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Studies on Steroids. XLV.[1] Synthesis of the Four Stereoisomers of 20,22-Dihydroxycholesterol[2]

Masuo Morisaki, Susumu Sato, and Nobuo Ikekawa

Laboratory of Chemistry for Natural Products, Tokyo Institute of Technology[3]

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The four stereoisomers of 20,22-dihydroxycholesterol **9**, **10**, **17** and **19** were synthesized from pregnenolone. Vinylation of the 3,5-cyclo derivative **1** of pregnenolone gave the [20*S*]-carbinol **2**, which was then oxidized with *m*-chloroperbenzoic acid to afford the [22*S*]-22,23-epoxide **3** and its [22*R*]-isomer **4** (7 : 2). Reaction of **3** and **4** with i-Bu_2CuLi followed by acid treatment yielded 20*R*,22*S*-dihydroxycholesterol **9** and its 20*R*,22*R*-isomer **10**, respectively. The latter triol **10** was more effectively prepared by a Grignard reaction on the [20*S*]-20-formyl-carbinol **14**, which was derived from pregnenolone THP ether **12**, through the 1,3-dithiane derivative **13**. Oxidation of 20-dehydrocholesterol acetate **16** with OsO_4 gave stereoselectively the 20*S*,22*S*-glycol **17** (R=Ac). Treatment **17** (R=Ac) with N-chlorosuccinimide-dimethylsulfide followed by reduction with $LiAlH_4$ afforded 20*S*,22*R*-dihydroxycholesterol **19** together with the 20*S*,22*S*-isomer **17** (R=H) (3 : 2).

Acid-catalyzed epoxide opening reactions of 20,22-epoxycholesterols were also discussed.

Keywords——cholesterol C-20, 22 lyase; pregnenolone; diisobutyl cuprous lithium; base-catalyzed epoxide migration; 1,3-dithiane; 20,22-epoxycholesterol

It has been well documented[4] that 20,22-dihydroxycholesterol is a key intermediate in the metabolic degradation of cholesterol to pregnenolone, the common biogenetic precursor of steroid hormones (Chart 1). For our studies[2] on the substrate specificity of cholesterol 20,22-lyase (cytochrome $P\text{-}450_{scc}$),[5] the four stereoisomers of 20,22-dihydroxycholesterol were required.

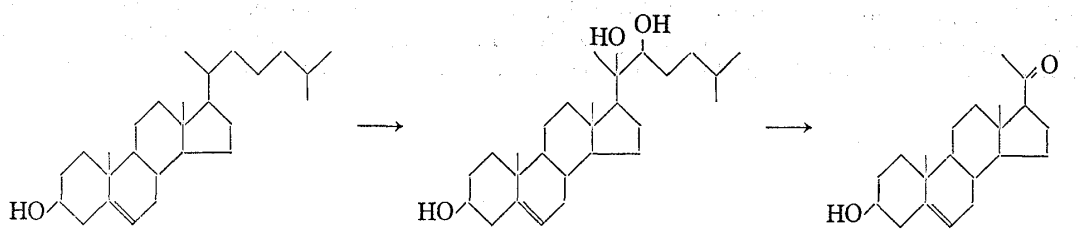

Chart 1

Although synthesis of [20*R*,22*R*]- and [20*R*,22*S*]-20,22-dihydroxycholesterols had already reported,[6] we have searched for an alternative method, especially for the more effective route to the [20*R*,22*R*]-glycol which would be, among the four stereoisomers, the most probable direct precursor of pregnenolne.[2]

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Addition of vinyl Grignard to 3α,5-cyclo-6β-methoxypregnan-20-one (**1**) gave the [20*S*]-20-vinyl alcohol **2** in 60% yield. The stereochemistry at C-20 rest on analogy to the previous observations on the nucleophilic addition reactions of 20-oxo-steroids.[7] Oxidation of the vinyl alcohol **2** with *m*-chloroperbenzoic acid yielded the [20*R*,22*S*]-22,23-epoxide **3** and its [20*R*,22*R*]-isomer **4** in a ratio of 7:2. The stereochemistry at C-22 as well as C-20 of each epoxide were deduced from their transformations to the known 20,22-glycols (**9** and **10**) as described below. The preferential formation of the [22*S*]-epoxide over the [22*R*]-isomer had been noted on the epoxidation of the analogous 20-vinyl system.[8,9]

Attempted couplings of the [20*R*,22*S*]-22,23-epoxide **3** with isobutyl magnesium bromide or isobutyl lithium gave a complex mixture of products, in which the expected 20,22-diol **7** was only scantily detected. Instead, besides the recovered epoxide, the Δ^{22}-20-ol **5** and the 20,22-epoxy-23-ol **6** were identified in the products. The epoxy alcohol **6** appeared to be formed by a base-catalyzed epoxide migration,[10] as suggested from the fact that the same compound was almost quantitatively obtained by treatment of the epoxide **3** with refluxing methanolic KOH.

The desired carbon-carbon bond formation was achieved, when the epoxide **3** was reacted with diisobutyl cuprous lithium in ether to afford the [20*R*,22*S*]-20,22-diol **7** in 90% yield. Acid treatment of **7** gave [20*R*,22*S*]-20,22-dihydroxycholesterol **9**.[6] When the 22,23-epoxide enriched with the 22*R*-isomer **4**, was similarly proceeded, [20*R*,22*R*]-20,22-dihydroxycholesterol **10**[6] was obtained through the 3,5-cyclo-derivative **8**.

To increase the yield of the [20*R*,22*R*]-triol **10**, the stereoselectivity of epoxidation on the olefin **2** should be reversed. For this purpose, the olefin **2** was subjected to iodoacetoxylation (I_2/silver acetate) or bromohydrination (N-bromosuccinimide/water) followed by base treatment. However, in both cases, the identified products were the 20,22-epoxy-23-halide **11** and the 20,22-epoxy-23-ol **6**. This may be rationalyzed by a sequence of reactions as depicted in Chart 2.

