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BIOACTIVE TERPENOIDS FROM OCTOCORALLIA, I. BIOACTIVE DITERPENOIDS: LITOPHYNOLS A AND B FROM THE MUCUS OF THE SOFT CORAL *LITOPHYTON* SP.

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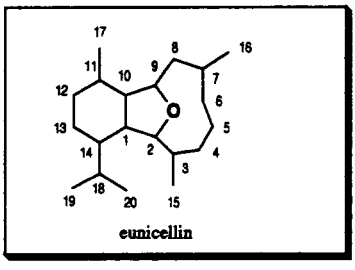
ABSTRACT.—Two new eunicellin-type diterpenoids, litophynols A [**1**] and B [**2**], and the known diterpenoids litophynins E [**3**], H [**11**], and I monoacetate [**12**], were isolated from the mucus secreted by the soft coral *Litophyton* sp. Their structures have been elucidated on the basis of spectral and single crystal X-ray analyses. These compounds have hemolytic activity.

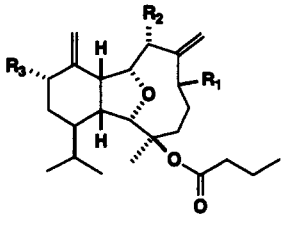
The litophynins, which are biologically active diterpenoids of the eunicellin class (1), have been isolated from the whole bodies of the soft coral *Litophyton* sp. (Octocorallia) collected from Sukumo Bay in Kochi Prefecture by Ochi *et al.* (2,3). This animal, named "Numeri-Tosaka" in Japanese, secretes much mucus from its surface when stimulated. In this paper, we report the structures of two new eunicellin-type diterpenoids, litophynols A [**1**] and B [**2**], isolated from the mucus of the soft coral *Litophyton* sp., which was collected from the Nichinan Coast in Miyazaki Prefecture. The absolute configuration of litophynin E [**3**] (4) was also determined, and the biological activities of these diterpenoids are discussed.

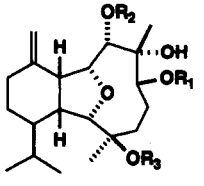
RESULTS AND DISCUSSION

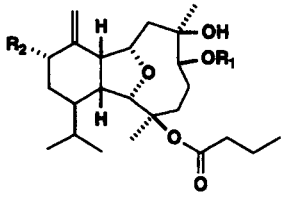
The Et_2O-soluble part of the EtOH extract obtained from the mucus of *Litophyton* sp. (4.2 kg) was subjected to Sephadex LH-20 and Si gel cc to furnish diterpenoid



eunicellin







1 R_1=OH, R_2=OH, R_3=H
4 R_1=OAc, R_2=OAc, R_3=H
5 R_1=OBz, R_2=OBz, R_3=H
11 R_1=OH, R_2=H, R_3=OH

2 R_1=H, R_2=H, R_3=$CO(CH_2)_2CH_3$
6 R_1=H, R_2=H, R_3=Ac
7 R_1=Bz, R_2=Bz, R_3=$CO(CH_2)_2CH_3$

3 R_1=H, R_2=H
8$_R$ R_1=(+)-MTPA, R_2=H
8$_S$ R_1=(−)-MTPA, R_2=H
12 R_1=H, R_2=OAc

fractions, which showed ichthyotoxic and hemolytic activities. These fractions were purified by reversed-phase column chromatography by mplc (RP-8) and hplc ($8NVC_{18}$) guided by hemolytic activity to give two novel eunicellin-type diterpenoids, litophynols A [**1**] and B [**2**], together with three known diterpenoids, litophynin E [**3**], H [**11**] (5), and litophynin I monoacetate [**12**] (6).

A molecular formula of $C_{24}H_{38}O_5$ for litophynol A [**1**] was determined by hreims data (*m/z* 406.2697 [M^+]). The ir spectrum of **1** exhibited absorptions due to hydroxy (3610 cm^{-1}) and ester (1730 cm^{-1}) functionalities. The ^{1}H- and ^{13}C-nmr spectra of **1** suggested the presence of one primary methyl, two secondary methyls, one tertiary methyl, six methylenes, four methines, four oxygen-bearing methines, one oxygen-bearing quaternary carbon, two exocyclic methylenes, and one ester (Table 1).

The fdms spectrum of **1** showed the presence of a butyloxy functionality [*m/z* 319 [M^+-87], *m/z* 71 ($C_3H_7CO^+$)]. Acetylation of **1** with Ac_2O/pyridine gave the diacetate [**4**]. These data indicated that **1** is a tricyclic diterpenoid possessing two hydroxy groups and one butyloxy group. Since diacetate **4** afforded crystals suitable for X-ray analysis, the determination of the relative stereostructure of **4** was conducted by this means. The results revealed the relative stereostructure of **4** as shown in Figure 1.

