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CHEMISTRY OF SPONGES, VI.[1] SCALARANE SESTERTERPENES FROM *HYATELLA INTESTINALIS*

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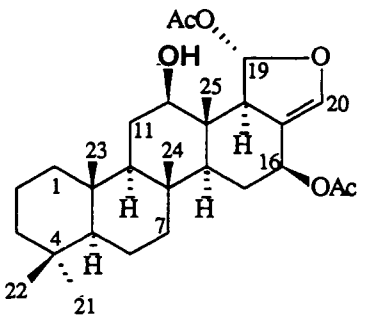
ABSTRACT.—The sponge *Hyatella intestinalis*, collected from shallow water, yields the known scalarane sesterterpenes heteronemin [**1**] and 12-*epi*-deoxoscalarin [**2**], and a new metabolite for which the structure 24-acetoxy-12-deacetyl-12-*epi*-deoxoscalarin [**4**] is elucidated.

Recently we reported (1) the isolation of a series of spongian diterpenoids from the sponge *Hyatella intestinalis* (Lamarck) (order: Dictyoceratida), collected off the Darwin Coast, Northern Australia. During attempts to reisolate further unidentified diterpenoids present in the sponge but not reported in the above paper, we have investigated the metabolites of *H. intestinalis* collected from shallower water within Darwin Harbor. In contrast to the earlier report, flash column chromatography and reversed-phase hplc of extracts of freeze-dried specimens yielded scalarane sesterterpenes rather than diterpenoids. One specimen yielded the known compounds heteronemin [**1**] (0.02%) (2–6) and 12-*epi*-deoxoscalarin [**2**] (0.04%) (7), while a second specimen gave **1** (0.01%), **2** (0.009%), and a new sesterterpene **4** (0.006%).

The structure of 24-acetoxy-12-deacetyl-12-*epi*-deoxoscalarin [**4**] was established from an examination of its high field (400 MHz) nmr spectra. The compound was assigned the formula $C_{27}H_{42}O_5$ from the ^{1}H-nmr (42 protons) and ^{13}C-nmr (27 carbons) spectra, and the hrms which, like that of other scalarane sesterterpenes, showed no molecular ion. Peaks corresponding to losses of H_2O (*m/z* 428.2924, $C_{27}H_{40}O_4$), and HOAc (*m/z* 368.2714, $C_{25}H_{36}O_2$) were observed. The formula together with an acetate methyl signal at δ 2.08 in the ^{1}H-nmr spectrum, a carbonyl signal at δ 170.9 in the ^{13}C-nmr spectrum, and a base peak in the mass spectrum at *m/z* 43 $[MeCO]^+$ indicated a sesterterpene with an acetate substituent. The presence of one double bond was evident from the ^{13}C-nmr spectrum (δ 134.6 s, 117.6 d), and, thus, a pentacyclic system was indicated. The ^{13}C-nmr spectrum also showed the presence of one doubly



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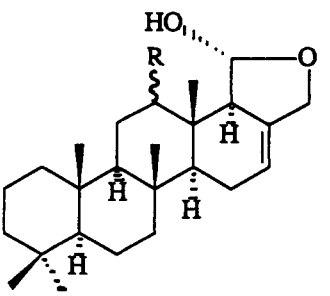

2 R=β-OAc
3 R=α-OAc

[1]For part V, see R.C. Cambie, P.R. Bergquist, and P. Karuso, *J. Nat. Prod.*, **51**, 1014 (1988).

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oxygenated carbon atom (δ 99.5 d) and three oxygenated carbon atoms (δ 81.1 d, 68.7 t, and 64.5 t). The presence of a two-proton signal in the ^{1}H-nmr spectrum which exchanged with D_2O indicated the presence of two hydroxyl groups. In view of the co-occurrence with heteronemin [**1**] and 12-*epi*-deoxoscalarin [**2**], a scalarane skeleton was indicated, and this was supported by signals in the ^{13}C-nmr spectrum at δ 56.6 (C-5), 58.6 (C-9), 54.2 (C-14), and 61.1 (C-18) which are reported (2,8) to be diagnostic of a normal scalarane framework.

