Stereochemistry of the Addition Reactions of Grignard Reagents to 20-Keto Steroids. Synthesis of 17α,20α-Dihydroxycholesterol[1]

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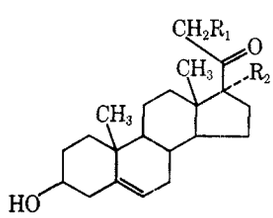
The stereochemistry of the addition reactions of Grignard reagents to 20-keto steroids and their 17α-hydroxy, 21-hydroxy, and 16α,17α-oxido derivatives has been determined by means of some stereospecific reactions, *e.g.*, hydroboration and osmylation. It has been shown that the 20-hydroxycholestanol (**9**) obtained by the addition of methylmagnesium bromide to 3β-acetoxy-21-nor-5α-cholestan-20-one (**11**) has the 20β-hydroxy configuration (as predicted by Cram's rule), since it was found to be identical with a synthetic sample prepared by hydroboration followed by oxidation of 5α-cholest-*cis*-17(20)-en-3β-ol (**8**). The 20α epimer **12** has been prepared by the addition of isohexylmagnesium bromide to 3β-acetoxy-5α-pregnan-20-one (**10**). The same stereochemical results were obtained with a 21-hydroxy-20-keto steroid. The reactions with the 17α-hydroxy derivatives, however, led to the opposite steric configurations at C-20. The 5,6-dihydro derivative of the product **15**, obtained by the addition of isohexylmagnesium bromide to 3β-acetoxy-17α-hydroxypregn-5-en-20-one (**13**), was identical with the osmylation product of **8**, which has the 20β-hydroxy configuration **16**. It was established that the compound known in the literature as "17α,20α-dihydroxycholesterol" is actually the 20β-hydroxy epimer. Authentic 17α,20α-dihydroxycholesterol (**14**) had been synthesized by the addition of methylmagnesium bromide to 3β-acetoxy-17α-hydroxy-21-norcholest-5-en-20-one (**23**). It has been shown by nmr spectroscopy that the Grignard reactions with 16α,17α-oxido-20 ketones are not stereospecific. The proportion of the 20α- and 20β-hydroxy epimers in the mixture depends on the Grignard reagent employed.

Several studies[2-4] in recent years have shown that 20α-hydroxycholesterol may serve as an intermediate in the catabolic pathway of cholesterol to pregnenolone and hence to other steroidal hormones. This led a number of investigators to synthesize this compound,[5,6] its 20 epimer,[6b] and two of its derivatives, (22*R*)-20α,22-dihydroxy-[7] and 17α,20α-dihydroxycholesterol.[8] The synthetic approach in all these cases has however been the same—namely the addition of a nucleophile to a 20-keto steroid. The nucleophile used in the synthesis of (22*R*)-20α,22-dihydroxycholesterol was hydrogen cyanide. All the other syntheses used different Grignard reagents as nucleophiles.



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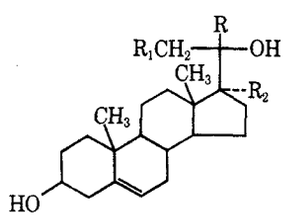

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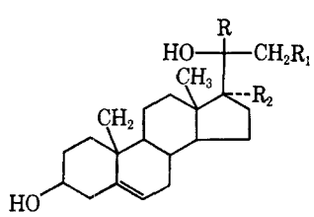

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Since a nucleophilic addition to the 20-keto group of **1** creates a new asymmetric center at C-20, the resulting tertiary alcohol may be represented by either of the structures **2** or **3**. Of these two structures, **2** (R = isohexyl; R_1, R_2 = H) has the 20α-hydroxy configuration[9] and corresponds to the absolute configuration of cholesterol at C-20 in which 20 H is α oriented. The structure **3** (R = isohexyl; R_1, R_2 = H) has the 20β-hydroxy configuration and may therefore be considered as a derivative of 20-isocholesterol. Heretofore the configuration at C-20 of these tertiary 20-hydroxy compounds has never been unequivocally established. We have now determined the stereochemical results of the addition of Grignard reagents to (1) the unsubstituted 20 ketones (**1**, R_1, R_2 = H; R_1 = isoamyl; R_2 = H), (2) 17α-hydroxy-20 ketones (**1**, R_1 = H; R_2 = OH), (3) 21-hydroxy-20 ketones (R_1 = OH; R_2 = H), and (4) 16α,17α-epoxy-20 ketones by means of some stereospecific reactions.

Reaction with Unsubstituted 20 Ketones.—Lieberman and coworkers[6b] have shown that the addition of isohexylmagnesium bromide to pregnenolone 3-acetate (**4**) gave exclusively one of the two possible 20-hydroxycholesterols and the addition of methylmagnesium bromide to 21-nor-20-keto-cholesterol 3-acetate (**5**) gave predominantly the other epimer. Since the usual methods (optical rotation and dehydration) for the determination of the absolute configuration at C-20 of the secondary alcohols belonging to the pregnane series are inapplicable in the case of these tertiary alcohols, they applied Cram's rule and assigned the 20α-hydroxy configuration **6** to the product isolated from the former reaction and the 20β-hydroxy configuration **7** to the major product isolated from the latter reaction. They also noted a significant difference in the nmr spectra of the two C-20 epimers. The chemical shift of the 21-methyl protons[10] of the 20α-hydroxy

(1) Supported by the Atomic Energy Commission Contract AT(30-1)918 and Grant AM-03419 from the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health.

(2) S. Solomon, P. Levitan, and S. Lieberman, *Rev. Can. Biol.*, **15**, 282 (1956).

(3) K. Shimizu, M. Hayano, M. Gut, and R. I. Dorfman, *J. Biol. Chem.*, **236**, 695 (1961).

(4) K. Shimizu, M. Gut, and R. I. Dorfman, *ibid.*, **237**, 699 (1962).

(5) V. Petrow and I. A. Stuart-Webb, *J. Chem. Soc.*, 4675 (1956).

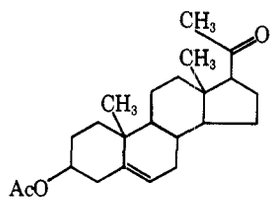
(6) (a) L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 34. (b) A. Mijares, D. I. Cargill, J. A. Glasel, and S. Lieberman, *J. Org. Chem.*, **32**, 810 (1967).

(7) K. Shimizu, M. Gut, and R. I. Dorfman, *J. Biol. Chem.*, **237**, 699 (1962). We are grateful to Professor A. Horeau, College de France, for the determination of the *R* configuration of C-22.

(8) K. Shimizu, *J. Biochem.* (Tokyo), **56**, 201 (1964).

(9) For definition of the configuration at C-20 of cholesterol and its 20-hydroxy derivatives, the Fieser convention (ref 6a, p 337) has been used in this paper.

(10) We have observed, however, that the nmr signal of the 21-methyl protons of the 20α-hydroxycholesterol appears at 1.21 ppm and that of the 20β-hydroxy epimer appears at 1.05 ppm in CCl_4 solution, whereas in deuteriochloroform they appear at 1.28 and 1.12 ppm, respectively.



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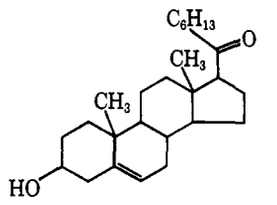

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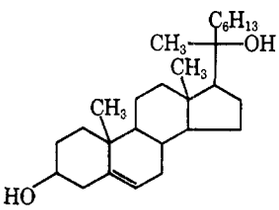

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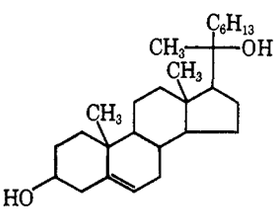

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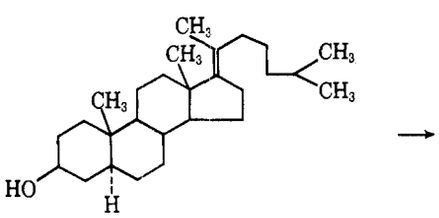
epimer (1.17 ppm) was downfield relative to that of the 21-methyl protons of the 20β-hydroxy epimer (1.00 ppm) by about 0.17 ppm. It was, however, concluded that, although a severe nonbonded interaction between the 12-methylene and the 21-methyl protons may result in the downfield shift of the 21-methyl protons of one of the epimers, *it may not be justified to assign the absolute configuration on this basis alone.* In view of the absence of a completely free rotation around the 17–20 single bond,[11] the application of Cram's rule cannot provide an unequivocal assignment either. We, therefore, sought a chemical method which would provide an unequivocal result.

