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Litophynin A and B, Two New Insect Growth Inhibitory Diterpenoids from the Soft Coral Litophyton sp.

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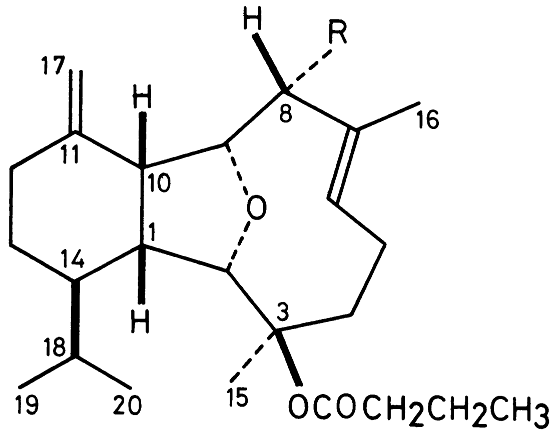
Two new diterpenoids, litophynin A and B, which exhibit insect growth inhibitory activity against the silkworm, Bombyx mori L., have been isolated from the soft coral Litophyton sp. Their structures have been fully characterized by extensive 2D NMR studies and molecular mechanics calculations.

Numerous investigations in recent years have shown that most marine invertebrates owe their survival to various chemical defensive strategies that are based on a number of biological activities of secondary metabolites, e.g. antimicrobial activity, ichthyotoxicity, feeding repellency, and antifouling activity.1) A possible extension of discoveries in such marine chemical ecology would be determination of applicability to agrochemical problems in insect control and plant growth regulation.1b) In our preliminary screening for biological activities of marine invertebrates, the crude extracts of a Litophyton sp. (Nephtheidae, Alcyonacea, Octocorallia) showed insect growth inhibitory activity against the silkworm, Bombyx mori L. We now wish to report the isolation of two new diterpenoids, designated litophynin A and B, which are responsible for the observed biological activity.2)

Litophyton sp. (2.0 kg) was collected using SCUBA from a shallow area of Sukumo Bay in August 1986. The frozen specimens were homogenized in methanol and left at room temperature for 24 hours. After filtration, the methanol solution was evaporated to an aqueous suspension and extracted with dichloromethane. Fractionation of the dichloromethane extract (6.25 g) by silica gel column chromatography and subsequent purification by reverse phase high-performance liquid chromatography (ODS column, MeOH - H_2O 9:1) gave litophynin A (93 mg) and B (40 mg). Isolation of the active principles was directed by using the artificial diet feeding bioassay.3)

Litophynin A (**1**) was obtained as a colorless oil: $[\alpha]_D^{20}$ -16.5° (c 0.23, EtOH); m/z 374.2822 (M^+, $C_{24}H_{38}O_3$ requires 374.2821), 286 ($M^+ - C_3H_7CO_2H$), and 71 ($C_3H_7C{\equiv}O^+$); ν_{max} (CCl_4) 3100, 1650, 890 (exocyclic methylene), and 1730 (ester) cm^{-1}. All the ^1H NMR signals for **1** could be unambiguously assigned by examination of the COSY and NOESY spectra in addition to simple homo-decoupling experiments (Table 1). A ^1H - ^{13}C COSY spectrum of **1** led to the straightforward assignments of the ^{13}C NMR signals except for those of fully substituted sp^2 carbon atoms (δ_C 124.90 and 146.78). These two signals, however, were easily characterized by comparison with those of analogues.4) These facts inevitably led to the gross structure 1 for litophynin A, which is closely related to cladiellin.[4a)] The presence of a butyric ester was evident from the aforementioned MS data (m/z 286 and 71) and NMR signals (δ_H 0.94, 1.62, and 2.23; δ_C 13.69, 18.66, 37.61, and 172.75). The appearance of two oxygen-bearing methine carbon signals at rather deshielded positions (δ_C 80.55 and 92.19) is characteristic for the transannular ether linkage of this type of compounds.[4)] The E geometry of the C_6 double bond was proved by the observation of an NOE between $H_5\beta$ and 16-Me. The stereochemical assignments were deduced from the examination of ^1H NMR data in conjunction with molecular mechanics calculations (MM2).[5)] Thus, vicinal H - H coupling constants assigned to protons at stereocenters well coincided with values calculated by the Karplus equation[6)] based on the dihedral angles in the MM2-optimized conformation (Table 2). Observations of NOEs ($H_2 \leftrightarrow H_{14}$, $H_2 \leftrightarrow H_{15}$, $H_2 \leftrightarrow H_{18}$, $H_8\beta \leftrightarrow H_{16}$, $H_9 \leftrightarrow H_{14}$, and $H_{10} \leftrightarrow H_{17}a$) were also consistent with the calculated conformation.[7)]

