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SYNTHESIS OF THE 11-OXYGENATED CHOLESTEROLS AND DERIVATIVES*

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Abstract—The Woodward (Grignard) synthesis of cholestanol has been extended to the preparation of cholesterol and related stenols by employing a Δ^5-3β-ol precursor, masking the 5,6-double bond of the Grignard product by epoxidation, eliminating the hydroxyl group at C-20 *via* the olefin, and regenerating the Δ^5-3β-ol system. This method has been used to prepare 11-ketocholesterol for the first time and, from it, the 11-hydroxycholesterols, the 11-keto- and 11β-hydroxycholestenones and some related compounds.

THIS WORK WAS UNDERTAKEN both because the 11-oxygenated cholesterols have not previously been prepared and because we believe that these new derivatives will be useful to those studying various aspects of cholesterol metabolism. For example, the adrenal does not 11-hydroxylate cholesterol itself even though the latter is a precursor of the adrenal steroids. Since the 11-oxygenated cholesterols are therefore abnormal substrates, it would be interesting to determine the fate of 11β-hydroxycholesterol (as a model) in the adrenal steroid biogenesis system and its effects, including inhibition, on other reactions occurring in this system.

We initially considered the method of Woodward *et al.*[1†] which involves a Grignard condensation between the appropriate 20-ketosteroid and *iso*-hexylmagnesium bromide with subsequent elimination of the C-20 hydroxyl group *via* the olefin. The method is attractive in that the pregnane precursor can be easily prepared, but it is limited to the synthesis of cholestanol and related stanols and suffers the serious disadvantage of generating a significant proportion of the 20-*iso* product with its attendant separation problem.

It would be useful to extend this method to the synthesis of cholesterol and related stenols. This we have done, in the specific case of 11-ketocholesterol, by the method indicated in Scheme I. In outline, 3β-acetoxypregn-5-ene-11,20-dione (**1**) is condensed with *iso*-hexylmagnesium bromide and the resulting 20-carbinol (**2**) is sequentially epoxidized and dehydrated, providing a mixture of epoxyolefins the chief component of

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†Chaudhuri[1] has developed a new synthesis of cholesterol which, while lengthy (eight steps from pregnenolone acetate), avoids the separation problem above. It appears unsuited for the preparation of the 11-oxygenated cholesterols since substitution of the appropriate 11-ketone in the Grignard condensation affords the desired product in only 10 to 15% yield.

which is the 5α,6α-oxido-$\Delta^{21,21}$-olefin **3**. Subsequent steps consist of catalytic reduction of the double bond, regeneration of the Δ^5-3β-ol system, and column chromatographic separation of the normal product (**4**) from its 20-*iso* companion (**5**).

The required 20-ketopregnene acetate (**1**) had been prepared earlier[2] by a method which involves some isomerization of the side chain, and for the present purpose was synthesized in another way as follows. Treatment of the readily available pregn-4-ene-3,11,20-trione (11-ketoprogesterone) with AcCl and Ac_2O[3] provided 3β-acetoxypregna-3,5-diene-11,20-dione, 85% yield. This conversion so exceeds that recorded for the preparation of progesterone 3-enol acetate (48%)[3] as to suggest that the conformational distortion induced by the C-11 carbonyl group promotes the reaction (the "conformational transmission" effect of Barton *et al.*[4]). Masking of the C-20 carbonyl group of the enol acetate was effected by converting it to the semicarbazone. This step was simplified by arranging for the prompt separation of the derivative in a crystalline, easily filtered form. It was necessary only to wash the product with water and MeOH and dry prior to the next step. Reduction of the enol acetate semicarbazone was carried out with excess $NaBH_4$ in alkaline EtOH aq, at about 0° to prevent reduction of the C-11 carbonyl group.[5] At the conclusion of this reaction the semicarbazone moiety was removed by mild acid hydrolysis and the neutral fraction recovered, acetylated and chromatographed. Overall yield of diketone **1** was 40 to 45%, based on 11-ketoprogesterone. This sequence may also be used to prepare 3β,11β-dihydroxypregn-5-en-20-one simply by allowing the reduction to proceed for several days at room temperature followed by processing as above.

The Grignard reaction was carried out under the conditions of Petrow and Stuart-Webb[6] except that reflux was extended to 6 hr. Recovery of the neutral fraction, followed by acetylation and chromatography, gave the desired 3β,20α-dihydroxycholest-5-en-11-one 3-acetate (**2**), 50% yield. The assigned structure is based on its elemental analysis and IR spectrum together with the fact that its reduction by the Barton modification of the Wolff-Kishner (W-K-B) method,[7] followed by acetylation and chromatography, gave the known cholest-5-ene-3β,20α-diol 3-acetate as sole product.*

Epoxidation of the Grignard product (**2**) proceeded nearly to completion using *m*-chloroperoxybenzoic acid in CH_2Cl_2 at room temperature. It was apparent that two oxides were formed and, although their separation is not essential for the purpose of this synthesis, it was easily effected on a small scale by partition chromatography of the corresponding 3β-ols. The more mobile oxide was judged to be the 5α,6α-isomer since its specific rotation (−23°) and that of its acetate (−26°) are, in order, more negative than those observed for the free alcohol (0°) and the acetate (−10°) of the less mobile 5β,6β-epimer. The 5α,6α : 5β,6β ratio was approximately 6 : 1.

