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FOUR NEW TRIHYDROXYLATED STEROLS FROM THE SPONGE *SPONGIONELLA GRACILIS*

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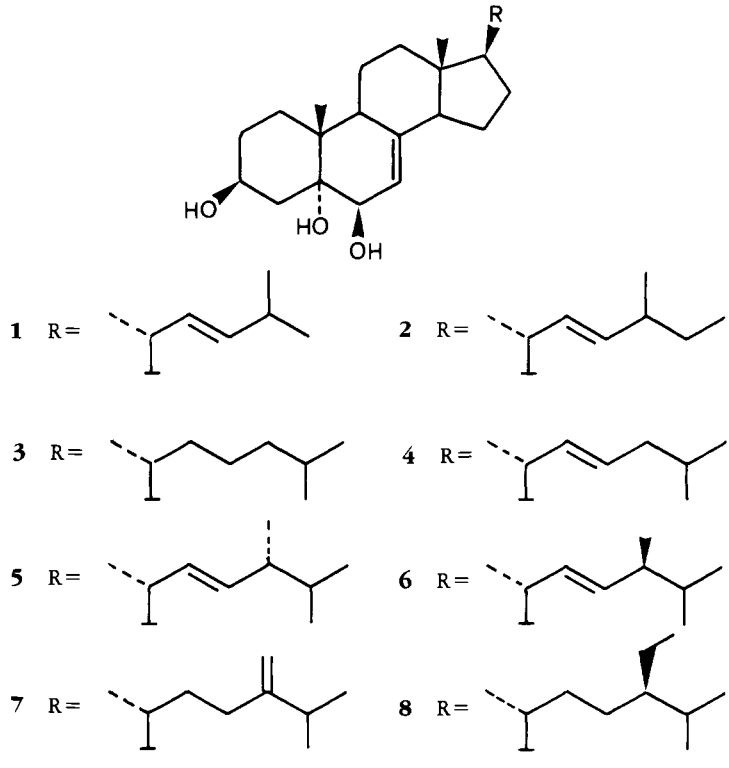
ABSTRACT.—Eight 3β,5α,6β-trihydroxysterols [**1-8**], of which four [**1**, **2**, **7**, and **8**] are new, have been isolated from the sponge *Spongionella gracilis* and their structures deduced by spectroscopic and chemical evidence.

Polyhydroxylated sterols are common metabolites in marine invertebrates such as gorgonians and alcyonarians (1-3), whereas they seem more rare in sponges (4-6). Recently we isolated from the sponge *Spongionella gracilis* Vosmaer (order Dyctioceratida, family Dysideidae) three new 3β,6α-dihydroxysterols (7). A further investigation of the lipidic extract of the same organism yielded a mixture of the 3β,5α,6β-trihydroxysterols described in this paper.

RESULTS AND DISCUSSION

Trihydroxysterols **1-8** were obtained by Si gel column chromatography of an Me_2CO extract of the sponge *S. gracilis* followed by reverse-phase hplc using different percentages of H_2O in MeOH. Compounds **3-6** have recently been isolated from the bryozoan *Myriapora truncata* as 3,6-diacetyl-derivatives (8); whereas **1**, **2**, **7**, and **8** are new, and their structure elucidation is described herein.

In the mass spectrum of the sterols **1-8** the molecular ion was absent. Peaks indicating successive losses of H_2O and peaks at *m/z* 287 (M^+-H_2O and side chain), 269 (M^+-$2H_2O$ and side chain), 251 (M^+-$3H_2O$ and side chain), 227 (M^+-$2H_2O$ and ring D



fission), and 209 (M^+-$3H_2O$ and ring D fission) suggested the presence of three hydroxyl groups and one double bond, all of them located in the rings A, B, and C.