That the new sesterterpene possessed the same substitution pattern as that of 12-*epi*-deoxoscalarin [**2**] was evident from the ^{1}H-nmr spectra. From the COSY spectrum the acetal proton (δ 5.31, d, J = 6.8 Hz) was coupled to a proton which resonated at δ 2.25. The olefinic proton (δ 5.49) exhibited allylic coupling to the same signal at δ 2.25, and it also showed long-range allylic coupling to a pair of geminal protons (δ 4.53, 4.22) attached to an oxygenated carbon. Coupling of another geminal pair of allylic protons (δ 2.37, 2.17) to each other and to a methine proton at δ 1.27 was also apparent. In addition, an AB system (δ 4.52, 4.30, 2d, J = 12.6 Hz) was readily identified, accounting for the second methylene group bearing oxygen. The remaining oxygenated carbon bore a proton which resonated at δ 3.57 (dd, J = 11.4, 4.3 Hz) and was coupled to protons at δ 1.76 and 1.47. These latter protons were in turn coupled to a signal at δ 1.04. These coupling patterns allowed assignment of all the ring protons in the partial structure for the C, D, and E rings (Figure 1).

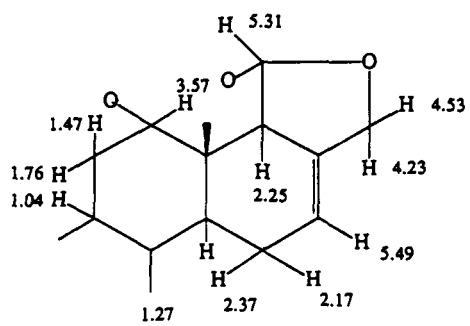
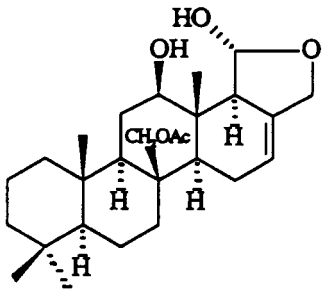


FIGURE 1. ^{1}H-nmr signals of the C, D, and E rings of compound **4**.

That the A and B rings bore no oxygenation was verified from the mass spectrum. Cimino *et al.* (7) noted that scalarin [**5**], 12-*epi*-scalarin [**6**], deoxoscalarin [**3**], and 12-*epi*-deoxoscalarin [**2**] all give "abundant ions at *m/z* 137 and 123 associated with the fragmentation of ring B". The same fragment ions at *m/z* 137 (21%) and 123 (26%) were also observed in the mass spectrum of **4**.

Comparison of the signal observed for H-19 (δ 5.31, br d, J = 6.8 Hz) in the ^{1}H-nmr spectrum of **4** with that of the same proton for 12-*epi*-deoxoscalarin [**2**] (δ 5.37, br d, J = 4 Hz) showed that in each case the C-19 substituent was a hydroxyl group rather than an acetate group. Similarly, comparison of the signal for H-12 (δ 3.57, dd, J = 11.4, 4.3 Hz) in the spectrum of **4** with the corresponding signal for 12-*epi*-deoxoscalarin (δ 4.67, dd, J = 10,4 Hz) showed that the functionality in **4** was a hydroxyl group rather than an acetoxyl group. However, the stereochemistry was unchanged inasmuch as the shape of the H-12 signal for deoxoscalarin [**3**] is reported as a "multiplet, W½ 4.5 Hz". Because only four methyl groups were present, in addition to the acetate methyl, one of the skeletal methyl groups must bear an acetoxyl function. As indicated



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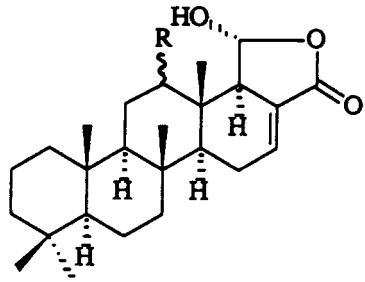
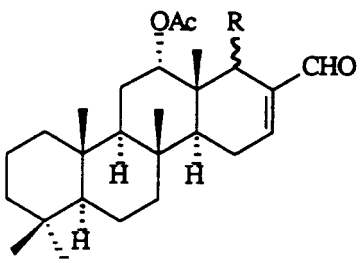