A satisfactory synthesis of [20*R*,22*R*]-20,22-dihydroxycholesterol **10** was attained in 80% yield by addition of isoamylmagnesium bromide to [20*R*]-20-formylpregn-5-ene-3β,20-diol **14** in a mixture of ethyl ether, tetrahydrofuran and benzene at 17°. The stereoselective formation of the [20*R*,22*R*]-glycol moiety has already been observed on the Grignard reaction on [20*R*]-20-formyl-20-ol system in the course of synthesis of ecdysone[11,12] and its analog.[13] The aldehyde **14** was conveniently prepared by addition reaction of 2-lithio-1,3-dithiane[14] to pregnenolone THP ether followed by treatment of the resulting adduct **13** with $HgCl_2$. The identical procedures have recently been used independently by Koreeda, *et al.*[15] in their synthesis of the [20*R*,22*R*]-glycol **10**. It may be noteworthy that the Grignard reaction on **14** in tetrahydrofuran at lower temperature (−18°) afforded the pinacolic rearrangement product **15** in a fair yield.

The other two isomers of 20,22-dihydroxycholesterol **17** and **19** were synthesized from (E)-20(22)-dehydrocholesterol acetate **16**.[16] As described previously,[17] oxidation of the

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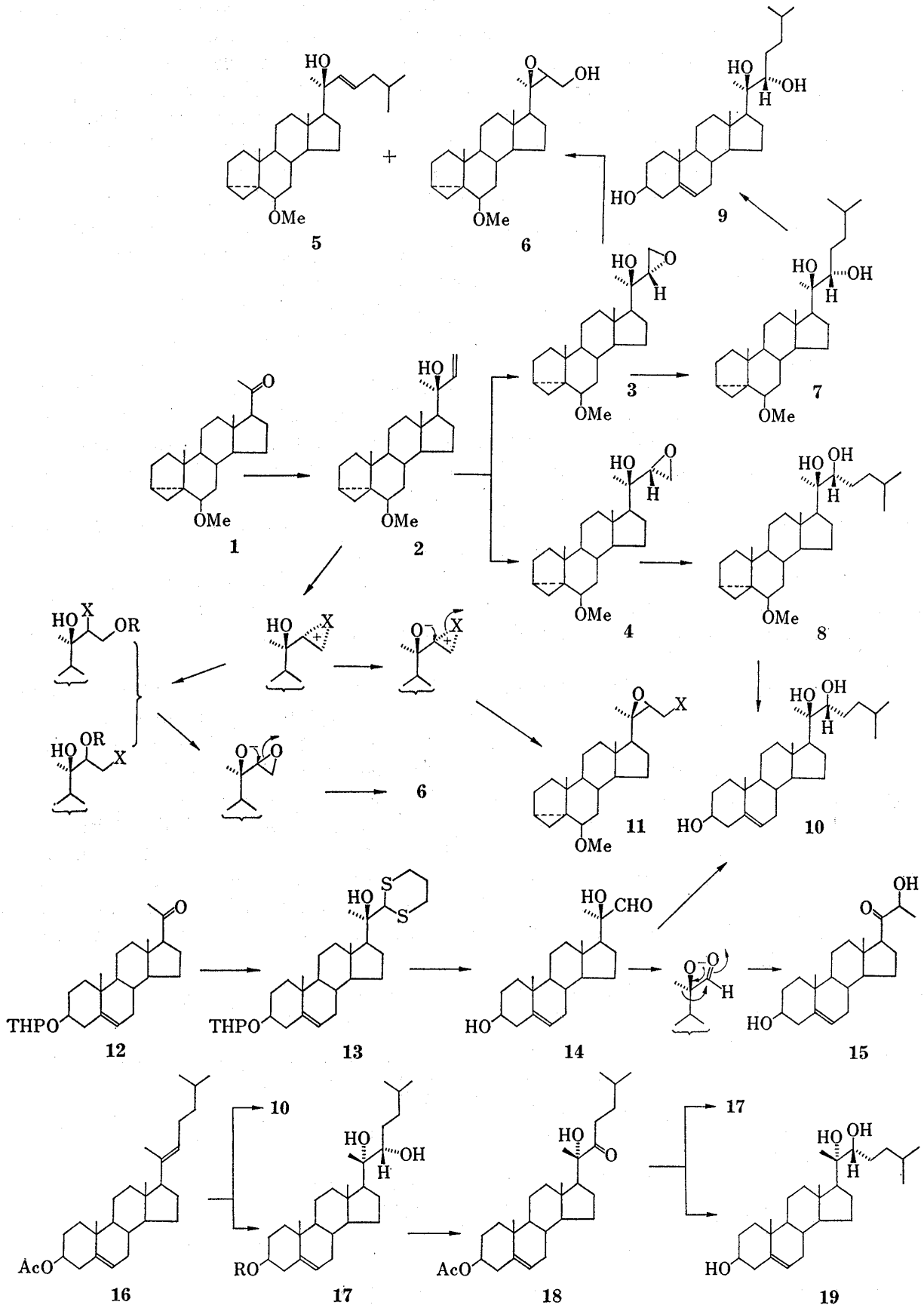

Chart 2

olefin **16** with OsO_4 gave a diastereoisomeric mixture (9: 1) of the 20,22-glycol which were resolved as their 22-benzoates by column chromatography on silica gel. Based on the previous results,[17] the [20*S*,22*S*]- and [20*R*,22*R*]- configurations were assigned to the less polar major and the more polar minor product, respectively. The subsequent hydrolysis of the major benzoate gave the [20*S*,22*S*]-triol **17** in 70% overall yield from the olefin **16**.

