Two New Diterpene Glycosides from the Soft Coral *Lemnalia bournei*

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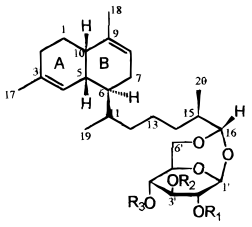
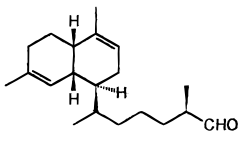
Two new bicyclic diterpene glycosides, lemnabourside B and C (**1**, **2**), have been isolated from the Chinese soft coral *Lemnalia bournei*. They are the monoacetate derivatives of lemnabourside (**3**) previously obtained from the same species. The structures of compounds **1** and **2**, which showed weak cytotoxicity against HepA, S_{180}A and EAC cells, respectively, were established from spectral data and chemical transformations.

In continuation of our studies of the Chinese soft coral *Lemnalia bournei* Roxas (Nephtheidae), two new minor diterpene glycosides, the lemnabourside B and C (**1**, **2**) were isolated. The two compounds are the monoacetate derivatives of the previously described lemnabourside (**3**), the major metabolite in *L. bournei*.[1]

Lemnabourside B (**1**) and C (**2**) were assigned the same formula ($C_{28}H_{44}O_7$) from EIMS and elemental analysis. The spectral data of compounds **1** and **2** were similar to those of compound **3** (Table 1), with the differences reflecting the monoacetylation of the sugar residue. The strong absorptions in the IR spectra of **1** and **2** at 1735 and 1720, respectively, and the ^{13}C NMR signals at δ 170.4 (s), 23.9 (q) and 171.8 (s), 24.1 (q) ppm indicated the presence of acetyl groups in these compounds. Peracetylation of compounds **1**, **2**, and **3** with anhydrous Ac_2O in pyridine gave the same triacetate **4** , while acid hydrolysis of **1**, **2**, and **3** yielded the same aglycon compound **5**,[1] as verified by comparison of the optical rotations and TLC R_f values of these semisynthetic products. A comparative study of the ^{1}H and ^{13}C NMR spectra of **1**, **2**, and **3** readily positioned the acetate group at C-3′ in **1** and at C-4′ in **2** as follows. The 3′ sugar methine proton in **1** was shifted downfield from δ 4.10 in **3** to δ 4.52 ppm in **1**, while the 3′ sugar carbon was also shifted downfield from δ 66.4 in **3** to δ 69.1 ppm in **1**. Similarly in **2**, the 4′ sugar methine proton was shifted from δ 3.68 in **3** to δ 4.56 ppm, and the 4′ carbon from δ 78.5 in **3** to δ 79.6 ppm.



1 $R_1 = R_3 = H$, $R_2 = CH_3CO$
2 $R_1 = R_2 = H$, $R_3 = CH_3CO$
3 $R_1 = R_2 = R_3 = H$
4 $R_1 = R_2 = R_3 = CH_3CO$



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These diterpene glycosides showed the following cytotoxicity against hepatoma ascites cells (HepA, IC_{50})— lemnabourside B (**1**) 33.5 μg/mL, lemnabourside C (**2**) 41.4 μg/mL, lemnabourside (**3**) 31.6 μg/mL; against sarcoma 180 ascites cells (S_{180}A, IC_{50}) **1** 40.0 μg/mL, **2** 186.9 μg/mL, **3** 27.4 μg/mL; against Ehrlich ascites carcinoma cells (EAC, IC_{50}) **1** 73.3 μg/mL, **2** 40.5 μg/mL, **3** 97.8 μg/mL.

Table 1. ^{1}H and ^{13}C NMR Spectral Data for Lemnabourside B (**1**), Lemnabourside C (**2**), and Lemnabourside (**3**)

	1		**2**		**3**	
C	^{1}H δ^a	^{13}C δ^b	^{1}H δ^a	^{13}C δ^b	^{1}H δ^c	^{13}C δ^b
1	1.12 m	25.4 t	1.12 m	25.6 t	1.12 m	25.1 t
	1.39 m		1.36 m		1.35 m	
2	1.81 m	31.0 t	1.83 m	31.5 t	1.83 m	30.8 t
	1.99 m		1.98 m		1.98 m	
3		134.6 s		134.5 s		134.4 s
4	5.42 br s	124.2 d	5.48 br s	124.2 d	5.48 d (4.9)	123.9 d
5	2.07 m	36.9 d	2.02 m	36.9 d	2.02 m	36.4 d
6	1.45 m	39.4 d	1.48 m	39.4 d	1.50 m	39.0 d
7	1.76 m	30.0 t	1.75 m	31.1 t	1.77 m	24.6 t
8	5.34 br s	121.8 d	5.38 br s	121.8 d	5.39 br s	121.5 d
9		136.4 s		136.4 s		136.5 s
10	1.95 m	39.9 d	1.93 m	39.9 d	1.93 m	39.6 d
11	1.76 m	32.1 d	1.70 m	32.1 d	1.75 m	31.9 d
12	1.19m	36.3 t	1.14 m	36.2 t	1.14 m	36.0 t
	1.24 m		1.22 m		1.22 m	
13	1.30 m	24.9 t	1.33 m	25.0 t	1.33 m	24.6 t
14	1.07 m	32.3 t	1.09 m	32.2 t	1.09 m	32.1 t
	1.42 m		1.45 m		1.44 m	
15	1.65m	38.3 d	1.65 m	38.4 d	1.64 m	37.9 d
16	4.53 m	102.0 d	4.57 m	101.6 d	4.57 d (4.8)	101.6 d
17	1.73 s	21.7 q	1.74 s	21.9 q	1.68 s	24.0 q
18	1.63 s	20.8 q	1.66 s	21.0 q	1.68 s	21.7 q
19	0.75 d (6.7)	13.6 q	0.78 d (6.7)	13.7 q	0.81 d (6.6)	13.4 q
20	0.86 d (6.6)	14.4 q	0.88 d (6.6)	14.4 q	0.90 d (6.6)	14.1 q
1′	4.91 d (7.3)	101.8 d	4.89 s	101.6 d	4.90 s	101.1d
2′	3.90 d (7.3)	78.5 d	3.90 d (7.8)	80.6 d	3.87 d (7.0)	80.1 d
3′	4.52 m	69.1 d	4.43 m	64.7 d	4.10 m	66.4 d
4′	3.71 m	77.0 d	4.56 m	79.6 d	3.68 m	78.5 d
5′	3.66 d (5.7)	74.6 d	3.65 d (4.6)	75.6 d	3.62 d (5.6)	76.3 d
6′	3.47 m	68.2 t	3.55 m	67.9 t	3.51 d (12)	67.6 t
	3.91 m		3.92 m		3.92 d (12)	
AcO	2.05 s	170.4 s	2.17 s	171.8 s		
		23.9 q		24.1 q		

