New Diterpene Lactones from the Sponge *Spongia matamata*

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Received November 19, 1997

Chemical investigation of the sponge *Spongia matamata*, collected in the western Pacific Island of Yap, has resulted in the isolation of a pair of new spongian diterpenoid epimers **2** and **3**, along with four other known spongians. The structures were determined by spectroscopic methods.

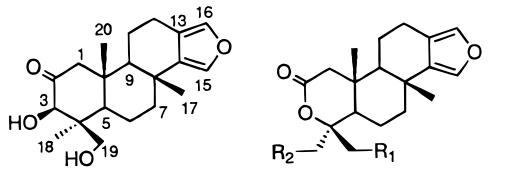
A broad array of sesqui-, di-, and sesterterpenoids have been isolated from sponges of the genus *Spongia*.[1] Prominent among these metabolites are a family of tetracyclic diterpenoids, the spongians, of which 3α,19-dihydroxyspongia-13(16),14-dien-2-one, **1**, is an example.[2] The members of this family vary primarily in the extent and pattern of oxidation at C-17 and ring A. A few of the metabolites have undergone oxidative cleavage of ring A to give lactones.[3,4] Some of the spongians have displayed antiviral and cytotoxic activities.[4,5] In connection with our studies on biologically active compounds from marine invertebrates, we have isolated two new spongian diterpenes, **2** and **3**, both of which possess ring A lactones, from *Spongia matamata* de Laubenfels, 1954, family Spongiidae, collected at Yap Island. The known compounds **1**,[6] **4**,[6] **5**,[4] and **6**[7] were also isolated. Lu and Faulkner have recently reported sesterterpenoids and a *seco*-sterol from *S. matamata* collected in Palau.[8] Spongian diterpenoids have also recently been isolated from nudibranchs,[9] which are believed to sequester various metabolites from their sponge diets and use them to inhibit predation.

The identity of the known compounds was established by comparisons of their spectral properties with those reported.[4,6,7] Compounds **2** and **3** were deduced to have the same molecular formula, $C_{19}H_{26}O_4$, on the basis of FAB MS data, *m*/*z* 319 $[M + 1]^+$, in addition to ^1H and ^{13}C NMR data. Comparison of the ^1H and ^{13}C data of **2** and **3** with those of **1** and **4**−**6** revealed that these compounds all belong to the same class and, furthermore, that all these metabolites share a common B, C, D ring structure. Hence, structural differences were confined to ring A.

The ^{13}C NMR spectrum of **2** contained signals for three oxygenated carbons at δ 171.5 (s), 87.4 (s), and δ 68.2 (t). The presence of the signal at δ 171.5, together with IR absorption at 1720 cm^{-1}, pointed to the presence of an ester in ring A, while IR absorption at 3280−3600 cm^{-1} indicated the presence of a hydroxyl group. The protonated carbon signals were first assigned from HMQC data, and then an HMBC experiment revealed long-range coupling between the methyl signal at δ 1.05 (H-20) and carbon signals at δ 35.8 (s, C-10), 44.1 (d, C-5), 52.9 (d, C-9), and 45.8 (t, C-1) (see Figure 1). The

2 **3**

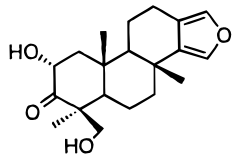
Figure 1. HMBC correlations in compounds **2** and **3**.



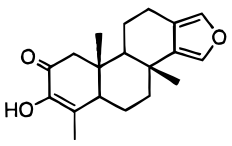
1

2 R_1=H R_2=OH

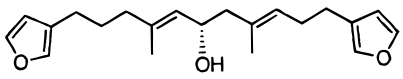
3 R_1=OH R_2=H



4



5



6

signals for the protons at C-1, δ 2.74 and 1.98, showed correlation to the carbonyl carbon resonance, δ 171.5, thus fixing this functionality at C-2. The proton methyl signal at δ 1.27 showed HMBC correlations with carbon signals at δ 87.4 (s) and an oxymethylene carbon at δ 68.2 and 44.1 (C-5). These correlations confirm that the signal at δ 87.4 is due to C-4 and that the oxymethylene is joined to C-4. The chemical shifts of the oxymethylene protons in **2**, δ 3.44 and 3.49, were consistent with those of a hydroxymethylene group, and hence, the ester linkage in ring A was via an oxygen at C-4 to give a six-membered lactone. The chemical shift of the oxymethylene proton signals in the alternate possible seven-membered-ring lactone are known to occur 0.5−1 ppm farther downfield.[5] The A/B ring fusion was assigned as trans because of the similarity of the carbon

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S0163-3864(97)00521-1 CCC: $15.00
Published on Web 03/26/1998

NMR shifts of C-20 with other compounds such as **1** and **4** and the coupling constants of H-5 (δ 2.02, J = 11.5, 2 Hz). An NOE enhancement was observed for the oxymethylene protons when H-5 was irradiated; hence, the oxymethylene group is assigned the α-configuration to give the structure depicted by **2**.

