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A NOVEL DITERPENE GLYCOSIDE FROM THE SOFT CORAL OF *LEMNALIA BOURNEI*

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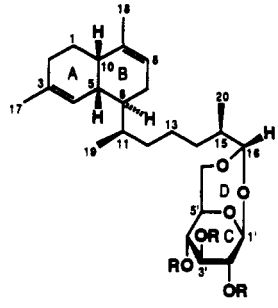
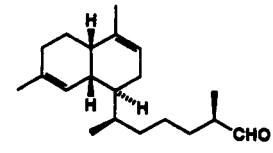
ABSTRACT.—A novel diterpene glycoside, lemnabourside [**1**], has been isolated from the soft coral, *Lemnalia bournei*. Lemnabourside [**1**] appears to possess an interesting structure because D-glucose is attached to a diterpene aldehyde through an acetal linkage. The structure was elucidated based on spectral methods including DQF-COSY, TOCSY, HMQC, HMBC and NOESY nmr experiments and by chemical transformations.

Soft corals (Alcyonacea) have proven to be rich sources of interesting secondary metabolites (1). As part of a program to study the chemical constituents of Chinese soft corals, we report here the structure of one of the constituents of *Lemnalia bournei* Roxas (Nephtheidae). The new compound, lemnabourside [**1**], appears to possess an interesting structure because D-glucose is attached to a diterpene aldehyde through an acetal linkage. A compound with a similar acetal linkage, whose full structure has not yet been determined, was previously reported from *Lemnalia digitata* (2).

The animals were sun-dried and extracted 3 times with EtOH. The combined extracts were chromatographed over a Si gel column with petroleum ether-EtOAc (2:1) to obtain lemnabourside [**1**], white needles, mp 90–90.5°, $[\alpha]^{22.5}D$ +33.3°. The molecular formula of **1** was



1 R=H
2 R=CH_3CO



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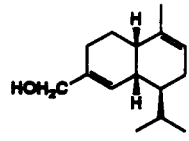

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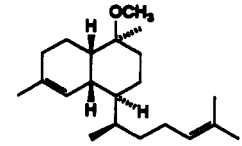

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determined as $C_{26}H_{42}O_6$ by mass spectrometry and elemental analysis. The eims of this compound shows a fragment representing a loss of $C_6H_{10}O_5$. This behavior, coupled with appropriate ^{1}H- and ^{13}C-nmr spectral signals, indicated the presence of a hexose component in compound **1**.

All ^{1}H- and ^{13}C-nmr data of **1** were unambiguously assigned by DQF-COSY, TOCSY, HMBC (3), HMQC (4) and NOESY experiments and are shown in Tables 1 and 2. The ^{1}H- and ^{13}C-nmr data and DEPT experiment suggested the presence of two trisubstituted double bonds [δ_H 5.48 (1H, m), 5.39 (1H, m); δ_C 136.5 (s), 123.9 (d), 134.4 (s), 121.5 (d)], and two acetal groups [δ_H 4.57 (1H, d, J=4.8 Hz), 4.90 (1H, m), δ_C 101.6 (d), 101.1 (d)]. The ir spectrum showed a strong hydroxyl absorption from 3480 cm^{-1} to 3550 cm^{-1} and ether absorption at 1050, and 1150 cm^{-1}. Resonance signals in the ^{1}H-nmr spectrum at δ 1.25 (1H), 1.50 (1H) and 2.04 (1H), together with eims peaks at m/z 432 $[M-H_2O]^+$, 414 $[M-2H_2O]^+$ and 396 $[M-3H_2O]^+$ also suggested the presence of three hydroxy

TABLE 1. ^{1}H-Nmr Data (600 MHz) for **1**.

