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Geodisterol, A Novel Polyoxygenated Sterol with an Aromatic A Ring from the Tropical Marine Sponge *Geodia* sp.

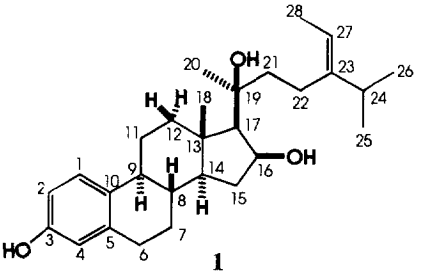
Gui-Yang-Sheng Wang and Phil Crews*

Department of Chemistry and Biochemistry, University of California, Santa Cruz, CA 95064

Abstract: Geodisterol (**1**), the first marine polyoxygenated sterol with an aromatic A ring, was isolated from the Indo-Pacific sponge *Geodia* sp. The structural and stereochemical features of **1** were based on the extensive analysis of 1D and 2D of it and MPA esters **2** and **3**. Copyright © 1996 Elsevier Science Ltd

For many years, sponges of the Choristida order have been high priority collection targets during our expeditions to coral reefs.[1] In this regard, Indo-Pacific sponges of the genus Geodia have been of special interest because the few prior studies on this group have afforded novel metabolites.[2] In spite of constant attempts, we were unable to locate members of this group until recently. In 1993, during fieldwork in Milne Bay Papua New Guinea, our SCUBA team collected and identified a massive purple sponge (coll. no. 93180, 3.8 kg wet wt. and heavily encrusted by a white tunicate) as a member of the *Geodia* genus. A detailed chemical study commenced once the crude extract was observed to be selectively active against a human colon solid tumor cell line.[3] Outlined below are the properties of geodisterol (**1**) which was isolated as the major component of the polar extract of this sponge.

The collected sponge was preserved, transported to our lab and extracted as previously described.[1] The combined oils obtained from the methanol extract were partitioned between aqueous methanol and hexane followed by aqueous methanol and methylene chloride. The CH_2Cl_2 partition fraction was chromatographed on silica gel using a CH_3OH-CH_2Cl_2 gradient. The 10% CH_3OH-CH_2Cl_2 fraction was next purified by normal phase HPLC to yield geodisterol (**1**) [108 mg, 0.083% dry wt., $[\alpha]_D$ +67° (*c* 0.31, CH_2Cl_2-CH_3OH 9:1)].



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The HRFABMS of **1** contained *m/z* peaks at 427.3054 $[M+H]^+$ and 409.3111 $[M-H_2O+H]^+$ which allowed the molecular formula to be established as $C_{28}H_{42}O_3$. The APT NMR spectra revealed the following carbon types: five methyl, seven methylene, ten methine and six quaternary residues for a total of $C_{28}H_{39}$. Half of the eight degrees of unsaturation were accounted for by the four double bonds observed by ^{13}C NMR.[4] The characteristic ^1H NMR shifts and *J*s associated with three of the double bonds suggested a trisubstituted phenol group (δ 7.06 d, 6.56 dd, 6.50 d) was present. Independent confirmation of this was provided by HMBC correlations from H1 to C3 and C5 and from H2 to C3, C4 and C10. The CH_3CH=C moiety was easily identified from other ^1H NMR multiplets. Firm evidence for the two additional alcohol groups was provided by preparing the diesters described below. The nature of ring B was established from HMBC correlations from H6 to C5, C7, C8 and C10; plus correlations from H9 to C8 and C10. The substitution pattern of the C and D rings was deduced from ^{13}C chemical shifts and HMBC cross-peaks including those from H11' to C9 and C12; from singlet $H_3$18 to C12, C13, C14 and C17; and from H15 to C14, C16 and C17. The additional ^1H-^1H COSY correlations to each of the protons in the sidechain attached to C19 justified this substructure containing C21 - C28. These data along with the HMBC correlations from H21' to C17, C19, and C20, and from H22 to C19, C23, C24 and C27 provided the final justification for the entire planar structure of **1**.