Litophynin B (2) was isolated as colorless needles: mp 99.5 - 100.5 °C, $[\alpha]_D^{20}$ -78.8° (c 0.19, EtOH), and displayed the spectral data quite similar to those of 1. ν_{max} (CCl_4) 3090, 1730, 1650, and 895 cm^{-1}; m/z 460.3219 (M^+, $C_{28}H_{44}O_5$ requires 460.3189), 372 ($M^+ - C_3H_7CO_2H$), 284 ($M^+ - 2C_3H_7CO_2H$), and 71; ^1H and ^{13}C NMR: Table 1. The ^1H and ^{13}C NMR spectra of 2, which were completely assigned by the same techniques described above, showed duplicate signals due to two butyric esters (δ_H 0.93:0.95, 1.63:1.64, and 2.26:2.28; δ_C 13.60:13.67, 18.49:18.62, 36.41:37.65, and 172.64:173.07) together with signals attributable to an oxygenated methine group (δ_H 4.85; δ_C 77.29). These facts and the MS fragment at m/z 284 revealed the presence of one additional butyric ester in 2. The location of the second butyric ester at $C_8\alpha$ was proved from the inspection of ^1H NMR data. Namely, the signal due to H_9 in 2 appears as a doublet instead of the doublet of doublets in 1, showing the absence of the coupling with $H_8\alpha$ which exists in 1. In agreement with this observation, the signal of the proton attached to a carbon atom bearing the butyric ester appears as a singlet. From the evidence outlined above, we proposed structure 2 for litophynin B.



1 R = H

2 R = $OCOCH_2CH_2CH_3$

Table 1. ^{13}C and ^{1}H NMR data of **1** and **2**

Position	**1**		**2**	
	δ_C a)	δ_H b)	δ_C a)	δ_H b)
1	46.68	2.11 (dd, 12.0, 6.5)	46.70	2.11 (dd, 11.8, 6.6)
2	92.19	3.77 (s)	92.87	3.85 (s)
3	87.05		86.77	
4	35.92	ca 1.62 (m)	36.41	ca 1.63 (m)
5	25.27	ca 2.20 (m)	25.30	ca 2.26 (m)
6	131.86	5.55 (br d, 8.0)	134.02	5.86 (br d, 7.2)
7	124.90		124.34	
8	38.43	α 2.44 (dd, 13.8, 5.4)	77.29	4.85 (s)
		β 2.02 (d, 13.8)		
9	80.55	4.03 (dd, 9.6, 5.4)	82.53	4.04 (d, 10.5)
10	47.44	2.82 (m)	46.88	2.87 (m)
11	146.78		145.66	
12	31.41	α 2.14 (m)	31.10	α 2.18 (m)
		β 2.26 (m)		β 2.25 (m)
13	25.38	α 1.75 (dddd, 12.8, 3.6, 3.6, 3.0)	25.47	α 1.76 (dddd, 12.8, 3.5, 3.5, 3.0)
		β 1.05 (dddd, 12.8, 12.8, 12.4, 3.8)		β 1.05 (dddd, 12.8, 12.8, 12.5, 3.4)
14	42.64	1.34 (dddd, 12.4, 12.0, 3.0, 2.6)	42.22	1.36 (dddd, 12.5, 11.8, 3.0, 3.0)
15	24.16	1.51 (br s)	24.66	1.45 (br s)
16	21.12	1.81 (s)	19.51	1.88 (d, 1.2)
17	110.27	4.74 (br s)	111.27	4.75 (br s)
		4.79 (t, 1.7)		4.84 (br s)
18	27.90	1.88 (m)	28.09	1.84 (m)
19	15.56	0.75 (d, 6.7)	15.45	0.75 (d, 6.8)
20	21.99	0.97 (d, 6.7)	21.90	0.97 (d, 6.8)
1′	172.75		172.64	
			173.07	
2′	37.61	2.23 (t, 7.3)	36.41	2.26 (t, 7.4)
			37.65	2.28 (t, 7.4)
3′	18.66	1.62 (sext, 7.3)	18.49	1.63 (sext, 7.4)
			18.62	1.64 (sext, 7.4)
4′	13.69	0.94 (t, 7.3)	13.60	0.93 (t, 7.4)
			13.67	0.95 (t, 7.4)