H	δ H, J (Hz)	DQF-COSY	HMBC	NOESY
1	1.12 (Ha)	Hb-1		
	m			
	1.35 (Hb)	Ha-1, H-2, H-10		
2	1.83 (Ha)	H-1, Hb-2		
	m		H-4, H-17	
	1.98 (Hb)	H-1, Ha-2		
3			H-17	
4	5.48 d, 4.86	H-5	H-2, H-17	H-11
5	2.02 ddd, 4.9, 10.0	H-4, H-6, H-10		H-10
6	1.50 m	H-5, H-7, H-11		
7	1.77 m	H-6, H-8		H-19
8	5.39 br s	H-7	H-18	
9			H-18, H-10	
10	1.93 m	H-5, Hb-1	Hb-1, H-2, H-4, H-18	H-5
11	1.75 m	H-6, H-12, H-19		H-4
12	1.14 (Ha)			
	m	H-11, H-13	H-11	
	1.22 (Hb)			
13	1.33 m	H-12, Ha-14, Hb-14	Ha-12, Ha-14	
14	1.09 (Ha)			
	m	H-13, H-15		
	1.44 (Hb)			
15	1.64 m	Ha-14, H-16, H-20	H-20	H-16
16	4.57 d, 4.8	H-15	H-20, H-1′	H-15, 20
				Ha-6′
17	1.68 s		H-2, H-4	
18	1.68 s		H-10	
19	0.81 d, 6.6	H-11		H-7
20	0.90 d, 6.6	H-15	H-15, H-16	H-16
1′.....	4.90 s		H-2′, H-4′, H-5′	
2′.....	3.87 d, 7.0	H-3′	H-1′	H-4′, 6′a
3′.....	4.10 dd, 7.0, 14.2	H-2′		
		H-4′		
4′.....	3.68 m	H-3′, H-5′	H-1′	H-2′
5′.....	3.62 d, 5.6	H-4′	H-1′, Ha-6′, Hb-6′	
6′.....	3.51 d, 11.6 (Ha)	Hb-6′		
	3.92 d, 11.9 (Hb)	Ha-6′		H-2′

[a]Chemical shifts shown in ppm relative to TMS as internal standard in $CDCl_3$ solution.

TABLE 2. ^{13}C-Nmr Data for Compounds **1** and **3** and Two Reference Compounds [14-Hydroxy-α-muurolene **4**, and Dictyotin D methyl ether **5**].

C	1[a]	3[a]	4[b]	5[c]
1	25.1 t	24.5 t	24.3 t	20.8 t
2	30.8 t	24.9 t	26.1 t	31.6 t
3	134.4 s	134.4 s	138.0 s	133.1 s
4	123.9 d	123.6 d	125.9 d	125.4 d
5	36.4 d	36.3 d	36.5 d	34.0 d
6	39.0 d	38.9 d	41.1 d	42.2 d
7	24.6 t	23.8 t	24.6 t	19.6 t
8	121.5 d	121.1 d	121.7 d	31.8 t
9	136.5 s	136.3 s	136.1 s	76.1 s
10	39.6 d	39.4 d	39.3 d	42.2 d
11	31.9 d	30.7 d	26.7 d	31.7 d
12	36.0 t	35.5 t	16.1 q	36.0 t
13	24.6 t	24.5 t	67.6 d	26.4 t
14	32.1 t	31.5 t	21.7 q	125.2 d
15	37.9 d	46.2 d	21.4 q	130.9 s
16	101.6 d	205.1 d		25.7 q
17	24.0 q	23.8 q		23.6 q
18	21.7 q	21.6 q		22.5 q
19	13.4 q	13.2 q		13.6 q
20	14.1 q	13.2 q		17.7 q
1′	101.1 d			
2′	80.1 d			
3′	66.4 d			
4′	78.5 d			
5′	76.3 d			
6′	67.6 t			

[a]Run at 90 MHz in $CDCl_3$, with TMS as internal standard.
[b]Run at 75.47 MHz in $CDCl_3$, with TMS as internal standard.
[c]Run at 22.5 MHz in $CDCl_3$, with $CDCl_3$ as internal standard.

groups. The acetylation of **1** with Ac_2O in pyridine led to a triacetate **2** (6), which also indicated the presence of three -OH groups. In addition, the ^{13}C-nmr and DEPT experiments of **1** indicated the presence of four methyl groups [δ_C 24.0 (q), 21.7 (q), 14.1 (q), 13.4 (q)], six methylenes [δ_C 36.0 (t), 32.1 (t), 30.8 (t), 25.1 (t), 24.6 (t) (2C)], one oxygenated methylene [δ_C 67.6 (t)], five methines [δ_C 39.6 (d), 39.0 (d), 37.9 (d), 36.4 (d), 31.9 (d)] and four oxygenated methines [δ_C 80.1 (d), 78.5 (d), 76.3 (d), 66.4 (d)].

