

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

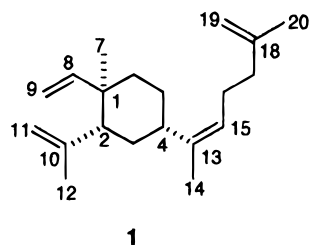
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A new cytotoxic lobane diterpene, ineleganene (**1**), was isolated from the Formosan soft coral *Sinularia ineleigans*. The structure of compound **1** was determined by 1D and 2D spectral analysis.

In the search for bioactive substances from marine organisms, the soft coral *Sinularia ineleigans* 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**).



The hexane-soluble fraction of *S. ineleigans* was chromatographed over Si gel to give a colorless oil, $[\alpha]_{\text{D}}^{25} +9.6^\circ$ (c 0.1, CHCl_3). HRFABMS and the DEPT spectrum of **1** established the molecular formula to be $\text{C}_{20}\text{H}_{32}$. 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, H₂-9), 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, H₂-11; 1.71, s, H₃-12), 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, H₃-14), 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, H₂-19; 1.62, s, H₃-20; 2.13, m, H₂-16; 2.08, m, H₂-17). 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, H₃-7) 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

Table 1. NMR Data of **1**^a

position	δ_{H} ; mult. ^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
3 α	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		3 α , 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		3 α , 15
15	5.13; m	124.3; d	13	16	14, 16
16	2.13; m	27.3; t	15	15, 17	3 α , 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.

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 $\mu\text{g/mL}$, respectively.

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 $^nJ_{\text{C-H}} = 8$ 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 F₂₅₄, 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 CH₂Cl₂ (2 L × 3). After removal of solvent in vacuo, the residue (40 g) was partitioned between CH₂Cl₂ and H₂O. The CH₂Cl₂-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.

Inelegantene (1): colorless oil (7 mg); [α]_D²⁵ +9.6° (*c* 0.10, CHCl₃); IR (KBr) ν_{\max} 3090, 2925, 1637 cm⁻¹; ¹H and ¹³C 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 C₂₀H₃₂, 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 assays were carried out according to the procedure described previously.⁶

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