The stereochemical elements of **1** were determined using several different types of data. The *trans* fusion of the B/C/D rings was established in steps. The two *J*=11.5 Hz couplings to H8 from H9 and H14 gave

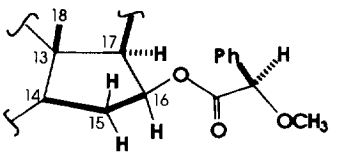

Figure 1. Conformation analysis of geodisterol (R)-MPA ester.

information about the B/C ring junction. The upfield ^{13}C NMR shift of Me18 (δ 14.3) indicated it was axial and attached to a *trans* fused C/D ring junction. The *cis* relationship between H16 (δ 4.62 ppm, ddd, *J*=7.5, 7.5, 4.5 Hz) and H17 (δ 1.30 ppm, d, *J*=7.5 Hz) was established on the magnitude of their mutual *J*-coupling. Preparation and analysis of the MPA (2-methoxyphenylacetic acid) esters of **1** was undertaken. The two derivatives, (*R*)-MPA ester **2** and (*S*)-MPA ester **3**, displayed the following shifts. The H15 and H17 chemical shifts of **2** were 2.39 and 1.40 ppm, and those of **3** were 2.58 and 1.32 ppm. Because H15 of (*R*)-MPA ester and H17 of (*S*)-MPA ester were shielded by the MPA phenyl group (Figure 1), the stereochemistry of C16 could be assigned as *S*.[5] The stereochemistry of C19 was determined by the strong dipolar coupling observed between $H_3$20 (δ 1.33 ppm) and the equatorial H12 (δ 2.24 ppm) in the NOESY spectrum. This indicated their close proximity and that C19 should be *S*-configuration.[6] A final set of peaks in the NOESY spectrum of **1** included dipolar couplings between H28 and H22 and between H27 and H24, $H_3$25, $H_3$26. These data indicated that the double bond between C23 and C27 was *E*.

Geodisterol (**1**) represents the first polyoxygenated sterol with an aromatic A ring isolated from marine organisms. While sterols with aromatic A rings have been obtained from terrestrial plants, animals and are ubiquituous as hormones (estradiol), all of those sterols have a small sidechain in comparison to **1**. To date several oxygenated sterols have been isolated from various marine organisms.[7] The only related polyoxygenated sterols from the same sponge genus are from *Geodia cydonium*, collected in the Bay of Naples. These compounds have a cholest-4-ene-3,6-dione frame with a different sidechain.[2d]

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4. ^1H NMR (500 MHz, $CDCl_3$-CD_3OD 2:1) δ (ppm) 7.06 (d, *J*=8.5 Hz, H1), 6.56 (dd, *J*=8.5, 2.5 Hz, H2), 6.50 (d, *J*=2.5 Hz, H4), 2.79 (m, H6), 2.77 (m, H6'), 1.85 (m, H7), 1.30 (m, H7')1.46 (dddd, *J*=11.5, 11.5, 11.0, 3.5 Hz, H8), 2.13 (dt, *J*=4.0, 11.5 Hz, H9), 2.21 (m, H11), 1.54 (m, H11'), 2.24 (m, H12), 1.39 (m, H12'), 1.06 (dt, *J*=7.5, 11.5 Hz, H14), 2.29 (dt, *J*=11.5, 7.5 Hz, H15), 1.37 (m, H15'), 4.62 (ddd, *J*=7.5, 7.5, 4.5 Hz, H16), 1.30 (d, *J*=7.5 Hz, H17), 1.14 (s, 3H, H18), 1.33 (s, 3H, H20), 1.82 (m, H21), 1.62 (dt, *J*=4.5, 12.5 Hz, H21'), 2.08 (dt, *J*=4.5, 12.5 Hz, H22), 2.01 (dt, *J*=4.5, 12.5 Hz, H22'), 2.22 (m, H24), 0.97 (d, *J*=6.0 Hz, 3H, H25), 0.97 (d, *J*=6.0 Hz, 3H, H26), 5.18 (q, *J*=6.0 Hz, H27), 1.58 (d, *J*=6.0 Hz, 3H, H28). ^{13}C NMR (125 MHz, $CDCl_3$-CD_3OD) δ (ppm) 125.9 (d, C1), 112.5 (d, C2), 154.3 (s, C3), 114.9 (d, C4), 137.6 (s, C5), 29.4 (t, C6), 27.5 (t, C7), 37.8 (d, C8), 43.7 (d, C9), 131.5 (s, C10), 26.3 (t, C11), 40.4 (t, C12), 43.1 (s, C13), 53.3 (d, C14), 36.5 (t, C15), 73.3 (d, C16), 59.6 (d, C17), 14.3 (q, C18), 76.8 (s, C19), 25.3 (q, C20), 42.6 (t, C21), 23.8 (t, C22), 146.1 (s, C23), 34.7 (d, C24), 21.7 (q, C25), 21.7 (q, C26), 115.7 (d, C27), 12.5 (q, C28).
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