Chem. Pharm. Bull. 42(3) 462—469 (1994) Vol. 42, No. 3

Synthesis of Oxygenated Cholesterols as Structural Mimics of Phorbol Ester-Type Tumor Promoters

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Received September 6, 1993; accepted October 28, 1993

We designed several oxygenated steroids in which functional groups including a hydrophobic group are arranged analogously to those of phorbol ester (12-*O*-tetradecanoylphorbol-13-acetate, TPA), with the aim of finding compounds with TPA-like activity, but having a different skeleton and a rigid conformation. The designed steroids, 1β,5α-dihydroxy-3β-hydroxymethylcholestan-6-one (4), 3β,5α-dihydroxycholestan-6-one (5), 3β-hydroxymethylcholestan-5α-ol-6-one (6) and 1β,3β,5α-trihydroxycholestan-6-one (7), were synthesized. A related oxygenated steroid isolated from soft coral, cholestane-1β,3β,5α,6β-tetrol (8), was also synthesized. Among these analogs, compound 7 showed weak TPA-like activities in three biological tests: inhibition of [^{3}H]TPA binding to protein kinase C and to cytosolic-nuclear tumor promoter-binding protein (CN-TPBP), and induction of differentiation of human promyelocytic leukemia cells (HL-60) to monocyte-like cells. On the other hand, compound 5 was found to be a specific ligand for CN-TPBP, but lacked the other TPA-like activities.

Keywords cholesterol; phorbol ester; tumor promoter; molecular design; oxygenated steroid

The diterpene ester tumor promoters, including 12-*O*-tetradecanoylphorbol-13-acetate (TPA, **1**) and 3-tetradecanoylingenol (3-TI, **2**), and the indolactam-based alkaloids, teleocidins (*e.g.*, teleocidin B-4, **3**), are known as TPA-type tumor promoters.[1] In addition to their potent skin tumor-promoting activity,[2] they act as growth modulators of a wide variety of cells.[3] These biological actions have been generally interpreted in terms of binding to and activating protein kinase C (PKC), which plays a major role in mediating signal transductions.[4] We have also proposed a nuclear receptor pathway.[5] The relationships between chemical structures of tumor promoters and biological activities provide an intriguing research target, because of the marked structural dissimilarities between the diterpene esters and teleocidins.[6] We have approached the problem by employing a computer-assisted molecular superposition and receptor mapping method based on spatial arrangements of physical and chemical properties rather than atomic positions.[7] Nevertheless, we have encountered difficulties in using TPA and teleocidins as the template molecules because of the flexibility of the hydrophobic region (tetradecanoyl group) of TPA and the conformational equilibrium of the nine-membered lactam ring of teleocidins.[8] Discovery of a new TPA-like compound having a different skeleton and a rigid conformation might overcome these problems, providing a new approach for analyzing the mechanism of tumor promotion and new possibilities for the creation of tumor promoter antagonists. In general, our strategy is to design new molecules with physico-chemically appropriate arrangements of polar or hydrophilic functional groups and hydrophobic regions on a structural framework of suitable size and shape, having regard to the structure of the parent molecule. Using this general concept, we have attempted to design and obtain new, synthetically accessible compounds which can substitute for TPA, for use in biological studies. In this paper, we report the synthesis of steroidal compounds whose functional group arrangements mimic those of the TPA molecule. If this approach is successful, the synthetic difficulties in obtaining phorbols would be circumvented, and structure–activity research would be promoted, because of the easy functionalization and rigid conformation of steroids. In addition, we wished to see whether any active compound among steroids which correspond to phorbols could be identified as an endogenous compound. Cholesterol is of particular interest in this context because it has a hydrophobic side chain.

Molecular Design of Oxygenated Steroids Target molecules were designed on the basis of hydrogen bonding directions of oxygenated functional groups (carbonyl at C-3, 4β-, 9- and 20-hydroxyl groups) of the TPA molecule. These functional groups were arranged intuitively on the A and B rings of the cholestane skeleton to give 1β,5α-dihydroxy-3β-hydroxymethylcholestan-6-one (**4**), as shown in Fig. 1. In this arrangement, the carbonyl on C-6 of **4** corresponds to the carbonyl on C-3 of phorbol as a hydrogen acceptor, and the 5α- and 1β-hydroxy and 3β-hydroxymethyl groups of **4** correspond to the phorbol 4β-, 9- and 20-hydroxyl groups as hydrogen donors, respectively. For superposing comparison, TPA may be better represented by **1a**. With regard to the role of hydrophobic moieties of the diterpene esters, it is suggestive that 3-TI, having a fatty acid ester at the C-3 position, has similar biological activities to phorbol, which has a fatty acid ester at the C-12 position. The fatty acid esters can plausibly be folded into a receptor cavity (as with the folded hydrophobic moiety of teleocidin-Bs). The direction of the hydrophobic region and the shape of the designed molecule (**4**) resemble those of TPA from a macroscopic viewpoint. Here, we should note that strict superposition of size and shape of the hydrophobic region is not necessarily important, because various teleocidins with different alkyl groups (including the synthetic analogs 7-decyl-[9] and 7-octylindolactam-V)[10] have almost the same activities. Therefore, such a macroscopic super-

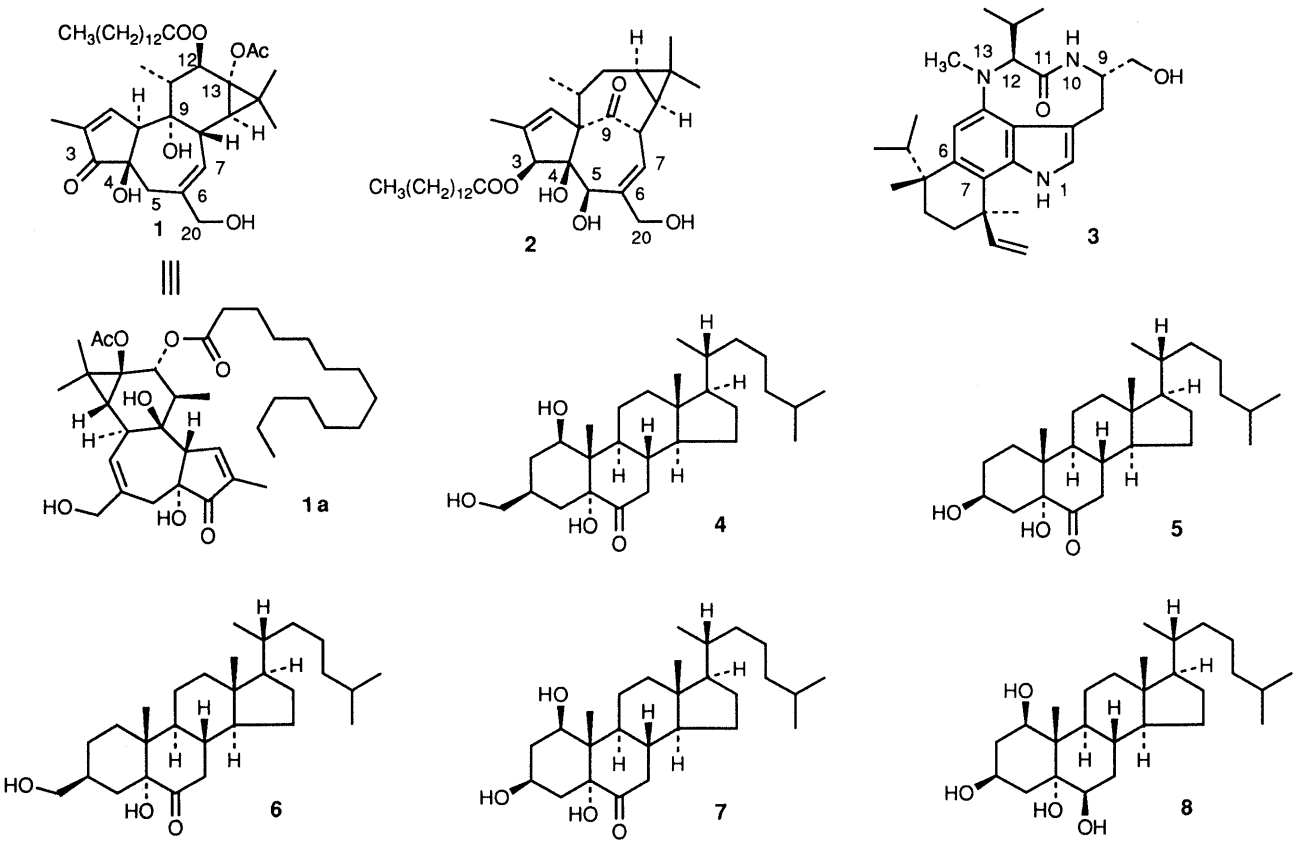

Fig. 1. TPA-Type Tumor Promoters **1**—**3** and the Designed Oxygenated Steroids **4**—**8**

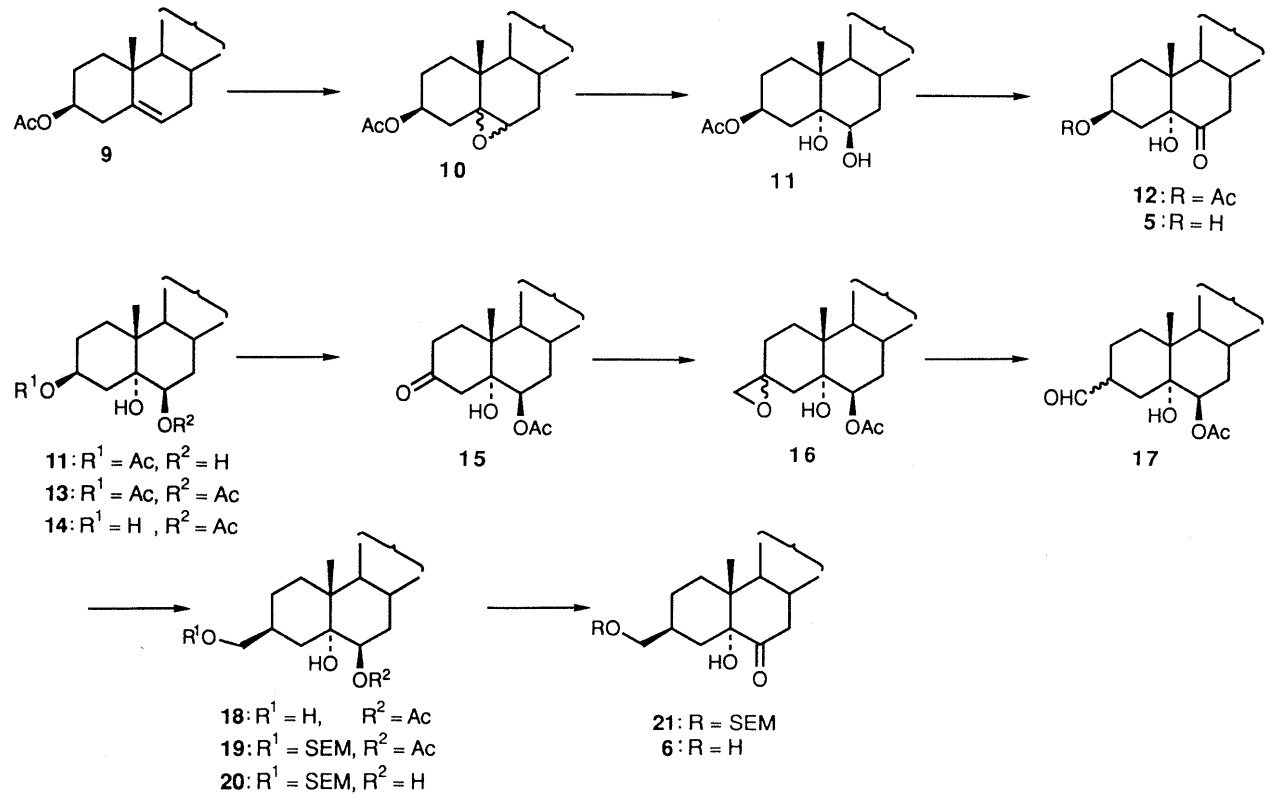

Fig. 2

position is not unreasonable. We then synthesized **4**, and three other analogs, 3β,5α-dihydroxycholestan-6-one (**5**), 3β-hydroxymethylcholestan-5α-ol-6-one (**6**) and 1β,3β,5α-trihydroxycholestan-6-one (**7**). A related oxygenated steroid, cholestane-1β,3β,5α,6β-tetrol (**8**), which has been isolated from the soft coral *Sarcophyton glaucum* by

Kobayashi *et al.*,[11] was also synthesized from cholesterol.

