Two New Spongian Diterpenes from *Coscinoderma mathewsi*

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Two new spongian diterpenes were isolated from the sponge, *Coscinoderma mathewsi*. One possesses a tricyclic skeleton bearing an aldehyde function, the other is a tetracyclic lactol. The structures of two compounds are elucidated by spectral method.

The marine sponge *Coscinoderma mathewsi* Lendenfeld (order Dictyoceratida, family Spongiidae) has been a source of various terpenoid compounds,[1−3] as have other members of this family.[4−7] In a continued investigation of this sponge, we have isolated three known diterpenes[7]—spongia-13(16),14-dien-19-oic acid (**1**), 15-oxospongi-13-en-19-oic acid (**2**), and 16-oxospongi-13-en-19-oic acid (**3**)—in addition to two new compounds, *ent*-13-norisocopalen-15-al-18-oic acid (**4**) and 15ξ-hydroxy-16-oxospongi-13-en-19-oic acid (**5**), which are the subject of this report.

The sponge was soaked in MeOH and the MeOH extract was partitioned between $CHCl_3$ and H_2O. The $CHCl_3$ layer was subjected to flash Si gel chromatography using a stepwise gradient of hexane/ethyl acetate. The 40% ethyl acetate fraction was further purified by ODS−HPLC using CH_3CN/H_2O (7:3). The known diterpenes **1** (major), **2**, and **3** (minor) were isolated in addition to two new spongian derivatives, *ent*-13-norisocopalen-15-al-18-oic acid (**4**) and 15-ξ-hydroxy-16-oxospongi-13-en-19-oic acid (**5**). The ^1H and ^{13}C NMR spectra (solvent, $CDCl_3$) of **2** and **3** were almost identical with the literature data except the splitting pattern of the H-15 methylene signal in **3**.[7] The H-15 signals of compound **3** at δ 4.74 and 4.64 appear as double doublets (J = 17.1, 2.7, 2.7 Hz and J = 17.1, 3.5, 1.5 Hz). The coupling constant of J = 17.1 Hz is the geminal coupling of H-15, while the small coupling constants might be long range couplings between H-15 and H-12 apparently due to the rigid skeleton of 16-oxospongi-13-en-19-oic acid (**3**). On the other hand, H-16 signals of compound **2** appear as doublets showing typical AB patterns (J = 17.0 Hz) at δ 4.59 and 4.52. (See Figure 1.)

The identity of *ent*-13-norisocopalen-15-al-18-oic acid (**4**) was established by comparison of its spectral properties with literature data.[4,7] Thus, the ^1H and ^{13}C NMR data of **4** are similar to those of **1**, **2**, and **3**, especially the three tertiary methyl groups (δ 1.25, 1.07, and 0.82, and δ_C 28.8, 21.2, and 14.1) and a carboxyl group (δ_C 181.1) in rings A and B were consistent with those of known spongian derivatives. The most significant difference between **4** and previously described spongian derivatives was observed in the aldehyde signal (δ 9.38 and δ_C 195.0) and double bond [δ 6.37, and δ_C 162.5 (d) and 137.6 (s)]. HMBC correlations between the olefinic signal at δ 6.37 (H-14) and carbon signals at δ_C 195.0 (C-15), 54.8 (C-9), 39.7 (C-7), and 23.3 (C-12) were observed. Also, the aldehyde proton signal at δ 9.38 (H-15) showed HMBC correlations with the olefinic carbon at δ_C 137.6 (C-13) and with the methylene carbon at δ_C 23.3 (C-12). These results proved that the aldehyde function is attached to the olefinic C-13 in ring C. This compound might be derived by decarbonylation of 15-oxospongi-13-en-19-oic acid (**2**). The molecular formula, $C_{19}H_{28}O_3$, of *ent*-13-norisocopalen-15-al-18-oic acid (**4**) was supported by HRFABMS data (*m/z* 303.1953 [M − H]$^-$).