Treatment of the OsO_4 oxidation product from the olefin **16** with N-chlorosuccinimide-dimethyl sulfide and then with triethylamine,[18] followed by fractional crytallization of the crude products, gave the [20*S*]-22-oxo-20-ol **18** (45% from **16**).

This ketone **18** was reduced with $LiAlH_4$ to give predominantly the [20*S*,22*R*]-glycol **19**, together with the [20*S*,22*S*]-isomer **17**. The product ratio was estimated to be 3: 2 by analysis of their 3,22-dibenzoates with high pressure liquid chromatography. The predominance of the [22*R*]-isomer, as opposed to the preferential formation of the [22*S*]-isomer in the similar reduction of the [20*R*]-20-hydroxy-22-ketone system,[15,19] may be rationalised by considering the "cyclic model" "cyclic model"[20] as shown in Chart 3.

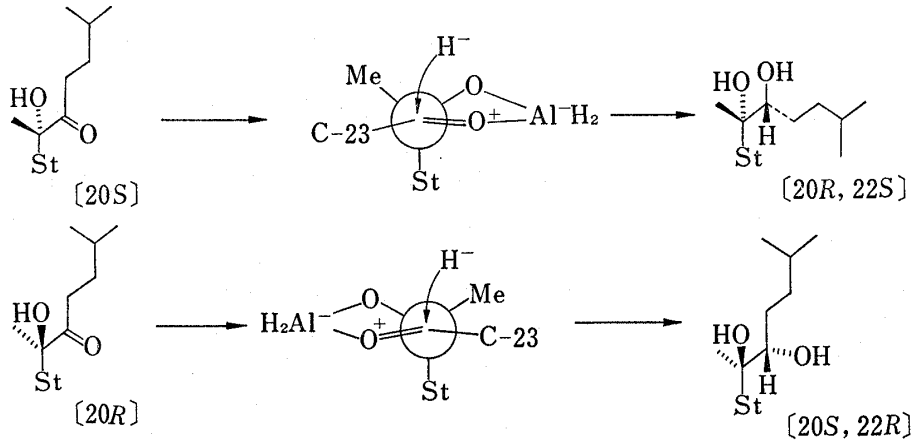

Chart 3

All four stereoisomers of 20,22-dihydroxycholesterol have now been in hand. These compounds would be also prepared by acid-catalyzed ring opening of the 20,22-epoxides (Chart 4), all stereoisomers of which have recently synthesized by three independent groups.[15,17,19] In this connection, it is interesting to note that Kraaipoel, *et al.*[21] have postulated, in pregnenolone biogenesis, an epoxide hydrase which would catalyze the formation of 20,22-dihydroxycholesterol from the hypothetical,[22] but improbable[22,23] precursor, 20,22-epoxycholesterol.

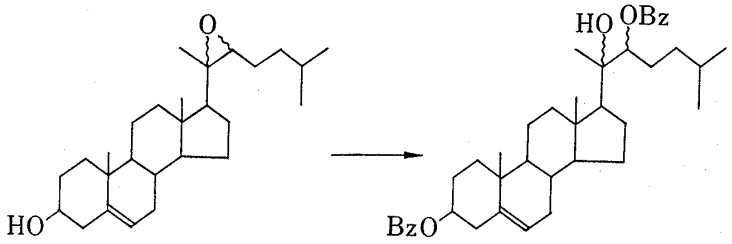

Chart 4

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23) S. Burstein, C.Y. Byon, H.L. Kimdall, and M. Gut, *Steroids*, **27**, 691 (1976).

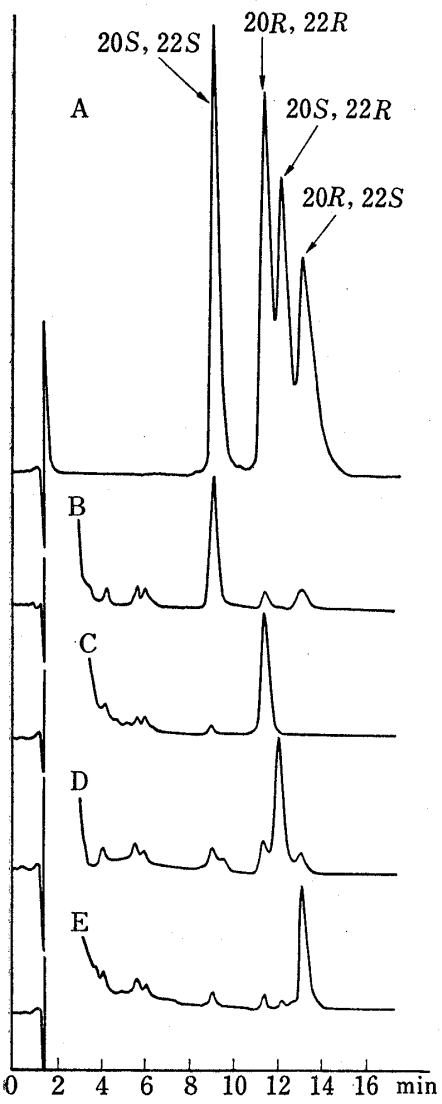

Fig. 1. High pressure liquid chromatography of cholest-5-ene-3β,20, 22-triol 3,22-dibenzoates produced by acid-catalyzed reaction of 20,22-epoxycholesterols followed by benzoylation, with Shimadzu-DuPont 830

Column, Zorbax SiL(25 cm×2.1 mm); mobile phase, CH_2Cl_2-*n*-hexane (10:1); pressure, 93 kg/cm^2. A, standard samples; B, product$_8$ from the 20*R*, 22*S*-epoxide; C, product from the 20*S*, 22*R*-epoxide; D, product from the 20*R*, 22*R*-epoxide; E, product from the 20*S*, 22*S*-epoxide.

