Biemnasterol, a New Cytotoxic Sterol with the Rare 22,25-Diene Side Chain, Isolated from the Marine Sponge Biemna sp.

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BIEMNASTEROL, A NEW CYTOTOXIC STEROL WITH THE RARE 22,25-DIENE SIDE CHAIN, ISOLATED FROM THE MARINE SPONGE *BIEMNA* SP.

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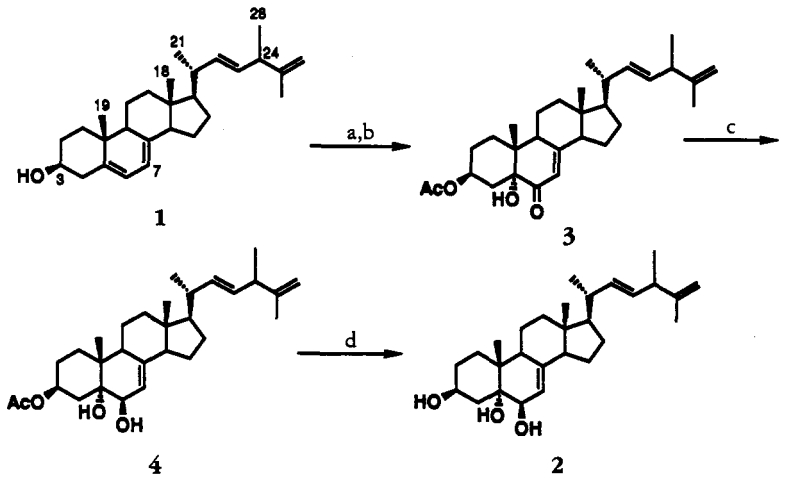
ABSTRACT.—Biemnasterol [**2**], a new sterol with a 22,25-diene side chain possessing cytotoxic activity, has been isolated from the Okinawan marine sponge *Biemna* sp. and the structure elucidated on the basis of spectroscopic data and chemical means.

A variety of unconventional sterols with unusual side chains and nuclei have been isolated from marine sponges (1–3). Among these was the sterol 24β-methylcholesta-5,7,22,25-tetraen-3β-ol [**1**], first isolated from a Hawaiian sponge *Ciocalypta* sp. as a major component of a sterol mixture (4). It contained the $\Delta^{5,7}$-sterol nucleus and interesting 22,25-diene side chain and provided strong evidence for the proposed intermediacy of a 22,25-diene in the biosynthesis of sterols (5). During our investigations on bioactive substances from Okinawan marine organisms (6–8), we recently examined extracts of the Okinawan sponge *Biemna* sp. and have isolated a new sterol, biemnasterol [**2**], possessing the rare 22,25-diene side chain, together with a known compound **1**. In this paper we describe the isolation and structure elucidation of compound **2**; its structure was confirmed by the chemical transformation of compound **1** into **2**.

The sponge *Biemna* sp. was collected at Unten Harbor, Okinawa Island and kept frozen until used. The MeOH extract was partitioned between EtOAc and 1M NaCl aqueous solution. The EtOAc-soluble fraction was subjected to Si gel flash cc eluted with hexane/EtOAc and $CHCl_3$/MeOH, followed by purification by a Sephadex LH-20 column to give biemnasterol [**2**] (0.0004%, wet wt) along with compound **1** (0.0015%).

