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Litophynins I and J, Two New Biologically Active Diterpenoids from the Soft Coral Litophyton sp.

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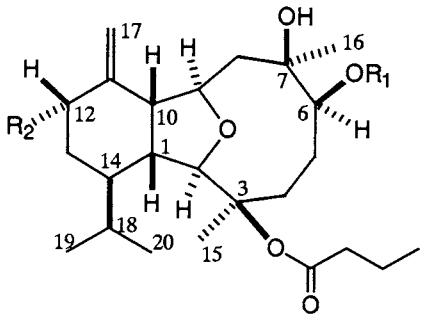
Two new diterpenoids, litophynins I and J, which exhibit molluscicidal and repellent activities against the muricid gastropod Drupella fragum, have been isolated from the soft coral Litophyton sp. Their structures were fully characterized by extensive 2D-NMR studies.

Our investigation of the soft coral Litophyton sp. has resulted in the isolation of a series of diterpenoids of eunicellin class which exhibit insect growth inhibitory activity against the silkworm, Bombyx mori L.[1] Recently, these insect growth inhibitors were also noted to possess molluscicidal and repellent activities against the muricid gastropods of the genus Drupella, which have caused environmental problems through feeding on polyps of stony corals to result in widespread destruction of scleractinian corals.[2] From the marine ecological point of view, therefore, we reexamined the methanol extract of Litophyton sp. Bioassay-guided purification of the extract has now led to the isolation of two new diterpenoids, named litophynins I and J. This paper deals with the structures of these new compounds.

Litophynin I (**1**) was isolated as fine needles (0.00048%, wet weight), mp 122.5-123.5°C, $[\alpha]_D^{20}$ +45.2° (c 0.58, $CHCl_3$), from the dichloromethane soluble fraction of the methanol extract of the frozen specimens through Sephadex LH-20 (MeOH) and silica gel (hexane/EtOAc) column chromatography, followed by reverse phase HPLC (ODS column, MeOH/H_2O 7:3). The molecular formula, $C_{24}H_{40}O_6$, was established by high resolution mass spectrum (m/z 424.2831, M^+, Δ +0.6 mmu). It showed IR absorptions indicative of hydroxyl (3410), ester (1730), and exocyclic methylene (3060, 1645, and 910 cm^{-1}) groups, and formed a diacetate **3**, $C_{28}H_{44}O_8$, colorless oil,[3] the IR spectrun of which still showed hydroxylic absorptions at 3600 and 3450 cm^{-1}, on acetylation with Ac_2O/pyridine. The close structural similarity between

3 and litophynin E acetate (4)[4] was revealed by the comparison of their spectral data. The ^{13}C NMR data of 3 included twenty signals compatible with the carbon frame work of 4. The difference between 3 and 4 resided solely in the presence of two secondary acetoxyl groups in 3 [δ_H 2.04, 2.07 (3H each, s), 5.48 (1H, t, J=3.4 Hz), and 5.64 (1H, brd, J=5.5 Hz); δ_C 21.36, 21.52, 72.89, 84.43, 170.31, and 171.77], one more than that of 4. A combination of the ^1H-^1H and ^1H-^{13}C COSY spectra together with partial spin decoupling studies allowed a complete assignment of all the proton and carbon resonances, leading to a gross structure 3 for the acetate. The location of the second acetoxyl group at $C_{12\alpha}$ in 3 was evident from the ^1H-^{13}C long-range coupling between 17-Hb and C_{12} and the coupling pattern of 12-H [δ 5.48 (t, J=3.4 Hz)]. The relative stereochemistry at remaining chiral centers of 3 was the same as that of 4 judging from the NOESY experiments, the results of which are depicted in 3a. Thus the structure 3 is assigned to the acetate, and hence the structure 1 to litophynin I.