Synthesis of the Oxygenated Steroids The target molecules were synthesized starting from cholesterol by several steps of regio- and stereoselective oxidation on the A and B rings and introduction of a C-1 unit at the 3-position of the molecule.

The syntheses of **5** and **6** are shown in Fig. 2. The synthesis of **5** was performed by the method of Yates and Stiver.[12] Treatment of cholesteryl acetate (**9**) with *m*-chloroperoxybenzoic acid (*m*CPBA) followed by acid-catalyzed hydrolysis with perchloric acid gave 3β-acetoxycholestane-5α,6β-diol (**11**), since oxidation with pyridinium chlorochromate (PCC) gave 3β-acetoxycholestan-5α-ol-6-one (**12**). The acetate was hydrolyzed with aqueous potassium hydroxide in methanol to afford **5** in an overall yield of 68% from **9**. For the synthesis of **6**, the introduction of a C-1 unit at the 3β-position was carried out by employing trimethylsulfoxonium ylide.[13] Compound **11** was converted to 6β-acetoxycholestane-3β,5α-diol (**14**) by acetylation at the 6β-hydroxyl group followed by selective hydrolysis of the 3β-acetoxy group in 94% yield. Oxidation of **14** with PCC gave 6β-acetoxycholestan-5α-ol-3β-one (**15**) in 87% yield. Treatment of **15** with trimethylsulfoxonium ylide in dimethyl sulfoxide (DMSO) gave a mixture of epoxides (**16**) (α-, β- 1 : 5) in 76% yield. The α- and β-mixture of epoxides was treated with boron trifluoride etherate to afford an α- and β-mixture of aldehydes (**17**) (47%), the mixture giving 6β-acetoxy-3β-hydroxymethylcholestan-5α-ol (**18**, 70%) and the 3α-isomer (13%) upon reduction with sodium borohydride. The stereochemistry at the 3-position was confirmed by a comparison of the ^{1}H-NMR spectrum with that of 1α-SEMO-3β-hydroxymethyl-6β-acetoxycholestan-5α-ol (**36**) (*vide infra*). Protection of the primary alcohol by a trimethylsilylethoxymethyl (SEM) group, deprotection of the acetyl group on 6β-hydroxy by reductive cleavage with diisobutylaluminum hydride (DIBAH), and PCC oxidation gave 3β-SEMO-cholestan-5α-ol-6-one (**21**) in an overall yield of 71%. Deprotection of the SEM group on the hydroxymethyl moiety afforded **6**.

The syntheses of **4**, **7** and **8** were carried out as illustrated in Fig. 3, starting from cholest-5-ene-1α,3β-diol (1α-hydroxycholesterol, **22**), which is readily available from cholesta-1,4,6-trien-3-one in a two-step procedure in 54% yield by a modified Barton's method.[14] After selective protection of the 3β-hydroxy group on **22**, transformation of the 1-hydroxy stereochemistry was carried out by the procedure of Mihailovic *et al.*[15] PCC oxidation of **22** followed by reduction with lithium tri(*tert*-butoxy)-aluminum hydride in tetrahydrofuran (THF) gave a 5 : 3 mixture of **22** and cholest-5-ene-1β,3β-diol (**25**), from which, upon protection of the 1-hydroxy group with SEM and chromatography, 1β-SEMO-cholest-5-en-3β-ol (**26**) (22%), a mixture of **26** and the 1α-SEMO-isomer (25%, 2 : 5) and a mixture of **25** and **22** (52%, 2 : 11) were isolated. The deprotected mixture of α- and β-SEMO-isomers, and

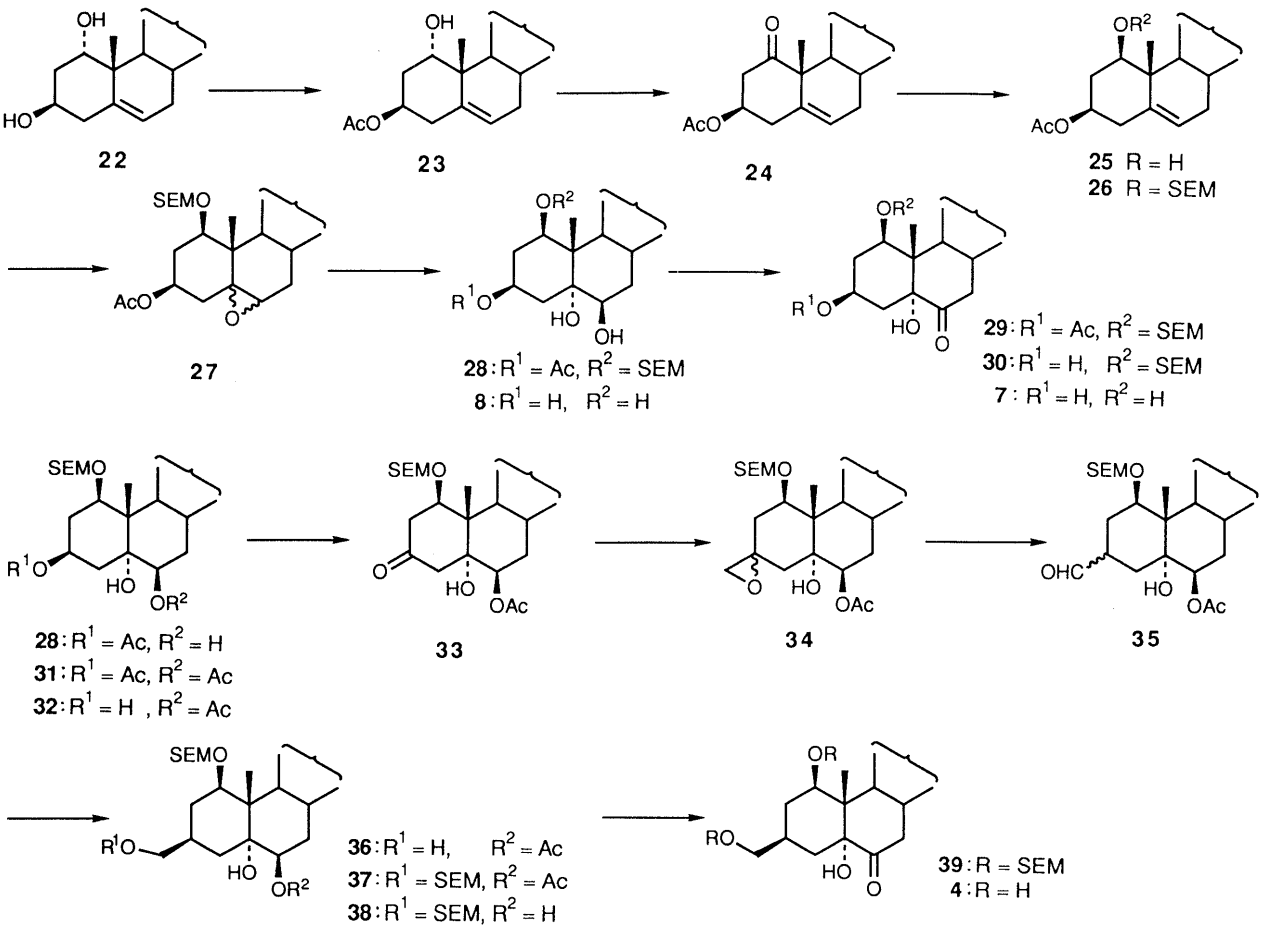

Fig. 3

the mixture of **25** and **22** were reoxidized to the starting ketone (**24**). Three recycles of the successive reactions gave **26** in 45% yield from the ketone (**24**). Compound **26** was converted to 1β-SEMO-3β-acetoxycholestane-5α,6β-diol (**28**) in 51% yield by the method described for the synthesis of **11**. Deprotection of the SEM and acetyl groups on **28** gave the natural product tetrol **8** (78%). The PCC oxidation, followed by deprotection of the SEM and acetyl groups gave **7** in an overall yield of 73% from **28**.

The synthesis of **4** from **28** was performed following the procedure described for the synthesis of **6**. After switching of the protective acetyl group from 3β-hydroxy to 6β-hydroxy, the PCC oxidation afforded 1β-SEMO-6β-acetoxycholestan-5α-ol-3-one (**33**) in 76% yield. Treatment of **33** with trimethylsulfoxonium ylide in DMSO gave a mixture of α- and β-epoxide (**34**) (1 : 5) in 66% yield, and this afforded a mixture of α- and β-aldehyde (**35**) (1 : 5) in 47% yield. The major aldehyde was identified as the 3β-formyl isomer from the proton nuclear Overhauser effect (NOE) difference spectrum of the mixture. Saturation of the 1α-proton (3.93 ppm) caused characteristic enhancement of the 3α-proton (2.86 ppm), indicating a 1,3-diaxial relationship. The mixture of the aldehydes (**35**) was reduced with sodium borohydride to give 1β-SEMO-3β-hydroxymethyl-6β-acetoxycholestan-5α-ol (**36**) in 63% yield, together with the 3α-hydroxymethyl isomer (13%). Protection of the primary alcohol on **36** with SEM followed by deprotection of the acetyl on 6β-hydroxy gave 1β-SEMO-3β-SEMOCH$_2$-cholestane-5α,6β-diol (**38**) (58%). The PCC oxidation and deprotection of the two SEM groups afforded **4** in 40% overall yield. The ^{13}C-NMR data for the oxygenated steroids **4**, **5**, **6**, **7** and **8** are listed in Table I.

TABLE I. ^{13}C-NMR Spectral Data for Compounds **4**, **5**, **6**, **7** and **8** (100 MHz, ppm, in Pyridine-d_5)[a]

Carbons	4	5	6	7	8
1	72.15	31.01	30.27	71.66	73.62
2	35.84	29.92	27.61	42.73	43.82
3	33.42	65.99	34.73	64.02	65.24
4	30.70	36.90	30.53	37.14	42.91
5	80.56	79.56	78.20	80.90	76.95
6	213.03	212.74	213.35	212.55	76.77
7	41.83	41.47	41.80	41.48	35.55
8	37.01	36.86	36.92	37.04	31.73
9	45.50	44.02	44.25	45.31	46.97
10	48.07	42.09	42.57	47.66	44.72
11	23.73	21.11	20.93	23.74	24.93
12	27.46	27.60	27.46	27.48	28.40
13	41.94	42.53	42.53	41.96	42.56
14[b]	55.81	55.61	55.63	55.81	56.85
15	24.14	23.37	24.05	24.02	24.72
16	40.10	39.37	39.40	40.05	41.28
17[b]	56.16	55.81	55.90	56.11	56.75
18	11.50	11.45	11.47	11.51	12.44
19	8.90	13.44	13.31	9.02	10.57
20	35.29	35.22	35.23	35.29	36.11
21	18.04	18.10	18.12	18.06	18.83
22	35.67	35.66	35.67	35.68	36.46
23	23.23	23.37	23.38	23.37	24.11
24	38.97	38.96	38.97	38.97	39.67
25	27.46	27.47	27.46	27.48	28.14
26	21.92	21.93	21.95	21.95	22.91
27	22.15	22.17	22.18	22.18	22.65
28	66.99		67.31		

a) All the assignments were confirmed by 2D CH shift correlation spectroscopy. b) These assignments may be interchanged.