Of the six degrees of unsaturation of the molecule, two belonged to double bonds, thus **1** was suggested as being tetracyclic. The 2D ^{1}H-^{1}H homo- and ^{1}H-^{13}C heteronuclear chemical shift correlations of **1** suggested the partial structures I and II (Figure 1). The DQF-COSY experiment showed the ^{1}H-^{1}H correlations for two large substructures as shown in Table 1. In the HMBC experiment, the correlations between H-17 and C-3, and H-4 and C-17 suggested that C-3 is connected to C-17 and C-4. The HMBC correlation between H-18 and C-10 indicated C-18 (Me) located at C-9. In addition, the HMBC experiment showed correlations between H-10 and C-9, H-17 and C-2, which indicated that C-9 was bonded to C-10 and C-2 to C-3. Thus substructure I was established as a 3,8-diene carbo-bicyclic diterpene with the same carbon skeleton as biflora-4,10(19),15-triene (7). Substructure II was deduced as a hexopyranose from the DQF-COSY experiment (Table 1 and Figure 1) and from the HMBC experiment.

In an attempt to examine the hexose and aglycone components of **1**, the compound was hydrolyzed with dilute H_2SO_4

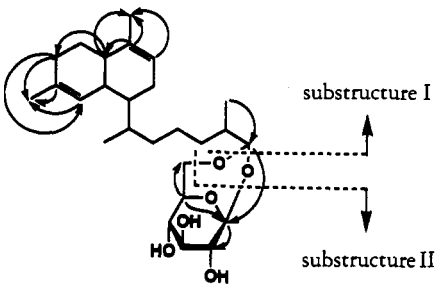

FIGURE 1. Substructures I and II (HMBC correlation C→H).

for 4 h in dioxane. Hydrolysis yielded a hexose, which was recognized as D-glucose by comparison with standard spectra (8,9) and optical rotation of $[\alpha]^{22.5}_{D}$ +52.3°, and an aglycone **3**, which was purified by chromatography over Si gel. The aglycone **3**, obtained as a colorless oil, had a molecular ion at *m/z* 288 consistent with the composition $C_{20}H_{32}O$. The ^{1}H-nmr and ^{13}C-nmr spectra of **3** indicated the presence of an aldehyde group [δ_H 9.62 (1H, d, J=2.8 Hz and δ_C 205.1 (d)], two trisubstituted double bonds [δ_H 5.48 (1H, br s) and 5.40 (1H, br s) and δ_C 136.3 (s), 134.4 (s), 123.6 (d) and 121.1 (d)], and four methyl groups [δ_H 1.72 (3H, br s), 1.69 (3H, br s), 1.07 (3H, d, J=7.6 Hz), 0.79 (3H, d, J=7.2 Hz) and δ_C 23.8 (q), 21.6 (q), 13.2 (q, 2C)], corresponding to the substructure I of compound **1**.

The HMBC correlation between H-1′ and C-16 indicated that the substructures I and II are connected at the C-16 and C-1′ positions. The presence of two acetals and three free hydroxyl groups in **1**, together with the further downfield chemical shift of C-6′ at δ_C 67.6, suggested that C-16 and O-6′ are connected to each other to form the second acetal bond and consequently a seven-membered ring D. An nOe observed between H-6′a and H-16 also indicated C-1′ and C-6′ to be attached to C-16 by two acetal linkages.

In the ^{1}H-nmr spectrum, the splitting pattern of H-5 was a doublet of double doublets with $J_1 \approx J_2$=4.9 Hz and J_3=10.0 Hz; one of the two coupling constants with J=4.9 Hz was due to the H-4, H-5 coupling, as it could be directly deduced from the spectrum. In the DQF-COSY and TOCSY spectra, H-5 showed cross-peaks with H-4 and H-10, as well as with H-6. Therefore, the other two were attributed to the H-5, H-10 (J=ca. 4.9 Hz) and H-5, H-6 (J=10.0 Hz) couplings. The large coupling constant (10.0 Hz) between H-5 and H-6 established the equatorial orientation of the side-chain at C-6. These values could only be justified by a relative cis-stereochemistry for H-5, H-10 and trans-stereochemistry for H-5, H-6 (10). These stereochemistries were supported by comparing the ^{13}C-nmr data of **1** and 14-hydroxy-α-muurolene **4** (Table 2) (10). The stereochemistry at C-11 in **1** could be determined by detailed analyses of the NOESY spectrum and comparing the ^{13}C nmr data for the eight carbon side-chain with dictyotin D methyl ether **5** (Table 2) (11). The side-chain of **1** does not freely rotate around the C-6/C-11 axis and it exists in a certain fixed conformation (11). Therefore, the nOe's observed between H-4 (δ 5.48) and H-11 (δ 1.75), H-19 (δ 0.81) and H-5, H-8 (δ 1.77) suggested the stereochemistry of C-11 as in Figure 2.

