Synthesis and Structure-Activity Relationships of Cytotoxic 7-Hydroxy Sterols

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SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF CYTOTOXIC 7-HYDROXY STEROLS

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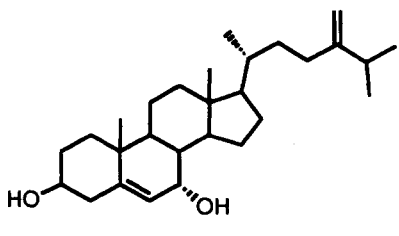
ABSTRACT.—The cytotoxic sterols **1** and **2**, previously isolated from *Pseudobersama mossambicensis*, have been synthesized in nine steps from stigmasterol, together with seven related sterols. Structure-activity relationships of these sterols in cytotoxicity and DNA-damaging assays are discussed.

As a part of our ongoing effort to isolate novel mechanism-based anticancer agents from plants, we recently obtained the bioactive methylenic sterol **1**, together with its epoxy derivative **2**, from *Pseudobersama mossambicensis*; the diol **3** was also isolated but is most probably an artifact arising from hydrolysis of **2** (1). These sterols showed significant activity in our yeast-based screen for DNA-damaging agents (1). However, only small amounts of the compounds were isolated, and larger quantities were needed to complete cytotoxicity testing. We thus elected to carry out a semisynthesis of sterols **1** and **2**, with the aim not only of providing material for additional testing but also of obtaining information on structure-activity relationships in this area.

RESULTS AND DISCUSSION

Our synthetic design took into account the fact that the readily available steroid stigmasterol [**4**] has been converted in four steps to the steroid **5** with the correct side-chain structure and absolute stereochemistry (2). Conversion of **5** to the key intermediate **8** was envisaged to proceed through modifications of standard methodology, and conversion of **8** to **1** and **2** could then be accomplished through the use of appropriate phosphorus and sulfur ylid chemistry (Scheme 1). This approach was realized as described below.

Conversion of stigmasterol **4** to the enone **5** was accomplished by Djerassi's method, and yielded enone **5** with physical data identical to those previously described (2). Hydrogenation of **5** over palladium on carbon in EtOAc gave the ketone **6** having ^{1}H- and ^{13}C-nmr spectra consistent with the assigned structure. In particular, signals for the $\Delta^{22(23)}$ protons and carbons were lacking, and an isolated carbonyl group was observed at



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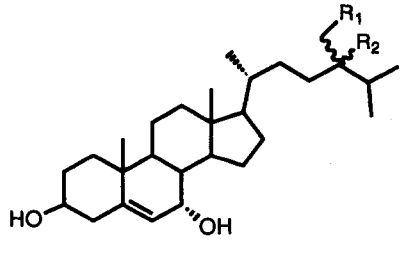
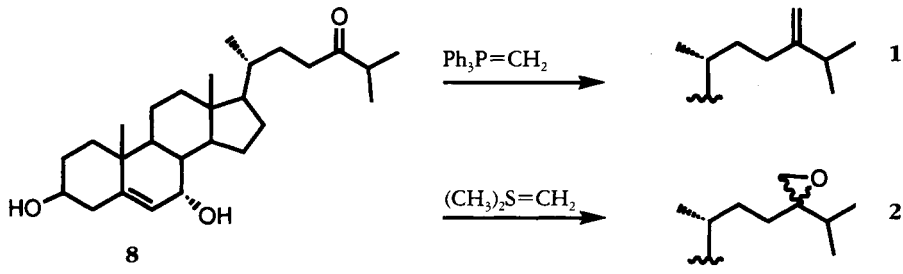


2 R_1, R_2=-O-
3 R_1=R_2=OH



SCHEME 1. Synthesis of **1** and **2** from a Common Intermediate.

215.2 ppm in its ^{13}C-nmr spectrum. Its mass spectrum showed a molecular ion at *m/z* 414.

Compound **6** was treated with HOAc in the presence of zinc acetate to open the cyclopropyl methyl ether protection system (3), and afforded the acetate **7**. The ^{1}H-nmr spectrum of **7** lacked the three cyclopropyl protons of **6** at 0.3–0.8 ppm, and showed instead a one-proton olefinic doublet at 5.37 ppm for C-6 and a three-proton singlet at 2.03 ppm for the C-3 acetate. The mass spectrum of **7** showed important ions at *m/z* 399 $[M-C_3H_7]^+$, and 382 $[M-60]^+$.