Biological Activities of the Oxygenated Steroids The TPA-type tumor promoters induce growth inhibition, cell adhesion and monocytic differentiation of human promyelocytic leukemia cells (HL-60).[16] All of the oxygenated steroids (**4**, **5**, **6**, **7**, **8**) caused more than 90% growth inhibition of HL-60 cells at the concentration of 5 μM. Among these compounds, **4** caused partial differentiation of HL-60 to macrophage-like cells at 5 μM. Compounds **6** and **7** showed the same activity at 10 μM. HL-60 cells were killed on treatment with **5** or **8** at 10 μM.

Inhibition of [^{3}H]TPA binding was used to determine the affinity of the oxygenated steroids for the PKC regulatory domain.[17] [^{3}H]TPA binding was inhibited by 21% in the presence of **7** (1000 fold excess), taking the inhibition by cold TPA (1000 fold) as 100%. In the same binding test, indolactam-V, which is the parent structure of teleocidin, showed 28% inhibition. The other analogs did not show any significant inhibition.

On the other hand, assay of inhibition of [^{3}H]TPA binding to cytosolic-nuclear tumor promoter-specific binding protein (CN-TPBP)[18] by the oxygenated steroids gave noteworthy results. All five analogs inhibited [^{3}H]TPA binding to CN-TPBP.[19] Extents of inhibition in the presence of the analogs (1000 fold) were as follows: 16% for **4**, 100% for **5**, 52% for **6**, 75% for **7** and 41% for **8**. Compound **5** is the strongest ligand for CN-TPBP among the oxygenated steroids, and the association constant (K_a) of the binding of **5** to CN-TPBP was estimated to be $5 \times 10^8\ M^{-1}$ based on the K_a value of TPA for the binding to CN-TPBP, which had been determined to be $1.4 \times 10^{10}\ M^{-1}$.

Compound **4**, which seems to mimic the TPA structure most closely, was suggested to have only a weak differentiation activity. On the other hand, the above results indicated that compound **7** has definite, though weak, TPA-like activities in all three tests employed. Compound **5** is a potent CN-TPBP-specific ligand, but does not inhibit [^{3}H]TPA binding to PKC. We gave the name yakkasteroids to those compounds which bind to CN-TPBP competitively with TPA.[19] Compound **5** has been reported to inhibit the growth of rat hepatoma cells at 33 μM[20] and to inhibit natural killer (NK) cell cytotoxicity at 5 μM.[21] It is interesting to speculate whether the specific binding of **5**, a possible metabolite of cholesterol, to CN-TPBP has any physiological relevance under normal conditions. Our working hypothesis is that **5** is a possible endogenous ligand of the nuclear receptor of phorbols and other tumor promoters. We are currently examining the validity of this proposal.

In conclusion, several oxygenated steroids have been prepared as mimics of phorbol esters, and have been shown to possess some TPA-like biological activities. The definite activities indicate that our strategy is a useful tool for designing new active molecules. The specific binding affinity of the oxygenated steroids to CN-TPBP might provide a basis for further work to separate the diverse activities of TPA-type tumor promoters and to analyze the mechanisms of tumor promotion.

Experimental

General Remarks Melting points were obtained on a Yanagimoto micro hot stage without correction. ^{1}H-NMR spectra were recorded with a JEOL JNM-FX-400 spectrometer (400 MHz), with tetramethylsilane (TMS) as an internal standard and chemical shifts are given in ppm as δ value from TMS. ^{13}C-NMR spectra were recorded with a JEOL JNM-FX-400 spectrometer (100 MHz), with $CDCl_3$ as an internal standard (77.0 ppm). Mass spectra were recorded on a JEOL JMS-D-300 for DI-Mass. Column chromatography was performed on silica gel (Merck 7734 or 9385 (flash chromatography)).

3β-Acetoxycholestan-5α-ol-6-one (12) The preparation of **12** was performed by the method of Yates and Stiver.[12] Cholesteryl acetate (4.29 g, 10 mmol) in dichloromethane (20 ml) was treated with 85% *m*CPBA (2.14 g, 10.5 mmol, 1.05 eq) in dichloromethane (30 ml) at room temperature for 0.5 h. The mixture was washed with 10% sodium hydrogensulfite solution (2 × 20 ml), then 4% sodium hydrogencarbonate solution (2 × 30 ml). After drying, the mixture was concentrated and was recrystallized from acetone–water (9 : 1) to give a 5 : 2 mixture of α- and β-epoxide (4.01 g, 90.2%) as colorless needles. Perchloric acid (70%, 0.35 ml) was added dropwise to a solution of the epoxide (1.14 g, 2.57 mmol) in acetone (50 ml) and water (3 ml). The solution was stirred at room temperature for 2 h, and water was added to precipitate the product. The collected product was washed with water and was crystallized from acetone–water (9 : 1) to give 1.11 g (92.9%) of 3β-acetoxycholestane-5α,6β-diol (**11**). Colorless fine needles, mp 206—208 °C (from ethyl acetate), $[\alpha]_D^{20}$ −16.5° (c = 1.28, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.68 (s, 3H, 18-CH_3), 0.86, 0.87 (s, 2 × 3H, 26, 27-CH_3), 0.90 (d, 3H, J = 6.6 Hz, 21-CH_3), 1.19 (d, 3H, J = 6.6 Hz, 19-CH_3), 2.03 (s, 3H, $OCOCH_3$), 2.17 (dd, 1H, J = 12.8, 11.0 Hz, 4β-H), 3.53 (s, 1H, 6α-H), 5.16 (m, 1H, 3α-H). *Anal.* Calcd for $C_{29}H_{50}O_4$: C 75.28; H, 10.89. Found: C, 75.08; H, 10.83. A solution of the diol (**11**, 480 mg, 1.04 mmol) in dichloromethane (20 ml) was added to a vigorously stirred suspension of PCC (560 mg, 2.6 mmol, 2.5 eq) and neutral, activity I aluminum oxide (Merck 70—230 mesh) (2.0 g) in dichloromethane (methanol free, 10 ml) under Ar at 0 °C. After stirring for 4 h at room temperature, the mixture was directly charged on a silica gel column and chromatographed (*n*-hexane–ethyl acetate, 4 : 1) to give **12** (455 mg, 95.2%). Colorless fine needles, mp 234—237 °C (from ethyl acetate), $[\alpha]_D^{20}$ −38.8° (c = 0.24, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.63 (s, 3H, 18-CH_3), 0.79 (s, 3H, 19-CH_3), 0.85, 0.86 (d, 2 × 3H, J = 6.6 Hz, 26,27-CH_3), 0.89 (d, 3H, J = 6.6 Hz, 21-CH_3), 1.98 (s, 3H, $OCOCH_3$), 2.08 (dd, 1H, J = 12.8, 4.8 Hz, 7β-H), 2.75 (t, 1H, J = 12.8 Hz, 7α-H), 3.43 (s, 1H, 5α-OH), 5.02 (m, 1H, 3α-H). ^{13}C-NMR ($CDCl_3$): 11.96 (18-C), 13.83 (19-C), 18.59 (21-C), 21.33 (11-C), 21.33 ($OCO\underline{C}H_3$), 22.50 (26-C), 22.77 (27-C), 23.87 (23-C), 26.21 (15-C), 27.96 (25-C), 28.05 (12-C), 29.48 (2-C), 32.22 (1-C), 35.73 (20-C), 36.08 (22-C), 37.30 (8-C), 39.40 (4-C), 39.55 (24-C), 39.55 (16-C), 41.71 (7-C), 42.47 (10-C), 43.08 (13-C), 44.19 (9-C), 56.16 (14-C), 56.25 (17-C), 70.78 (3-C), 80.18 (5-C), 171.08 ($O\underline{C}OCH_3$), 212.62 (6-C). *Anal.* Calcd for $C_{29}H_{48}O_4$: C, 75.61; H, 10.50. Found: C, 75.49; H, 10.41.

3β,5α-Dihydroxycholestan-6-one (5) A 2 N potassium hydroxide solution (1.5 ml, 4.0 eq) was added to a solution of the acetate (**12**, 350 mg, 0.76 mmol) in methanol (50 ml) at room temperature. The mixture was stirred for 2 h at room temperature. After evaporation, the mixture was partitioned between ethyl acetate and water. The aqueous layer was extracted with ethyl acetate. Combined organic layers were washed with brine, dried and concentrated. The crude residue was chromatographed on silica gel (*n*-hexane–ethyl acetate, 1 : 2) to give 256 mg (80.5%) of **5**, colorless fine needles. mp 231—233 °C (from ethyl acetate), $[\alpha]_D^{20}$ −34.8° (c = 0.40, CH_3OH). ^{1}H-NMR ($CDCl_3$): 0.64 (s, 3H, 18-CH_3), 0.81 (s, 3H, 19-CH_3), 0.85, 0.86 (d, 2 × 3H, J = 6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J = 6.6 Hz, 21-CH_3), 2.02 (br d, 1H), 2.13 (dd, 1H, J = 12.5, 4.7 Hz, 7β-H), 2.71 (t, 1H, J = 12.5 Hz, 7α-H), 3.97 (m, 1H, 3α-H). The ^{13}C-NMR (pyridine-d_5) data are listed in Table I. *Anal.* Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.07. Found: C, 77.16; H, 11.01.

6β-Acetoxycholestane-5α,3β-diol (14) The diol acetate (**11**, 2.55 g, 5.51 mmol) in acetic anhydride (12 ml) was refluxed for 1 h. The mixture was concentrated *in vacuo* and the residue was dissolved in dichloromethane (100 ml). The solution was washed with saturated $NaHCO_3$ aqueous solution (2 × 30 ml), dried over $MgSO_4$ and concentrated to give the diacetate (**13**, 2.68 g, 96.5%). Next, 3.0 g (5.94 mmol) of **13** was dissolved in ether (120 ml), and to this solution was added a 0.8 M solution of potassium hydroxide in methanol (8.17 ml, 1.1 eq) at 0 °C. The solution was stirred for 3 h at 0 °C, then acetic acid was added (0.7 ml). The solution was washed with water, and saturated $NaHCO_3$ aqueous solution, dried and concentrated to give 2.67 g (97.1%) of **14**. mp 141—143 °C (from ethanol), $[\alpha]_D^{20}$ −22.5° (c = 1.00, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.68 (s, 3H, 18-CH_3), 0.86, 0.87 (d, 2 × 3H, J = 6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J = 6.6 Hz, 21-CH_3), 1.14 (s, 3H, 19-CH_3), 2.07 (s, 3H, $OCOCH_3$), 4.08 (m, 1H, 3α-H), 4.72 (s, 1H, 6α-H). *Anal.* Calcd for $C_{29}H_{50}O_4$: C, 75.28; H, 10.89. Found: C, 75.12; H, 10.91.