The four 20,22-epoxides were thus, separately treated with $HClO_4$ in tetrahydrofuran and the resulting 20, 22-glycols were analyzed as their 3,22-dibenzoates by high pressure liquid chromatography which clearly resolves the four stereoisomers (Fig. 1). Fig. 1 illustrates that almost single stereoisomer of the 20,22-glycol was produced from the respective 20,22-epoxide. The stereochemistry of the glycol formed was conveniently determined by cochromatography with authentic samples. The results indicate that the hydroxide anion reacted regioselectively on C-20 in any of the 20,22-epoxide, with inversion of the configuration, and this mode of ring opening is in marked contrast to the assumed[21] action of the epoxide hydrase which might catalyze a nucleophilic attack of the hydroxide anion on the sterically less hindered carbon,[24] *i.e.* C-22.

Hikino, *et al.*[13] have pointed out that mass spectra of some stereoisomers of 20,22-dihydroxycholestanol showed peaks at *m/e* 419 for M$^+$—1 but not those for M$^+$. All the four stereoisomers of 20,22-dihydroxycholesterols **9**, **10**, **17** and **19** presently prepared have no M$^+$ peak but only M$^+$—1 peak (*m/e* 417). Even their tris-trimethylsilyl ethers showed peaks at *m/e* 633 (M$^+$—1) but no M$^+$ peak.

Our recent experiments[2] have shown that, among the four stereoisomers of 20,22-dihydroxycholesterol, only the 20*R*,22*R* and the 20*R*,22*S* isomers were effectively converted to pregnenolone by incubations with cholesterol 20,22-lyase, and the former was a better substrate than the latter.

Experimental

Melting points were determined with a hot-stage microscope and are uncorrected. Nuclear magnetic resonance (NMR) spectra were obtained with a Hitachi R-24A spectrometer for solutions in $CDCl_3$. Chemical shifts (δ) are given in ppm downfield from internal TMS and coupling constants (*J*) expressed in Hz. MS were run on a Shimadzu-LKB 9000S instrument. Column chromatography was effected with silica gel (Wakogel C-200). Thin-layer chromatography (TLC) was carried out with Merk precoated Kieselgel 60 F_{254} (0.25 mm thick). "The usual work-up" refers to extraction with organic solvent, washing to neutrality, drying ($MgSO_4$), filtration, and evaporation under vacuum. The following abrreviations are used: THF, tetrahydrofuran; s, singlet; d, doublet; q, quartet; m, multiplet.

3α,5-Cyclo-pregnan-6β-ol-20-one Methyl Ether (1)——A solution of pregn-5-en-3β-ol-20-one tosylate (54.7 g) in a mixture of pyridine (240 ml) and methanol (2.5 l) was refluxed for 3 hr. Methanol was evaporated off and the residue was diluted with water. The usual work-up using ethyl acetate for extraction and crystallization from *n*-hexane gave the methyl ether **1** (30.7 g), mp 123—126°, δ 0.65 (3H, s, 13-Me), 1.00 (3H, s, 10-Me), 2.08 (3H, s, 20-Me), 2.76 (1H, m, 6-H), 3.27 (3H, s, MeO), *m/e* 330 (M$^+$), 315 (M–Me), 298 (M–MeOH), 283 (298—Me), 255 (C-17, 20 cleavage with loss of MeOH). *Anal.* Calcd. for $C_{22}H_{34}O_2$: C, 79.95; H, 10.37. Found: C, 80.20; H, 10.36.

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[20*S*]-3α,5-Cyclo-norchol-22-ene-6β,20-diol 6-Methyl Ether (2)——A solution of vinyl bromide (25 ml) in THF (250 ml) was added to magnesium during 30 min, and the mixture was refluxed for 30 min. To this Grignard reagent a solution of the methyl ether **1** (21.0 g) in THF (450 ml) was dropwise added with maintaining the temperature below 5°. The mixture was stirred vigorously at 5° for 1.5 hr and then 17° for 48 hr. Addition of the saturated solution of aqueous NH_4Cl an the usual work-up gave amorphous powder (22.5 g). A portion (10 g) of the crude product was chromatographed on silica gel column. Elution with benzene–ethyl acetate (50: 1) and crystallization from *n*-hexane gave the vinyl alcohol **2** (6.0 g), mp 94—96°, δ 0.87 (3H, s, 13-Me), 1.02 (3H, s, 10-Me), 1.32 (3H, s, 20-Me), 2.74 (1H, m, 6-H), 3.28 (3H, s, MeO), 4.87 (1H, dd, J=2 and 9, 23-Ha), 5.10 (1H, dd, J=2 and 14, 23-Hb), 5.94 (1H, dd, J=9 and 14, 22-H), *m/e* 358 (M^+), 343 (M–Me), 326 (M–MeOH), 255 (M–MeOH with C-17, 20 cleavage). *Anal.* Calcd. for $C_{24}H_{38}O_2$: C, 80.39; H, 10.68. Found: C, 80.49; H, 10.76.