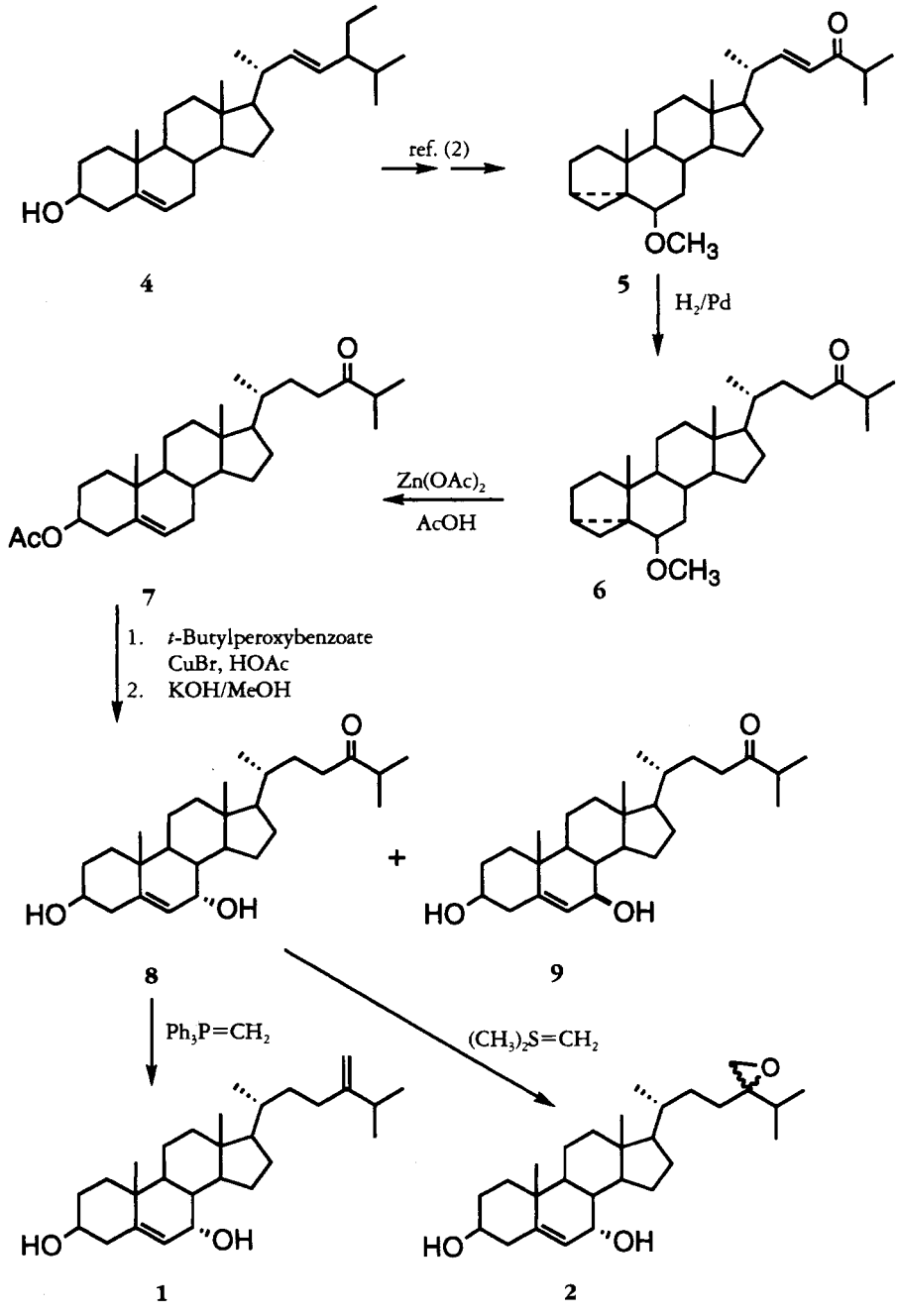
Allylic acetoxylation of **7** was carried out using *tert*-butylperoxybenzoate in the presence of cuprous bromide in HOAc (4) to yield a mixture of C-7 acetates. Although the literature states that the α-product is formed exclusively in similar situations, in our hands this method generally gave a 1.5:1 ratio of α and β forms. The mixture of diacetates was subjected to hydrolysis using 5% KOH in MeOH to yield the diols **8** and **9** which could be separated by Si gel column chromatography.

The ir spectrum of **8**, obtained from **7** in 16% yield, exhibited a broad OH stretch at 3380 cm^{-1}, and a carbonyl stretch at 1710 cm^{-1}. The ^{1}H-nmr spectrum of **8** revealed a 1H doublet of doublets (J=5.2 and 1.2 Hz) at δ 5.60, corresponding to the vinylic C-6 proton. An H-7α proton was present, represented by a 1H multiplet at δ 3.85, and a 1H multiplet at δ 3.60 was assigned to H-3α. A 1H septet (J=6.9 Hz) was seen at δ 2.60, which is typical for H-25 when C-24 bears a carbonyl group (2). Two overlapping doublets centered at δ 1.08 (J=7.0 Hz) were assigned to H_3-26 and H_3-27. Other methyl signals included a singlet at δ 0.92, a doublet (J=6.4 Hz) at δ 0.92, and a singlet at δ 0.68. The ^{13}C-nmr spectrum of **8** indicated the expected number of carbons, including a carbonyl resonance at δ 215.3. The mass spectrum of **8** showed a base peak at *m/z* 398 $[M-H_2O]^+$, and another significant peak at *m/z* 380 $[M-2H_2O]^+$.

The yield of pure intermediate **8**, while relatively low, was better than that achieved when pursuing an alternative route, which involved the protection of the C-24 carbonyl group as a ketal, allylic oxidation at C-7, and then stereoselective reduction of the resulting carbonyl group with L-Selectride. This was followed by removal of the ketal and saponification of the C-3 acetate. Direct acetoxylation of **7** not only saved steps, but it also had the advantage of giving reasonable amounts of the 7β-OH material, **9**, which was then used to generate the β analogs of **1** and **2** for the structure-activity relationship study.

With the diol **8** in hand, both **1** and **2** could readily be prepared (Scheme 2). For the synthesis of olefin **1**, the precursor **8** was treated with triphenyl phosphonium methylide in DMSO [formed *in situ* using Ph_3PCH_3Br and NaH in DMSO (5)] to give **1**. The ir spectrum of **1** showed no carbonyl stretch, and its $[\alpha]_D$ value was in good agreement with that for the natural product. Its ^{1}H- and ^{13}C-nmr spectra, and its other physical properties (tlc, mp) were also identical with those of the natural product.

