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SESTERTERPENES FROM A PACIFIC SPONGE, *CARTERIOSPONGIA FLABELLIFERA*

FRANCIS J. SCHMITZ* and JAMES C. CHANG

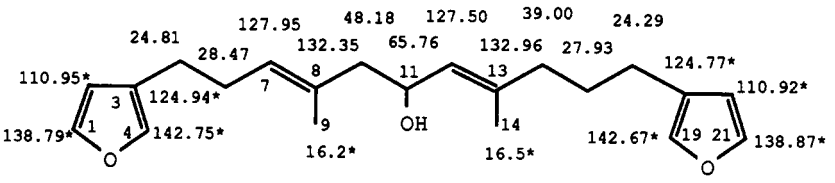
Department of Chemistry, University of Oklahoma, Norman, Oklahoma 73019

ABSTRACT.—12,13-Didehydrofurospongin-1 [**1**] and 16β-acetoxy-24-methyl-12,24-dioxosclaran-25-al [**2**], C_{21} and C_{26} sesterterpenes, were isolated from the sponge *Carteriospongia flabellifera* collected on the Great Barrier Reef, and their structures were determined by spectral analysis. The occurrence of these compounds falls into previously noted chemotaxonomic patterns.

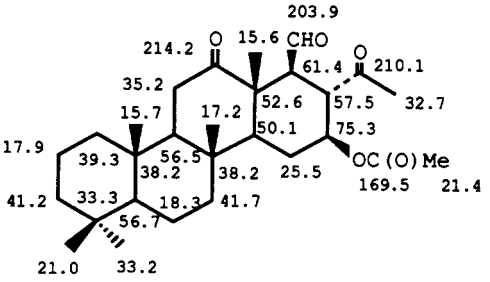
Chemotaxonomic studies have revealed that the Demospongia orders Dictyoceratida and Dendroceratida are characterized by the presence of specific classes of terpenes (1). Two of the more diagnostic terpene classes in the family Spongiidae, order Dictyoceratida, are truncated C_{21} difurano-sesterterpenes and tetracyclic homosesterterpenes (1–3). In the course of our continuing search for antitumor compounds from marine organisms, we have examined one specimen of a sponge from the Great Barrier Reef, Australia, identified as *Carteriospongia flabellifera* (Bowerbank) (= *Phyllospongia*), family Spongiidae. Extracts of this sponge have yielded two sesterterpenes consistent with the previous chemotaxonomic correlations, a didehydro analog of a C_{21} difuran described earlier and a tetracyclic homosesterterpene whose structure has been suggested earlier (4,5) but for which no substantiating data have ever been reported to our knowledge. Trace quantities of the known chlorinated metabolite **3** (6) were also isolated. This metabolite is totally unexpected for a *Carteriospongia* species (1), and it seems most likely that its occurrence in this case is due to inclusion of a small piece of *Dysidea herbacea* in our sponge sample, inasmuch as these two kinds of sponges look very similar in the field.

RESULTS AND DISCUSSION

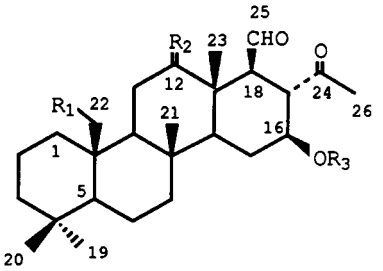
Hexane extracts of a freeze-dried specimen of *C. flabellifera* collected at Myrmedon Reef, Great Barrier Reef, Australia, were chromatographed repeatedly over Si gel to yield three metabolites, **1–3**. The formula $C_{21}H_{28}O_3$ was established for **1** from low resolution mass analysis, *m/z* 310 $[M-18]^+$, combined with ^{13}C-nmr data [DEPT spectrum (7)] that unequivocally confirmed the presence of 21 carbons and 27 protons. The Ft-ir spectrum showed only very weak, broad absorption for a hydroxyl group (ca. 3400 cm^{-1}), but the presence of a secondary hydroxyl group was established by the observation of an exchangeable proton nmr signal at 1.57 ppm that was coupled to a multiplet at 4.43 ppm. ^1H-nmr data further indicated the presence of two β-substituted furan rings: broad singlets at 6.26, 7.20, and 7.34 ppm, each integrating for two protons and coupled as expected for a β-substituted furan. In the ^1H-nmr spectrum of **1** in $CDCl_3$ containing a few drops of CD_3OD, each of these signals appeared as a multiplet with very small *J*s, but due to overlap of the signals for like protons in the two furan rings, multiplicities were not clear. Decoupling experiments confirmed that H-1 (2) was coupled to both H-2 (20) and H-4 (19); H-4 (19) also showed allylic coupling to H-5 (17). Further evidence for the furan moieties came from the broad band and DEPT ^{13}C-nmr spectra that showed two closely spaced pairs of doublets at 142.67, 142.75, and 138.79, 138.87 for α-furan carbons and showed doublets at 110.01, 110.92 and a pair of singlets at 124.77, 124.94 ppm for β-furan carbons. The two furan rings and a secondary hydroxyl group confirm the three oxygens postulated in the formula gleaned from the low resolution spectrum. The nmr signals for the non-furanoid protons were