6β-Acetoxycholestan-5α-ol-3-one (15) A solution of the diol (**14**, 2.13 g, 4.6 mmol) in dichloromethane (50 ml) was added to a vigorously stirred suspension of PCC (1.49 g, 6.9 mmol, 1.5 eq) and neutral, activity I aluminum oxide (Merck 70—230 mesh) (5.0 g) in dichloromethane (methanol free, 25 ml) under Ar at 0 °C. After stirring for 6 h at room temperature, the mixture was directly charged on a silica gel column and chromatographed (*n*-hexane–ethyl acetate, 10 : 3) to give **15** (1.83 g, 86.3%). mp 162—164 °C (from *n*-hexane–ethyl acetate), $[\alpha]_D^{20}$ −13.2° (c = 1.00, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.71 (s, 3H, 18-CH_3), 0.86, 0.87 (d, 2 × 3H, J = 6.6 Hz, 26,27-CH_3), 0.91 (d, 3H, J = 6.6 Hz, 21-CH_3), 1.32 (s, 3H, 19-CH_3), 1.72 (m, 1H, 1β-H), 1.95 (m, 1H, 1α-H), 2.03 (d, 1H, J = 15.0 Hz, 4α-H), 2.09 (s, 3H, $OCOCH_3$), 2.08 (dd, 1H, J = 12.8, 4.8 Hz, 7β-H), 2.38 (m, 2H, 2α,β-H), 2.91 (d, 1H, J = 15.0 Hz, 4β-H), 4.67 (s, 1H, 6α-H). ^{13}C-NMR ($CDCl_3$): 12.08 (18-C), 15.88 (19-C), 18.62 (21-C), 21.16 (11-C), 21.42 ($OCO\underline{C}H_3$), 22.53 (26-C), 22.79 (27-C), 23.82 (23-C), 24.14 (15-C), 27.96 (25-C), 28.19 (12-C), 30.53 (8-C), 31.35 (7-C), 33.59 (1-C), 35.78 (20-C), 36.10 (22-C), 37.77 (2-C), 38.82 (10-C), 39.46 (24-C), 39.75 (16-C), 42.61 (13-C), 45.21 (9-C), 48.83 (4-C), 55.57 (14-C), 56.13 (17-C), 76.88 (6-C), 80.18 (5-C), 170.18 ($O\underline{C}OCH_3$), 211.34 (3-C). *Anal.* Calcd for $C_{29}H_{48}O_4$: C, 75.61; H, 10.50. Found: C, 75.33; H, 10.40.

6β-Acetoxy-3β-hydroxymethylcholestan-5α-ol (18) Sodium hydride (60% purity in oil, 34.6 mg, 0.864 mmol, 1.2 eq) was washed with *n*-hexane and air was evacuated from the container and replaced with argon. After addition of trimethylsulfoxonium iodide (190 mg, 0.864 mmol, 1.2 eq), DMSO was added to the mixture under Ar at room temperature. After 15 min at room temperature, a solution of the ketone (**15**, 332 mg, 0.72 mmol) in THF (1.75 ml) was added to the solution. Stirring was continued at room temperature for 0.5 h. The mixture was diluted with water and extracted with dichloromethane (2 × 30 ml). The combined organic layers were washed with water, dried and concentrated. The crude residue was chromatographed on silica gel (*n*-hexane–ethyl acetate, 4 : 1) to give the α-epoxide **16** (261 mg, 76.3%). mp 114—116 °C (from *n*-hexane–ethyl acetate), $[\alpha]_D^{20}$ −28.4° (c = 1.25, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.69 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2 × 3H, J = 6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J = 6.6 Hz, 21-CH_3), 1.16 (s, 3H, 19-CH_3), 1.70 (br d, 1H, J = 11.0 Hz, 7α-H), 1.84 (m, 1H, 1α,β-H), 2.02 (br d, 1H, J = 12.1 Hz, 2β-H), 2.05 (s, 3H, $OCOCH_3$), 2.14 (dd, 1H, J = 13.5, 4.8 Hz, 4α-H), 2.55, 2.59 (d, 2 × 2H, J = 4.4 Hz, epoxide methylene), 2.96 (s, 1H, 5α-OH), 4.73 (s, 1H, 6α-H). ^{13}C-NMR ($CDCl_3$): 12.14 (18-C), 15.76 (19-C), 18.65 (21-C), 20.81 (11-C), 21.45 ($OCO\underline{C}H_3$), 22.53 (26-C), 22.79 (27-C), 23.87 (23-C), 24.11 (15-C), 27.99 (25-C), 28.22 (12-C), 28.49 (2-C), 30.59 (8-C), 31.11 (7-C), 31.23 (1-C), 35.84 (20-C), 36.16 (22-C), 38.00 (4-C), 39.05 (10-C), 39.49 (24-C), 39.90 (16-C), 42.67 (13-C), 44.98 (9-C), 50.82 (28-C, epoxide methylene), 55.72 (14-C), 56.16 (17-C), 58.00 (3-C), 74.90 (5-C), 76.68 (6-C), 170.09 ($O\underline{C}OCH_3$). The epoxide (**16**, 416 mg, 0.88 mmol) was dissolved in 1% boron trifluoride etherate in THF (4.0 ml, BF_3, 0.44 mmol, 0.5 eq) at 0 °C under Ar. The solution was allowed to stand for 15 min at room temperature. Quenching was done by the addition of saturated $NaHCO_3$ aqueous solution and the mixture was diluted with water and dichloromethane. After separation, the aqueous layer was extracted with dichloromethane (2 × 20 ml). The combined organic layers were washed with brine, dried and concentrated. The crude residue was chromatographed on silica gel (*n*-hexane–ethyl acetate 7 : 2) to give a mixture of α- and β- aldehydes (**17**, 194 mg, 46.6%). To a solution of the aldehydes (194 mg, 0.409 mmol) in THF (5 ml) was added sodium borohydride (31.0 mg, 0.82 mmol, 2.0 eq) followed by ethanol (2 ml) at 0 °C with stirring. Stirring was continued for 30 min at 0 °C, then the reaction was quenched by the addition of 5% aqueous acetic acid. The mixture was diluted with water (10 ml) and ethyl acetate (30 ml). After separation, the aqueous layer was extracted with ethyl acetate (2 × 10 ml). The combined organic layers were washed with brine, dried and concentrated. The crude residue was chromatographed on silica gel (*n*-hexane–ethyl acetate, 5 : 4 then 1 : 1) to give the 3α-hydroxymethyl isomer (26.2 mg, 13.4%, less polar isomer) and the 3β-hydroxymethyl isomer (**18**, 136.5 mg, 70.1%, more polar isomer). mp 128—131 °C (from *n*-hexane–ethyl acetate), $[\alpha]_D^{20}$ −30.7 °C (c = 1.05, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2 × 3H, J = 6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J = 6.6 Hz, 21-CH_3), 1.09 (s, 3H, 19-CH_3), 1.27

(br d, 1H, J=12.7 Hz, 7β-H), 1.67 (br d, 1H, J=12.7 Hz, 7α-H), 1.81 (m, 1H), 2.00 (m, 2H, 1α-, 3α-H), 2.06 (s, 3H, $OCOCH_3$), 3.49 (ABX, 2H, $C\underline{H}_2OH$), 4.69 (s, 1H, 6α-H). ^{13}C-NMR ($CDCl_3$): 12.14 (18-C), 16.23 (19-C), 18.62 (21-C), 20.84 (11-C), 21.51 ($OCO\underline{C}H_3$), 22.50 (26-C), 22.76 (27-C), 23.87 (23-C), 24.05 (15-C), 27.96 (25-C), 28.16 (12-C), 28.16 (C-1), 30.73 (8-C), 31.70 (7-C), 32.60 (2-C), 34.41 (4-C), 34.73 (3-C), 35.78 (20-C), 36.13 (22-C), 38.85 (10-C), 39.46 (24-C), 39,90 (16-C), 42.67 (13-C), 45.36 (9-C), 55.81 (14-C), 56.16 (17-C), 68.24 ($C\underline{H}_2OH$), 73.56 (5-C), 76.59 (6-C), 170.56 ($O\underline{C}OCH_3$). *Anal.* Calcd for $C_{30}H_{52}O_4$: C, 75.58; H, 10.99. Found: C, 75.33; H, 10.88.

3β-SEMOCH$_2$-6β-acetoxycholestan-5α-ol (19) A solution of trimethylsilylethoxymethyl chloride (SEMCl, 99 mg, 0.6 mmol, 2.6 eq) in dry dichloromethane (0.5 ml) was added to a solution of **18** (108 mg, 0.227 mmol) in 1.3 ml of dry dichloromethane containing diisopropylethylamine (146.4 mg, 1.13 mmol, 5.0 eq) at room temperature. The mixture was allowed to stand at room temperature for 1 h. The reaction was quenched by the addition of saturated $NaHCO_3$ aqueous solution. The mixture was diluted with water (20 ml) and dichloromethane (20 ml). After separation, the aqueous layer was extracted with dichloromethane (2 × 20 ml). The combined organic layers were dried and concentrated. The crude residue was chromatographed on silica gel (*n*-hexane–ethyl acetate, 6 : 1) to give **19** (128.4 mg, 93.4%), amorphous gum. $[\alpha]_D^{20}$ −24.3° (c=0.92, $CHCl_3$). MS m/z: 606 (M^+). ^{1}H-NMR ($CDCl_3$): 0.02 (s, 9H, $(CH_3)_3Si$), 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2 × 3H, J=6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J=6.6 Hz, 21-CH_3), 0.95 (t, 2H, J=8.4 Hz, CH_2Si), 1.09 (s, 3H, 19-CH_3), 1.27 (br d, 1H, J=12.7 Hz, 7β-H), 1.58 (br d, 1H, J=12.7 Hz, 7α-H), 1.66 (t, 1H, J=11.4 Hz, 4β-H), 1.80 (m, 1H, 12α-H), 2.00 (br d, 1H, J=11.0 Hz), 2.07 (s, 3H, $OCOCH_3$), 3.38 (m, 2H, $C\underline{H}_2OH$), 3.60 (t, 2H, J=8.4 Hz, $SiCH_2C\underline{H}_2O$–), 4.46 (s, 2H, –OCH_2O–), 4.66 (s, 1H, 6α-H).

