A Furanoid Labdane Diterpene from *Potamogeton nodosus*

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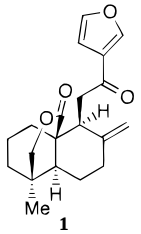
A new furanoid diterpene, 15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide (**1**) was isolated from an ethanolic extract of *Potamogeton nodosus*. Its structure was elucidated by the usual spectroscopic methods, including 2D NMR techniques. Compound **1** was found to exhibit moderate inhibitory activity against a number of both Gram-positive and Gram-negative bacteria.

Potamogeton nodosus Poir. (syn. *P. indicus* Roxb.), belonging to the Potamogetonaceae, is a submerged aquatic herb having creeping root stocks.[1] It grows on the littoral zones of lakes and ditches in Bangladesh and is also distributed in parts of India, Sri Lanka, Myanmar, and Malaysia.[2] In the Ayurvedic system, *P. nodosus* has been reported to be effective in treating cancer, tuberculosis, acne, and cough.[3] Previous chemical investigations on different species of *Potamogeton* have shown the presence of alkaloids,[4,5] flavonoids,[4,5] and a labdane diterpenoid.[6] As a part of our continuing studies on medicinal plants of Bangladesh, we have examined *P. nodosus* and isolated a furanoid labdane diterpene (**1**). In this paper, we report the structure of **1** and its antibacterial activity.



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Solvent−solvent partitioning[7] of the cold ethanolic extract of *P. nodosus* followed by purification with column chromatography afforded a new furanoid labdane diterpene (**1**) as a yellow amorphous powder, together with β-stigmasterol. The structure of **1** was determined as follows. The molecular formula was suggested as $C_{20}H_{24}O_4$ by high-resolution mass spectrometry. The IR spectrum showed bands demonstrative of a furan ring (1570, 1505, 878 cm^{-1}) and two carbonyl groups (1730 1680 cm^{-1}). More detailed analysis was performed by NMR measurements. The typical low-field signals at δ 6.81 (H-14), 7.44 (H-15), and 8.20 (H-16) in the ^{1}H-NMR spectrum suggested the presence of a 3-substituted furan unit, which was conjugated with a carbonyl group.[8] In the HMBC[9] spectrum, the furan protons showed long-range correlations with the ketone carbon at 194.76 ppm at C-12. Two olefinic protons appeared at 4.80 and 4.62 ppm and were assigned to an exomethylene group. On the basis of the careful analysis of its PFG-DQFCOSY,[10] HMQC,[11] and HMBC data (Table 1), the structure of **1** was suggested as 15,-16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide. The isolated methylene protons at 4.05 and 4.21 ppm gave long-range correlations with the carbonyl carbon at 173.56 ppm (C-20); hence, the presence of a lactone ring was inferred, and these methylene protons were assigned to the δ-position of the δ-valerolactone moiety. HMBC correlations from the methyl protons (H-18) to the oxygenated methylene carbon C-19 and H-1ax, and H-5 and H-9 to the carbonyl carbon in the lactone (C-20), supported the presence of a 20,19-olide functionality.[6,12] The relative stereochemistry was established by 1D PFG-selective ROESY[13] and NOE differential experiments. Strong NOEs were observed between H-1ax and H-9 and H-5 and H-9, respectively. Thus, the configuration of the decalin system was established as *trans*, and the side chain at C-9 as equatorial. The other observed NOEs are summarized in Table 1. From these NOE data, together with analysis of coupling constant values, all ^{1}H-NMR signals could be assigned as shown in Table 1.

This is the first reported isolation of 15,16-epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide (**1**) from a natural source, although this compound has previously been prepared by a semisynthetic method.[12] The ^{13}C-NMR data of the isolated **1** are basically identical with those of the semisynthetic substance, but the reported assignments[12] of C-1, C-3, C-7, and C-14 should be revised as shown in Table 1, according to our present investigation.[14]

Compound **1** exhibited moderate antibacterial activity against *Bacillus cereus, B. subtilis, Shigella boydii, S. shiga, S. sonnei, Staphylococcus aureus*, and *Streptococcus faecalis*; the zones of inhibition produced were 13, 16, 8, 12, 10, 14, and 12 mm, respectively. On the other hand, the zones of inhibition produced by the standard antibiotic, doxycycline, were 31, 34, 38, 37, 30, 35, and 35 mm, respectively.