Dehydration of the tertiary hydroxyl group at C-20 traditionally has been effected with refluxing AcOH. But the reaction proceeds slowly and is accompanied by some hydrolysis of the acetoxyl group. $POCl_3$ in pyridine at room temperature was wholly

* Saponification of the mother liquor, followed by chromatography of the neutral fraction on a partition-type column, gave a small fraction well separated from and more mobile than the diol derived from **2**. Judging from the elemental analysis of its crystalline acetate, it is an isomer of **2**. But W-K-B reduction gave an amorphous product clearly different from the known 3β,20β-diol 3-acetate. It is generally agreed[8,9] that the Woodward (Grignard) method furnishes 20α-ols exclusively.

inactive, but $SOCl_2$ in pyridine at *ca.* 0° rapidly effected dehydration without altering the epoxide ring.*

In reducing the mixture of olefins we were limited, by the sensitivity of the epoxide ring, to catalysts of moderate activity. Hydrogenation at atmospheric pressure in EtOAc solution using 5% Pd/C proved satisfactory. Later it was found that AcOH could be used as solvent without disrupting the oxide group but, contrary to the view of Sondheimer and Mechoulam,[10] this did not increase the yield of the desired product (**4**).

Regeneration of the Δ^5-3β-acetoxy system was carried out with the reagent of Cornforth *et al.*[12] This was complicated in a minor way by the generation of some material more polar (less mobile) than the desired products (**4**, **5**) and which was unaffected by treatment with Ac_2O–pyridine. It seems likely that this by-product results from reductive elimination of the iodine atom of the intermediate iodohydrin, thus leaving a hydroxyl group (of unknown configuration) at the C-5 position. This view appeared to be confirmed by the observation (Fudge *et al.*[13]) that brief treatment of the Cornforth product with $SOCl_2$ in pyridine wholly converted the by-product into the **4**, **5** mixture.

Following column chromatography of the final mixture, the separated products (**4** and **5**) were characterized, and hence distinguished, by conversion to the corresponding 11-deoxystenols. Thus the more mobile acetate (**5**) gave, on reduction by the W-K-B method, 20-*iso*cholesterol as the sole product and may therefore be designated 3β-acetoxy-20-*iso*cholest-5-en-11-one. Similarly reduced, the polar product (**4**) gave cholesterol itself and therefore is 3β-acetoxycholest-5-en-11-one or, trivially, 11-ketocholesterol acetate. The mass spectra of the acetates were essentially identical; in each case the molecular ion was not seen and the most prominent peak was *m/e* 382 (M-60). As has been noted for the 20-*iso*cholesterol–cholesterol pair,[10] the specific rotations of **5** (−32°) and **5a** (−27°) are, respectively, slightly more negative than those of **4** (−10°) and **4a** (0°). (The utility and limitations of this and other criteria as a means of distinguishing between members of normal : 20-*iso* pairs will be discussed at a later point in this paper.)

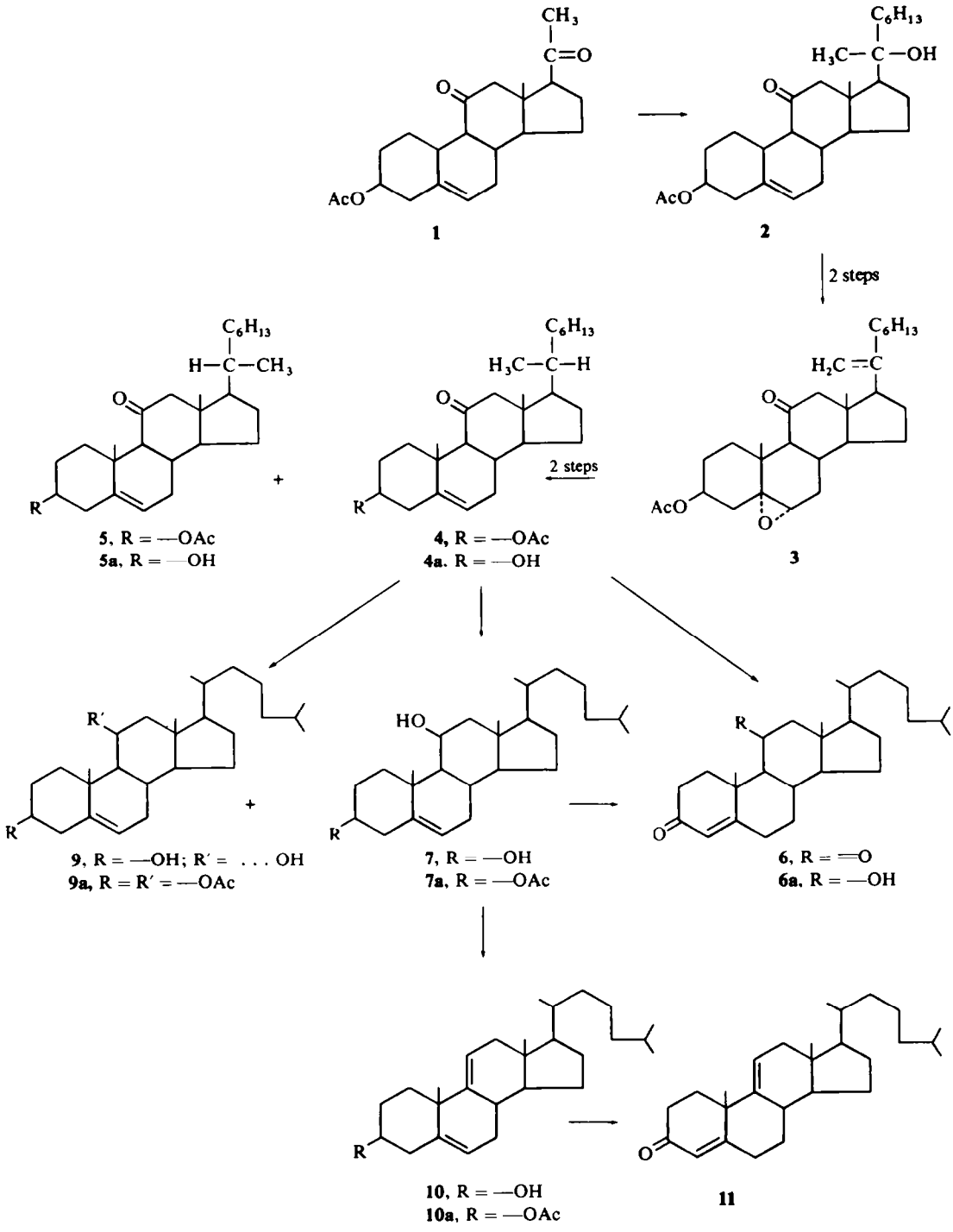
As Scheme I illustrates, a number of other compounds were prepared from 11-ketocholesterol or its acetate in order to confirm their structures and logically extend the series. All were prepared without difficulty using well-known reactions.