The absolute configuration of **1** was determined by application of the cd exciton chirality method (7). Perbenzoylation of **1** afforded a dibenzoate [**5**]. The cd spectrum of **5** showed a positive chirality ($[\theta]_{238}$ +76600, $[\theta]_{224}$ −53000) due to the C-6 and C-8 benzoates. These data suggested that the absolute configurations of C-6 and C-8 were *S* and *R*, respectively (see Figure 2).

The molecular formula of litophynol B [**2**] was determined to be $C_{24}H_{40}O_6$, by hreims data (*m/z* 424.2827 [M^+]). Compound **2** exhibited similar spectral features to those of **1** except for signals due to C-6–C-7–C-8. The ^{13}C-^{1}H long-range COSY nmr spectrum revealed that the quaternary methyl signal δ_H 1.25 (3H, s, Me-16) had correlations with two oxygen-bearing methine signals δ_C 79.7 (d, C-8) and δ_C 77.3 (d, C-6). Furthermore, the oxygen-bearing methine signal δ_H 3.55 (1H, d, J=9.2 Hz, H-8) correlated with another oxymethine signal δ_H 3.88 (1H, dd, J=5.9 and 8.9 Hz, H-9) in the ^{1}H-^{1}H COSY spectrum. These data suggested that litophynol B [**2**] had a contiguous triol system in its 9-membered ring.

The relative stereochemistry of **2** was deduced by comparison of ^{1}H-nmr spectral data for sclerophytin C [**6**] (8), which was isolated from another soft coral *Sclerophytum capitalis*. This comparison indicated that the relative stereochemistries at C-6, -7, and -8 of **2** were the same as those of **6**. The absolute configuration of **2** was determined in the same manner as for **1**. Thus, the cd spectrum of the dibenzoate derivative [**7**] of **2** showed a positive chirality ($[\theta]_{237}$ +33000, $[\theta]_{221}$ −42000) due to the C-6 and C-8 benzoates, which suggested that the absolute configurations of C-6 and C-8 in **2** were *S* and *S*, respectively (see Figure 3).

The absolute configuration of litophynin E [**3**], which was isolated by Ochi *et al.*, has not yet been determined. We have investigated its absolute configuration at this time. The (+)-(*R*)-MTPA (**8***R*) and (−)-(*S*)-MTPA (**8***S*) esters of **3** were prepared, and a modified Mosher method (9) was applied to these MTPA esters. The positive and negative $\Delta\delta$ values were found on the right and left sides of the MTPA planes, respectively, indicating an *S* configuration for C-6. Thus, the absolute configuration of litophynin E was determined (see Figure 4).

The absolute configurations of these compounds were further confirmed as follows. Ozonolysis of **2** and **3** gave monooxo derivatives **9** and **10**, respectively (Scheme 1). Their structures were suggested by the ir absorptions due to the ketone group at C-11 (1715 cm^{-1}; **9**, 1720 cm^{-1}; **10**). The cd spectra of **9** and **10** showed similar negative Cotton

TABLE 1. ^{13}C-Nmr and ^{1}H-Nmr Data for Litophynols A [**1**] and B [**2**], Litophynin E [**3**], and Sclerophytin C [**6**].

Position	Compound							
	1		**2**		**3**		**6**	
	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)	δ_C	δ_H (*J*, Hz)
1	44.0 (d)		45.2 (d)		45.5 (d)	2.19 dd (4.0, 7.0)	45.0 (d)	
2	91.4 (d)	3.75 s	91.9 (d)	3.60 s	92.1 (d)	3.58 s	91.4 (d)	3.58 s
3	84.6 (s)		86.1 (s)		86.5 (s)		86.2 (s)	
4	35.2 (t)		35.2 (t)		36.2 (t)		34.5 (t)	
5	28.7 (t)		29.6 (t)		30.5 (t)		29.5 (t)	
6	66.8 (d)	4.72 dd (4.0, 11.0)	77.3 (d)	4.61 d (6.0)	80.2 (d)	4.58 d (6.0)	77.0 (d)	4.61 d (6.4)
7	152.1 (s)		79.8 (s)		76.9 (s)		79.6 (s)	
8	77.4 (d)	4.16 s	79.7 (d)	3.55 d (9.0)	45.9 (t)	1.81 dd (4.0, 15.0) 1.91 dd (11.0, 15.0)	79.5 (d)	3.54 d (8.8)
9	83.7 (d)	4.16 d (10.5)	81.2 (d)	3.88 dd (6.0, 9.0)	78.3 (d)	4.14 ddd (4.0, 7.0, 11.0)	81.1 (d)	3.86 dd (6.3, 8.8)
10	48.0 (d)	2.84 dd (8.0, 10.5)	52.9 (d)	3.29 t (6.5)	53.7 (d)	2.97 t (7.0)	52.5 (d)	3.25 dd (6.4, 6.5)
11	146.0 (s)		148.8 (s)		147.6 (s)		148.6 (s)	
12	31.6 (t)		31.7 (t)		31.5 (t)		31.6 (t)	
13	25.3 (t)		24.9 (t)		24.6 (t)		24.8 (t)	
14	44.4 (d)	1.28 br t	43.9 (d)		44.0 (d)		43.7 (d)	
15	22.2 (q)	1.61 s	23.1 (q)	1.41 s	23.2 (q)	1.40 s	23.0 (q)	1.40 s
16	118.2 (t)	5.22 s, 5.52 s	17.6 (q)	1.25 s	22.7 (q)	1.16 s	17.7 (q)	1.23 s
17	111.6 (t)	4.65 s, 4.81 s	110.3 (t)	4.79 s, 4.89 s	109.4 (t)	4.65 s, 4.69 s	109.9 (t)	4.78 s, 4.87 s
18	27.5 (d)	1.87 m	29.1 (d)		29.1 (d)		29.0 (d)	
19	15.5 (q)	0.75 d (6.5)	16.1 (q)	0.80 d (6.5)	15.7 (q)	0.79 d (6.5)	16.2 (q)	0.80 d (6.5)
20	21.9 (q)	0.97 d (7.0)	22.0 (q)	0.97 d (7.0)	22.0 (q)	0.97 d (7.0)	21.9 (q)	0.96 d (6.5)
1′	172.6 (s)		172.4 (s)		172.3 (s)		169.5 (s)	
2′	37.4 (t)		37.4 (t)		37.4 (t)		22.7 (q)	2.01 s
3′	18.5 (t)		18.4 (t)		18.4 (t)			
4′	13.6 (q)	0.92 t (7.5)	13.7 (q)	0.98 t (7.5)	13.7 (q)	0.99 t (7.5)		