Epoxidation of the Vinyl Carbinol (2)——To a stirred solution of the vinyl carbinol **2** (138 mg) in CH_2Cl_2 (5 ml) was added *m*-chloroperbenzoic acid (114 mg) at 0°. After stirring for 24 hr at 17°, the mixture was diluted with CH_2Cl_2 and washed with 2 N NaOH. The usual work-up gave an amorphous material (129 mg), a part (22 mg) of which was chromatographed on TLC developed with benzene–ethyl acetate (15: 1) for 6 times to give the less polar [22*S*]-epoxide **3** (14 mg) and the more polar [22*R*]-epoxide **4** (4 mg). **3**: mp 172—174° (from *n*-hexane–acetone), δ 0.89 (3H, s, 13-Me), 1.01 (3H, s, 10-Me), 1.37 (3H, s, 20-Me), 2.5—3.1 (4H, m, 6, 22- and 23-H_4) and 3.26 (3H, s, OMe), *m/e* 374 (M^+), 359 (M–Me), 342 (M–MeOH), 331 (C-20, 22 cleavage), 299 (331−MeOH), 255 (M–MeOH with C-17, 20 cleavage). *Anal.* Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.94; H, 10.34. **4**: δ 0.89 (3H, s, 13-Me), 1.01 (3H, s, 10-Me), 1.24 (3H, s, 20-Me), 2.5—3.1 (4H, m, 6, 22- and 23-H_4) and 3.29 (3H, s, OMe).

Reaction of the Epoxide 3 with iso-Butyllithium——To a suspension of lithium metal (190 mg) in ethyl ether (40 ml) was added at −25° a solution of isobutyl bromide (1.89 g) in ethyl ether (10 ml) under argon during 15 min. After stirring at −25° for 1 hr a solution of the epoxide **3** (0.93 g) in ethyl ether (80 ml) added at −20°. Stirring was continued at −10° for 30 min and then at 20° for 15 min. Addition of cold saturated solution of NH_4Cl and the usual work-up using ethyl acetate for extraction gave colorless crystals (0.99 g), which was chromatographed on silica gel. Elution with benzene–ethyl acetate (50: 1) gave the 22-ene-20-ol **5** (74 mg), δ 0.88 (3H, s, 13-Me), 0.89 (6H, d, J=6, 25-Me_2), 1.03 (3H, s, 10-Me), 1.33 (3H, s, 20-Me), 2.79 (1H, m, 6-H), 3.34 (3H, s, OMe), 5.59 (2H, m, 22, 23-H_2). The bis-trimethylsilyl ether of **5** showed *m/e* 486 (M^+), 471 (M–Me), 439 (M–Me–MeOH), 396 (M–TMSOH), 381 (M–TMSOH–MeOH). Continued elution with benzene/ethyl acetate (15: 1) gave the recovered epoxide **3** (750 mg).

20,22-Oxido-3α,5-cyclo-norcholane-6β,23-diol 6-Methyl Ether (6)——(a) Further elution with benzene-ethyl acetate (5: 1) on the above chromatography gave the epoxide **6** (47 mg), mp 131—134° (from acetone–*n*-hexane), δ 0.82 (3H, s, 13-Me), 1.02 (3H, s, 10-Me), 1.32 (3H, s, 20-Me), 2.5—3.1 (2H, m, 6- and 22-H_2), 3.31 (3H, s, OMe), 3.74 (2H, d, J=6, 23-H_2), *m/e* 374 (M^+), 359 (M–Me), 342 (M–MeOH), 312 (M–MeOH with C-22, 23 cleavage). *Anal.* Calcd. for $C_{24}H_{38}O_3$: C, 76.96; H, 10.23. Found: C, 76.98; H, 10.15. Acetylation of **6** with acetic anhydride/pyridine gave the corresponding acetate, which was in turn reduced with $LiAlH_4$ to give 3α,5-cyclo-norcholane-6β,20,23-triol 6-methyl ether, δ 0.88 (3H, s, 13-Me), 1.02 (3H, s, 10-Me), 1.35 (3H, s, 20-Me), 2.75 (1H, m, 6-H), 3.31 (3H, s, OMe), 3.8 (2H, m, 23-H_2). The bis-trimethylsilyl ether of this diol showed *m/e* 520 (M^+), 505 (M–Me), 488 (M–MeOH), 473 (M–Me–MeOH), 430 (M–TMSOH), 403 (cleavage of C-20, 22), 371 (M−403−MeOH).

(b) A mixture of [20*R*,22*S*]-22,23-epoxide **3** (100 mg) and 5% methanolic KOH (10 ml) was refluxed for 3.5 hr. The usual work-up using ethyl acetate for extraction gave the epoxide **6** (99 mg), which was identified with an authentic sample described above in respect of TLC and NMR.

[20*R*,22*S*]-3α,5-Cyclo-cholestane-6β,20,22-triol 6-Methyl Ether (7)——A solution of isobutyl bromide (2.2 ml) in ethyl ether (5 ml) was added at −25° to a suspension of Li metal (280 mg) in ethyl ether (10 ml) under argon during 15 min. After stirring at −25° for 1 hr, the solution was added to CuI (1.92 g) in ethyl ether (5 ml). To this freshly prepared solution of diisobutyl cuprous lithium, was added a solution of the epoxide **3** (374 mg) in ethyl ether (50 ml) at −25° under argon and the mixture was stirred at this temperature for 4 hr. Addition of saturated solution of NH_4Cl and the usual work-up using ethyl ether for extraction, gave colorless crystals (450 mg), which was chromatographed on silica gel. Elution with benzene–ethyl acetate (100: 3) afforded the 20, 22-diol **7** (390 mg), mp 161—162° (from acetone), δ 0.88 (6H, d, J=6, 25-Me_2), 0.93 (3H, s, 13-Me), 1.03 (3H, s, 10-Me), 1.27 (3H, s, 20-Me), 2.78 (1H, m, 6-H), 3.25 (1H, m, 22-H), and 3.32 (3H, s, OMe), *m/e* 414 (M–H_2O), 399 (414−Me), 382 (414−MeOH), 331 (C-20, 22 cleavage), 299 (331−MeOH). *Anal.* Calcd. for $C_{28}H_{48}O_3$: C, 77.72; H, 11.18. Found: C, 77.46; H, 11.11.