The molecular formula of the most abundant sterol [**5**], established as $C_{28}H_{46}O_3$ by hrms on the ion at *m/z* 412 (M^+-H_2O), indicated it to be a di-unsaturated C_{28}-sterol. The ^{13}C-nmr spectrum of **5** indicated the presence of three sp^3 carbons attached to oxygen atoms, two secondary at δ 67.6 and 74.3 and one tertiary at δ 76.2, and showed signals for four olefinic carbons at δ 120.5 (d), 132.2 (d), 136.2 (d), and 141.6 (s). Its ^{1}H nmr spectrum contained one-proton signals centered at δ 4.08 (bm) and 3.63 (bs), consistent with the presence of two secondary carbinol methines. The broad methine multiplet at δ 4.08 had the normal complexity for the 3α-carbinol proton of an A/B *trans*-steroid (9). This unusually downshifted signal is typical of 3β-hydroxysterols bearing a 5α-hydroxyl group. Two double doublets at δ 2.14 (J=12.8 and 12.8 Hz) and 1.78 (J=12.8 and 4.9 Hz), mutually coupled and coupled with the 3α-proton at δ 4.08, were assigned, respectively, to the 4-H_{ax} and 4-H_{eq} protons next to the C-5 substituted position. The ^{1}H nmr spectrum showed, in addition, an olefinic proton at δ 5.35 (bd, J=4.9 Hz) coupled with the broad singlet at δ 3.63. These data and the agreement of the C-18 methyl resonance at δ 0.59 with the value expected for a Δ^7-sterol suggested a 7-ene-3β,5α,6-triol structure. The downshift of the C-19 methyl signal at δ 1.50 in the spectrum recorded in pyridine-d_5 was indicative of the β-orientation of the C-6 hydroxyl group (10). The ergosterol-type side chain of **5** was deduced from ^{13}C- (11) and ^{1}H nmr spectra (13). Thus, the structure of this sterol was formulated as (22*E*,24*R*)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol [**5**].

Sterol **5** was obtained by $LiAlH_4$ reduction of $\Delta^{7,22}$-ergostadiene-3β,5α-diol-6-one-3-acetate (12). This reaction afforded also, as a minor product, the 6α epimer of **5**. The ^{1}H nmr of the synthetic 6β epimer was superimposable on that of the natural sterol **5**, and the two compounds had identical $[\alpha]^{25}$D values, confirming the stereochemical assignment of compound **5**. The 6α epimer showed, as expected, differences in the chemical shift values of the 4-Heq, 6-H, 7-H, 18-H_3, and 19-H_3 and had a different $[\alpha]^{25}$D from compound **5**.

The close similarity of the mass and ^{1}H-nmr spectra of compound **6** ($C_{28}H_{46}O_3$) with those of **5** suggested that the two compounds must be C-24 epimers, assuming cholestane stereochemistry at C-20. The 21-H_3 doublet in the ^{1}H-nmr spectrum of sterol **6** appeared upshifted at δ 1.02 when compared to the corresponding 21-H_3 signal (δ 1.04) for the sterol **5**. The structure of **6** was, therefore, formulated as (22*E*,24*S*)-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol.

Comparison of the ^{1}H-nmr spectra of **5** and **6** with those of **1-4**, **7**, and **8** showed almost identical chemical shift values for 3-H, 4-H_2, 6-H, 7-H, 18-H_3, and 19-H_3 indicating that all components of the sterol mixture possessed identical nuclei but differed in the side chain. For all these sterols the stereochemistry at C-20 has been assigned on the assumption that they all belong to the cholestane series.

Sterol **3** had a molecular formula $C_{27}H_{46}O_3$. The ions at *m/z* 287 (M^+-C_8H_{17}-H_2O), 269 (M^+-C_8H_{17}-$2H_2O$), and 251 (M^+-C_8H_{17}-$3H_2O$) established the presence of a C_8H_{17} saturated side chain. The presence of overlapping doublets at δ 0.87 (J=6.6 Hz, 26-H_3 and 27-H_3) and a doublet at δ 0.93 (J=6.6 Hz, 21-H_3) in the ^{1}H-nmr spectrum of **3** suggested a cholesterol type side chain (13). The assigned structure is, thus, 5α-cholest-7-ene-3β,5,6β-triol.