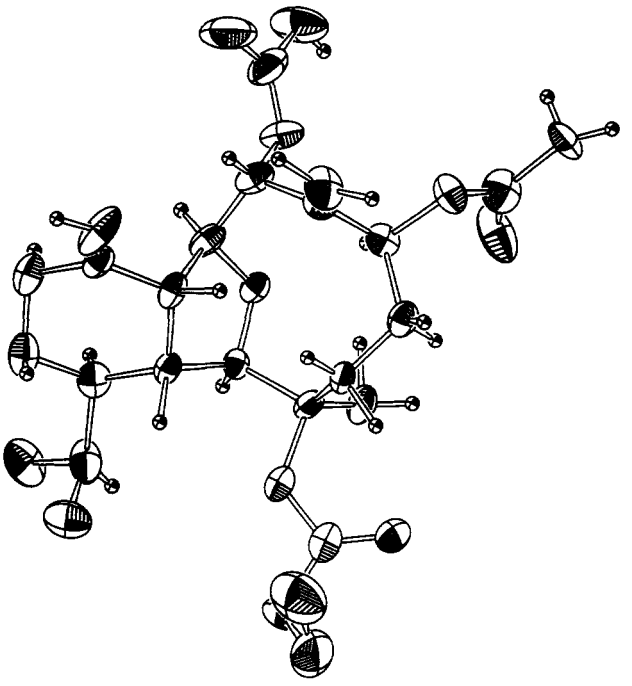


FIGURE 1. ORTEP view of **4**.

effect curves at 294 nm ($\Delta\epsilon$ ext -0.7; **9**) and at 297 nm ($\Delta\epsilon$ ext -0.5; **10**), and application of the octant rule (10) to their cd spectra supported the absolute configuration determined as described above.

We investigated the constituents of the animal bodies, from which the mucus was removed. Five eunicellin-type diterpenoids (**1–3**, **11**, and **12**) were also obtained in low yield compared with the mucus. Litophynols A [**1**] and B [**2**], litophynins E [**3**] and H [**11**], and I monoacetate [**12**] were positive in a hemolytic reaction test, and crude diterpenoid fractions exhibited ichthyotoxicity (IC_{100} 20 ppm). This suggests that this soft coral holds eunicellin-type diterpenoids in its mucus for the purpose of defense against predators.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Yanaco micro-melting point

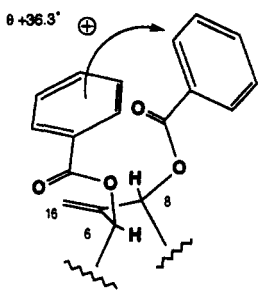


FIGURE 2. The dihedral angle between C-6 and C-8 in benzoate was examined by nOe experiments, *J* values, and MM2 (13) calculations.

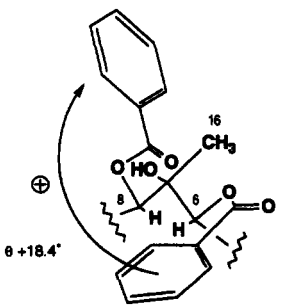


FIGURE 3. The dihedral angle between C-6 and C-8 in benzoate was examined by nOe experiments, *J* values, and MM2 (13) calculations.

