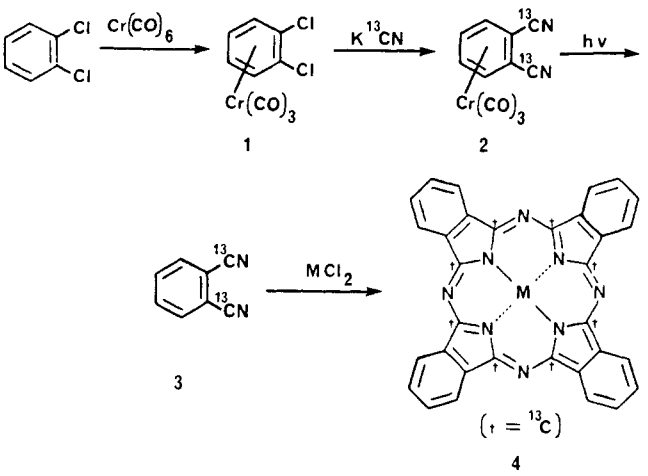

quently contains this impurity. Reaction of **3** with nickel(II) chloride[1] in quinoline gave the required phthalocyanine complex **4** (M = Ni) (63%). Spectroscopic data for both **3** and **4** were fully in accord with the required high level of isotopic enrichment. The copper complex **4** (M = Cu) was prepared in exactly the same way. It is clear from these results that (arene)tricarbonylchromium chemistry is particularly useful in labeled phthalocyanine construction.

Experimental Section

Melting points were determined by using a Reichert Jung hot-stage microscope. IR data were obtained on a Mattson spectrometer. Solution ^1H and ^{13}C NMR spectra were obtained with a Varian VXR-400 instrument and CP-MAS ^{13}C NMR spectra with a Varian XLA-300 instrument. Mass spectra were recorded by using a VG-70-250SE instrument.

1,2-Di[^{13}C]cyanobenzene (3). Potassium [^{13}C]cyanide[6] (99% ^{13}C, 0.91 g, 15 mmol) and 18-crown-6 (3.84 g, 14 mmol) were dissolved in DMSO (16 mL) under nitrogen. (η^6-1,2-Dichlorobenzene)tricarbonylchromium[3] (2.0 g, 7.1 mmol) was added, and the solution was stirred for 3 h, under nitrogen, in the dark. An equal volume of water was added to this solution on ice, and the resulting mixture was extracted with CH_2Cl_2 (5 × 30 mL). The red-orange extract was exposed to either bright sunlight or a UV light source (Hg vapor arc lamp) for ca. 3 days in order to decomplex **2**. The mixture was filtered through Celite daily to remove the green flocculent, oxidized-chromium precipitate and expedite the photodecomplexation. When the filtrate appeared clear and colorless, the solution volume was reduced to a minimum by rotary evaporation and cold H_2O (50 mL) was added. The resulting tan-white precipitate was filtered off, dissolved in hot ethanol (20 mL), and decolorized with a small amount of charcoal. After filtering, warm H_2O (30 mL) was added to the ethanolic solution, which was chilled on ice. The resulting white crystalline precipitate was filtered and recrystallized from hot ethanol/H_2O (1:1) to give **3** (0.50 g, 63%): mp 139–141 °C; IR (KBr) 3079 (w), 3040 (w), 2177 (s, C≡N), 1483 (m), 1355 (m), 1277 (s), 1221 (w), 1206 (w), 1206 (w), 1145 (sh), 1118 (s), 965 cm^{-1} (m) (the C≡N stretch in unlabeled 1,2-dicyanobenzene appears at 2232 cm^{-1}); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.78 (m); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 115.32; mass spectrum (EI) m/e 130 ($M^{+\cdot}$).

Nickel [5,7,12,14,19,21,26,28-$^{13}C_8$]Phthalocyanine (4) (M = Ni). 1,2-Di[^{13}C]cyanobenzene (**3**) (0.50 g) and $NiCl_2 \cdot 6H_2O$ (0.50 g) in quinoline (6 mL) were heated to 180 °C and maintained at that temperature for 15 h. The resulting solid mass was broken up in acetone, filtered, and washed with acetone and H_2O. The purple crude material was sublimed at ca. 400 °C (10^{-3} Torr) to give **4** (M = Ni) (0.35 g, 63%) as lustrous, purple needles: IR (KBr) 2928, 2851, 1383, 1375, 1363, 1362, 1332, 1288, 1261, 1163, 1119, 1079, 1021, 906, 803, 775, 749, 722 cm^{-1}; UV-vis (1,2,4-trichlorobenzene) λ_{max} 672 (log ϵ = 5.00), 640 (4.51), 604 (4.53), 336 nm (4.66); ^{13}C CP-MAS NMR (75 MHz) δ 143.6; mass spectrum (EI) m/e 578 ($M^{+\cdot}$).

