A Cytotoxic Lobane Diterpene from the Formosan Soft Coral *Sinularia inelegans*

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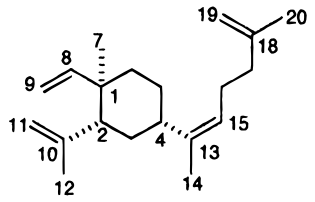
A new cytotoxic lobane diterpene, ineleganene (**1**), was isolated from the Formosan soft coral *Sinularia inelegans*. The structure of compound **1** was determined by 1D and 2D spectral analysis.

In the search for bioactive substances from marine organisms, the soft coral *Sinularia inelegans* Tixier-Durivault (family Alcyoniidae) was studied because the hexane extracts showed significant cytotoxicity in A549 (human lung adenocarcinoma) and P-388 (mouse lymphocytic leukemia) cell cultures as determined by standard procedures.[1,2] Bioassay-guided fractionations resulted in the isolation of a new cytotoxic lobane diterpene, ineleganene (**1**).



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The hexane-soluble fraction of *S. inelegans* was chromatographed over Si gel to give a colorless oil, $[\alpha]^{25}_D$ +9.6° (*c* 0.1, $CHCl_3$). HRFABMS and the DEPT spectrum of **1** established the molecular formula to be $C_{20}H_{32}$. Thus, 5 degrees of unsaturation were determined for **1**. ^1H and ^{13}C NMR spectral data (Table 1) showed that the structure of **1** contained four olefin groups, including a vinyl (δ_C 150.4, d, C-8; 109.8, t, C-9; δ_H 5.82, dd, J = 17.7, 10.2 Hz, H-8; 4.88 d, J = 10.2 Hz, 4.91 d, J = 17.7 Hz, H_2-9), an isopropenyl group (δ_C 147.8, s, C-10; 112.1, t, C-11, 24.7, q, C-12; δ_H 4.59, br s, 4.82, br s, H_2-11; 1.71, s, H_3-12), a methyl-bearing trisubstituted double bond (δ_C 131.6, s, C-13; 124.3, d, C-15; 25.6, q, C-14; δ_H 5.13, m, H-15; 1.69, s, H_3-14), and an isopentenyl group (δ_C 107.1, t, C-19; 154.5, s, C-18; 17.7, q, C-20; 34.9, t, C-17; 27.3, t, C-16; δ_H 4.74 br s, 4.79 br s, H_2-19; 1.62, s, H_3-20; 2.13, m, H_2-16; 2.08, m, H_2-17). These facts, in combination with the molecular formula, suggested the occurrence of one ring. The presence of the vinyl and isopropenyl groups together with a tertiary methyl group (δ_C 16.6, q, C-7; δ_H 1.01, s, H_3-7) is reminiscent of a 3-isopropenyl-4-methyl-4-vinylcyclohexane-1-yl moiety that is reported in lobane-type diterpenoids,[3−5] and this partial structure was confirmed by COSY, HMQC, and HMBC spectra. Further, the trisubstituted olefin and the isopentenyl groups were connected by HMBC and NOESY correlations to form a 1,5-dimethyl-1(*E*),5-hexadiene-1-yl side chain, which was attached at the α (equatorial) position of C-4. Compound **1** exhibited cytotoxicity against A549 and P-388 cell lines with GI_{50} values of 3.63 and 0.20 μg/mL, respectively.

Table 1. NMR Data of **1**[a]

position	δ_H; mult. J[b]	δ_C; mult.[c]	HMBC	COSY	NOESY
1		40.4; s			
2	2.01; dd; 12.1, 5.0	52.9; d	3, 6, 7, 10, 11, 12		11
3α	1.51; m	33.4; t	4, 5, 13	4	7
β	1.58; m				
4	1.92; m	44.5; d	6, 14	3	
5	2.14; m	26.8; t		6	
6	1.46; m	39.8; t	1, 2, 4, 7	5	8
7	1.01; s	16.6; q	2, 6, 8		3α, 9, 11, 12, 14
8	5.82; dd; 17.7,10.2	150.4; d	6, 7	9	6, 9
9	4.88; d, 10.2 4.91; d; 17.7	109.8; t	1, 8	8	7, 8
10		147.8; s			
11	4.59; br s 4.82; br s	112.1; t	2, 12	12	2, 3, 7
12	1.71; s	24.7; q	2, 10, 11	11	7
13		131.6; s			
14	1.69; s	25.6; q	13, 15		3α, 15
15	5.13; m	124.3; d	13	16	14, 16
16	2.13; m	27.3; t	15	15, 17	3α, 15
17	2.08; m	34.9; t	16, 18, 19, 20	16	19
18		154.5; s			
19	4.74; br s 4.79; br s	107.1; t	17, 18		17
20	1.62; s	17.7; q	18		

[a] Spectra recorded in $CDCl_3$. [b] J values in Hz. [c] Multiplicity deduced by DEPT and indicated by usual symbols.

Experimental Section

General Experimental Procedures. Optical rotation was determined on a JASCO DIP-181 polarimeter. The IR spectrum was recorded on a Hitachi 26-30 spectrophotometer. ^1H and ^{13}C NMR spectra were recorded with a Bruker AMX 400 NMR spectrometer at 400 and 100.6 MHz, respectively, in $CDCl_3$ using TMS as internal standard. The HMBC experiment was obtained using HMBC (optimized for $^nJ_{C-H}$ = 8 Hz) pulse sequences with a pulse gradient. EIMS spectra were obtained with a JEOL JMS-SX/SX 102A mass spectrometer at 70 ev. Si gel 60 (Merck, 230−400 mesh) was used for column chromatography; precoated Si gel plates (Merck, Kieselgel 60 F_{254}, 0.25 mm) were used for TLC analysis.

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Animal Material. The soft coral *S. inelegans* was collected at Green Island off Taiwan in September 1997, at a depth of 12 m and was stored for 1 day in a freezer until extraction. A voucher specimen NSUGN-1024 (identified by Prof. Chang-Feng Dai) was deposited in the Department of Marine Resources, National Sun Yat-sen University, Taiwan.

Extraction and Isolation. *S. inelegans* specimens (wet wt 2.1 kg) were freeze-dried to give 980 g of a solid, which was extracted with CH_2Cl_2 (2 L × 3). After removal of solvent in vacuo, the residue (40 g) was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2-soluble fraction was further partitioned between *n*-hexane and 10% aqueous MeOH. The *n*-hexane-soluble fraction was chromatographed over Si gel 60 using *n*-hexane and *n*-hexanes−EtOAc mixtures (the final ratio, 1:3) of increasing polarity. Elution by *n*-hexane afforded fractions containing **1**. Compound **1** was finally purified by Sephadex LH-20 chromatography using *n*-heptane as eluting solvent.

Ineleganene (1): colorless oil (7 mg); $[\alpha]^{25}_D$ +9.6° (*c* 0.10, $CHCl_3$); IR (KBr) ν_{max} 3090, 2925, 1637 cm^{-1}; ^1H and ^{13}C NMR, see Table 1; EIMS *m*/*z* 272 $[M]^+$ (0.3), 258 (0.3), 244 (0.2), 230 (1), 216 (0.4), 147 (11), 133 (15), 121 (19), 41 (100); HREIMS *m*/*z* 272.2496 (calcd for $C_{20}H_{32}$, 272.2498).

Cytotoxicity Testing. P-388 cells were supplied by Prof. J. M. Pezzuto, University of Illinois at Chicago; A549 and HT-29 were purchased from the American Type Culture Collection. Cytotoxicity asssays were carried out according to the procedure described previously.[6]

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