5 R=α-OAc
6 R=β-OAc

above, from mass spectral evidence it was neither of the methyl groups on ring A (C-21, C-22) nor the methyl group at the A/B ring junction (C-23), and, thus, nOe difference spectroscopy was used to determine whether the acetate was on C-24 or C-25. On irradiation of H-19 (δ 5.31) a 1.3% nOe was observed to a methyl group (δ 0.89). This could only be the C-25 methyl group; therefore the acetate group must be on C-24. Since an nOe was observed from H-19 to the C-25 methyl group, the hydroxy group at C-19 had to be α-oriented.

The remaining stereochemistry was determined by the following observations: When H-12 (δ 3.57) was irradiated, a 3.6% nOe was observed to H-18 showing that H-12 was α-oriented, i.e., the C-12 hydroxyl group was β. H-12 also showed an nOe to H-9. When H-18 (δ 2.25) was irradiated, a 4.4% nOe to H-14 (δ 1.27) and a 1.6% nOe to H-12 (δ 3.57) were observed, and thus H-12, H-14, and H-18 were all α. When the C-25 methyl group (δ 0.89) was irradiated, a 3.2% nOe to H-19 (δ 5.31) was observed. NOe's were also observed from the C-25 methyl group to the C-24 methylene group (δ 4.53, 2.2%; 4.30, 2.6%) and to the acetate methyl group (1.1%), thereby confirming that H-19 was β-oriented and that the acetate functionality was indeed at C-24.

^{13}C-nmr assignments for all carbon atoms of **4** were determined by comparison with those made previously for known related compounds (2,4–7). Crews and Bescansa (2) note that in scalaradial [**7**] the ^{13}C-nmr shift for C-25 is 15.3 ppm, whereas in 12-*epi*-scalaradial [**8**] C-25 resonates at 11.0 ppm due to shielding of the equatorial substituent at C-12. The observed carbon shift for C-25 in the compound **4** (8.9 ppm) is clearly characteristic of the 12β stereochemistry.



7 R=β-CHO
8 R=α-CHO

The isolation of sesterterpenes from *H. intestinalis* obtained from shallow water specimens is unexpected and indicates either that the sponge can metabolize different compounds depending on depth and locality or that the morphology of the species is more complex than hitherto supposed. A careful comparison of the physical characteris-

tics of reference specimens from both localities provides no sound basis for the creation of distinct species.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mp's are uncorrected and were determined on a Kofler hot-stage microscope. The optical rotation was determined on a Perkin-Elmer 241 polarimeter. Uv spectra were measured in EtOH solutions on a Varian DMS100 spectrometer and ir spectra for CCl_4 solutions on a Shimadzu IR-27G spectrometer. Eims were measured with a Varian-MAT CH7 mass spectrometer equipped with a DS30 data system. High resolution ms were obtained on a A.E.I. MS9 instrument ($m/\Delta m$ 104) interfaced with an A.E.I. mass spectrometry data system DS30. ^{1}H-nmr spectra (60 MHz) were measured in $CDCl_3$ in Varian T60 or JEOL FX-60 spectrometers, while ^{13}C-nmr spectra were measured in $CDCl_3$ with a JEOL FX-60 spectrometer (15 MHz). High field nmr spectra were obtained using a Bruker AM400 spectrometer operating at 400.13 MHz for ^{1}H and 100.62 MHz for ^{13}C.

Hplc was carried out with a Waters Associates 6000A solvent delivery system; for preparative separations Merck Hibar LiChrosorb SI-60 (7μm) or LiChrosorb RP-18 (7μm) 25 by 1 cm columns were used with r.i. detection. Hexane had bp 66.5–68°.