Copper [5,7,12,14,19,21,26,28-$^{13}C_8$]Phthalocyanine (4) (M = Cu). Reaction of **3** with $CuCl_2$ gave **4** (M = Cu) (28%): IR (KBr) 2962, 2917, 1383, 1375, 1363, 1332, 1331, 1284, 1261, 1162, 1116, 1077, 1022, 891, 802, 775, 748, 718 cm^{-1}; UV-vis (1,2,4-trichlorobenzene) λ_{max} 677 (log ϵ = 5.16) 648, (4.40), 610 (4.47), 345 nm (4.68), mass spectrum (EI) m/e 583, 584, 585 ($M^{+\cdot}$).

Acknowledgment. We thank the National Science Foundation (DMR-85-19233) and G. D. Searle and Company for support of our research and the National Institutes of Health for the purchase of a 400-MHz NMR spectrometer (RR-02314) and a high-resolution mass spectrometer (RR-03245) used in this work.

Registry No. **1**, 70140-19-1; **2**, 120771-51-9; **3**, 120788-97-8; **4** (M = Ni), 99476-50-3; **4** (M = Cu), 120771-52-0; 18-crown-6, 17455-13-9; potassium [^{13}C]cyanide, 25909-68-6; 1,2-dichlorobenzene, 95-50-1.

(6) Potassium [^{13}C]cyanide (99% label) was purchased from the Aldrich Chemical Company.

Three New Polyhydroxylated Sterols with the 5β-Configuration from the Sponge *Dysidea etheria*[1]

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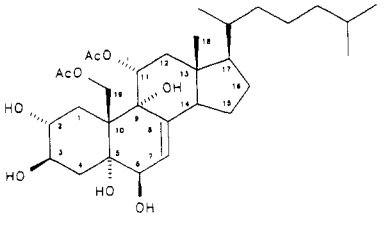
We recently reported the isolation of eight new, highly oxygenated sterols from the sponge *Dysidea etheria*, represented by **1**.[2] In the course of examining slightly less polar fractions of the extract for less oxidized or more highly acylated steroids, we uncovered a series of sterols analogous to those disclosed earlier, but distinguished by a 5β skeleton (cis AB ring juncture). It appears that herbasterol, a secosteroid isolated from *Dysidea herbacea*,[3] and a series of coprostanols from *Petrosia ficiformis*[4] are the only other examples of 5β sterols found in the phylum Porifera. The isolation, structure elucidation, and initial biological testing of three new 5β steroids with seven oxygen functionalities comprise this report.



As was noted earlier,[2] solvent partitioning[5] of the crude organic extracts of *D. etheria*, collected in Bermuda, yielded a chloroform-soluble fraction with a high concentration of highly functionalized steroids. Gel permeation chromatography (Sephadex LH-20, Bio-Beads S-X4) of

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Table I. ^{13}C Nuclear Magnetic Resonance Data Comparison[a]

carbon no.	1[b]	2	3	4	5[c]
1	35.4	42.0	42.0	42.1	36.2
2	73.1	65.0[d]	65.0[d]	65.0[d]	68.2[d]
3	72.4	69.3[d]	69.4[d]	69.4[d]	69.9[d]
4	40.2	46.7	46.7	46.7	35.9
5	77.8	79.6	79.8	79.7	80.0
6	71.3	70.0	70.0	70.0	201.1
7	122.7	125.9	126.0	126.0	120.3
8	143.8	141.4	141.5	141.5	165.0
9	76.5	78.1	78.2	78.2	46.2
10	49.7	51.0	51.0	51.0	45.5
11	73.2	68.9	68.8	68.9	69.6
12	46.5	42.7	42.8	42.8	44.0
13	43.2	43.0	43.0	43.1	48.2
14	51.2	51.7	51.7	51.7	48.2
15	23.4	23.6	23.6	23.7	31.7
16	28.2	28.2[e]	28.3	28.4	21.4
17	56.3	56.5	56.4	56.4	49.4
18	13.0	12.6	12.6	12.6	17.4
19	65.1	62.9	62.8	62.9	18.8
20	36.5	36.4[g]	36.9	36.7	76.8
21	18.9	18.9	18.9	19.0	21.4
22	36.3	36.3[g]	33.8	34.0	76.8
23	24.1	24.2	31.0	26.8	30.1
24	39.7	39.7	39.3	46.3	37.0
25	28.3	28.3[e]	31.7	29.3	28.2
26	22.6[d]	22.6[f]	17.7	19.8	22.4
27	22.9[d]	22.9[f]	20.4	19.2	23.4
28			15.6	23.3	
29				12.5	

