Lemnaflavoside, a New Diterpene Glycoside from the Soft Coral *Lemnalia flava*

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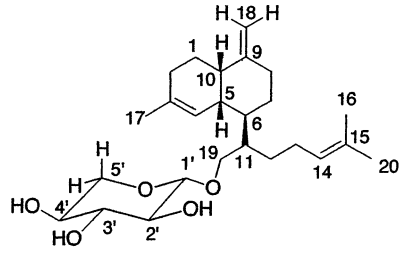
Received March 11, 2002

Lemnaflavoside (**1**) and three monoacetates (**2**−**4**) have been isolated from the Indo-Pacific soft coral *Lemnalia flava*, collected off Mombasa, Kenya. The structure of the new glycoside was elucidated by interpretation of MS and 2D NMR data. The sugar of the diterpene glycoside was found to be D-xylose.

Soft corals (Alcyonacea) have proven to be a rich source of isoprenoids.[1] As part of our continuing program to discover bioactive compounds from marine invertebrates,[2,3] we have studied the chemical composition of several soft corals collected on Shelly Reef, off Mombasa, Kenya. Clumps of colonies of *Lemnalia flava* May, 1898 (Nephtheidae) are commonly found at this site, at a depth of 10−12 m. To the best of our knowledge, this is the first chemical study of *L. flava*.

We hereby report the isolation and structure elucidation of four closely related new diterpene glycosides (**1**−**4**) isolated from *L. flava*. Silica gel chromatography of the two similar petroleum ether and EtOAc extracts, eluted with a gradient of EtOAc in petroleum ether, afforded the four glycosides (**1**−**4**). Of the four, the major compound, **1**, designated lemnaflavoside (1%, dry wt), eluted last with petroleum ether/EtOAc (2:3).



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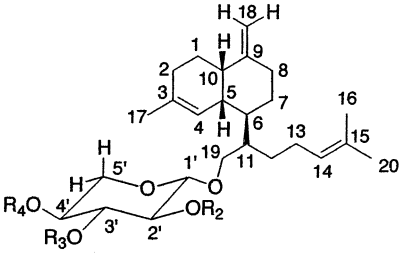

2 R_2 =Ac ; R_3 = R_4 = H
3 R_3 =Ac ; R_2 = R_4 = H
4 R_4 =Ac ; R_3 = R_2 = H
5 R_2 = R_3 = R_4 = Ac

Compound **1** exhibited a molecular ion at *m*/*z* 420 in the EIMS. The ^{13}C NMR resonances (Table 1) revealed the presence of 25 carbon atoms, of which three were methyls, nine methylenes (including one sp^2 methylene and two oxymethylene groups, C-19 and C-5′), 10 methines (including two sp^2 and four oxymethine carbons), and three quaternary sp^2 carbon atoms. In combination with a DEPT 135 experiment, these data accounted for a total of 25 carbon atoms and 37 protons. The above data, together with the possibility of preparing a triacetate (**5**), vide infra, suggested a glycoside structure (for **1**), a pentose diterpene with a formula $C_{25}H_{40}O_5$, which was corroborated by the HREIMS data (Experimental Section). The presence of three double bonds, as concluded from the ^{13}C NMR data, and a pyranose ring accounted for 4 of the 6 degrees of unsaturation inherent in the molecular formula. Therefore, we determined the diterpene to be bicyclic. The existence of a pyranose ring was supported by NMR resonances for the anomeric carbon atom (δ_C 102.7 ppm and δ_H 4.16 ppm), three oxymethine carbons (δ_C 72.1 d, 74.6 d, 69.1 d), and one oxymethylene carbon (δ_C 63.7 t), as well as a base peak, in the mass spectrum of **1**, at *m*/*z* 287 [M − $C_5H_9O_4$]$^+$, consistent with the aglycone. β-Xylopyranose, in which all four oxygen substituents are equatorial, was suggested for the sugar of **1**, since the axial configuration of H-1′−H-4′ was inferred from the 6.7−8.9 Hz axial−axial coupling constants of these four ring protons (Table 1). Also, in agreement with the xylopyranose ring were the coupling constants of H_2-5′ and the carbon C-1′ to C-5′ resonances.[4] Subsequently, acid hydrolysis of lemnaflavoside (**1**) indeed afforded xylose, which, after mutarotation overnight in H_2O at room temperature, gave a positive $[\alpha]_D$ +15°, establishing the D-configuration of this aldopentose.[5]

