A Biomimetic Approach to the Synthesis of an Antiviral Marine Steroidal Orthoester

José-Luis Giner* and Juan A. Faraldos

Department of Chemistry, SUNY-ESF, Syracuse New York 13210

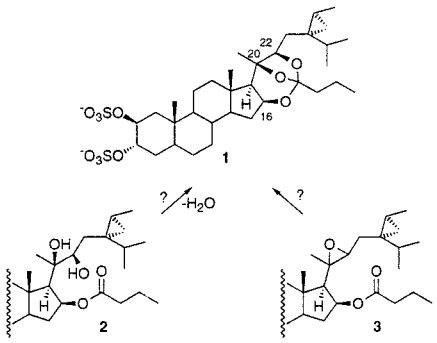
jlginer@syr.edu

Received November 29, 2001

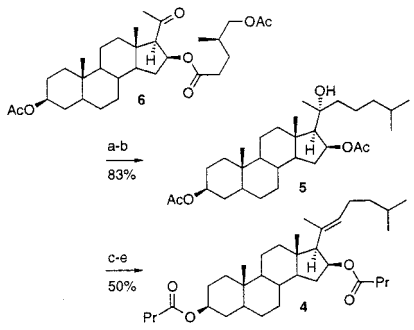
Abstract: Orthoesterol B, a marine natural product exhibiting antiviral activities, contains a [3.2.1]-bicyclic orthobutyrate bridging the steroid side chain and ring D. A biosynthetic reaction was proposed by which rearrangement of an epoxy ester results in the formation of the orthoester moiety. Steroidal model compounds incorporating 16-butyroxy and 20,22-epoxy groups were synthesized from tigogenin and were shown to rearrange to orthoesters under mild acidic catalysis.

The synthesis of complex natural products is carried out with great efficiency, under mild conditions by the organisms that produce them. Consideration of the ways nature builds molecules can be instructive to organic chemists in the design of new laboratory syntheses, as is best exemplified by the biomimetic synthesis of tropinone by Robert Robinson,[1] and by W. S. Johnson's use of the cationic polyene cyclization in the synthesis of steroids.[2,3] We describe herein a biomimetic approach toward the synthesis of orthoesterol B (**1**), a natural orthoester from the sponge *Petrosia weinbergi* that exhibits activity against the feline leukemia virus, mouse influenza virus, and mouse coronavirus.[4]

Although our interest in orthoesterol B was sparked by the biosynthetically interesting cyclopropyl steroid side chain,[5] the origins of the orthoester presented an intriguing puzzle for biogenetic analysis. Biosynthetically, formation of the orthoester by dehydrative cyclization of an ester diol (**2**) is a possibility with precedent in the proposed origin of resiniferatoxin from proresiniferatoxin.[6] However, the oxygen atoms found at carbons 20 and 22 could also originate from an epoxide (**3**), in which case no loss or addition of atoms would be necessary. Epoxides have been implicated in many biosynthetic tranformations, including the polyolefin cyclization of 2,3-epoxysqualene that initiates steroid biosynthesis.[7] Furthermore, steroids bearing epoxy substitution in the proximal part of the side chain, such as the antitumor aragusterol A, are known from sponges.[8] On the basis of these considerations, a model compound was designed and constructed to test whether the orthoester moiety could be formed in vitro from the rearrangement of an epoxy ester.



Scheme 1[a]



[a] (a) $(CH_3)_2CHCH_2CH_2CH_2MgBr$; (b) Ac_2O/pyr; (c)$POCl_3$/pyr; (d) $LiAlH_4$; (e) $(PrCO)_2O$/pyr.

The synthesis of olefinic ester **4** was readily accomplished via the intermediacy of steroidal alcohol **5** from tigone diacetate (**6**) which was prepared by Marker degradation (Scheme 1).[9] Peracid epoxidation of **4** led to a of mixture of two epoxy esters. While measuring the NMR spectrum, one of the two epoxides (**7**) was found to rearrange cleanly to an orthoester due to traces of acid in the $CDCl_3$. Observation of an NOE between positions 16 and 23 indicated that the orthoester had the 20*R*,22S configuration (**8**), the C-22 epimer of the natural configuration. On the basis of a mechanism involving 6-*exo* cyclization with inversion at the proximal center of epoxide ring (**9**, Scheme 2), the reactive epoxide (**7**) was considered to have the 20*S*,22*S* configuration. Intramolecular quenching of dioxycarbenium ion **10** by the newly generated hydroxyl group yields the bicyclic

(1) (a) Robinson, R. *J. Chem. Soc.* **1917**, *111*, 762−768. (b) Robinson, R. *J. Chem. Soc.* **1917**, *111*, 876−899.

(2) (a) Gravestock, M. B.; Johnson, W. S.; McCarry, B. E.; Parry, R. J.; Ratcliffe, B. E. *J. Am. Chem. Soc.* **1978**, *100*, 4274−4282. (b) Johnson, W. S.; Plummer, M. S.; Reddy, S. P.; Bartlett, W. R. *J. Am. Chem. Soc* **1993**, *115*, 515−521.

