

CHEMISTRY OF SPONGES, IV.¹ SPONGIAN DITERPENES FROM *HYATELLA INTESTINALIS*

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ABSTRACT.—In addition to known spongian diterpenes **1**, **2**, and **3**, the new compounds 3 β -acetoxy-19-hydroxyspongia-13(16),14-dien-2-one [**4**], 19-hydroxyspongia-13(16),14-dien-3-one [**5**], and 2 α ,19-dihydroxyspongia-13(16),14-dien-3-one [**10**] have been isolated from the Dictyoceratid sponge *Hyatella intestinalis*.

In a continuation of our investigation (1) of sponges of the order Dictyoceratida we have investigated the metabolites of *Hyatella intestinalis* (Lamarck), collected off the Darwin Coast, Northern Australia. Extensive chromatography of a pentane extract of the freeze-dried sponge afforded a series of compounds, all of which exhibited spectral characteristics expected for a β , β -disubstituted furan moiety, viz., an absorption in the uv spectrum at ca. 240 nm (ϵ 1400–1700); two ir absorption bands at ca. 1030 and 885 cm^{-1} ; two mutually coupled doublets ($J = 1.0$ – 1.5 Hz) in the region δ 7.0–7.15 of the ¹H-nmr spectrum; and four ¹³C-nmr signals in the aromatic region (two quaternary and two tertiary carbons), one of which (C-13) is further upfield (ca. δ 119) than the other three (ca. δ 135–138). Thus, the compounds appeared to be diterpenoids of the spongian type (2).

The structures of spongia-13(16),14-diene [**1**], 3 β ,19-dihydroxyspongia-13(16),14-dien-2-one [**2**], and 3 β ,19-diacetoxyspongia-13(16),14-dien-2-one [**3**] were assigned from an examination of their ir, ¹H-nmr, ¹³C-nmr, and mass spectra and were confirmed by comparison of their physical and spectral properties with those recorded for the compounds from species of *Spongia* (2,3).

Compound **4** was assigned the formula C₂₂H₃₀O₅ from the hrms, which showed a molecular ion at m/z 374.2132 as well as fragments corresponding to the successive loss of COCH₂, 3 \times Me, and H₂O. This new compound showed spectral properties similar to those of both **2** and **3**. In particular, the ir spectrum showed hydroxyl (3560 cm^{-1}), carbonyl (1743, 1726 cm^{-1}), and acetate (1225 cm^{-1}) absorptions, while the ¹H-nmr spectrum showed the presence of three tertiary methyl groups (δ 1.25, 1.21, 0.92). The latter spectrum also showed a one-proton singlet at δ 5.01 similar to that observed for the CHOAc proton in **3** and two mutually coupled one-proton doublets ($J = 12$ Hz) with chemical shifts (δ 3.78, 3.46) very close to those observed for the CH₂OH group of **2**. Moreover, two mutually coupled one-proton doublets at δ 2.65 and 2.20 ($J = 12$ Hz) indicated a similar A-ring substitution pattern to that of compounds **2** and **3**. The ¹³C-nmr spectrum showed the presence of an acetate group (δ 169.5, 20.7) and one other carbonyl group (δ 203.2) in a six-membered ring (4), as well as two oxygen-bearing carbons (δ 84.9, 63.7) and three methyl groups (δ 25.7, 23.3, 16.9).

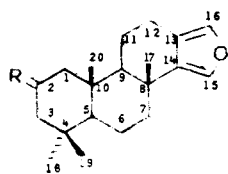
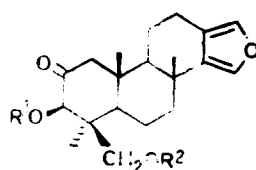
Comparison of the spectra (Table 1) of **4** with those of **2** and **3** and with published data (3) for the related compounds **7** and **8** showed that (a) its C-2 carbonyl resonance was much closer to those of **3** and **8** in which C-3 bears an acetoxy substituent than to those of **2** and **7** in which C-3 bears a hydroxy substituent, and (b) the two C-19 proton

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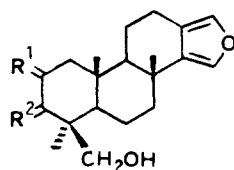
TABLE 1. Selected ^{13}C - and ^1H -nmr Data.