[20*R*,22*R*]-3α,5-Cyclo-cholestane-6β,20,22-triol 6-Methyl Ether (8)——A solution of isobutyl bromide (2.2 ml) in ethyl ether (5 ml) was added at −25° to a suspension of Li metal (290 mg) in ehtyl ether (10 ml) under argon during 15 min. After stirring at −25° for 1 hr, the solution was added to CuI (1.9 g) in ethyl ether (5 ml). A solution of the 22,23-epoxide enriched with [22*R*]-isomer **4** (400 mg) in ethyl ether (50 ml) was added to the freshly prepared solution of iso-Bu_2CuLi at −25° under argon and the mixture was stirred at this temperature for 1 hr. Addition of saturated solution of NH_4Cl and the usual work-up using ethyl ether for extraction, gave colorless crystals (392 mg), which was chromatographed on silica gel column. Elution with benzene–ethyl acetate (100: 1) afforded the 20*R*,22*S*-diol **7** (37 mg). Further elution with

benzene–ehtyl acetate (50: 1) afforded a mixture (138 mg) of the 20*R*,22*S*-diol **7** and its 20*R*,22*R*-isomer **8**. Continued elution with the same solvent gave the 20*R*,22*R*-diol **8** (75 mg), δ 0.90 (6H, d, J=6, 25-Me_2), 0.94 (3H, s, 13-Me), 1.03 (3H, s, 10-Me), 1.20 (3H, s, 20-Me), 2.77 (1H, m, 6-H), 3.1—3.6 (1H, m, 22-H), 3.32 (3H, s, –OMe), *m/e* 414 (M–H_2O), 399 (414–Me), 382 (414–MeOH), 331 (C-20, 22 cleavage).

[20*R*,22*S*]-Cholest-5-ene-3β,20,22-triol (9)——A solution of the methyl ether **7** (170 mg) in THF (2 ml) was added at 0° to a stirred mixture of dimethylsulfoxide (3 ml), water (5 ml) and 60% $HClO_4$ (0.2 ml). Stirring was continued at 0° for 1 hr and then 20° for 4 days. The usual work-up using ethyl acetate for extraction gave an amorphous material (147 mg). Crystallization from methanol gave the triol **9** (91 mg), mp 187—189° (lit.[5] mp 187—189°), δ 0.87 (3H, s, 13-Me), 0.90 (6H, d, J=6, 25-Me_2), 1.00 (3H, s, 10-Me), 1.26 (3H, s, 20-Me), 3.1—3.9 (2H, m, 3, 22-H_2) and 5.4 (1H, m, 5-H), *m/e* 417 (M−1), 400 (M–H_2O), 385 (400 −Me), 382 (M–2H_2O), 367 (382−Me), 349 (367−H_2O), 317 (C-20, 22 cleavage), 299 (317−H_2O), 273 (C-17, 20 cleavage). Heating of **9** with trimethylsilylimidazole at 80° for 1 hr gave the corresponding tris-TMS ether, *m/e* 633 (M−1), 619 (M–Me), 529 (619−TMSOH), 461 (C-20, 22 cleavage), 371 (461−TMSOH), 345 (C-17, 20 cleavage), 281 (371−TMSOH).

[20*R*,22*R*]-Cholest-5-ene-3β,20,22-triol (10)——(a) A solution of isoamyl bromide (0.65 ml) in ethyl ether/benzene (1: 1) (10 ml) was added to magnesium (125 mg) and the mixture was stirred at 17° for 30 min and then refluxed for 30 min. To this Grignard reagent (5 ml) was added dropwise a solution of the aldehyde **14** (173 mg) in a mixture of THF (4 ml), ethyl ether (3 ml) and benzene (3 ml) at 0° during 15 min and the whole mixture was stirred at 17° for 15 hr. Addition of saturated solution of aqueous NH_4Cl, the usual work-up using ethyl acetate for extraction and crystallization from $CHCl_3$ gave the 20,22-glycol **10** (167 mg), mp 179—180° (lit,[5] mp 178—180°), δ 0.88 (6H, d, J=6, 25-Me_2), 0.89 (3H, s, 13-Me), 1.01 (3H, s, 10-Me), 1.20 (3H, s, 20-Me), 3.2—3.8 (2H, m, 3 and 22-H), 5.35 (1H, m, 6-H).

(b) A solution of the methyl ether **8** (42 mg) in THF (0.5 ml) was added at 0° to a stirred mixture of dimethylsulfoxide (5 ml), water (1.0 ml) and 60% $HClO_4$ (250 μl). Stirring was continued at 0° for 1 hr and then 20° for 4 days. The usual work-up using ethyl acetate for extraction gave an amorphous material (45 mg). The product was chromatographed on TLC developed with benzene–ethyl acetate (3: 1) for 3 times to give the [20*R*,22*R*]-3β,20,22-triol **10** (20 mg).

Iodoacetoxylation of the Vinyl Alcohol 2 followed by Base Treatment——To a stirred solution of the vinyl alcohol **2** (250 mg) in acetic acid (6 ml), were added silver acetate (250 mg) and then iodine (190 mg). The mixture was stirred at 30° for 2 hr. The usual work-up using CH_2Cl_2 for extraction gave an oil (305 mg), which was chromatographed on silica gel column. Elution with benzene gave the iodo epoxide **11** (X=I) (184 mg), δ 0.81 (3H, s, 13-Me), 1.01 (3H, s, 10-Me), 1.29 (3H, s, 20-Me), 2.4—3.5 (4H, m, 6, 22, 23-H_4), 3.25 (3H, s, OMe), *m/e* 484 (M^+). Further elution with benzene–ethyl acetate (50: 1) afforded the 22,23-iodoacetate (107 mg), δ 0.83 (3H, s, 13-Me), 1.02 (3H, s, 10-Me), 1.35 (3H, s, 20-Me), 2.05 (3H, s, OAc), 2.5—3.4 (m, H adjacent to I), 3.26 (3H, s, OMe), 3.6—4.5 (m, H adjacent to OAc). The iodo acetate (50 mg) was stirred with K_2CO_3 (50 mg) in methanol (4 ml) at 25° for 24 hr to give the epoxy alcohol **6** (42 mg), which showed the identical behaviors in TLC, NMR and MS with those of the authentic sample mentioned above.