The epoxide **2** was formed as a mixture of diastereomers by treating **8** with



SCHEME 2. Synthesis of **1** and **2** from Stigmasterol [**4**].

dimethylsulfonium methylide in DMSO (formed *in situ* using trimethyl sulfonium iodide and NaH in DMSO) (6). Compound **2** had the same R_f value on tlc (both normal and reversed-phase) in several solvent systems as the natural product, and it had the same retention time on a reversed-phase hplc column. The ir spectrum of **2** showed the absence of a carbonyl stretch. Its ^{1}H-nmr spectrum revealed that the H(C-25) septet at δ 2.60 was now distorted and shifted upfield, merging with the two-proton multiplet seen for the C-23 protons. A new two-proton multiplet centered at δ 2.56 was attributed to the C-

28 methylene protons. In the naturally occurring product, these protons are represented by a doublet of doublets at δ 2.53 and 2.60. The fact that these signals for the synthetic product appear as a complex multiplet is rationalized on the basis of the presence of diastereomers at C-24. Multiple doublets are also seen for the C-26 and C-27 methyl groups at δ 0.88–0.96. The remaining major ^{1}H-nmr signals are identical to those of the authentic sample. The ^{13}C-nmr spectrum of **2** compares well with that for the natural product, the difference being that doublets are seen for carbons 17, 20, 22, 23, 25, 26, and 27 as would be expected with a mixture of diastereomers at position 24 (1). The mass spectrum of **2** was similar to that for the authentic sample, showing significant peaks at *m/z* 412 $[M-H_2O]^+$, 394 $[M-2H_2O]^+$.

Compound **2**, prepared as a mixture of diastereomers, ran as a single spot on tlc in several solvent systems. Attempts at separation of the diastereomers by hplc using both reversed-phase columns and a normal-phase chiral column were unsuccessful. ^{1}H- and ^{13}C-nmr assignments were made based on comparison with authentic samples, and are listed in Tables 1 and 2. ^{13}C-Nmr Attached-Proton Test experiments were run on both compounds in order to confirm multiplicities.

The 7β-hydroxyl analogs of **1** and **2** were prepared by exactly the same methods as were the target sterols. Thus, treatment of **9** with triphenylphosphonium methylide in DMSO at 60° yielded the 3β,7β-diol-24(28) olefin **10**. The ir spectrum of **10** showed

TABLE 1. Selected ^{1}H-Nmr Chemical Shifts (400 MHz) of Sterols **1** and **2** in $CDCl_3$.[a]

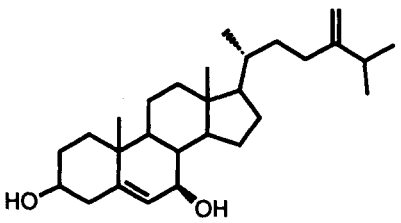
Proton	1	2
H-3	3.59 m	3.59 m
H-6	5.61 dd (5.2, 1.6)	5.61 dd (5.2, 1.6)
H-7	3.85 m	3.85 m
H_3-18	0.69 s	0.68 s
H_3-19	0.99 s	0.99 s
H-21	0.96 d (6.8)	0.95 d (6.8)
H_3-26	1.03 d (6.8)	0.88–0.95 pair of doublets
H_3-27	1.02 d (6.8)	0.88–0.95 pair of doublets
H_2-28	4.65 s, 4.71 s	2.56 m

[a]Chemical shifts (relative to TMS) are in ppm and coupling constants in Hz.

TABLE 2. ^{13}C-Nmr Chemical Shifts (100.57 MHz) of Sterols **1** and **2** in $CDCl_3$.[a]

Carbon	1	2	Carbon	1	2
1	37.0	37.0	15	24.3	24.3
2	31.4	31.4	16	28.2	28.2
3	71.3	71.3	17	55.6	55.52, 55.48
4	42.2	42.2	18	11.6	11.7
5	146.2	146.3	19	18.2	18.2
6	123.8	123.8	20	35.7	35.8, 35.7
7	65.3	65.3	21	18.7	18.7
8	37.4	37.5	22	34.6	28.2, 28.0
9	42.2	42.3	23	30.8	30.1, 29.7
10	37.5	37.4	24	156.8	62.7
11	20.7	20.7	25	33.8	31.6, 32.1
12	39.2	39.1	26	21.8	17.7, 17.9
13	42.0	42.0	27	21.9	18.4, 18.6
14	49.4	49.4	28	105.9	50.5

[a]In ppm from internal TMS.



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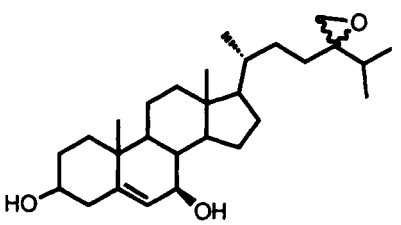

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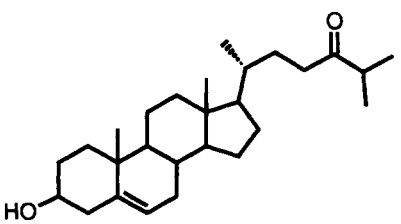

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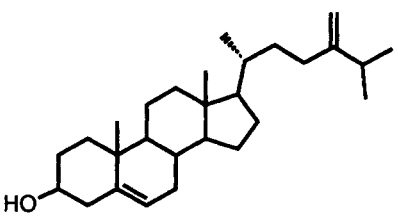

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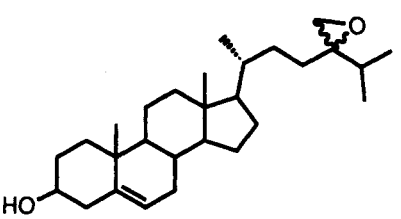

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the absence of a carbonyl stretch, and its ^{1}H-nmr spectrum showed the same two 1H singlets at δ 4.71 and 4.65 for the C-28 protons as seen for its epimer. The only observable differences in the ^{1}H-nmr spectrum from that of **1** (and the natural product) was for the H-7 and H_3-18 protons. In the β compound, the H-7 proton resonated at δ 5.29 and appeared as a broad singlet, and the H_3-18 methyl signal was shifted upfield slightly to δ 1.05. The ^{13}C-nmr spectrum and mass spectrum of **10** were similar to those of **1**.