The conformation of rings C and D could also be determined by analyses of NOESY experiments. The signal enhancements arising from the interaction of H-2′ and H-4′ and also of H-6a′ indicated H-2′, H-4′ and C-6′ to be axial (β) with respect to ring C, and H-6a′ to be axial (β) with respect to ring D. The orienta-

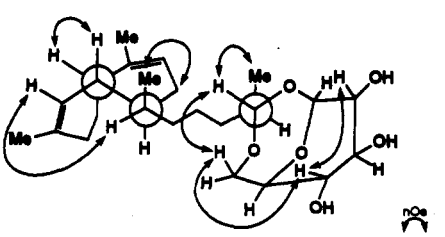

FIGURE 2. NOe interactions for **1**.

tions (α and β) refer to 2′ β-H. Thus ring C was in the boat form. The presence of the two acetal bonds established that C-16 does not rotate around the C-16/C-1′ or the C-16/C-6′ axis, so H-16 exists in only one fixed stereochemistry of orientation either α or β with respect to ring D. However, the nOes observed between H-16 and H-6a′ suggested H-16 to be β with respect to ring D, with ring D also in the boat form. The relative stereochemistry of C-15 could be determined by analysis of the ^{1}H-nmr spectrum of **1**, whose coupling constant between H-15 and H-16 was 4.8 Hz. This *J* value suggested that H-15 and H-16 are in a threo relationship (12). The absolute stereochemistry of C-15 and C-16 in compound **1** could be deduced as 15*R*,16*S* from the β-D-glucose component, but the absolute stereochemistry at C-5, C-6, C-10, and C-11 could not be determined.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H- and ^{13}C-nmr spectra were recorded on Bruker-AMX-600MHz and JEOL Fx-900 instruments. A VG analytical ZAB mass spectrometer, a Nicolet 5Dx-FTIR spectrometer, and a Perkin-Elmer 240 elemental analyzer were also used. Optical rotations were measured with a Perkin-Elmer 241 polarimeter.

ANIMAL MATERIAL.—The fresh soft coral *Lemnalia bournei* was collected by hand from the South China Sea near the Xisha Islands. A voucher specimen is deposited in the Research Center of Organic Natural Products, Zhongshan University, Guangzhou, People's Republic of China.

EXTRACTION AND ISOLATION.—The specimens (2 kg) were extracted with EtOH 3 times. The extracts were combined and chromatographed over a column of Si gel eluted with petroleum ether stepwise by increasing the concentration of EtOAc. The fractions eluted with 35% EtOAc gave lemnabourside [**1**] (28 g), as roughly 1.5% dry weight of the soft coral.

Lemnabourside [**1**].—White needles, mp 90–90.5°, $[\alpha]^{22.5}$D +33.3° (*c*=0.030, EtOH); ir (KBr) 3480–3550 (vs), 2930, 1640, 1550, 1450, 1380, 1150, 1115, 1050 cm^{-1}; *anal.* found: C 69.50%, H 9.60%; calcd for $C_{26}H_{42}O_6$, C 69.29%, H 9.39%; ms *m/z* 450 $[M]^+$ (6), 432 (2), 414 (1), 396 (1), 288 (31), 189 (20), 161 (73), 145 (19), 119 (33), 105 (100).

