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Nanolobatolide, a New C_{18} Metabolite from the Formosan Soft Coral *Sinularia nanolobata*

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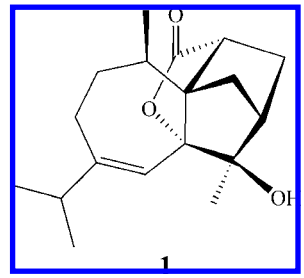
ABSTRACT



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Nanolobatolide (1), possessing a novel C_{18} molecular structure, was isolated from the Formosan soft coral *Sinularia nanolobata.* The structure of 1 was established by extensive spectroscopic study and confirmed by X-ray diffraction analysis. A plausible biosynthetic pathway of 1 was proposed. Nanolobatolide (1) has been found to possess significant anti-neuroinflammatory and neuroprotective activities.

Formosan soft corals of the genus *Sinularia* have been proven to be rich sources of novel metabolites.[1−5] Our previous chemical investigation on the soft coral *Sinularia nanolobata* (Verseveldt), collected along the coast of the most southern tip of Taiwan, has led to the isolation of new norsesquiterpenoids, diterpenoids, and a novel norditerpenoid.[6] Our current chemical investigation on a new collection of *S. nanolobata*, collected in a different region of Taiwan, has led to the isolation of a novel C_{18} terpenoid-related compound nanolobatolide (**1**). The structure of the new natural product was determined by extensive spectroscopic analysis and further confirmed by a single-crystal X-ray diffraction analysis.

The soft coral *S. nanolobata* (1.0 kg, wet weight) was collected by hand using scuba off the coast of the northeastern region of Taiwan in May 2004 and stored in a freezer before extraction. A voucher specimen was deposited in the

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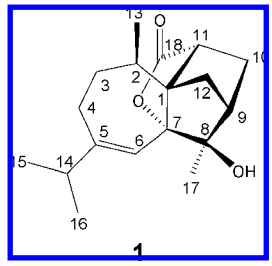
Department of Marine Biotechnology and Resources, National Sun Yat-sen University (specimen no. 200405-5). The freeze-dried organism was minced and extracted exhaustively with EtOH. The organic extract was concentrated under reduced pressure to an aqueous suspension, which was partitioned between *n*-hexane and H_2O and then between EtOAc and H_2O. The EtOAc-soluble layer was concentrated by rotary evaporator to a residue (5.6 g) which was subjected to column chromatography over silica gel, using *n*-hexane, *n*-hexane and EtOAc mixture of increasing polarity, and finally pure EtOAc to yield 21 fractions. Fraction 9, eluted with *n*-hexane−EtOAc (1:5), was further purified by silica gel column chromatography to afford **1** (5.5 mg).



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Nanolobatolide (**1**), $[\alpha]^{25}_D$ +11.4 (*c* 0.72, $CHCl_3$), was obtained as colorless crystals, mp 102−103 °C, and had the molecular formula $C_{18}H_{26}O_3$ as determined by HRESIMS (*m*/*z* calcd 313.1780, found 313.1782 $[M + Na]^+$), requiring six degrees of unsaturation. The infrared spectrum of **1** showed absorptions of hydroxyl (ν_{max} 3444 cm^{-1}), carbonyl group of a γ-lactone (ν_{max} 1756 cm^{-1}), and isopropyl methyls (ν_{max} 1392, 1379 cm^{-1}). The ^{13}C NMR spectroscopic data of **1** (Table 1) revealed the presence of 18 carbon signals, which were identified by the assistance of DEPT spectrum into four methyls, four sp^3 methylenes, four sp^3 methines, one sp^2 methine, and three sp^3 quaternary carbons (including two oxygenated ones resonating at δ 98.3 and 80.4). The remaining two carbon signals appearing in the lower field were due to the quaternary carbons of one carbonyl group (δ 178.3) and one olefin (δ 152.7). From the ^1H NMR spectrum of **1**, the resonances of one olefinic proton (δ 5.33, s) and four methyls (δ 1.28, s; 1.05, d, J = 7.0 Hz; 1.04, d, J = 7.0 Hz; 0.93, d, J = 6.5 Hz) were also observed, revealing the terpenoidal character of **1**.