Atom	Compound				
	2	3	4	7	8
C-2	209.8	202.6	203.2	212.8	202.5
C-3	84.1	83.0	84.9	76.0	77.4
C-19	63.9	64.6	63.7	66.6	65.9
OCOMe	—	170.5/170.3	169.5	—	170.4/168.7
H-3	4.01	5.00	5.01	4.71	5.46
H-19	3.68/3.34	4.08	3.46/3.78	3.76/3.54	4.03

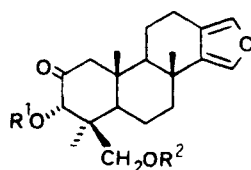
doublets had an identical pattern to those of the C-19 hydroxy compounds **2** and **7** but a different pattern from that of the C-19 acetoxy compounds **3** and **8** where they appear as a singlet at δ 4.0–4.1. The compound, therefore, possessed a hydroxy group at C-19; thus, its structure was either **4** or **9** which differed only in stereochemistry at C-3. The stereochemistry was established from the following observations: (a) the C-3 resonance occurred at δ 84.9, much closer to the corresponding signal in **2** and **3**, in which the C-3 substituent is β , than to those in **7** and **8** in which the C-3 substituent is α ; (b) the C-19 resonance occurred closer to the corresponding signals in **2** and **3** than to those in **7** and **8**; and (c) the C-3 proton singlet possessed a chemical shift (δ 5.01) similar to that of **3** in which the C-3 acetoxy substituent is β , but different from the downfield signal (δ 5.46) of **8** where the acetoxy group is α . This evidence established that the C-3 acetoxy group was β and that the compound was **3 β -acetoxy-19-hydroxyspongia-13(16),14-dien-2-one **4**. Acetylation of **4** gave the diacetate **3**.**

**1** R=H₂

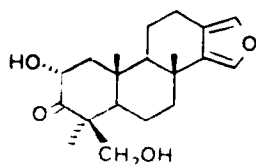
2 R¹=R²=H
3 R¹=R²=Ac
4 R¹=Ac, R²=H



5 R¹=H₂, R²=O
6 R¹=O, R²=H₂



7 R¹=R²=H
8 R¹=R²=Ac
9 R¹=Ac, R²=H

**10**

The hrms of compound **5** established the molecular formula $C_{20}H_{28}O_3$ and showed fragment ions due to the successive losses of CH_2O , Me, and H_2O . The ir spectrum exhibited bands at $3400\text{--}3500\text{ cm}^{-1}$ (OH), and $1757, 1705\text{ cm}^{-1}$ (CO), while the 1H - and ^{13}C -nmr spectra revealed the presence of three methyl groups, a C-19 hydroxymethyl group, and a ketonic group in a six-membered ring. From comparisons with the other metabolites two structures, viz. **5** and **6**, were possible, which differed only in the position of the keto group in ring A. Structure **6** could be eliminated because the 1H -nmr spectrum did not show the two pairs of geminally coupled doublets expected of a ring-A ketone flanked by two isolated methylene groups. Rather, the spectrum showed a four-proton spin system in which three ddd patterns were apparent. A signal at δ 2.64 exhibited couplings of 15.8 (geminal), 9.9 (eq-ax), and 5.2 Hz (eq-eq), a typical pattern for an equatorial proton (H-2 α). Another equatorial proton signal with couplings of 13.4 (geminal), 8.7 (eq-ax), and 5.2 Hz (eq-eq) occurred further upfield at δ 2.05 and was, therefore, assigned to H-1 β . A third signal at δ 2.43 exhibited couplings of 15.8 (geminal), 8.8 (eq-ax), and 10.0 Hz (ax-ax) and was assigned to an axial proton H-2 β . The remaining signal for H-1 α was partially hidden under a complex multiplet in the region δ 1.54–1.80. Attempts to record a 1H - 1H correlation spectrum or to substitute the enolate protons with deuterium (**5**) were unsuccessful owing to the instability of the compound. However, from the spectral parameters above, the structure could be assigned as 19-hydroxyspongia-13(16),14-dien-3-one [**5**].