Sterol **4** had a molecular formula $C_{27}H_{44}O_3$ and a C_8H_{15} side chain containing one double bond. Its ^{1}H-nmr spectrum contained a doublet at δ 0.88 (6H, J=6.6 Hz) due to the isopropyl group attached to the normal side chain and a doublet at δ 1.03 (3H, J=6.6 Hz), which is typical of a 20-Me group in a Δ^{22}-sterol. The ^{1}H-nmr spectrum included two olefinic protons that appeared as two multiplets centered at δ 5.32 and

5.22 attributable to the 22-H and 23-H protons, respectively. In a double resonance experiment irradiation at δ 2.05 (tentatively the frequency of 20-H) caused the multiplet at δ 5.32 to collapse to a doublet (J=14.7 Hz) and the 21-H_3 proton doublet to a singlet, thus, establishing the configuration of the Δ^{22} double bond to be *E* [from the value (14.7 Hz) of the 22-H/23-H coupling constant]. Comparison of the ^{1}H-nmr spectrum of this sterol with those of sterols having a similar side chain (13) suggested the structure of (22*E*)-5α-cholesta-7,22-diene-3β,5,6β-triol.

The first new trihydroxylated sterol **1** had the molecular formula $C_{26}H_{42}O_3$. The presence of a C_7H_{13} monounsaturated side chain was indicated by the ions at *m/z* 287, 269, and 251. In the ^{1}H-nmr spectrum the protons from C-20 to C-25/C-26 were readily interrelated by spin decoupling experiments. Irradiation at δ 2.03 (tentatively the frequency of 20-H) collapsed the doublet at δ 1.01 (J=6.9 Hz, 21-H_3) to a singlet and the 22-H double doublet at δ 5.16 (J=15.7 and 8.9 Hz) to a doublet. The one-proton multiplet at δ 2.18 (25-H) was coupled with the doublet at δ 0.94 (J=6.9 Hz, 26-H_3 and 27-H_3) and with the 23-H double doublet at δ 5.28 (J=15.7 and 6.9 Hz). Consequently, the structure of this sterol was established as (22*E*)-24-nor-5α-cholesta-7,22-diene-3β,5,6β-triol. The configuration of the Δ^{22} double bond was established as *E* from the value (15.7 Hz) of the 22-H/23-H coupling constant.

The new sterol **2** had a molecular formula $C_{27}H_{44}O_3$ and a C_8H_{15} monounsaturated side chain. Spin decoupling experiments established the segment C-21 to C-28. Irradiation at δ 2.03 (tentatively the frequency of 20-H) collapsed the methyl doublet at δ 1.03 (J=6.6 Hz, 21-H_3) into a singlet and simplified the multiplet centered at δ 5.20 (22-H) to a doublet (J=15.4 Hz). Likewise, irradiation at δ 1.92 (tentatively the frequency of 24-H) collapsed the methyl doublet at δ 0.94 (J=6.6 Hz, 28-H_3) into a singlet and caused the multiplet at δ 5.16 (23-H) to transform into a doublet (J=15.4 Hz). The presence in the ^{1}H-nmr spectrum of a triplet at δ 0.85 (J=7.3 Hz, 26-H_3) for a terminal ethyl group suggested a (22*E*)-27-nor-24-methyl-5α-cholesta-7,22-diene-3β,5,6β-triol structure for this sterol. The Δ^{22} configuration was assigned as *E* on the basis of the value (15.4 Hz) of the 22-H/23-H coupling constant.

The new sterol **7** had a molecular formula $C_{28}H_{46}O_3$ and a C_9H_{17} side chain containing one double bond. Its ^{1}H-nmr spectrum showed two broad singlets at δ 4.72 (28-H) and 4.66 (28-H), a signal at δ 2.23 (septet, J=6.9 Hz, 25-H) coupled with the two methyl doublets at δ 1.03 (J=6.9 Hz, 26-H_3 or 27-H_3) and 1.02 (J=6.9 Hz, 27-H_3 or 26-H_3), and a methyl doublet at δ 0.96 (J=6.9 Hz, 21-H_3). These data suggested the 24-methylene type side chain for the sterol **7**. The assigned structure is, therefore, 24-methylene-5α-cholest-7-ene-3β,5,6β-triol.

The last new sterol **8** had molecular formula $C_{29}H_{50}O_3$ and a $C_{10}H_{21}$ saturated side chain. On the basis of its ^{1}H-nmr spectrum that showed the presence of three methyl groups at δ 0.84 (J=6.9 Hz, 26-H_3 or 27-H_3), 0.82 (J=6.9 Hz, 27-H_3 or 26-H_3), and 0.94 (J=6.9 Hz, 21-H_3) and one methyl triplet at δ 0.85 (J=7.9 Hz, 29-H_3), the structure of (24*R*)-24-ethyl-5α-cholest-7-ene-3β,5,6β-triol was assigned to this sterol. The configuration at C-24 was assigned as *R* by comparison of the ^{1}H-nmr spectrum of **8** with those of authentic samples of sitosterol and clionasterol. The side chain ^{1}H nmr data are in agreement with those of sitosterol.