Bromohydrination of the Vinyl Alcohol 2 followed by Base Treatment——N-Bromosuccinimide (40 mg) was added at 0° to a stirred solution of the vinyl alcohol **2** (70 mg) in THF–H_2O (4: 1) (2.0 ml) during 10 min. Stirring was continued at 0° for 2.5 hr and then at 30° for 4 hr. The usual work-up using CH_2Cl_2 for extraction gave an oil (92 mg), which was chromatographed on silica gel column. Elution with benzene gave the bromoepoxide **11** (X=Br) (49 mg), δ 0.83 (3H, s, 13-Me), 1.02 (3H, s, 10-Me), 1.35 (3H, s, 20-Me), 2.4—3.7 (4H, m, 6, 22, and 23-H_4), 3.26 (3H, s, OMe). Further elution with CH_2Cl_2–acetone (10: 1) gave the 22,23-bromohydrin (20 mg), δ 0.90 (3H, s, 13-Me), 1.01 (3H, s, 10-Me), 1.36 (3H, s, 20-Me), 2.6—4.5 (4H, m, 6, 22, and 23-H_4), 3.26 (3H, s, OMe). The bromohydrin (20 mg) was stirred with K_2CO_3 (20 mg) in methanol (2 ml) at 25° for 48 hr to give the epoxy alcohol **6** (14 mg) identified with the anthentic sample as mentioned above.

[20*R*]-20-(1,3-Dithianyl-2)-pregn-5-ene-3β,20-diol 3-Tetrahydropyranyl Ether (13)——1,3-Dithiane was prepared by the published method.[14] To a stirred solution of 1,3-dithiane (3.6 g) in THF (20 ml), was added 20 ml of *n*-butyl lithium solution (0.1 g/ml of *n*-hexane) at −18° under argon and the mixture was stirred at 17° for 1.5 hr. To the resulting 2-lithio-1,3-dithiane solution, was added dropwise a solution of pregnenolone tetrahydropyranyl ether (**12**) (5.70 g) in THF (20 ml) at −18° during 20 min, and the mixture was stirred at 17° for 18 hr. Addition of 2 N HCl, the usual work-up using ethyl acetate for extraction and crystallization from *n*-hexane–acetone gave the 20-carbinol **13** (6.65 g), mp 205—208°, δ 0.87 (3H, s, 13-Me), 1.00 (3H, s, 10-Me), 1.42 (3H, s, 20-Me), 2.7—3.0 (4H, m, H_4 adjacent to S), 3.2—4.1 (3H, m, 3-H and 5′-H_2 of THP group), 4.15 (1H, s, 22-H), 4.72 (1H, m, 2′-H of THP group), 5.35 (1H, m, 6-H). *Anal.* Calcd. for $C_{30}H_{48}O_3S_2$: C, 69.18; H, 9.29. Found: C, 69.01; H, 9.29.

[20*R*]-20-Formylpregn-5-ene-3β,20-diol (14)——A mixture of the dithiane derivative **13** (1.01 g), mercuric chloride (2.7 g) and 80% aqueous acetonitrile (100 ml) was refluxed under argon for 4 hr. The precipitate was removed by decantation and the upper solution diluted with ethyl acetate, was washed with 5 M ammonium acetate. The usual work-up followed by crystallization from *n*-hexane–acetone gave the aldehyde **14** (0.53 g), mp 184—188°, δ 0.80 (3H, s, 13-Me), 1.00 (3H, s, 10-Me), 1.34 (3H, s, 20-Me), 3.5 (1H, m, 3-H), 5.37 (1H, m, 6-H), 9.60 (1H, s, CHO), *m/e* 346 (M^+), 328 (M–H_2O), 317 (C-20,22-cleavage), 313 (328−Me), 299

(317−H_2O), 273 (C-17, 20 cleavage), 255 (273−H_2O). *Anal.* Calcd. for $C_{22}H_{34}O_3$: C, 76.24; H, 9.89. Found: C, 76.06; H, 9.80.

20-Oxo-21,23-bisnorchol-5-ene-3β,22-diol (15)——A solution of isoamyl bromide (0.15 ml) in THF (1 ml) was added to magnesium (36 mg) and the mixture was stirred at 17° for 1.5 hr. To this Grignard reagent was added dropwise a solution of the aldehyde **14** (87 mg) in THF (5 ml) at −18° during 10 min and the whole mixture was stirred at 17° for 20 hr. Addition of saturated solution of aqueous NH_4Cl, and the usual work-up using ethyl acetate for extraction gave the crude product which was chromatographed on silica gel column. Elution with benzene–ethyl acetate (100: 1) afforded a mixture (79 mg) of the triol **10** and the ketol **15.** The ketol **15** (35 mg) was isolated by crystallization from hexane–acetone. **15**: mp 174—177°, $\nu_{max}^{CHCl_3}$ 1700 cm^{-1}, δ 0.81 (3H, s, 13-Me), 1.03 (3H, s, 10-Me), 1.35 (3H, d, J=6, 23-Me), 3.4 (1H, m, 3-H), 4.2 (1H, q, J=6, 22-H), 5.37 (1H, m, 6-H). *Anal.* Calcd. for $C_{22}H_{34}O_3$: C, 76.26; H, 9.89. Found: C, 76.21 H, 10.00. Trimethylsilylation of **15** with trimethylsilyl imidazole gave the tris-TMS ether, *m/e* 562 (M^+), 547 (M–Me), 472 (M–TMSOH), 457 (472−Me), 345 (C-17, 20 cleavage), 255 (345−TMSOH).