Determination of Absolute Configuration at C-20.— For this purpose, we synthesised the *cis*-$\Delta^{17(20)}$-5α-cholestenol[12] (**8**) from the corresponding *cis*-$\Delta^{17(20)}$-



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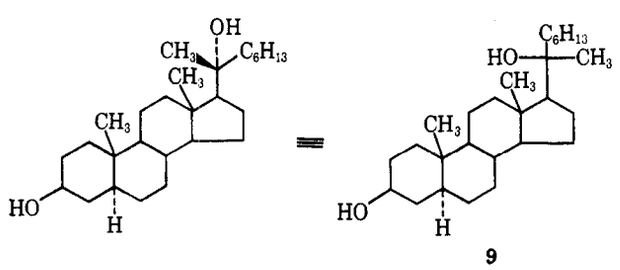


9

nitrile[13] obtained by dehydration of the cyanohydrin of 3β-acetoxy-5α-pregnan-20-one. The $\Delta^{17(20)}$ steroid **8** was then hydroborated by treatment with a 1 *M* solution of diborane in tetrahydrofuran at 0–5° for 2 hr and the resulting borane was oxidized with 30% hydrogen peroxide. The major crystalline product obtained after chromatography was found to be a 20-hydroxy compound **9** from the following considerations.

(11) S. Rakhit and C. R. Engel, *Can. J. Chem.*, **40**, 2163 (1962).

(12) The *cis* nomenclature refers to the relationship of the 18- and 21-methyl groups of **8**. Proof of the *cis* geometry around the 17–20 double bond of **8** and details of its synthesis have been described in another paper: N. K. Chaudhuri, R. Nickolson, J. G. Williams, and M. Gut, *J. Org. Chem.*, **34**, 3767 (1969).

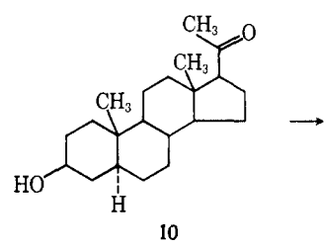
(13) N. K. Chaudhuri and M. Gut, *J. Amer. Chem. Soc.*, **87**, 3737 (1965).

Its mass spectrum showed the molecular ion peak at *m/e* 404 and the ir spectrum of its acetylated derivative showed the presence of a nonacetylated hydroxyl group. The chemical shift of the nmr signal of its 21-methyl protons (a singlet at 67 cps) was considerably downfield relative to that of the 21-methyl protons of cholesterol and cholestanol (50–51 cps). In case of 17α hydroxylation the nmr signal of the 21-methyl protons would have appeared as a doublet at a higher field. A small amount of an oil was also isolated. Its nmr spectrum suggests a mixture of a 20-hydroxy and a 17α-hydroxy compound.

The 20β-hydroxy configuration of **9** was derived from the following considerations. Brown and coworkers[14] have shown that the alcohols obtained by hydroboration of olefins are formed chiefly by *cis* addition from the less hindered side. In the case of a $\Delta^{17(20)}$ steroid the less hindered side has been found to be the "α" side from the results of the hydroboration experiments of Olivetto and coworkers[15] and the osmylation experiments of Reichstein and coworkers,[16] Sarett,[17] and Fieser and Fieser.[18]

For correlation studies, we first attempted to prepare the two epimeric 20-hydroxycholestanols by catalytic hydrogenation of the corresponding 20-hydroxycholesterols, obtained by addition of the Grignard reagents to suitable 20-keto steroids, following the procedure of Lieberman and coworkers. But the hydrogenation (in the presence of platinum and acetic acid) of the 5–6 double bond was found to be accompanied by considerable hydrogenolysis of the 20-hydroxyl group. A similar hydrogenolysis of a tertiary 17β-hydroxy and a tertiary 17β-dimethylamino steroid has been observed by others.[19] Therefore we started with the 5–6-dihydro derivatives of the 20-keto steroids and condensed them with the proper Grignard reagents to obtain the two epimeric 20-hydroxycholestanols.

The 20-hydroxycholestanol **12** obtained by the condensation of isohexylmagnesium bromide with 3β-



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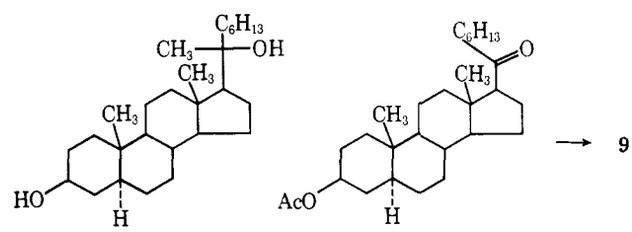


12 11 → 9

(14) H. C. Brown, "Hydroboration," W. A. Benjamin Publishing Co., New York, N. Y., 1962, p 129.

(15) A. M. Krubeiner and E. P. Olivetto, *J. Org. Chem.*, **31**, 24 (1966); A. M. Krubeiner, N. Gottfried, and E. P. Olivetto, *ibid.*, **33**, 1715 (1968).

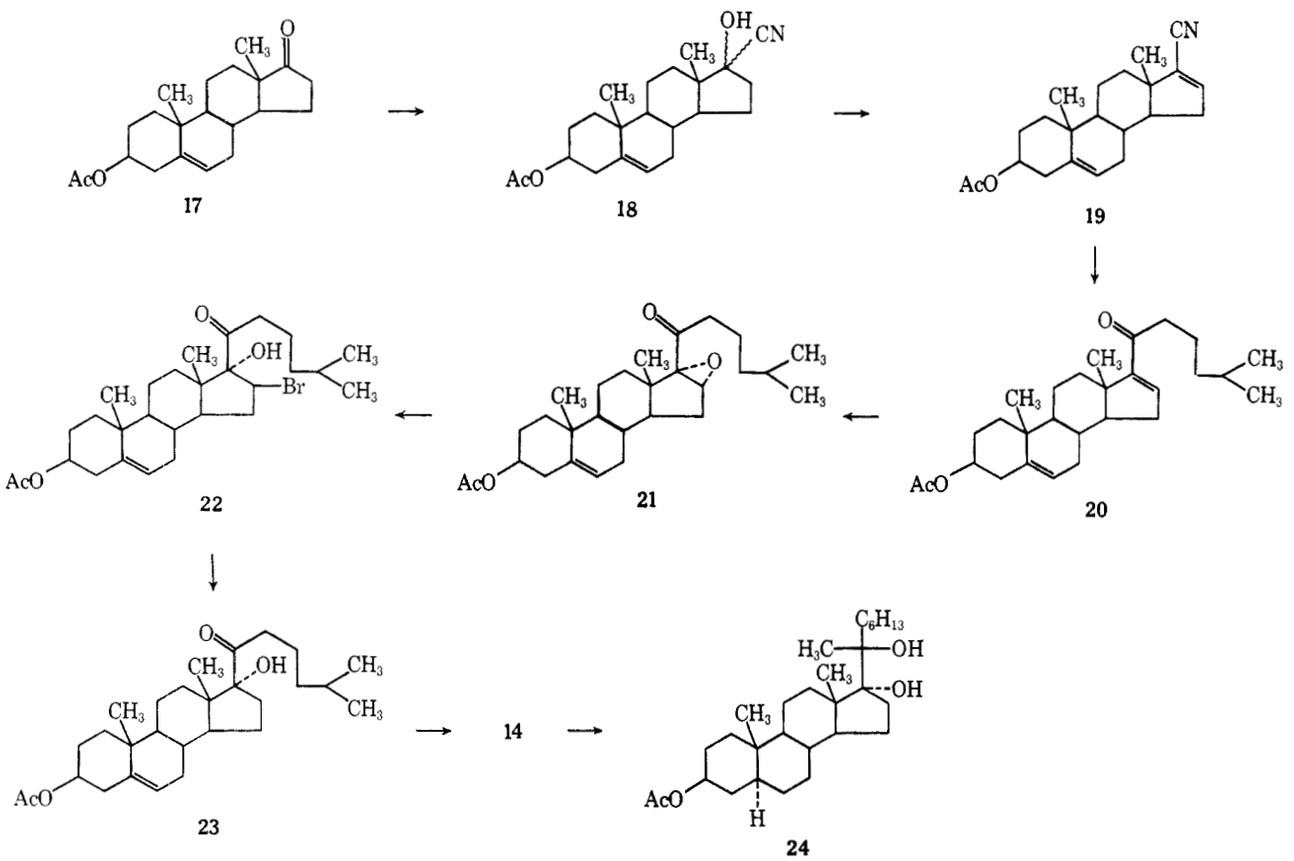
(16) H. Reich, M. Suter, and T. Reichstein, *Helv. Chim. Acta*, **23**, 170 (1940).