(3) For more recent examples of biomimetic syntheses, see: (a) Wallace, G. A.; Heathcock, C. H. *J. Org. Chem.* **2001**, *66*, 450−454. (b) Heathcock, C. H. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 14323−14327. (c) Baldwin, J. E.; Claridge, T. D. W.; Culshaw, A. J.; Heupel, F. A.; Lee, V.; Spring, D. R.; Whitehead, R. C. *Chem. Eur. J.* **1999**, *5*, 3153−3161. (d) Baldwin, J. E.; Claridge, T. D. W.; Culshaw, A. J.; Heupel, F. A.; Lee, V.; Spring, D. R.; Whitehead, R. C.; Boughtflower, R. J.; Mutton, I. M.; Upton, R. J. *Angew. Chem., Int. Ed.* **1998**, *37*, 2661−2663. (e) Rainier, J. D.; Smith, A. B. *Tetrahedron Lett.* **2000**, *41*, 9419−9423.

(4) Koehn, F. E.; Gunasekera, M.; Cross, S. S. *J. Org. Chem.* **1991**, *56*, 1322−1325.

(5) (a) Giner, J.-L.; Gunasekera, S. P.; Pomponi, S. A. *Steroids* **1999**, *64*, 820−824. (b) Giner, J. L. *Chem. Rev.* **1993**, *93*, 1735−1752. (c) Giner, J. L.; Djerassi, C. *J. Am. Chem. Soc.* **1991**, *113*, 1386-1393. (d) Giner, J. L.; Zimmerman, M. P.; Djerassi, C. *J. Org. Chem.* **1988**, *53*, 5895−5902.

(6) Hergenhahn, M.; Adolf, W.; Hecker, E. *Tetrahedron Lett.* **1975**, 1595−1598.

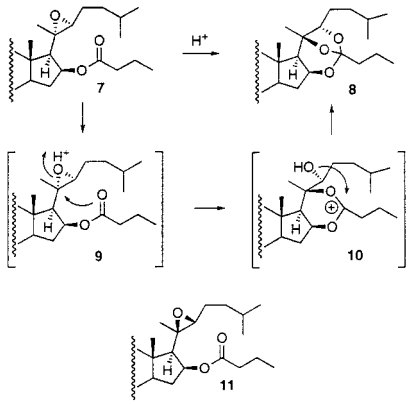
(7) (a) Corey, E. J.; Russey, W. E.; Ortiz de Montellano, P. R. *J. Am. Chem. Soc.* **1966**, *88*, 4750−4751. (b) van Tamelen, E. E.; Willet, J. D.; Clayton, R. B.; Lord, K. E. *J. Am. Chem. Soc.* **1966**, *88*, 4752−4754.

(8) Iguchi, K.; Fujita, M.; Nakaoka, H.; Mitome, H.; Yamada, Y. *Tetrahedron Lett.* **1993**, *34*, 6277−6280.

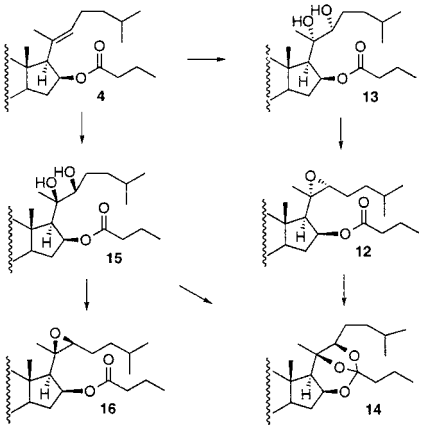
(9) (a) Marker, R. E.; Turner, D. L.; Wagner, R. B.; Ulshafer, P. R.; Crooks, H. M., Jr.; Wittle, E. L. *J. Am. Chem. Soc.* **1941**, *63*, 774−777. (b) Marker, R. E.; Wagner, R. B.; Ulshafer, P. R.; Wittbecker, E. L.; Goldsmith, D. P. J.; Ruof, C. H. *J. Am. Chem. Soc.* **1947**, *69*, 2167−2230.

10.1021/jo011111z CCC: $22.00
Published on Web 03/14/2002

Scheme 2



Scheme 3

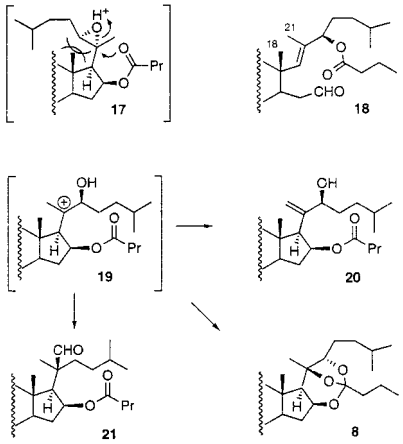


orthoester. The isomeric epoxide **11** did not react and remained unchanged under similar conditions for months.