Thus, in addition to 3β,6α-dihydroxysterols, the sponge *S. gracilis* contains 3β,5α,6β-trihydroxysterols. The origin of these sterols is unknown. They could derive from the $\Delta^{5,7}$-sterols present in large amount in the sponge (14).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra were run on a Perkin-Elmer Model 399 spectrophotometer. ^{1}H-nmr spectra were recorded on a Bruker WM-500 spectrometer in $CDCl_3$ solutions. ^{13}C-nmr experiments were performed on a Bruker WM-250 spectrometer: all chemical shifts are given in

ppm with TMS as internal reference (δ=0). Low resolution mass spectra were recorded at 70 eV on an AEI 30 instrument. High resolution mass spectra were obtained on an AEI MS 902 spectrometer. Reverse phase hplc was performed on a Varian 2010 instrument equipped with a differential refractometer using Hibar Supersphere (3μ, 4×250 mm) and Hibar RP-18 (7μ, 250×10 mm) columns. Melting points were determined on a Kofler apparatus and are uncorrected. Optical rotations were taken on a Perkin-Elmer Model 141 polarimeter with a 10 cm cell.

ISOLATION OF **1-8** FROM *S. GRACILIS*.—The sponge *S. gracilis* was collected in the Bay of Naples and supplied by the Zoological Station of Naples. A voucher specimen is on file at our laboratories. Fresh sponge (25 g dry wt after extraction) was cut into small pieces and extracted three times with Me_2CO at room temperature for 3 days. Solvent was evaporated, and the resulting aqueous phase was extracted with Et_2O (4×50 ml). The organic extracts were combined, dried over Na_2SO_4, and evaporated to yield an oily residue (2.77 g). The oil was chromatographed on a Si gel column (200 g) eluted with increasing amounts of MeOH in $CHCl_3$. The fractions eluted with $CHCl_3$-MeOH (95:5) contained the mixture of trihydroxysterols **1-8** homogeneous by tlc. The sterol mixture was fractionated by reverse phase hplc using MeOH-H_2O (85:15) as eluent to obtain pure samples of **1** (0.8 mg), **3** (1.0 mg), **7** (1.0 mg), and **8** (1.2 mg), and mixtures of **2**, **4**, **5**, and **6**. Further separations using MeOH-H_2O (8:2) afforded pure samples of **2** (1.0 mg), **4** (1.2 mg), **5** (1.8 mg), and **6** (1.4 mg).

Compound **1**.—Mp 215-217° (MeOH); ir ($CHCl_3$) 3460 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 5.35 (1H, bd, J=4.9 Hz, 7-H), 5.16 (1H, dd, J=15.7 and 8.9 Hz, 22-H), 5.28 (1H, dd, J=15.7 and 6.9 Hz, 23-H), 4.08 (1H, m, 3-H), 3.63 (1H, bs, 6-H), 2.18 (1H, m, 25-H), 2.14 (1H, dd, J=12.8 and 12.8 Hz, 4-Hax), 1.78 (1H, dd, J=12.8 and 4.9 Hz, 4-Heq), 1.08 (3H, s, 19-H_3), 1.01 (3H, d, J=6.9 Hz, 21-H_3), 0.94 (6H, d, J=6.9 Hz, 26-H_3 and 27-H_3), 0.59 (3H, s, 18-H_3); ms *m/z* (rel. int.) 384 (M^+-H_2O, 21), 369, (M^+-H_2O and CH_3, 21), 366 (M^+-$2H_2O$, 26), 351 (M^+-$2H_2O$ and CH_3, 100), 287 (M^+-side chain and H_2O, 10), 269 (M^+-side chain and H_2O, 54), 251 (M^+-side chain and H_2O, 70), 227 (M^+-side chain-$2H_2O$ and 42, 38), 225 (M^+-side chain-$3H_2O$ and 27, 22), 209 (M^+-side chain-$3H_2O$ and 42, 27); hrms *m/z* 384.3034 ($C_{26}H_{40}O_2$ requires 384.3027).