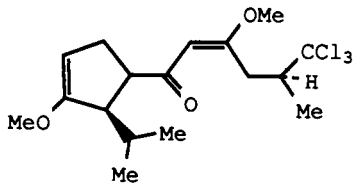
1



2



	R_1	R_2	R_3
4	OAc	=O	Ac
5	H	<OH H	H



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quite well resolved, and from decoupling results and a $^1H/^1H$ COSY spectrum (7), the remaining skeleton could be deduced as indicated by the coupling data (see Experimental). Allylic coupling between H-10 and H-7 and between H-12 and H-15 provided the evidence for making connections across the respective intervening quaternary carbons. Furan **1** is, thus, 12,13-didehydrofurospongin-1. [Compound numbering is according to Cimino *et al.* (8)]. Furospongin-1 and tetradehydrofurospongin-1 have been isolated previously from sponges of the family Spongiidae (9,10).

Compound **2**, $C_{28}H_{42}O_5$ by high resolution mass analysis, exhibited ir absorption for ester (1748 cm^{-1}) and ketone or aldehyde groups (1713 cm^{-1}), while ^{13}C-nmr data confirmed the presence of two ketones (210.13, 214.9), one aldehyde (203.85), and one acetate group (169.56 ppm; ^1H-nmr absorption, 2.03 ppm). The window for the DEPT spectrum did not extend to the carbonyl region, but the intensity of the 203.85 ppm peak was approximately twice that of the other carbonyl peaks, which is consistent with the faster relaxation expected for the proton-bearing aldehyde carbon. Because there were no olefinic carbons, **2** was confirmed to be tetracyclic. From comparison of the ^{13}C- (DEPT) and ^1H-nmr data of **2** with that reported (2,5) for **4** and **5**, it could be inferred that **2** and **4** have the same structure with the exception that **2** has a methyl group at C-10 whereas in **4** there is a acetoxymethyl group at this position. A compound assigned structure **2** has previously been reported without substantiating spectral or physical data (4,5). The proton sequence from H-15 to H-18 was confirmed by decoupling and $^1H/^1H$ COSY data (see Experimental). There is no coupling evident be-

tween H-18 and H-25; this coupling is also lacking in the spectrum of **4** (5). The signals for H-11α, H-11β, and H-9 were also identified. Using the skeleton name suggested by Kazlauskas *et al.* (5), keto aldehyde **2** is 16β-acetoxy-24-methyl-12,24-dioxosclaran-25-al.

A chlorinated metabolite isolated in trace amounts was identified as **3** by comparison of its ^{1}H- and ^{13}C-nmr data with literature values (6).

The occurrence of **1** and **2** in a species of *Carteriospongia* is consistent with previous chemotaxonomic correlations. The presence of **3** in this sponge is not expected and seems likely to be due to contamination of our sponge sample with a piece of *Dysidea herbacea*, as mentioned above.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are uncorrected. Infrared spectra were taken on a Nicolet 8000 FT-IR (200 SXV) spectrophotometer, optical rotations on a Perkin-Elmer 141 Polarimeter, and nmr spectra on a Varian XL-300 spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C. Signals are reported in ppm downfield from internal TMS. High resolution mass spectra were taken on a CEC (DuPont, Monrovia) 110 instrument, and low resolution mass spectra were taken on a Hewlett-Packard 5985B spectrometer. The chromatographic adsorbent used was Brinkmann Si gel (type 60). Hplc separations were carried out on an Applied Science 5μ, 10 mm × 25 cm Si gel column.

