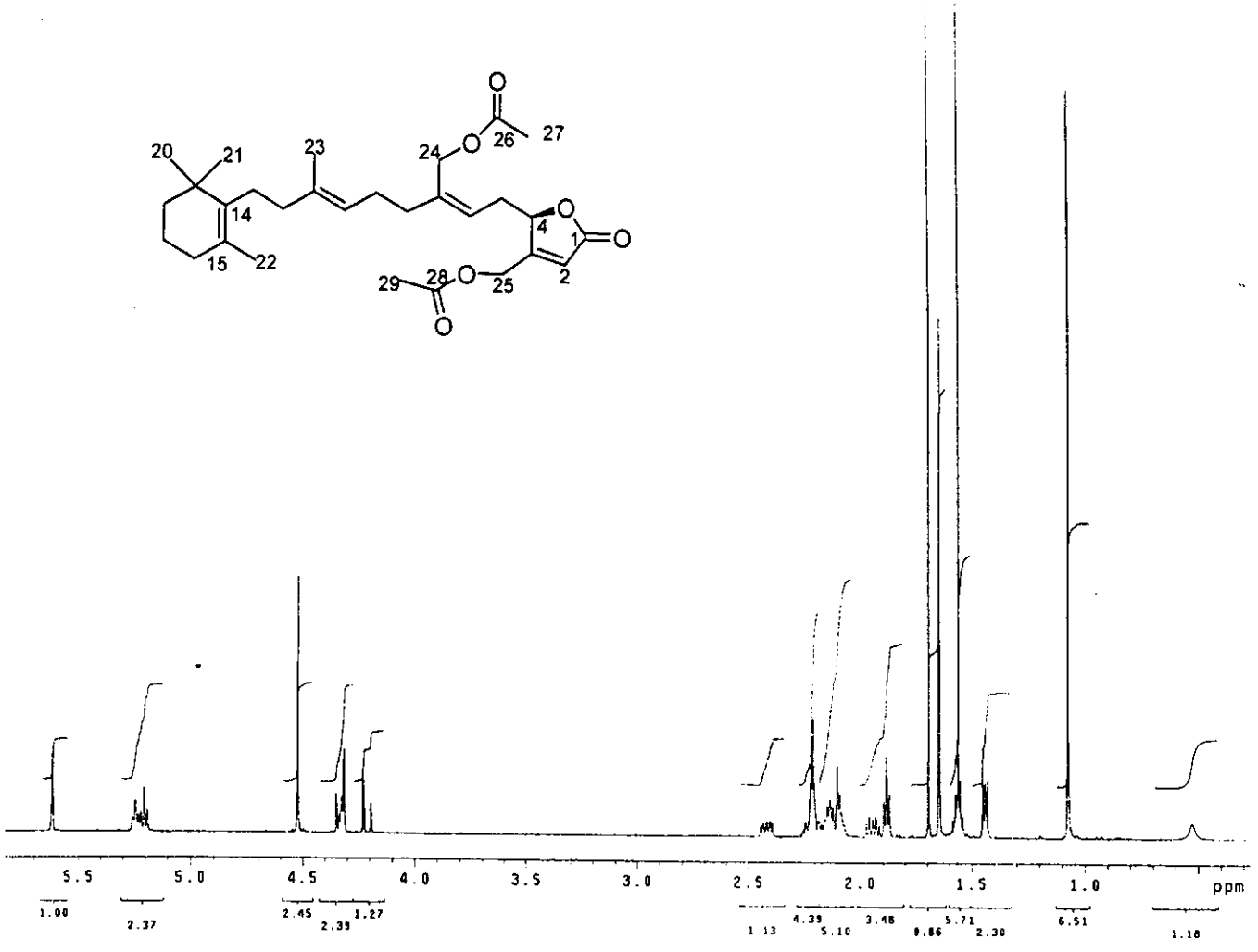


Figure 21 ^{1}H NMR spectrum of **45** (500 MHz; C_6D_6)

3.2.2.3 The structure elucidation of 46

Compound **46** was re-crystallized as a white needle crystal (7 mg) from the mixture of isopropanol:hexane (1:5). It was isolated from hexane-soluble material of the second-expedition specimen by successive chromatographic techniques using SiO_2 column (EtOAc:acetone:hexane = 20:5:75) followed by semi-preparative normal phase HPLC (SiO_2, isocratic 2% isopropanol in hexane; UV detector, 220 nm), respectively.