15-ξ-Hydroxy-16-oxospongi-13-en-19-oic acid (**5**) displays an acetal proton signal at δ 6.06 (δ_C 97.5) and exchangeable proton signals at δ 7.76 and 7.52. These exchangeable proton signals have a integral ratio of approximately 5:1. Except for them, its ^1H and ^{13}C NMR spectral data were very similar to those of 15α-methoxy-16-oxospongi-13-en-19-oic acid (**6**) recently isolated from *Spongia matamata*.[7] The different appearance of exchangeable proton signals might be due to 15α- and β-epimers. This was confirmed by addition of D_2O, which resulted in separation of the acetal proton (δ 6.06, br s) into δ 6.08 and 6.02, in the ratio of ca. 1:5. An HMBC experiment showed the correlations from H-12 (δ 2.22) to the olefinic quaternary carbon signals at δ_C 168.5 (C-14) and 126.0 (C-13), but unfortunately, the correlation between the acetal signal at δ 6.06 and the carbon signals was ambiguous. The HRFABMS data (*m/z* 347.1850 [M − H]$^-$) revealed the molecular formula, $C_{20}H_{28}O_5$, of 15-α(and 15β)-hydroxy-16-oxospongi-13-en-19-oic acid (**5**) (Figure 2).

According to Li et al.,[7] spongian derivatives **2**, **3**, and **6** are natural products. It might be considered that compounds **2** and **3** were produced by oxidation of **1**, and subsequently H-15 of **3** was substituted by a hydroxy or methoxy group to give compound **5** or **6**. So, we examined whether the decomposition of spongia-13(16),14-dien-19-oic acid (**1**) occurs with methanol in the presence of trifluoroacetic acid (TFA). Though the reaction was carried out at reflux for 3 h, the starting material (**1**) was quantitatively recovered. Compound **1** is very stable. When compound **2** was treated with sodium methoxide at room temperature for 24 h, **2** was recovered. Thus, we also consider that **5** and **6** are not artifacts.

Experimental Section

Animal Material. A specimen of grayish black sponge was collected at Paliker Pass, Pohnpei, June 19, 1990, and kept frozen prior to being freeze-dried. The sponge is *Coscinoderma mathewsi* Lendenfeld (order Dictyoceratida, family Spongiidae). A voucher specimen has been deposited at the Natural History Museum, London, U.K. (BMNH 1996:6:6:1).

Extraction and Isolation. The freeze-dried sponge (220 g) was soaked in MeOH (3 L), and the MeOH extract (66 g) was partitioned by the solvent system of $CHCl_3$−H_2O−MeOH (7:5:1, 1.95 L). A part (9.8 g) of $CHCl_3$ layer (20 g) was subjected to flash Si gel chromatography with a stepwise gradient solvent system of 1.0 L each: hexane−ethyl acetate (10:0), (6:4), (2:8), (0:10); $CHCl_3$−MeOH−H_2O (8:2:0.1), and

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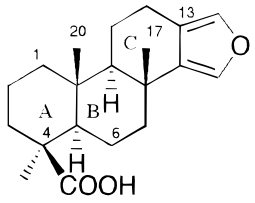
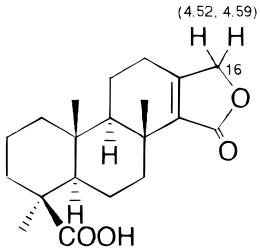


Spongia-13(16),14-dien-19-oic acid (**1**)



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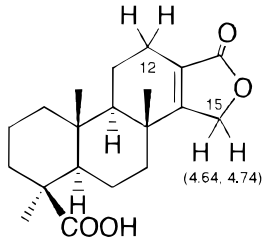
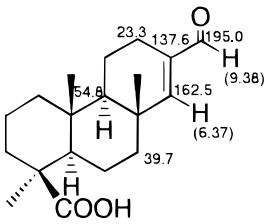
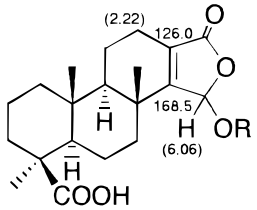


16-Oxospongi-13-en-19-oic acid (**3**)

Figure 1.



ent-13-Norisocopalen-15-al-18-oic acid (**4**)



15-ξ-Hydroxy-16-oxospongi-13-en-19-oic acid (**5**) R=H

15-α-Methoxy-16-oxospongi-13-en-19-oic-acid (**6**) R=Me

Figure 2.