The COSY and HMBC (Figure 1) correlations were further used to establish the molecular skeleton of **1**. From the $^1H-^1H$ COSY spectrum of **1**, it was possible to establish three proton sequences from H_2-4 to H_3-13 through H_2-3 and H-2; H-11 to H_2-12 through H_2-10 and H-9; and H_3-15 to H_3-16 through H-14. The HMBC experiment used to connect the above substructures was found to show the following key correlations: H-2 to C-1 and C-12; H_2-3 to C-1; H_2-4 to C-5; H-6 to C-1 and C-4; H-9 to C-1, C-7, and C-8; H_2-10 to C-8, C-9 and C-18; H-11 to C-1, C-9, C-10 and C-18; H_3-13 to C-1, C-2, and C-3; H-14 to C-5, both H_3-15 and H_3-16 to C-5 and C-14, and H_3-17 to C-7, C-8, and C-9. The ring juncture proton, H-11, should be positioned at the α carbon to the carbonyl group due to its appearance at lower field (δ 2.64). The quaternary carbon C-1 (δ 61.9) was confirmed to be the spiro carbon atom by the HMBC correlations from H-2, H-6, H-9, H-11and H-12 to this carbon. By consideration of molecular formula, IR absorption of the carbonyl group at 1756 cm^{-1}, and the chemical shifts of C-8 (δ 80.4), C-7 (δ 98.3), C-18 (δ 178.3), and H_3-17 (δ 1.28), it was suggested that C-8 is the hydroxylated carbon bearing a methyl group and C-7 is the sp^3 oxygenated carbon of the γ-lactone. Thus, the planar structure of **1** was established. The relative structure of nanolobatolide (**1**) was determined by the analysis of NOE correlations (Figure 2) and was further confirmed by a single-crystal X-ray diffraction analysis (Figure 3).[7]

A plausible biosynthetic pathway for **1** from a guaiane-type[8,9] precursor was proposed as shown in Scheme 1. The hypothetical guaiane **4**, which formally functions as the diene

Table 1. ^1H and ^{13}C NMR Chemical Shifts for Compound **1** (δ in ppm Relative to TMS)

C/H	^1H[a]	^{13}C[b]
1		61.9 (C)
2	1.97, m, 1H	27.8 (CH)
3α	1.42, m, 1H	32.8 (CH_2)
3β	1.78, m, 1H	
4α	2.52, m, 1H	26.4 (CH_2)
4β	2.02, m, 1H	
5		152.7 (C)
6	5.33, s, 1H	115.8 (CH)
7		98.3 (C)
8		80.4 (C)
9	2.28, d, 1H (3.5)[c]	46.2 (CH)
10α	1.86, d, 1H (10.5)	31.9 (CH_2)
10β	2.04, m, 1H	
11	2.64, d, 1H (10.5)	44.7 (CH)
12α	2.42, d, 1H (11.0)	33.5 (CH_2)
12β	1.46, d, 1H (11.0)	
13	0.93, d, 3H (6.5)	18.3 (CH_3)
14	2.37, m, 1H	37.9 (CH)
15	1.04, d, 3H (7.0)	21.4 (CH_3)
16	1.05, d, 3H (7.0)	20.9 (CH_3)
17	1.28, s, 3H	20.3 (CH_3)
18		178.3 (C)

[a] Spectrum recorded at 500 MHz in $CDCl_3$. [b] 125 MHz in $CDCl_3$. [c] *J* values (in Hz) in parentheses.

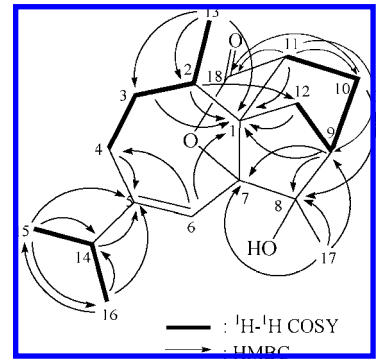


Figure 1. Key $^1H-^1H$ COSY and HMBC correlations of **1**.