Thus oxidation of **4a** by the Oppenauer method[14] gave cholest-4-ene-3,11-dione (11-ketocholestenone, **6**), easily recognized from its UV and IR spectra. This confirmed the presence of the Δ^5-3β-ol system in the former.

Reduction of the C-11 carbonyl group of **4** was best effected with LAH. The product was assigned the structure cholest-5-ene 3β, 11β-diol (11β-hydroxycholesterol, 7) on the basis of its ability to form a monoacetate only (**7a**) and on the UV and IR spectral properties of its Oppenauer oxidation product, namely 11β-hydroxycholest-4-en-3-one (11β-hydroxycholestenone, **6a**).

* No reliable information is available on the composition of the olefinic mixture obtained in such cases since as many as five olefins ($\Delta^{20,21}$, *cis* and *trans* $\Delta^{20,22}$ and *cis* and *trans* $\Delta^{17,20}$) can be formed, all displaying similar chromatographic properties. In the above case some information was obtained by dehydrating the pure 5α,6α-oxide of the diol acetate **2**. Following column chromatography, an apparently homogeneous crystalline product was recovered in *ca.* 50% yield. Judging from its elemental analysis and IR spectrum [bands at 1640 and 890 cm^{-1} (terminal methylene[10]) and at 871 cm^{-1} (epoxide ring[11])], it is the 5α,6α-oxido-$\Delta^{20,21}$-olefin **3**.

SCHEME I


CH3
C=O
O
AcO
1
C6H13
H3C—C—OH
2
2 steps
C6H13
H—C—CH3
5, R = —OAc
5a, R = —OH
C6H13
H3C—C—H
2 steps
4, R = —OAc
4a, R = —OH
C6H13
H2C=C
3
R′
9, R = —OH; R′ = . . . OH
9a, R = R′ = —OAc
HO
7, R = —OH
7a, R = —OAc
6, R = =O
6a, R = —OH
10, R = —OH
10a, R = —OAc
11

The 3β,11β-diol acetate **7a** was further characterized by its ready dehydration in $SOCl_2$–pyridine[15] to the $\Delta^{5,9(11)}$-diene acetate **10a**. The structure of this product was apparent from its elemental analysis and IR spectrum, but was also confirmed by its catalytic reduction to the previously prepared 5α-cholest-9(11)-en-3β-ol acetate[16] and by Oppenauer oxidation of the corresponding 3-ol (**10**) to the $\Delta^{4,9(11)}$-diene-3-one **11**.

Preparation of cholest-5-ene-3β,11α-diol (11α-hydroxycholesterol, **9**) was easily accomplished by reducing 11-ketone **4** with sodium in 1-propanol.[17] The assigned structure is based on the observations that the product is a diol but different from **7**, and that it furnished a diacetate (**9a**) on treatment with Ac_2O–pyridine. The Oppenauer oxidation product of the diol could not be crystallized either as the free ketol or as its 11-acetate, but further oxidation of the free ketol with CrO_3–pyridine[18] gave a crystalline product identical with the reference sample of cholest-4-ene-3,11-dione (**6**).