ISOLATION OF SESTERTERPENES.—*H. intestinalis* (28.2 g) was collected from Darwin Harbor, and a type sample of this specimen has been lodged at the Northern Territory Museum of Arts and Sciences, Darwin, under the registry number 2159. A freeze-dried sample was extracted (Soxhlet) with hexane for 4 h, and the extract was concentrated to give an oily solid (1.64 g). The extract was flash chromatographed on Si gel, and the column was eluted with hexane containing increasing amounts of EtOAc to afford 13 fractions. Fraction 4 contained carotenes, and fractions 9–10 contained sterols. Reversed-phase hplc (90% MeOH) of fractions 11–12 gave **1** (5 mg, 0.02%), and **2** (12 mg, 0.04%). Similar treatment of a further sample (31.9 g) of the sponge gave **4** (2 mg, 0.006%), **1** (5 mg, 0.01%), and **2** (3 mg, 0.009%).

HETERONEMIN [**1**].—The compound crystallized as needles, mp 185–187° $[\alpha]^{20}D$ −102° (c = 0.2, MeOH) [lit. (4) 183–184°]; found $[M-HOAc]^+$ 428.2939, calcd for $C_{27}H_{40}O_4$, $[M-HOAc]^+$ 428.2926; uv λ max (EtOH) 223 nm (ε 1400); ir (CCl_4) ν max 3530 (OH), 1742 (OAc), 1722 (OAc), 1690 (C=C), 1380, 1357, 1231, 725 cm^{-1}; ^{1}H nmr δ_H ($CDCl_3$) 6.77 (d, J = 1.5 Hz, H-19), 6.17 (t, J = 3 Hz, H-20), 5.38 (dd, J = 4, 10 Hz, H-16), 3.46 (dd, J = 4.6, 5.1, H-12), 2.42 (br s, H-18), 2.10 (s, 2 × OAc), 0.89 (s, Me), 0.83 (s, 2Me), 0.80 (s, 2Me); ^{13}C nmr δ ($CDCl_3$) 171.3 (*C*OMe), 170.0 (*C*OMe); 135.3 (C-20), 114.4 (C-17), 101.7 (C-19), 80.5 (C-12), 69.3 (C-16), 64.2 (C-18), 58.8 (C-9), 56.5 (C-5), 54.7 (C-14), 42.7 (C-13), 42.0 (C-3), 41.8 (C-7), 39.9 (C-1), 38.1 (C-10), 37.4 (C-8), 33.3 (C-21), 33.1 (C-4), 28.0 (C-15), 27.2 (C-11), 21.4 ($COCH_3$), 21.3 (*C*OMe), 21.1 (C-22), 18.6 (C-6), 18.2 (C-2), 17.4 (C-24), 16.4 (C-23), 8.8 (C-25); ms *m/z* $[M-HOAc]^+$ 428 (9%), $[C_{25}H_{38}O_3]^+$ 386.2818 (10), $[C_{25}H_{36}O_2]^+$ 368.2729 (39), $[C_{25}H_{34}O]^+$ 350.2617 (25), $[C_{24}H_{31}O]^+$ 335.2377 (10), 244 (7), $[C_{14}H_{23}]^+$ 191.1807 (64), 145 (49), 133 (81), 95 (42), 69 (50), 43 (100).