The preparation of the 24,28-epoxy-3β,7β-dihydroxy analog **11** was carried out by treating **9** with dimethylsulfonium methylide in DMSO at 0°, then allowing the mixture to warm to room temperature. The ir spectrum of **11** indicated the absence of a carbonyl stretch. Its ^{1}H-nmr spectrum was similar to that of its epimer **2**, with the exception of H-7, where a broad singlet was seen at δ 5.29. Its ^{13}C-nmr spectrum and mass spectrum were similar to those for **2**. Selected ^{1}H-nmr chemical shifts for compounds **9** and **10** are listed in Table 3.

In addition to the 7-hydroxysterols **1**, **2**, **10**, and **11**, the C-7 desoxy analogs were also prepared in order to determine the effect of the C-7 hydroxyl group on activity.

3β-Hydroxycholest-5-en-24-one [**12**] was prepared by simple hydrolysis of **7** using 5% KOH in MeOH at room temperature. Its ^{1}H-nmr spectrum showed the absence of an acetate group, and the H-3 proton was shifted upfield 1.1 ppm to δ 3.53. Its mass spectrum indicated an $[M]^+$ of *m/z* 400, and showed other significant peaks at *m/z* 382 $[M-H_2O]^+$, and 71 $[C_4H_7O]^+$.

TABLE 3. Selected ^{1}H-Nmr Chemical Shifts (400 MHz) of Sterols **10** and **11** in $CDCl_3$.[a]

Proton	**10**	**11**
H-3	3.55 m	3.55 m
H-6	5.29 br s	5.29 br s
H-7	3.84 m	3.84 m
H_3-18	0.70 s	0.69 s
H_3-19	1.05 s	1.05 s
H-21	0.96 d (6.4)	0.95 d (6.8)
H_3-26	1.03 d (6.8)	0.80–0.98 pair of doublets
H_3-27	1.02 d (6.8)	0.80–0.98 pair of doublets
H_2-28	4.65 s, 4.71 s	2.57 m

[a]Chemical shifts (relative to TMS) are in ppm and coupling constants (in parentheses) in Hz.

3β-Hydroxyergost-5,24(28)-diene [**13**] was prepared from **7** as in the previously discussed Wittig olefinations. Thus, **7** was treated with an excess of triphenylphosphonium methylide in dry DMSO to yield the desired product **13**. The ir spectrum of **13** showed no carbonyl stretch, and its ^{1}H-nmr spectrum showed the same two doublets for the H-28 methylene protons as seen in the cases of **1** and **10**. The ^{1}H-nmr spectrum also lacked an acetate resonance; there was no need for a separate hydrolysis step as the ylide conveniently performs that process. The mass spectrum of **13** showed the expected parent ion.

The 24,28 epoxide **14** of this series was formed from **12** with dimethylsulfonium methylide in DMSO as for the 7-hydroxy compounds. This compound showed no carbonyl stretch in its ir spectrum, and its ^{1}H-nmr spectrum was similar to that of the 7-hydroxy compounds, lacking only the low-field C-7 proton. Its mass spectrum indicated the expected parent ion.

All of the sterols prepared were assayed in the rad 52 and rad$^+$ DNA-damaging bioassays, and the results are presented in Table 4. Selected compounds were also tested for cytotoxicity to Vero cells, and these results are also reported in Table 4.

The synthetic sterols **1** and **2** showed DNA-damaging activity comparable to the authentic natural products. It is noted that with regard to the 7α-OH compounds, there appears to be an inverse correlation between activity in the yeast assay and Vero cell culture activity. Thus, the 7α-OH sterol which is most active in the DNA-damaging assay is the epoxide **2**, with an IC_{12} value of 0.2 μg/ml, but the compound is inactive in the Vero cell assay, with an IC_{50} of >100 μM. The second most active 7α-OH compound

TABLE 4. Bioactivity of Synthetic Sterols.

Sterol	Description	IC_{12} (μg/ml) rad 52[a]	Cytotoxicity to Vero cells IC_{50} (μM)
1	3β,7α-diol-24(28)-ene	7	58
2	3β,7α-diol-24,28-epoxide	0.2	>100
8	3β,7α-diol-24-one	14	9.9
9	3β,7β-diol-24-one	>500	21
10	3β,7β-diol-24(28)-ene	>8000	31
11	3β,7β-diol-24,28-epoxide	>8000	16
12	3β-OH-24-one	>8000	NT[b]
13	3β-OH-24(28)-ene	>8000	NT
14	3β-OH-24,28-epoxide	>8000	NT

[a]All sterols were inactive against the wild-type strain RAD$^+$.
[b]Not tested.

in the yeast assay is the olefin **1**, which shows an IC_{12} of 7 μg/ml; it is weakly active in the Vero cell assay, with an IC_{50} of 58 μM. The C-24 ketone **8**, the least active of the three in the DNA-damaging assay with an IC_{12} of 14 1μg/ml, is the most active in the Vero cell culture with an IC_{50} of 9.9 μM.