Along with the xylose spin system (measured in $CDCl_3$), the ^{1}H NMR spectrum of **1** in C_6D_6 exhibited a second spin system,[6] accounting for all of the other protons of **1**. Interpretation of the COSY, TOCSY, and, for the determination of geminal proton pairs, HMQC experiments gave, as presented in Table 1, a complete assignment of all of the diterpene protons of **1**, establishing a bicyclo[4.4.0]-decane structure, namely, a prenyl cadinene[7] (or prenyl murrolene).[8] Unambiguous confirmation of the planar structure (of **1**) came from the long-range CH correlations deduced from the HMBC experiment (Table 1). Furthermore, on the basis of the decalin ^{13}C chemical shifts, a *cis* configuration was suggested for the diterpene portion. The ^{13}C resonances were in good agreement with the values of dictyotin D methyl ether[9] and lemnabourside,[10] vide infra, and differed from the δ_C values for the bridge C atoms in the *trans*-decalin of γ-cadinene (δ_C 49.2, 41.6).[11] Further support for the *cis*-decalin stereochemistry, as well as for the preferred conformation of the system, came from *J* values in the ^{1}H NMR spectra. Irradiation of H-4 (δ 5.75) in a selective 1D TOCSY experiment,[12] with a TOCSY mixing time of 5 ms, brought out only H-5 as a broad

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10.1021/np0201016 CCC: $22.00
Published on Web 08/24/2002

Table 1. NMR Data of Lemnaflavoside (**1**) at 25 °C in C_6D_6[a,c]

atom #	**1** δ_C[b]	**1** δ_H (mult., *J* in Hz)	**1** HMBC[d]	δ_C of dictyotin D − methyl ether[9]	δ_C of lemnabourside[10]
1	25.0 (t)	1.98, 1.46 (m)		20.8	25.1 (t)
2	30.8 (t)	1.98, 1.86 (m)	4, 17	31.6 (t)	30.8 (t)
3	133.0 (s)		17	133.1 (s)	134.4 (s)
4	123.7 (d)	5.75 (bd, 4.0)	17	125.4 (d)	123.9 (d)
5	36.6 (d)	2.21 (bd, 13.6)	19b	34.0 (d)	36.4 (d)
6	40.1 (d)	1.74 (m)	4, 19a, 19b	42.2 (d)	39.0 (d)
7	27.9 (t)	1.70, 1.10 (m)		19.6 (t)	24.6 (t)
8	31.8 (t)	2.20, 2.15 (m)	18a, 18b	31.8 (t)	121.5 (d)
9	152.5 (s)		18a, 18b	76.1 (s)	136.5 (s)
10	42.8 (d)	2.49 (dm, 10.8)	4, 18a, 18b	42.2 (d)	39.6 (d)
11	36.6 (d)	2.17 (m)		31.7 (d)	31.9 (d)
12	30.5 (t)	1.70, 1.40 (m)	14, 19a, 19b	36.0 (t)	
13	25.9 (t)	2.12, 2.11 (m)	14	26.3 (t)	
14	124.1 (d)	5.25 (t, 6.7)	12a, 12b, 16, 20	125.2 (d)	
15	130.1 (s)		13a, 13b, 16, 20	130.9 (s)	
16	25.7 (q)	1.70 (s)	14, 20	25.7 (q)	
17	23.9 (q)	1.65 (s)	4	23.6 (q)	
18	106.3 (t)	4.70 (s), 4.60 (s)		22.5 (q)	
19	69.8 (t)	3.84 (dd, 9.8, 4.9)	1′, 12a, 12b	13.6 (q)	
		3.14 (dd, 9.8, 8.7)		17.7 (q)	
20	17.6 (q)	1.60 (s)	14, 16		
1′[c]	102.7 (d)	4.16 (d, 6.9)	2′, 3′, 5′a,b, 19b		
2′	72.1 (d)	3.30 (dt, 7.9, 6.9)	3′		
3′	74.6 (d)	3.44 (t, 7.9)	1′, 2′, 4′, 5′a,b		
4′	69.1 (d)	3.58 (ddd, 8.7, 7.9, 4.7)	2′, 5′b		
5′	63.7 (t)	3.87 (dd, 11.8, 4.7)	1′, 3′		
		3.18 (dd, 11.8, 8.7)			

[a] Bruker ARX-500 instrument. [b] Multiplicities were determined by DEPT and HMQC experiments. [c] Interpretation of the sugar unit was carried out in $CDCl_3$. [d] a and b refer to the low-field and high-field resonance of a geminal pair, respectively.