The ESI mass spectrum of **46** (Figure 72) shows a molecular peak at *m/z* 481 ($[M+Na]^+$). Together with the numbers of carbons and protons observed from the ^{13}C and ^{1}H spectra (Figures 22 and 23), a molecular formula of $C_{27}H_{38}O_6$ was suggested. Six of the nine-degree of unsaturation calculated from the molecular formula of **46** are taken up in four carbon-carbon double bonds and two carbonyl carbons; thus this molecule is composed of three ring systems. The IR spectrum (Figure 73) exhibits the absorption bands at ν 3430 cm^{-1} for a hydroxyl group and at ν 1790, 1770 and 1235 cm^{-1} for ester functionalities. The UV spectrum (Figure 74) shows the absorption maximum at λ 224 nm (log ε 3.81).

Whereas both ^{13}C and ^{1}H NMR spectra of **46** (Figures 22 and 23) are not entirely identical to those of **44** and **45**, the western part of the structure, i.e. the hexenyl cyclohexene part (C-8 - C-23), was able to be observed. Thus, the three compounds evidently share similar structural unit, suggesting the possibility that all the three belong to the same chemical family. The eminent differences

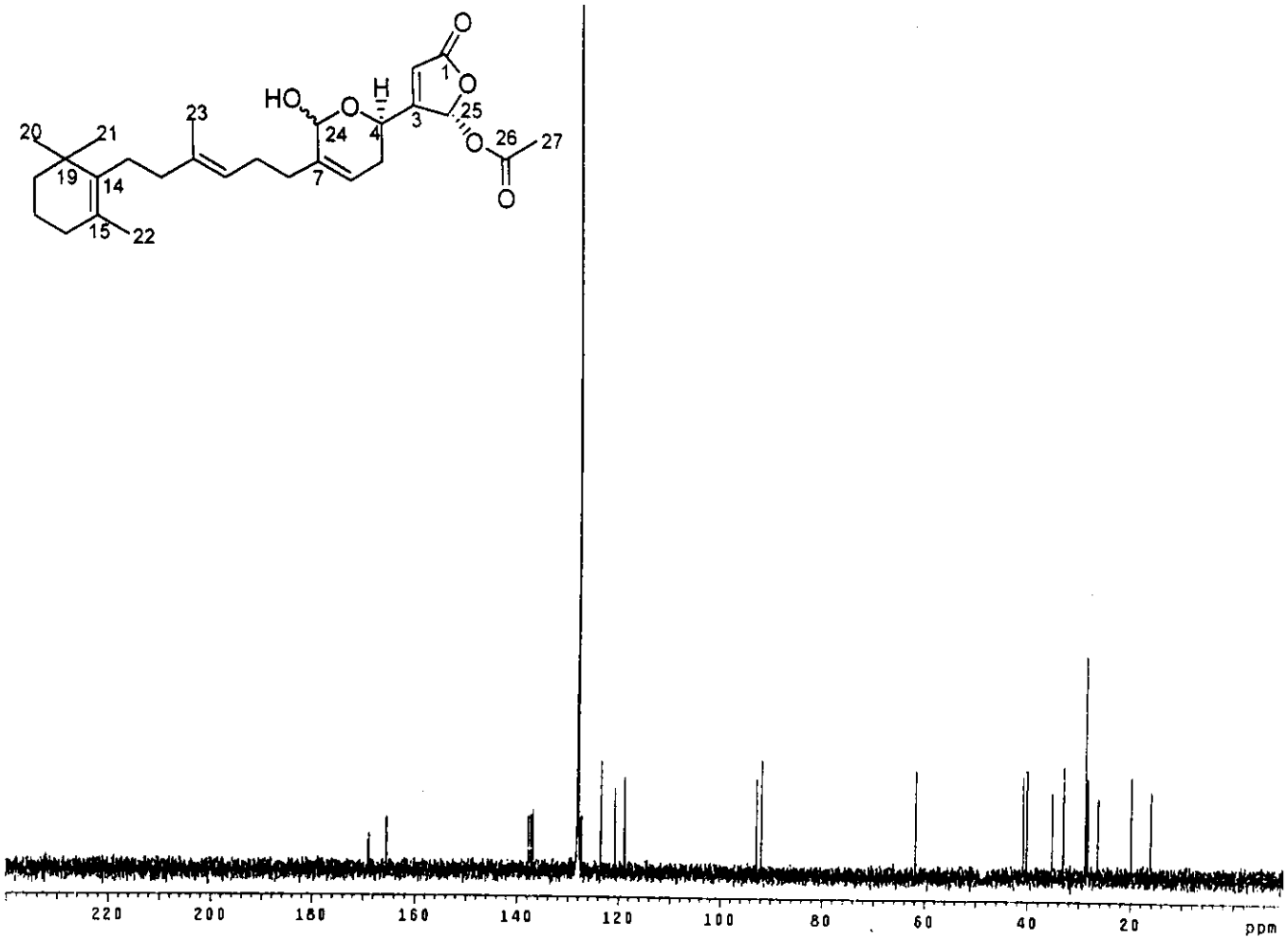


Figure 22 ^{13}C NMR spectrum of **46** (125 MHz; C_6D_6)

