Anal. Calcd for $C_{12}H_{15}NO_3S$: C, 56.90; H, 5.79; S, 12.66. Found: C, 56.79; H, 6.00; S, 12.53.

Determination of the Enantiomeric Purity of (*R*)- and (*S*)-Thiorphan. A magnetically stirred solution of 89.5 mg (353 μmol) of (±)-thiorphan, 44 μL (51 mg, 360 μmol) of boron trifluoride–diethyl etherate in 5 mL of anhydrous methanol was heated at 50 °C for 4 h (until no starting material remained by TLC analysis). The mixture was diluted with dichloromethane, washed with 1 M aqueous potassium carbonate, water, and brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford 102 mg (108% mass balance) of (±)-thiorphan methyl ester as a pale yellow oil.

A mixture of 27 mg (100 μmol) of (±)-thiorphan methyl ester, 20 mg (100 μmol) of (*R*)-1-[(1-naphthyl)ethyl]isocyanate (**13**), and 10 mg of anhydrous potassium carbonate in 2 mL of benzene was heated at 80 °C for 4 h (until no starting material remained by TLC analysis). The mixture was diluted with dichloromethane, washed with water and brine, dried over anhydrous magnesium sulfate, and concentrated in vacuo to afford 50 mg (106% mass balance) of a mixture of thiourethane diastereomers **14** and **15**. The unfractionated product was analyzed by HPLC (Regis, 4.6 mm × 25 cm, Pirkle chiral phase covalently bound to 5-μm aminopropyl silica gel; 84:16 isooctane/isopropyl alcohol; 4.0 mL/min; k'(**14**) = 8.16, k'(**15**) = 11.91; α = 1.46).

The reactions were repeated separately with (2*R*)- and (2*S*)-thiorphan. HPLC analysis of the unfractionated product obtained from (2*R*)-thiorphan (*R*)-**1** showed a ≥95:5 ratio of **14** to **15**. Likewise, HPLC analysis of the unfractionated product obtained from (2*S*)-thiorphan (*S*)-**1** showed a ≤5:95 ratio of **14** to **15**. Therefore, the enantiomeric ratio of both (2*R*)- and (2*S*)-thiorphan is ≥95:5.

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Registry No. (*R*)-**1**, 95908-99-9; (*S*)-**1**, 95909-00-5; (±)-**1**, 76721-89-6; (±)-**1** methyl ester, 83960-22-9; **3a**, 77943-39-6; **3b**, 95841-14-8; **4a**, 17016-83-0; **4b**, 95798-31-5; **5a**, 3970-13-6; **5b**, 15960-81-3; **8a**, 95798-32-6; **8b**, 95798-33-7; **9a**, 95798-34-8; **9b**, 95798-35-9; (*R*)-**10**, 95841-15-9; (*S*)-**10**, 95798-36-0; (*R*)-**11**, 95798-37-1; (*S*)-**11**, 95798-38-2; (*R*)-**12**, 95841-16-0; (*S*)-**12**, 95841-17-1; (*R*)-**13**, 42340-98-7; **14** (isomer 1), 95864-07-6; **14** (isomer 2), 95864-08-7; (1*S*,2*R*)-norephedrine hydrochloride, 40626-29-7; diphenyl carbonate, 102-09-0; (*S*)-valinol, 2026-48-4; diethyl carbonate, 105-58-8; benzyl mercaptan, 100-53-8; *s*-trioxane, 110-88-3; 3-phenylpropanoyl chloride, 645-45-4; glycine benzyl ester *p*-toluenesulfonate, 1738-76-7; enkephalinase, 70025-49-9.

Stereoselective Synthesis and Solvolytic Behavior of the Isomeric 7-Dehydrocholesterol 5,6-Oxides

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Cholesterol oxide hydrolase is a recently described mammalian enzyme which catalyzes the hydration of Δ^5-sterol oxides to 5,6-glycols in the liver. As the isomeric 7-dehydrocholesterol 5,6-oxides represent useful mechanistic probes of the action of the enzyme, synthetic procedures were sought for the stereoselective preparation of these unstable epoxides. Direct epoxidation of 7-dehydrocholesterol with peracid in the presence of aqueous buffer stereoselectively provided the α-oxide **2b** in good yield. Synthesis of the β-oxide **12** proved more difficult in that attempted formation of an intermediate bromohydrin with appropriate stereochemistry proved unsatisfactory. The finding that 7α-bromocholesteryl benzoate undergoes selective β-epoxidation and that the desired Δ^7-double bond could be formed by treatment with potassium *tert*-butoxide resulted in the successful synthesis of the β-oxide **12**. Both epoxides undergo cis addition of benzoic acid in chloroform at the allylic carbon and trans addition of 2-mercaptoethanol in base at the same position. Hydrolytic reactions prove to be more complex. Aqueous acid hydrolysis of the α-oxide **2b** produced triol **5a** and dienediol **6**, which can further dehydrate to the trienol **7**. Under identical conditions the β-oxide **12** hydrolyzes to a single product. Both epoxides, particularly the β-oxide **12**, proved to be effective inhibitors of cholesterol oxide hydrolase.

Recently, a new rat liver microsomal epoxide hydrolase capable of catalyzing the metabolism of cholestrol 5,6-oxide and other Δ^5-sterol oxides to 5,6-glycols was reported.[1,2] This newly described cholesterol oxide hydrolase is antigenically distinct from the microsomal epoxide hydrolase (EC 3.3.2.3) that catalyzes the hydrolysis of arene oxides[3,4] to *trans*-dihydrodiols and has none of this catalytic activity. In an investigation[5] of the properties of cholesterol oxide hydrolase, the need for the title compounds arose since these unsaturated epoxides have the potential (1) to be used for a spectrophotometric assay of the enzyme; (2) to determine how changes in the geometry and reactivity of these substrates affect the catalytic activity; and (3) to provide information as to whether the mechanism of hydrolysis proceeds by way of a carbocation intermediate or via nucleophilic displacement by hydroxide.