In order to extend the earlier comparison of 11-ketocholesterol with its 20-*iso* companion to other normal : 20-*iso* pairs and thus derive physical criteria whereby members of such pairs can be distinguished, additional companions were prepared giving, in all, 20-*iso* compounds corresponding to **4a**, **4**, **7**, **7a**, **6** and **6a**. These comparative studies gave the following results. First, M_D normal→*iso* increments were, in order, −108, −99, −74, −68, −38 and + 11. These values indicate that the 20-*iso* member has a slightly more negative rotation in simple stemols but that changes elsewhere in the molecule, notably conversion of the Δ^5-3β-ol structure to the Δ^4-3-keto system, reduce or reverse such differences. Second, distinction on the basis of IR spectra must be regarded uncertain since only differences in band *intensities* were observed. Third, in five pairs the melting point of the 20-*iso* member was, on the average, six degrees lower than the normal member; in the sixth case (the 7a-*iso* pair) the *iso* member melted fifteen degrees higher. Fourth, the 20-*iso* member of a given pair is always, if sometimes very slightly, chromatographically more mobile than the normal member (TLC; Experimental section). While the above differences have some utility, it is recognized that NMR spectroscopy is the most reliable method for making these distinctions.

After this work was completed, it was noted that the specimen of 11-ketocholestenone (**6**), which had been obtained as colourless rods *ca.* 6 months earlier, had become faintly yellow. Further examination showed that its m.p. had fallen about 6° and its specific rotation 7°. Recrystallization afforded colorless rods with the original constants, but TLC analysis of the yellow mother liquor showed that one or more UV-absorbing, very polar, neutral components were present. While we have no doubt as to the identity and purity of the original specimen, a new sample was prepared from **4a** as before. It had the same constants as originally observed for the pure diketone **6** as did the sample of **6** prepared from the 3β,11α-diol **9**. Understandably, all the remaining compounds prepared in this series were re-examined at this point; all were unchanged.

The TLC evidence suggests that the deterioration of the diketone **6** is chiefly autoxidative in type, characterized by the insertion of hydroxyl or hydroperoxyl groups into the nucleus of side chain. Our reason for mentioning this observation is to indicate two structural features which may be influential. The first is that the molecular rotation (M_D, + 751) and the rotational increment (Δ) for the introduction of the C-11 carbonyl group (+ 414) in **6** are higher than we have observed for other 11-ketones in this or other series. Granted that these constants are "abnormally" high, it seems reasonable to

suggest that they reflect a degree of conformational distortion. The second, and more interesting, feature is that the corresponding 20-*iso* compound (20-*iso*cholest-4-ene-3,11-dione, m.p. 104–105°, α_D +179°, M_D +713) is perfectly stable. This demonstrates that the observed deterioration of **6** requires some interaction between the C-11 carbonyl group and the (normal) side chain.

EXPERIMENTAL

Reactions were examined by TLC (silica gel, IB-F) to evaluate composition and chromatography systems. Most mixtures were fractionated on columns of silica gel (Davison, grade 923); the effluent was collected fractionally and examined by TLC. Unless otherwise indicated, systems consisted of EtOAc–*iso*octane mixtures. Each is indicated in the text by a number which corresponds to one of the following compositions [in each case the number is followed (in parenthesis) by that volume of EtOAc which, diluted to 25 ml with *iso*octane, comprises the system]: **1** (9), **2** (4), **3** (2), **4** (7), **5** (3), **6** (0·93), **7** (1·2), **8** (5), and **9** (2·5 ml). Compounds lacking a UV chromophore were detected by spraying the developed plates with a 20% ethanolic solution of *p*-TsOH, heating to 120°, and examining under a lamp emitting maximally at about 360 mμ.

M.ps were obtained on a Fisher–Johns apparatus and are uncorrected. Optical rotations were determined in $CHCl_3$ solution in a Zeiss 0·005° photoelectric polarimeter at 25 ± 2° and at a concentration of around 1%. UV spectra were obtained in MeOH solution in a Zeiss RPQ 20A recording instrument and IR spectra as KBr dispersions in a Beckman IR-8 spectrophotometer. Elemental analyses were performed by Alfred Bernhard, West Germany.

Preparation of 3β-*acetoxypregn*-5-*ene*-11,20-*dione* (**1**). A solution of 11-ketoprogesterone (30 g, Upjohn Co., recrystallized from MeOH) in AcCl (900 ml) and Ac_2O (600 ml) was refluxed gently in a N_2 atmosphere for 3 hr. After removal of solvents *in vacuo*, the neutral residue was crystallized from CH_2Cl_2–MeOH, furnishing 29 g (85%) of pure 3β-*acetoxypregna*-3,5-*diene*-11,20-*dione*, m.p. 143–145°; α_D + 15°; λ_{max} 233 mμ, ε = 19,300; ν_{max} 3040 (w), 1755, 1710–1700, 1671, 1640 cm^{-1} (w), absent OH. (Calc. for $C_{23}H_{30}O_4$ (370·47): C, 74·56; H, 8·16; CH_3CO, 11·62. Found: C, 74·42; H, 8·20; CH_3CO, 10·45%).