Hrms and ^{13}C -nmr measurements supported a molecular formula of $C_{20}H_{28}O_4$ for compound **10**, while its ir spectrum showed the presence of both hydroxyl (3400 cm^{-1}) and ketonic (1715 cm^{-1}) functionalities. The 1H -nmr spectrum indicated the presence of three methyl groups, while mutually coupled doublets at δ 4.14 and 3.65 ($J = 11$ Hz) implied the presence of a hydroxymethylene substituent. A doublet of doublets at δ 4.62 ($J = 12.6, 6.6$ Hz) corresponded to an axial hydroxymethine proton adjacent to a single methylene group and to a quaternary carbon in a six-membered ring. The ^{13}C -nmr spectrum showed the presence of two oxygenated carbon atoms (δ 69.9, 65.6) and a ketonic carbon (δ 214.0). Comparison with the spectra of **2**, **3**, and **4** indicated that C-2, C-3, and C-19 were the oxygenated carbon atoms. Inasmuch as the compound was different from **2** and **7** in which C-3 bears a hydroxy group and C-2 bears an oxo group, the structure was assigned as 2 α ,19-dihydroxyspongia-13(16),14-dien-3-one [**10**]. The stereochemistry of the hydroxy group was defined from the fact that H-2 participated in axial-axial ($J = 12.6$ Hz) and axial-equatorial ($J = 6.6$ Hz) coupling to H-1 α and H-1 β , respectively. As expected, the C-19 protons occurred at lower field than in **2** as a result of the close proximity of the carbonyl group, while the C-1 protons occurred at higher field.

Other related compounds obtained from the sponge will be the subject of a further communication.

H. intestinalis yields diterpenoid metabolites similar to those obtained from species of *Spongia* (**2**, **3**). Its metabolites are of interest because, in addition to diol and triol derivatives of spongia, the sponge also yields their probable biosynthetic precursor **1**.

EXPERIMENTAL

ISOLATION OF DITERPENOIDS.—A freeze-dried sample of *H. intestinalis* (Lamarck) (Darwin Museum Register No. 2466) (27 g) was extracted (Soxhlet) with pentane for 8 h, and the extract was concentrated to yield an oily solid (2.24 g). The extract was chromatographed on Si gel (50 g), and the column was eluted with CH_2Cl_2 containing increasing proportions of Et_2O to afford 17 fractions. Preparative tlc (hexane) of fraction 2 (0.24 g) yielded **1** (50 mg), and preparative tlc (EtOAc-hexane, 1:10) of fractions 3–6 (0.56 g) followed by normal phase hplc (THF/hexane) yielded **3** (5 mg). Recrystallization of fractions 8–11 (0.60 g) yielded **2** (0.12 g), while preparative tlc (EtOAc-hexane, 1:5) of the mother liquors gave after further preparative tlc (Me_2CO -hexane, 1:1) **5** (32 mg) and (Me_2CO -hexane, 1:1) **4** (40 mg). Preparative tlc (C_6H_6 - Et_2O , 2:1) of fractions 12–17 (0.16 g) gave **10** (25 mg).