MeOH to yield six fractions. The 40% ethyl acetate fraction (1.39 g) was again subjected to flash Si chromatography using hexane−ethyl acetate (7:3 and 0:10). The 30% ethyl acetate fraction was separated by Si gel HPLC using 20% ethyl acetate in hexane to afford **1** (9.9×10^{-3} %, based on dry wt). The second fraction was further purified by ODS−HPLC, again with CH_3CN-H_2O (7:3), to give **2** and **3** (9.0×10^{-3} % and 8.7×10^{-3} %).

***ent*-13-Norisocopalen-15-al-18-oic acid (4):** 2.0×10^{-3} %, colorless oil; $[\alpha]_D$ − 41° [*c* 0.029, MeOH−$CHCl_3$ (1:1)]; IR (KBr disk) ν_{max} 2924, 2851, 1686, 1676 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 9.38 (1H, s, H-15), 6.37 (1H, s, H-14), 2.41 (1H, dd, *J* = 18.0, 6.1 Hz, H-12e), 2.11 (1H, m, H-12a), 1.25 (3H, s, H-17), 1.13 (1H, dd, *J* = 12.6, 2.7 Hz, H-5), 1.07 (3H, s, H-16), 0.82 (3H, s, H-19); ^{13}C NMR δ 195.0 (d, C-15), 181.1 (s, C-18), 162.5 (d, C-14), 137.6 (s, C-13), 57.2 (d, C-5), 54.8 (d, C-9), 43.7 (s, C-4), 39.8, 39.7 (t, C-1, C-7), 38.1 (s, C-10), 38.0 (t, C-3), 37.0 (s, C-8), 28.8 (q, C-17), 23.3 (t, C-12), 21.2 (q, C-16), 19.9 (t, C-6), 19.0 (t, C-2), 16.9 (t, C-11), 14.1 (q, C-19); HRFABMS (matrix, glycerol) *m*/*z* 303.1953 $[M - H]^-$, (calcd for $C_{19}H_{27}O_3$, 303.1960).

15-ξ-Hydroxy-16-oxospongi-13-en-19-oic acid (5): 2.6×10^{-3} %, amorphous solid; $[\alpha]_D$ −46° [*c* 0.12, MeOH−$CHCl_3$ (1:1)]; IR (KBr disk) ν_{max} 3285, 2924, 2851, 1734, 1709, 1695 cm^{-1}; ^1H NMR (DMSO-d_6, 270 MHz) δ 7.76, 7.52 (1H, br s, 15-OH), 6.06 (1H, br s, H-15), 2.22 (1H, dd, *J* = 17.8, 5.6 Hz, H-12e), 1.16 (3H, s, H-17), 1.11 (3H, s, H-18), 0.77 (3H, s, H-20); ^{13}C NMR δ 178.7 (s, C-19), 171.2 (s, C-16), 168.5 (s, C-14), 126.0 (s, C-13), 97.5 (d, C-15), 56.2 (d, C-5), 54.7 (d, C-9), 43.1 (s, C-4), 38.4 (s, C-10), 37.8, 37.8 (t, C-1, C-3), 36.6 (s, C-8), 36.1 (t, C-7), 28.7 (q, C-18), 21.1 (t, C-12), 20.1 (q, C-17), 19.4 (t, C-11), 18.9 (t, C-6), 16.8 (t, C-2), 14.1 (q, C-20); HRFABMS (matrix, glycerol) *m*/*z* 347.1850 $[M - H]^-$, (calcd for $C_{20}H_{27}O_5$, 347.1859).

Reaction of 1 and MeOH in the Presence of TFA. Spongia-13(16),14-dien-19-oic acid (**1**) (3.1 mg) in 10% TFA in MeOH (2 mL) was refluxed for 3 h; the solvent was evaporated in vacuo. The residue was separated by Si gel−HPLC using hexane−ethyl acetate (9:1) to yield the starting material **1** quantitatively.

Reaction of 2 and Sodium Methoxide in Methanol. Compound **2** (4.8 mg) in 2.5% sodium methoxide solution (0.5 mL) was allowed to stand overnight. After neutralization with methanolic oxalic acid solution, the solvent was evaporated. The residue was partitioned between ether and water, and the ether extract was checked by NMR, and it proved **2** was recovered unchanged.

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