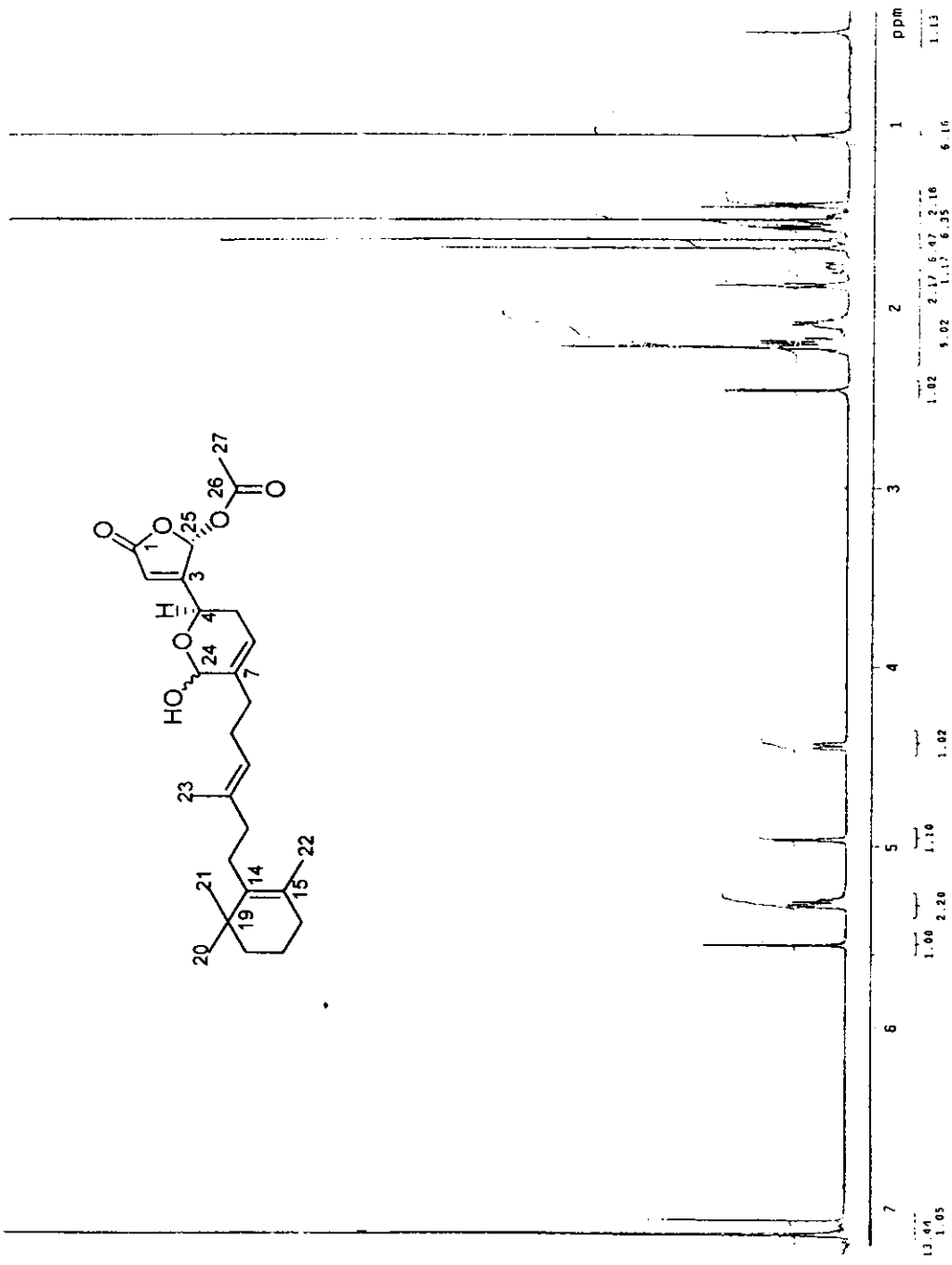


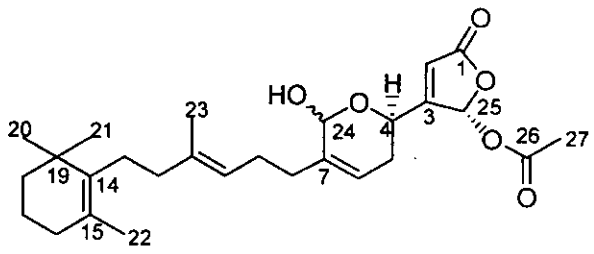
Figure 23 ^{1}H NMR spectrum of **46** (500 MHz; C_6D_6)

observed in the ^{1}H NMR spectrum of **46** include the signal of two olefinic protons, two acetals, one carbonol proton, two methylene protons and one acetate methyl. Connecting all of these signals by means of HMBC spectral analysis, led to the establishment of two structural moieties.

The first moiety was proposed as an α,β-unsaturated γ-lactone ring, which is composed of the signals at δ 5.55 (dd, J = 1.0, 2.0 Hz, H-2) coupled to the signal at δ 7.07 (br.s, H-25), and one carbonyl and one β carbons resonating at δ 169.1 (C-1) and 165.4 (C-3), respectively, as determined by the analysis of HMBC spectrum (Figure 77). The chemical shifts of 7.07 ppm (H-25) and 92.8 ppm (C-25), characteristic to the acetal functionality, suggest that this