(17) L. H. Sarett, *J. Biol. Chem.*, **162**, 601 (1948).

(18) L. F. Fieser and M. Fieser, *Experientia*, **4**, 285 (1948).

(19) D. F. Morrow, M. E. Butler, and E. C. Y. Huang, *J. Org. Chem.*, **30**, 579 (1965); see also footnote 15 therein.

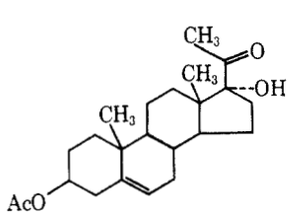
SCHEME I



acetoxy-5α-pregnan-20-one (**10**) was found to be different from **9** and therefore must have the 20α-hydroxy configuration. Whereas the 20-hydroxycholestanol obtained by the condensation of 3β-acetoxy-21-nor-5α-cholestan-20-one (**11**) with methylmagnesium bromide was identical with **9** (melting point, ir, and nmr) which has the 20β-hydroxy configuration. It may therefore be concluded that the configuration of the 20-hydroxy compounds, obtained by addition of Grignard reagents to 20-keto steroids, may be derived by application of Cram's rule, as originally assumed by Lieberman and coworkers.[6b]

The 20-keto-21-nor-5α-cholestanol 3-acetate (**11**) used in the above reaction was prepared by catalytic reduction of the unsaturated ketone **20** described in a later section (Scheme I). Although our compound had a slightly higher melting point (93–96°) than that prepared from 3β-hydroxy-5α-etianic acid by previous workers,[20] the structure **11** was confirmed by its nmr spectrum. The 18-methyl peak appeared at 35.5 cps which is comparable with that of 3β-acetoxy-5α-pregnan-20-one (**10**) at 36 cps. The alternative structure having a 17α side chain (in the case of hydrogen addition from the β face) would cause a larger downfield shift of the 18-methyl resonance.[21]

Reaction with 17α-Hydroxy-20 Ketones.—In 1964, Shimizu,[8] with the aim of synthesizing 17α,20α-dihydroxycholesterol (**14**), subjected 17α-hydroxypregnenolone 3-acetate (**13**) to the action of isohexylmagnesium bromide. The configuration of the resulting product was assumed to be 20α-hydroxy in analogy with the reaction of pregnenolone 3-acetate with isohexylmagnesium bromide, but, in view of the fact that the stereochemistry of the major product obtained by metal hydride reduction of 17α-hydroxy-20 ketones[22] is just the opposite of that of the major product obtained by similar reductions of 17-unsubstituted 20 ketones (*cf.* ref 11), it seemed that Shimizu was not justified in making his assumption without any proof in favor of the assigned structure **14**. We therefore undertook the



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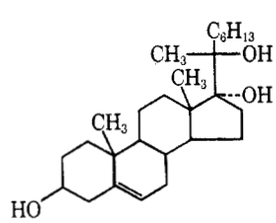

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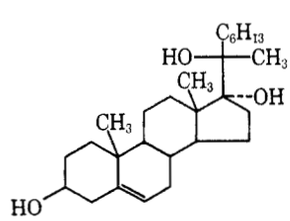

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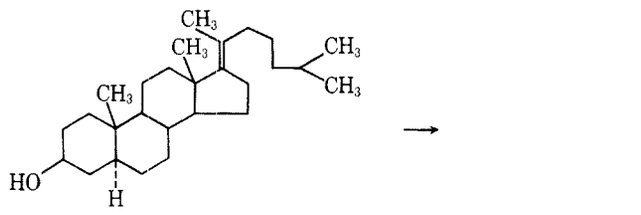
determination of the stereochemistry at C-20 of his compound. Treatment of the $\Delta^{17(20)}$ compound **8** with osmium tetroxide in a pyridine solution gave an osmate ester which was reduced with lithium aluminum hydride and the product was acetylated with acetic anhy-

(20) P. Kurath and M. Capezzuto, *J. Amer. Chem. Soc.*, **78**, 3527 (1956).

(21) A. I. Cohen and S. Rock, Jr., *Steroids*, **3**, 243 (1964); M. B. Rubin and E. C. Blossey, *J. Org. Chem.*, **29**, 1932 (1964).

(22) Reference 6a, p 567 and references therein.

dride in the presence of pyridine at room temperature and chromatographed over alumina. The major crystalline product was found by mass spectrometric analysis to have the molecular ion peak at *m/e* 462 and two unacylated hydroxyl groups. This showed that the product was a 17,20-dihydroxy compound and it must therefore be the 17α,20β-dihydroxy-20-iso-5α-cholestanol 3-acetate (**16**) because osmylation of $\Delta^{17(20)}$ steroids is known[14-16] to take place in a *cis* manner, chiefly from the less hindered "α" side. A small



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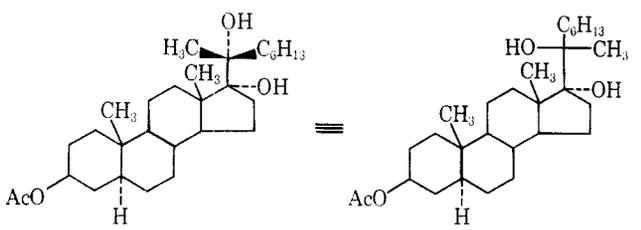

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amount of a monohydroxy diacetate (mp 126–127°) was also isolated from the earlier chromatographic fractions. We think that this compound was formed by the osmylation of a small amount of the $\Delta^{20(22)}$ isomer which was present in our sample of the $\Delta^{17(20)}$ compound **8**. For correlation studies we catalytically reduced the 5–6 double bond of the 17,20-dihydroxycholesterol 3-acetate, prepared by following the procedure of Shimizu, and found that it was identical in all respects (melting point, ir, nmr, and mass spectrum) with our synthetic sample of 3β,17α,20β-trihydroxy-20-iso-5α-cholestane 3-acetate (**16**). This shows that the assignment of the 17α,20α-dihydroxycholesterol structure (**14**) to the Grignard reaction product by Shimizu[8] is incorrect and must be revised to 17α,20β-dihydroxy-20-isocholesterol (**15**). It may also be concluded that the stereochemistry of the products obtained by addition of Grignard reagents to 17α-hydroxy-20 ketones is opposite to that obtained from C-17-unsubstituted 20 ketones.