[a] 400 MHz in $CDCl_3$, TMS as internal reference. Coupling constants are given in Hertz in parentheses. [b] 90 MHz in $CDCl_3$, TMS as internal standard. [c] 600 MHz in $CDCl_3$, TMS as internal reference.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker MSL-400 and a JEOL Fx-90Q 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. TLC used GF_{254} produced by Shandong Marine Chemical Plant in China.

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Animal Material. The fresh soft coral *L. bournei* was collected by hand from the South China Sea near the Xisha Islands. A voucher specimen (no. 87009) is deposited in the Research Center of Organic Natural Products, Zhongshan University, Guangzhou, China.

Extraction and Isolation. The specimens (2 kg) were extracted with EtOH × 3. The extracts were combined and chromatographed over a column of Si gel eluted with petroleum ether (60−90) stepwise by increasing the concentration of EtOAc. The fractions eluted with 20% EtOAc were concentrated, the residue was dissolved in Me_2CO and the batyl alcohol crystals separated out, and the mother solution was repeatedly separated by column chromatograph on Si gel to give pure lemnabourside B (**1**, 265 mg) and lemnabourside C (**2**, 480 mg). TLC [C_6H_6−EtOAc (1:1)] showed R_f of compound **1** 0.39, **2** 0.55, and **3** 0.18.

Lemnabourside B (1): amorphous solid, mp 66.5−67.5 °C; $[\alpha]^{22.5}_D$ +10.3° (*c* 0.029, EtOH); IR (KBr) ν_{max} 3450, 2930, 1735, 1650, 1450, 1240, 1145, 1026 cm^{-1}; EIMS *m/z* 492 [M^+] (13), 474 (4), 288 (35), 189 (23), 161 (94), 105 (100); *anal.* C 68.18%, H 8.91%, calcd for $C_{28}H_{44}O_7$, C 68.27%, H 9.00%.

Lemnabourside C (2): amorphous solid, mp 69.0−70.2 °C; $[\alpha]^{22.5}_D$ +25.0° (*c* 0.020, EtOH);IR (KBr) ν_{max} 3500, 2920, 1720, 1650, 1440, 1260, 1145, 1025 cm^{-1}; EIMS *m/z* 492 [M^+] (10), 474 (2), 288 (32), 205 (35), 189 (20), 161 (91), 105 (100); *anal.* C 68.21%, H 8.87%, calcd for $C_{28}H_{44}O_7$, C 68.27%, H 9.00%.

Acetylation of 1 and 2. Ac_2O (1 mL) was added to portions (49 mg, 0.1 mmol) of **1** and **2** in pyridine (1.25 mL) and the solutions refluxed (5 min). The solutions were cooled and kept at room temperature overnight, then quenched with excess cold H_2O and extracted with $CHCl_3$ (325 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 the solvent yielded the crude triacetate **4**. Purification by preparative Si gel TLC developed with C_6H_6−EtOAc (1:1) gave a colorless solid **4** (40 mg, 70% from **1**, and 43 mg, 75% from **2**).

Triacetate 4 : mp 54.5−56.0 °C; $[\alpha]^{22.5}_D$ +6.91° (*c* 0.025, EtOH).[1]

Acid Hydrolysis of 1 and 2. Individual portions (49 mg, 0.1 mmol) of compounds **1** and **2** were added to a solution consisting of dioxane (4 mL) and 1.0 N H_2SO_4 (4 mL). The solution was refluxed (4 h), cooled, and extracted with $CHCl_3$ (3 × 20 mL). The combined $CHCl_3$ extracts were washed with H_2O and 5% $NaHCO_3$, dried over anhydrous $MgSO_4$, filtered, and the solvent removed under reduced pressure to yield a light orange oil, aglycon **5**. Purification by preparative Si gel TLC developed with C_6H_6−EtOAc (2:1) gave colorless oil **5** (25 mg, 85% from **1**, and 23 mg, 80% from **2**). The aqueous layer was neutralized with $BaCO_3$ to pH 7, filtered, and the solvent was removed under reduced pressure to yield D-glucose (16 mg) as a light orange solid; $[\alpha]^{22.5}_D$ +52.0° (*c* 0.022, H_2O).

Aglycon 5: $[\alpha]^{22.5}_D$ −19.6° (*c* 0.020, $CHCl_3$).

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References and Notes

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