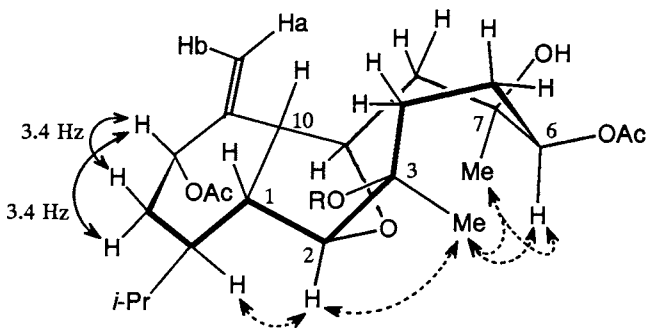
Litophynin J (2) was obtained as fine needles (0.00087%, wet weight), $C_{24}H_{38}O_5$ (m/z 406.2722, M^+, Δ +0.2 mmu), mp 120.0-121.5°C, $[\alpha]_D^{20}$ +5.9° (c 0.51, $CHCl_3$), from the less polar fraction. It also showed IR absorptions indicative of hydroxyl (3615, 3425), ester (1735), and exocyclic methylene (3060, 1640, and 910 cm^{-1}) groups, and formed a monoacetate 5, $C_{26}H_{40}O_6$, colorless oil,[5] which displayed spectral data similar to those of 3. The only significant difference in their ^1H and ^{13}C NMR data was the replacement of the monoacetylated vic-glycol system at C_6-C_7 in 3 by a grouping $-\overset{\overset{O}{\|}}{C}-\overset{\overset{CH_3}{|}}{CH}-$ [δ_H 1.06 (3H, d, J=6.7 Hz) and 2.68 (1H, m); δ_C 15.42, 40.62, and 213.23] in 5. Observations of NOEs between 7-H and 9-H and among 2-H, 3-Me, and 4α-H defined the relative stereochemistry at C_7 as depicted in 5a.



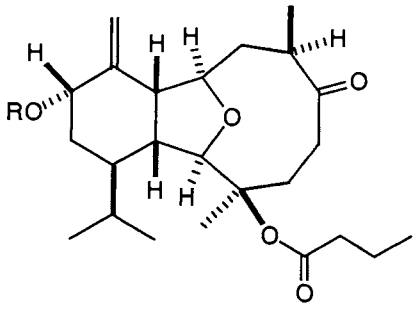
1 R_1 = H, R_2 = OH

3 R_1 = Ac, R_2 = OAc

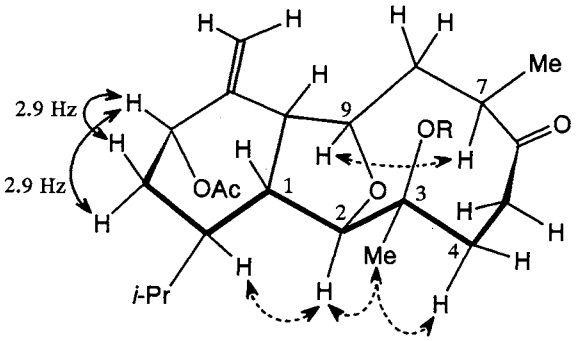
4 R_1 = Ac, R_2 = H



3a



2 R = H
5 R = Ac



NOE
J
R = COC_3H_7

5a

From the evidence outlined above, we proposed the structure **5** for the acetate and, consequently, the structure **2** for litophynin J.

Both litophynins I and J possess significant molluscicidal and repellent activities[6] against the muricid gastropod Drupella fragum. At 30 ppm concentration, they exhibit 100% mortality to the snail within 24 hours. They are also repellent to the gastropod when impregnated on filterpaper by 45 $\mu g/cm^2$. These compounds in combination with a wide variety of compounds stored in skin glands of Litophyton sp., which is devoid of physical means of defense, appear to be the foundation of a chemical defense adaptation to survive in predator-rich environments.