SPONGIA-13(16),14-DIENE [1].—The compound crystallized from $\text{CH}_2\text{Cl}_2/\text{MeOH}$ as flakes, mp 115–117°, $[\alpha]_D^{21}$ +29.7° ($c = 0.3$, CHCl_3) [lit. (3) mp 115–116°, $[\alpha]_D - 32.7^\circ$]; uv λ max (CHCl_3) 241 nm (ϵ 1510); ir ν max (KBr) 1035, 894 cm^{-1} (furan); ^1H nmr δ 7.03 (br s, H-15, H-16), 1.23 (s, Me), 0.98 (s, Me), 0.87 (6H, 2Me); ^{13}C nmr δ 137.7 (C-14), 136.6 (C-16), 134.9 (C-15), 119.8 (C-13), 56.7 (C-5), 56.3 (C-9), 42.1 (C-4), 41.2 (C-7), 40.0 (C-3), 37.6 (C-10), 34.3 (C-8), 33.4 (C-1, C-2), 26.3 (C-17), 21.5 (C-18), 20.7 (C-6), 18.8 (C-11), 18.6 (C-12), 18.1 (C-19), 16.4 (C-20); ms m/z $[\text{M}]^+$ 286 (42%), $[\text{M} - \text{Me}]^+$ 271 (100), 253 (42), 187 (40), 175 (50), 147 (54), 137 (68), 123 (20).

3 β ,19-DIHYDROXYSPONGIA-13(16),14-DIEN-2-ONE [2].—The compound crystallized from $\text{CH}_2\text{Cl}_2/\text{hexane}$ as needles, mp 160–163°, $[\alpha]_D^{21}$ +17.4° ($c = 0.4$, CHCl_3) [lit. (2) mp 157–158.5°, $[\alpha]_D + 18.7^\circ$]; uv λ max 240 nm (ϵ 1470); ir ν max (KBr) 3400 br (OH), 1715 (CO), 1030, 885 cm^{-1} (furan); ^1H nmr δ (400 MHz) 7.11 (d, $J = 1.3$ Hz, H-16), 7.06 (d, $J = 1.3$ Hz, H-15), 4.01 (s, H-3), 3.68 (d, $J = 12$ Hz, H-19a), 3.34 (dd, $J = 12$ Hz, 1 Hz, H-19b), 3.00 (br s, OH), 2.74 (d, $J = 12.6$ Hz, H-1a), 2.11 (d, $J = 12.6$ Hz, H-1b), 1.41 (s, Me), 1.19 (s, Me), 0.83 (s, Me); ^{13}C nmr δ 209.8 (C-2), 136.9 (C-16), 136.3 (C-14), 135.1 (C-15), 119.0 (C-13), 84.1 (C-3), 63.9 (C-19), 55.8 (C-9), 55.2 (C-5), 52.7 (C-1), 49.3 (C-4), 43.0 (C-10), 40.6 (C-7), 34.4 (C-8), 25.6 (C-17), 24.0 (C-18), 20.5 (C-12), 18.7 (2C, C-6, C-11), 17.3 (C-20); ms m/z $[\text{M}]^+$ 332 (49%), $[\text{M} - \text{Me}]^+$ 317 (15), $[\text{M} - 2\text{Me}]^+$ 302 (37), $[\text{M} - 3\text{Me}]^+$ 287 (41), $[287 - \text{H}_2\text{O}]^+$ 269 (51), 147 (100).