For further confirmation of the above results and also for our biochemical studies,[23] we synthesized 17α,20α-dihydroxycholesterol (**14**) by the addition of methylmagnesium bromide to 17α-hydroxy-21-nor-20-ketocholesterol 3-acetate (**23**). Its synthesis from 3β-acetoxy-5-androsten-17-one (**17**) is described in Scheme I. The α,β-unsaturated nitrile **19**, prepared by dehydrating the cyanohydrin **18** of dehydroepiandrosterone acetate (**17**), was converted into the α,β-unsaturated ketone **20** in about 40% yield. The structure **20** was confirmed by its uv, ir, and nmr spectra. The introduction of the 17α-hydroxy group required for the preparation of **23** from **20** *via* the epoxide **21** and the bromhydrin **22** was effected by essentially the same method as that described by Julian, *et al.*,[24] for the preparation of 17α-hydroxypregnenolone from 16-dehydropregnenolone 3-acetate. The structure **23** was confirmed by comparing its nmr spectrum with that of 17α-hydroxypregnenolone 3-acetate (**13**). The nmr signals of the 18- and 19-methyl protons of both of these ketones appeared at about 42 and 61 cps in deuteriochloroform and at 30 and 58 cps in dimethyl sulfoxide-d_6, respectively.

The crude product, obtained from the reaction of methylmagnesium bromide with **23**, was acetylated with acetic anhydride and pyridine at room temperature and then chromatographed over alumina. Elution with a benzene–ethyl acetate mixture yielded the 3-acetate of **14** as the main product (yield about 45%).

The assignment of structure **14** to our synthetic compound was confirmed by oxidation experiments. As in the case of Shimizu's diol **15**, the 3-acetate of **14** on oxidation with periodate as well as lead tetraacetate yielded 3β-acetoxy-5-androsten-17-one (**17**), confirming the presence of the normal steroid nucleus and of two hydroxyl groups, one at the 17 and the other at the 20 position. The mass spectra of the two compounds **14** and **15** were also identical with respect to the fragmentation pattern. Evidently, **14** is the 20 epimer of **15**. The physical constants of **14** and **15**, their 3-acetyl derivatives and the 5α,6-dihydro derivatives of the 3-acetates (**24** and **16**) are given in the Table I.

TABLE I

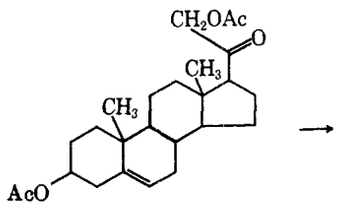
Compound	Mp, °C	Nmr peaks in cps 18 Me	21 Me
14	165–167	51	78
15	178–180	54	73.5
3-Acetate of **14**	176–178	52	78
3-Acetate of **15**	160–162	54.5	74
24	175–177	50	77.5
16	150–152	52	73.5

The most significant difference between each epimeric pair is exhibited by their nmr spectra. The chemical shifts due to the 21-methyl protons of the 17α,20α-diols are 4 cps downfield relative to those of the epimeric 17α,20β-diols although these shifts are not so large as in the case of the 20-epimeric 20-hydroxycholesterols and 20-hydroxycholestanols (which are about 10 cps).

Reaction with 21-Hydroxy-20 Ketones.—To study this reaction, we treated 21-acetoxypregnenolone 3-acetate (**25**) with isohexylmagnesium bromide. The reaction product was then acetylated with acetic anhydride in the presence of pyridine at room temperature and purified by chromatography to yield the diacetate **26** (R = $COCH_3$). The triol **26** (R = H) prepared by base hydrolysis or by lithium aluminum hydride hydrogenolysis could not be readily crystallized. In order to determine the stereochemistry at the 20 position, the triol **26** (R = H) was treated with *p*-toluenesulfonyl chloride in the presence of pyridine. The crude ditosyl derivative **27** was then boiled with aqueous acetone to hydrolyze the homoallylic 3-tosyl group and chromatographed over alumina. An oily product was obtained the infrared spectrum of which was devoid of any tosyl absorption. It was presumably the epoxide **28**, formed during the chromatography over

(23) S. Burstein, H. L. Kimball, N. K. Chaudhuri, and M. Gut, *J. Biol. Chem.*, **243**, 4417 (1968).

(24) P. L. Julian, E. W. Meyer, W. J. Karpel, and I. R. Waller, *J. Amer. Chem. Soc.*, **72**, 5145 (1950).



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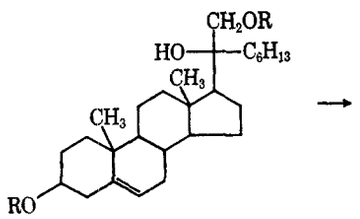

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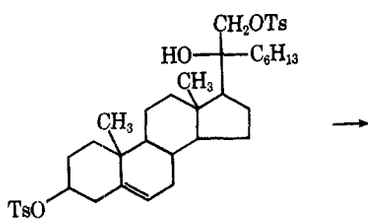

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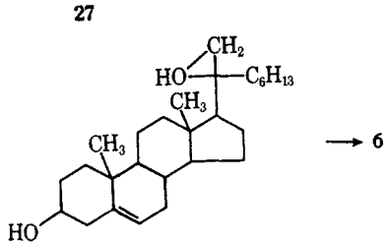


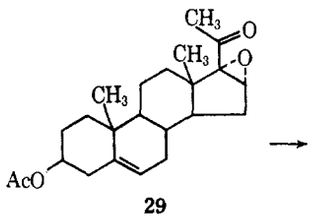
28

basic alumina. It was then reduced with lithium aluminum hydride and the crystalline product, obtained after chromatography, was found to be identical with 20α-hydroxycholesterol (**6**). Since there is no possibility of inversion of configuration at C-20 during the conversion of **26** to **6**, it may be concluded that the steric result of the addition of Grignard reagents to 21-hydroxy-20 ketones is the same as that of the unsubstituted 20 ketones.

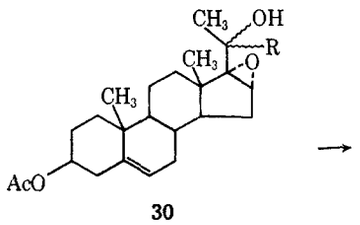
Reaction with 16α,17α-Epoxy-20 Ketones.—Finally, we investigated the reaction of 16α,17α-oxidopregnenolone 3-acetate (**29**) with three Grignard reagents, methyl-, ethyl-, and isohexylmagnesium bromide. It has been given in a patent[25] that the 16,17-epoxy group activates the 20-keto group of **29** as a result of which the addition of methylmagnesium bromide is very fast (being over in 10 min at −10°), compared to the corresponding reaction with pregnenolone 3-acetate. We repeated the above reaction and reduced the resulting epoxy alcohol **30** (R = CH_3) with lithium aluminum hydride. The triol **31** (R = CH_3) thus obtained was identical with that obtained by treating 17α-hydroxypregnenolone 3-acetate with methylmagnesium bromide.[26] The nmr spectrum of **31** (R = CH_3) showed two 21-methyl peaks (78 cps and 81.5 cps), proving thereby that the rotation around the 17–20 single bond is restricted. It may here be mentioned that the 20-

(25) P. L. Julian, J. W. Cole, E. W. Meyer and W. J. Karpel, U. S. Patent 2,887,478 (1959).

(26) M. Uskoković, M. Gut, and R. I. Dorfman, *J. Amer. Chem. Soc.*, **81**, 4561 (1959).

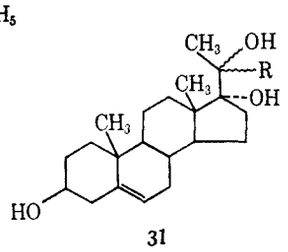


29



30

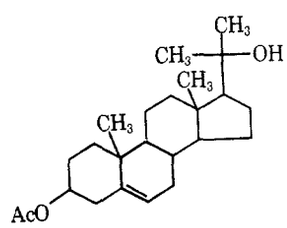
a, R = isohexyl
b, R = C_2H_5



31

a, R = isohexyl
b, R = C_2H_5

carbinol **32** behaved similarly. Two 21-methyl peaks (72 and 79 cps) were observed in its nmr spectrum. The downfield shift of one of the methyl groups of both **31** (R = CH_3) and **32** may be due to the nonbonded interaction of its protons with the 12-methylene protons.