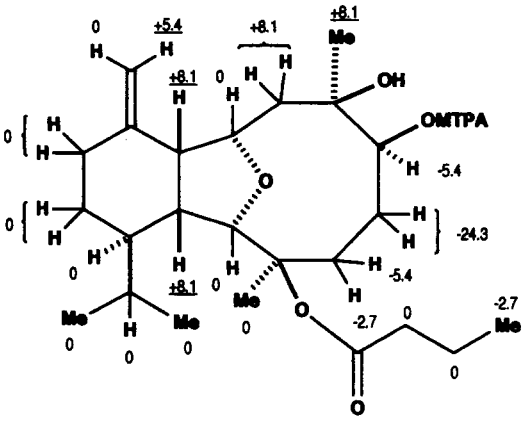
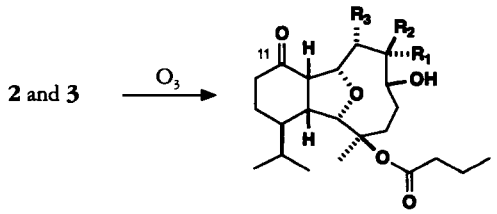


FIGURE 4. Δδ Values ($\Delta\delta=\delta_S-\delta_R$ in Hz at 270 MHz) obtained for MTPA esters $\mathbf{8}_R/\mathbf{8}_S$.

apparatus and are uncorrected. Spectra were recorded on the following instruments: specific optical rotations, Jasco DIP-370 digital polarimeter; circular dichroism, Jasco J-600 spectrophotometer; ir, Jasco IR-810 spectrophotometer; eims/hreims, JEOL JMS-DX-300/JMA-3500 data system, accelerating potential of 3 kV, ionizing potential of 30 eV, sample temperature of 200–250°; nmr, JEOL FX-270 (270 MHz) spectrometer in $CDCl_3$. X-ray crystallographic measurements were made on a Rigaku RASA-5R automatic single-crystal X-ray structure analysis system. Normal and reversed-phase tlc were performed with Merck Si gel 60 F_{254} and RP-8 F_{254}, respectively. Cc was carried out with Pharmacia Sephadex LH-20, Merck Si gel 60 (0.063–0.200 μm), and Fuji Davison Si gel BW-300. Hplc and mplc separations were performed on a Jasco BIP 1 hplc pump and a RID-300 RI detector. The columns were Merck LiChroprep RP-8 (40–63 μm) and Waters radial pak 8NVC$_{18}$ (4 μm) (RCM 8×10).

COLLECTION AND TAXONOMY.—The soft coral is probably an undescribed species of the genus *Litophyton* (phylum Cnidaria, class Anthozoa, subclass Octocorallia, order Alcyonaceae, family Nephtheidae), and was collected from the rocky coast of Nango-cho, Miyazaki Prefecture, Japan, at 3 m deep, in November 1989. It has a short sterile stalk and bush-like capitulum. Unfortunately, the base and the lower part of the stalk were missing. The capitulum was covered with non-retractile monomorphic zooids, 0.3 mm in diameter, 1.2 mm in height including tentacles. Sclerites of the stalk cortex are warty spindles, 0.03×0.17 mm. The stalk interior contained a few spindles, 0.02×0.11 mm. The capitulum was free from sclerites. The color of the colony is creamy white when stored in alcohol. The present specimen is closely related to *Litophyton viridis* (May, 1898) in the absence of the sclerite in the capitulum, but is different in the size of sclerite at the stalk. A voucher specimen is deposited in the Wakayama Prefecture Museum of Natural History (Catalog No. 1994-INV-0004).

EXTRACTION AND ISOLATION.—The mucus (4.2 kg) was separated from the animal (7.9 kg, wet wt) and extracted with EtOH (14 liters). The EtOH layer was evaporated *in vacuo* and the residue partitioned between H_2O (1 liter) and Et_2O (1.8 liter). The Et_2O layer was evaporated *in vacuo* to give 20.6 g of extract. Part of this (11.5 g) was chromatographed on a Sephadex LH-20 column with $CHCl_3$-MeOH (1:1) as eluent to yield three fractions: fractions 1 (941.9 mg), 2 (10.43 g), and 3 (146.0 mg). Fraction 2 was



9 R_1=OH, R_2=CH_3, R_3=OH
10 R_1=CH_3, R_2=OH, R_3=H

SCHEME 1

chromatographed on a Si gel column. Elution was performed with *n*-hexane-EtOAc (4:1→EtOAc) to yield two main fractions which exhibited hemolytic activity: fraction 4 (352.6 mg) and fraction 5 (275.8 mg). Fraction 4 was chromatographed on LiChroprep RP-8 with 85% aqueous MeOH, followed by column chromatography on Si gel (BW-300) with *n*-hexane-EtOAc (3:2) to give pure litophynin E [**3**] (33.3 mg). Fraction 5 was chromatographed on LiChroprep RP-8 with 85% aqueous MeOH to give two hemolytic fractions: fraction 6 (95.2 mg) and fraction 7 (35.6 mg). Fraction 6 was subjected to hplc ($8NVC_{18}$) with 70% aqueous MeOH to give litophynol B [**2**] (36.2 mg), litophynin H [**11**] (7.8 mg), and litophynin I monoacetate [**12**] (5.0 mg). Fraction 7 was chromatographed on Si gel (BW-300) with *n*-hexane-EtOAc (1:2) to give litophynol A [**1**] (26.8 mg).