position was linked to the acetoxy group (δ 169.1, C-26 and 19.9, C-27 in ^{13}C NMR; and δ 1.53, s, 3H, H-27 in ^{1}H NMR). The HMBC correlation from C-26 to H-25 confirms this connectivity.

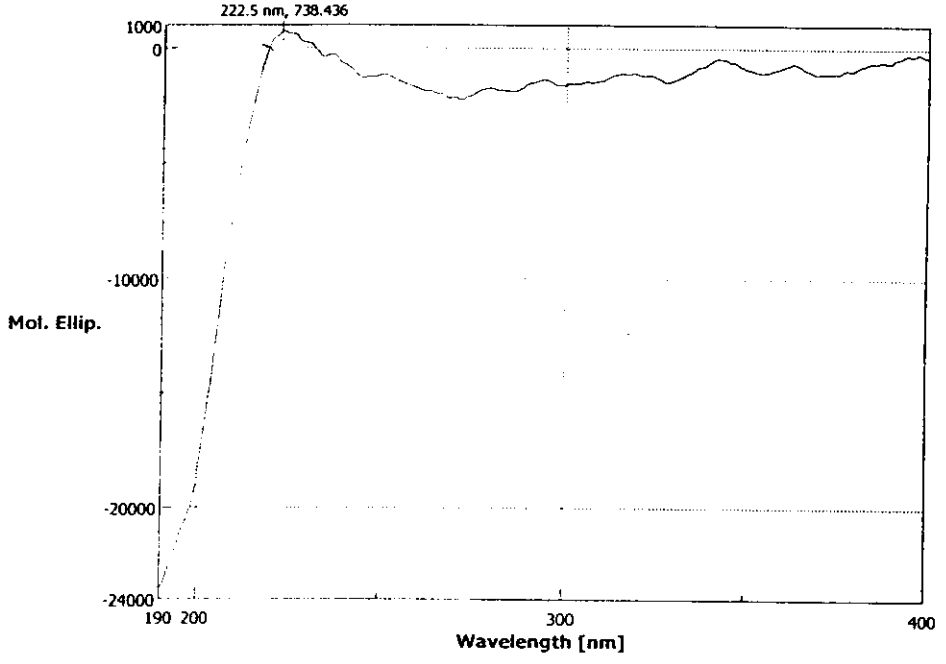
The other moiety is a δ-hydroxy lactone ring, composed of the signal at δ 4.46 (ddd, J = 2.0, 3.2, 11.4 Hz, H-4), which then further couples to the signal at δ 1.51 (m, H-5a) and 1.78 (br.ddd, J = 3.4, 11.4, 17.0 Hz, H-5b), then to one olefinic proton at δ 5.33 (m, H-6), and finally to the other acetal proton at δ 4.98 (d, J = 4.8 Hz, H-24). Additionally, the ^{1}H-^{1}H COSY spectrum (Figure 75) also shows a transverse coupling between the signal of H-24 and an exchangeable proton at δ 2.45 (d, J = 4.8 Hz, 24-OH). The coupling constant of 11.4 Hz of H-4 indicates the orientation of H-4 as axial. The allylic coupling between H-4 and H-2 suggests the