3β-SEMOCH$_2$-cholestane-5α,6β-diol (20) A 1.5 M solution of DIBAH in toluene (0.49 ml, 0.735 mmol, 3.5 eq) was added to a solution of the acetate (**19**, 128.4 mg, 0.212 mmol) in THF (4 ml) at −40 °C under Ar with stirring. Stirring was continued for 2 h at −40 °C to 0 °C. Quenching was done by the addition of methanol (1 ml) followed by 0.5 N potassium and sodium tartrate (10 ml), and the mixture was diluted with ethyl acetate (20 ml). After separation, the aqueous layer was extracted with ethyl acetate (20 ml). Combined organic layers were washed with brine, dried and concentrated. The crude residue was chromatographed on silica gel (*n*-hexane–ethyl acetate, 3 : 1) to give **20** (108 mg, 90.4%). mp 90—91 °C (from *n*-hexane–ethyl acetate), $[\alpha]_D^{20}$ −28.2° (c=1.30, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.02 (s, 9H, $Si(CH_3)_3$), 0.67 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2 × 3H, J=6.6 Hz, 26,27-CH_3), 0.89 (d, 3H, J=6.6 Hz, 21-CH_3), 0.94 (t, 2H, J=7.0 Hz, $SiCH_2$), 1.13 (s, 3H, 19-CH_3), 1.32 (m, 1H, 4β-H), 1.45 (m, 2H, 7α,β-H), 1.82 (m, 1H, 12α-H), 1.92 (t, 1H, J=11.4 Hz, 4β-H), 1.98 (br d, 1H, J=12.0 Hz, 11β-H), 2.09 (m, 1H, 3α-H), 3.41 (m, 2H, $C\underline{H}_2OH$), 3.51 (br s, 1H, 6α-H), 3.61 (t, 2H, J=7.0 Hz, $SiCH_2C\underline{H}_2O$–), 4.65 (s, 2H, –OCH_2O–). ^{13}C-NMR ($CDCl_3$): −1.41 ($(CH_3)_3Si$), 12.14 (18-C), 16.69 (19-C), 18.09 ($SiCH_2$), 18.65 (21-C), 20.90 (11-C), 22.53 (26-C), 22.79 (27-C), 23.87 (23-C), 24.10 (15-C), 24.43 (2-C), 27.99 (25-C), 28.19 (12-C), 30.24 (8-C), 32.63 (3-C), 32.92 (1-C), 34.82 (7-C), 35.11 (4-C), 35.78 (20-C), 36.16 (22-C), 38.76 (10-C), 39.49 (24-C), 39.96 (16-C), 42.73 (13-C), 45.97 (9-C), 55.98 (17-C), 56.25 (14-C), 64.92 ($SiCH_2\underline{C}H_2O$–), 73.44 ($C\underline{H}_2OH$), 74.31 (5-C), 76.33 (6-C), 95.01 (–OCH_2O–). *Anal.* Calcd for $C_{34}H_{64}O_7Si$: C, 66.62; H, 10.52. Found: C, 66.39; H, 10.41.

3β-SEMOCH$_2$-cholestan-5α-ol-6-one (21) The diol **20** (95 mg, 0.168 mmol) was converted into 80.6 mg (85.1%) of **21** by a method similar to that used for the preparation of **12** with PCC (109 mg, 0.51 mmol, 3 eq) and aluminum oxide (350 mg). Amorphous gum, $[\alpha]_D^{20}$ −27.5° (c=1.00, $CHCl_3$). MS m/z: 562 (M^+). ^{1}H-NMR ($CDCl_3$): 0.02 (s, 9H, $Si(CH_3)_3$), 0.63 (s, 3H, 18-CH_3), 0.75 (s, 3H, 19-CH_3), 0.85, 0.86 (d, 2 × 3H, J=6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J=6.6 Hz, 21-CH_3), 0.93 (t, 2H, J=7.0 Hz, $SiCH_2$), 1.70 (m, 1H, 8-H), 1.95 (m, 1H, 3α-H), 2.12 (dd, 1H, J=13.2, 4.7 Hz, 7β-H), 2.74 (t, 1H, J=13.2 Hz, 7α-H), 3.40 (m, 2H, $C\underline{H}_2OH$), 3.59 (t, 2H, J=7.0 Hz, $SiCH_2C\underline{H}_2O$–), 4.64 (s, 2H, –OCH_2O–). ^{13}C-NMR ($CDCl_3$): −1.41 ($(CH_3)_3Si$), 12.02 (18-C), 13.92 (19-C), 18.09 ($SiCH_2$), 18.61 (21-C), 21.19 (11-C), 22.53 (26-C), 22.79 (27-C), 23.90 (23-C), 23.90 (2-C), 24.14 (15-C), 27.99 (25-C), 28.05 (12-C), 30.21 (1-C), 30.68 (4-C), 32.34 (3-C), 35.73 (20-C), 36.10 (22-C), 37.39 (8-C), 39.46 (24-C), 39.64 (16-C), 42.15 (7-C), 42.96 (10-C), 43.08 (13-C), 44.83 (9-C), 56.16 (17-C), 56.42 (14-C), 65.00 ($SiCH_2\underline{C}H_2O$–), 73.18 ($C\underline{H}_2OH$), 79.36 (5-C), 95.01 (–OCH_2O–), 212.88 (C-6).

3β-Hydroxymethylcholestan-5α-ol-6-one (6) Perchloric acid (0.2 ml) was added to a solution of **21** (40 mg, 0.071 mmol) and tetra-*n*-butylammonium fluoride (92.7 mg, 0.355 mmol, 5.0 eq) in THF (1 ml) at 0 °C with stirring. The mixture was allowed to stand for 1 h at room temperature. The reaction was quenched by the addition of saturated $NaHCO_3$ (4 ml), and the mixture was extracted with ethyl acetate (3 × 7 ml). The combined organic layers were washed with brine, dried and concentrated. The crude residue was chromatographed on silica gel (*n*-hexane–ethyl acetate, 2 : 3) to give **6** (21.2 mg, 69.0%). mp 196—197 °C (from *n*-hexane–ethyl acetate), $[\alpha]_D^{20}$ −34.8° (c=0.58, CH_3OH). ^{1}H-NMR ($CDCl_3$): 0.64 (s, 3H, 18-CH_3), 0.76 (s, 3H, 19-CH_3), 0.86, 0.87 (d, 2 × 3H, J=6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J=6.6 Hz, 21-CH_3), 0.99 (t, 1H, J=9.5 Hz, 22-H), 1.71 (m, 1H, 8-H), 1.85 (m, 1H), 2.02 (m, 1H), 2.13 (dd, 1H, J=13.2, 4.7 Hz, 7β-H), 2.75 (t, 1H, J=13.2 Hz, 7α-H), 3.51 (t, 2H, J=5.6 Hz, $C\underline{H}_2OH$). ^{1}H-NMR (pyridine-d_5): 0.62 (s, 3H, 18-CH_3), 0.86 (s, 3H, 19-CH_3), 0.88 (d, 2 × 3H, J=6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J=6.6 Hz, 21-CH_3), 1.75 (m, 1H, 8-H), 1.86 (m, 1H), 1.98 (d, 1H, J=6.6 Hz), 2.06 (t, 1H, J=13.1 Hz), 2.13 (dd, 1H, J=13.0, 3.8 Hz), 2.26 (m, 1H, 7β-H), 2.52 (m, 1H, 3α-H), 3.18 (t, 1H, J=13.1 Hz, 7α-H), 3.80 (m, 2H, $C\underline{H}_2OH$). The ^{13}C-NMR (pyridine-d_5) data are listed in Table I. *Anal.* Calcd for $C_{28}H_{48}O_3$; C, 77.73; H, 11.18. Found: C, 77.38; H, 11.20.

3β-Acetoxycholest-5-en-1α-ol (23) Acetic anhydride (5.43 g, 53.28 mmol, 3.0 eq) was added to a solution of the diol (**22**)[16] (7.15 g, 17.76 mmol) in pyridine (50 ml) at room temperature. After 2 h, further acetic anhydride (5.43 g, 53.28 mmol, 3.0 eq) was added to the solution and the mixture was allowed to stand for 3 h. After addition of water (10 ml), the mixture was concentrated *in vacuo*. The residue was partitioned between ethyl acetate (300 ml) and water (20 ml). After drying, the organic layer was concentrated. The resulting residue was chromatographed on silica gel (*n*-hexane–ethyl acetate, 9 : 2 then 2 : 5) to give the diacetate (0.61 g, 7.1%), 3β-acetoxy-(**23**, 5.21 g, 66.0%) and starting material (**22**, 1.20 g, 16.8%). The diacetate was hydrolyzed to **22**. The recovered **22** was acetylated as described above. The 3β-acetylation of **22** yielded **23** (6.45 g, 81.6% in total). mp 165—167 °C (lit[16] mp 166—168 °C), $[\alpha]_D^{20}$ −44.7° (c=1.28, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.67 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2 × 3H, J=6.6 Hz, 26,27-CH_3), 0.91 (d, 3H, J=6.6 Hz, 21-CH_3), 1.04 (s, 3H, 19-CH_3), 1.83 (dt, 1H, J=13.5, 2.0 Hz, 2β-H), 2.00 (m, 2H, 7α, β-H), 2.03 (s, 3H, $OCOCH_3$), 2.12 (m, 1H, 2α-H), 2.34 (br t, 1H, J=13.5 Hz, 4β-H), 2.46 (br dd, 1H, J=13.5, 2.2 Hz, 4α-H), 3.86 (br s, 1H, 1β-H), 5.03 (sept, 1H, J=5.1 Hz, 3α-H), 5.61 (d, 1H, J=5.2 Hz, 6H). *Anal.* Calcd for $C_{29}H_{48}O_3$: C, 78.33; H, 10.88. Found: C, 78.06; H, 10.91.

3β-Acetoxycholest-5-en-1-one (24) The alcohol **23** (5.78 g, 13.0 mmol) was converted into 5.58 g (97.0%) of **24** by a method similar to that used for the preparation of **12** with PCC (7.01 g, 32.5 mmol, 2.5 eq) and aluminum oxide (27.1 g). mp 144—147 °C (lit[16] mp 146—148 °C), $[\alpha]_D^{20}$ −12.8° (c=1.00, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.67 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2 × 3H, J=6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J=6.6 Hz, 21-CH_3), 1.25 (s, 3H, 19-CH_3), 1.98 (m, 2H, 7α,β-H), 2.03 (s, 3H, $OCOCH_3$), 2.46 (br t, 1H, J=13.1 Hz, 4β-H), 2.62 (br dd, 1H, 4α-H), 2.63 (dd, 1H, J=13.1, 12.5 Hz, 2β-H), 2.72 (dd, 1H, J=13.1, 5.9 Hz, 2α-H). 4.92 (m, 1H, 3α-H), 5.65 (d, 1H, J=5.1 Hz, 6H). ^{13}C-NMR ($CDCl_3$): 11.93 (18-C), 18.44 ($OCOCH_3$), 18.62 (21-C), 21.16 (19-C), 22.39 (11-C), 22.53 (26-C), 22.79 (27-C), 23.79 (23-C), 24.22 (15-C), 27.96 (25-C), 28.11 (12-C), 31.17 (7-C), 31.76 (8-C), 35.75 (20-C), 36.10 (22-C), 37.13 (2-C), 39.46 (24-C), 39.46 (16-C), 42.29 (13-C), 42.70 (9-C), 43.84 (4-C), 52.77 (10-C), 56.07 (17-C), 56.53 (14-C), 70.05 (3-C), 79.36 (5-C), 126.60 (C-6), 134.53 (5-C), 170.09 ($OCOCH_3$), 210.40 (1-C), *Anal.* Calcd for $C_{29}H_{46}O_3$: C, 78.68; H, 10.47. Found: C, 78.34; H, 10.33.