Compound **2**.—Mp 235-237° (MeOH); ir ($CHCl_3$) 3460 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 5.36 (1H, bd, J=4.9 Hz, 7-H), 5.18 (2H, m, 22-H and 23-H), 4.08 (1H, m, 3-H), 3.63 (1H, bs, 6-H), 2.14 (1H, dd, J=12.8 and 12.8 Hz, 4-Hax), 2.03 (submerged by other signals, 20-H), 1.92 (submerged by other signals, 24-H), 1.78 (1H, dd, J=12.8 and 4.9 Hz, 4-Heq), 1.09 (3H, s, 19-H_3), 1.03 (3H, d, J=6.6 Hz, 21-H_3), 0.94 (3H, d, J=6.6 Hz, 28-H_3), 0.85 (3H, t, J=7.3 Hz, 26-H_3), 0.60 (3H, s, 18-H_3); ms *m/z* (rel. int.) 398 (M^+-H_2O, 37), 383 (M^+-H_2O and CH_3, 25), 380 (M^+-$2H_2O$, 46), 365 (M^+-$2H_2O$ and CH_3, 100), 287 (11), 269 (56), 251 (90), 227 (41), 225 (28), 209 (34); hrms *m/z* 398.3175 ($C_{27}H_{42}O_2$ requires 398.3184).

Compound **3**.—Mp 227-229° (MeOH); ir ($CHCl_3$) 3460 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 5.36 (1H, bd, J=4.9 Hz, 7-H), 4.08 (1H, m, 3-H), 3.63 (1H, bs, 6-H), 2.14 (1H, dd, J=12.8 and 12.8 Hz, 4-Hax), 1.78 (1H, dd, J=12.8 and 4.9 Hz, 4-Heq), 1.09 (3H, s, 19-H_3), 0.93 (3H, d, J=6.6 Hz, 21-H_3), 0.87 (6H, d, J=6.6 Hz, 26-H_3 and 27-H_3), 0.59 (3H, s, 18-H_3); ms *m/z* (rel. int.) 400 (M^+-H_2O, 28), 3.85 (M^+-H_2O and CH_3, 26), 382 (M^+-$2H_2O$, 37), 367 (M^+-$2H_2O$ and CH_3, 100), 287 (12), 269 (36), 251 (75), 227 (42), 225 (26), 209 (32).

Compound **4**.—Mp 234-236° (meOH); ir ($CHCl_3$) 3460 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 5.36 (1H, bd, J=4.9 Hz, 7-H), 5.32 (1H, m, 23-H or 22-H), 5.22 (1H, m, 22-H or 23-H), 4.08 (1H, m, 3-H), 3.63 (1H, bs, 6-H), 2.14 (1H, dd, J=12.8 and 12.8 Hz, 4-Hax), 1.78 (1H, dd, J=12.8 and 4.9 Hz, 4-Heq), 1.08 (3H, s, 19-H), 1.03 (3H, d, J=6.6 Hz, 21-H_3), 0.88 (6H, d, J=6.6 Hz, 26-H_3 and 27-H_3), 0.60 (3H, s, 18-H_3); ms *m/z* (rel. int.) 398 (M^+-H_2O, 30), 383 (M^+-H_2O and CH_3, 25), 380 (M^+-$2H_2O$, 36), 367 (M^+-$2H_2O$ and CH_3, 100), 287 (13), 269 (36), 251 (70), 227 (42), 225 (26), 209 (30); hrms *m/z* 398.3188 ($C_{27}H_{42}O_2$ requires 398.3184).