Compound **3** has the same molecular formula as **2**, and NMR data of the two compounds differed only slightly in the signals for the ring A portion of the molecule. HMBC data confirmed the same connectivity as for compound **2** (see Figure 1), and the proton and carbon-13 NMR shifts for the oxymethylene group, 3.43, 3.94/65.6, respectively, again pointed to a free primary alcohol group. Hence, **2** and **3** appeared to differ only in stereochemistry, and this was confirmed when irradiation of the H-20 signal at δ 1.02 caused an NOE effect on one of the oxymethylene protons, δ 3.43. Hence, **3** was confirmed to be the C-4 epimer of **2**. The proton signals for the two H-19 protons of **3** were dramatically different: δ 3.94 was a sharp doublet (J = 12.0 Hz), while δ 3.43 was a broad doublet that changed to a sharp doublet, J = 12.0 Hz, upon proton exchange. This indicates a restricted conformation in which there is significant coupling between only one of the methylene protons and the hydroxyl proton. This could be due to hydrogen bonding of the OH to one of the ester oxygens.

The brine shrimp lethality test[10] was carried out for the purified compounds. Compound **2** was inactive, and the remaining metabolites showed mild toxicity with LC_{50} values of approximately 50−100 μg/mL.

Experimental Section

General Experimental Procedures. IR spectra were obtained on a Bio-Rad Win-IR instrument. FAB mass spectra were obtained on a VG ZAB spectrometer, and NMR spectra were measured on a Varian VXR-500 instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C). Preparative HPLC was performed using a Phenomenex C-18 column (250 × 10 mm) with UV detection. Flash chromatography was carried out on Si gel 60-H (230−400 mesh).

Animal Material. The sponge *S. matamata* de Laubenfels, 1954 (order Dictyoceratida, family Spongiidae) was collected at Yap Island, Federated States of Micronesia, in August 1995. A voucher specimen is maintained at the Department of Chemistry, University of Oklahoma (2-YA-95), and the Natural History Museum, London, U.K. (BMNH 1997.9.20.6).

Isolation. Sponges were frozen for shipment to Oklahoma and then stored in MeOH at 5 °C until workup. The material was extracted twice with MeOH and twice with CH_2Cl_2−MeOH (1:1), dry wt 67 g. The extracts were concentrated and combined to give a residue that was dissolved in 300 mL of 10% aqueous MeOH. The solution was partitioned against hexane (3 × 300 mL), and the resulting aqueous solution was diluted to 30% H_2O in MeOH and partitioned against CH_2Cl_2 (3 × 300 mL). The aqueous MeOH phase was concentrated in vacuo, and the aqueous concentrate was extracted with *n*-BuOH (3 × 120 mL). Only the residue from the CH_2Cl_2 extract (1.3 g) showed brine shrimp lethality, and this was therefore fractionated on an open Si gel column using increasing amounts of EtOAc in hexane as eluent. The resulting 16 fractions were further purified by either open-column chromatography or reversed-phase HPLC using MeOH−H_2O mixture as eluent to furnish compounds **1** (1.0 g), **2** (4.0 mg), **3** (3.2 mg), **4** (2.3 mg), **5** (23 mg), and **6** (12 mg).