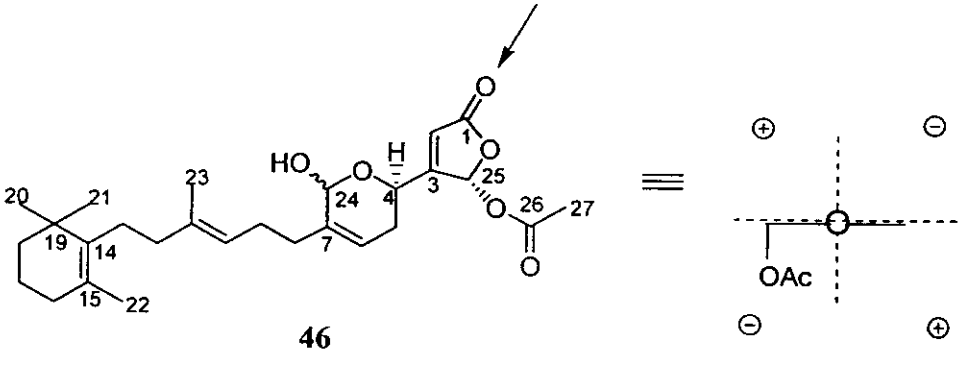
connection between the two moieties as shown. Finally the hexenyl cyclohexene portion of C-8-C-25 was linked to C-7 and structure of **46** was established as shown. The NMR spectral data are summarized in Table 7.



46

Compound **46** was identified as a known compound, manoalide-25-acetate. The proton and carbon assignments of **46** were confirmed by comparison with the data reported by Cambie and Craw (1988). The absolute configuration at C-25 of **46** was determined by the CD exciton chirality method. As the CD spectrum of **46** (Figure 24) reveals the first negative Cotton effect ($[\theta]_{200}$ –20000), indicating the *S* configuration at C-25. On the other hand, the absolute configuration on the pyranol ring is unable to be determined directly. Here, the configuration on C-4 was proposed as *R* by comparison of the positive specific rotation ($[\alpha]_D^{25}$ +25°) along with the biosynthetic consideration of natural manoalide family (Amoo, Bernardo and Weigele, 1988; Butler and Capon, 1992; Kobayashi *et al.*, 1994). We proposed the structure of **46** with the first report on the absolute configuration at C-25 as 4*R*,25*S*-manoalide-25-acetate.