To a hot solution of the above enol acetate (25·9 g) in a mixture of pyridine (133 ml) and MeOH (560 ml), a solution of semicarbazine hydrochloride (78 g) in water (130 ml) was added in one portion with vigorous stirring. The product separated almost immediately as small needles. After standing at room temp overnight, the product was recovered by filtration, washed with water and MeOH and dried *in vacuo* over anhydrous $CaCl_2$ to yield 27·5 g of 3β-*acetoxypregna*-3,5-*diene*-11-*one* 20-*semicarbazone* as a white powder. A sample crystallized from CH_2Cl_2–MeOH had the constants: m.p. > 300° (dec.); α_D −10°; λ_{max} 231 mμ, ε = 34,000; ν_{max} 3505, 3400, 3260–3210, 3180–3130, 1760, 1715–1685 cm^{-1}. (Calc. for $C_{24}H_{33}O_4N_3$ (427·52): C, 67·42; H, 7·78; N, 9·83. Found: C, 67·31; H, 7·87; N, 9·97%).

To a solution of the crude semicarbazone (27·2 g) in a mixture of EtOH and $CHCl_3$ (800 ml each, chilled to around 0°), a cold solution of $NaBH_4$ (8 g) in a mixture of EtOH (720 ml) and 1 N NaOH (80 ml) was added in one portion. After thorough mixing, the mixture was maintained at 0° for 2 hr. Excess hydride was decomposed with AcOH and the solution concentrated *in vacuo* to *ca.* ⅓ its original volume. The residue was dissolved in EtOH aq and sufficient conc HCl was added to adjust the pH of the solution to around 1·5. After warming on the steam bath, the solution was allowed to stand for 2 hr at room temp, reconcentrated and worked up in the usual way. The residue was treated with 20 ml each of Ac_2O and pyridine at room temp overnight, after which the neutral product was recovered and chromatographed on a 90 × 880 mm column prepared and developed with system 1. The desired fraction furnished 11·5 g of 3β-*acetoxypregn*-5-*ene*-11,20-*dione* (**1**) as stout needles from MeOH, m.p. 136–137°; α_D +39°; ν_{max} 1729, 1710–1700, 1670 (w) cm^{-1}, absent OH. (Calc. for $C_{23}H_{32}O_4$ (372·49): C, 74·16; H, 8·66. Found: C, 74·21; H, 8·73%). Reported:[2] m.p. 133·5–167° (15% 17α-isomer). Saponification of a sample of the acetate in the usual fashion and crystallization from MeOH gave 3β-*hydroxypregn*-5-*ene*-11,20-*dione*, m.p. 173·5–174·5°; α_D +52°; ν_{max} 3480–3410, 1710–1695 cm^{-1}. (Calc. for $C_{21}H_{30}O_3$ (330·45): C, 76·32; H, 9·15; O, 14·53. Found: C, 76·30; H, 9·13; O, 14·29%). Reported:[2] m.p. 166·2–167·0°.

If this preparation is modified by allowing the reduction to proceed for several days at room temp and chromatographing as above, a lower yield of 3β,11β-*dihydroxypregn*-5-*en*-20-*one acetate* is obtained as needles from acetone, m.p. 182–182·5°; α_D + 15°; ν_{max} 3490, 1735, 1710–1700, 1690 cm^{-1}. Reported:[2] m.p. 179–180°. The corresponding free compound, 3β,11β-*dihydroxypregn*-5-*en*-20-*one*, crystallized from

acetone-n-hexane as needles, m.p. 188·5–189·5°; α_D +24°; ν_{max} 3480–3440, 1690 cm^{-1}. Reported:[2] m.p. 187–189·5°.

3β,20α-Dihydroxycholest-5-en-11-one-3-acetate (**2**) *from* **1**. To a flask containing Mg turnings (4·8 g) and dry ether (around 50 ml) was added *iso*hexyl bromide[19] (29 ml). After reaction had subsided, most of the ether was replaced with dry benzene and a solution of 3β-acetoxy-5-ene-11,20-dione (12 g in benzene) was added dropwise with stirring over 30 min. The suspension was refluxed for 6 hr and chilled, after which the complex was decomposed with sat NH_4Cl aq. After usual work-up, the neutral fraction was treated with Ac_2O–pyridine as above and chromatographed on a 70 × 890 mm column prepared and developed with system 2. The band of interest furnished 6·2 g of needles from acetone-n-hexane, m.p. 140·5–141·5°; α_D −31°; ν_{max} 3580 (plus broad shoulder at 3550–3400), 1725, 1715–1705, 1672 (w) cm^{-1}. (Calc. for $C_{29}H_{46}O_4$ (458·66): C, 75·94; H, 10·11. Found: C, 76·10; H, 10·02%).

Cholest-5-ene-3β,20α-diol-3-acetate from **2**. To a solution of Na (80 mg) in diethylene glycol (4 ml), dry hydrazine (2·2 ml) was added (this amount is just sufficient to provide a mixture which refluxes at 140–145°). To this system, cooled, 50 mg of the Grignard product was added. After a 12 hr reflux, sufficient hydrazine was distilled out of the system to raise the temperature to around 210° where it was maintained for 22 hr. The neutral fraction was recovered in the usual manner, acetylated, and chromatographed on a 12 × 580 mm column prepared and developed with system 3. Crystallization of the recovered product from MeOH gave 34 mg of needles, m.p. 158·5–159°; α_D −62°; ν_{max} 3590, 3540–3420, 1725 cm^{-1}. On admixture with an authentic preparation of cholest-5-ene-3β,20α-diol-3-acetate (m.p. 156–156·5°; α_D −60°) the m.p. was 157–157·5°. The IR spectra of the isolated and reference diol acetates were identical.