Encouraged by the efficient conversion of epoxy ester **7** to orthoester **8**, we set out to syntheize the 20*S*,22*R*-epoxide (**12**) required to obtain the natural 20*R*,22*R* configuration of the orthoester. Osmylation of olefin **4** proceeded predominantly (75%) from the less hindered face to give the 20*S*,22*S*-diol **13** (Scheme 3), which was treated with mesyl chloride followed by potassium carbonate. The epoxide (**12**) thus obtained rearranged smoothly to an orthoester that was shown by measurement of an NOE between H-16 and H-22 to have the natural configuration (**14**). The ^{1}H and ^{13}C NMR data of the relevant portions of **14** were in good agreement with those reported for orthoesterol B (**1**).[4]

The minor diol (**15**) was cyclized in the same way to diasteromeric epoxide **16**. Although the two 20*S*-epoxy esters **7** and **12** cyclized to orthoesters under relatively mild acid catalysis, the two 20*R*-epoxy esters **11** and **16** were much less reactive. NMR measurements of the rates of reaction using TFA in purified $CDCl_3$ showed that the disappearance of the epoxy esters followed pseudo-first-order kinetics. Whereas in 1.3 mM TFA the rearrangement of the 20*S*-epoxy esters **7** and **12** proceeded with $t_{1/2}$'s of 12.4 and 11.4 min, respectively, the 20*R*-epoxy esters **11** and **16** exhibited $t_{1/2}$'s of 13.6 min in 13 mM and 26 mM TFA, respectively. In addition to exhibiting much slower rates of isomerization, epoxy esters **11** and **16** did not yield the expected orthoesters. This is believed to be a consequence of steric interactions between the 18-methyl and the side chain. Instead of reacting with backside attack of the protonated epoxide (**17**, Scheme 4), the 20*R*,22*R*-epoxy ester **11** rearranged to a ring D

Scheme 4



secosteroid aldehyde (**18**), the structure of which was shown to have the 17(20)*E*-configuration from NOE between the 18- and 21-methyl groups. The rearrangement of the 20*R*,22*S*-epoxy ester (**16**) apparently proceeds via the intermediacy of a tertiary carbenium ion centered at C-20 (**19**). The products of this reaction include the allylic alcohol (**20**, 40%), an aldehyde (**21**, 20%) arising from 1,2-alkyl migration, as well as the 20*R*,22*S*-orthoester (**8**, 40%). In this unusual case, orthoester formation proceeds with retention of configuration rather than inversion at C-20.

To model the alternative biosynthetic reaction whereby an ester diol (**2**) leads to the orthoester (**1**), the two ester diols obtained from osmylation (**13** and **15**) were treated with acid. The 20*R*,22*R*-diol (**15**) analogous to **2** cyclized in the presence of 1.3 mM TFA in $CDCl_3$ to the 20*R*,-22*R*-orthoester (Scheme 3, **14**) with a $t_{1/2}$ of 45 min. However, even after several hours in 83 mM TFA, the 20*S*,-22*S*-diol (**13**) showed no reaction other than partial transesterification of the 16-butyrate to the 22-position. Formation of orthoester **14** from both diols **13** and **15** by different reactions (Scheme 3) confirms that epoxy ester−orthoester rearrangement occurs with inversion at C-20.

These experiments support our biogenetic theory of epoxy ester−orthoester rearrangement in the biosynthesis of orthoesterol B. When steric hindrance does not interfere with the generation of a productive conformation, the epoxy ester−orthoester rearrangement proceeds efficiently under mild acid catalysis. Evidence for a biosynthetic role for this reaction is provided by the case of the petuniasterone orthoesters, which were isolated together with their epoxy ester precursors from *Petunia* species.[10] Nevertheless, the alternative formation of the orthoester from an ester diol, while less efficient under our conditions, cannot be conclusively ruled out without biosynthetic experiments with isotopic labeling of the oxygens.

Experimental Section

General Methods. NMR spectra were acquired using Bruker Avance-300 and Bruker Avance-600 instruments using $CDCl_3$ as the solvent unless specified otherwise. LRMS (EI) data were obtained using a Hewlett-Packard 5989B mass spectrometer; HRMS (EI) and all CI data were obtained using a MicroMass 70-VSE mass spectrometer; LRFAB data were obtained using a MicroMass ZAB−SE mass spectrometer; and HRFAB data were

(10) Elliger, C. A.; Benson, M.; Haddon, W. F.; Lundin, R. E.; Waiss, A. C., Jr.; Wong, R. Y. *J. Chem. Soc., Perkin Trans. 1* **1988**, 711−717.

obtained using a MicroMass 70-SE−4F mass spectrometer. TLC was performed on aluminum backed plates coated with a 0.25 mm layer of silica gel 60 F254.

The acid catalyzed rearrangements were carried out in NMR tubes using various concentrations of TFA in 0.8 mL of dry $CDCl_3$. The reactions were followed at 30 °C by ^{1}H NMR (300 MHz) spectroscopy. The rate of disappearance of substrate over time was measured by integration of isolated NMR signals. All reactions were stopped by evaporation with a stream of N_2. Products were isolated by preparative TLC.

Tigone Diacetate (6). This compound was obtained in 93% yield though Marker degradation of tigogenin acetate (1.89 g). ^{1}H NMR (300 MHz) 5.35 (m, 1H, C-16), 4.63 (m, 1H, C-3), 3.85 (d, J = 6.0 Hz, 2H, C-26), 2.43 (m, 1H, C-15), 2.30 (m, 4H, C-15, C-17 and C-23), 2.01 (s, 3H, C-21), 2.00 (s, 3H, AcO), 1.97 (s, 3H, AcO), 0.98 (s, 3H, C-18), 0.88 (d, J = 6.6 Hz, 3H, C-27), 0.79 (s, 3H, C-19). ^{13}C NMR (75 MHz) 205.31, 172.83, 170.94, 170.50. 74.28, 73.43, 68.62, 66.58, 54.19, 53.83, 44.56, 42.45, 38.06, 36.60, 35.48, 34.93, 34.30, 33.86, 31.93, 31.82, 31.68, 30.58, 28.27, 28.19, 27.32, 21.31, 20.78, 20.41, 16.32, 13.53, 12.11.