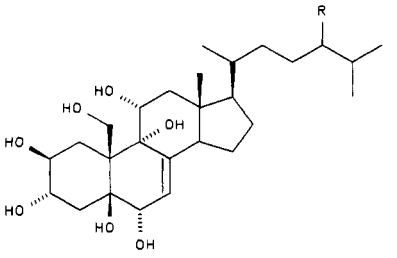
[a] Spectra recorded in pyridine-d_5. [b] Reference 2. [c] Reference 6. [d] Assignments may be reversed in each column.

this material, followed by low-pressure silica gel chromatography, gave eight fractions, seven of which contained varying amounts of sterols. Reversed-phase (C_{18}) HPLC of the third fraction gave three major steroid fractions, each still a mixture. Final purification was achieved by use of a β-cyclodextrin HPLC column, and **2**–**4** were obtained as amorphous white solids.

^1H and ^{13}C NMR analyses suggested that the three compounds possessed functionality patterns identical with those in **1** and differed only in the steroid side chain. As might be expected, molecular weight information was not forthcoming from electron-impact mass spectrometry because of facile losses of water, but fast atom bombardment mass spectrometry in a glycerol matrix laced with RbI provided substantial $(M + Rb)^+$ ions. This approach, linked with the ^{13}C NMR data, elicited a molecular formula, $C_{27}H_{46}O_7$, for the first compound in the group, indicating no acetylation and a cholestane side chain. A ^1H–^1H COSY experiment enumerated the spin–spin relationships in **2** and, along with ^1H–^{13}C correlation experiments, revealed that the functionalities were distributed just as they were in **1** and its congeners.[2] Curiously, however, the proton and carbon shifts in the A and B rings were substantially altered from those in **1**. The resonances of C-1 and C-4 were both shifted to lower field by ~6.5 ppm relative to **1**, while C-2 and C-3 were shifted to higher field by 3–8 ppm. The ring juncture carbons, C-5 and C-10, were slightly deshielded (1–2 ppm), and C-19 was shifted upfield by more than 2 ppm. These aberrations from **1** were consistent with a cis AB ring juncture. The C-19 shift could be due to anisotropic shielding by the C-5 hydroxyl group's nonbonding electrons; the C-2 and C-3 chemical shifts of **2** correspond well with those of the phytoecdysone muristerone (**5**)[6] (see Table I).

The remaining stereochemical assignments for **2** were derived largely from decoupling and difference NOE experiments. Irradiation of the signal at δ 4.65 (overlapping signals for H-6 and H-19) resulted in a 3.5% enhancement of H-1β at δ 2.55. Two large couplings (geminal and axial–axial) to H-1β necessitated an equatorial (β) hydroxyl at C-2. There was no enhancement of either proton at C-4, corroborating the 5β-hydroxyl assignment, and the NOE between H-6 and H-7 was a barely discernible 1%; these facts support placement of H-6 in the β position. The stereochemistry of the C-3 hydroxyl was assigned as α because one of the H-4 protons appeared as a degenerate doublet of doublets at δ 2.09 (t, J = 11.7), indicating geminal and axial–axial couplings of equal magnitude. Irradiation at this proton resonance (difference NOE) elicited a 7.6% enhancement of its geminal partner at δ 2.55, revealing a large (geminal) and small (axial–equatorial) coupling; a small NOE to H-2α was also observed, supporting all the assignments inferred for the A ring. Despite some chemical shift changes incurred by the cis AB ring juncture, the couplings from H-11 to H-12 remained unchanged, indicating the same stereochemistry as found in **1** and its analogues. Sterol **2**, therefore, was identified as 5β-cholest-7-ene-2β,3α,5β,6α,9α,11α,19-heptol.