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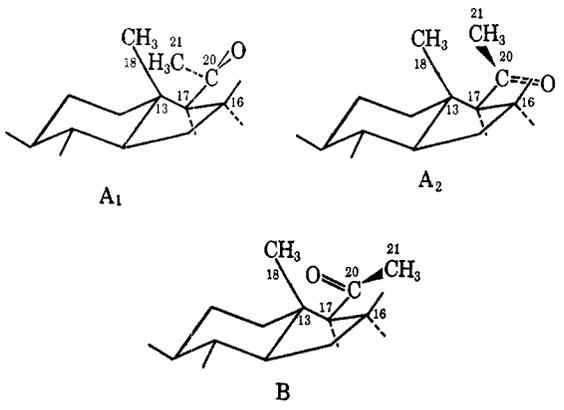
When the epoxy ketone **29** was treated with either ethylmagnesium bromide or isohexylmagnesium bromide and the resulting epoxy alcohols were reduced with lithium aluminum hydride, a mixture of 17α,20-diols was obtained in each case, as evidenced by the nmr spectra of these 17α,20-diols which showed two methyl peaks (73 and 77 cps in the case of the ethyl, and 74 and 78 cps in the case of the isohexyl). However, judging from the intensities of the 21-methyl peaks, the ratios of the two 20 epimers in the mixture were different. In the case of the ethyl compound,[27] the 20α-hydroxy epimer was formed to a greater extent (ratio of α/β being 3:2), whereas in the case of the isohexyl, the 20β-hydroxy compound was predominant (ratio of α/β being 1:3).

Discussion of the Results

The stereochemical results which are discussed above may be explained by consideration of the conformation of the 17β side chain as suggested by Rakhit and Engel[11] who explained the similar results of lithium aluminum hydride reductions of 20-keto

(27) The authentic 20-hydroxy epimers have been described in another paper, N. K. Chaudhuri and M. Gut, *J. Org. Chem.*, **34**, 3754 (1969).



A_1 A_2 B

steroids. Thus the conformation of the 17-unsubstituted ketones (including 21-hydroxy and acetoxy derivatives) is such[28] (either A_1 or A_2 type) that it is conducive to the formation of 20α-hydroxy compounds on reaction with Grignard reagents by an attack from the less hindered side. Although the 17α-hydroxy ketones exist to a considerable extent in conformation A_2, because of the strong hydrogen bonding[29] between the 17α-hydroxy group and the 20-carbonyl group, complex formation with Grignard reagents may drive the side chain into conformation B and lead to the formation of 20β-hydroxy compounds (*cf.* ref 11). The geometry of the side chain of the 16α,17α-epoxy compound is not the same as in the case of ring-D-saturated steroids. It may be such that the formation of both isomers is possible.

Experimental Section

Melting points are uncorrected. Nmr spectra were obtained in deuteriochloroform solution (unless otherwise stated) on a 60-Mc Varian Associates DA-60 spectrometer using tetramethylsilane as reference. Mass spectra were determined on a Varian Associates M-66 mass spectrometer. Optical rotations were measured with a Hilger–Watts III polarimeter. Ir spectra of all solid compounds were determined as KBr pellets.

20-Iso-5α-cholestane-3β,20β-diol (9). A. By Hydroboration.—To an ice-cold solution of 950 mg of **8** in 30 ml of dry tetrahydrofuran was added 8 ml of commercially available diborane solution in tetrahydrofuran (1 *M* as BH_3) with stirring. Cooling and stirring was continued for 1 hr. The solution was brought to room temperature after an additional hr and carefully decomposed by the addition of a small quantity of water. A dilute solution of sodium hydroxide was added until the pH of the solution was 9–10. The reaction flask was then cooled in an ice bath and 10 ml of 30% hydrogen peroxide was added slowly with stirring during 15 min. Stirring was continued for 1 hr and the organic material was extracted with ethyl acetate. The ethyl acetate extract was washed successively with water and brine. Evaporation of the solvent, after drying over sodium sulfate, gave an oil which was chromatographed over alumina. The eluate with 5–10% ethyl acetate in benzene gave an oily material which was twice crystallized from methanol: mp 125–127°; nmr data 48.5 (18 CH_3), 50 (19 CH_3), 67.5 (21 CH_3), doublet centered at 53 (26, 27-methyl) cps; mass spectrum *m/e* 404 (M^+, 3%), 389 (M − CH_3, 5%), 386 (M − H_2O, 25%), 371 (386 − CH_3, 389 − H_2O, 10%), 368 (386 − H_2O, 5%), 353 (371 − H_2O, 368 − CH_3, 3%), 319 (M − C_6H_{13}, base peak), 301 (319 − H_2O, 75%), 283 (301 − H_2O, 7%), 276 (M − $C_8H_{17}O$ + 1, 50%), 258 (276 − H_2O, 40%).

The succeeding fractions from the chromatogram gave an oil, the nmr signals of which appeared at 40 (18 Me), 48.5 (19 Me), and a doublet centered at 53 (21, 26, 27 methyls).

B. By Grignard Reaction.—An ethereal solution of methylmagnesium bromide was prepared from 5 g of methyl bromide and 1.2 g of magnesium turnings in 25 ml of dry ether. To this solution was added with ice cooling and stirring a benzene solution of 4.3 g of 21-nor-20-keto-5α-cholestanol 3-acetate (**20**) in 100 ml of dry benzene. The mixture was stirred at room temperature for 1 hr and refluxed for 6 hr. It was decomposed by adding an ice-cold solution of ammonium chloride, whereby the benzene layer separated and the aqueous solution was then extracted with ethyl acetate. The organic solution was washed with a solution of sodium carbonate and water and evaporated after drying over sodium sulfate. The crude oily residue showed no carbonyl band in its ir spectrum. It was purified by crystallization from methanol to give 3.7 g of 20β-hydroxy-20-iso-5α-cholestanol. Recrystallization from methanol gave plates: mp 125–127°; nmr data 48.5 (18 CH_3), 50 (19 CH_3), 67 (21 CH_3), and doublet centered at 53 (26, 27 methyl) cps; same mass spectrum as that of the compound prepared by hydroboration.

Anal. Calcd for $C_{27}H_{48}O_2$: C, 80.14; H, 11.96. Found: C, 80.25; H, 11.75.

The 3-acetate was prepared by treatment of the 3-hydroxy compound with acetic anhydride and pyridine and crystallized from methanol: mp 86–88°; nmr signals 49 (18 CH_3), 51 (19 CH_3), a doublet centered at 53 (26, 27 methyl), 68 (21 CH_3), and 123 (acetate methyl) cps; peaks (high mass) in mass spectrum 446 (M^+, 1%), 431 (M^+ − CH_3, 3%), 428 (M^+ − H_2O, 5%), 413 (431 − H_2O, 428 − CH_3, 3%), 371 (431 − HOAc, 2%), 361 (M − C_6H_{13}, 55%), 343 (361 − H_2O, 25%), 301 (361 − HOAc, 40%), 283 (301 − H_2O, 7%), 258 (M^+ − $C_8H_{17}O$ − HOAc + 1, base peak).

Anal. Calcd for $C_{29}H_{50}O_3$: C, 77.97; H, 11.28. Found: C, 78.13; H, 11.41.

3β-Acetoxy-5α-pregnan-20-one (10).—A solution of 20 g pregnenolone acetate in 200 ml of ethyl acetate was stirred with 2 g of 10% palladized charcoal in an atmosphere of hydrogen. It was worked up in the usual way after the absorption of hydrogen had ceased. The product was crystallized from methanol: mp 150–152° (lit.[30] 150–152°); nmr data 36.5 (18 CH_3), 49.5 (19 CH_3), 125.5 cps (21 CH_3), and no vinylic proton.