3 β ,19-DIACETOXYSPONGIA-13(16),14-DIEN-2-ONE [3].—The compound crystallized from THF-hexane (1:4) as needles, mp 197–199°, $[\alpha]_D^{21}$ +45.9° ($c = 0.5$, CHCl_3) [lit. (2) mp 195–198°, $[\alpha]_D + 45.2^\circ$]; uv λ max (CHCl_3) 239 nm (ϵ 1600); ir ν max (KBr) 1734, 1721 (CO), 1240, 1229 (OAc), 1029, 886 cm^{-1} (furan); ^1H nmr δ (400 MHz) 7.12 (d, 1.1 Hz, H-16), 7.08 (d, $J = 1.1$ Hz, H-15), 5.00 (s, H-3), 4.08 (s, 2H, H-19), 2.69 (d, $J = 12.5$ Hz, H-1a), 2.20 (d, $J = 12.5$ Hz, H-1b), 2.18 (s, COCH₃), 2.07 (s, COCH₃), 1.22 (s, 6H, 2Me), 0.95 (s, Me); ^{13}C nmr δ 202.6 (C-2), 170.5 (COMe), 170.3 (COMe), 137.1 (C-16), 136.4 (C-14), 135.2 (C-15), 119.2 (C-13), 83.0 (C-3), 64.6 (C-19), 56.1 (2C, C-5, C-9), 54.2 (C-1), 46.3 (C-4), 42.7 (C-10), 40.9 (C-7), 34.5 (C-8), 25.7 (C-17), 23.7 (C-18), 21.0 (C-6), 20.6 (COCH₃), 20.5 (COCH₃), 19.8 (C-12), 18.7 (C-11), 16.6 (C-20); ms m/z $[\text{M}]^+$ 416 (36%), $[\text{M} - \text{Me}]^+$ 401 (4), $[\text{M} - \text{Me} - \text{HOAc}]^+$ 341 (8), $[341 - \text{HOAc}]^+$ 281 (21), 147 (17), 43 (100).

3 β ,19-ACETOXY-19-HYDROXYSPONGIA-13(16),14-DIEN-2-ONE [4].—The compound crystallized from Me_2CO -hexane (1:3) as prisms, mp 216–218°, $[\alpha]_D + 34.0^\circ$ ($c = 0.15$, CHCl_3) (Found: $[\text{M}]^+$ 374.2134, $\text{C}_{22}\text{H}_{30}\text{O}_5$ requires $[\text{M}]^+$ 374.2093); uv λ max 240 nm (ϵ 1660); ir ν max (KBr) 3560 (OH), 1743, 1726 (CO), 1225 (OAc), 1030, 887 cm^{-1} (furan); ^1H nmr δ (400 MHz) 7.11 (d, $J = 1.5$ Hz, H-16), 7.07 (d, $J = 1.3$ Hz, H-15), 5.01 (s, H-3), 3.78 (d, $J = 12$ Hz, H-19a), 3.46 (d, $J = 12$ Hz, H-19b), 2.65 (br d, $J = 12$ Hz, H-1a), 2.22 (s, COCH₃), 2.20 (d, $J = 12$ Hz, H-1b), 1.25 (s, Me), 1.21 (s, Me), 0.92 (s, Me); ^{13}C nmr δ 203.2 (C-2), 169.5 (COMe), 137.0 (C-16), 136.4 (C-14), 135.1 (C-15), 119.1 (C-13), 84.9 (C-3), 63.7 (C-19), 56.2 (C-9), 55.9 (C-5), 54.0 (C-1), 48.1 (C-4), 42.8 (C-10), 40.8 (C-7), 34.5 (C-8), 25.7 (C-17), 23.3 (C-18), 20.7 (COCH₃), 20.5 (C-6), 19.2 (C-12), 18.7 (C-11), 16.9 (C-20); ms m/z $[\text{M}]^+$ 374 (10%), $[\text{M} - \text{COCH}_3]^+$ 332 (22); $[332 - \text{Me}]^+$ 317 (8), $[317 - \text{Me}]^+$ 302 (29), $[302 - \text{Me}]^+$ 287 (34), $[287 - \text{H}_2\text{O}]^+$ 269 (51).