Epoxidation of **2**. To a solution of 3β,20α-dihydroxycholest-5-en-11-one (5·5 g, 12 mmole) in CH_2Cl_2 (15 ml), 2·15 g (12·4 mmole) of *m*-chloroperoxybenzoic acid (crystallized from EtOAc-n-hexane after preliminary purification[20]) in CH_2Cl_2 (20 ml) was added in one portion. The solution was kept at *ca.* 15° for 30 min, then at room temp for 2 hr. Examination of the recovered neutral fraction by TLC (system 4) showed about 90% conversion of **2** to a more polar component.

In a separate experiment, a 1 g sample of **2** was epoxidized as above, and the neutral fraction allowed to stand overnight at room temp in MeOH aq containing excess NaOH. The recovered mixture of 5,6-oxido-3-ols was chromatographed on a 54 × 890 mm column in which Celite served as the support for the stationary phase of the system toluene, 650; *iso*octane (2,2,4-trimethylpentane), 1350; MeOH, 1600; water, 400 ml. The *mobile oxide* was crystallized from MeOH to furnish 780 mg of needles, m.p. 199–200°; α_D −23°; ν_{max} 3500–3400, 1705, 870 cm^{-1}. (Calc. for $C_{27}H_{44}O_4$ (432·62): C, 74·95; H, 10·25. Found: C, 74·82; H, 10·27%). Its *acetate* crystallized from MeOH, m.p. 185–186°; α_D −26°; ν_{max} 3530, 1728, 1700, 871 cm^{-1}. (Calc. for $C_{29}H_{46}O_5$ (474·66): C, 73·38; H, 9·77. Found: C, 73·35; H, 9·82%). The *polar oxide* was well separated from its epimer and provided 120 mg of needles from MeOH aq, m.p. 157–158°; α_D 0°; ν_{max} 3520–3420, 1700, 875 cm^{-1}. (Calc. for $C_{27}H_{44}O_4$ (432·62): C, 74·95; H, 10·25. Found: C, 74·87; H, 9·99%). Its *acetate* crystallized as needles from EtOAc-n-hexane, m.p. 163–164°; α_D −10°; ν_{max} 3515, 1735–1720, 1700, 875 cm^{-1}. (Calc. for $C_{29}H_{46}O_5$ (474·66): C, 73·38; H, 9·77. Found: C, 73·46; H, 9·79%).

Dehydration of epoxide mixture. To a solution of the epoxidation products derived from 5·5 g of **2** in pyridine (50 ml, cooled in an ice bath), freshly distilled $SOCl_2$ (12·5 ml) was added in one portion with good mixing. After 15 min in the ice bath, the mixture was cautiously poured into a stirred ice–brine mixture after which the products were extracted by EtOAc and processed as usual. We separated the mixture of epoxyolefins from small amounts of very mobile contaminants by chromatography on a short, wide column (54 × 460 mm, system 5). This is probably unnecessary and even unwise since epoxides deteriorate on long exposure to silica gel.

Catalytic reduction of epoxyolefins. To a solution of the dehydration products in EtOAc (50 ml), 5% Pd/C (Engelhard Industries, 2 g) was added and the suspension gently shaken in H_2 for 2 hr. Catalyst was removed by filtration and the filtrate concentrated to dryness *in vacuo*.

Regeneration of the Δ^5*-3β-acetoxy system*. To a solution of NaI (10·2 g) and anhyd NaOAc (3·36 g) in AcOH (20 ml) and water (2·5 ml) at 15°, powdered Zn (10·2 g) was added with stirring followed by the above reduction mixture in the minimum volume of AcOH. After stirring at 15–20° for 1 hr, the mixture was diluted with brine and extracted with EtOAc. The neutral residue was dissolved in pyridine (35 ml), chilled in ice, and treated with $SOCl_2$ (8·8 ml) as above. The recovered neutral residue (5·1 g) was an orange glass.

Column chromatographic separation of **4** *and* **5**. Analysis of the final reaction residue by TLC showed that little could be discerned in addition to a closely associated pair of products. Since R_f differences

between the mobile (**5**) and polar (**4**) components of this pair do not exceed 0·03 in an appropriate TLC system, it was apparent that a column of unusual efficiency would be required to effect their separation on a large scale. Ultimately it was found that by increasing column length and substantially decreasing both the polarity and rate of flow of the mobile phase, excellent separations could be obtained. As an example, the above residue was applied to a 80 × 1500 mm column prepared and developed with system 6. The flow rate was about 60 ml per hr and the total development time about one month. [This is less tedious than it may appear since fractional collection of the effluent is required only during emergence of the mobile component (**5**) and the small mobile–polar mixture; also, some speeding up of the process can be gained by increasing slightly the polarity of the system as soon as the pure polar component (**4**) appears]. After delineating the intermediate (mixture) band by TLC (system 7), the mobile, intermediate and, finally, the polar fractions were separately pooled. Of these, only the intermediate fraction (50 mg) was fully dried.