a) 100 MHz, in $CDCl_3$, TMS as int. standard.

b) 400 MHz, in $CDCl_3$, TMS as int. standard. Multiplicities and J values (in Hz) are given in parentheses.

Table 2. Selected dihedral angles and calculated vicinal H - H coupling constants for the MM2-optimized structure of **1**

	Dihedral angle / deg	J_{calcd} / Hz[a)]	J_{obsd} / Hz
H_1-C_1-C_2-H_2	104.2	0.5	≈ 0
H_1-C_1-C_{10}-H_{10}	39.5	5.1	6.5
H_1-C_1-C_{14}-H_{14}	-170.3	12.2	12.0
H_9-C_9-C_8-H_8a	-7.6	8.6	5.4
H_9-C_9-C_8-H_8b	111.9	1.0	≈ 0
H_9-C_9-C_{10}-H_{10}	-163.0	11.4	9.6
H_{13}a-C_{13}-C_{14}-H_{14}	64.3	1.4	3.0
H_{13}b-C_{13}-C_{14}-H_{14}	-177.0	12.5	12.8
H_{14}-C_{14}-C_{18}-H_{18}	72.9	0.5	2.6

a) $J = J_0 \times \cos^2\phi - 0.28$ ($J_0 = 12.8$ Hz for $90° \leqq \phi \leqq 180°$, $J_0 = 9.0$ Hz for $0° \leqq \phi \leqq 90°$).[6)]

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1) For recent articles, see: a) P. A. Keifer, K. L. Rinehart, Jr., and I. R. Hooper, J. Org. Chem., 51, 4450 (1986); b) J. H. Cardellina II, Pure Appl. Chem., 58, 365 (1986); c) K. Iguchi, K. Mori, M. Suzuki, H. Takahashi, and Y. Yamada, Chem. Lett., 1986, 1789; d) P. Crews, L. V. Manes, and M. Boehler, Tetrahedron Lett., 27, 2797 (1986); e) T. M. Zabriskie, J. A. Klocke, C. M. Ireland, A. H. Marcus, T. F. Molinski, D. J. Faulkner, C. Xu, and J. C. Clardy, J. Am. Chem. Soc., 108, 3123 (1986); f) B. Carté, M. R. Kernan, E. B. Barrabee, and D. J. Faulkner, J. Org. Chem., 51, 3528 (1986).

2) The ED_{50}-values, the effective doses for 50% growth inhibition, of litophynin A and B against *Bombyx mori* L. were 12 ppm and 2.7 ppm, respectively.

3) The second instar larvae of the silkworm were used as the test insects. The procedures for the diet preparation and bioassay were the same as those reported by Asano et al.: S. Asano, E. Kuwano, and M. Eto, Appl. Entomol. Zool., 19, 212 (1984).

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6) Y. Nawata and Y. Iitaka, Tetrahedron, 39, 1133 (1983) and references cited therein.

7) The calculated distances (Å) for each couple of hydrogen atoms are as follows: H_2-H_{14} 2.91, H_2-H_{15} 2.64, H_2-H_{18} 2.28, $H_{8}\beta$-H_{16} 2.64, H_9-H_{14} 2.21, and H_{10}-H_{17}a 2.36.

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