HEMOLYTIC ACTIVITY.—Performed with a 2% erythrocyte suspension: fresh rabbit blood (1 ml) was washed three times with 0.1 M phosphate-buffered saline of pH 6.8 (PBS), and resuspended in 50 ml of 0.85% saline. Sample solutions were subjected to tlc with a suitable developing solvent, and the 2% erythrocyte suspension was sprayed on the plate. After 15 min, hemolysins were observed as pale red spots.

ICHTHYOTOXICITY.—Ichthyotoxicity assays were conducted using the mosquito fish, *Oryzias latipes*. Fractions 2, 4, and 5 were assayed at 20 ppm by dissolving the appropriate amount in 1 ml of EtOH. Control tests were carried out with each test run. The toxicity was evaluated as times for lethality to occur. Fractions 4 and 5 killed fish within 24 h at 20 ppm.

Litophynol A [**1**].—Amorphous solid; mp 131–132°; $[\alpha]^{28}D$ +19.2° (c=1.1, $CHCl_3$); ir ($CHCl_3$) ν max 3610, 3100, 1730, 1655, 910 cm^{-1}; fdms *m/z* 406 $[M^+]$, 389 [M−17], 319 [M−87]; hreims *m/z*, found $[M^+]$ 406.2697 ($C_{24}H_{38}O_5$ requires 406.2718); ^1H nmr and ^{13}C nmr, see Table 1.

Acetylation of **1**.—Litophynol A [**1**] (13.9 mg) was dissolved in pyridine (1.5 ml) and added to (Ac_2O) (1.5 ml). The reaction mixture was stood for 24 h at room temperature, and was then poured into excess H_2O (50 ml). The aqueous layer was extracted with $CHCl_3$ (20, 10, 10 ml). The $CHCl_3$ layer was evaporated *in vacuo* to yield the crude extract, which was treated by chromatography on Si gel using *n*-hexane-EtOAc (3:1) as eluent to yield a pure diacetate [**4**] (10.0 mg) as colorless plates; $[\alpha]^{27}D$ −22.4° (c=0.9, $CHCl_3$); fdms *m/z* 490 $[M]^+$, 431 [M−59], 403 [M−87]; ^1H nmr (270 MHz, $CDCl_3$) δ 0.76 (3H, d, J=6.9 Hz, CH_3-19), 0.91 (3H, t, J=7.6 Hz, H-4′), 0.97 (3H, d, J=7.3 Hz, CH_3-20), 1.65 (3H, s, H-15), 1.97 (3H, s, $COCH_3$), 2.06 (3H, s, $COCH_3$), 2.95 (1H, dd, J=11.1 and 7.8 Hz, H-10), 3.82 (1H, s, H-2), 4.17 (1H, d, J=10.7 Hz, H-9), 4.72 (1H, s, H-17), 4.86 (1H, t, J=1.7 Hz, H-17), 5.19 (1H, s, H-8), 5.49 (1H, s, H-16), 5.66 (1H, s, H-16), 5.79 (1H, dd, J=11.0 and 4.1 Hz, H-6).

X-RAY CRYSTAL STRUCTURE ANALYSIS OF **4**.—Crystal data: $C_{28}H_{42}O_7$; mol wt=490.64, orthorhombic, space group $P2_12_12_1$, No. 19, a=18.80 (2), b=23.04 (2), c=6.54 (1) Å (from 15 orientation reflections, 9.2°<2θ<11.1°), V=2832 (7) Å3, Z=4, Dc=1.151g/cm^3, m(MoKα radiation, λ=0.71069 Å). Intensity data were recorded on a Rigaku AFC 5R diffractometer [MoKα radiation, ω−2θ scans, θ max=55.1°, scanwidth (1.42+0.30 tan θ°)]. The intensities of three standard reflections re-measured every 150 reflections during data collection to monitor crystal stability, indicated that significant deterioration occurred (overall intensity loss = 0.5%). From a total of 3210 measurements, those 1200 reflections with $I>3.00\sigma(I)$ were retained for the analysis. Lorentz-polarization corrections were applied.

The crystal structure was solved by direct methods (SIR88) (12). The non-hydrogen atoms were refined anisotropically. The final cycle of full-matrix least-squares refinement was based on 1200 observed reflections and 388 variable parameters and converged with unweighted and weighted agreement factors of: R=0.067, Rw=0.076, GOF=2.73.

Crystallographic calculations were performed on a Micro-VAX 3200 by use of the TEXSAN Structure Analysis Software. In the least-squares iterations, $\Sigma\omega(|Fo|-|Fc|)2$, $\omega=4Fo^2/\sigma^2(Fo^2)$ was minimized.