The direct epoxidation of $\Delta^{5,7}$-steroids has produced varying results (Scheme I). In an attempt to determine the number of double bonds in ergosterol (**1a**), Windaus and Luttringhaus[6] treated the sterol with perbenzoic acid. With excess reagent, exactly 3 mol of peracid were consumed by the $\Delta^{5,7,22}$ double bonds. However, when only 1 mol of the peracid was used, triol monobenzoate **4a** was obtained rather than the expected monooxide **2a**. Reaction

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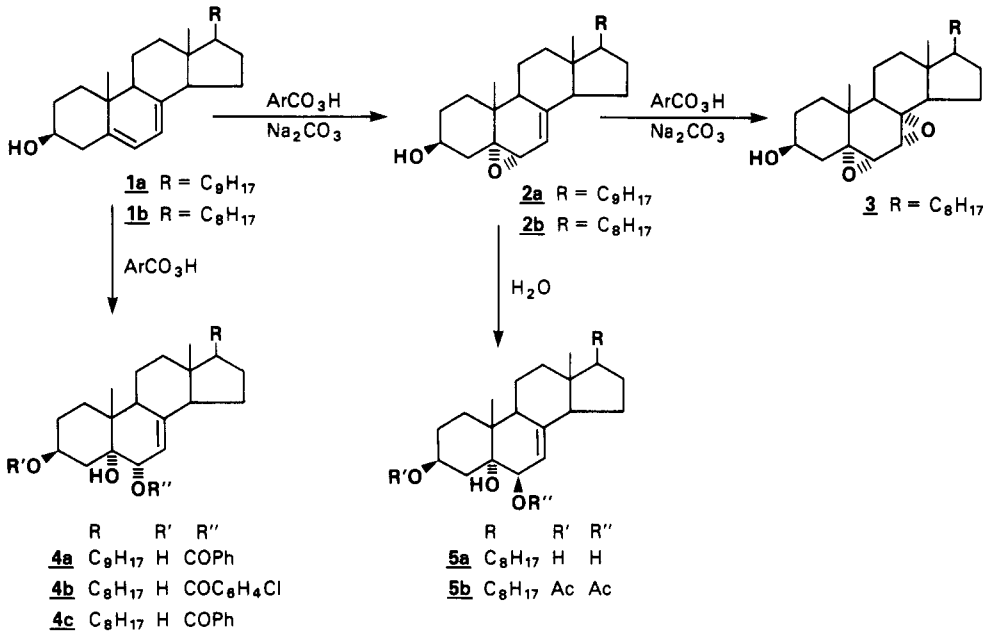
0022-3263/85/1950-1835$01.50/0

Scheme I



of peracids with isomers of ergosterol, principally lumisterol, produced similar products. Early attempts[7] at epoxidation of lumisterol in chloroform with perbenzoic acid yielded only the triol monobenzoate. Lumisterol 5,6-oxide could be prepared,[8] however, by oxidation with perbenzoic acid in benzene solution at 5 °C. Mayor and Meakins[9] demonstrated that oxidation of lumisterol or its acetate with perbenzoic acid produced a 5β,6β-epoxide which is subsequently cleaved to the triol monobenzoate. Cragg and Meakins[10] found that oxidation of the 9α-lumisterol and 9β-ergosterol with perbenzoic acid was surprisingly fast and that the orientation of attack by the peracid was dependent on the stereochemistry of the angular methyl groups in that epoxidtion occurs on the face opposite the C-19 methyl group. Generally, triol monobenzoates were isolated rather than the epoxides since the allylic monooxide initially formed is sensitive to attack by the benzoic acid in the reaction medium. In order to prevent decomposition of highly reactive vinyl epoxides, the monoepoxidation of conjugated dienes has been attempted in the presence of acid scanvengers. Thus, in the synthesis of homoallylic alcohols, Crandall et. al[11] made the intermediate monoepoxides of cyclopentadiene, cyclohexadiene, and cyclooctadiene cleanly with peracid in the presence of solid sodium carbonate. The use of similar solid buffers in peroxidations with pertrifluoroacetic acid is well documented.[12]

Results and Discussion

In the present synthesis of the α-oxide **2b**, we expected the oxidation of 7-dehydrocholesterol with peracids to parallel that of ergosterol and its stereoisomers. Thus, reaction of 7-dehydrocholesterol (**1b**) with *m*-chloroperbenzoic acid in methylene chloride yielded the triol monobenzoate **4b** as the major product. The presence of solid sodium carbonate in the reaction medium did not prevent decomposition of the initially formed monooxide. However, peracid oxidation in a biphasic mixture of aqueous sodium carbonate/methylene chloride allowed isolation of the 5α,6α-monooxide **2b** in very good yield (89%, Scheme I). The epoxidation is extremely fast, and the reaction conditions are milder than those reported[13] for epoxidation of 7-dehydrocholesterol acetate. Confirmation of the structure **2b** was obtained by hydrolysis of the epoxide to the triol **5a** and subsequent acetylation to the known[14] triol 3,6-diacetate **5b**. Spectral evidence for the epoxidation of the 5,6-double bond rather than the 7,8-double bond was obtained from examination of the NMR spectrum of **2b** at 500 MHz. Through application of resolution enhancement[15] and difference spectroscopy[16] techniques, the olefinic proton was shown to be coupled allylically to two protons neither of which was the readily identifiable 4β-proton. When excess peracid is used in the presence of sodium carbonate the diepoxide **3** is obtained.