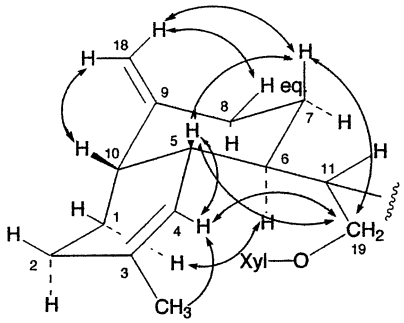

Figure 1. Key NOEs for lemnaflavoside (**1**) (NOESY).

doublet (J = 13.6 Hz). Since $J_{4,5}$ is 4 Hz and $J_{5,10}$ is ca. 2 Hz, the 13.6 Hz coupling has to be between H-5 and H-6. Therefore, both H-5 and H-6 must be axial. The latter J values define the relative configuration of C-5, C-6, and C-10, three of the four chiral centers of the diterpene portion (Figure 1). Moreover, the axial position of H-5, toward the second, disubstituted ring, also defines the conformation of the *cis*-decalin system of **1**, as depicted in Figure 1. The latter stereochemistry is in full agreement with the measured NOEs, as shown in Figure 1. NOEs were also measured between the oxymethylene H_2-19 and H-4, axial H-5 and axial H-7 (Figure 1). However, no clear NOEs could be measured between H-11 or H-12 and decalin protons, making it impossible to conclude the configuration of the fourth chiral center C-11. Tentatively, on the basis of biogenetic considerations, the 11*R** configuration of lemnabourside[10] is suggested for lemnaflavoside (**1**). Lemnabourside was isolated from *Lemnalia bournei*[10] and has the same diterpene skeleton as **1**, yet it differs in the number and position of the double bonds, degree of oxidation, and sugar unit. Lemnaflavoside was found to be cytotoxic to sea urchin embryos; the results will be published elsewhere.

In addition to lemnaflavoside (**1**), three minor compounds (**2**−**4**) were obtained from the 25−30% EtOAc/petroleum ether fractions. Repeated chromatography afforded minute amounts of **2**−**4** (0.007−0.05%, dry wt), all three of which had NMR data very similar to those of **1**. Common to all three was the same aglycone as that in **1** and an additional acetate group.

The downfield shift of H-2′, H-3′, or H-4′ in compounds **2**, **3**, and **4** ($\Delta\delta$ 1.44, 1.37, and 1.26 respectively), together with interpretation of the COSY experiments determined the three compounds to be the 2′-, 3′-, and 4′-acetate derivatives of **1**, respectively. Microacetylation of the monoacetates with a 1:1 mixture of Ac_2O/pyridine at room temperature overnight afforded the same triacetate (**5**) as obtained from **1**.

Lemnaflavoside and lemnabourside are among the few diterpenes isolated from *Lemnalia* species, most of the identified isoprenoids being sesquiterpenes. We have previously obtained the same diterpene skeleton as in **1** from cyclization of obscuronatin, a *Xenia* metabolite.[13]

Experimental Section

General Experimental Procedure. Optical rotations were obtained with a Jasco P-1010 polarimeter. IR spectra were obtained with a Bruker FTIR Vector 22 spectrometer. EIMS were recorded on a Fisons Autospec Q instrument. ^1H and ^{13}C NMR spectra were recorded on Bruker ARX500 and ARX400 spectrometers. All chemical shifts are reported with respect to TMS ($\delta_H = 0$) and C_6D_6 ($\delta_C = 128.0$) or $CDCl_3$ ($\delta_C = 77.0$).

Animal Material. *Lemnalia flava* was collected on Shelly Reef, off Mombasa, Kenya, using scuba at the depth of 10−12 m in February 2001. Clumps of these colonies are commonly found at this site. The colonies are arborescent, up to 10 cm in height, and light brown in color. The identification of this species matches the taxonomic description, as provided by Verseveldt (1969). The genus *Lemnalia* includes several species that are in need of revision. *L. flava* was previously recorded from Zanzibar, Aldabra, and Madagascar. A voucher sample, KB368, is deposited in the Zoological Museum, Tel-Aviv University (one of us, Y.B., who is the manager of the

museum, identified the soft coral). The collected sample was immediately frozen and kept at −20 °C until processed.