The mobile fraction give, from acetone–MeOH, 1·85 g of 3β-*acetoxy*-20-iso*cholest*-5-*en*-11-*one* (**5**) as prisms, m.p. 92·5–93·5°; α_D −32°; ν_{max} 1732, 1702, 1670 (w) cm^{-1}, absent OH. (Calc. for $C_{29}H_{46}O_3$ (442·66): C, 78·68; H, 10·48. Found: C, 78·68; H, 10·34%). Saponification of **5** in the usual manner gave 3β-*hydroxy*-20-iso*cholest*-5-*en*-11-*one* (**5a**) as needles from MeOH aq, m.p. 134·5–135°; α_D −27°; ν_{max} 3270, 1705, 1675 (w) cm^{-1}. (Calc. for $C_{27}H_{44}O_2$ (400·62): C, 80·94; H, 11·07. Found: C, 81·25; H, 10·92%).

The polar fraction gave, from acetone–MeOH, 1·59 g of 3β-*acetoxycholest*-5-*en*-11-*one* (**4**) as prisms, m.p. 103·5–104°; α_D −10°; ν_{max} 1740, 1700, 1675 (w) cm^{-1}. (Calc. for $C_{29}H_{46}O_3$ (442·66): C, 78·68; H, 10·48. Found: C, 78·57; H, 10·33. Saponification furnished 3β-*hydroxycholest*-5-*en*-11-*one* (**4a**) as platelets from MeOH aq, m.p. 137·5—138°; α_D0°; ν_{max} 3390, 1700, 1674 (w) cm^{-1}. (Calc. for $C_{27}H_{44}O_2$ (400·62): C, 80·94; H, 11·07. Found: C, 80·82; H, 10·94%).

W-K-B reduction of **4** *and* **5**. 50 mg samples of 3β-acetoxycholest-5-en-11-one (**4**) and 3β-acetoxy-20-*iso*cholest-5-en-11-one (**5**) were reduced as above. The neutral fraction was chromatographed on a 12 × 580 mm column prepared and developed with system 8. The material recovered from **4** yielded 32 mg of platelets from acetone–MeOH, identified as cholesterol: m.p. 148·5–149·5°, α_D −39°; acetate, m.p. 115–116°, α_D −47°. Crystallixation of the residue from **5** provided 27 mg of needles, identified as 20-*iso*cholesterol: m.p. 154–155°, α_D −42°; acetate, m.p. 125–126°, α_D −53°. The IR spectra of the recovered stenols and their acetates were identical with those obtained from reference samples.

Oxidation of **4a** *to cholest*-4-*ene*-3,11-*dione* (**6**). To a solution of 3β-hydroxycholest-5-en-11-one (600 mg) in acetone (4·5 ml, freshly distilled from anhyd K_2CO_3 and benzene (10 ml, freshly distilled from P_2O_5), 510 mg of newly prepared and well dried aluminium t-butoxide was added. After 8 hr reflux the neutral fraction was recovered in the usual way. Since analysis by TLC showed that the produce was poorly separated from small amounts of unoxidized **4a**, the mixture was treated with Ac_2O–pyridine (to facilitate separation) and chromatographed on a 30 × 815 mm column prepared and developed with system 2. This furnished 85 mg of **4** as a very mobile component and, well separated from it, a larger polar band. Crystallization of the latter from MeOH gave 460 mg of needles, m.p. 118–119°; α_D + 189°; λ_{max} 238 mμ, ε = 15,750; ν_{max} 1702, 1670, 1619 cm^{-1}. (Calc. for $C_{27}H_{42}O_2$: C, 81·35; H, 10·62. Found: C, 81·31; H, 10·44%).

Cholest-5-*ene*-3β,11β-*diol* (**7**) *and its acetate* (**7a**) *from* **4**. To a solution of 3β-acetoxycholest-5-en-11-one (500 mg) in THF (25 ml, distilled from LAH), 350 mg of the hydride was added in one portion and the suspension refluxed for 3 hr. After decomposing excess reagent by the sequential addition of EtOAc, water and HCl, the neutral fraction was recovered in the usual way and chromatographed on a 30 × 870 mm column prepared and developed with system 8. Crystallization of the recovered product from MeOH aq gave 410 mg of needles, m.p. 162–162·5°; α_D −34°; ν_{max} 3520–3370 and 3280–3250 cm^{-1}, no absorption in carbonyl region. (Calc. for $C_{27}H_{46}O_2$ (402·64): C, 80·63; H, 11·52. Found: C, 80·46; H, 11·52%). Treatment of a small portion of the product with Ac_2O–pyridine, followed by the usual work-up, gave the 3-*acetate* (**7a**) as needles from MeOH, m.p. 143–144°; α_D −38°; ν_{max} 3495, 1715, 1675 cm^{-1}. (Calc. for $C_{29}H_{48}O_3$ (444·67): C, 78·33; H, 10·88. Found: C, 78·41; H, 10·64%).