19-HYDROXYSPONGIA-13(16),14-DIEN-3-ONE [5].—The compound crystallized from Me_2CO -hexane (1:3) as prisms, mp 143–144°, $[\alpha]_D^{21}$ +18.8° ($c = 0.6$, CHCl_3) (Found: $[\text{M}]^+$ 316.2036, $\text{C}_{20}\text{H}_{28}\text{O}_3$ requires $[\text{M}]^+$ 316.2039); uv λ max (CHCl_3) 240 nm (ϵ 1700); ir ν max 3400–3500 (OH), 1757, 1705 (CO), 1035, 883 cm^{-1} (furan); ^1H nmr δ 7.11 (d, $J = 1.5$ Hz, H-16), 7.07 (d, $J = 1.2$ Hz, H-15), 4.00 (d, $J = 11$ Hz, H-19a), 3.49 (d, $J = 11$ Hz, H-19b), 2.64 (ddd, $J = 15.8, 9.9, 5.2$ Hz, H-2 α), 2.43 (ddd, $J = 15.8, 8.8, 10.0$ Hz, H-2 β), 2.05 (ddd, $J = 13.4, 8.7, 5.2$ Hz, H-1 β), 1.54–1.80 (m, 8H), 1.30 (s, Me), 1.21 (s, Me), 0.94 (s, Me); ^{13}C nmr δ 220.7 (C-3), 136.9 (C-16), 136.5 (C-14), 135.2 (C-15), 119.4 (C-13), 65.6 (C-19), 55.4 (C-9), 55.1 (C-5), 50.9 (C-4), 40.2 (C-7), 38.9 (C-10), 36.7 (C-1), 34.1 (C-8), 34.0 (C-2), 25.4 (C-17), 22.2 (C-18), 20.7 (C-6), 19.4 (C-12), 19.0 (C-11), 17.0 (C-20); ms m/z $[\text{M}]^+$ 316 (2%), $[\text{M} - \text{CH}_2\text{O}]^+$ 286 (58), $[286 - \text{Me}]^+$ 271 (100), $[271 - \text{H}_2\text{O}]^+$ 253 (18), 147 (96).

ACETYLYATION OF COMPOUND 4.—Compound 4 (3.4 mg) was acetylated with pyridine (1.5 ml), Ac_2O (0.1 ml), and 4-dimethylaminopyridine (1.0 mg) at 20° for 17 h. Work-up and extraction with CH_2Cl_2 gave 3 β ,19-diacetoxyspongia-13(16),14-dien-2-one [3] (4.5 mg, 100%) (correct tlc, ms, and ^1H -nmr spectrum).

2 α ,19-DIHYDROXYSPONGIA-13(16),14-DIEN-3-ONE [10].—The compound crystallized from $\text{MeOH}/\text{H}_2\text{O}$ as prisms, mp 180–182°, $[\alpha]_D - 48^\circ$ ($c = 0.1$, CHCl_3) (Found: $[\text{M}]^+$ 332.1982, $\text{C}_{20}\text{H}_{28}\text{O}_4$ requires $[\text{M}]^+$ 332.1980); uv λ max (CHCl_3) 240 nm (ϵ 1500); ir ν max (KBr) 3400 (OH), 1715 (CO), 1026, 885 cm^{-1} (furan); ^1H nmr δ (400 MHz) 7.08 (d, $J = 1.3$ Hz, H-16), 7.05 (d, $J = 1.3$ Hz, H-15), 4.62 (dd, $J = 12.6, 6.6$ Hz, H-2), 4.14 (d, $J = 11$ Hz, H-19a), 3.65 (d, $J = 11$ Hz, H-19b), 1.29 (s, Me),

1.26 (s, Me), 1.25 (s, Me); ^{13}C nmr δ 214.0 (C-3), 136.9 (C-16), 136.6 (C-14), 135.0 (C-15), 119.3 (C-13), 69.9 (C-2), 65.6 (C-19), 58.6 (C-5), 55.8 (C-9), 54.5 (C-4), 49.4 (C-1), 41.1 (C-7), 38.0 (C-10), 34.3 (C-8), 26.4 (C-17), 20.5 (C-6), 20.0 (C-6), 19.3 (C-18), 18.8 (C-11), 17.6 (C-20); *ms m/z* $[\text{M}]^+$ 332 (8%), $[\text{M} - \text{CH}_2\text{O}]^+$ 302 (29), $[\text{302} - \text{Me}]^+$ 287 (40), $[\text{287} - \text{H}_2\text{O}]^+$ 269 (26), 185 (23), 43 (100).

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