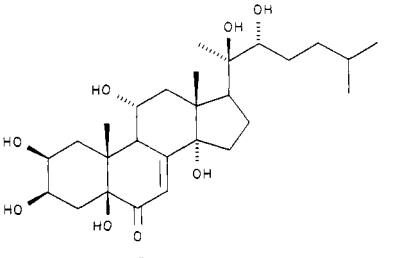
The other two compounds in this new series were found to have molecular weights of 496 and 510 daltons, respectively, indicating side-chain homologues of **2** containing C-24 methyl (**3**) and C-24 ethyl (**4**) groups. The remainder of the spectral data were quite similar to those of **2**, and the structure elucidation followed the pattern established here and in our previous work.[2]



2 R=H

3 R=CH_3

4 R=CH_2CH_3



5

It is clear from our work and disclosures by the Faulkner[3] and Schmitz[7] groups that highly functionalized sterols are widespread in the sponge genus *Dysidea*, but what role they play in the sponge remains undetermined. Their high level of functionality makes it unlikely that they contribute to the unique cell membrane structure in the sponge; similar sterols do not appear common in other members of the Demospongiae. The similarities between these compounds and the crustecdysones could imply a function of feeding deterrence to potential crustacean predators. An extension of this line of thought leads to consideration

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that they could be kairomones which induce settling and metamorphosis in larvae of the dorid nudibranchs known to be associated with the genus *Dysidea*.[8-11]

The presence of both the 5α,6β and 5β,6α hydroxylated sterol series in the same sponge suggests a 5α,6α epoxide precursor to both groups. Such a compound could undergo either electrophilic or nucleophilic ring opening to give rise to both sets of steroids. Since Djerassi has suggested[4] that 5β-stanols are most likely the product of bacterial conversion of 5α-stanols in *P. ficiformis*, it is possible that **2-4** are derived from bacterial conversion of appropriate precursors in *D. etheria*. This sponge does contain a substantial community of bacteria.[12]

Although the trans-AB steroid **1** was moderately cytotoxic,[2] the 5β steroid **3** was inactive (IC_{50} = 26 μg/mL in the KB assay, >10 μg/mL PS). Neither group of steroids elicited any reproducible effect upon larvae of the tobacco hornworm, *Manduca sexta*, whether incorporated into an agar-based diet (250 ppm) or injected subcutaneously (100-μg dose).

Experimental Section

General. One-dimensional NMR spectra were recorded on a Bruker WM-250 spectrometer; COSY and HMQC 2D experiments were done on a Varian VXR-500 instrument; chemical shifts are reported in δ units relative to tetramethylsilane (δ = 0) with pyridine-d_5 as the solvent and internal standard. ^{13}C NMR and ^1H NMR assignments were made by using 2D COSY and 2D HMQC sequences and by comparison of chemical shift data with those in the literature. Mass spectra were determined on a VG-7070 EHF mass spectrometer in the fast atom bombardment (FAB) mode, using a 0.14 M RbI in glycerol matrix to obtain $(M + Rb)^+$ adduct ions. Optical rotations were obtained on a Perkin-Elmer 241 MC polarimeter. All HPLC separations were carried out on a Perkin-Elmer series 3B liquid chromatograph. A Knauer differential refractometer was used for detection in all HPLC steps.

Collection, Extraction, and Initial Fractionation. The collection, extraction, partitioning, and initial chromatographic separations of *D. etheria* have been reported in detail.[2]

Isolation of 2-4. The third fraction from an isocratic LPLC silica gel column (Whatman LPS-2, 2.5 × 25 cm), using $CHCl_3$-iPrOH-MeOH (15:5:1) as eluent, was dissolved in MeOH and subjected to semipreparative HPLC on a Hamilton PRP-1 (C_{18}) column (30 × 0.7 cm) using CH_3CN-H_2O (1:1) as the mobile phase. Further purification of the three major fractions from this separation was achieved by HPLC on an analytical β-cyclodextrin HPLC column (ASTEC, 250 × 4.6 mm), with different mixtures of CH_3CN-H_2O (4:1, **2**), (1:1, **3** and **4**).