11β-*Hydroxycholest*-4-*en*-3-*one* (**6a**) *from* **7**. A 200 mg sample of cholest-5-ene-3β,11β-diol was oxidized with the Oppenauer reagent as in the preparation of **6** from **4a**. The neutral fraction was chromatographed on a 25 × 710 mm column prepared and developed with system 8. Crystallization of the recovered product from CH_2Cl_2–MeOH furnished 145 mg of long needles, m.p. 191–192°; α_D + 127°; λ_{max} 243 mμ, ε = 16,400; ν_{max} 3450, 1650, 1618 cm^{-1}. (Calc. for $C_{27}H_{44}O_2$ (400·62): C, 80·94; H, 11·07. Found: C, 81·12; H, 10·89%).

Cholesta-5,9(11)-*diene*-3β-*ol* (**10**) *and its acetate* (**10a**) *from* **7a**. To a solution of cholest-5-ene-3β,11β-

diol 3-acetate (360 mg) in pyridine (3·6 ml, chilled in ice), $SOCl_2$ (0·72 ml) was added in one portion. After 10 min at 0° the neutral product was recovered as above, saponified (in order to increase the polarity of the product) and chromatographed on a 30 × 675 mm column (system 2). Crystallization of recovered product from acetone aq gave 260 mg of needles, m.p. 120–120·5°; α_D −12°; ν_{max} 3500–3380, 3050 (w), 1650–1640 (w) cm^{-1}. (Calc. for $C_{27}H_{44}O$ (384·62): C, 84·31; H, 11·53. Found: C, 84·13; H, 11·28%). Treatment of a portion of the diene with Ac_2O–pyridine, followed by crystallization of the product from acetone–MeOH, provided the *acetate* (**10a**) as needles, m.p. 97·5–98·5°; α_D −10°; ν_{max} 3050 (w), 1732, 1645 (w) cm^{-1}. (Calc. for $C_{29}H_{46}O_2$ (426·66): C, 81·63; H, 10·87. Found: C, 81·39; H, 10·61%).

Catalytic reduction of **10a**. 100 mg of cholesta-5,9(11)-diene-3β-ol acetate was reduced as in the previous example. After removal of catalyst, crystallization from MeOH provided 70 mg of *5α-cholest-9(11)-en-3β-ol acetate* as needles, m.p. 105–106°; α_D +16°; ν_{max} 3060, 1735 cm^{-1}. Reported:[16] m.p. 105°; α_D +22·5°. Saponification of the acetate, followed by crystallization of the neutral product from MeOH aq, gave *5α-cholest-9(11)-en-3β-ol* as needles, m.p. 121–122°; α_D +26°; ν_{max} 3460, 3060 cm^{-1}. Reported:[16] m.p. 122–123°; α_D +27°.

Cholesta-4,9(11)-diene-3-one (**11**) *from* **10**. A 100 mg sample of cholesta-5,9(11)-diene-3β-ol was oxidized by the Oppenauer method as in the preparation of **6** from **4a**. Chromatography of the recovered neutral fraction on a 20 × 745 mm column (system 9), followed by crystallization of the recovered product from MeOH, gave 82 mg of needles, m.p. 114–115°; α_D +85°; λ_{max} 240 mμ, ε = 17,000; λ_{max} 3080 (w), 3039 (w), 1675, 1615 cm^{-1}. (Calc. for $C_{27}H_{42}O$ (382·61): C, 84·75; H, 11·07. Found: C, 84·57; H, 10·79%).

Cholest-5-ene-3β,11α-diol (**9**) *and its diacetate* (**9a**) *from* **4**. To a solution of 3β-acetoxycholest-5-en-11-one (500 mg) in hot 1-propanol (25 ml), a total of 3 g Na was quickly added and the mixture refluxed for 30 min. After excess Na was converted to the alcoholate by MeOH, the solution was concentrated to near-dryness *in vacuo*, diluted with brine, and extracted with CH_2Cl_2. Direct crystallization of the product from MeOH aq yielded 410 mg of needles, m.p. 150·5–151°; α_D −41°; ν_{max} 3500–3300 cm^{-1}, no carbonyl absorption in carbonyl region. (Calc. for $C_{27}H_{46}O_2$ (402·64): C, 80·63; H, 11·52. Found: C, 80·40; H, 11·28. The *diacetate* (*9a*) was prepared as in the previous examples and crystallized from MeOH as platelets, m.p. 138–139°; α_D −59°; ν_{max} 1745–1730 (split) cm^{-1}, absent OH absorption. (Calc. for $C_{31}H_{50}O_4$ (486·71): C, 76·50; H, 10·36. Found: C, 76·32; H, 10·23%).

Oxidation of a 100 mg sample of diol **9** by the Oppenauer method, followed by chromatography on a 16 × 675 mm column (system 4), gave 85 mg of an UV-absorbing, chromatographically homogeneous product which resisted crystallization. To a solution of this residue in pyridine (1 ml), CrO_3 (85 mg) in pyridine (2 ml) was added. After 12 hr at room temp, the neutral residue was recovered and the product crystallized from MeOH to yield 65 mg of needles, m.p. 117·5–118·5°. Its identity as *cholest-4-ene-3,11-dione* (**6**) is based on m.p. and IR spectral comparisons with the authentic diketone.

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