Compound 2: white powder; $[\alpha]_D$ +12.8° (*c* 0.2, $CHCl_3$); IR ($CHCl_3$) υ_{max} 3600−3200 (br), 1720, 1690−1684 (br) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.06 (1H, s, H-15), 7.02 (1H, s, H-16), 3.49, 3.44 (each 1H, J = 12.0 Hz, H-18), 2.80, 2.45 (each 1H, m, H-12), 2.74 (1H, d, J = 16.5 Hz, H-1β), 2.11 (1 H, dd, J = 11.0, 4 Hz, H-7), 2.02 (1H, dd, J = 11.5, 2 Hz, H-5), 1.98 (1H, d, J = 16.5 Hz, H-1α), 1.68 (1H, m, H-7), 1.65 (m, H-6), 1.64−1.67 (m, H-11), 1.47 (1H, d, J = 10.0 Hz, H-6), 1.34 (1H, t, J = 7 Hz, H-9), 1.27 (3H, s, H-19), 1.22 (3H, s, H-17), 1.05 (3H, s, H-20); ^{13}C NMR ($CDCl_3$) δ 45.8 (C-1), 171.5 (C-2), 87.4 (C-4), 44.1 (C-5), 20.6 (C-6), 39.0 (C-7), 33.8 (C-8), 52.9 (C-9), 35.8 (C-10), 18.2 (C-11), 19.7 (C-12), 119.0 (C-13), 136.1 (C-14), 135.1 (C-15), 137.1 (C-16), 25.4 (C-17), 68.2 (C-18), 20.3 (C-19), 16.8 (C-20); FAB-MS *m/z* 319 $[M + 1]^+$, 301 $[M + 1 - H_2O]^+$.

Compound 3: white powder; $[\alpha]_D$ +15.2° (*c* 0.27, $CHCl_3$); IR ($CHCl_3$) 3700−3250 (br), 1712, 1670−1683 (br) cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.08 (1H, s, H-15), 7.04 (1H, s, H-16), 3.94 (1H, d, J = 12.0 Hz, H-19), 3.43 (1H, br d, J = 12.0 Hz, H-19), 2.82 (1H, d, J = 17.0 Hz, H-1β), 2.80, 2.45 (each 1H, m, H-12), 2.16 (1H, d, J = 5.5 Hz, H-7), 1.95 (1H, d, J = 17.0 Hz, H-1α), 1.72 (1H, m, H-5), 1.58−1.66 (5H, m, H-6, H-7 and H-11), 1.48 (3H, s, H-18), 1.28 (1H, t, J = 7.0 Hz, H-9), 1.20 (3H, s, H-17), 1.02 (3H, s, H-20); ^{13}C NMR ($CDCl_3$) δ 46.3 (C-1), 170.0 (C-2), 87.2 (C-4), 52.8 (C-5), 19.8 (C-6), 39.6 (C-7), 33.8 (C-8), 53.2 (C-9), 36.4 (C-10), 18.2 (C-11), 20.3 (C-12), 118.9 (C-13), 135.9 (C-14), 135.1 (C-15), 137.1 (C-16), 25.4 (C-17), 26.2 (C-18), 65.6 (C-19), 17.1 (C-20); FAB-MS *m/z* 319 $[M + 1]^+$.

Acknowledgment. This work was supported by Department of Commerce, NOAA, Sea Grant Project NA66RG0172. We thank the Coral Reef Research Foundation for use of its facilities and the Government of Chuuk, Federated States of Micronesia, for permission to collect specimens.

References and Notes

(1) Faulkner, D. J. *Nat. Prod. Rep.* **1997**, *14*, 259−302 and references therein.
(2) Kazlauskas, R.; Murphy, P. T.; Wells. R. J.; Noack, K.; Oberhansli, W. E.; Schonholzer, P. *Aust. J. Chem.* **1979**, *32*, 867−880.
(3) Hirsch, S.; Kashman, Y. *J. Nat. Prod.* **1988**, *51*, 1243−1245.
(4) Gunasekera, S. P.; Schmitz, F. J. *J. Org. Chem.* **1991**, *56*, 1250−1253.
(5) Kohmoto, S.; McConnell, O. J.; Wright, A.; Cross, S. *Chem. Lett.* **1987**, 1687- 1690.
(6) Cambie, R. C.; Craw, P. A.; Stone, M. J. *J. Nat. Prod.* **1988**, *51*, 293−297.
(7) Kobayashi, M.; Chavakula, R.; Murata, O.; Sarma, N. S. *Chem. Pharm. Bull.* **1992**, *40*, 599−601.
(8) Lu, Q.; Faulkner, D. J. *J. Nat. Prod.* **1997**, *60*, 195−198.
(9) Fontana, A.; Mollo, E.; Ricciardi, D.; Fakhr, I.; Cimino, G. *J. Nat. Prod.* **1997**, *60*, 444−448.
(10) McLaughlin, J. L.; Chang, C. J; Smith, D. L. In *Studies in Natural Products Chemistry*; Rahman, A., Ed.; Elsevier: Amsterdam, 1991; pp 383−405.

NP9705219