A New Cytotoxic Sterol Methoxymethyl Ether from a Deep Water Marine Sponge *Scleritoderma* sp. cf. *paccardi*

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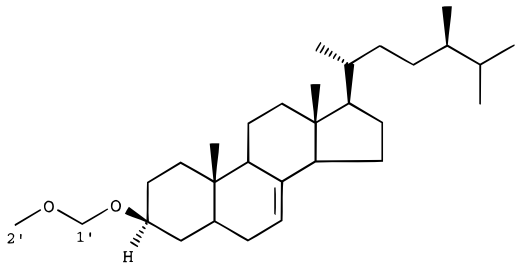
24(*R*)-Methyl-5α-cholest-7-enyl 3*β*-methoxymethyl ether (**1**), a new sterol ether, has been isolated from a deep-water marine sponge *Scleritoderma* sp. cf. *paccardi*. Compound **1** exhibited *in vitro* cytotoxicity against the cultured murine P-388 tumor cell line with an IC_{50} of 2.3 *μ*g/mL. The isolation and structure elucidation of **1** by NMR spectroscopy is described.

Numerous new sterols have been isolated from marine organisms. Although most have side chains that are polyoxygenated or alkylated,[1] few naturally occurring sterols are known to contain a methyl ether.[2,3] In this paper, we report the isolation of a cytotoxic new sterol ether (**1**) from the deep-water sponge *Scleritoderma* sp. cf. *paccardi* Schmidt (Scleritodermiidae). To our knowledge, this is the first report of a novel compound isolated from this genus.



Sterol ether (1)

In our search for biologically active substances from marine organisms, we found that an EtOH extract from a deep-water marine sponge *Scleritoderma* sp. cf. *paccardi* inhibited the growth of the yeast *Candida albicans*. This sponge contains a high proportion of silicious spicules and relatively little organic material. This makeup resulted in a low yield of organic extract and relatively little material with which to pursue the chemical investigation. The *C. albicans*-active compound was tentatively identified as a peptide by NMR studies but decomposed on further purification. In the course of these studies a new sterol ether (**1**) was isolated and shown to be cytotoxic against P-388 tumor cells.

An EtOH extraction of the frozen sponge yielded an extract that was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction on column chromatography over Si gel followed by HPLC furnished a new sterol ether (**1**). The HREIMS of **1** calculated to a molecular formula of $C_{30}H_{50}O_2$. The IR spectrum indicated the presence of an ether group (1105, 1038 cm^{-1}) and absence of hydroxyl groups. From a combination of APT, DEPT, and HMQC experiments, the 30 resonance lines in the carbon spectrum were assigned to two quaternary carbons, one protonated and one nonprotonated SP^2-hybridized carbon, seven methines, one oxygen-bearing methine, 10 methylenes, one oxygen-bearing methylene, six methyls, and one oxygen-bearing methyl. The ^1H NMR spectrum indicated two methyl singlets and four methyl doublets typical of a sterol: *δ* 0.51 (s, C18), 0.76 (d, J = 6.5 Hz, C28), 0.78 (s, C19), 0.79 (d, J = 7.0 Hz, C27), 0.83 (d, J = 7.0 Hz, C26), and 0.90 (d, J = 6.5 Hz, C21). The presence of a 3*β* oxygenation was indicated by a broad methine multiplet at *δ* 3.47 ($W_{1/2}$ = 24 Hz). The downfield shift of the above chemical shift value is consistent[3,4] with the shielding from the methoxymethyl ether group (*δ* 4.66, 2H, s, C1′; 3.35, 3H, s, C2′). In the COSY NMR spectrum, the broad multiplet signal for H3 showed coupling to H4 protons at *δ* 1.83 and 1.05, which were in turn coupled to H5 at *δ* 1.35. In the LRCI mass spectrum, an intense peak was observed at m/z 383, indicating a loss of a CH_3OCH_2O- group from the molecular ion. The mass spectrum also indicated loss of mass units m/z 15, 31, and 45 from the molecular ion corresponding to the loss of CH_3, OCH_3, and CH_3OCH_2- fragments, respectively. These observations confirmed the presence of a CH_3OCH_2O group in the molecule. The results from an HMBC NMR experiment indicated long-range C−H correlations from H3 to C5 (*δ* 40.3), C1 (35.1) and C1′ (94.6), from H1′ to C3 (76.1) and C2′ (55.1). These results unambiguously established the position of the methoxymethyl ether group in the molecule. The only olefinic proton (*δ* 5.13 br s) was assigned to C7 based on the following observations. In the COSY spectrum it showed coupling to H6 protons (*δ* 1.73, 1.24), which were in turn coupled to H5 (1.35). The long-range C−H correlations observed from H7 to C5 (*δ* 40.3), C9 (49.5), and C14 (55.1) confirmed the assignment. Comparison of the ^{13}C-NMR data for positions C5 (*δ* 40.3) and C10 (34.4) with other known compounds confirmed an A/B *trans* ring junction.[5]

The side-chain structure was determined by MS data and ^1H NMR analysis and was confirmed by long-range C−H correlations. A major fragment was observed at m/z 255 (23%) due to the loss of a C_9H_{19} + H from the fragment ion at m/z 383 (98%) and thus suggested a conventional saturated steroidal C9-side chain. The long-range C−H correlations observed from H21 (*δ* 0.90) to C17 (56.2); H26 (0.83) and H27 (0.79) to C24 (38.9); and H28 (0.76) to C25 (32.4) confirmed the presence of a typical 24-methyl C9 side chain. The configuration at C24 was assigned as 24*R* by comparison with the ^1H

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NMR data reported for (24*R*)-3β-methoxy-24-methyl-5α-cholest-7-ene.[3] Combination of the above data established the structure of **1** as 24(*R*)-methyl-5α-cholest-7-en-3β-methoxymethyl ether. To the best of our knowledge, this is the first report of a methoxymethyl ether sterol from a natural source. Compound **1** was discovered to inhibit the in vitro proliferation of murine P-388 leukemia cell line with an IC_{50} value of 2.3 μg/mL.