(20*R*)-3*β*,16*β*-Diacetoxycholestan-20-ol (5). 1-Bromo-4-methylpentane (4 mL, 28.2 mmol) was slowly added (ca. 1 h) with stirring to 852 mg of Mg (35.5 mmol) in 15 mL of dry Et_2O under N_2, at room temperature. After stirring for 1 h, a solution of **6** (850 mg, 1.6 mmol) in 10 mL of dry Et_2O was added slowly. After 15 min, the mixture was poured into water and extracted with EtOAc. Drying over Na_2SO_4 and evaporation under reduced pressure gave a white solid that was immediately saponified (K_2CO_3/MeOH) at room-temperature overnight. Evaporation of MeOH under reduced pressure gave a residue that was taken up with Et_2O, filtered through silica gel, and evaporated under reduced pressure. Acetylation (Ac_2O/pyr) at room temperature overnight and evaporation under reduced pressure gave an oily residue that was purified by silica gel chromatography using a gradient of 2.5−5% EtOAc/hexane yielding 667 mg product (83%). The assignment of (20*R*)-configuration is based on the production of the (*E*)-$\Delta^{17(20)}$ olefin during anti-elimination (see below). TLC R_f = 0.43 (hexane/EtOAc 4:1); ^{1}H NMR (300 MHz) 5.33 (m, 1H, C-16), 4.66 (m, 1H, C-3), 2.96 (br s, 1H, OH), 2.43 (m, 1H, C-15), 2.08 (s, 3H, AcO), 2.02 (s, 3H, AcO), 1.27 (s, 3H, C-21), 1.11 (s, 3H, C-18), 0.87 (d, J = 6.6 Hz, 6H, C-26 and C-27), 0.83 (s, 3H, C-19); ^{13}C NMR (151 MHz) 170.7, 169.5, 78.0, 77.8, 75.8, 73.7, 60.0, 54.4, 54.2, 44.7, 44.5, 43.6, 40.3, 39.7, 36.7, 35.5, 35.0, 34.3, 34.0, 31.7, 28.5, 27.9, 27.5, 26.5, 22.6, 22.4, 21.7, 21.5, 21.0, 14.7, 12.2; MS (EI) m/z 487 ($M^+ - H_2O + 1$, 30), 427 (13), 419 (73), 367 (3), 359 (100), 341 (64), 316 (56), 299 (95); MS (FAB) m/z 527 (90, M^+ + Na), 487 (9), 427 (14), 329 (26), 301 (24), 177-(100). HRMS (FAB) m/z 527.3711 (calcd for $C_{31}H_{52}O_5$+Na, 527.3712).

(*E*)-3*β*,16*β*-Dibutyryloxycholest-20(22)-ene (4). To a solution of **5** (508 mg, 1.01 mmol) in dry pyridine (10 mL) was slowly added $POCl_3$ (0.75 mL, 8.04 mmol). After 24 h at 45 °C, the reaction was extracted with Et_2O and water, and the organic layer was dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give 481 mg (98%) of three isomeric olefins: (*E*)-$\Delta^{20(22)}$ (55%), Δ^{20} (25%), and (*E*)-$\Delta^{17(20)}$ (20%). This mixture was fractionated by preparative TLC using hexane/EtOAc 39:1. For characterization of these olefins, see Supporting Information.

A stirred solution of (*E*)-3*β*, 16*β*-diacetoxycholest-20(22)-ene (189.9 mg, 0.39 mmol) in 10 mL of dry Et_2O was treated with $LiAlH_4$ (150 mg, 2.73 mmol) at room temperature under N_2. After 10 min, water was carefully added, and the solution was extracted with EtOAc. After drying and evaporating, the resulting oily residue was esterified using *n*-butyric anhydride (1.5 mL, 9.2 mmol) in 5 mL of pyridine at reflux for 3 h. Evaporation under reduced pressure gave 193.3 mg (92%) of **4**: TLC R_f = 0.52 (hexane/EtOAc 9:1); ^{1}H NMR (300 MHz) 5.31 (m, 2H, C-16 and C-22), 4.69 (m, 1H, C-3), 2.35 (dt, J = 13.5 and 7.5 Hz, 1H, C-15), 2.24 (t, J = 7.5 Hz, 2H, 16-butyrate CH_2CO), 2.22 (t, J = 7.5 Hz, 2H, 3-butyrate CH_2CO), 1.71−1.56 (m, 4H, 3 and 16-butyrate CH_3CH_2), 1.63 (s, 3H, C-21), 0.95 (s, 3H, C-18), 0.95 (t, J = 7.5 Hz, 3H, 16-butyrate CH_3CH_2), 0.94 (t, J = 7.5 Hz, 3H, 3-butyrate CH_3CH_2), 0.89 (d, J = 6.6 Hz, 6H, C-26 and C-27), 0.85 (s, 3H, C-19), 0.69 (m, 1H, C-9); ^{13}C NMR (75 MHz) 173.1, 173.0, 130.6, 128.5, 75.4, 73.2, 63.0, 54.5, 54.4, 44.6, 42.9, 38.9, 38.4, 36.7, 36.6, 36.5, 35.5, 34.7, 34.7, 33.9, 31.7, 28.4, 27.6, 27.4, 26.0, 22.5, 22.4, 20.6, 18.5, 18.3, 18.0, 14.8, 13.6, 13.5, 12.1; MS (FAB) m/z (relative intensity) 542 (7, M^+), 541 (18, $M^+ - 1$), 455 (46), 367 (53), 343 (23), 295 (25), 255 (25), 215 (34), 145 (52), 133 (52), 121(57), 119 (67), 109 (65), 107 (100), 104 (88); HRMS (FAB) m/z 541.4258 (calcd for $C_{35}H_{58}O_4 - 1$, 541.4256).