[20*S*,22*S*]-Cholest-5-ene-3β,20,22-triol (17,R=H)——Oxidation of the $\Delta^{20(22)}$-olefin **16** was carried out as described previously.[17] The crude product (730 mg) was kept in a mixture of benzoyl chloride (0.28 ml) and pyridine (2 ml) at 15° for 18 hr. The usual work-up using CH_2Cl_2 for extraction gave the 22-benzoate, which was chromatographed on silica gel column. Elution with benzene gave the [20*S*,22*S*]-benzoate (690 mg) mp 168—170° (from methanol), δ 0.85 (3H, d, J=6, 25-Me_2), 0.98 (3H, s, 13-Me), 1.02 (3H, s, 10-Me), 2.00 (3H, s, OAc), 4.5 (1H, m, 3-H), 5.4 (2H, m, 6- and 22-H_2). Further elution with benzene–ethyl acetate (10: 1) gave the [20*R*,22*R*]-benzoate (70 mg), mp 288—290° (from methanol), δ 0.86 (6H, d, J=6, 25-Me_2), 0.92 (3H, s, 13-Me), 1.07 (3H, s, 10-Me), 1.39 (3H, s, 20-Me), 2.00 (3H, s, OAc), 4.5 (1H, m, 3-H), 4.9—5.5 (2H, m, 6- and 22-H_2). Heating of the [20*S*,22*S*]-benzoate (224 mg) with a mixture of 5% methanolic KOH (8 ml) and benzene (1 ml) at 70° for 30 min gave the [20*S*,22*S*]-triol **17** (126 mg), mp 169—171° (from acetone), δ 0.86 (3H, s, 13-Me), 0.89 (6H, d, J=6, 25-Me), 0.99 (3H, s, 10-Me), 1.05 (3H, s, 20-Me), 3.2—4.0 (2H, m, 3 and 22-H_2), 5.38 (1H, m, 6-H). $C_{27}H_{44}O_2$ (M–H_2O) requires *m/e* 400.334. Found: 400.332.

[20*S*]-22-Oxo-cholest-5-ene-3β,20-diol 3-Acetate (18)——To a suspension of N-chlorosuccinimide (458 mg) in toluene (5 ml) was added dimethyl sulfide (250 μl) at 0° under argon. To this cooled (−18°) mixture, was added a solution of the 20,22-diol 3-acetate (632 mg), the crude OsO_4 oxidation product from the olefin **16** described above, in a mixture of toluene (2 ml) and THF (2 ml) during 10 min. After stirring for 3 hr at −25°, triethylamine (0.4 ml) was added and the mixture was stirred at 15° for 5 min. The usual work-up using ethyl ether for extraction gave colorless powder (633 mg), which was crystallized twice from acetone gave the [20*S*]-22-ketone **18** (396 mg), mp 160—161°, δ 0.80 (3H, s, 13-Me), 0.93 (6H, d, J=6, 25-Me_2), 0.98 (3H, s, 10-Me), 1.31 (3H, s, 20-Me), 2.01 (3H, s, OAc), 4.6 (1H, m, 3-H), 5.4 (1H, m, 6-H), *m/e* 443 (M−Me), 398 (M–AcOH), 359 (C-20, 22 cleavage), 341 (359−H_2O), 299 (359−AcOH). *Anal.* Calcd. for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.17; H, 10.12.

[20*S*,22*R*]-Cholest-5-ene-3β,20,22-triol (19)——To a solution of the ketone **18** (50 mg) in THF (5 ml) was added $LiAlH_4$ (30 mg) and the mixture was stirred at 17° for 30 min. The usual work-up using ethyl acetate for extraction gave colorless powder (45 mg). The crude triol was benzoylated in the usual manner to give the 3,22-dibenzoate (69 mg). High pressure liquid chromatography (see Fig. 1) of this benzoate indicated the presence of [20*S*,22*R*]- and [20*S*,22*S*]-isomers in a ratio of 3: 2. The more polar benzoate (24 mg) was isolated by preparative thin-layer chromatography developed with benzene for 4 times, followed by crystallization from *n*-hexane–acetone. Hydrolysis of the benzoate with 5% methanolic KOH (2 ml) followed by crystallization from *n*-hexane–acetone gave the [20*S*,22*R*]-triol **19** (10 mg), mp 173—174°, δ 0.89 (3H, s, 13-Me), 0.90 (6H, d, J=6, 25-Me_2), 1.01 (3H, s, 10-Me), 3.2—3.9 (2H, m, 3- and 22-H_2), 5.4 (1H, m, 6-H). $C_{27}H_{44}O_2$ (M–H_2O) requires *m/e* 400.334. Found: 400.332.

Acid Treatment of 20,22-Epoxycholesterol followed by Benzoylation——[20*S*,22*S*]-20,22-Epoxycholesterol[17] (8 mg) was kept in a mixture of 3% $HClO_4$ (320 μl) and THF (2 ml) at 17° for 24 hr and then at 45° for 4 hr. The crude product was stirred in a mixture of benzoyl chloride (10 μl) and pyridine (0.3 ml) at 17° for 3 hr. The usual work-up gave the benzoate (11 mg), which was subjected to analysis with high pressure liquid chromatography (Fig. 1). The other three stereoisomers of the 20,22-epoxide[17] were similarly treated. The standard samples of the 3,22-dibenzoate were prepared by benzoylation of the triols **9, 10, 17** and **19** in the usual manner.

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