5β-Cholest-7-ene-2β,3α,5β,6α,9α,11α,19-heptol (2): yield, 1 × 10^{-2}% dry wt; mp 230-250 °C dec; $[\alpha]^{25}_D$ +23.1° (*c* 0.78, EtOH); ^1H NMR (250 MHz, pyridine-d_5, exchanged with MeOH-d_4) δ 0.75 (3 H, s, H-18), 0.84 (6 H, d, *J* = 6.6, H-26,27), 0.92 (3 H, d, *J* = 5.3, H-21), 1.08 (3 H, m, H-23,24), 1.28 (6 H, m, H-16,17,20,22,23), 1.52 (3 H, m, H-15,25), 1.83 (1 H, m, H-16), 2.10 (1 H, t, *J* = 11.6, H-4α), 2.55 (2 H, m, H-1β, H-4β), 2.76 (1 H, dd, *J* = 11.9, 23.9, H-12α), 2.85 (1 H, m, H-14), 2.99 (1 H, m, H-12β), 3.20 (1 H, dd, *J* = 2.7, 10.0, H-1α), 4.69 (3 H, m, H-6, and H-19 methylene protons—coalesced AB pattern), 4.82 (2 H, m, H-2,3), 5.38 (1 H, dd, *J* = 4.1, 11.9, H-11), 5.71 (1 H, s, H-7); FAB-MS, *m/z* 567 $(M + Rb^+, 1.7)$.

24-Methyl-5β-cholest-7-ene-2β,3α,5β,6α,9α,11α,19-heptol (3): yield, 1 × 10^{-2}% dry wt; mp 240-260 °C dec; $[\alpha]^{25}_D$ +18.2° (*c* 0.28, EtOH); ^1H NMR δ 0.75 (3 H, s, H-18), 0.78 (3 H, d, H-27), 0.79 (3 H, d, H-28), 0.82 (3 H, d, *J* = 6.9, H-26), 0.92 (3 H, d, *J* = 4.4, H-21), 1.0-1.6 (11 H), 1.84 (1 H, m, H-16), 2.10 (1 H, t, *J* = 11.7, H-4α), 2.57 (2 H, m, H-1β, H-4β), 2.82 (1 H, dd, *J* = 11.9, 23.9, H-12α), 2.86 (1 H, m, H-14), 2.99 (1 H, m, H-12β), 3.20 (1 H, dd, *J* = 3.0, 13.1, H-1α), 4.68 (3 H, m, H-6, and H-19 methylene protons—coalesced AB pattern), 4.83 (2 H, m, H-2,3), 5.38 (1 H, dd, *J* = 4.7, 12.1, H-11), 5.71 (1 H, s, H-7); FAB-MS, *m/z* 581 $(M + Rb^+, 0.7)$.

24-Ethyl-5β-cholest-7-ene-2β,3α,5β,6α,9α,11α,19-heptol (4): yield, 1 × 10^{-2}% dry wt; mp 235-245 °C dec; $[\alpha]^{25}_D$ +17.7° (*c* 1.3, EtOH); ^1H NMR δ 0.75 (3 H, s, H-18), 0.8124 (9 H, m, H-26,27,29), 0.94 (3 H, d, *J* = 4.1, H-21), 1.0-1.6 (13 H), 1.83 (1 H, m, H-16), 2.11 (1 H, t, *J* = 11.7, H-4α), 2.56 (2 H, m, H-1β), 2.76 (1 H, dd, *J* = 12.0, 23.8, H-12α), 2.86 (1 H, m, H-14), 2.98 (1 H, m, H-12β), 3.20 (1 H, dd, *J* = 3.0, 13.2, H-1α), 4.67 (3 H, m, H-6, and H-19 methylene protons—coalesced AB pattern), 4.82 (2 H, m, H-2,3), 5.37 (1 H, dd, *J* = 4.4, 12.2, H-11), 5.71 (1 H, s, H-7); FAB-MS, *m/z* 595 $(M + Rb^+, 1.1)$.

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Registry No. **2**, 120711-48-0; **3**, 120711-49-1; **4**, 120711-50-4.

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A Subtotal Synthesis of Methynolide via an Electrophilic Spirocyclization Reaction

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As the aglycon of the simplest member of the macrolide class of antibiotics, methynolide, **1**, has served as a proving ground in the development of stereochemical methods for synthesis of this family of natural products.[1-8] Most routes to methynolide involve the assembly of two optically active fragments in order to control the relationship between the stereocenters in the right half of the molecule (C-2 to C-6) and those in the left (C-10 and C-11). Exceptions are the synthesis reported by Vedejs et al.,[6] in which the C-10 and C-11 stereocenters are introduced on a macrocyclic intermediate, and the formal synthesis by Ireland et al.,[4] in which they are assembled on the rigid, spirocyclic framework of intermediate **2**.

We have developed a number of methods for the stereocontrolled construction of cyclic ethers via electrophilic cyclization of unsaturated alcohols and derivatives.[9-11]

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