20α-Hydroxy-5α-cholestanol (12).—An ethereal solution of isohexylmagnesium bromide was prepared from 6.6 g of isohexyl bromide and 0.96 g of magnesium turnings in 20 ml of dry ether. To this was added with stirring and cooling a solution of 3.6 g of 5α-pregnanolone 3-acetate (**10**) in 80 ml of dry benzene. The mixture was stirred at room temperature for 1 hr and refluxed for 6 hr. It was worked up as described above for the 20β-hydroxy compound. The crude oil obtained was crystallized from methanol and recrystallized from acetone–methanol to give 3.2 g of 20α-hydroxy-5α-cholestanol: mp 141–143°; nmr data 48.5 (18 CH_3), 50 (19 CH_3), 75 (21 CH_3) cps; same mass spectrum as that of 20β-hydroxy isomer reported above.

Anal. Calcd for $C_{27}H_{48}O_2$: C, 80.14; H, 11.96. Found: C, 80.32; H, 11.98.

The 3-acetate was prepared by treatment with pyridine and acetic anhydride and crystallized from methanol: mp 119–121°; nmr data 49 (18 CH_3), 50 (19 CH_3), 75 (21 CH_3), 120 (acetate methyl), and doublet centered at 53 (26, 27 methyl); same mass spectrum as that of the 20β-hydroxy isomer.

3β,17α,20β-Trihydroxy-20-iso-5α-cholestane 3-Acetate (16) by Osmylation of $\Delta^{17(20)}$-5α-Cholestenol (8).—To a solution of 933 mg of $\Delta^{17(20)}$-cholestenol in 20 ml of dry pyridine was added 1 g of osmium tetroxide dissolved in 10 ml of dry pyridine. The solution was diluted with 30 ml of dry tetrahydrofuran and allowed to stand at room temperature. After 6 hr, an aqueous solution of sodium bisulfite was added and stirred at room temperature for 1 hr and then extracted with ethyl acetate. Thin layer chromatography showed that very little of the osmate ester was hydrolyzed by this method. Evaporation of the solvent gave 1.4 g of a purple crystalline solid. It was purified by chromatography over alumina, when 800 mg of a solid was obtained. This was dissolved in 30 ml of dry tetrahydrofuran and 500 mg of lithium aluminum hydride was added to the solution. The reaction mixture was stirred at room temperature for 30 min and then decomposed with water and 15% sodium hydroxide solution. Inorganic solid was filtered off and washed with benzene. The organic solution was washed with water and brine. The solution was dried over sodium sulfate and solvent removed. The residual oil (650 mg) was acetylated with acetic anhydride and pyridine at room temperature. After working up in the usual way, the acetylated material was chromatographed over basic alumina

(28) L. P. Kier, *J. Med. Chem.*, **11**, 915 (1968).

(29) R. N. Jones, P. Humphries, F. Herling, and K. Dobriner, *J. Amer. Chem. Soc.*, 74, 2820 (1952).

(30) Ch. Meystre and K. Miescher, *Helv. Chim. Acta*, **29**, 33 (1946).

(Alcoa). Elution with 2–3% ethyl acetate in benzene gave 50 mg of a material which was crystallized from ethanol: mp 126–127°; nmr data 50 (18 CH_3), 59 (19 CH_3), 64 (21 CH_3), 121 (3β-acetate methyl), and 126 (22-acetate methyl) cps, side-chain methyl peaks merged with 18- and 19-methyl peaks; mass spectrum *m/e* 444 (M − HOAc, 4%), 429 (444 − CH_3, 4%), 426 (444 − H_2O, 10%), 411 (429 − H_2O, 7%), 361 (M − $C_6H_{12}OAc$, base peak), 343 (361 − H_2O, 50%), 301 (361 − HOAc, 80%), 283 (301 − H_2O, 20%).

Anal. Calcd for $C_{31}H_{52}O_5$: C, 73.76; H, 10.38. Found: C, 73.88; H, 10.30.

Elution with 10% ethyl acetate in benzene gave 450 mg of a material which was crystallized from hexane: mp 150–152°; $[\alpha]^{22}D$ 0 ± 2° (*c* 1.4 in chloroform) (lit.[8] 140–142°). Its melting point was not depressed upon admixture with a sample obtained from the reaction of isohexyl magnesium bromide with 17α-hydroxypregnenolone 3-acetate followed by catalytic hydrogenation as described later.

17α,20β-Dihydroxy-20-isocholesterol (15).—This was prepared by treating 6.4 g of 17α-hydroxypregnenolone 3-acetate with isohexylmagnesium bromide, prepared from 6.6 g of isohexyl bromide, and 1 g of magnesium. The mixture was stirred at room temperature for 1 hr and refluxed for 8 hr. The use of a lesser amount of Grignard reagent and reduction in refluxing time, led to a much better yield of the product than that reported in the literature.[8] After decomposing with an ammonium chloride solution, the reaction mixture was extracted with ethyl acetate. On concentration, about 500 mg of unreacted 17α-hydroxypregnenolone (identified by melting point and ir) crystallized out and was removed by filtration. The solid obtained from the mother liquor was crystallized from acetone, whereby 3.5 g 17α,20β-dihydroxy-20-isocholesterol was obtained: mp 179–180°; $[\alpha]^{23}D$ −55 ± 3° (*c* 2.1 in ethanol) (lit.[8] 174–176°; $[\alpha]D$ −53°); nmr data 54 (18 Me), 60.5 (19 Me), doublet centered at 53 (26, 27 methyl), 73.5 (21 Me), and 320 (6 H) cps; mass spectrum, *m/e* 418 (M^+, 0.5%), 403 (M − CH_3, 1.2%), 400 (M − H_2O, 5%), 385 (400 − CH_3, 1%), 382 (400 − H_2O, 1.5%), 367 (382 − CH_3, 385 − H_2O, 2%), 349 (367 − H_2O, 1%), 333 (M − C_6H_{13}, 2%), 315 (333 − H_2O, 8%), 297 (315 − H_2O, 7%), 289 (M − $C_8H_{17}O$, 75%), 271 (289 − H_2O, base peak), 253 (271 − H_2O, 75%).

The 3-acetate, prepared in the usual way, was crystallized from methanol, mp 159–160°. On recrystallization from hexane, needles were obtained: mp 160–162° (lit.[8] 159–160°); nmr data 54.5 (18 Me), 61.5 (19 Me), doublet centered at 53 (26, 27 methyl), 74 (21 Me), 122 (acetate methyl), and 325 (6 H) cps.

3β,17α,20β-Trihydroxy-20-iso-5α-cholestane 3β-Acetate (16).—A solution of 200 mg of 17α,20β-dihydroxy-20-isocholesterol 3-acetate in 20 ml of acetic acid was stirred with 20 mg of platinum oxide in an atmosphere of hydrogen until the absorption of hydrogen ceased. The reaction product was crystallized from hexane: mp 150–152°; nmr data 50 (19 CH_3), doublet at 53 (26, 27 methyl), 73.5 (21 Me), 121 (acetate methyl) cps, and no vinylic proton; mass spectrum *m/e* 462 (M^+, 1%), 444 (M − H_2O, 10%), 429 (M − CH_3, 1%), 426 (M − H_2O, 1%), 411 (429 − H_2O, 426 − CH_3, 1%), 377 (M − C_6H_{13}, 4%), 368 (2.5%), 359 (377 − H_2O, 6.5%), 333 (M − $C_8H_{17}O$, 65%), 315 (333 − H_2O, 50%), 299 (359 − HOAc, 8%), 292 (8%), 288 (10%), 273 (333 − HOAc, 60%), 255 (273 − H_2O, 315 − HOAc, base peak), etc.

Anal. Calcd for $C_{29}H_{50}O_4$: C, 75.28; H, 10.89. Found: C, 75.51; H, 11.03.

Cyanohydrin of Dehydroepiandrosterone Acetate (18) and the Unsaturated Nitrile 19.—These were prepared according to the published procedure[31] from dehydroepiandrosterone acetate.