Biemnasterol [**2**] was obtained as a colorless needle; the molecular formula was indicated to be $C_{28}H_{44}O_3$ by hrfabms (*m/z* 429.3346 $[M+H]^+$, Δ −2.3 mmu) and contained seven degrees of unsaturation. The ^{13}C-nmr spectrum showed the resonances attributed to six olefinic carbons, two sp^3 oxymethines, one oxygenated sp^3 quaternary carbon, seven sp^3 methylenes, five sp^3 methines, two sp^3 quaternary carbons, and five methyls. These signals corresponded well to those observed in the ^{13}C spectrum of compound **1** except for the presence of an additional sp^3 oxymethine and an oxygenated sp^3 quaternary carbon and the absence of two olefinic carbons. The positions of the two oxygenated carbons were deduced as C-5 and C-6 on the basis of ^{1}H-^{1}H COSY cross peaks for H-6/H-7 and HMBC correlations for H-6/C-5, H-6/C-7, H-6/C-8, H-7/C-5, H-7/C-9, and H-7/C-14. From these observations, biemnasterol [**2**] was inferred to be the 5,6-dihydroxy derivative of **1**. The structure of biemnasterol [**2**], including the stereochemistry of the hydroxyl groups, was firmly established by chemical transformation of compound **1** into **2** according to a literature method (Scheme 1) (9). Treatment of the acetate of **1** with $Na_2Cr_2O_7$ afforded an α-hydroxyl ketone **3**, which was reduced with $NaBH_4$ to give a 1,2-diol **4** as a major product. Deprotection of the 3-acetoxyl group of **4** furnished the triol **2**. The eims, ^{1}H-nmr, and ir spectral data as well as the optical rotation of compound **2** thus prepared from **1** were identical with those of compound **2** of the natural specimen. The structure of biemnasterol was, therefore, concluded to be 24β-methylcholesta-7,22,25-tirene-3β,5α,6β-triol [**2**].



SCHEME 1. a: Ac_2O/pyridine, room temperature, 24 h. b: $Na_2Cr_2O_7 \cdot 2H_2O/Ac_2O$/HOAc/$C_6H_6$, 0°, 4h. c: $NaBH_4$/THF/iPrOH, room temperature, 48 h. d: 0.5 M KOH/MeOH, room temperature, 14 h.

Biemnasterol [**2**] exhibited cytotoxicity against murine lymphoma L1210 and human epidermoid carcinoma KB cells in vitro, with IC_{50} values of 3.0 and 1.3 μg/ml, respectively.

EXPERIMENTAL

GENERAL METHODS.—Optical rotations were recorded on a JASCO DIP-4 digital polarimeter. Uv and ir spectra were taken on JASCO Ubest-35 and JASCO Report-100 spectrometers, respectively. ^{1}H- and ^{13}C-nmr spectra were recorded on JEOL JMN GX-270 and EX-400 spectrometers. Eims and fabms were obtained on a JEOL JMS DX-303 and a JEOL HX-110 spectrometer, respectively. Wako C-300 Si gel was used for cc, and tlc was carried out on Merck Si gel GF_{254}.

SPONGE MATERIAL.—The sponge *Biemna* sp. (Order Poecilosclerida; Family Desmacellidae; Gray, 1867), collected by netting at Unten Harbor, Okinawa Island, was kept frozen until used. The specimen was a very dark brown to purple black sponge when preserved, with some foreign material in the mesohyl. Skeleton a loose unispicular or bispicular reticulation of styles without fibre development. Numerous large sigmas throughout the mesohyl. Styles 552–612×12–13 μm; sigmas 96 μm, rephides 210 μm long. The voucher specimen (SS-857) was deposited at the Faculty of Pharmaceutical Sciences, Hokkaido University.

ISOLATION.—The MeOH extract of the sponge (1.7 kg, wet wt) was evaporated under reduced pressure, and the residue (36 g) was partitioned between EtOAc (400 ml×3) and 1M NaCl (400 ml). A portion (432 mg) of the EtOAc-soluble material (877 mg) was subjected to Si gel flash cc (50×3 cm) with gradient elution of EtOAc in hexane (0–100%) and MeOH in $CHCl_3$ (50–100%). The fraction (29 mg) eluted with 20% EtOAc in hexane was further purified by Sephadex LH-20 column (50% MeOH in $CHCl_3$; 120×2.5 cm) to give compound **1** (23 mg). The fraction (71 mg) eluted with 50% MeOH in $CHCl_3$ was separated by the second Si gel column (30×1.5 cm) eluted with 0–100% MeOH in $CHCl_3$. The fraction (9 mg) eluted with 10% MeOH in $CHCl_3$ was subjected to a Sephadex LH-20 column (50% MeOH in $CHCl_3$; 120×2.5 cm) to give compound **2** (3.1 mg).