Experimental Section

General Experimental Procedures. Silica gel 60 PF_{254} was used for TLC while silica gel (60−120 mesh)

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Table 1. NMR Data for **1** at 150 MHz (^{13}C) and 600 MHz (^{1}H) in $CDCl_3$

carbon	δ_C	Proton(s)	δ_H (J in Hz)	DQF-COSY	HMBC	NOE
1	36.34	1eq	2.08 (m)	H-1ax,2eq,2ax	C-2,3,5,10	
		1ax	1.48 (m)	H-1eq,2eq,2ax	C-9,10,20	
2	20.84	2eq	1.74 (m)	H-1eq,1ax,3eq,3ax	C-1,3,4	
		2ax	1.68 (m)			
3	41.01	3eq	1.68(m)			
		3ax	1.48 (m)	H-2ax,2eq,19a[a]	C-2,4,5,19	
4	33.43					
5	49.15	5	1.66 (m)	H-6eq,19b[a]	C-19,20	
6	27.58	6eq	2.10 (m)	H-5,7eq,7ax	C-7,8,10	
		6ax	1.30 (dddd, 13.2,13.2,13.2,4.4)	H-5,7eq,7ax	C-5,7	H-19a,7eq
7	36.28	7eq	2.45 (ddd, 13.2,4.4,2.4)	H-6eq,6ax	C-5,6,8,9,10	
		7ax	2.22 (ddd, 13.2,13.2,4.4)	H-6eq,6ax	C-5,6,8,9,10	H-5,6eq,9
8	146.54					
9	46.13	9	2.85 (dd, 8.8,3.4)	H-11a,11b,17a[a],17b[a]	C-1,7,8,10,11, 12,17,20	H-1ax,5,7ax 11a,11b
10	50.40					
11	38.09	11a	3.80 (dd, 18.1,8.8)	H-9	C-8,9,10,12	
		11b	3.35 (dd, 18.1,3.4)	H-9	C-8,9,10,12	
12	194.76					
13	127.98					
14	108.64	14	6.81 (d, 1.5)	H-15,16	C-12,13,15,16	H-11a,15
15	144.11	15	7.44 (dd, 1.5,1.5)	H-14,16	C-13,14,16	
16	147.37	16	8.20 (br s)	H-14,15	C-13,14,15	
17	107.84	17a	4.80 (s)	H-7ax[a],9a	C-7,9	H-7eq,17b
		17b	4.62 (s)	H-7ax[a],9[a]	C-7,8,9	H-11a,17a
18	23.60	18	0.94 (s)	H-19b[a]	C-3,4,5,19	H-3eq,3ax,5, 6eq,19a,19b
19	76.64	19a	4.21 (dd, 11.7,2.4)	H-3ax[a]	C-3,4,18,20	H-6ax,18
		19b	4.05 (d, 11.7)	H-5[a],18[a]	C-3,4,5,20	H-3eq,18
20	173.56					

[a] Observed long-range coupling. [b] Observed NOEs in 1D selective ROESY and/or NOE differential experiments.

was used for column chromatography. The optical rotation was measured on a JASCO DIP-370 polarimeter using a sodium lamp (589 nm D line). The IR spectrum was recorded on a Valor-III FT-IR spectrometer. NMR experiments were recorded on a JEOL JNM A600 spectrometer at 600 and 150 MHz for ^{1}H- and ^{13}C NMR, respectively. The sample was dissolved in $CDCl_3$, and chemical shifts were referenced to TMS for ^{1}H NMR and to the residual solvent peak for ^{13}C NMR. Inverse-detected heteronuclear correlations were measured using the HMQC (optimized for $^{1}J_{CH} = 145$ Hz) and HMBC (optimized for $^{n}J_{CH} = 8.3$ Hz) pulse sequences with a pulsed-field gradient. The FABMS spectrum was measured on a JEOL JMS HX-110A spectrometer.

Plant Material. Whole plants of *P. nodusus* were collected at Alamdanga, Chuadanga, Bangladesh, in March 1995. The specimen was identified by staff of the Bangladesh National Herbarium, Dhaka, where a voucher specimen has been deposited (DACB Accession No. 27,564).

Extraction and Isolation. Air-dried whole plants (1 kg) were powdered and extracted with 95% EtOH. After evaporation of the solvent, the crude extract was subjected to the Kupchan partitioning process.[7] The *n*-hexane fraction (1.0 g) was further separated by column chromatography over silica gel using solvents of increasing polarity. Elution of the column with C_6H_6–$CHCl_3$ (1:4) followed by $CHCl_3$ (100%) afforded β-stigmasterol[15] (37 mg) and the diterpene **1** (20 mg), respectively.

15,16-Epoxy-12-oxo-8(17),13(16),14-labdatrien-20,19-olide (1): yellow amorphous powder; $[\alpha]^{25}_D$ +32.3° (*c* 0.2, $CHCl_3$); IR (neat) ν_{max} 3020, 2940, 1730, 1680, 1650, 1570, 1505, 1140, 1050, 900, 878 cm^{-1}; ^{1}H- and ^{13}C-NMR data, see Table 1; HRFABMS *m/z* calcd for $C_{20}H_{25}O_4$ $[M + H]^+$ 329.1753, found 329.1756.

Antibacterial Activity. The antibacterial activity was determined by the standardized disk diffusion technique[16] using doxycycline as a reference standard.

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