Acetylation of Lemnabourside [**1**].—Ac_2O (0.8 ml) was added to a 50-mg sample of **1** in 1 ml pyridine, and refluxed for 5 min. The solution was cooled and kept at room temperature overnight, then quenched with excess cold H_2O and extracted with $CHCl_3$ (3×40 ml). The combined $CHCl_3$ extracts were washed with 0.5 N HCl, distilled H_2O, and 5% $NaHCO_3$ solution, then dried over anhydrous $MgSO_4$. Removal of solvent under reduced pressure yielded the crude triacetate **2**, 55 mg. Purification by prep. tlc on Si gel developed with C_6H_6-EtOH (1:1) yielded **2**, as a colorless solid, mp 54–55.5°; $[\alpha]^{22.5}$D +6.82° (*c*=0.02, EtOH); ir (KBr) 3400, 2810, 1750, 1440, 1365, 1240, 1220, 1125, 1100, 1035 cm^{-1}; ms *m/z* 576 $[M]^+$ (2), 289 (9.5), 229 (15), 189 (26), 161 (93), 145 (23), 133 (33), 119 (46), 105 (100); ^{1}H nmr ($CDCl_3$) δ 5.48 (br s, H-4), 5.32 (br s, H-8), 4.92 (m, H-16), 4.98 (d, *J*=4.8 Hz, H-1′), 5.43 (m, H-2′), 5.75 (m, H-3′), 4.58 (m, H-4′), 3.80 (m, H-5′), 3.52 (m, H-6a′), 4.02 (m, H-6b′), 2.05 (s, 6H), 2.10 (s, 3H); ^{13}C nmr ($CDCl_3$) δ 136.0 (s, C-3), 134.0 (s, C-9), 123.6 (d, C-4), 121.2 (d, C-8), 101.9 (d, C-16), 97.9 (d, C-1′), 77.3 (d, C-2′), 75.2 (d, C-5′), 72.0 (d, C-4′), 67.4 (t, C-6′), 65.5 (d, C-3′), 39.2 (d, C-10), 38.6 (d, C-6), 37.6 (d, C-15), 36.0 (d, C-5), 35.6 (t, C-12), 31.4 (2C, d, C-11, t, C-14), 30.5 (t, C-2), 24.8 (t, C-1), 24.3 (t, C-13), 23.5 (t, C-7), 20.4 (q, C-17), 17.9 (q, C-18), 13.9 (q, C-20), 13.0 (q, C-19), 169.7 (s), 169.6 (s), 169.4 (s), 21.4 (q), 20.4 (q), 17.9 (q).

Acid Hydrolysis.—Lemnabourside **1** (50 mg) was added to a solution consisting of 4 ml dioxane and 4 ml 1.0 N H_2SO_4. The solution was refluxed for 4 h, cooled and extracted with $CHCl_3$ (3×20 ml). The combined $CHCl_3$ extracts were washed with H_2O and 5% $NaHCO_3$, then dried over anhydrous $MgSO_4$, filtered, and the solvent removed under reduced pressure to yield a light orange oil, diterpenoid **3**, 26 mg. Purification by chromatography over Si gel yielded **3**, as a colorless oil, $[\alpha]^{22.5}$D −19.2° (*c*=0.02, $CHCl_3$); ir (KBr) 3070, 2930, 1720, 1670, 1650, 1500, 1380, 915 cm^{-1}; ms *m/z* 288 $[M]^+$ (5), 185 (15), 175 (18), 161 (40), 159 (70), 145 (23), 119 (43), 105 (100); ^{1}H nmr ($CDCl_3$) δ 5.48 (br s, H-4), 5.40 (br s, H-8), 4.62 (d, *J*=2.8 Hz, H-16), 1.72 (br s, 3H, H-17), 1.69 (br s, 3H, H-18), 1.07 (d, *J*=7.6 Hz, 3H, H-20), 0.79 (d, *J*=7.2 Hz, 3H, H-19).

The H_2O layer was neutralized with $BaCO_3$ to pH7, filtered, and the solvent was removed under reduced pressure to yield D-glucose, 20 mg, as a light orange solid. $[\alpha]^{22.5}$D +52.3° (*c*=0.02, H_2O; ^{1}H nmr (D_2O, DSS, 90 MHz) δ 5.24 (d), 5.00 (s), 3.80, 3.68, 3.44; ^{13}C nmr (D_2O, dioxane, 90 MHz) δ 98.96 (C-1β), 95.17 (C-1α), 79.09 (C-5β), 78.82 (C-3β), 77.24 (C-2β), 76.08 (C-2α), 75.53 (C-3α, C-5α), 72.53 (C-4α, C-4β), 63.86 (C-6β), 63.70 (C-6α).

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