Attempted synthesis of the isomeric 7-dehydrocholesterol 5β,6β-oxide (**12**) by formation of an intermediate halohydrin proved to be futile. Reaction of 7-dehydrocholesterol or its acetate with acetyl hypobromite[17] yielded only a complex mixture of highly colored products. Reaction with NBS in a Me_2SO–water solvent system, a method[18] which forms 1,2-adducts from conjugated dienes in good yields, also produced a complex mixture of products.

Since the formation of the β-oxide via the halohydrin route was unsuccessful, a new strategy was attempted which involved introduction of the 7,8-double bond after formation of the epoxide (Scheme II). It is well-known[19] that allylic bromination of cholesterol esters (cf. 8) yields the 7α-bromide as the major product. These 7-bromocholesterol esters are key intermediates in the synthesis

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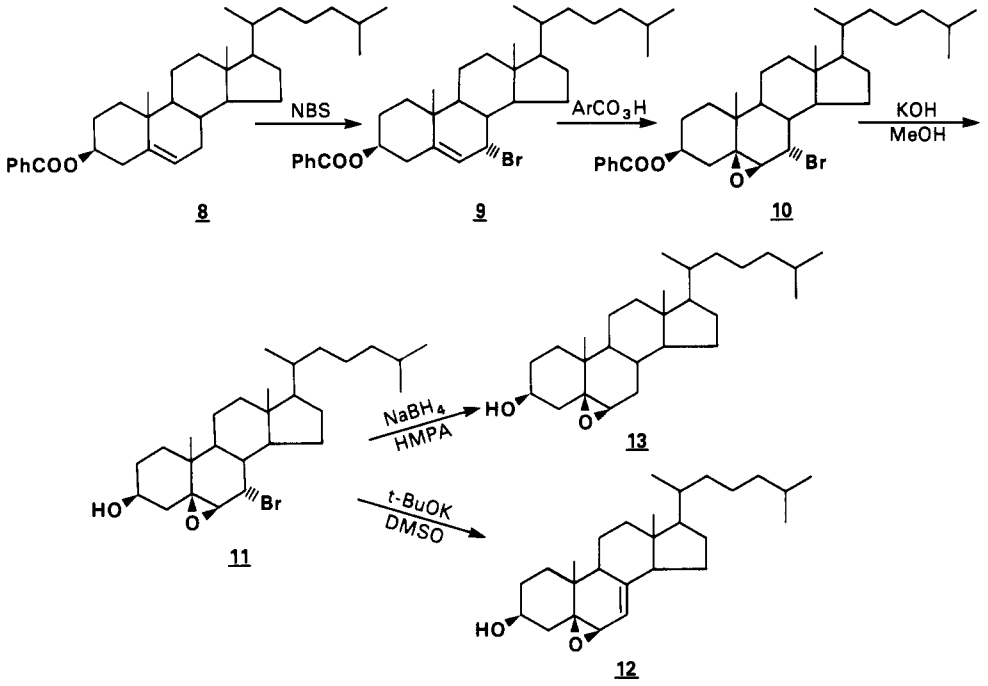
Scheme II



of 7-dehydrocholesterol.[20] Direct epoxidation of the 7-bromocholesteryl ester followed by dehydrobromination was hoped to yield the desired unsaturated oxide. The essential requirement in the sequence is that epoxidation by peracid occurs on the more hindered β-face of the molecule. It was reasoned that steric bulk[21] and/or electrostatic repulsion[22] of the bromine atom could force attack on the β-face rather than normal attack at the α-face.[19] Reaction of 7α-bromocholesteryl benzoate (**9**) with *m*-chloroperbenzoic acid in benzene for 6 h yielded epoxide **10** (Scheme II). Comparison of chemical shift changes for the C18- and C19-methyl groups in cholesterol α- and β-oxides with the 7α-bromocholesterol oxide **11** obtained on hydrolysis of **10** indicated that peracid attack occurred primarily on the β-face of **9**. This was confirmed by hydrogenolysis of the bromo epoxide **11** with sodium borohydride in HMPA[23a] to the well-known cholesterol β-oxide **13**[23b].

The final step in the synthesis of the desired unsaturated β-oxide **12** consisted of dehydrobromination. In the synthesis of 7-dehydrocholesterol the 7-bromocholesterol ester **9** is efficiently dehydrobrominated with collidine or trimethyl phosphite.[20] Although use of these reagents on the bromo epoxide **11** failed, potassium *tert*-butoxide in Me_2SO[24] effected the elimination smoothly to give the desired unsaturated oxide **12**.

Since there is some confusion in the older steroid literature[8] as to which products are expected from ergosterol 5,6-oxides under a given set of hydrolysis conditions, we examined in detail the stereochemical course of ring opening of the unsaturated cholesterol oxides **2b** and **12** and found the product distribution to be very dependent upon reaction condtions. Reaction of the initially formed oxide **2b** with the byproduct *m*-chlorobenzoic acid in methylene chloride to yield the triol monoester **4b** has already been described. In separate experiments, benzoic acid was added to samples of the α- and β-oxides in $CDCl_3$, and the reaction was followed by NMR. After 1 nearly all of the epoxide was consumed. In both cases the major product of the reaction was a triol monobenzoate (**4c**, Scheme III, and **14**, Scheme IV) derived by cis-opening of the epoxide at the allylic carbon by benzoic acid. Presumably the products arise via collapse of the intimate ion pair obtained after acid-catalyzed ring opening of the epoxide.