EXTRACTION AND ISOLATION.—Specimens of the sponge *C. flabellifera* were collected at approximately −10 m at Myrmedon Reef, Great Barrier Reef, Australia, and were freeze-dried. Dry, ground sample (261 g) was soaked successively in hexane (7 days), $CHCl_3$ (3 days), and *n*-BuOH (3 days). Evaporation of solvents yielded 2.1 g, 7.3 g, and 4.3 g of dark green residues, respectively. The hexane fraction was resolved using flash cc and step gradient elution: $CHCl_3$, then 40% EtOAc/$CHCl_3$, then a direct change to 20% MeOH/$CHCl_3$; 8 fractions (10–20 ml) were collected. Rechromatography of fractions 3, 4, and 5 using hplc [Me_2CO-hexane (1:9)] yielded three pure compounds, **1** (0.7 mg), **2** (7.4 mg), and **3** (traces).

12,13-Didehydrofurospongin 1 [**1**].—Ir (neat) 3200–3500 (brd), 3140 (vs), 1500, 1450, 1380, 1160, 1030 (vs), 875 (vs), 780 (vs) cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 1.64 (3H, d, J = 1.1, H-9), 1.67 (3H, d, J = 1.4, H-14), 1.70 (2H, m, H-16), 2.04 (2H, dd, J = 7.0, 7.7, H-15), 2.15 (2H, d, J = 6.6, H-10), 2.29 (2H, br q, J = 7.3, H-6), 2.39 (2H, t, J = 7.4, H-17), 2.48 (2H, t, J = 7.3, H-5), 4.43 (1H, ddt, J = 2.4, 8.3, 6.6, H-11), 5.16 (1H, dq, J = 8.3, 1.4, H-12), 5.27 (1H, tq, J = 7.1, 1.2, H-7), 6.26 (2H, br s, H-2 and H-20), 7.20 (2H, br s, H-4 and H-19), 7.34 (2H, br s, H-1 and H-21); ^{13}C nmr ($CDCl_3$; 70 eV low resolution eims) *m/z* (rel. int.) $[M-18]^+$ 310 (1), 179 (76), 161 (22), 150 (78), 135 (46), 97 (53), 95 (58), 85 (21), 83 (20), 82 (39), 81 (100).

16β-Acetoxy-24-methyl-12,24-dioxosclaran-25-al [**2**].—Mp 227–229°; $[\alpha]_D$ +135° (c = 0.74, $CHCl_3$); ir (neat) 1748 (vs), 1713 (vs), 1226 (vs); ^{1}H nmr ($CDCl_3$, 300 MHz) δ 0.82 (3H, s), 0.86 (3H, s), 0.88 (3H, s), 1.04 (3H, s, H-21), 1.19 (3H, s, H-23), 1.25 (1H, dd, J = 1.9, 12.8, H-9), 1.40 (m, H-15), 1.76 (1H, dt, J = 14.2, 2.1), 2.03 (3H, s, OAc), 2.12 (1H, m, H-15), 2.30 (3H, s, OAc), 2.36 (1H, dd, J = 1.9, 12.8, H-11α), 2.66 (1H, dd, J = 12.9, 12.9, H-11β), 3.1 (1H, t, J = 11.4, H-17β), 3.25 (1H, d, J = 11.8, H-18α), 4.62 (1H, m, H-16α), 9.95 (1H, s, H-25); low resolution eims (70 eV), *m/z* (rel. int.) $[M]^+$ 458 (10), 370 (29), 327 (22), 206 (13), 192 (23), 191 (63), 178 (25), 177 (33), 165 (32), 149 (22), 137 (44), 136 (29), 135 (47); hrms observed *m/z* $[M]^+$ 458.3037; calcd for $C_{28}H_{42}O_5$, 458.3032.

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