Extraction. The freeze-dried animal (20 g) was homogenized and extracted with petroleum ether and then with EtOAc (3 × 50 mL). The combined filtered extracts were evaporated under reduced pressure to give a brown gum (760 mg). This gum was subjected to normal-phase flash chromatography on Si gel. The fraction eluted with 40% EtOAc in petroleum ether gave compound **1** (200 mg, 1% dry wt, R_f = 0.35, Si gel, EtOAc). Repeated flash chromatography on Si gel of the 25−30% EtOAc/petroleum ether afforded compounds **2**−**4** (1.5 mg, 0.007%; 10 mg, 0.05%; and 1.5 mg, 0.007%, respectively, R_f = 0.73, 0.75, 0.74, respectively, Si gel, EtOAc).

Lemnaflavoside (1): amorphous material; $[\alpha]_D$ +12° (*c* 0.9, MeOH); IR (neat) ν_{max} 3420, 2930, 2358, 1645, 1045 cm^{-1}; ^{1}H and ^{13}C NMR, see Table 1; EIMS *m*/*z* (%) 420 (M^+, 15), 288 ($M^+ - C_5H_9O_4$,100), 253 (65), 160 (35); HREIMS 420.2873, calcd for $C_{20}H_{40}O_5$, 420.2877.

Acetylation of 1. 1 (1.5 mg) in a mixture of 1:1 Ac_2O/pyridine (1 mL) was left at room temperature for 24 h. After removal of the solvents, the residue was taken through a Si gel column to afford the 2′,3′,4′-triacetate of **1** (**5**): ^{1}H NMR (C_6D_6) δ 5.75 (1H, bd, *J* = 4.0 Hz, H-4), 5.40 (1H, t, *J* = 9.0 Hz, H-3′), 5.26 (1H, dd, *J* = 9.0, 7.1 Hz, H-2′), 5.21 (1H, t, *J* = 6.7 Hz, H-14), 5.03 (1H, m, H-4′), 4.24 (1H, d, *J* = 7.1 Hz, H-1′), 4.00 (1H, dd, *J* = 9.4, 4.9 Hz, H-5a′), 3.85 (1H, dd, *J* = 11.7, 5.3 Hz, H-19a), 3.20 (1H, dd, *J* = 9.4, 6.1 Hz, H-5b′), 3.05 (1H, dd, *J* = 11.7, 9.3, H-19b), 1.84 (3H, s, CH_3COO), 1.70 (3H, s, CH_3COO), 1.66 (3H, s, CH_3), 1.62 (3H, s, CH_3), 1.58 (3H, s, CH_3COO), 1.55 (3H, s, CH_3); EIMS *m*/*z* (%) 546 (M^+, 10), 502 ($M^+ - 42$, 15), 486 ($M^+ - Ac$, 60), 460 (15), 287 ($M^+ - C_{11}H_{15}O_7$, 100); HREIMS 546.3191 (M^+), calcd for $C_{31}H_{46}O_8$, 546.3194.

Hydrolysis of 1. 1 (15 mg) was dissolved in 4:1 MeOH/HCl (5 mL) and refluxed for 2.5 h. After neutralization with NH_4OH to pH 8, EtOAc (20 mL) and water (20 mL) were added. The sample was lyophilized and the residue applied to a short flash RP-18 column to afford xylose (7 mg) eluting with water: $[\alpha]_D$ +15° (*c* 0.07, H_2O), identical to an authentic sample of D-xylose.[5]

Compound 2: white oil; IR ν_{max} ($CHCl_3$) 3415, 2930, 1690, 1050 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.52 (1H, bd, *J* = 4.0 Hz, H-4), 5.07 (1H, t, *J* = 6.7 Hz, H-14), 4.74 (1H, t, *J* = 6.3 Hz, H-2′), 4.47 (1H, d, *J* = 6.3 Hz, H-1′), 4.06 (1H, dd, *J* = 11.9, 4.0 Hz, H-5a′), 3.94 (1H, dd, *J* = 9.4, 4.5 Hz, H-19a), 3.71 (1H, m, H-4′), 3.62 (1H, dd, *J* = 7.3, 6.3 Hz, H-3′), 3.38 (1H, dd, *J* = 11.9, 7.3 Hz, H-5b′), 3.25 (1H, dd, *J* = 9.4, 7.2 Hz, H-19b); CIMS *m*/*z* (%) 463 (MH^+, 30), 445 (10), 345 (30), 278 (80), 175 (acetyl xylose, 100).