Cleavage of the oxides in aqueous media is more complex. Both **2b** and **12** display a UV absorption in the region of 220–225 nm. This absorption arrises from the bathochromic shift of the Δ^7-olefin caused by the proximity of the epoxide oxygen. Marked time-dependent changes occur in their spectra at low pH. In the case of the β-oxide **12**, the absorbance maximum at 221 nm decreases with time without the appearance of any other absorptions in the region of 200–400 nm. This is consistent with cleavage of the epoxide ring to form a triol. In an investigation of the reaction on a preparative scale, the NMR spectrum of the reaction product displayed a pair of doublets at 5.64 and 5.88 ppm with a vicinal coupling constant (J = 10 Hz) due to a cis-substituted olefin. The NMR data taken together with the lack of any UV absorption above 210 nm indicated that 1,4-addition had occurred to produce cholest-6-ene-3β,5,8-triol (**15**, Scheme IV). Analysis of the NMR spectrum and examination of Driedig models suggest that the 8-hydroxyl group has β-stereochemistry.[25]

The α-oxide **2b** behaves quite differently in aqueous acid (Scheme III). In addition to a decrease in the absorption maximum at223 nm of the oxide with time, a new ab-

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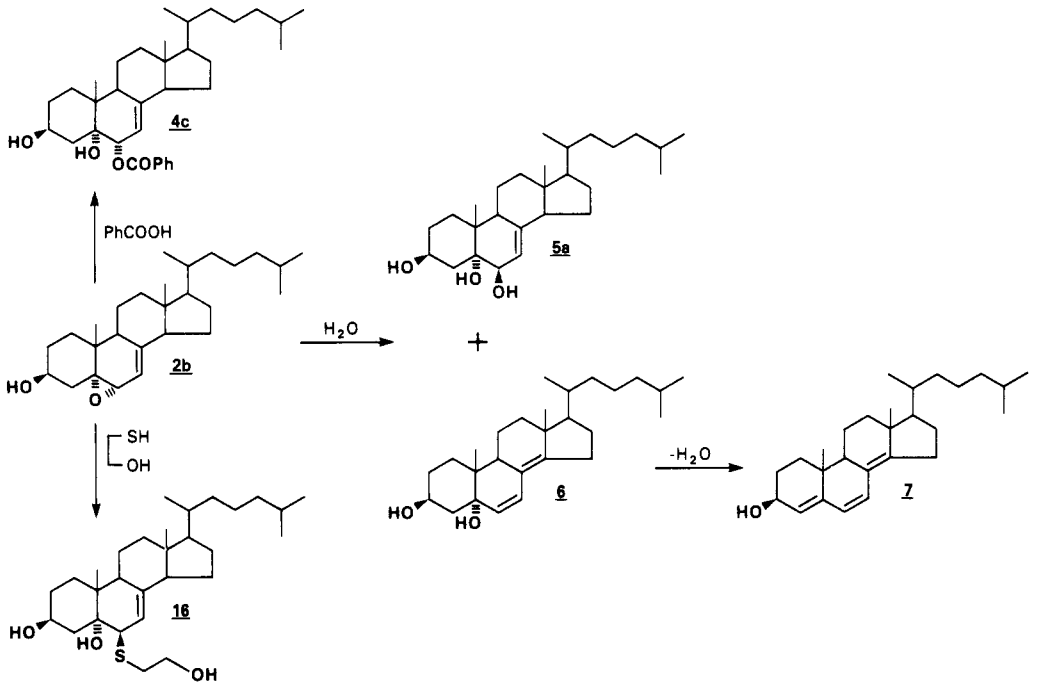
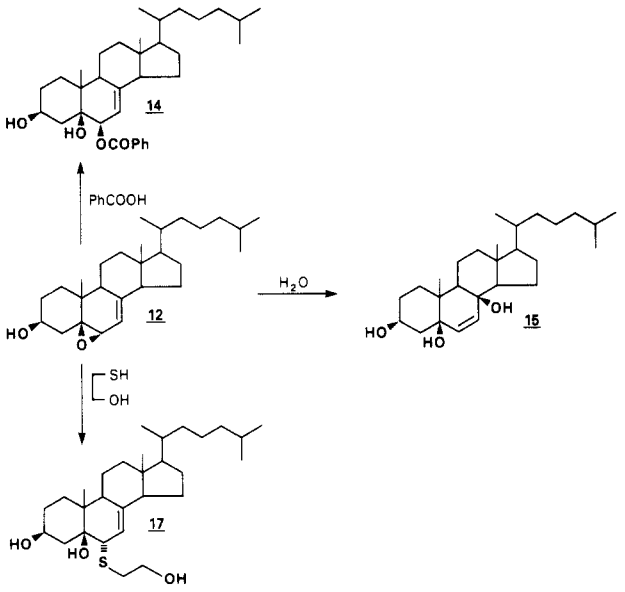
(25) The NMR spectrum of triol **15** contains, in addition to signals from the C-3 proton and the two cis olefinic protons, two multiplets downfield from the methylene envelope: a double triplet at 2.76 ppm and a doubled quartet at 2.23 ppm. Decoupling established the identity of these signals as being due to the 1β proton and the 11β proton, respectively. A plasubile explanation for the observed deshielding of these protons is through-space deshielding by the hydroxyl groups. In addition, the significant shielding of the C-3 proton relative to the saturated cholestane-3,5,6-triols is presumably due to the shielding cone of the olefin on the α-face of the molecule.