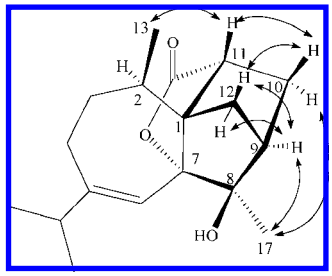


Figure 2. Key NOESY correlations of **1**.

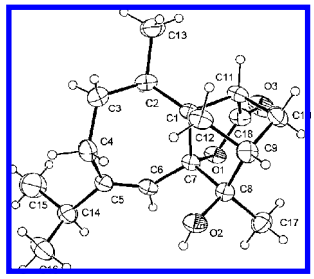
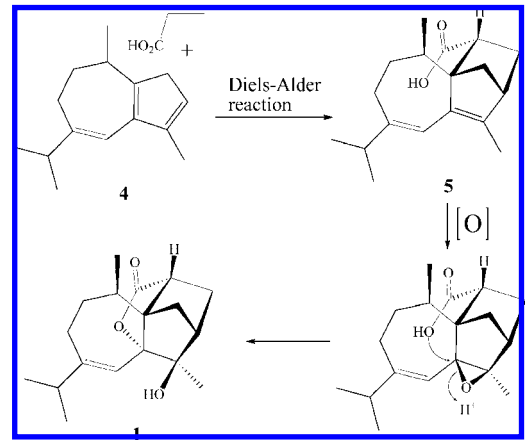


Figure 3. Molecular structure of **1** based on X-ray diffraction.

of a Diels−Alder reaction, might react with acrylic acid to afford the tricyclic carboxylic acid **5**. Epoxidation of **5** in the double bond of the five-membered ring, the following acid-catalyzed ring-opening of the epoxide, and finally the lactonization could lead to the formation of **1**. However, the possibility that **1** was biosynthesized via a degradation step of a related diterpene resulting in the loss of two carbons should not be excluded.

The antineuroinflammatory activity of **1** against the accumulation of pro-inflammatory iNOS and COX-2 proteins in microglial cells stimulated with INF-γ was evaluated according to a previous study.[10] It was found that at 10 μM compound **1** did not show cytotoxicity against microglial cells but significantly reduced the iNOS expression to 45.5 ± 11.4%, relative to the control cells treated with INF-γ only. In neuroprotective assay using 6-OHDA (6-hydroxydopamine)-induced neurotoxicity in neuroblastoma SH-SY5Y, a human dopaminergic neuron often used for study of Parkinson's disease,[11,12] the neuroprotective effect of **1** against the damage of 6-OHDA toward SH-SY5Y cells was measured by a method reported previously.[13] It was observed that the cytotoxicity of 6-OHDA on SH-SY5Y cells was significantly reduced by pretreatment of **1** at various concentrations. The relative neuroprotective activities of **1** at 0.01, 0.1, 1, and 10 μM were 41.4 ± 9.7, 66.8 ± 14.0, 72.0 ± 10.1, and 83.3 ± 7.1%, respectively. From the neurological activity results, we suggest that further investigation of **1** for its therapeutic potential for neurodegenerative diseases is worthwhile.

Scheme 1. Proposed Biosynthetic Pathway for **1**



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Supporting Information Available: ^{1}H and ^{13}C NMR, ^{1}H−^{1}H COSY, HMQC, HMBC, and NOESY spectra and X-ray crystallographic data (CIF) for **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Crystallographic data for nanolobatolide (**1**) have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 690527. Copies of the data can be obtained, free of charge, on application to the Director, CCDC, 12 Union Road, Cambridge CB2 1EZ, U.K. (fax: +44(0)-1223−336033 or e-mail: deposit@ccdc.cam.ac.uk).

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