3β-Acetoxy-21-nor-5,16-cholestadien-20-one (20).—A solution of isohexylmagnesium bromide was prepared from 32 g of isohexyl bromide and 5 g of magnesium turnings in 100 ml of dry ether. The solution was cooled in ice–water and to it was added with stirring a solution of 32 g of the unsaturated nitrile **19** in 250 ml of dry benzene. The mixture was stirred at room temperature for 1 hr and then refluxed for 6 hr. It was decomposed by pouring over a mixture of ice and concentrated hydrochloric acid. The precipitated imine hydrochloride was filtered and washed with cold ether. It was then dissolved in acetic acid and diluted with water until turbidity and heated on a steam bath for 5 min. After 30 min the solution was diluted with water and extracted with ethyl acetate. The ethyl acetate extract was washed with a cold 2 *N* sodium hydroxide solution to neutrality, dried, and evaporated under reduced pressure. The residue was crystallized from methanol to give 12 g of the unsaturated ketone (mp 123–126°) and recrystallized from acetone–hexane: mp 125–126°; λ_{max}^{MeOH} 240 mμ (ε 13,000); infrared bands at 6.05 (conjd C=O), strong band at 6.3 (conjd C=C) μ; nmr data 55.5 (18 Me), 63 (19 Me), doublet centered at 53 (26, 27 methyl), 323 (6 H), and triplet at 402 (16 H) cps.

Anal. Calcd for $C_{26}H_{40}O_2$: C, 81.20; H, 10.48. Found: C, 81.32; H, 11.03.

The 3-acetate, prepared by treatment with acetic anhydride and pyridine was crystallized from methanol, mp, 99–100°.

Anal. Calcd for $C_{28}H_{42}O_3$: C, 78.82; H, 9.92. Found: C, 79.13; H, 9.70.

3β-Acetoxy-21-nor-5α-cholestan-20-one (11).—A solution of 5 g of 3β-acetoxy-21-nor-5,16-cholestadien-20-one (**20**) in 100 ml of ethyl acetate and 500 mg of 10% palladized charcoal was stirred in an atmosphere of hydrogen until the absorption of hydrogen ceased. After the work-up the material was crystallized from methanol: mp 93–96°; nmr data 35.5 (18 Me), 49 (19 Me), doublet centered at 53 (26, 27 methyl) and 121 (acetate methyl) cps, and no vinylic proton peak.

Anal. Calcd for $C_{28}H_{46}O_3$: C, 78.09; H, 10.77. Found: C, 77.88; H, 10.90.

16α,17α-Oxido-21-norcholest-5-en-20-one (21).—A solution of 11 g of the unsaturated ketone **20** in 400 ml of methanol was cooled to 10°, and treated successively with 25 ml of 4 *N* sodium hydroxide solution and 45 ml of 30% hydrogen peroxide solution. The resulting precipitate was dissolved by the addition of 400 ml of methanol. The solution was kept at 0–5° for 20 hr and then diluted with water. The precipitated steroid was separated by filtration. The solids were washed with water and recrystallized from methanol: mp 126–127°; carbonyl band at 5.87 μ in the ir spectrum; nmr data 61 (18 Me), 63 (19 Me), doublet centered at 52 cps (26, 27 methyl), 220 (16 H), and 320 (6 H) cps.

Anal. Calcd for $C_{26}H_{40}O_3$: C, 77.95; H, 10.07. Found: C, 78.16; H, 10.15.

The 3-acetate, prepared in the usual way, was crystallized from methanol, mp 103–104°.

Anal. Calcd for $C_{28}H_{42}O_4$: C, 75.97; H, 9.56. Found: C, 76.18; H, 9.67.

3β-Acetoxy-16β-bromo-17α-hydroxy-21-nor-5-cholesten-20-one (22).—A solution of 11 g of the epoxy acetate **21** in 50 ml acetic acid was treated at 0° with a solution of 10 g of hydrogen bromide in 20 ml acetic acid. The solution was kept 5 min at 0° and 5 min at room temperature, then a solid crystallized out. After 30 min the solid was separated by filtration, washed with cold water and air dried at room temperature to give 10 g of the bromohydrin. The filtrate on dilution gave an additional 2 g of the material. A small amount of the bromohydrin was crystallized from methanol, mp 155–157°.

3β-Acetoxy-17α-hydroxy-21-norcholest-5-en-20-one (23).—To a solution of 5 g of the bromohydrin **22** in 100 ml of methanol was added 1.25 g ammonium acetate and 0.5 g of 5% palladized charcoal and the mixture was shaken in a hydrogen atmosphere. The uptake of hydrogen was terminated in 2 hr and then the catalyst was removed by filtration. The solution was diluted with water and extracted with ethyl acetate. The organic solution was washed with water, dried and evaporated under reduced pressure. The residue was crystallized from methanol to give 4 g of the 17-hydroxy ketone: mp 170–172°; infrared bands at 5.8 (acetate), 5.9 (C=O), 2.6 and 2.8 (OH) μ; nmr data 41 (18 Me), 60 (19 Me), doublet centered at 50 (26, 27 methyl), 120 (acetate methyl), and 322 (6 H) cps; nmr data [in $(CH_3)_2SO_2$-d_6 solution], 29 (18 Me), 58 (19 Me), doublet centered at 50 (26, 27 methyl), 118 (acetate methyl), 303 (17 OH), and 320 (6 H) cps.

Anal. Calcd for $C_{28}H_{44}O_4$: C, 75.63; H, 9.97. Found: C, 75.58; H, 9.87.

17α,20α-Dihydroxycholesterol (14).—To a solution of methylmagnesium bromide made from 8.6 g of methyl bromide and 2.16 g of magnesium in 50 ml of ether was added with stirring at room temperature, a solution of 6.7 g of 3β-acetoxy-17α-hydroxy-21-norcholest-5-en-20-one (**23**) in 200 ml of benzene. After stirring at room temperature for 1 hr, the mixture was refluxed for 8 hr. After the usual work-up the crude material was acetylated and chromatographed over alumina. The earlier fractions obtained by elution with 5% ethyl acetate in benzene exhibited ketonic bands in their ir spectra. The later fractions obtained by elution

(31) A. Butenandt and J. Schmidt-Thome, *Ber.*, **71**, 1487 (1938); **72**, 182 (1939).

with 9–10% ethyl acetate in benzene were free from ketonic materials. Nonketonic fractions were combined and twice crystallized from methanol to give 3 g of material: mp 176–178°; $[\alpha]^{23}D$ −59 ± 3° (*c* 1.5 in ethanol); nmr data 52 (18 Me), 62 (19 Me), 78 (21 Me), doublet centered at 53 (26, 27 methyl), 121 (acetate), and 322 (6 H) cps.

Anal. Calcd for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.34; H, 10.41.

17α,20α-Dihydroxycholesterol (**14**) was prepared by treatment of the above acetate with 5% methanolic potassium hydroxide at room temperature for 16 hr. The solid was crystallized from methylene chloride: mp 166–168°; nmr data 51 (18 CH_3), doublet centered at 53 (26, 27 methyl), 60.5 (19 Me), 78 (21 Me), and 120 (6 H) cps; same mass spectrum as that of the 20β epimer.

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.61; H, 11.07.

3β,17α,20α-Trihydroxy-5α-cholestane 3-Acetate (24).—This was prepared by catalytic (platinum) hydrogenation of 17α,20α-dihydroxycholesterol in the same way as described for the 20β epimer. The product was crystallized from hexane: mp 175–177°; $[\alpha]^{23}D$ −1 ± 2° (*c* 0.9 in chloroform); nmr data 50 (18 and 19 Me), doublet centered at 52 (26, 27 methyl), 77 (21 Me), 120 (acetate methyl) cps, and no vinylic proton peak; mass spectrum same as that of the 20β epimer.

Anal. Calcd for $C_{29}H_{50}O_4$: C, 75.28; H, 10.89. Found: C, 75.48; H, 11.02.

Oxidation of 17α,20α-Dihydroxycholesterol 3-Acetate. A. By Lead Tetraacetate.—To a solution of 250 mg of the steroid in 50 ml glacial acetic acid were added 500 mg of lead tetraacetate and the solution was left at room temperature for 48 hr. It was then diluted with water and extracted with ethyl acetate. The ethyl acetate solution was washed with cold sodium carbonate solution and water, dried over sodium sulfate, and evaporated to dryness. The residue was chromatographed over alumina. The material (120 mg) obtained by elution with 2% ethyl acetate in benzene had ir and nmr spectra identical with those of dehydroepiandrosterone acetate.