Scheme III



Scheme IV



sorption maximum at 253 nm appears. After a longer period of time the absorption band at 253 nm decreases, and a new absorption band at 286 nm appears. The absorption band at 253 nm suggests the presence of a 6,8-(14)-diene moiety while the absorption band at 286 nm indicates a 4.6.8(14)-trienic system.[26] To determine if any 5,6-glycol which has no UV absorption is formed, the reaction was carried out on a preparative scale. An NMR of the crude material indicated the presence of two major compounds. Included in the NMR absorptions were two olefinic doublets at 5.49 and 6.21 ppm with vicinal cis coupling constants (J = 10 Hz). The crude product was separated into two components by preparative TLC. The lower R_f component corresponded to the 5,6-glycol **5a**. NMR splitting patterns for the H-3 proton, which is dependent on C-5 stereochemistry, and the H-6 proton are consistent with *trans*-5α,6β-diol stereochemistry. The NMR of the higher R_f component contained two olefinic doublets at 5.86 and 6.18 ppm (J = 10 Hz). The observed difference in chemical shift of the doublets with respect to the crude material before chromatography, the presence of an additional olefinic absorption at 5.41 ppm, and a change in UV absorption from 253 to 286 nm suggest that dehydration of the diene diol **6** to the triene monoalcohol **7** occurred during chromatography. No UV absorbance change was observed when the triol **5a** was added to aqueous acid, indicating that it is not an intermediate in the formation of the diene diol **6**.

The difference in product distribution in the acid-catalyzed hydrolysis of the oxides **2b** and **12** may be a consequence of stereochemical factors and carbocation stability. It is evident from the fact that only one product is obtained in the hydrolysis of the β-oxide **12** that a full allylic carbocation intermediate is not formed. Otherwise, 1,2-adduct formation would also have been observed. Apparently anchimeric assistance by the double bond with concomitant attack at C-8 by water is far more favorable than the equatorial approach of a water molecule on the protonated epoxide.

In the hydrolysis of the α-oxide **2b**, the data is consistent with more advanced C–O bond cleavage in which a free allylic carbocation may be formed. Water may approach C-6 to form the trans-diaxial product **5a**, and loss of the C-14 proton to yield the diene **6** has precedence in the fact that acid-catalyzed isomerizations of $\Delta^{5,7}$-steroidal dienes also yielded 6,8(14)-dienes via an allylic carbocation intermediate.[27,28] Thus, there is no common intermediate in the acid-catalyzed hydrolysis of **2b** and **12**.

In contrast to the numerous products obtained from both oxides in aqueous acid, addition of nucleophiles, such

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(28) An alternate explanation for the product formation in the acid-catalyzed hydrolysis of the α-oxide **2b** is that both products may arise from simultaneous and separate pathways involving back-side displacement of the protonated epoxide to form the 1,2-glycol **5a**, and an *all-trans* configuration where a concerted elimination of the C-14 proton and anchimeric assistance of the double bond to cleave the protonated epoxide occurs to form the diene **6**.

as 2-mercaptoethanol, under basic conditions yields only 1,2-adducts. The reaction is conveniently folloed by NMR. Both oxides were dissolved in separate NMR tubes in deuteriomethanol containing sodium deuteriomethoxide, generated in situ by addition of sodium hydride. Addition of an approximately molar amount of 2-mercaptoethanol to the α-oxide **2b** resulted in an immediate and complete formation of the thioether **16** (Scheme III). Addition of thiol to the β-oxide **12** to give thioether **17** (Scheme IV) was only 80% complete after 2 h. For both oxides, the mercaptoethanol reacted to give exclusively trans products. The difference in reactivity may be a reflection of the fact that the α-oxide **2b** is better able to undergo trans diaxial opening with the nucleophile in the transition state.

Preliminary studies[29] on the inhibition of the hydration of cholesterol 5,6α-oxide catalyzed by liver microsomal cholesterol oxide hydrolase have shown that (a) the I_{50} for the α-oxide **2b** is 4.5 μM which is comparable to the K_m value for cholesterol 5,6α-oxide; (b) the I_{50} for β-oxide **12** is 0.048 μM which is a factor of 2 lower than that for 5,6α-iminocholestanol, the most effective inhibitor for cholesterol oxide hydrolase known to date[5,30]; and (c) there is a parallel loss of activity toward hydrolysis of **12** with covalent binding to the enzyme. The possibility of specific covalent modification of the active site of cholesterol oxide hydrolase by any of these compounds is currently under investigation in our laboratory.

Experimental Section

^{1}H NMR spectra were run at 220 and 500 MHz and the signals are reported in parts per million downfield from internal tetramethylsilane. Melting points were obtained in capillaries and are reported uncorrected. UV spectra were recorded in acetonitrile solution.

5α,6α-Epoxycholest-7-en-3β-ol (2b). 7-Dehydrocholesterol (Aldrich, 1.0 g, 2.6 mmol) and sodium carbonate (0.55 g, 5.2 mmol) were dissovled in a mixture of methylene chloride (25 mL) and water (25 mL). *m*-Chloroperbenzoic acid (0.62 g, 3.6 mmol, 85% technical grade) dissolved in methylene chloride (10 mL) was added dropwise to the rapdily stirring mixture over a period of 1 min. After continued stirring for 10 min, the organic layer was washed with aqueous sodium sulfite and bicarbonate and dried over anhydrous sodium carbonate. Recrystallization rom acetone afforded white needles: 0.92 g (89%); mp 144–146 °C; $[\alpha]_D$ −112° (*c* 0.59, $CHCl_3$); IR ($CHCl_3$) 3620, 3440, 2955, 2875, 1643, 1465, 1382, 1038, 898, 868 cm^{-1}; UV λ_{max} 223 (ε 9200); NMR δ 0.55 (s, 3 H, C-18), 1.02 (s, 3 H, C-19), 3.00 (d, 1 H, *J* = 4 Hz, H-6), 3.91 (m, 1 H, H-3), 5.43 (br s, 1 H, H-7); MS, *m/e* (relative intensity) 400 (2), 382 (37), 364 (100), 349 (33), 251 (49), 197 (37); HRMS for $C_{27}H_{44}O_2$ calcd 400.3343, found 400.3351.