Benzoylation of **1**.—Litophynol A [**1**] (2.5 mg) was dissolved in pyridine (5 μl) and added to benzoyl chloride (8 μl). The reaction mixture was stood for 24 h at room temperature, and was then poured into excess H_2O (50 ml). The aqueous layer was extracted with $CHCl_3$ (15, 10, 10 ml). The $CHCl_3$ layer was evaporated *in vacuo* to yield a crude extract, which was subjected to hplc [radial pak $8NVC_{18}$ (4 μm)] using 90% aqueous MeOH as eluent to yield a pure dibenzoate [**5**] (0.6 mg) which exhibited: fdms *m/z* 614 $[M]^+$, 493 [M−121]; ^1H nmr (270 MHz, $CDCl_3$) δ 0.78 (3H, d, J=6.6 Hz), 0.95 (3H, t, J=7.3 Hz), 0.98 (3H, d, J=6.6 Hz), 1.77 (3H, s), 3.08 (1H, dd, J=10.9 and 7.6 Hz), 3.90 (1H, s), 4.36 (1H, d, J=9.9 Hz), 4.83 (1H, s), 4.93 (1H, s), 5.57 (1H, s), 5.65 (1H, s), 5.85 (1H, s), 6.33 (1H, dd, J=11.2 and 4.0 Hz), 7.0–8.0 (10H, m); cd ($c=6.0\times10^{-6}$, MeOH), $\Delta\epsilon$ =+23.2 (λ ext = 238 nm), $\Delta\epsilon$ =−16.1 (λ ext = 224 nm).

Litophynol B [**2**].—Colorless oil; $[\alpha]^{28}D$ −17.6° (c=3.1, $CHCl_3$); ir ν max ($CHCl_3$) 3590, 3090, 1735, 1650, 905 cm^{-1}; fdms *m/z* 424 $[M]^+$, 406 [M−18], 337 [M−87]; hreims *m/z* found $[M]^+$ 424.2827 ($C_{24}H_{40}O_6$ requires 424.2823); ^1H nmr and ^{13}C nmr, see Table 1.

Benzoylation of **2**.—Litophynol B [**2**] (3.1 mg) was benzoylated as described above and purified with hplc [radial pak 8NVC$_{18}$ (4 μm)] using 90% aqueous MeOH as eluent to yield a pure dibenzoate [**7**] (2.5 mg) which exhibited: fdms *m/z* 632 [M]$^+$, 511 [M−121]; ^{1}H nmr (270 MHz, CDCl$_3$), 0.78 (3H, d, *J*=6.9 Hz), 0.99 (3H, d, *J*=6.9 Hz), 1.07 (3H, t, *J*=7.6 Hz), 1.27 (3H, s), 1.43 (3H, s), 3.51 (1H, t, *J*=7.0 Hz), 3.90 (1H, s), 3.72 (1H, s), 4.34 (3H, m), 5.61 (1H, d, *J*=9.6 Hz), 6.03 (1H, dd, *J*=2.4 and 5.4 Hz), 7.4–8.1 (10H, m); cd (*c*=2.5×10^{-5}, MeOH), Δε = +9.9 (λ ext = 237 nm), Δε = −12.8 (λ ext = 221 nm).

Litophynin E [**3**].—Colorless oil; [α]^{27}D +12.6° (*c*=3.1, CHCl$_3$); ir ν max (CHCl$_3$) 3600, 3100, 1735, 1650, 900 cm^{-1}; fdms *m/z* 408 [M]$^+$, 390 [M−18], 321 [M−87]; hreims *m/z* found [M]$^+$ 408.2869 (C$_{24}$H$_{40}$O$_5$ requires 408.2874); ^{1}H nmr and ^{13}C nmr, see Table 1.

Preparation of (R)- *and* (S)-*MTPA esters of litophynin E* [**3**].—Litophynin E [**3**] (8.0 mg, 19.6 μmol), (+)-MTPA acid (11.4 mg, 48.7 mmol), dicyclohexylcarbodiimide (10.7 mg, 51.9 μmol), and 4-(dimethylamino)pyridine (10.3 mg, 84.3 μmol) were dissolved in CH_2Cl_2 (5 ml). The reaction mixture was stirred in an atmosphere of N_2 for 113 h at room temperature, and the residue obtained after evaporation of the solvent was treated by chromatography on Si gel using *n*-hexane-Me$_2$CO (95:5) as eluent to yield the pure (*R*)-MTPA [**8**$_R$] (5.1 mg, 64%) and (*S*)-MTPA esters [**8**$_S$] (35%).