(20*S*,22*S*)-3*β*,16*β*-Dibutyryloxy-20,22-epoxycholestane (7) and (20*R*,22*R*)-3*β*,16*β*-Dibutyryloxy-20,22-epoxycholestane (11). Steroidal olefin **4** (43 mg, 0.08 mmol) was treated with 55 mg of mCPBA (70−75%, ca. 0.23 mmol) in 2 mL of CH_2Cl_2. After 1 h, the mixture was partitioned between 5% NaOH and EtOAc. The organic layer was filtered through a pad of silica gel and evaporated under reduced pressure. The crude product was separated by preparative TLC (hexane/EtOAc 9:1) to yield **7** (16.0 mg, 36%) and **11** (25.4 mg, 57%).

7: TLC R_f = 0.42 (hexane/EtOAc 9:1); ^{1}H and ^{13}C NMR: see tables; MS (FAB) m/z (relative intensity) 559 (M^+ + 1, 19), 471 (5), 383 (4), 343 (21), 155 (51), 135 (67), 119 (100), 102 (62); HRMS (FAB) m/z 559.4364 (calcd for $C_{35}H_{58}O_5$ + 1, 559.4362).

11: TLC R_f = 0.36 (hexane/EtOAc 9:1); ^{1}H and ^{13}C NMR: see tables; MS (EI) m/z (relative intensity) 559 (M^+ + 1, 6), 471 (16), 455 (20), 415 (41), 387 (18), 343 (18), 71 (100); HRMS (FAB) m/z 581.4182 (calcd for $C_{35}H_{58}O_5$ + Na, 581.4181).

(20*R*,22*S*)-3*β*-Butyryloxycholestane-16*β*,20,22-orthobutyrate (8). Isomerization of epoxy ester **7** (4.0 mg) in 1.3 mM TFA/$CDCl_3$ (see general methods) gave the orthoester in quantitative yield. TLC R_f = 0.83 (hexane/EtOAc 4:1); ^{1}H and ^{13}C NMR: see tables; MS (FAB) m/z 559 (M^+ + 1, 17), 471 (20), 383 (6), 343 (12), 257 (36), 155 (54), 135 (70), 119 (100); HRMS (FAB) m/z 559.4356 (calcd for $C_{35}H_{58}O_5$ + 1, 559.4362).

(20*S*,22*S*)-3*β*,16*β*-Dibutyryloxycholestane-20,22-diol (13) and (20*R*,22*R*)-3*β*,16*β*-Dibutyryloxycholestane-20,22-diol (15). To a stirred solution of olefin **4** (88 mg, 0.16 mmol) in 2 mL of Et_2O containing 70 μL of pyridine was added 1 mL of a 0.33 M solution of OsO_4/benzene at room temperature. After 45 min, the solvents were evaporated with a steam of N_2. The resulting brown osmate was immediately hydrolyzed by adding 8.5 mL of water, 13 mL of pyridine, and 750 mg of $Na_2S_2O_5$ (3.95 mmol) at room temperature. After 12 h, the solution was extracted with EtOAc. The organic layer was washed with 10% HCl and brine, dried (Na_2SO_4), and evaporated under reduced pressure. Preparative TLC using hexane/EtOAc 3:1 gave starting material **4** (16 mg, 18%), (20*S*, 22*S*)-diol **13** (45 mg, 48%), and (20*R*, 22*R*)-diol **15** (15 mg, 16%).