Compound **5**.—$[\alpha]^{25}D$ −75 (*c* 0.16, pyridine); mp 246-248° (MeOH); ir ($CHCl_3$) 3460 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 5.35 (1H, bd, J=4.9 Hz, 7-H), 5.20 (1H, dd, J=14.8 and 6.9 Hz, 23-H), 5.15 (1H, dd, J=14.8 and 7.9 Hz, 22-H), 4.08 (1H, m, 3-H), 3.63 (1H, bs, 6-H), 2.14 (1H, dd, J=12.8 and 12.8 Hz, 4-Hax), 1.78 (1H, dd, J=12.8 and 4.9 Hz, 4-Heq), 1.08 (3H, s, 19-H_3), 1.04 (3H, d, J=6.9 Hz, 21-H_3), 0.91 (3H, d, J=6.9 Hz, 28-H_3), 0.84 (3H, d, J=6.9 Hz, 26-H_3 or 27-H_3), 0.82 (3H, d, J=6.9 Hz, 27-H_3 or 26-H_3), 0.59 (3H, s, 18-H_3); ^1H nmr (pyridine-d_5, 250 MHz), δ 5.20 (2H, m, 22-H and 23-H), 5.08 (1H, bs, 7-H), 4.80 (1H, m, 3-H), 4.29 (1H, bs, 6-H), 3.00 (1H, dd, J=12.8 and 12.8 Hz, 4-H_{ax}), 1.50 (3H, s, 19-H_3), 1.06 (3H, d, J=6.9 Hz, 21-H_3), 0.95 (3H, d, J=6.9 Hz, 28-H_3), 0.86 (6H, d, J=6.9 Hz, 26-H_3 and 27-H_3), 0.65 (3H, s, 18-H_3); ^{13}C nmr (pyridine-d_5, 62.9 MHz) δ 141.6 (C-8), 136.2 (C-22), 132.2 (C-23), 120.5 (C-7), 76.2 (C-5), 74.3 (C-6), 67.6 (C-3), 56.2 (C-17), 55.3 (C-14), 43.8 (C-13), 43.8 (C-9 or C-24), 43.2 (C-24 or C-9), 41.9 (C-4), 40.8 (C-20), 40.0 (C-12), 38.1 (C-10), 33.9 (C-2), 33.4 (C-25), 32.6 (C-1), 28.5 (C-16), 23.5 (C-15), 22.5 (C-11),

21.5 (C-21), 20.2 (C-26 or C-27), 19.9 (C-27 or C-26), 18.8 (C-19), 17.9 (C-28), 12.6 (C-18). Side-chain carbon assignments are based on literature data (11). Nuclear carbon assignments were facilitated using 5α-cholestane-3β,5,6β-triol as a model compound (11). Assignments were confirmed by DEPT experiments. Ms *m/z* (rel. int.) 412 (M^+-H_2O, 31), 397 (M^+-H_2O and CH_3, 20), 394 (M^+-$2H_2O$, 30), 379 (M^+-$2H_2O$ and CH_3, 100), 287 (11), 269 (58), 251 (75), 227 (42), 225 (27), 209 (21).

Compound **6**.—Mp 245-247° (MeOH); ir ($CHCl_3$) 3460 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 5.35 (1H, bd, *J*=4.9 Hz, 7-H), 5.20 (1H, dd, *J*=14.8 and 6.9 Hz, 23-H), 5.15 (1H, dd, *J*=14.8 and 7.9 Hz, 22-H) 4.08 (1H, m, 3-H), 3.63 (1H, bs, 6-H), 2.14 (1H, dd, *J*=12.8 and 12.8 Hz, 4-Hax), 1.78 (1H, dd, *J*=12.8 and 4.9 Hz, 4-Heq), 1.08 (3H, s, 19-H_3), 1.02 (3H, d, *J*=6.9 Hz, 21-H_3), 0.91 (3H, d, *J*=6.9 Hz, 28-H_3), 0.84 (3H, d, *J*=6.9 Hz, 26-H_3 or 27-H_3), 0.82 (3H, d, *J*=6.9 Hz, 27-H_3 or 26-H_3), 0.59 (3H, s, 18-H_3); ms *m/z* (rel. int.) 412 (M^+-H_2O, 30), 397 (M^+-H_2O and CH_3, 20), 394 (M^+-$2H_2O$, 30), 379 (M^+-$2H_2O$ and CH_3, 100), 287 (10), 269 (60), 251 (75), 227 (40), 225 (25), 209 (20); hrms *m/z* 412.3353 ($C_{28}H_{44}O_2$ requires 412.3341).