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References

1) M. Ochi, K. Yamada, K. Futatsugi, H. Kotsuki, and K. Shibata, Heterocycles, **32**, 29 (1991) and references cited therein.
2) J. T. Moyer, W. K. Emerson, and M. Ross, The Nautilus, **96**, 69 (1982); L. M. Boucher, Bull. Mar. Sci., **38**, 9 (1986).
3) **3**: ^1H NMR (400 MHz, $CDCl_3$) δ 0.80 and 0.96 (3H each, d, J=7.0 Hz, 19- and 20-H_3), 0.99 (3H, t, J=7.3 Hz, 4'-H_3), 1.19 and 1.41 (3H each, s, 16- and 15-H_3), 1.67 and 1.69 (1H each, sext, J=7.3 Hz, 3'-H_2), 1.72 (1H, m, 14-H), 1.83 (1H, m, 18-H), 2.04 and 2.07 (3H each, s, 2Ac), 2.28 and 2.33 (1H each, t, J=7.3 Hz, 2'-H_2), 2.28 (1H, m, 1-H), 3.03 (1H, t, J=7.2 Hz, 10-H), 3.72 (1H, s, 2-H), 4.38 (1H, dd, J=14.7 and 7.3 Hz, 9-

H), 4.94 (1H, s, 17-Ha), 5.15 (1H, d, J=1.5 Hz, 17-Hb), 5.48 (1H, t, J=3.4 Hz, 12-H), and 5.64 (1H, brd, J=5.5 Hz, 6-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.70 ($C_{4'}$), 15.38 (C_{19}), 18.46 ($C_{3'}$), 21.36 and 21.52 (2Ac), 21.67 (C_{20}), 23.16 (C_{15}), 23.74 (C_{16}), 28.53 (C_{18}), 28.68 (C_{13}), 29.19 (C_5), 35.71 (C_4), 36.51 (C_{14}), 37.48 ($C_{2'}$), 44.83 (C_1), 46.21 (C_8), 51.97 (C_{10}), 72.89 (C_{12}), 75.48 (C_7), 79.26 (C_9), 84.43 (C_6), 86.58 (C_3), 91.33 (C_2), 116.55 (C_{17}), 143.04 (C_{11}), 170.31 and 171.77 (2Ac), and 172.16 ($C_{1'}$).

4) M. Ochi, K. Yamada, K. Futatsugi, H. Kotsuki, and K. Shibata, Chem. Lett., **1990**, 2183.

5) **5**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 and 0.94 (3H each, d, J=6.9 Hz, 19- and 20-H_3), 1.00 (3H, t, J=7.4 Hz, 4'-H_3), 1.06 (3H, d, J=6.7 Hz, 16-H_3), 1.47 (3H, s, 15-H_3), 1.69 and 1.70 (1H each, sext, J=7.4 Hz, 3'-H_2), 1.75 (1H, brt, J=2.7 Hz, 14-H), 1.83 (1H, m, 18-H), 1.96 (3H, s, Ac), 2.27 (1H, m, 1-H), 2.32 and 2.34 (1H each, t, J=7.4 Hz, 2'-H_2), 2.68 (1H, m, 7-H), 3.09 (1H, dd, J=10.0 and 7.3 Hz, 10-H), 3.74 (1H, s, 2-H), 4.24 (1H, ddd, J=10.0, 5.1, and 4.9 Hz, 9-H), 4.97 and 5.23 (1H each, d, J=1.7 Hz, 17-H_2), and 5.49 (1H, t, J=2.9 Hz, 12-H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 13.70 ($C_{4'}$), 14.99 (C_{19}), 15.42 (C_{16}), 18.53 ($C_{3'}$), 21.39 (Ac), 21.57 (C_{20}), 22.74 (C_{15}), 27.51 (C_{18}), 28.94 (C_{13}), 33.04 (C_4), 35.78 (C_{14}), 37.49 ($C_{2'}$), 37.67 (C_8), 38.46 (C_5), 40.62 (C_7), 45.14 (C_1), 49.45 (C_{10}), 73.12 (C_{12}), 78.91 (C_9), 84.62 (C_3), 90.90 (C_2), 118.60 (C_{17}), 141.82 (C_{11}), 169.93 (Ac), 172.45 ($C_{1'}$), and 213.23 (C_6).

6) The procedures for the bioassays with D. fragum will be discussed elsewhere together with the details of the both activities for diterpenoids of eunicellin class as well as many types of compounds isolated from marine invertebrates.

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