13: TLC R_f = 0.59 (hexane/EtOAc 3:1); ^{1}H NMR (600 MHz) 5.36 (m, 1H, C-16), 4.67 (1H, m, C-3), 3.86 (d, J = 10.4 Hz, 1H, C-22), 2.44 (dt, J = 13.6 and 7.7 Hz, 1H, C-15), 2.29 (t, J = 7.4 Hz, 2H, 16-butyrate CH_2CO), 2.22 (t, J = 7.4 Hz, 2H, 3-butyrate CH_2CO), 1.98 (dt, J = 12.5 and 3.2 Hz, 2H, C-12), 1.79 (dd, J = 12.5 and 3.1 Hz, 1H, C-2), 1.70 (dt, J = 13.4 and 3.5 Hz, 1H, C-1), 1.65, (m, 2H, 16-butyrate CH_3CH_2), 1.62 (m, 2H, 3-butyrate CH_3CH_2), 1.12 (s, 3H, C-21), 1.06 (s, 3H, C-18), 0.95 (t, J = 7.4 Hz, 3H, 16-butyrate CH_3CH_2), 0.92 (t, J = 7.4 Hz, 3H, 3-butyrate CH_3CH_2), 0.88 (d, J = 6.6 Hz, 3H, C-26), 0.88 (d, J = 6.6 Hz, 3H, C-27), 0.81 (s, 3H, C-19), 0.65 (m, 3H, C-9); ^{13}C NMR (151 MHz) 173.2 (s), 171.9 (s), 78.5 (s), 78.2 (d), 75.1 (d), 73.2 (d), 57.8 (d), 54.3 (d), 53.9 (d), 44.5 (d), 43.4 (s), 39.8 (t), 36.7 (t), 36.6 (t), 36.6 (t), 35.9 (t), 35.4 (s), 35.0 (t), 34.3 (d), 33.9 (t), 31.5 (t), 28.9 (t), 28.3 (2C, d and t), 27.4 (t), 22.6 (q), 22.5 (q), 21.2 (q), 20.8 (t,), 18.5 (t), 18.5 (t), 14.6 (q), 13.6 (q), 13.6 (q), 12.1 (q); MS (EI) m/z (relative intensity) 577 (M^+ + 1, 12), 559 (100), 471 (88), 387 (64), 369 (32), 343 (88), 299 (97), 281 (54), 257 (65); MS (FAB) m/z (relative intensity) 599 (M^+ + Na, 39), 329 (17), 177 (100); HRMS (FAB) m/z 599.4287 (calcd for $C_{35}H_{60}O_6$ + Na, 599.4287).

15: TLC R_f = 0.50 (hexane/EtOAc 3:1); ^{1}H NMR (600 MHz) 5.24 (m, 1H, C-16), 4.69 (m, 1H, C-3), 3.35 (dd, J = 2.2 and 9.6 Hz, 1H, C-22), 2.46 (dt, J = 13.7 and 7.6 Hz, 1H, C-15), 2.29 (t, J = 7.4 Hz, 2H, 16-butyrate CH_2CO), 2.24 (t, J = 7.3 Hz, 2H, 3-butyrate CH_2CO), 2.16 (dt, J = 12.5 and 3.2 Hz, 1H, C-12), 1.80 (dd, J = 12.5 and 3.1 Hz, 1H, C-2), 1.72 (dt, J = 13.4 and 3.5 Hz, 1H, C-1), 1.63, (m, 4H, 3 and 16-butyrate CH_3CH_2), 1.20 (s, 3H, C-21), 1.11 (s, 3H, C-18), 0.96 (t, J = 7.4 Hz, 3H, 16-butyrate CH_3CH_2), 0.93 (t, J = 7.4 Hz, 3H, 3-butyrate CH_3CH_2), 0.89 (d, J = 6.4 Hz, 3H, C-26), 0.88 (d, J = 6.3 Hz, 3H, C-27), 0.83 (s, 3H, C-19), 0.68 (1H, m, C-9); ^{13}C NMR (151 MHz) 173.2 (s), 171.6 (s), 78.9 (s), 77.2 (d), 76.0 (d), 73.2 (d), 57.0 (d), 54.1 (d), 54.1 (d), 44.6 (d), 44.0 (s), 40.2 (t), 36.7 (t), 36.6 (t), 36.6 (t), 36.4 (t), 35.4 (s), 35.3 (t), 34.3 (d), 33.9 (t), 31.5 (t), 29.6 (t), 28.3

(t), 28.2 (d), 27.4 (t), 22.8 (q), 22.3 (q), 20.9 (t), 19.7 (q), 18.5 (t,), 18.5 (t), 14.5 (q), 13.6 (q), 13.5 (q), 12.1 (q); MS (EI) *m/z* (relative intensity) 559 (17, $M^+ - H_2O + 1$), 475 (70), 387 (85), 299 (100). MS (FAB) *m/z* (relative intensity) 599 ($M^+ + Na$, 60), 329 (26), 177 (100); HRMS (FAB) *m/z* 599.4288. (calcd for $C_{35}H_{60}O_6 + Na$, 599.4287)

(20*S*,22*R*)-3*β*,16*β*-Dibutyryloxy-20,22-epoxycholestane (12) and (20*R*,22*S*)-3*β*,16*β*-Dibutyryloxy-20,22-epoxycholestane (16). Methanesulfonyl chloride (240 *μ*L, 3.23 mmol) was slowly added to a stirred solution of **13** (40 mg, 0.07 mmol) in 1.5 mL of anhydrous pyridine at 0 °C. After 10 min, the solution was stirred at room temperature for another 30 min. The mixture was partitioned between 10% HCl and Et_2O. The organic layer was dried (Na_2SO_4) and concentrated with a steam of N_2. The product was purified by preparative TLC with hexane/EtOAc 4:1 to give 43 mg of pure (20*S*,22*S*)-22-mesylate (95%). Analogously, the (20*R*,22*R*)-22-mesylate (16 mg) was synthesized from **15** in 94% yield. For characterization of the 22-mesylates, see Supporting Information.