7α-Bromocholesteryl Benzoate (9). Bromocholesteryl benzoate was synthesized (47% yield) in the manner described by Bernstein et. al:[20] mp 140–143 °C [lit.[31] mp 144.5–145.5 °C]; $[\alpha]_D$ −193.2° (*c* 1.24, benzene) [lit.[31] $[\alpha]_D$ −199°, benzene]; NMR δ 0.73 (s, 3 H, C-18), 1.10 (s, 3 H, C-19), 4.70 (br s, 1 H, H-7), 4.98 (m, 1 H, H-3), 5.80 (d, 1 H, *J* = 5 Hz, H-6), 7.3-8.2 (m, 5 H, benzoate).

5β,6β-Epoxy-7α-bromocholestan-3β-ol Benzoate (10). 7α-Bromocholesteryl benzoate (6.2 g, 10.9 mmol) and *m*-chloroperbenzoic acid (2.5 g, 14.6 mmol, 85% tech) were dissolved in benzene (100 mL) and left at room temperature for 6 h. The organic solution was washed with aqueous sodium sulfite and sodium bicarbonate and dried over sodium sulfate. The crude material was recrystallized from acetone to give the product as white needles: 5.3 g (84% yield); mp 160–161 °C; $[\alpha]_D$ −45.7° (*c* 1.45, $CHCl_3$); NMR δ 0.69 (s, 3 H, C-18), 1.10 (s, 3 H, C-19), 3.46 (d, 1 H, *J* = 4 Hz, H-6), 4.51 (t, 1 H, *J* = 4 Hz, H-7), 5.11 (m, 1 H, H-3); MS (CI–NH_3), *m/e* (relative intensity) 587 (6), 585 (6), 465 (6), 463 (7), 383 (100). Anal. Calcd for $C_{34}H_{49}BrO_3$: C, 69.73; H, 8.43; Br, 13.64. Found: C, 69.68; H, 8.29; Br,13.56.

5β,6β-Epoxy-7α-bromocholestan-3β-ol (11). The ester **10** was saponified by refluxing in methanolic KOH for 30 min. The product was washed with water, dried over Na_2SO_4, and recrystallized from methanol: mp 118–120 °C; $[\alpha]_D$ −71° (*c* 0.3, $CHCl_3$); NMR δ 0.67 (s, 3 H, C-18), 1.03 (s, 3 H, C-19), 3.37 (d, 1 H, *J* = 3 Hz, H-6), 3.78 (m, 1 H, H-3), 4.48 (t, 1 H, *J* = 3 Hz, H-7); MS, *m/e* (relative intensity) 482 (4), 480 (3), 401 (100), 383 (85); HRMS calcd (M^+ − Br) 401.3419, found 401.3411.

5β,6β-Epoxycholest-7-en-3β-ol (12). 7α-Bromocholesterol 5β,6β-oxide (11) (320 mg, 0.66 mmol) was dissolved in Me_2SO (10 mL). Potassium *tert*-butoxide (150 mg, 1.34 mmol) was added, and the mixture was heated at about 55 °C for 1 h. The reaction mixture was cooled, diluted with ether, and washed with water, and the organic phase was dried over anhydrous sodium carbonate to yield a white powder (208 mg, 78%): mp 105–108 °C; $[\alpha]_D$ +301° (*c* 0.485, $CHCl_3$); IR ($CHCl_3$) 3615, 3470, 2955, 2875, 1666, 1466, 1377, 1040, 848 cm^{-1}; UV λ_{max} 221 (ε 8300); NMR δ 0.52 (s, 3 H, C-18), 1.17 (s, 3 H, C-19), 2.99 (d, 1 H, *J* = 4.2 Hz, H-6), 3.74 (m, 1 H, H-3), 5.44 (br d, 1 H, *J* = 4.2 Hz, H-7); *m/e* (relative intensity) 400 (89), 382 (41), 364 (100), 349 (34), 287 (79), 251 (89), 197 (64); HRMS for $C_{27}H_{44}O_2$ calcd 400.3343, found 400.3342.

5,6;7,8-Diepoxycholestan-3β-ol (3). 7-Dehydrocholesterol (1.0 g, 2.6 mmol) and sodium carbonate (2.2 g, 20.8 mmol) were dissolved in a mixture of methylene chloride (30 mL) and water (30 mL). To the vigorously stirring solution was added *m*-chloroperbenzoic acid (2.48 g, 14.4 mmol) dissolved in methylene chloride (20 mL). Stirring was continued at room temperature overnight. The organic layer was washed with aqueous sodium sulfite and bicarbonate and dried over $MgSO_4$: mp 138–141 °C; NMR δ 0.76 (s, 3 H, C-18), 1.18 (s, 3 H, C-19), 3.00 (d, 1 H, *J* = 3 Hz, H-6), 3.16 (d, 1 H, *J* = 3 Hz, H-7), 3.84 (m, 1 H, H-3); MS, *m/e* (relative intensity) 416 (6), 398 (12), 380 (11), 365 (7), 315 (14), 152 (100); HRMS for $C_{27}H_{44}O_3$ calcd 416.3290, found 416.3307.