(R)-*MTPA ester* [**8**$_R$].—^{1}H nmr (270 MHz, CDCl$_3$) δ 0.78 (3H, d, *J*=6.9 Hz), 0.98 (3H, d, *J*=6.9 Hz), 1.00 (3H, t, *J*=7.3 Hz), 1.13 (3H, s), 1.28 (1H, m), 1.39 (3H, s), 1.57 (2H, m), 1.72 (2H, m), 1.73 (1H, m), 1.87 (2H, m), 2.17 (1H, m), 2.18 (1H, m), 2.32 (2H, m), 2.69 (1H, dd, *J*=14.5 and 7.6 Hz), 3.00 (1H, t, *J*=7.3 Hz), 3.58 (3H, s, MTPA), 3.66 (1H, s), 4.15 (1H, m), 4.66 (1H, s), 4.69 (1H, s), 5.86 (1H, d, *J*=5.0 Hz), 7.41 (3H, m), 7.54 (2H, m).

(S)-*MTPA ester* [**8**$_S$].—^{1}H nmr (270 MHz, CDCl$_3$) δ 0.79 (3H, d, *J*=6.8 Hz), 0.98 (3H, d, *J*=6.8 Hz), 0.99 (3H, t, *J*=7.3 Hz), 1.16 (3H, s), 1.28 (1H, m), 1.40 (3H, s), 1.48 (2H, m), 1.68 (2H, m), 1.76 (1H, m), 1.90 (2H, m), 2.15 (1H, m), 2.21 (1H, m), 2.32 (2H, m), 2.68 (1H, ddd, *J*=15.3, 8.5, and 3.1 Hz), 3.03 (1H, t, *J*=7.1 Hz), 3.52 (3H, s, MTPA), 3.66 (1H, s), 4.15 (1H, m), 4.66 (1H, s), 4.71 (1H, s), 5.84 (1H, d, *J*=5.0 Hz), 7.40 (3H, m), 7.55 (2H, m).

Ozonolysis of litophynol A [**2**] *and litophynin E* [**3**].—Ozone was bubbled through a solution of **2** (5.8 mg) in 1 ml of MeOH at −78° until the solution colored pale blue. The residue obtained after evaporation of the solvent was treated with dimethyl sulfide (10 μl) for 12 h, and then poured into excess H_2O (50 ml). The aqueous layer was extracted with $CHCl_3$ (10, 5, 5 ml). The $CHCl_3$ layer was evaporated *in vacuo* to yield a crude extract, which was treated by chromatography on Si gel using *n*-hexane-Me$_2$CO (1:1) as eluent to yield the pure oxo-derivative of **2** [**9**] (1.5 mg, 26%) and the oxo-derivative of **3** [**10**] (1.9 mg, 53%).

Oxo-derivative **9**.—Ir ν max (CHCl$_3$) 3600, 1735, 1720 cm^{-1}; cd (*c*=1.6×10^{-4}, MeOH), Δε = −0.5 (λ ext = 297 nm); fabms (positive ion, *m/z*) 409 [M−17, base peak]; ^{1}H nmr (270 MHz, CDCl$_3$) δ 0.90 (3H, d, *J*=6.6 Hz), 0.97 (3H, t, *J*=7.3 Hz), 1.05 (3H, d, *J*=6.6 Hz), 1.27 (3H, s), 1.48 (3H, s), 2.56 (1H, dt, *J*=4.6 and 17.2 Hz), 2.70 (1H, ddd, *J*=3.3, 8.9, and 8.9 Hz), 3.11 (1H, dd, *J*=4.4 and 7.9 Hz), 3.17 (1H, d, *J*=4.6 Hz), 3.51 (1H, dd, *J*=4.6 and 9.6 Hz), 3.81 (1H, d, *J*=3.3 Hz), 4.15 (1H, dd, *J*=4.6 and 9.6 Hz), 4.57 (1H, br d, *J*= 7.6 Hz); ^{13}C nmr (67.5 MHz, CDCl$_3$) δ 44.6 (d, C-1), 90.6 (d, C-2), 86.0 (s, C-3), 34.0 (t, C-4), 29.3 (t, C-5), 77.4 (d, C-6), 78.6 (s, C-7), 75.8 (d, C-8), 80.2 (d, C-9), 57.2 (d, C-10), 213.2 (s, C-11), 38.0 (t, C-12), 22.1 (t, C-13), 42.3 (d, C-14), 23.2 (q, C-15), 17.9 (q, C-16), 28.8 (d, C-18), 16.7 (q, C-19), 21.9 (q, C-20), 172.1 (s, C-1′), 37.3 (t, C-2′), 18.3 (t, C-3′), 13.6 (q, C-4′).