1β-SEMO-3β-acetoxycholest-5-ene (26) A solution of the ketone **24** (5.40 g, 12.2 mmol) in THF (100 ml) was treated with 1.0 N lithium tri(*tert*-butoxy)aluminum hydride in THF (36.6 ml, 36.6 mmol, 3.0 eq) at −40 °C with stirring under Ar. Stirring was continued for 2 h at −40 °C to 0 °C. Quenching was done by the addition of 5% aqueous acetic acid (250 ml). The mixture was extracted with ethyl acetate (3 × 100 ml). The organic layers were washed with brine, dried and concentrated to give the crude 1-alcohol, which was treated with SEMCl (10.1 g, 61.0 mmol, 5.0 eq) in dichloromethane (100 ml) in the presence of diisopropylethylamine (15.8 g, 122 mmol, 10.0 eq) for 1.5 h at room temperature and worked up in the usual way. The resulting mixture of 1α- and 1β-SEMO isomers and 1α- and 1β-hydroxy isomers was chromatographed on silica gel. *n*-Hexane–ethyl acetate (12 : 1) eluted 1β-SEMO-3β-acetoxycholest-5-ene (**26**) first (1.55 g, 22.1%). The next fraction, eluted with *n*-hexane–ethyl acetate (12 : 1), contained a 5 : 2 mixture (1.74 g, 24.8%)

of 1α- and 1β-SEMO (**26**) isomers (estimated from ^{1}H-NMR data). The final *n*-hexane–ethyl acetate (9 : 2) eluate gave a mixture (11 : 2) of 1α- and 1β-hydroxy (**25**) isomers (2.80 g, 51.6%). The mixture of 1α- and 1β-SEMO- isomers was deprotected to give the 1α- and 1β-hydroxy isomers. The combined 1α- and 1β-hydroxy isomers were reoxidized with PCC to give **24**. Three recycles of the procedure afforded 3.16 g (45.1%) of **26** from **24** in total. Amorphous gum, $[\alpha]_D^{20}$ −10.3° (c=1.50, $CHCl_3$). MS m/z: 574 (M^+). ^{1}H-NMR ($CDCl_3$): 0.02 (s, 9H, $Si(CH_3)_3$), 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.89 (d, 3H, J=6.6 Hz, 21-CH_3), 0.95 (m, 2H, $SiCH_2$–), 1.06 (s, 3H, 19-CH_3), 1.65 (br q, 1H, J=12.1 Hz, 2β-H), 1.80 (m, 1H), 1.96 (m, 2H, 7α, β-H), 2.03 (s, 3H, $OCOCH_3$), 2.18 (m, 1H, 2α-H), 2.31 (m, 1H, 4α,β-H), 3.31 (dd, 1H, J=11.7, 4.4 Hz, 1α-H), 3.51 (m, 2H, $SiCH_2C\underline{H}_2O$–), 4.60 (m, 1H, 3α-H), 4.63, 4.74 (d, 2×2H, J=7.0 Hz, –OCH_2O–), 5.60 (d, 1H, J=5.1 Hz, 6-H). ^{13}C-NMR ($CDCl_3$): −1.35 ($Si(CH_3)_3$), 12.05 (18-C), 14.10 ($OCOCH_3$), 18.18 ($SiCH_2$-), 18.62 (21-C), 21.31 (19-C), 22.53 (26-C), 22.79 (27-C), 23.79 (23-C), 23.96 (11-C), 24.40 (15-C), 28.02 (25-C), 28.08 (12-C), 31.38 (7-C), 33.21 (8-C), 33.65 (2-C), 35.78 (20-C), 36.16 (22-C), 38.32 (24-C), 39.52 (16-C), 40.54 (4-C), 42.00 (10-C), 42.50 (13-C), 50.41 (9-C), 56.36 (17-C), 56.86 (14-C), 65.76 ($SiCH_2C\underline{H}_2O$–), 70.32 (3-C), 83.19 (1-C), 93.81 (OCH_2O–), 126.42 (C-6), 136.69 (5-C), 170.29 ($OCOCH_3$).

1β-SEMO-3β-acetoxycholestane-5α,6β-diol (28) Compound **26** (3.20 g, 5.56 mmol) was converted to a 3 : 2 mixture of α- and β-epoxide (**27**, 2.85 g, 86.7%) by the same method as that used for the preparation of **11**. The epoxides (2.84 g, 4.8 mmol) were hydrolyzed with perchloric acid (1.03 ml, 12.0 mmol, 2.5 eq) in acetone (90 ml) at room temperature for 2 h to give 1.70 g (58.0%) of **28**. Amorphous gum, $[\alpha]_D^{20}$ +7.23° (c=1.15, $CHCl_3$). MS m/z: 608 (M^+). ^{1}H-NMR ($CDCl_3$); 0.02 (s, 9H, $Si(CH_3)_3$), 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.89 (d, 3H, J=6.6 Hz, 21-CH_3), 0.95 (m, 2H, $SiCH_2$–), 1.20 (s, 3H, 19-CH_3), 1.5—1.7 (m, 2H, 7α,β-H), 1.70 (m, 2H, 2α,4α-H), 1.80 (m, 1H, 12α-H), 1.90 (m, 1H, 1β-H), 1.97 (dt, 1H, 16β-H), 2.02 (s, 3H, $OCOCH_3$), 2.11 (t, 1H, J=11.8 Hz, 4β-H), 3.50 (br s, 1H, 6α-H), 3.52, 3.64 (m, 2H, $SiCH_2C\underline{H}_2O$–), 4.64, 4.71 (d, 2×2H, J=6.6 Hz, –OCH_2O–), 5.13 (m, 1H, 3α-H). ^{13}C-NMR ($CDCl_3$): −1.44 ($Si(CH_3)_3$), 10.83 (19-C), 12.14 (18-C), 18.09 ($SiCH_2$–), 18.56 (21-C), 21.33 ($OCOCH_3$), 22.53 (26-C), 22.79 (27-C), 23.46 (11-C), 23.96 (23-C), 24.46 (15-C), 27.99 (25-C), 27.99 (12-C), 30.78 (8-C), 34.00 (2-C), 34.29 (7-C), 35.84 (20-C), 36.16 (22-C), 37.16 (C-4), 39.49 (24-C), 40.63 (16-C), 42.18 (10-C), 43.81 (13-C), 46.26 (9-C), 55.98 (17-C), 56.54 (14-C), 65.73 ($SiCH_2C\underline{H}_2O$–), 68.68 (3-C), 76.27 (6-C), 76.77 (5-C), 80.39 (1-C), 94.75 (OCH_2O–), 170.88 ($OCOCH_3$).

Cholestane-1β,3β,5α,6β-tetrol (8) Deprotection of the SEM by the same procedure as used for the preparation of **6** followed by the usual base-catalyzed hydrolysis of the acetate gave **8** (77.2 mg, 78.1%) from **28** (137.8 mg, 0.226 mmol). **8**: Colorless needles, mp 261—263 °C (from ethanol–*n*-hexane), $[\alpha]_D^{20}$ −5.6° (c=1.02, CH_3OH). ^{1}H-NMR ($CDCl_3$): 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J=6.6 Hz, 21-CH_3), 1.16 (s, 3H, 19-CH_3), 1.50 (m, 2H, 2β,7β-H), 1.60 (m, 2H, 4α,2α-H), 2.00 (m, 2H, 8-, 12α-H), 2.11 (t, 1H, J=12.5 Hz, 4β-H), 2.15 (m, 1H, 2α-H), 3.52 (br d, 1H, J=3.7 Hz, 6α-H), 4.02 (m, 1H, 1α-H), 4.14 (m, 1H, 3α-H). The ^{13}C-NMR (pyridine-d_5) data are listed in Table I. *Anal.* Calcd for $C_{27}H_{48}O_4$: C, 74.26; H, 11.08. Found: C, 74.02; H, 10.91.

1β-SEMO-3β-acetoxycholestan-5α-ol-6-one (29) The diol **28** (44.7 mg, 0.0734 mmol) was converted into 43.0 mg (96.5%) of **29** by the same method as that used for the preparation of **12** with PCC (40 mg, 0.184 mmol, 2.5 eq) and aluminum oxide (150 mg). **29**: Amorphous gum, $[\alpha]_D^{20}$ −18.5° (c=0.42, $CHCl_3$). MS m/z: 606 (M^+). ^{1}H-NMR ($CDCl_3$): 0.01 (s, 9H, $Si(CH_3)_3$), 0.63 (s, 3H, 18-CH_3), 0.82 (s, 3H, 19-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.89 (d, 3H, J=6.6 Hz, 21-CH_3), 0.95 (m, 2H, $SiCH_2$–), 1.57 (t, 1H, J=11.4 Hz, 2α-H), 1.65 (m, 1H, 8-H), 1.80 (t, 1H, J=12.1 Hz, 4β-H), 2.03 (s, 3H, $OCOCH_3$), 2.08 (dd, 1H, J=12.8, 4.4 Hz, 7β-H), 2.22 (dt, 1H), 2.33 (m, 1H, 2β-H), 2.78 (t, 1H, J=12.8 Hz, 7α-H), 3.30 (s, 1H, 5α-OH), 3.52, 3.64 (m, 2H, $SiCH_2C\underline{H}_2O$–), 4.01 (dd, 1H, J=11.4, 5.1 Hz, 1α-H), 4.63, 4.73 (d, 2×2H, J=6.6 Hz, –OCH_2O–), 5.01 (m, 1H, 3α-H). ^{13}C-NMR ($CDCl_3$): −1.46 ($Si(CH_3)_3$), 9.75 (19-C), 11.94 (18-C), 18.06 ($SiCH_2$–), 18.53 (21-C), 21.25 ($OCOCH_3$), 22.53 (26-C), 22.76 (27-C), 23.47 (11-C), 23.96 (23-C), 24.22 (15-C), 27.87 (12-C), 27.99 (25-C), 32.40 (2-C), 33.74 (4-C), 35.78 (20-C), 36.10 (22-C), 37.36 (8-C), 39.43 (24-C), 40.25 (16-C), 41.56 (7-C), 42.38 (10-C), 45.18 (9-C), 47.61 (13-C), 56.42 (14-C), 56.51 (17-C), 66.00 ($SiCH_2C\underline{H}_2O$–), 68.19 (3-C), 79.13 (1-C), 81.03 (5-C), 94.75 (OCH_2O–), 170.91 ($OCOCH_3$), 211.71 (6-C).

1β,3β,5α-Trihydroxycholestan-6-one (7) Deprotection of the SEM group of **29** by the same method as that used for the preparation of **6** followed by the usual base-catalyzed hydrolysis of the acetate gave **7** (77.2 mg, 78.1%) from **29** (137.8 mg, 0.226 mmol). **7**: Colorless needles, mp 250—253 °C (from ethanol–*n*-hexane), $[\alpha]_D^{20}$ −40.9° (c=0.24, CH_3OH). ^{1}H-NMR ($CDCl_3$): 0.66 (s, 3H, 18-CH_3), 0.80 (s, 3H, 19-CH_3), 0.87 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.91 (d, 3H, J=6.6 Hz, 21-CH_3), 1.69 (dq, 1H, J=10.6, 4.4 Hz, 8-H), 1.79 (t, 1H, J=12.0 Hz, 4β-H), 1.88 (br d, 1H, 2α-H), 2.05 (m, 2H), 2.15 (m, 2H, 2β,7β-H), 2.69 (t, 1H, J=12.8 Hz, 7α-H), 4.03 (m, 1H, 3α-H), 4.16 (m, 1H, 1α-H). The ^{13}C-NMR (pyridine-d_5) data are listed in Table I. *Anal.* Calcd for $C_{27}H_{46}O_4$: C, 74.61; H, 10.67. Found: C, 74.44; H, 10.60.