B. By Sodium Periodate.—To a solution of 250 mg of the steroid in 25 ml of methanol was added 20 ml of a solution of 250 mg of sodium metaperiodate and the pH was adjusted to 4.5 by the addition of a few drops of dilute sulfuric acid and kept at room temperature for 4 days. On working up 127 mg of dehydroepiandrosterone acetate was obtained.

3β,20α,21-Trihydroxycholesterol 3,21-Diacetate (26).—A benzene solution of 6.0 g of 21-acetoxypregnenolone 3-acetate (**25**) was added to an ethereal solution of isohexylmagnesium bromide prepared from 13.0 g of isohexyl bromide and 2.2 g of magnesium. The solution was refluxed for 6 hr and then decomposed with an ammonium chloride solution. After work-up in the usual way, the reaction product was acetylated with acetic anhydride and pyridine and then chromatographed on a basic alumina (Alcoa) column. Upon removal of the solvent from the fractions eluted with 5% ethyl acetate in benzene a solid was obtained (3.4 g) which was crystallized from methanol: mp 149–150°; ir spectrum showed bands at 2.6 (OH), 5.8 and 8.15 (acetate) μ; nmr data 52.5 (18 Me), doublet centered at 53 (26, 27 methyl), 60.5 (19 Me), 120 (acetate methyl), and 124 (acetate methyl) cps.

Anal. Calcd for $C_{31}H_{50}O_5$: C, 74.06; H, 10.03. Found: C, 73.88; H, 10.21.

20α-Hydroxycholesterol (6) from 26.—To 1 g of compound **26** was added 20 ml of 5% methanolic potassium hydroxide solution and this was heated on a steam bath for 2 hr. After the usual work-up, the 3,20,21-triol was obtained as a noncrystallizable solid. It was dissolved in 20 ml pyridine, 2 g of *p*-toluenesulfonyl chloride was added, and the solution left at room temperature for 16 hr. An oil was obtained after the usual work-up. Its ir spectrum showed bands at 2.7 (OH), 8.4 and 8.5 (tosylate) μ. The oil was dissolved in 10 ml of acetone and 2 ml of water was added to it. The solution was then heated on a steam bath. The oil obtained after work-up showed the absence of any band in the ir spectrum due to the tosyl group. It was dissolved in 25 ml of tetrahydrofuran and 500 mg of lithium aluminum hydride was added to it. The mixture was refluxed for 6 hr. The product obtained after work-up was chromatographed on an alumina column. After removal of solvent from the 10% ethyl acetate in benzene eluate, a solid was obtained which was crystallized from acetone–methanol: mp 138–140° (lit.[6b] 136–137°); nmr data 52 (18 Me), 16 (19 Me), doublet centered at 53 (26, 27 methyl), and 76.5 (21 Me) cps.

Reaction of 3β-Acetoxy-16α,17α-oxido-5-pregnen-20-one (29) with Isohexylmagnesium Bromide. Formation of 30a.—An ethereal solution of isohexylmagnesium bromide was prepared from 0.72 g magnesium in 20 ml of dry ether and 5.2 g of isohexyl bromide. The solution was cooled in an ice–salt mixture and to it was added, with stirring, a solution of 3.72 g of **29** in 100 ml of benzene during 5 min. The resultant slurry was vigorously stirred with continued cooling for 10 min and then poured into a mixture of ice and aqueous hydrochloric acid. The mixture was then extracted with ethyl acetate and the ethyl acetate solution was washed with water, dried over sodium sulfate, and evaporated. The residue was crystallized from methanol, mp 135–137°. The thin layer chromatogram showed only one spot due to the product; no spot due to the starting material was observed. The infrared spectrum showed bands at 2.7 (OH) and 5.78 (acetate) μ; nmr data doublet at 53 (J = 6 cps; 26, 27 CH_3), 55 (18 CH_3 of one isomer), 59 (18 CH_3 of the second isomer), 62 (19 CH_3), 76 (21 CH_3 of one isomer), 79 (21 CH_3 of the second isomer), 122 (acetate CH_3), doublet at 206 ($J_{16\beta H,15\beta H}$ = 4 cps, $J_{16\beta H,15\alpha H}$ = 0; 16β H) and 320–326 (6 H) cps.

Anal. Calcd for $C_{29}H_{46}O_4$: C, 75.94; H, 10.11. Found: C, 76.12; H, 10.02.

Reaction of 29 with Ethylmagnesium Bromide. Formation of 30b.—This reaction was carried out in exactly the same way as described above for the corresponding reaction of **29** with isohexylmagnesium bromide. The crude product was crystallized from acetone, mp 139–142°. The thin layer chromatogram, however showed only one spot. The ir spectrum showed bands at 2.7 (OH) and 5.78 (acetate) μ; nmr data broad band at 55–59 (18 and 23 CH_3), 62.5 (19 CH_3), 78 and 79 (21 CH_3 of two isomers), 122 (acetate CH_3), doublet at 206 ($J_{16\beta H}$, $J_{15\beta H}$ = 4 cps, $J_{16\beta H,15\alpha H}$ = 0; 16β H) and 320–326 (6 H) cps.

Anal. Calcd for $C_{25}H_{38}O_4$: C, 74.59; H, 9.52. Found: C, 74.37; H, 9.68.

Reduction of 30a and 30b with Lithium Aluminum Hydride. Formation of 31a and 31b.—To a suspension of 0.5 g of lithium aluminum hydride in 50 ml of dry ether was added with stirring a solution of 1.5 g of **30a** or **30b** in 25 ml of benzene. The mixture was stirred for 30 min at room temperature and refluxed for 3 hr. After the usual work-up the solvents were removed to give a solid residue.

The product **31a** was crystallized from acetone: mp 160–170°; nmr data 52 (18 CH_3 of 20α-OH isomer), 54 (18 CH_3 of 20β-OH isomer), doublet at 53 (J = 6 cps; 26, 27 CH_3's), 61 (19 CH_3), 74 (21 CH_3 of the 20β-OH isomer), 78 (21 CH_3 of the 20α-OH isomer), 122 (acetate CH_3), and 322 (6 H) cps.

Anal. Calcd for $C_{27}H_{46}O_3$: C, 77.46; H, 11.08. Found: C, 77.30; H, 10.95.

The product **31b** was converted into its 3-acetyl derivative by treatment with acetic anhydride and pyridine. The crude product obtained after the usual work-up was crystallized from acetone: mp 188–193° (*cf.* ref 27 which gives 201–204° for the 20α-hydroxy isomer, 191–194° for the 20β-hydroxy isomer); nmr data 52.5 (18 CH_3 of the 20α isomer), 54.5 (18 CH_3 of the 20β isomer), 62 (19 CH_3), 73 (21 CH_3 of the 20β-hydroxy isomer), 77 (21 CH_3 of the 20α-hydroxy isomer), 122 (acetate CH_3), and 320–326 (6 H) cps.

Anal. Calcd for $C_{25}H_{40}O_4$: C, 74.21; H, 9.97. Found: C, 74.30; H, 9.88.

Registry No.—22-acetate of the $\Delta^{20(22)}$ isomer of **8**, 21927-86-6; **9**, 21902-65-8; 3-acetate of **9**, 21902-66-9; **11**, 21902-67-0; **12**, 21902-68-1; **14**, 382-78-5; 3-acetate of **14**, 21955-14-6; **15**, 17913-42-7; **16**, 21902-71-6; **20**, 21927-87-7; free base of **20**, 21902-72-7; **21**, 21902-73-8; free base of **21**, 21902-74-9; **22**, 21902-75-0; **23**, 21902-76-1; **24**, 21927-88-8; **26**, 21902-77-2; **30a**, 21902-78-3; **30b**, 21902-79-4; **31a**, 21902-80-7; **31b**, 21902-81-8.

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