46

Figure 24 The CD spectrum of **46**

Table 7 NMR data (500 MHz for ^1H; in C_6D_6) of **44**, **45** and **46**

position	δ_H (mult.; *J* in Hz)			δ_C (mult.)		
	44	**45**	**46**	**44**	**45**	**46**
1	-	-	-	170.6 (s)	170.7 (s)	169.1 (s)
2	5.62 (ddd, 1.6, 1.6, 3.4)	5.62 (ddd, 1.6, 1.8, 3.4)	5.55 (dd, 1.0, 2.0)	117.2 (d)	117.7 (d)	118.8 (d)
3	-	-	-	164.6 (s)	164.9 (s)	165.4 (s)
4	4.26 (m)	4.33 (m)	4.46 (ddd, 2.0, 3.2, 11.4)	80.5 (d)	80.7 (d)	61.8 (d)
5	Ha, 1.93 (ddd, 6.5, 7.3, 15.3) Hb, 2.26 (ddd, 5.0, 7.3, 15.3)	Ha, 1.94 (ddd, 6.9, 7.3, 15.1) Hb, 2.41 (ddd, 4.4, 7.3, 15.1)	Ha, 1.51 (m) Hb, 1.78 (br.ddd, 3.2, 11.4, 17.0)	30.7 (t)	30.5 (t)	28.3 (t)
6	5.23 (t, 7.3)	5.21 (t, 7.3)	5.33 (m)	121.1 (d)	122.4 (d)	120.7 (d)
7	-	-	-	139.2 (s)	138.9 (s)	137.8 (s)
8	Ha, 2.06 (m) Hb, 2.17 (m)	Ha, 2.09 (m) Hb, 2.13 (m)	2.08 (m)	28.8 (t)	35.6 (t)	33.1 (t)
9	2.23 (m, 2H)	2.19 (m, 2H)	2.18 (m, 2H)	28.3 (t)	28.4 (t)	28.4 (t)
10	5.26 (dd, 6.2, 6.9)	5.24 (ddd, 1.1, 6.2. 7.1)	5.30 (ddd, 0.9, 6.2, 6.9)	123.2 (d)	123.4 (d)	123.5 (d)
11	-	-	-	137.2 (s)	136.8 (s)	136.9 (s)

Table 7 (cont.)

position	δ_H (mult.; *J* in Hz)			δ_C (mult.)		
	44	**45**	**46**	**44**	**45**	**46**
12	2.19 (m, 2H)	2.20 (m, 2H)	2.20 (m, 2H)	40.8 (t)	40.8 (t)	40.8 (t)
13	2.05 (m, 2H)	2.10 (m, 2H)	2.21 (m, 2H)	26.9 (t)	27.0 (t)	26.4 (t)
14	-	-	-	137.2 (s)	137.3 (s)	137.3 (s)
15	-	-	-	127.3 (s)	127.3 (s)	127.3 (s)
16	1.88 (dd, 6.2, 6.4, 2H)	1.88 (dd, 6.2, 6.4, 2H)	1.88 (dd, 6.2, 6.4, 2H)	33.0 (t)	33.0 (t)	33.0 (t)
17	1.56 (m, 2H)	1.57 (m, 2H)	1.56 (m, 2H)	19.9 (t)	20.0 (t)	19.9 (t)
18	1.44 (m, 2H)	1.44 (m, 2H)	1.44 (m, 2H)	40.2 (t)	40.2 (t)	40.1 (t)
19	-	-	-	35.2 (s)	35.2 (s)	35.2 (s)
20	1.07 (s, 3H)	1.07 (s, 3H)	1.06 (s, 3H)	28.8 (q)	28.8 (q)	28.8 (q)
21	1.07 (s, 3H)	1.07 (s, 3H)	1.06 (s, 3H)	28.8 (q)	28.8 (q)	28.8 (q)
22	1.63 (s, 3H)	1.65 (s, 3H)	1.63 (s, 3H)	20.0 (q)	20.0 (q)	20.0 (q)
23	1.67 (s, 3H)	1.65 (s, 3H)	1.68 (s, 3H)	16.2 (q)	16.2 (q)	16.2 (q)
24	Ha, 4.43 (d, 12.4)	4.52 (s)	4.98 (d, 4.8)	67.4 (t)	61.4 (t)	91.8 (d)
	Hb, 4.49 (d, 12.4)					

Table 7 (cont.)