Oxo-derivative **10**.—Ir ν max (CHCl$_3$) 3600, 1735, 1715 cm^{-1}; cd (*c*=1.9×10^{-4}, MeOH), Δε = −0.7 (λ ext = 294 nm); fabms (positive ion, *m/z*) 393 [M−17, base peak]; ^{1}H nmr (270 MHz, CDCl$_3$) δ 0.89 (3H, d, *J*=6.9 Hz), 0.97 (3H, t, *J*=7.3 Hz), 1.05 (3H, d, *J*=6.9 Hz), 1.16 (3H, s), 1.50 (3H, s), 2.70 (1H, ddd, *J*=3.0, 7.9, and 7.9 Hz), 2.77 (1H, dd, *J*=4.6 and 7.6 Hz), 3.76 (1H, d, *J*=3.0 Hz), 4.51 (1H, br d, *J*=8.3 Hz), 4.59 (1H, dt, *J*=4.2 and 11.6 Hz); ^{13}C nmr (67.5 MHz, CDCl$_3$) δ 45.7 (d, C-1), 90.7 (d, C-2), 86.0 (s, C-3), 34.1 (t, C-4), 30.3 (t, C-5), 78.1 (d, C-6), 76.3 (s, C-7), 46.1 (t, C-8), 77.2 (d, C-9), 57.9 (d, C-10), 210.6 (s, C-11), 37.8 (t, C-12), 22.6 (t, C-13), 42.7 (d, C-14), 23.4 (q, C-15), 22.6 (q, C-16), 28.9 (d, C-18), 17.0 (q, C-19), 22.0 (q, C-20), 172.3 (s, C-1′), 37.3 (t, C-2′), 18.4 (t, C-3′), 13.7 (q, C-4′).

Litophynin H [**11**].—Amorphous solid; [α]^{27}D +10.6° (*c*=0.5, CHCl$_3$); ir ν max (CHCl$_3$) 3600, 1730, 1650, 920 cm^{-1}; fdms *m/z* 406 [M]$^+$ 389 [M−17], 319 [M−87]; hreims *m/z* found [M]$^+$ 406.2740 (C$_{24}$H$_{38}$O$_5$ requires 406.2717); ^{1}H nmr (270 MHz, CDCl$_3$) 0.76 (3H, d, *J*=6.6 Hz), 0.92 (3H, t, *J*=7.4 Hz), 0.99 (3H, d, *J*=6.6 Hz), 1.64 (3H, s), 3.07 (1H, dd, *J*=10.6 and 8.3 Hz), 3.79 (1H, s), 4.41 (2H, m), 4.54 (1H, dd, *J*=10.4 and 4.1 Hz), 4.86 (1H, d, *J*=1.3 Hz), 5.11 (1H, s), 5.45 (1H, s); ^{13}C nmr (67.5 MHz, CDCl$_3$) δ 43.6 (d, C-1), 90.2 (d, C-2), 84.7 (s, C-3), 35.6 (t, C-4), 28.5 (t, C-5), 72.2 (d, C-6), 151.0 (s, C-7), 38.5 (t, C-8), 81.9 (d, C-9), 45.8 (d, C-10), 146.6 (s, C-11), 71.7 (d, C-12), 31.1 (t, C-13), 35.9 (d, C-14), 22.1 (q, C-15), 116.6 (t, C-16), 116.0 (t, C-17), 26.9 (d, C-18), 15.5 (q, C-19), 21.7 (q, C-20), 172.2 (s, C-1′), 37.4 (t, C-2′), 18.6 (t, C-3′), 13.6 (q, C-4′).

Litophynin I monoacetate [**12**].—Colorless oil; $[\alpha]^{27}D$ +40.1° (c=0.3, $CHCl_3$); ir ν max ($CHCl_3$) 3600, 1740, 1655, 910 cm^{-1}; fdms *m/z* 466 $[M]^+$, 449 [M−17], 406 [M−60], 379 [M−87]; hreims *m/z* found $[M]^+$ 466.2962 ($C_{26}H_{42}O_7$ requires 466.2928); ^1H nmr (270 MHz, $CDCl_3$) δ 0.82 (3H, d, J=6.9 Hz), 0.96 (3H, d, J=6.9 Hz), 0.98 (3H, t, J=7.4 Hz), 1.17 (3H, s), 1.45 (3H, s), 2.07 (3H, s), 2.97 (1H, t, J=6.6 Hz), 3.69 (1H, d, J=1.7 Hz), 4.39 (1H, m), 4.58 (1H, d, J=5.9 Hz), 4.97 (1H, s), 5.11 (1H, s), 5.15 (1H, s), 5.45 (1H, dd, J=5.0 and 2.6 Hz); ^{13}C nmr (67.5 MHz, $CDCl_3$) δ 44.3 (d, C-1), 90.5 (d, C-2), 86.4 (s, C-3), 35.3 (t, C-4), 30.5 (t, C-5), 79.4 (d, C-6), 77.2 (s, C-7), 45.9 (t, C-8), 78.9 (d, C-9), 51.5 (d, C-10), 143.0 (s, C-11), 72.6 (d, C-12), 28.9 (t, C-13), 37.0 (d, C-14), 23.3 (q, C-15), 22.7 (q, C-16), 115.3 (t, C-17), 28.8 (d, C-18), 16.0 (q, C-19), 21.8 (q, C-20), 172.2 (s, C-1′), 37.4 (t, C-2′), 18.4 (t, C-3′), 13.7 (q, C-4′), 170.3 (s, Ac), 21.5 (q, Ac).

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