Experimental Section

General Experiment Procedures. IR spectra were obtained on a Midac FTIR M 1200 instrument. ^{13}C NMR spectra were measured on a Bruker AM360 instrument. ^1H and all 2D spectra were measured on a Bruker AMX−500 instrument. The ^1H chemical shifts were assigned using a combination of data from COSY, TOCSY, and HMQC experiments. Similarly, ^{13}C chemical shifts were assigned on the basis of APT, DEPT, and HMQC experiments. The MS were obtained on a Kratos MS-80RFA mass spectrometer at the Chemical Instrumentation Center, Yale University.

Collection and Taxonomy. The sponge was collected by the Johnson-Sea-Link I manned submersible on 12 Nov 1985, from a depth of 293 m, from Cay Bokel, Turneffe Islands, Belize. The sponge is a small fungiform knob, restricted at the base, and with an apical oscular depression. The upper surface is hairy with long projecting spicules, and longitudinal subsurface canals are visible on the sides of the sponge. The texture is stony. The color in life and in EtOH is cream. The skeleton is a very dense reticulation of highly warty monocrepid desmas. Extremely irregular, faintly roughened microstrongyles pack the ectosome, which is pierced by long hair-like oxea. Sigmaspires are scattered throughout the mesohyl matrix. The sample is closely comparable to *Scleritoderma* sp. cf. *paccardi* (Order Spirophorida, lithistid family Scleritodermiidae) as described by van Soest and Stentoff.[6] A taxonomic voucher specimen is deposited in the Natural History Museum, London, U.K. (BMNH 1985.11.12.4).

Extraction and Isolation. The EtOH extract of the sponge (150 g) was partitioned between EtOAc and H_2O. The EtOAc-soluble fraction (0.07 g) was then column chromatographed on SiO_2 using hexane/CH_2Cl_2 step gradient. Rechromatography of the sterol fraction eluted with 60% hexane/CH_2Cl_2 on HPLC (Si, 5μ, 25 × 1 cm) with 50% hexane/CH_2Cl_2 gave the sterol ether **1** (0.003% yield, wet weight of powder) as a gum.

Sterol ether (**1**): $[\alpha]^{24}_D$ 240 (c = 0.03, CH_2Cl_2); IR (neat) ν max 2950, 1461, 1375, 1147, 1105, 1038, cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.13 (1H, br s, H-7), 4.66 (2H, s, H-1′), 3.47 (1H, m, H-3), 3.35 (3H, s, H-2′), 2.01 (1H, m, H-12), 1.90 (1H, m, H-16), 1.83 (1H, m, H-4), 1.82 (1H, m, H-2), 1.78 (1H, m, H-14), 1.75 (1H, m, H-1), 1.73 (1H, m, H-6), 1.62 (1H, m, H-9), 1.55 (1H, m, H-11),1.52 (1H, m, H-15), 1.52 (1H, m, H-25), 1.46 (1H, m, H-11), 1.41 (1H, m, H-2), 1.37 (1H, m, H-15), 1.35 (1H, m, H-5), 1.32 (1H, m, H-1), 1.32 (1H, m, H-22), 1.30 (1H, m, H-20), 1.25 (2H, m, H-23), 1.25 (1H, m, H-16), 1.24 (1H, m, H-6), 1.21 (1H, m, H-12), 1.20 (1H, m, H-17), 1.20 (1H, m, H-24), 1.05 (1H, m, H-4), 1.05 (1H, m, H-22), 0.90 (3H, d, J = 6.5 Hz, Me-21), 0.83 (3H, d, J = 7.0 Hz, Me-26), 0.79 (3H, d, J = 7.0 Hz, Me-27), 0.78 (3H, s, Me-19), 0.76 (3H, d, J = 6.5 Hz, Me-28), 0.51 (3H, s, Me-18); ^{13}C NMR ($CDCl_3$) δ 139.6 (s, C-8), 117.4 (d, C-7), 94.6 (t, C-1′), 76.1 (d, C-3), 56.2 (d, C-17), 55.1 (d, C-14), 55.1 (q, C-2′), 49.5 (d, C-9),43.4 (s, C-13), 40.3 (d, C-5), 39.6 (t, C-12), 38.9 (d, C-24), 37.2 (t, C-4), 36.3 (d, C-20), 35.1 (t, C-1), 34.4 (s, C-10), 33.7 (t, C-22), 32.4 (d, C-25), 30.4 (t, C-23), 29.8 (t, C-6), 28.7 (t, C-2), 27.9 (t, C-16), 23.0 (t, C-15), 21.5 (t, C-11), 20.2 (q, C-26), 18.8 (q, C-21), 18.3 (q, C-27), 15.4 (q, C-28), 12.9 (q, C-19), 11.8 (q, C-18); HREIMS m/z 444.397, Δ 2 mmu for $C_{30}H_{52}O_2$; LRCIMS (ace/isob) m/z (rel int) 444 (100), 429 (13), 399 (9), 383 (98), 313 (10), 255 (23).

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