The 7β-OH analogs **10** and **11**, which are completely inactive in the yeast assay, showed moderate cytotoxic activity in the Vero cell assay, indicating that they act by a different mechanism of action than their α-counterparts. The 3-OH sterols **12–14**, regardless of their side-chain funtionalities, were inactive in the yeast assay; they were not tested in the Vero cell cultures. The bioassay results of the synthetic sterols are given in Table 4.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were determined on a Kofler hot-stage apparatus and are uncorrected. Optical rotations were taken in $CHCl_3$ solution with a Perkin-Elmer Model 241 polarimeter. The ^1H- and ^{13}C-nmr spectra were recorded on a Varian Unity 400 spectrometer at 400 and 100.57 MHz, respectively, with TMS as an internal standard. Flash chromatography was performed using Si gel Merck G60 (230–400 mesh), prep. tlc with Si gel GF_{254} plates (Analtech, 500 μm, 20×20 cm), and reversed-phase prep. tlc with Whatman PLKC18F linear K reversed-phase (1000 and 250 μm, 20×20 cm) plates. Hplc experiments were carried out on a Waters apparatus equipped with photodiode array and refractive index detectors. Hplc columns used were a Whatman Partisil 10 ODS-3 (4.6 mm i.d.×25 cm), and a Daicel Chemical Chiralcel OD 31–10 (3 mm i.d.×25 cm).

6β-METHOXY-3α,5-CYCLO-CHOLESTAN-24-ONE [**6**].—A mixture of 100 mg (0.24 mmol) of enone **5** (2) and 15 mg of 10% palladium on carbon in 10 ml of EtOAc was treated with H_2 for 2 h. The mixture was filtered, the solvent evaporated, and the crude mixture was purified by Si gel cc eluting with 6% EtOAc in hexane to yield 90 mg (91%) of **5**, mp 91° ($CHCl_3$/MeOH); $[\alpha]_D$ +32° (*c*=0.20, $CHCl_3$); ir ν max (KBr) 2930, 2870, 1710, 1470, 1380, 1290, 1180, 1100, 1015, 970 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.28 (3H, s), 2.76 (1H, t, *J*=2.8 Hz), 2.60 (1H, sept., *J*=6.9 Hz), two overlapping d at 1.08 (*J*=6.9 Hz), 1.01 (3H, s), 0.90 (3H, d, *J*=5.6 Hz), 0.71 (3H, s), 0.64 (1H, m), 0.42 (1H, m); ^{13}C nmr δ 215.2, 82.5, 56.7, 56.5, 56.2, 48.1, 43.5, 42.9, 40.9, 40.4, 37.4, 35.6, 35.4, 35.1, 33.5, 30.6, 29.9, 28.4, 25.1, 24.3, 22.9, 21.6, 19.4, 18.6, 18.5, 18.4, 13.2, 12.2; eims *m/z* $[M]^+$ 414.3510 ($C_{28}H_{46}O_2$ requires 414.3498), 399 (60), 382 (65), 359 (100), 255 (25), 213 (30), 107 (40).

3β-ACETOXY-5-CHOLESTEN-24-ONE [**7**].—A mixture of 150 mg (0.36 mmol) of ketone **6**, 1.2 g of freshly fused zinc acetate, and 30 ml of glacial HOAc was stirred at reflux (120°) for 2 h. After the mixture was cooled, 40 ml cold H_2O was added and this was extracted with hexane-C_6H_6 (50:50). The organic extracts were combined and washed with H_2O, 5% $NaHCO_3$, and finally brine, then dried over anhydrous Na_2SO_4. The solvent was removed, leaving 187 mg of a crude yellow solid. This was subjected to Si gel cc, eluting with hexane-CH_2Cl_2 (50:50), to yield 145 mg (93%) of **7**, mp 133° ($CHCl_3$/MeOH); $[\alpha]_D$ −46° (*c*=0.22, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.37 (1H, d, *J*=4.8 Hz), 4.60 (1H, m), 2.61 (1H, sept., *J*=7.0 Hz), 1.08 (6H, two overlapping d, *J*=7.0 Hz), 1.02 (3H, s), 0.92 (3H, d, *J*=6.4 Hz), 0.68 (3H, s); ^{13}C nmr ($CDCl_3$) δ 215.3, 170.4, 139.5, 122.5, 73.9, 56.6, 55.8, 49.9, 42.3, 40.7, 39.6, 38.0, 37.1, 36.9, 36.5, 35.3, 31.8, 31.7, 29.7, 28.0, 27.7, 24.2, 21.4, 20.9, 19.2, 18.4, 18.3, 18.2, 11.8; eims *m/z* $[M-AcOH]^+$ 382.3234 ($C_{27}H_{42}O$ requires 382.3235), 367 (15), 296 (17), 255 (17), 213 (18), 147 (28), 145 (26), 107 (24).