1β-SEMO-6β-acetoxycholestane-5α,3β-diol (32) The diol **28** (1.60 g, 2.63 mmol) was converted into 1.28 g (80.0%) of **32** by the same method as that used for the preparation of **14**. Amorphous gum, $[\alpha]_D^{20}$ −7.1° (c=0.90, $CHCl_3$). MS m/z: 608 (M^+), ^{1}H-NMR ($CDCl_3$): 0.02 (s, 9H, $Si(CH_3)_3$), 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.89 (d, 3H, J=6.6 Hz, 21-CH_3), 0.95 (m, 2H, $SiCH_2$–), 1.16 (s, 3H, 19-CH_3), 1.45 (d, 1H, J=4.5 Hz, 2α-H), 1.60 (m, 1H, 7-H), 1.80 (m, 1H, 12α-H), 1.89 (t, 1H, J=11.3 Hz, 4β-H), 1.97 (m, 1H, 11β-H), 2.08 (s, 3H, $OCOCH_3$), 2.32 (m, 1H, 2β-H), 3.52, 3.64 (m, 2H, $SiCH_2C\underline{H}_2O$–), 3.84 (dd, 1H, J=11.3, 4.7 Hz, 1α-H), 4.09 (m, 1H, 3α-H), 4.66 (s, 1H, 6α-H), 4.65, 4.72 (d, 2×2H, J=6.6 Hz, –OCH_2O–). ^{13}C-NMR ($CDCl_3$): −1.44 ($Si(CH_3)_3$), 10.59 (19-C), 12.17 (18-C), 18.15 ($SiCH_2$–), 18.53 (21-C), 21.42 ($OCOCH_3$), 22.53 (26-C), 22.79 (27-C), 23.47 (11-C), 23.84 (23-C), 24.40 (15-C), 27.99 (12-C), 27.99 (25-C), 31.08 (7-C), 31.40 (8-C), 35.81 (20-C), 36.13 (22-C), 38.00 (2-C), 39.49 (24-C), 40.63 (16-C), 42.18 (10-C), 43.87 (13-C), 46.18 (9-C), 55.96 (17-C), 56.39 (14-C), 64.68 (3-C), 65.79 ($SiCH_2C\underline{H}_2O$–), 75.83 (6-C), 77.20 (5-C), 80.33 (1-C), 94.72 (OCH_2O–), 170.47 ($OCOCH_3$).

1β-SEMO-6β-acetoxycholestan-5α-ol-3-one (33) The diol **32** (1.25 g, 2.05 mmol) was converted into 1.16 g (92.7%) of **33** by the same method as that used for the preparation of **15** with PCC (1.32 g, 6.16 mmol, 3.0 eq) and aluminum oxide (3.7 g). Amorphous gum, $[\alpha]_D^{20}$ −18.0° (c=1.20, $CHCl_3$). MS m/z: 606 (M^+). ^{1}H-NMR ($CDCl_3$): 0.01 (s, 9H, $Si(CH_3)_3$), 0.70 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.89 (d, 3H, J=6.6 Hz, 21-CH_3), 0.95 (m, 2H, $SiCH_2$–), 1.29 (s, 3H, 19-CH_3), 1.62 (m, 2H, 7-H), 1.81 (m, 1H, 12α-H), 1.88 (m, 1H, 11β-H), 2.00 (m, 1H, 16β-H), 2.00 (d, 1H, J=11.0 Hz, 4β-H), 2.08 (s, 3H, $OCOCH_3$), 2.40 (dd, 1H, J=15.4, 9.2 Hz, 2β-H), 2.90 (dd, 1H, J=15.4, 4.1 Hz, 2α-H), 2.94 (d, 1H, J=15.2 Hz, 4α-H), 3.50, 3.64 (m, 2H, $SiCH_2C\underline{H}_2O$–), 4.07 (dd, 1H, J=9.2, 5.2 Hz, 1α-H), 4.67 (m, 1H, 6α-H), 4.63, 4.69 (d, 2×2H, J=6.6 Hz, –OCH_2O–). ^{13}C-NMR ($CDCl_3$): −1.44 ($Si(CH_3)_3$), 10.45 (19-C), 12.11 (18-C), 18.12 ($SiCH_2$–), 18.56 (21-C), 21.39 ($OCOCH_3$), 22.53 (26-C), 22.79 (27-C), 23.29 (11-C), 23.90 (23-C), 24.46 (15-C), 27.99 (12-C), 27.99 (25-C), 31.00 (7-C), 31.23 (8-C), 35.84 (20-C), 36.13 (22-C), 39.49 (24-C), 40.45 (16-C), 42.09 (10-C), 44.04 (13-C), 46.03 (2-C), 46.32 (9-C), 55.84 (17-C), 56.42 (14-C), 66.00 ($SiCH_2C\underline{H}_2O$–), 76.48 (6-C), 77.20 (5-C), 81.58 (1-C), 94.96 (OCH_2O–), 170.06 ($OCOCH_3$), 207.51 (3-C).

1β-SEMO-6β-acetoxy-3β-hydroxymethylcholestan-5α-ol (36) The 3-ketone **33** (1.0 g, 1.65 mmol) was converted into the α-epoxide **34** (671 mg, 65.6%) by a method similar to that used for the preparation of **16** with sodium hydride (98 mg, 2.45 mmol, 1.5 eq) and trimethysulfoxonium iodide (540 mg, 2.45 mmol, 1.5 eq). **34**: $[\alpha]_D^{20}$ +2.4° (c=0.29, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.01 (s, 9H, $Si(CH_3)_3$), 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J=6.6 Hz, 21-CH_3), 0.95 (m, 2H, $SiCH_2$–), 1.15 (s, 3H, 19-CH_3), 1.60 (m, 2H, 7-α, β-H), 1.80 (m, 1H, 12α-H), 1.88 (m, 1H, 11β-H), 1.97 (m, 1H, 16β-H), 2.07 (s, 3H, $OCOCH_3$), 2.12 (dd, 1H, J=15.0, 11.5 Hz, 2β-H), 2.57, 2.59 (d, 2×2H, J=5.9 Hz, epoxide methylene), 3.49, 3.64 (m, 2×1H, –$SiCH_2C\underline{H}_2O$–), 3.74 (m, 1H), 4.05 (dd, 1H, J=9.2, 5.9 Hz, 1α-H), 4.68 (s, 1H, 6α-H), 4.65, 4.69 (d, 2×1H, –OCH_2O–), ^{13}C-NMR ($CDCl_3$): −1.44 ($Si(CH_3)_3$), 10.13 (19-C), 12.14 (18-C), 18.12 ($SiCH_2$–), 18.53 (21-C), 21.45 ($OCO\underline{C}H_3$), 22.53 (26-C), 22.79 (27-C), 23.49 (11-C), 23.87 (23-C), 24.46 (15-C), 27.99 (25-C), 27.99 (12-C), 30.91 (7-C), 31.26 (8-C), 35.87 (20-C), 36.08 (2-C), 36.13 (22-C), 38.03 (4-C), 39.49 (24-C), 40.60 (16-C), 42.12 (10-C), 44.39 (13-C), 45.97 (9-C), 49.74 (28-C, epoxide methylene), 55.84 (17-C), 56.39 (14-C), 56.71 (3-C), 65.73 (–$SiCH_2\underline{C}$-H_2O–), 76.18 (5-C), 76.80 (6-C), 80.27 (1-C), 95.04 (–OCH_2O–), 170.09 ($O\underline{C}OCH_3$). The epoxide (325 mg, 0.525 mmol) was converted into a 1 : 5 mixture (152.5 mg, 46.9%) of 3α- and 3β-aldehydes by the method described for the preparation of **17**. β-Aldehyde **17**: ^{1}H-NMR ($CDCl_3$):

0.01 (s, 9H, $Si(CH_3)_3$), 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.90 (d, 3H, J=6.6 Hz, 21-CH_3), 0.95 (m, 2H, $SiCH_2$–), 1.10 (s, 3H, 19-CH_3), 1.95 (t, 1H, J=13.0 Hz, 4β-H), 2.07 (s, 3H, $OCOCH_3$), 2.86 (m, 1H, 3α-H), 3.51, 3.67 (m, 2×1H, –$SiCH_2C\underline{H}_2O$–), 3.93 (dd, 1H, J=9.1, 5.9 Hz, 1α-H), 4.68 (s, 1H, 6α-H), 4.72 (d, 2×1H, J=6.6 Hz, –OCH_2O–), 9.62 (s, 1H, –CHO). The major isomer was characterized as the 3β-aldehyde (**35**) by examination of the proton NOE difference spectrum of the mixture of the aldehydes. Saturation of the 1α-proton (3.93 ppm) caused characteristic enhancement of the 3α-proton (2.86 ppm). The mixture (152.2 mg, 0.245 mmol) was reduced with sodium borohydride by the same method as that used for the preparation of **18** to give **36** (96.5 mg, 63.2%) and the 3α-isomer (20.2 mg, 13.2%). Amorphous gum, $[\alpha]_D^{20}$ −12.7° (c=1.44, $CHCl_3$). MS m/z 622 (M^+). ^{1}H-NMR ($CDCl_3$): 0.01 (s, 9H, $Si(CH_3)_3$), 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.89 (d, 3H, J=6.6 Hz, 21-CH_3), 0.94 (m, 2H, $SiCH_2$–), 1.11 (s, 3H, 19-CH_3), 1.55 (m, 2H, 7-H), 1.80 (m, 1H, 12α-H), 2.07 (s, 3H, $OCOCH_3$), 3.49 (m, 3H, $C\underline{H}_2OH$ and $SiCH_2C\underline{H}_2O$–), 3.66 (m, 1H, $SiCH_2C\underline{H}_2O$–), 3.88 (dd, 1H, J=11.0, 4.8 Hz, 1α-H), 4.66 (s, 1H, 6α-H), 4.72 (d, 2×2H, J=6.6 Hz, –OCH_2O–). ^{13}C-NMR ($CDCl_3$): −1.43 ($Si(CH_3)_3$), 10.48 (19-C), 12.21 (18-C), 18.15 ($SiCH_2$–), 18.55 (21-C), 21.52 ($OCOCH_3$), 22.53 (26-C), 22.80 (27-C), 23.61 (11-C), 23.87 (23-C), 24.40 (15-C), 27.99 (12-C), 27.99 (25-C), 31.11 (2-C), 31.40 (7-C), 31.40 (8-C), 32.81 (3-C), 34.58 (4-C), 35.84 (20-C), 36.15 (22-C), 39.49 (24-C), 40.70 (16-C), 42.18 (10-C), 44.35 (13-C), 46.47 (9-C), 56.04 (17-C), 56.43 (14-C), 65.69 ($SiCH_2C\underline{H}_2O$–), 67.78 (CH_2OH), 77.09 (5-C), 81.18 (1-C), 94.58 (OCH_2O–), 170.38 ($OCOCH_3$).