Compound **1**.—^{1}H nmr ($CDCl_3$) δ_H 5.58 (1H, dd, J=5.5 and 2.6 Hz, H-6), 5.39 (1H, dd, J=5.5 and 2.6 Hz, H-7), 5.22–5.27 (2H, m, H-22 and H-23), 4.68–4.72 (2H, m, H_2-26), 3.62 (1H, m, H-3), 2.72 (1H, m, H-24), 2.45 (1H, ddd, J=8.0, 4.4, and 2.0 Hz, H_a-4), 2.27 (1H, m, H_b-4), 2.08 (2H, m, H-20 and H_a-12), 1.97 (1H, m, H-9), 1.86–1.92 (3H, m, H-14, H_a-1, and H_a-2), 1.75 (1H, m, H_a-16), 1.73 (1H, m, H_a-15), 1.67 (3H, s, H_3-27), 1.62 (2H, m, H_2-11), 1.59 (1H, m, H_b-2), 1.25–1.36 (5H, m, H_b-1, H_b-12, H_b-15, H_b-16, and H-17), 1.08 (3H, d, J=6.6 Hz, H_3-28), 1.03 (3H, d, J=6.6 Hz, H_3-21), 0.94 (3H, s, H_3-19), 0.63 (3H, s, H_3-18); ^{13}C nmr ($CDCl_3$) δ_C 38.4 (t, C-1), 32.0 (t, C-2), 70.4 (d, C-3), 40.8 (t, C-4), 141.3 (s, C-5), 119.6 (d, C-6), 116.3 (d, C-7), 139.8 (s, C-8), 46.3 (d, C-9), 37.0 (s, C-10), 21.1 (t, C-11), 39.1 (t, C-12), 42.9 (s, C-13), 54.5 (d, C-14), 23.5 (t, C-15), 28.3 (t, C-16), 55.7 (d, C-17), 12.0 (q, C-18), 16.3 (q, C-19), 40.2 (d, C-20), 20.9 (q, C-21), 131.6 (d, C-22), 135.7 (d, C-23), 43.6 (d, C-24), 149.8 (s, C-25), 108.8 (t, C-26), 20.6 (q, C-27), 18.9 (q, C-28); eims *m/z* $[M]^+$ 394 (100), 361 (75), 335 (40), 271 (25), 253 (35).

Biemnasterol [**2**].—Colorless solid: mp 241–242°; $[\alpha]^{19}D$ −7.6° (*c*=0.43, MeOH); uv (MeOH) λ max 204 (ε 12700); ir (KBr) ν max 3400, 2950 cm^{-1}; ^1H nmr (CD_3OD) δ_H 5.30 (1H, m, H-7), 5.25 (2H, m, H-22 and H-23), 4.70 (2H, m, H_2-26), 3.96 (1H, m, H-3), 3.53 (1H, m, H-6), 2.71 (1H, m, H-24), 1.66 (3H, s, H_3-27), 1.08 (3H, d, *J*=7.0 Hz, H_3-21), 1.05 (3H, s, H_3-19), 1.03 (3H, d, *J*=6.6 Hz, H_3-28), 0.64 (3H, s, H_3-18); ^{13}C nmr ($CDCl_3$) δ_C 30.8 (t, C-1), 32.0 (t, C-2), 67.7 (d, C-3), 39.2 (t, C-4), 76.0 (s, C-5), 73.7 (d, C-6), 117.6 (d, C-7), 143.9 (s, C-8), 43.8 (d, C-9), 37.1 (s, C-10), 22.9 (t, C-11), 39.5 (t, C-12), 43.5 (s, C-13), 54.7 (d, C-14), 22.0 (t, C-15), 27.8 (t, C-16), 56.0 (d, C-17), 12.3 (q, C-18), 18.8 (q, C-19), 40.2 (d, C-20), 20.9 (q, C-21), 135.5 (d, C-22), 131.8 (d, C-23), 43.6 (d, C-24), 149.8 (s, C-25), 108.8 (t, C-26), 20.6 (q, C-27), 18.9 (q, C-28); eims *m/z* (rel. int. %) $[M-H_2O]^+$ 410 (50), $[M-2H_2O]^+$ 392 (30), 377 (35), 287 (10), 269 (25), 251 (30), 123 (85), 43 (100); hrfabms *m/z* 429.3346 (calcd for $C_{28}H_{45}O_3$ $[M+H]^+$ 429.3369).