3β,7α-DIHYDROXY-5-CHOLESTEN-24-ONE [**8**].—A mixture of 80 mg (0.18 mmol) acetate **7**, 80 mg CuBr (0.56 mmol) and 156 mg *tert*-butylperoxybenzoate (0.80 mmol) in 1.0 ml of AcOH under an Ar atmosphere was stirred at 120° for 0.5 h. The mixture was cooled to room temperature, diluted with C_6H_6, filtered, and washed with H_2O, Na_2CO_3, and H_2O again. The C_6H_6 was evaporated and the crude mixture was subjected to hydrolysis using 2% KOH in MeOH. Separation of the 7α-OH and 7β-OH compounds was achieved by Si gel chromatography using 100% Et_2O as eluent. This yielded 12 mg (16%) of **8**, mp 120–122° (MeOH); $[\alpha]_D$ −60° (*c*=0.10, $CHCl_3$); ir ν max (KBr) 3380, 2930, 1700, 1560, 1470, 1380, 1135, 1060, 950, 825, 790 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.60 (1H, dd, *J*=3.8 and 1.4 Hz), 3.85 (1H, br s), 3.58 (1H, m), 2.60 (1H, sept., *J*=6.9 Hz), 2.46 (1H, m), 1.08 (6H, two overlapping d, *J*=6.9 Hz), 0.99 (3H, s), 0.92 (3H, d, *J*=6.4 Hz), 0.68 (3H, s); ^{13}C nmr ($CDCl_3$) δ 215.3, 146.2, 123.8, 71.3, 65.3, 55.6, 49.4, 42.2, 42.1, 41.9, 40.8, 39.2, 37.5, 37.4, 37.03, 37.00, 35.3, 31.4, 29.8, 28.1, 24.2, 20.7, 18.5, 18.3, 18.26, 18.20, 11.6; eims *m/z* $[M]^+$416.3305 ($C_{27}H_{44}O_3$ requires 416.3290), $[M-H_2O]^+$ 398.3179 ($C_{27}H_{42}O_2$ requires 398.3185), $[M-2H_2O]^+$ 380.3079 ($C_{27}H_{40}$ requires 380.3079), 271 (8), 145 (9), 73 (62), 55 (13).

3β,7β-DIHYDROXY-5-CHOLESTEN-24-ONE [**9**].—From the above procedure, 8 mg (10%) of **8** was obtained, mp 139° (MeOH); [α]D +10° (*c*=0.18, $CHCl_3$); ir ν max (KBr) 3380, 2325, 1705, 1560, 1460, 1380, 1140, 1055, 950, 820, 790 cm^{-1}; ^1H nmr ($CDCl_3$) δ 5.29 (1H, br s), 3.84 (1H, m), 3.54 (1H, m), 2.60 (1H, sept., *J*=6.9 Hz), 2.46 (1H, m), 1.08 (6H, two overlapping d, *J*=6.9 Hz), 0.99 (3H, s), 0.92 (3H, d, *J*=6.4 Hz), 0.68 (3H, s); ^{13}C nmr ($CDCl_3$) δ 215.2, 143.4, 125.5, 73.3, 71.4, 55.9, 55.3, 48.3, 42.9, 41.7, 40.9, 40.8, 39.5, 37.2, 36.9, 36.4, 35.3, 31.6, 29.9, 28.4, 26.3, 21.1, 19.1, 18.5, 18.31, 18.26, 11.8.

3β,7α-DIHYDROXYERGOST-5,24(28)-DIENE [**1**].—Under Ar, 6 mg (0.024 mmol) NaH in 0.5 ml dry DMSO was heated to 70° for 45 min, then cooled to room temperature. Ph_3PCH_3Br (86 mg, 0.24 mmol) in 2 ml DMSO was added and the mixture stirred at room temperature for 10 min. Compound **8** (10 mg, 0.024 mmol) in 0.3 ml DMSO was added and the mixture was stirred at 60° for ca. 1 h. The mixture was cooled, diluted with Et_2O, and washed repeatedly with H_2O. The Et_2O layer was dried over Na_2SO_4, evaporated and after reversed-phase prep. tlc (developing with 95% aqueous MeOH), yielded 7.7 mg (78%) of **1**, mp 194–195° (MeOH); [α]D −88° (*c*=0.21, $CHCl_3$); ir ν max (KBr) 3395, 2930, 1630, 1560, 1540, 1520, 1500, 1460, 1380, 1100, 1050, 950, 930 cm^{-1}; ^1H nmr, see Table 1; ^{13}C nmr, see Table 2; eims *m/z* $[M]^+$ 414.3486 ($C_{28}H_{46}O_2$ requires 414.3498), $[M-H_2O]^+$ 396.3392 ($C_{28}H_{44}O$ requires 396.3392), 378 (15), 312 (13), 269 (10), 143 (12), 107 (15).

3β,7α-DIHYDROXY-24,28-EPOXYERGOST-5-ENE [**2**].—Under Ar, 81 mg NaH in 10 ml dry DMSO was heated to 70° for 45 min. This was cooled to room temperature and diluted with 10 ml of dry THF. By syringe, 0.2 ml of this solution was removed and added to an Ar-filled two-neck flask. This was cooled to 0° and 7 mg $(CH_3)_3SI$ was added slowly in a minimal volume of DMSO. The resulting mixture was stirred for 1 min at 0° and 7 mg of **8** in THF was added. This was stirred at 0° for ca. 1 min, then allowed to warm to room temperature. The reaction was judged complete in 1 h and the mixture was worked up with Et_2O/H_2O. The Et_2O layer was dried over Na_2SO_4, evaporated, and after reversed-phase prep. tlc (developing with 95% aqueous MeOH), yielded 4.5 mg (63%) of **2**, mp 172–174° (MeOH); [α]D −63° (*c*=0.15, $CHCl_3$); ir ν max (KBr) 3370, 2920, 2555, 1440, 1270, 1170, 1010, 690 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr, see Table 2; eims *m/z* $[M-H_2O]^+$ 412.3325 ($C_{28}H_{44}O_2$ requires 412.3341), $[M-2H_2O]^+$ 394.3229 ($C_{28}H_{42}O$ requires 394.3235), 380 (10), 364 (5), 157 (40), 143 (50), 119 (55), 105 (55), 91 (60), 81 (75), 69 (70).