Compound **7**.—Mp 244-246° (MeOH); ir ($CHCl_3$) 3460 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 5.36 (1H, bd, *J*=4.9 Hz, 7-H), 4.72 (1H, bs, 28-H), 4.66 (1H, bs, 28-H), 4.08 (1H, m, 3-H), 3.63 (1H, bs, 6-H), 2.23 (1H, septet, *J*=6.9 Hz, 25-H), 2.14 (1H, dd, *J*=12.8 and 12.8 Hz, 4-Hax), 1.78 (1H, dd, *J*=12.8 and 4.9 Hz, 4-Heq), 1.09 (3H, s, 19-H_3), 1.03 (3H, d, *J*=6.9 Hz, 26-H_3 or 27-H_3), 1.02 (3H, d, *J*=6.9 Hz, 27-H_3 or 26-H_3), 0.96 (3H, d, *J*=6.9 Hz, 21-H_3), 0.59 (3H, s, 18-H_3); ms *m/z* (rel. int.) 412 (M^+-H_2O, 15), 397 (M^+-H_2O and CH_3, 18), 394 (M^+-$2H_2O$, 25), 379 (M^+-$2H_2O$, and CH_3, 100), 287 (7), 269 (24), 267 (29), 251 (63), 227 (78), 225 (53), 209 (61); hrms *m/z* 412.3335 ($C_{28}H_{44}O_2$ requires 412.3341).

Compound **8**.—Mp 254-256° (MeOH); ir ($CHCl_3$) 3460 cm^{-1}; ^1H nmr ($CDCl_3$, 500 MHz) δ 5.36 (1H, bd, *J*=4.9 Hz, 7-H), 4.08 (1H, m, 3-H), 3.63 (1H, bs, 6-H), 2.14 (1H, dd, *J*=12.8 and 12.8 Hz, 4-Hax), 1.78 (1H, dd, *J*=12.8 and 4.9 Hz, 4-Heq), 1.08 (3H, s, 19-H_3), 0.94 (3H, d, 21-H_3), 0.85 (3H, t, *J*=7.9 Hz, 29-H_3), 0.84 (3H, d, *J*=6.9 Hz, 26-H_3 or 27-H_3), 0.82 (3H, d, *J*=6.9 Hz, 27-H_3 or 26-H_3), 0.59 (3H, s, 18-H_3); ms *m/z* (rel. int.) 428 (M^+-H_2O, 31), 413 (M^+-H_2O and CH_3, 18), 410 (M^+-$2H_2O$, 33), 395 (M^+-$2H_2O$ and CH_3, 100), 287 (12), 269 (56), 251 (75), 227 (40), 225 (25), 209 (20); hrms *m/z* 428.3670 ($C_{29}H_{48}O_2$ requires 428.3654).

SYNTHESIS OF **5** AND ITS 6α-EPIMER.—The title compounds were obtained as described by Fieser *et al.* (12) starting from $\Delta^{7,22}$-ergostadiene-3β,5α-diol-6-one-3-acetate with $LiAlH_4$ reduction at room temperature for 15 min affording a mixture of 6β- and 6α-epimers in the ratio of 3:1, which were separated by hplc on a Hibar RP-18 (7μ, 250×10 mm) column using MeOH as eluent. The 6β-epimer [[α]^{25}D −72, *c* 0.9 pyridine; reported (12) −82; **5**, −75] showed chemical and physical properties identical to those of natural **5**; 6α-epimer: [α]^{25}D +14.9 (*c* 0.8, pyridine); mp 245-246° (MeOH); ^1H nmr ($CDCl_3$, 250 MHz) δ 5.22 (1H, dd, *J*=14.8 and 6.9 Hz, 23-H), 5.16 (1H, dd, *J*=14.8 and 7.9 Hz, 22-H), 5.02 (1H, bs, 7-H), 4.01 (1H, m, 3-H), 3.97 (1H, bs, 6-H), 2.22 (1H, dd, *J*=12.8 and 4.9 Hz, 4-Heq), 1.02 (3H, d, *J*=6.9 Hz, 21-H_3), 0.97 (3H, s, 19-H_3), 0.91 (3H, d, *J*=6.9 Hz, 28-H_3), 0.84 (3H, d, *J*=6.9 Hz, 26-H_3 or 27-H_3), 0.82 (3H, d, *J*=6.9 Hz, 27-H_3 or 26-H_3), 0.56 (3H, s, 18-H_3).

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