The (20*S*,22*S*)-22-mesylate thus obtained (40.3 mg, 0.06 mmol) was treated with 50 mg of K_2CO_3 in 1 mL of MeOH for 10 min with stirring at room temperature. The mixture was extracted with water and hexane/EtOAc 4:1, filtered though a pad of silica gel, and concentrated with a stream of N_2 to give epoxide **12** (34.4 mg, 100%). Analogously, epoxide **16** (7.1 mg) was quantitatively obtained starting from the (22*R*)-mesylate (8.2 mg).

Epoxide 12: TLC $R_f = 0.61$ (hexane/EtOAc 9:1); ^1H and ^{13}C NMR: see tables; MS (EI) *m/z* (relative intensity) 559 ($M^+ + 1$, 9), 558 (M^+, 3), 471 (18), 388 (17), 347 (21), 275 (12), 257 (20), 71 (100); MS (FAB) *m/z* (relative intensity) 581 ($M^+ + Na$, 60), 329 (26), 177 (100); HRMS (FAB) *m/z* 581.4182 (calcd for $C_{35}H_{58}O_5 + Na$, 581.4181).

Epoxide 16: TLC $R_f = 0.55$ (hexane/EtOAc 9:1); ^1H and ^{13}C NMR: see tables; MS (FAB) *m/z* (relative intensity) 581 (60, $M^+ + Na$), 329 (26), 177 (100); HRMS (FAB) *m/z* 581.4182 (calcd for $C_{35}H_{58}O_5 + Na$, 581.4181).

(20*R*,22*R*)-3*β*-Butyryloxycholestane-16*β*,20,22-orthobutyrate (14). Isomerization of epoxy ester **12** (5.0 mg) in 1.3 mM TFA/$CDCl_3$ (see general methods) gave the orthoester in quantitative yield. Alternatively, cyclization of diol **15** (4.3 mg) under the same conditions also gave orthoester **14**. TLC $R_f = 0.79$ (hexane/EtOAc 3:1); ^1H and ^{13}C NMR: see tables; MS (EI) *m/z* (relative intensity) 559 ($M^+ + 1$, 10), 487 (15), 471 (75), 413 (47), 388 (91), 347 (89), 259 (42), 241 (45), 215 (42), 71 (100); HRMS (FAB) *m/z* 581.4182 (calcd for $C_{35}H_{58}O_5 + Na$, 581.4141).

Rearrangement of Epoxy Ester 11. Treatment of **11** (4.0 mg) with 13 mM TFA/$CDCl_3$ (see general methods) gave ring D secosteroid **18** (3.5 mg, 87% yield).

(*E*,22*R*)-3*β*,22-Dibutyryloxy-(16,17)-secocholest-17(20)-ene-16-carboxaldehyde (18): TLC $R_f = 0.41$ (hexane/EtOAc 4:1); ^1H NMR (600 MHz) 9.70 (br d, $J = 2.8$ Hz, 1H, 16-CHO), 5.24 (s, 1H, C-17), 4.94 (t, $J = 7.0$ Hz, 1H, C-22), 4.70 (m, 1H, C-3), 2.25 (t, $J = 7.4$ Hz, 2H, 22-butyrate CH_2CO), 2.24 (t, $J = 7.5$ Hz, 2H, 3-butyrate CH_2CO), 1.89 (m, 1H, C-12), 1.81 (m, 1H, C-2), 1.75 (dt, $J = 13.4$ and 3.6 Hz, 1H, C-1), 1.71 (s, 3H, C-21), 1.07 (s, 3H, C-18), 0.93 (t, $J = 7.4$ Hz, 3H, 3-butyrate CH_3CH_2), 0.93 (t, $J = 7.4$ Hz, 3H, 22-butyrate CH_3CH_2), 0.86 (d, $J = 6.6$ Hz, 6H, C-26 and C-27), 0.79 (s, 3H, C-19); ^{13}C NMR (151 MHz) 201.5 (d), 172.1 (s), 171.7 (s), 138.0 (d), 132.1 (s), 80.2 (d), 72.0 (d), 52.1 (d), 45.4 (t), 44.8 (d), 42.9 (d), 38.8 (s), 37.1 (t), 36.2 (d), 35.5 (t), 35.5 (t), 35.4 (t), 34.4 (s), 33.5 (t), 32.7 (t), 31.1 (t), 29.2 (t), 27.3 (t), 26.6 (d), 26.2 (t), 21.5 (q), 21.3 (q), 19.2 (t), 18.4 (q), 17.4 (t), 17.4 (t), 12.5 (q), 12.5 (q), 11.9 (q), 11.1 (q); MS (EI) *m/z* 559 ($M^+ + 1$, 2), 558 (M^+, 2), 530 (1), 471 (66), 413 (28), 388 (56), 347 (44), 259 (39), 241 (43), 107 (36), 95 (32), 71 (100); MS (FAB) *m/z* 581 ($M^+ + Na$, 24), 329 (19), 301 (18), 177 (100); HRMS (FAB) *m/z* 581.4182 (calcd for $C_{35}H_{58}O_5 + Na$, 581.4181). The *E*-configuration was determined by NOE enhancement of C-18 when C-21 is irradiated. The 22*R*-configuration is based on the probable mechanism.

Rearrangement of Epoxy Ester 16. Treatment of **16** (4.0 mg) with 26 mM TFA/$CDCl_3$ (see general methods) gave orthoester **8** (1.6 mg, 40%), allylic alcohol **20** (1.6 mg, 40%), and aldehyde **21** (0.8 mg, 20%).