Hydrogenolysis of 11. The bromo epoxide **11** (211 mg) was dissolved in HMPA (100 mL) and heated to 85 °C with stirring. Sodium borohydride (66 mg) was added, and heating was continued for 6 h. The resultant mixture was diluted with ether and washed with water, and the organic layer was dried over Na_2CO_3. The cholesterol 5β,6β-oxide (**13**) obtained in 48% yield after preparative TLC was identical with authentic material as evidenced by NMR and mixture melting point.

Reaction of 5α,6α-Epoxycholest-7-en-3β-ol (2b) with Water. Phosphate buffer (25 mL, 0.1 M, pH 4.4) was added to 7-dehydrocholesterol 5α,6α-oxide (**2b**, 300 mg) dissolved in tetrahydrofuran (25 mL) and stirred at room temperature for 5 min. The product was extracted into ether, and the ether was dried over sodium sulfate. The crude material, which contained a triol (**5a**, 70%) and a diene diol (**6**, 30%) as indicated by NMR, was suspended in hot hexane and filtered. The diene component contained in the filtrate was purified by HPLC on a Waters radial-compression silica colum (8 × 100 mm) using 20% dioxane/hexane as solvent; flow rate = 2.2 mL/min, t_R = 10 min: $[\alpha]_D$ +73.4° (*c* 0.5, $CHCl_3$); UV λ_{max} 253 (ε 15 000); NMR δ 0.77 (s, 3 H, C-19), 0.91 (s, 3 H, C-18), 4.08 (m, 1 H, H-3), 5.49 (d, 1 H, *J* = 10 Hz, H-6 or H-7), 6.21 (d, 1 H, *J* = 10 Hz, H-7 or H-6); MS, *m/e* (relative intensity) 400 (2), 382 (25), 364 (91), 349 (45), 251 (100), 197 (56). The hexane-insoluble material was recrystallized from ethyl acetate to give the triol **5a**: mp 230 °C dec. (lit.[32] mp 240–242 °C); $[\alpha]_D$ −60.4° (*c* 0.27, pyridine) (lit.[32] −60°, pyridine); NMR δ 0.59 (s, 3 H, C-18), 1.08 (s, 3 H, C-19), 3.62 (m, 1 H, $W_{1/2}$ = 10 Hz, H-6), 4.07 (m, 1 H, $W_{1/2}$ = 22 Hz, H-3), 5.34 (m, 1 H, $W_{1/2}$ = 10Hz, H-7); MS, *m/e* (relative intensity) 400 (21), 382 (50), 364 (96), 349 (48), 251 (94), 197 (77), 43 (100); HRMS calcd (M^+ − H_2O) 400.3343, found 400.3343.

Attempted separation of the above two components by preparative TLC (silica) using 20% dioxane/cyclohexane yielded only a triol (**5a**, R_f 0.03) and a triene (**7**, R_f 0.47): UV λ_{max} 286; NMR δ 0.86 (s, 3 H, C-19), 0.91 (s, 3 H, C-18), 4.30 (t, 1 H, *J* = 7.5 Hz, H-3), 5.41 (s, 1 H, H-4), 5.86 (d, 1 H, *J* = 10Hz, H-6 or H-7), 6.18

(29) The substrate (cholesterol 5,6α-oxide) concentration was kept well above the K_m value, and incubation conditions were as described in ref 5.

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(d, 1 H, J = 10Hz, H-7 or H-6).

Reaction of 5β,6β-Epoxycholest-7-en-3β-ol (12) with Water. 7-Dehydrocholesterol 5β,6β-oxide (**12**, 30 mg) was treated in the manner described above for **2b**. NMR of the crude material, which displayed no UV absorptions in the region of 200-400 nm, indicated the presence of only one component, a triol (**15**): mp 191-195 °C; $[\alpha]_D$ −20° (c 0.18, pyridine); NMR δ 0.66 (s, 3 H, C-18), 1.17 (s, 3 H, C-19), 2.76 (dt, 1 H, J = 4, 14, 14, Hz, H-1β), 3.46 (m, 1 H, H-3), 5.64 (d, 1 H, J = 10 Hz, H-6 or H-7), 5.88 (d, 1 H, J = 10 Hz, H-7 or H-6); MS, m/e (relative intensity) 400 (9), 382 (14), 364 (91), 349 (37), 287 (8), 251 (100), 197 (67); HRMS calcd ($M^+ - H_2O$) 400.3343, found 400.3328.

Reaction of Oxides 2b and 12 with Benzoic Acid. Thirty milligrams of each unsaturated oxide was dissolved in chloroform in separate tubes, benzoic acid (6 mg each) was added, and the reactions were followed by NMR. The α-oxide **2b** gave predominantly the 5α-hydroxy-6α-benzoate (**4c**) after recrystallization from benzene: mp 161–163 °C; NMR δ 0.61 (s, 3 H, C-18), 1.02 (s, 3 H, C-19), 3.93 (m, 1 H, $W_{1/2}$ = 22 Hz, H-3), 5.05 (br s, 1 H, $W_{1/2}$ = 6.5 Hz, H-6), 5.50 (br s, 1 H, $W_{1/2}$ = 6.5 Hz, H-7); MS, m/e (relative intensity) 486 (3), 471 (5), 400 (15), 382 (27), 364 (44), 349 (23), 251 (33), 197 (33), 122 (45), 105 (100). Reaction of the β-oxide **12** with benzoic acid gave exclusively **14**: $[\alpha]_D$ −56.8 (c 1.30, $CHCl_3$); NMR δ 0.59 (s, 3 H, C-18), 1.15 (s, 3 H, C-19), 4.09 (m, 1 H, $W_{1/2}$ = 8 Hz, H-3), 5.11 (br d, 1 H, J = 5.5 Hz, H-6), 5.42 (br d, 1 H, J = 5.5 Hz, H-7); MS, m/e (relative intensity) 504 (3), 486 (8), 471 (13), 400 (68), 382 (29), 364 (61), 287 (39), 122 (64), 105 (100); HRMS calcd ($M^+ - H_2O$) 504.3603, found 504.3567.