position	δ_H (mult.; *J* in Hz)			δ_C (mult.)		
	44	**45**	**46**	**44**	**45**	**46**
25	Ha, 4.15 (dd, 1.6, 16.2) Hb, 4.25 (dd, 1.6, 16.2)	Ha, 4.21 (ddd, 0.6, 1.8, 16.2) Hb, 4.33 (dd, 1.6, 16.2)	7.07 (br.s)	58.9 (t)	59.0 (t)	92.8 (d)
26	-	-	-	169.8 (s)	170.1 (s)	168.7 (s)
27	1.71 (s, 3H)	1.69 (s, 3H)	1.52 (s, 3H)	20.4 (q)	20.4 (q)	19.9 (q)
28	-	-	-	169.1 (s)	169.1 (s)	-
29	1.53 (s, 3H)	1.56 (s, 3H)	-	19.8 (q)	19.8 (q)	-
24-OH	-	-	2.45 (d, 4.8)	-	-	-

Table 8 HMBC correlations ($^{13}C \longrightarrow {}^{1}H$) of **44** and **45**

Position	HMBC correlation ($^{13}C \longrightarrow {}^{1}H$)	
	44	**45**
1	H-2, H-4	H-2, H-4, H-25a, H-25b
2	H-25a, H-25b	H-25a, H-25b
3	H-2, H-5a, H-5b, H-6, H-25a, H-25b	H-2, H-5a, H-5b, H-25a, H-25b
4	H-2, H-5a, H-5b, H-25a, H-25b	H-2, H-5a, H-5b,H-6, H-25a, H-25b
5	H-4, H-6	H-4, H-6
6	H-4, H-5a, H-5b, H-8a, H-8b, H-9, H-24a, H-24b	H-4, H-5a, H-5b, H-24
7	H-5a, H-5b, H-8a, H-8b, H-9, H-24a, H-24b	H-5a, H-5b, H-8a, H-8b, H-24
8	H-6, H-24a, H-24b	H-6, H-24
9	-	H-23
10	H-8a, H-8b, H-12, H-13, H-23	H-8a, H-8b, H-9, H-13, H-23
11	-	H-9, H-13, H-23
12	H-23	-
13	H-10	H-10
14	H-12, H-13, H-16, H-20, H-21	H-12, H-16, H-18, H-20, H-21, H-22
15	H-16, H-17, H-22	H-12, H-16, H-17, H-20, H-21, H-22
16	H-17, H-18, H-22	H-17, H-18
17	-	-
18	H-16, H-20, H-21	H-16, H-20, H-21
19	H-16, H-17, H-18, H-20, H-21	H-13, H-16, H-17, H-18, H-20, H-21, H-22
20	H-18, H-21	H-18, H-21
21	H-18, H-20	H-18, H-20
22	-	H-16

Table 8 (cont.)

Position	HMBC correlation ($^{13}C \longrightarrow ^{1}H$)	
	44	**45**
23	H-10	H-10
24	H-6	H-6
25	-	-
26	H-24a, H-24b, H-27	H-24, H-27
27	-	-
28	H-25a, H-25b, H-29	H-25a, H-25b, H-29
29	-	-

3.3 Biological activities of the isolated compounds

All the eight sesterterpenes isolated from the sponge *Brachiaster* sp. were assessed for antituberculosis activity against *Mycobacterium tuberculosis* ($H_{37}Ra$) using the Microplate alamar blue assay, and for cytotoxic activity against four cell lines, including MCF-7, HeLa, HT-29 and KB, using SRB assay. The results are presented in Table 9. The MICs and IC_{50} values were obtained as a microgram per milliliter unit, and later converted to a micromolar unit, which is preferable for potency comparison.

The results demonstrate that all compounds exhibit antituberculosis activity against *M. tuberculosis* with MICs ranging from 3 to 117 μM (1.56-50 μg/mL). Compounds **40**, **18**, **41** and **46** show the most potent antituberculosis activity with MICs of 3-7 μM, whereas compounds **42**, **43**, **44** and **45** are mildly active. On the other hand, the significant cytotoxicity was observed only with compounds **18** and **46**, with IC_{50} in all targeted cell lines lower than 1 μg/mL. Among all the isolated compounds reported, **18** was the only agent previously tested elswhere for antituberculosis activity with MIC in a comparable potency of 6.25 μg/mL (El Sayed *et al.*, 2000). Here, the antituberculosis activity of the other seven compounds is first reported along with their cytotoxicity against cancer cell lines.