(22*S*)-3*β*,16*β*-Dibutyryloxycholest-20-en-22-ol (20): TLC $R_f = 0.58$ (hexane/EtOAc 4:1); ^1H NMR (600 MHz) 5.21, (m, 1H, C-16), 5.00 (s, 1H, C-21), 4.96 (s, 1H, C-21), 4.71 (m, 1H, C-3), 3.90 (t, $J = 6.8$ Hz, 1H, C-22), 2.27 (t, $J = 7.4$ Hz, 2H, 16-butyrate CH_2CO), 2.24 (t, $J = 7.4$ Hz, 2H, 3-butyrate CH_2CO), 1.81 (br d, $J = 12.6$ Hz, 1H, C-2), 1.26 (s, 3H, C-18), 0.94 (t, $J = 7.4$ Hz, 3H, 3-butyrate CH_3CH_2), 0.93 (t, $J = 7.4$ Hz, 3H, 16-butyrate CH_3CH_2), 0.87 (t, $J = 6.5$ Hz, 6H, C-26 and C-27), 0.85 (s, 3H, C-19), 0.70 (m, 1H, C-9); ^{13}C NMR (151 MHz) 173.8, 172.1, 145.9, 114.7, 78.7, 72.1, 53.2, 52.6, 52.5, 43.5, 42.6, 37.1, 35.8, 35.6, 35.5, 34.5, 33.9, 33.8, 32.9, 32.1, 31.4, 30.8, 28.6, 27.4, 26.9, 26.3, 21.5 (2C), 19.4, 17.4, 17.0, 14.5, 12.6, 12.5, 11.1; MS (EI) *m/z* 558 (M^+, 3), 540 (M^+-18, 9), 470 (100), 452 (10), 414 (10), 400 (27), 388 (11), 343 (25), 215 (22), 107 (21), 71 (35); HRMS (FAB) *m/z* 581.4182 calcd for $C_{35}H_{58}O_5 + Na$, 581.4181). The 22*S*-configuration is based on the probable mechanism.

(20*S*)-3*β*,16*β*-Dibutyryloxy-22-norcholestane-20-carboxaldehyde (21): TLC $R_f = 0.70$ (hexane/EtOAc 4:1); ^1H NMR (600 MHz) 9.95 (s, 1H, 22-CHO), 5.24 (m, 1H, C-16), 4.69 (m, 1H, C-3), 2.44 (dt, $J = 13.5$ and 7.5 Hz, 1H, C-15), 2.24 (t, $J = 7.5$ Hz, 2H, 16-butyrate CH_2CO), 2.23 (t, $J = 7.5$ Hz, 2H, 3-butyrate CH_2CO), 2.00 (dt, $J = 12.0$ and 3.3 Hz, 1H, C-12), 1.80 (br d, $J = 12.8$ Hz, 1H, C-2), 1.71 (dt, $J = 13.2$ and 3.3 Hz, 1H, C-1), 1.64 (m, 4H, 3 and 16-butyrate CH_3CH_2), 1.16 (s, 3H, C-18), 0.95 (t, $J = 7.4$ Hz, 3H, 16-butyrate CH_3CH_2), 0.94 (t, $J = 7.4$ Hz, 3H, 16-buryrate CH_3CH_2), 0.93 (s, 3H, C-21), 0.86 (d, $J = 6.5$ Hz, 6H, C-26 and C-27), 0.82 (s, 3H, C-19), 0.66 (m, 1H, C-9); ^{13}C NMR (75 MHz) 207.4 (d, CHO), 173.3 (s), 172.8 (s), 75.1 (d), 73.2 (d), 61.7 (d), 54.2 (d), 54.1 (d), 52.2 (s), 44.6 (d), 43.8 (s), 40.4 (t), 36.6 (t), 36.6 (t), 35.5 (s), 35.0 (t), 34.8 (d), 34.0 (t), 33.2 (t), 32.6 (t), 31.5 (t), 28.6 (d), 28.3 (t), 27.4 (t), 22.6 (q), 22.4 (q), 20.8 (t), 20.0 (q), 18.6 (t), 18.4 (t), 15.9 (q), 13.6 (q), 13.6 (q), 12.2 (q); MS (EI) *m/z* 559 ($M^+ + 1$, 0.3), 529 (4), 471 (4), 443 (29), 427 (21), 400 (11), 371 (100), 343 (41), 283 (25), 255 (21), 215 (14), 107 (16), 71 (48); MS (CI, CH_4) *m/z* 557, ($M^+ - 1$, 4), 471 (25), 383 (50), 343 (100), 255 (25); HRMS (CI, CH_4) *m/z* 557.4201 (calcd for $C_{35}H_{58}O_5 - 1$, 557.4206). The 20*S*-configuration is based on the probable mechanism.

Acknowledgment. We thank the American Society of Pharmacognosy and the Petroleum Research Fund, administered by the American Chemical Society, for financial support.

Supporting Information Available: Kinetics data for the rearrangements, characterization data for the dehydration products of **5** and the mesylates of **13** and **15**, ^1H and ^{13}C NMR data for epoxides **7**, **11**, **12**, **16** and orthoesters **8** and **14**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO011111Z