Compound 3: an oil; IR ν_{max} (neat) 2930, 1690, 1645 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.58 (1H, bd, *J* = 4.0 Hz, H-4), 5.25 (1H, t, *J* = 6.7 Hz, H-14), 4.81 (1H, t, *J* = 6.8 Hz, H-3′), 4.29 (1H, d, *J* = 6.8 Hz, H-1′), 4.04 (1H, dd, *J* = 11.8, 4.5 Hz, H-5a′), 3.96 (1H, dd, *J* = 9.6, 4.6 Hz, H-19a), 3.77 (1H, m, H-4′), 3.52 (1H, t, *J* = 6.8 Hz, H-2′), 3.30 (1H, dd, *J* = 11.8, 8.9 Hz, H-5b′), 3.26 (1H, dd, *J* = 9.6, 7.3 Hz, H-19b), 2.49 (1H, bd, *J* = 10.8, H-10), 2.18 (3H, s, CH_3COO), 1.60 (3H, s, CH_3), 1.59 (3H, s, CH_3), 1.52 (3H, s, CH_3); ^{13}C NMR ($CDCl_3$) δ 173.0 (s), 153.7 (s), 134.3 (s), 131.5 (s), 124.5 (d), 124.1 (d), 107.0 (t), 103.3 (d), 77.0 (d), 71.1 (t), 71.0 (d), 68.7 (d), 64.9 (t), 43.6 (d), 40.9 (t), 39.4 (d), 37.2 (d), 31.9 (t), 30.8 (t), 30.6 (t), 27.8 (t), 26.0 (t), 25.7 (q), 25.0 (t), 23.9 (q), 21.0 (q), 17.7 (q); CIMS *m*/*z* (%) 463 (MH^+, 35), 445 ($MH^+ - H_2O$, 10), 345 (20), 273 (65), 175 (monoacetylated xylose moiety, 100).

Compound 4: white oil; IR ν_{max} ($CHCl_3$) 3420, 2930, 1690, 1048 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 5.54 (1H, bd, *J* = 4.0 Hz, H-4), 5.08 (1H, t, *J* = 6.7 Hz, H-14), 4.84 (1H, m, H-4′), 4.42 (1H, d, *J* = 7.3 Hz, H-1′), 4.09 (1H, dd, *J* = 11.8, 4.4 Hz, H-5a′), 3.95 (1H, dd, *J* = 9.6, 4.2 Hz, H-19a), 3.75 (1H, t, *J* = 6.8 Hz, H-3′), 3.52 (1H, t, *J* = 6.8 Hz, H-2′), 3.39 (1H, dd, *J* = 11.8, 6.8 Hz, H-5b), 3.29 (1H, dd, *J* = 9.6, 7.2 Hz, H-19b); CIMS *m*/*z* (%) 463 (MH^+, 25), 445 (15), 345 (15), 273 (70), 175 (acetyl xylose, 100).

Acknowledgment. This work was supported by PharmaMar, Madrid. We thank A. Zverdling, Coral Farm Ltd., Mombasa, for his generous technical help.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **2002**, *19*, 1−48, and previous reports in this series.
(2) Loya, S.; Rudi, A.; Kashman, Y.; Hizi, A. *J. Biochem.* **1999**, *344*, 85−92.
(3) Rudi, A.; Yosief, T.; Loya, S.; Hizi, A.; Schleyer, M.; Kashman, Y. *J. Nat. Prod.* **2001**, *64*, 1451−1453.
(4) Agrawal, P. K. *Phytochemistry* **1992**, *31*, 3307−3330.
(5) Budavari, S. Editor, *The Merck Index*, 12th ed.; Merck Res. Labs: NJ, 1996; p 10220.
(6) All of the protons of the second spin system were in the geminal, vicinal, or allylic positions.
(7) Nakanishi, K., Ed. *Natural Products Chemistry*; Academic Press: NY, 1974; Vol. 1, p 163.
(8) Kashman, Y.; Rudi, A.; Gutman-Naveh, N. *Tetrahedron* **1978**, *34*, 1227−1229.
(9) Ishitsuka, M. O.; Kusumi, T.; Ichikawa, A.; Kakisawa, H. *Phytochemistry* **1990**, *29*, 2605−2609.
(10) Zhang, M.; Long, K.; Wu, H.; Ma, K. *J. Nat. Prod.* **1994**, *57*, 155−160.
(11) Sakurai, H.; Hosomi, A.; Saito, M.; Sasaki, K.; Ignchi, H.; Sasaki, J.; Araki, Y *Tetrahedron* **1983**, *39*, 883−894.
(12) Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355−360.
(13) Kashman, Y.; Groweiss, A. *J. Org. Chem.* **1980**, *45*, 3814−3824.

NP0201016