3β-Acetoxy-5α-hydroxy-24β-methylcholesta-7,22,25-trien-6-one [**3**].—Compound **1** (8.1 mg) was acetylated with Ac_2O (0.5 ml) and pyridine (2 ml). After evaporation of the solvent, the crude product was dissolved in HOAc (1 ml), Ac_2O (2 ml), and C_6H_6 (1 ml), to which $Na_2Cr_2O_7 \cdot 2H_2O$ (6.2 mg) was added at 0° and stirred for 4 h at 0°. After addition of H_2O (2 ml), the reaction mixture was extracted with EtOAc and the organic phase was purified by a Si gel column [20×1.5 cm; hexane-EtOAc (5:1)] to give **3** (4.3 mg): $[\alpha]^{19}D$ −5.0° (*c*=0.10, $ClCH_2CH_2Cl$); ir (KBr) ν max 3400, 2900, 1720, 1680, 1250 cm^{-1}; ^1H nmr ($CDCl_3$) δ_H 5.65 (1H, m, H-7), 5.24–5.28 (2H, m, H-22 and H-23), 5.10 (1H, m, H-3), 4.70 (2H, m, H_2-26), 2.72 (1H, m H-24), 2.03 (3H, s, Ac), 1.68 (3H, s, H_3-27), 1.08 (3H, d, *J*=6.9 Hz), 1.03 (3H, d, *J*=6.6 Hz), 0.96 (3H, s, H_3-19), 0.60 (3H, s, H_3-18); eims *m/z* (rel. int. %) $[M]^+$ 468 (8), $[M-HOAc]^+$ 408 (6), 390 (20), 374 (18), 251 (20), 123 (90), 43 (100).

3β-Acetoxy-24β-methylcholesta-7,22,25-triene-5α,6β-diol [**4**].—$NaBH_4$ (1.5 mg) was added to the solution of compound **3** (3.4 mg) in iPrOH (1 ml), and the mixture was stirred at room temperature for 48 h. Then H_2O (1 ml) was added, and the reaction mixture was extracted with EtOAc. The organic phase was purified by a Si gel column [20×1.0 cm; hexane-EtOAc (2:1)] to give **4** (1.1 mg) (10): $[\alpha]^{21}D$ +40° (*c*=0.11, $CHCl_3$); ir (KBr) ν max 3450, 2950, 1720, 1270 cm^{-1}; ^1H nmr ($CDCl_3$) δ_H 5.32–5.35 (1H, m, H-7), 5.24–5.27 (2H, m, H-22 and H-23), 5.15 (1H, m, H-3), 4.70 (2H, m, H_2-26), 3.61 (1H, m, H-6), 2.71 (1H, m, H-24), 2.03 (3H, s, Ac), 1.68 (3H, s, H_3-27), 1.09 (3H, s, H_3-19), 1.08 (3H, d, *J*=6.6 Hz), 1.02 (3H, d, *J*=6.6 Hz), 0.60 (3H, s, H_3-18); eims *m/z* (rel. int. %) $[M-H_2O]^+$ 452 (38), $[M-H_2O-HOAc]^+$ 392 (100), 377 (43), 363 (55), 269 (60), 251 (25), 123 (95).

24β-Methylcholesta-7,22,25-triene-3β,5α,6β-triol [**2**].—Compound **4** (1.1 mg) was treated with 0.5 M KOH/MeOH (1.5 ml) for 14 h at room temperature. After usual workup, compound **2** (0.9 mg) was obtained, whose ir, ^1H-nmr, eims, and $[\alpha]D$ data were all identical with those of a natural specimen of biemnasterol [**2**].

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