3β,7β-DIHYDROXYERGOST-5,24(28)-DIENE [**10**].—Under Ar, 4.3 mg (0.18 mmol) NaH in 0.3 ml dry DMSO was heated to 70° for 45 min, then cooled to room temperature. Ph_3PCH_3Br (64 mg, 0.18 mmol) in 1.5 ml DMSO was added to the mixture and stirred at room temperature for 10 min. Compound **9** (7 mg, 0.018 mmol) in 0.2 ml DMSO was added and the mixture was stirred at 60° for ca. 1 h. The mixture was cooled, diluted with Et_2O, and washed repeatedly with H_2O. The Et_2O layer was dried (Na_2SO_4), evaporated, and after reversed-phase prep. tlc (developed with 95% aqueous MeOH), yielded 4.1 mg (59%) of **10**, mp 152–153° [lit. (7) 149–151°] (MeOH), [α]D +19° (*c*=0.16, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.29 (1H, br s), 4.71 (1H, s), 4.65 (1H, s), 3.84 (1H, m), 3.55 (1H, m), 1.05 (3H, s), 1.03 (3H, d, *J*=6.8 Hz), 1.01 (3H, d, *J*=6.8 Hz), 0.95 (3H, d, *J*=6.4 Hz), 0.70 (3H, s); ^{13}C nmr ($CDCl_3$) δ 156.8, 143.5, 125.5, 106.1, 73.3, 71.4, 55.9, 55.3, 48.2, 42.9, 41.7, 40.9, 39.5, 36.9, 36.4, 35.7, 34.7, 33.8, 31.6, 31.0, 28.5, 26.3, 22.0, 21.8, 21.1, 19.1, 18.7, 11.8; eims *m/z* $[M]^+$ 414.3489 ($C_{28}H_{46}O_2$ requires 414.3498), $[M-H_2O]^+$ 396.3392 ($C_{28}H_{44}O$ requires 396.3392), 378 (9), 312 (12), 143 (8), 107 (11).

3β,7β-DIHYDROXY-24,28-EPOXYERGOST-5-ENE [**11**].—Under Ar, 3 mg (0.12 mmol) NaH in 2.5 ml DMSO was heated to 70° for 45 min, then cooled to room temperature, and 2.5 ml THF was added. A 1.0-ml portion of this solution was removed by syringe, and added to an Ar-filled two-neck flask. This was cooled to 0° and 5 mg (0.024 mmol) $(CH_3)_3SI$ in a minimal volume of DMSO was added slowly. The resulting mixture was stirred at 0° for 1 min, and 5 mg (0.012 mmol) of **9** in THF was added. This was stirred at 0° for ca. 1 min, then allowed to warm to room temperature. After 1 h, the reaction was worked up with H_2O/Et_2O. The Et_2O layer was dried (Na_2SO_4), evaporated, and after reversed-phase prep. tlc (developing with 95% aqueous MeOH), yielded 3.2 mg (63%) of **11**, mp 157–158° (MeOH); [α]D +14° (*c*=0.13, $CHCl_3$); ^1H nmr ($CDCl_3$) δ 5.29 (1H, br s), 3.84 (1H, m), 3.55 (1H, m), 2.57 (2H, m), 1.05 (3H, s), 0.80–0.98 (6H, four overlapping d), 0.69 (3H, s); ^{13}C nmr ($CDCl_3$) δ 143.5, 125.4, 73.3, 71.4, 62.7, 55.9, 55.2, 55.0, 50.5, 48.2, 42.9, 41.7, 40.9, 39.5, 36.9, 36.4, 35.8, 35.7, 32.0, 31.7, 31.6, 30.3, 30.2, 28.5, 28.4, 27.7, 26.3, 21.0, 19.1, 18.7, 18.6, 18.4, 18.2, 17.9, 17.7, 11.8; eims *m/z* $[M]^+$ 430.3462 ($C_{28}H_{46}O_3$ requires 430.3447), $[M-H_2O]^+$ 412.3337 ($C_{28}H_{44}O_2$ requires 412.3341), $[M-2H_2O]^+$ 394.3236 ($C_{28}H_{42}O$ requires 394.3236), 353 (5), 312 (7), 269 (10), 211 (12), 143 (35), 95 (38), 55 (51).

3β-HYDROXYCHOLEST-5-EN-24-ONE [**12**].—Acetate **7** (110 mg, 0.25 mmol) in 5% methanolic KOH was stirred at room temperature for 2 h. The solvent was removed under vacuum, and the resultant mixture was taken up in CH_2Cl_2, washed repeatedly with H_2O, then dried over Na_2SO_4. After reversed-phase prep. tlc using 90% aqueous MeOH as eluent, 98 mg (98%) of **12** was obtained, mp 127–129° ($CHCl_3$/MeOH) [lit. (8) 129–130°]; ^1H nmr ($CDCl_3$) δ 5.34 (1H, m), 3.53 (1H, m), 2.60 (1H, sept., *J*=6.9 Hz), 1.08 (6H,

two overlapping d, J=6.9 Hz), 0.99 (3H, s), 0.90 (3H, d, J=6.8 Hz), 0.67 (3H, s); ^{13}C nmr ($CDCl_3$) δ 215.5, 140.7, 135.6, 127.6, 121.7, 71.8, 56.7, 55.9, 50.0, 42.3, 42.2, 40.8, 39.7, 37.2, 36.5, 35.4, 31.9, 31.6, 29.8, 28.1, 24.2, 21.1, 19.4, 18.5, 18.4, 18.3, 11.9; eims *m/z* [M]$^+$ 400.3341 ($C_{27}H_{44}O_2$ requires 400.3341), [M−H_2O]$^+$ 382.3242 ($C_{27}H_{42}O$ requires 382.3236), 367 (41), 314 (60), 312 (53), 234 (50), 213 (80), 71 (95).