1β-SEMO-3β-SEMOCH$_2$-cholestan-5α,6β-diol (38) The diol **36** (108.3 mg, 0.174 mmol) was converted into 104.4 mg (84.5%) of **38** by the same method as that used for the preparation of **20**. **38**: Amorphous gum, $[\alpha]_D^{20}$ −18.0° (c=0.92, $CHCl_3$). MS m/z: 710 (M^+). ^{1}H-NMR ($CDCl_3$): 0.01 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$), 0.68 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.88 (d, 3H, J=6.6 Hz, 21-CH_3), 0.95 (m, 4H, $SiCH_2$–), 1.14 (s, 3H, 19-CH_3), 1.55 (m, 2H, 7-H), 1.80 (m, 1H, 12α-H), 1.95 (m, 3H, 2, 11, 16-H), 1.98 (t, 1H, 13.5 Hz, 4β-H), 2.16 (m, 1H, 3α-H), 3.37 (dd, 1H, J=9.5, 6.8 Hz, CH_2OH), 3.44 (dd, 1H, J=9.5, 5.5 Hz, CH_2OH), 3.45 (s, 1H, 6α-H), 3.52 (m, 1H, $SiCH_2C\underline{H}_2O$–), 3.60 (dd, 2H, J=9.1, 7.7 Hz, $SiCH_2C\underline{H}_2O$–), 3.64 (m, 1H, $SiCH_2C\underline{H}_2O$–), 3.84 (dd, 1H, J=11.5, 4.8 Hz, 1α-H), 4.62 (d, 1H, J=6.6 Hz, –OCH_2O–), 4.65 (s, 2H, –OCH_2O–), 4.66 (s, 1H, 6α-H), 4.70 (d, 1H, J=6.6 Hz, –OCH_2O–). ^{13}C-NMR ($CDCl_3$): −1.41 ($Si(CH_3)_3$), 10.91 (19-C), 12.20 (18-C), 18.09 ($SiCH_2$–), 18.56 (21-C), 22.53 (26-C), 22.80 (27-C), 23.67 (11-C), 23.87 (23-C), 24.46 (15-C), 27.99 (12-C), 27.99 (25-C), 30.68 (3-C), 30.85 (8-C), 31.49 (2-C), 34.53 (7-C), 34.49 (4-C), 35.84 (20-C), 36.13 (22-C), 39.49 (24-C), 40.75 (16-C), 42.21 (10-C), 44.22 (13-C), 46.88 (9-C), 56.13 (17-C), 56.51 (14-C), 65.00 ($SiCH_2C\underline{H}_2O$–), 65.65 ($SiCH_2C\underline{H}_2O$–), 72.94 (CH_2OH), 76.07 (5-C), 77.06 (6-C), 81.26 (1-C), 94.49 (OCH_2O–), 95.04 (OCH_2O–).

1β-SEMO-3β-SEMOCH$_2$-cholestan-5α-ol-6-one (39) The diol **38** (88 mg, 0.124 mmol) was converted into 79.4 mg (90.5%) of **39** by a method similar to that used for the preparation of **12** with PCC (80 mg, 0.37 mmol, 3 eq) and aluminum oxide (260 mg). **39**: Colorless needles mp 132—132.5 °C (from n-hexane–ethyl acetate), $[\alpha]_D^{20}$ −5.1° (c=0.90, $CHCl_3$). ^{1}H-NMR ($CDCl_3$): 0.01 (s, 9H, $Si(CH_3)_3$), 0.02 (s, 9H, $Si(CH_3)_3$), 0.63 (s, 3H, 18-CH_3), 0.78 (s, 3H, 19-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.88 (d, 3H, J=6.6 Hz, 21-CH_3), 0.92 (m, 4H, $SiCH_2$–), 1.62 (m, 2H) 1.80 (m, 1H, 12α-H), 2.00 (m, 3H, 3, 16, 2α-H), 2.10 (dd, 1H, J=13.1, 4.8 Hz, 7β-H), 2.18 (dt, 1H, J=11.3, 3.6 Hz, 9-H), 2.78 (t, 1H, J=13.1 Hz, 7α-H), 2.16 (m, 1H, 3α-H), 3.36 (t, 1H, J=9.6 Hz, CH_2OH), 3.42 (dt, 1H, J=9.6, 6.8 Hz, CH_2OH), 3.52 (m, 1H, $SiCH_2C\underline{H}_2O$–), 3.60 (dt, 2H, J=9.1, 7.7 Hz, $SiCH_2C\underline{H}_2O$–), 3.63 (m, 1H, $SiCH_2C\underline{H}_2O$–), 3.94 (dd, 1H, J=11.5, 4.8 Hz, 1α-H), 4.64 (s, 2H, –OCH_2O–), 4.66 (d, 1H, J=6.6 Hz, –OCH_2O–), 4.72 (d, 1H, J=6.6 Hz, –OCH_2O–). ^{13}C-NMR ($CDCl_3$): −1.41 ($Si(CH_3)_3$), −1.34 ($Si(CH_3)_3$), 9.77 (19-C), 11.96 (18-C), 18.09 ($SiCH_2$–), 18.53 (21-C), 22.53 (26-C), 22.79 (27-C), 23.64 (11-C), 23.90 (23-C), 24.22 (15-C), 27.87 (12-C), 27.99 (25-C), 30.41 (3-C), 30.76 (4-C), 31.40 (2-C), 35.75 (20-C), 36.10 (22-C), 37.30 (8-C), 39.46 (24-C), 40.34 (16-C), 41.94 (7-C), 42.38 (13-C), 45.65 (9-C), 47.90 (10-C), 56.36 (17-C), 56.60 (14-C), 65.06 ($SiCH_2C\underline{H}_2O$–), 65.88 ($SiCH_2C\underline{H}_2O$–), 72.71 (CH_2OH), 79.95 (1-C), 81.15 (5-C), 94.57 (OCH_2O–), 95.04 (OCH_2O–), 212.15 (6-C). *Anal.* Calcd for $C_{40}H_{78}O_6Si_2$, C: 67.55, H: 11.05. Found, C: 67.18, H: 10.99.

1β,5α-Dihydroxy-3β-hydroxymethylcholestan-6-one (4) Compound **39** (35 mg, 0.049 mmol) was converted into **4** by the same procedure as that used for the preparation of **6**. mp 260—270 °C (dec.), $[\alpha]_D^{20}$ −31.9° (c=0.15, CH_3OH). ^{1}H-NMR (pyridine-d_5): 0.67 (s, 3H, 18-CH_3), 0.85, 0.86 (d, 2×3H, J=6.6 Hz, 26,27-CH_3), 0.91 (d, 3H, J=6.6 Hz, 21-CH_3), 1.18 (s, 3H, 19-CH_3), 1.48 (m, 2H, 11, 25-H), 1.75 (m, 2H, 15α,12β-H), 1.88 (m, 2H, 8,2β-H), 2.06 (br d, 1H, J=12.5 Hz), 2.15 (t, 1H, J=13.9 Hz, 4β-H), 2.32 (m, 2H, 4α, 7β-H), 2.40 (m, 1H, 2α-H), 2.70 (m, 1H, 3α-H), 2.85 (m, 2H, 9-, 15β-H), 3.27 (t, 1H, J=12.4 Hz, 7α-H), 3.85 (d, 2H, J=6.0 Hz, CH_2OH), 4.83 (dd, 1H, J=11.0, 4.8 Hz, 1α-H). The ^{13}C-NMR (pyridine-d_5) data are listed in Table I. *Anal.* Calcd for $C_{28}H_{48}O_4$: C, 74.95; H, 10.78: Found: C, 74.81; H, 10.82.

Acknowledgments We thank Dr. Fujiki (Saitama Cancer Center Research Institute) for a critical reading of the manuscript. This work was supported in part by Grants-in-Aid for General Research and for Cancer Research from the Ministry of Education, Science and Culture, Japan and by a grant from the Uehara Memorial Life Science Foundation.

References and Notes

1) H. Fujiki, M. Suganuma, *Envir. Carcino. Revs. (J. Envir. Sci. Hlth.)*, **C7**, 1 (1989) and references cited therein.
2) E. Hecker, W. Adolf, M. Hergenhahn, R. Schmidt, B. Sorg, "Cellular Interaction by Environmental Tumor Promoters," ed. by H. Fujiki, E. Hecker, R. E. Moore, T. Sugimura, B. Weinstein, Japan Scientific Societies' Press, Tokyo/VNU Science Press, Utrecht, 1984, p. 3; H. Fujiki, M. Mori, M. Nakayasu, M. Terada, T. Sugimura, R. E. Moore, *Proc. Natl. Acad. Sci. U.S.A.*, **78**, 3872 (1981); H. Fujiki, M. Suganuma, T. Tahira, A. Yoshioka, M. Nakayasu, Y. Endo, K. Shudo, T. Sugimura, "Cellular Interaction by Environmental Tumor Promoters," ed. by H. Fujiki, E. Hecker, R. E. Moore, T. Sugimura, B. Weinstein, Japan Scientific Societies' Press, Tokyo/VNU Science Press, Utrecht, 1984, p. 37.
3) E. Hecker, "Carcinogenesis: A Comprehensive Survey, Mechanisms of Tumor Promotion and Cocarcinogenesis," Vol. 2, ed. by T. J. Slaga, A. Sivak, R. K. Boutwell, Raven, New York, 1978, p. 11.
4) M. Castagna, Y. Takai, K. Kaibuchi, K. Sano, U. Kikkawa, Y. Nishizuka, *J. Biol. Chem.*, **257**, 7847 (1982); J. H. Exton, *ibid.*, **265**, 1 (1990).
5) Y. Hashimoto, K. Shudo, *Biochem. Biophys. Res. Commun.*, **166**, 1132 (1990).
6) A. M. Jeffery, R. M. J. Liskamp, *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 241 (1986); P. A. Wender, K. F. Koehler, N. A. Sharkey, M. L. Dell'aquila, P. M. Blumberg, *ibid.*, **83**, 4214 (1986).
7) A. Itai, Y. Kato, N. Tomioka, Y. Iitaka, Y. Endo, M. Hasegawa, K. Shudo, H. Fujiki, S. Sakai, *Proc. Natl. Acad. Sci. U.S.A.*, **85**, 3688 (1988).
8) Y. Endo, M. Hasegawa, A. Itai, K. Shudo, M. Tori. Y. Asakawa, S. Sakai, *Tetrahedron Lett.*, **26**, 1069 (1985); Y. Endo, K. Shudo, A. Itai, M. Hasegawa, S. Sakai, *Tetrahedron*, **42**, 5905 (1986).
9) Y. Endo, Y. Sato, K. Shudo, *Tetrahedron*, **43**, 2241 (1987).
10) Commercially available (LC Service Corp., Woburn, Mass, U.S.A.).
11) M. Kobayashi, T. Hayashi, K. Hayashi, M. Tanabe, T. Nakagawa, H. Mitsuhashi, *Chem. Pharm. Bull.*, **31**, 1848 (1983).
12) P. Yates, S. Stiver, *Can. J. Chem.*, **65**, 2203 (1987).
13) E. J. Corey, M. Chaykovsky, *J. Am. Chem. Soc.*, **87**, 1353 (1965).
14) A. Furst, L. Labler, W. Meier, *Helv. Chim. Acta*, **64**, 1870 (1981).
15) M. Lj. Mihailovic, Lj. Lorenc, V. Pavlovic, J. Kalvoda, *Tetrahedron*, **33**, 441 (1977).
16) H. Fujiki, M. Mori, M. Nakayasu, M. Terada, T. Sugimura, *Biochem. Biophys. Res. Commun.*, **90**, 976 (1979).
17) A. Jeng, N. Sharkey, P. Blumberg, *Cancer Res.*, **46**, 1966 (1986).
18) Y. Hashimoto, K. Shudo, *Jpn. J. Cancer Res.*, **82**, 665 (1991).
19) CN-TPBP binding of the oxygenated steroids and a structure–activity study have been reported: Y. Endo, Y. Hashimoto, H. Fukasawa, K. Shudo, *Biochem. Biophys. Res. Commun.*, **193**, 1529 (1993).
20) J. Valisolalao, B. Luu, G. Ourisson, *Tetrahedron*, **39**, 2779 (1983).
21) O. Kucuk, J. Stoner-Picking, S. Yachnin, L. I. Gordon, R. M. Willasms, L. J. Lis, M. P. Westerman, *Cellular Immunol.*, **139**, 541 (1992).