6β-[(2-Hydroxyethyl)thio]cholest-7-ene-3β,5α-diol (16). Sodium hydride (20 mg), washed with hexane and dried under nitrogen, was added to deuteriomethanol (1 mL). The α-oxide **2b** (30 mg) was then added, followed by a few drops of deuteriobenzene to aid in solubility. After the NMR spectrum was recorded, 2-mercaptoethanol (10 μL) was added and the spectrum was reobtained at various time intervals. At completion of the reaction, the mixture was dissolved in ether, and the organic phase was washed with water and dried over sodium sulfate to give the thioether **16** in quantitiative yield: mp 157–161 °C; NMR δ 0.59 (s, 3 H, C-18), 1.02 (s, 3 H, C-19), 2.77 (t, 2 H, J = 7 Hz, CH_2S-), 2.91 (m, 1 H, H-6), 3.73 (t, 2 H, J = 7 Hz, CH_2O-), 4.18 (m, 1 H, H-3), 5.30 (m, 1 H, H-7); MS, m/e (relative intensity) 460 (11), 442 (4), 427 (4), 415 (2), 397 (3), 383 (10), 365 (100); HRMS calcd ($M^+ - H_2O$) 460.3375, found 460.3416.

6α-[(2-Hydroxyethyl)thio]cholest-7-ene-3β,5β-diol (17). The β-oxide **12** is treated with 2-mercaptoethanol in the manner described above for the α-oxide **2b** to yield the thioether **17** in nearly quantitative yield: NMR δ 0.55 (s, 3 H, C-18), 0.95 (s, 3 H, C-19), 2.89 (m, 2 H, CH_2S-), 3.59 (br s, 1 H, $W_{1/2}$ = 8 Hz, H-6), 3.82 (m, 2 H, CH_2O-), 4.18 (br s, 1 H, $W_{1/2}$ = 7.5 Hz, H-3), 5.12 (br s, 1 H, $W_{1/2}$ = 6.5 Hz, H-7); MS, m/e (relative intensity) 460 (100), 442 (38), 427 (81), 415 (45), 397 (68), 383 (61), 365 (81); HRMS calcd ($M^+ - H_2O$) 460.3375, found 460.3459.

Registry No. **1b**, 434-16-2; **2b**, 95841-65-9; **3**, 95864-11-2; **4b**, 95841-66-0; **4c**, 63139-17-3; **5a**, 15361-40-7; **6**, 95841-67-1; **7**, 95841-68-2; **8**, 604-32-0; **9**, 26048-46-4; **10**, 95841-69-3; **11**, 95841-70-6; **12**, 95841-71-7; **13**, 4025-59-6; **14**, 95841-72-8; **15**, 95910-37-5; **16**, 95841-73-9; **17**, 95841-74-0; benzoic acid, 65-85-0; 2-mercaptoethanol, 60-24-2; cholesterol oxide oxidase, 55467-47-5.

Acyclic Stereocontrol through the Dianionic Claisen Rearrangement of β-Hydroxy Esters: Synthesis of (±)-Botryodiplodin

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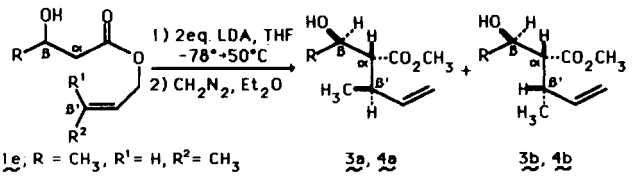
Dianionic Claisen rearrangements of (*E*)- and (*Z*)-butenyl β-hydroxy esters afford moderate stereoselection in the construction of highly functionalized acyclic systems. The reaction proceeds with remote stereocontrol and the resulting products display three contiguous chiral centers, C(α) and C(β′) stereocenters being established in the rearrangement (cf. **1z** → **3b**). Experiments are described which unambiguously establish that each dianionic Claisen proceeds with excellent diastereoface selectivity [C(α),C(β) stereocontrol] and moderate chair/boat selectivity [C(α),C(β′) stereocontrol]. Application of this Claisen protocol to a synthesis of the mycotoxin botryodiplodin is also described (**1z** → **8b**).

Development of methodology for achieving stereocontrol in the construction of acyclic systems is an important objective in current synthetic study.[1] In this regard, the regio- and stereochemically reliable Claisen rearrangement[2] has proven invaluable, particularly in self-immolative[3] transfer of chirality.[4] However, Claisen rearrangement protocols which utilize the asymmetry of a remote stereocenter to induce chirality at the prochiral termini of the rearranging 1,5-diene have received little attention.[5,6]

Scheme I



1e, R = CH_3, R^1 = H, R^2 = CH_3
1z, R = CH_3, R^1 = CH_3, R^2 = H
2e, R = $CH(CH_3)_2$ R^1 = H, R^2 = CH_3
2z, R = $CH(CH_3)_2$, R^1 = CH_3, R^2 = H

3a, **4a**
3a,b, R = CH_3
4a,b, R = $CH(CH_3)_2$

3b, **4b**

In a preliminary communication, we reported the discovery of a dianionic β-hydroxy ester Claisen rearrangement which uses remote stereocontrol to regulate the introduction of three contiguous chiral centers on an acyclic skeleton.[7] While our preliminary work established the

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