3β-HYDROXYERGOST-5,24(28)-DIENE [**13**].—Under Ar, 1.1 mg (0.046 mmol) NaH in 0.5 ml dry DMSO was stirred at 70° for 45 min, then cooled to room temperature. To this solution 16 mg (0.046 mmol) of Ph_3PCH_3Br in 0.5 ml of DMSO was added and stirred for 10 min. Then 10 mg (0.023 mmol) of **7** was added in 0.2 ml DMSO and the mixture was stirred at 60° for ca. 2 h. The mixture was cooled, diluted with Et_2O, and washed repeatedly with H_2O. The Et_2O layer was dried (Na_2SO_4), evaporated, and after prep. tlc (eluting with 30% EtOAc in hexane), yielded 7 mg (71%) of **13**, mp 148–149° ($CHCl_3$) [lit. (8) 149–151°]; ^{1}H nmr ($CDCl_3$) δ 5.34 (1H, m), 4.71 (1H, s), 4.65 (1H, s), 3.53 (1H, m), 2.57 (2H, m), 1.05 (3H, s), 1.03 (3H, d, J=6.8 Hz), 1.01 (3H, d, J=6.8 Hz), 0.95 (3H, d, J=6.5 Hz), 0.68 (3H, s); eims *m/z* 398 (5), 383 (5), 314 (55), 299 (20), 271 (25), 229 (20), 213 (25), 145 (35), 133 (20), 105 (30), 91 (40), 81 (35), 69 (100).

3β-HYDROXY-24,28-EPOXY-ERGOST-5-ENE [**14**].—Under Ar, 11 mg of NaH in 2.5 ml dry DMSO was heated to 70° for 45 min, then cooled to room temperature and diluted with 2.5 ml of dry THF. A portion (0.5 ml) of this solution was removed and added to an Ar-filled two-neck flask and cooled to 0°. $(CH_3)_3SI$ (9.4 mg) in 0.5 ml DMSO was added and stirred for 10 min. Sterol **7** (10 mg, 0.023 mmol) was added in a minimal volume of THF and the mixture was stirred at 0° for 1 min, then allowed to warm to room temperature and stirred for 2 h. The reaction was worked up with Et_2O and H_2O. After drying the Et_2O layer over Na_2SO_4, the crude product was subjected to prep. tlc, eluting with 30% EtOAc in hexane to yield 3.6 mg (36%) of **14**, mp 118–119° ($CHCl_3$/MeOH) [lit. (9) 116°]; ir ν max (KBr) 3370, 2920, 2850, 1440, 1270, 1170, 1120, 1015, 690 cm^{-1}; ^{1}H nmr ($CDCl_3$) δ 5.34 (1H, m), 3.53 (1H, m), 2.57 (2H, m), 1.05 (3H, s), 0.80–0.98 (6H, four overlapping d), 0.69 (3H, s); ^{13}C nmr ($CDCl_3$) δ 140.7, 121.6, 71.8, 62.7, 55.9, 55.8, 50.4, 50.1, 42.33, 42.30, 39.8, 37.2, 36.5, 35.8, 35.7, 32.0, 31.90, 31.87, 31.70, 31.66, 30.3, 30.2, 29.7, 28.2, 28.1, 27.8, 24.2, 21.1, 19.4, 18.64, 18.61, 18.35, 18.17, 17.9, 17.7, 11.8; eims *m/z* 414 (40), 396 (35), 381 (20), 329 (15), 314 (25), 303 (15), 271 (35), 213 (30), 161 (40), 145 (50), 133 (40), 119 (35), 105 (50), 95 (55), 81 (70), 69 (60).

BIOASSAYS.—Yeast bioassays were carried out by determining the growth-inhibition activity against the mutant strain of *Saccharomyces cerevisiae* RS322YK (rad 52). Activity is expressed as that concentration necessary to produce a zone of inhibition 12 mm in diameter (IC_{12}), as previously described (1). Cytotoxicity measurements against Vero cells were determined by standard procedures at SmithKline Beecham Pharmaceuticals.

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LITERATURE CITED

1. A.A.L. Gunatilaka, G. Samaranayake, D.G.I. Kingston, G. Hofmann, and R.K. Johnson, *J. Nat. Prod.*, **55**, 1648 (1992).
2. G.D. Anderson, T.J. Powers, C. Djerassi, J. Fayos, and J. Clardy, *J. Am. Chem. Soc.*, **97**, 388 (1975).
3. J.A. Steel and E. Mosettig, *J. Org. Chem.*, **28**, 571 (1963).
4. L. Starka and K. Syhora, Czech. Patent 100,325, July 15, 1961; *Chem. Abstr.*, **57**, 11270f (1962).
5. D.H.R. Barton, D.M. Harrison, G.P. Moss, and D.A. Widdowson, *J. Chem. Soc. (C)*, 775 (1970).
6. E.J. Corey and M. Chaykovsky, *J. Am. Chem. Soc.*, **87**, 1353 (1965).
7. J.A. Findlay and A.D. Patil, *Can. J. Chem.*, **63**, 2406 (1985).
8. D.R. Idler and U.H.M. Fagerlund, *J. Am. Chem. Soc.*, **77**, 4142 (1955).
9. U.H.M. Fagerlund and D.R. Idler, *J. Org. Chem.*, **21**, 372 (1956).

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