12-*epi*-DEOXOSCALARIN [**2**].—The compound crystallized from EtOH as needles, mp 202–204° [lit. (7) 192–194°]; found $[M-H_2O]^+$ 412.2994, calcd for $C_{27}H_{40}O_3$, $[M-H_2O]^+$ 412.2978; uv λ max (EtOH) 196 (ε 5300), 260 nm (ε 160); ir (CCl_4) ν max 3450 (OH), 1735, 1710 (CO), 1235, 1032, 905 cm^{-1}; ^{1}H nmr δ_H ($CDCl_3$) 5.48 (m, H-16), 5.39 (d, J = 4 Hz, H-19), 4.69 (dd, J = 4, 10 Hz, H-12), 4.42 (d, J = 12 Hz, H-20), 4.10 (d, J = 12 Hz, H-20), 2.78 (br s, OH), 2.22 (br m, H-18), 2.04 (s, OAc), 0.94 (s, Me), 0.89 (s, Me), 0.84 (s, 2Me), 0.81 (s, Me); ^{13}C nmr δ ($CDCl_3$) 170.9 (*C*OMe), 136.2 (C-17), 116.5 (C-16), 99.9 (C-19), 82.6 (C-12), 68.4 (C-20), 61.5 (C-18), 58.3 (C-9), 56.5 (C-5), 53.9 (C-14), 42.0 (C-7), 41.5 (C-3), 39.7 (C-1), 38.0 (C-13), 37.5 (C-10), 37.4 (C-8), 33.3 (C-21), 33.3 (C-4), 23.6 (C-11), 22.2 (C-15), 21.4 (C-22), 21.3 ($COCH_3$), 18.5 (C-6), 18.1 (C-2), 16.6 (C-23), 16.6 (C-24), 9.9 (C-25); ms *m/z* $[M-H_2O]^+$ 412 (34%), $[M-HOAc=C_{25}H_{38}O_2]^+$ 370.2875 (14), $[M-H_2O-HOAc=C_{25}H_{36}O]^+$ 352.2767 (29), $[C_{24}H_{33}O]^+$ 337.2552 (21), $[C_{24}H_{36}]^+$ 324.2795 (6), $[C_{23}H_{33}]^+$ 309.2572 (4), 257 (6), 231 (7), 215 (89), $[C_{14}H_{23}]^+$ 191.1797 (16), 147 (42), 133 (38), 119 (39), 109 (33), 95 (51), 81 (54), 69 (60), 55 (54), 43 (100).

24-ACETOXY-12-DEACETYL-12-*epi*-DEOXOSCALARIN [**4**].—The compound was obtained as a waxy oil: found $[M-H_2O]^+$ 428.2924, $C_{27}H_{42}O_5$ requires $[M-H_2O]^+$ 428.2926; uv λ max (EtOH) 196 (ε 5200); ir ($CHCl_3$) ν max 3430 br (OH), 1730 (OAc), 1460, 1370, 1240, 1015, 905 cm^{-1}; ^{1}H nmr δ_H (400 MHz, $CDCl_3$) 5.49 (m, H-16), 5.31 (br d, J = 6.8 Hz, H-19), 5.42 (d, J = 12.6 Hz, H-24b), 4.23 (br d, J = 12.2 Hz, H-20b), 3.89 (br s, OH), 3.57 (dd, J = 11.4, 4.3 Hz, H-12), 2.08 (s, $COCH_3$), 0.90 (s, Me), 0.89 (s, 25-Me), 0.84 (s, Me), 0.80 (s, Me); ^{13}C nmr δ ($CDCl_3$) 170.9 (*C*OMe), 134.6 (C-17), 117.6 (C-16), 99.5 (C-19), 81.1 (C-12), 68.7 (C-20), 64.5 (C-24), 61.1 (C-18), 58.6 (C-9), 56.6 (C-5), 54.2 (C-14), 41.9 (C-3), 40.3 (C-13 or C-8), 40.1 (C-1), 40.0 (C-8 or C-13), 37.2 (C-10), 36.8 (C-7), 33.2 (C-4 and C-21), 25.7 (C-11), 23.4 (C-15), 21.2 ($COCH_3$ and C-22), 18.7 (C-2 or C-6), 18.6 (C-6 or

C-2), 16.4 (C-23), 8.9 (C-25); ms *m/z* $[M-H_2O]^+$ 428 (75), 413 (8), 410 (8), 400 (4), 382 (5), 368 (4), 353 (26), 337 (12), 335 (13), 309 (12), 215 (14), 189 (19), 147 (21), 137 (21), 135 (30), 134 (22), 133 (29), 123 (26), 121 (24), 119 (30), 109 (29), 107 (30), 105 (27), 95 (39), 91 (32), 81 (41), 69 (57), 55 (46), 43 (100).

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