Evidently, among the five scalaranes, the furanoid ring E significantly affects the potency of antituberculosis activity. It was reported by Crews and Bescansa (1986) that the oxygen functionality in the vicinity of C-19 and C-20 of

scalaranes may be a structural feature required for biotoxicity. Particularly, the 19-acetal moiety seem to influence antituberculosis potency; i.e., the MICs of **40**, **18** and **41**, which bear the 19-acetal moiety, are ranging from 3 to 6 μM, whereas the other two scalaranes are much less active. Besides, the oxygenated pattern of C-12 possibly assert the complementarity of such activity to the certain extent. However, with only two pairs of such structure (**18** versus **41** and **42** versus **43**), an absolute conclusion is yet unable to be drawn.

In the manoalide-family, compound **46** shows the most potent antituberculsis activity (MIC 7 μM), whereas **44** and **45** show moderate activity (MICs 26 and 51 μM, respectively). It was observed that several natural products containing α,β-unsaturated γ-lactone moiety exhibit interesting chemotherapeutic activities, including antituberculosis and cytotoxic activities, due to the alkylation by conjugate additions with essential biological nucleophiles, i.e., proteins and nucleic acid (Cantrell *et al.*, 2001).

Whereas, most of the isolated sesterterpenes reported have showed a good correlations between cytotoxic and antituberculosis activities, i.e., those with potent cytotoxicity are as well antituberculosis active and vice versa, interestingly, 12-deacetoxy-scalarin acetate (**40**) exhibit the otherwise potency. The compound is strongly antituberculosis active (MIC 4 μM), but slightly cytotoxic against all targeted cell lines (IC_{50} higher than 12 μM). Such selectivity indicate the potential of scalarane-type sesterterpenes as antituberculosis agents, and probably other

chemotherapeutic agents, especially when the appropriate structural modification (s) are carried out.

Table 9 Antituberculosis and cytotoxic activities of compounds **40**, **18**, **41**, **42**, **43**, **44**, **45** and **46**

Compounds	Anti-TB[a] MIC; μg/mL	Cytotoxicity (IC_{50} ± SEM; μg/mL)			
		MCF-7	HT-29	HeLa	KB
12-Deacetoxy-scalarin acetate (**40**)	1.56 (4)[b]	> 5 (> 12)[b]	> 5 (> 12)[b]	> 5 (> 12)[b]	> 5 (> 12)[b]
Heteronemin (**18**)	1.56 (3)[b]	0.14±0.04 (0.29±0.08)[b]	0.1-0.25 (0.2-0.51)[b]	0.1-0.25 (0.2-0.51)[b]	0.1-0.25 (0.2-0.51)[b]
Heteronemin acetate (**41**)	3.125 (6)[b]	3.42±0.16 (6.45±0.30)[b]	> 5 (> 9)[b]	NT[c]	> 5 (> 9)[b]
12-Epi-19-deoxyscalarin (**42**)	50 (117)[b]	> 5 (> 12)[b]	> 5 (> 12)[b]	2.5-5 (5.8-12)[b]	1.29±0.68 (3.01±1.59)[b]
12-Deacetyl-12-epi-19-deoxyscalarin (**43**)	6.25 (16)[b]	> 5 (> 13)[b]	> 5 (> 13)[b]	> 5 (> 13)[b]	> 5 (> 13)[b]
E-Neomanoalide diacetate (**44**)	25 (51)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]
Z-Neomanoalide diacetate (**45**)	12.5 (26)[b]	2.91±0.06 (5.99±0.12)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]	> 5 (> 10)[b]
Manoalide-25-acetate (**46**)	3.125 (7)[b]	$0.12\pm0.42\times10^{-2}$ $(0.26\pm0.92\times10^{-2})$[b]	0.35±0.06 (0.76±0.13)[b]	0.77±0.14 (1.68±0.39)[b]	0.29±0.05 (0.63±0.11)[b]

[a] Isoniazid and kanamycin sulfate were used as standard drugs (MICs 0.3-0.7 and 3-9 μM, respectively)

[b] The numbers in parentheses denote the potency in μM (with SEM, if applicable)

[c] not tested