Hydroxylation of Δ^4-Steroids by Osmium Tetraoxide; Stereochemistry and Substituent Effects

By **James R. Bull** * and **Jan Floor**, National Chemical Research Laboratory, Council for Scientific and Industrial Research, P.O. Box 395, Pretoria 0001, South Africa

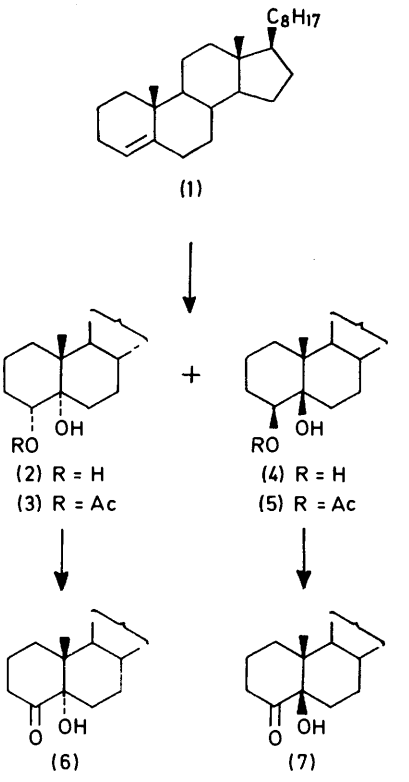
Hydroxylation of cholest-4-ene by osmium tetraoxide leads preferentially to the 4β,5β-diol: this preference is even more pronounced in the case of 3α-acetoxycholest-4-ene. By contrast, α-attack is favoured in the case of 3β-acetoxycholest-4-ene. Similar results have been obtained in an analogous 19-nor series. but with a slightly greater proportion of β-attack in all comparable cases. It is suggested that the ring A conformation of the reactant or a derived complex is the primary factor determining the stereoselectivity of the reaction. The major role of a proximate substituent is to anchor the appropriate conformation favouring α- or β-attack. This argument is supported by the results of hydroxylation of 2α- and 2β-acetoxycholest-4-ene.

ALTHOUGH the reaction of osmium tetraoxide (in stoicheiometric proportion, or as catalyst in the presence of hydrogen peroxide) with Δ^4-steroids has been exploited for many years as a convenient route to *cis*-4,5-diols, the reported results [1-12] show certain discrepancies in stereoselectivity and configurational assignments.

A careful study [1] of the *cis*-hydroxylation of cholest-4-en-3-one resulted in positive identification of the two 3-oxo-4,5-diols obtained; these assignments have been corroborated in subsequent work on Δ^4-3-ketones [2,3] and other conjugated Δ^4-steroids.[3-5] The results obtained from *cis*-hydroxylation of the isolated 4,5-double bond in steroids are more ambiguous. The reaction of osmium tetraoxide with cholest-4-ene (1) was first reported [6] to give a low yield of the 4α,5α-diol (2), but it has since been shown [7,8] that it proceeds efficiently to give a mixture of the 4α,5α- (2) and the 4β,5β-diol (4) in the ratio *ca.* 5 : 1 (it was reasonably assumed that the former isomer is preponderant). However, an oblique reference has been made [9] to the incorrect stereochemical assignment of cholestane-4,5-diol derivatives, although details of the hydroxylation procedure and isomer distribution are not given. In a more recent publication,[10] the major diol is assigned the 4β,5β-structure (4) without comment.

It has been demonstrated [9,11] that hydroxylation of 3β-substituted Δ^4-steroids with osmium tetraoxide affords the corresponding 4α,5α-diols. By contrast, the major product derived from 7β-hydroxycholest-4-ene is the 4β,5β,7β-triol.[12]

We have re-examined the reaction of osmium tetraoxide with cholest-4-ene (1) in pyridine at 25 °C. After 48 h, reductive work-up afforded a product which was chromatographed to give, in the ratio 24 : 76, the diols (2) and (4), notable for the similarity in their physical properties. The assignments given here were verified by conversion of each diol into the 4-acetates [6,13] [(3) or (5)] and the 4-ketone [1,6] [(6) or (7)]; these products have been securely identified through other reaction pathways.



The result is surprising since it is generally assumed [14] that the stereochemical preference in formation of the osmate ester of an endocyclic steroidal olefin will be similar to that in most other *cis*-additions. Instead, the result is reminiscent of the medium-dependent outcome of catalytic hydrogenation [14,15] of cholest-4-ene (1), and

[1] J. F. Eastham, G. B. Miles, and C. A. Krauth, *J. Amer. Chem. Soc.*, 1959, **81**, 3114.
[2] A. D. Tait. *Steroids*. 1972, **20**. 531.
[3] T. Kubota. K. Yoshida. and F. Wanatabe, *Chem. and Pharm. Bull. (Japan)*. 1966, **14**, 1426.
[4] T. Kubota, K. Yoshida, F. Hayashi. and K. Takeda, *Chem. and Pharm. Bull. (Japan)*, 1965, **13**. 50.
[5] T. Kubota and F. Hayashi, *Tetrahedron*, 1967, **23**, 995.
[6] D. N. Jones, J. R. Lewis. C. W. Shoppee, and G. H. R. Summers. *J. Chem. Soc.*, 1955, 2876.
[7] G. H. Whitham and J. A. R. Wickramasinghe. *J. Chem. Soc.*, 1964. 1655.
[8] C. W. Davey, E. L. McGinnis. J. M. McKeown, G. D. Meakins. M. W. Pemberton. and R. N. Young. *J. Chem. Soc.* (*C*), 1968, 2674.
[9] M. Nussim and Y. Mazur. *Tetrahedron*, 1968, **24**. 5337.
[10] E. T. J. Bathurst, J. M. Coxon, and M. P. Hartshorn, *Austral. J. Chem.*, 1974, **27**. 1505.
[11] D. Baldwin. J. R. Hanson, and A. M. Holton, *J.C.S. Perkin I*, 1973, 2687.
[12] A. R. Davies and G. H. R. Summers, *J. Chem. Soc.* (*C*), 1966, 1012.
[13] D. Lavie, Y. Kashman, and E. Glotter, *Tetrahedron*, 1966, **22**. 1103.
[14] D. N. Kirk and M. P. Hartshorn, 'Steroid Reaction Mechanisms,' Elsevier. Amsterdam. 1968, ch. 3.
[15] C. W. Shoppee. B. D. Agashe, and G. H. R. Summers, *J. Chem. Soc.*, 1957, 3107: M. C. Dart and H. B. Henbest, *ibid.*, 1960. 3563.

suggests that analogous steric demands or modified reactant conformations are involved during attack by osmium tetraoxide. Although it may be possible to gain further evidence for this analogy by varying the conditions under which *cis*-hydroxylation of the 4,5-double bond is carried out, it was considered that a distinction between the factors influencing the direction of attack could more readily be made through studying the effect of proximate substitution. Accordingly, hydroxylations were carried out under identical conditions with osmium tetraoxide, upon a series of Δ^4-steroids differing in ring A substitution. The relative yields of hydroxylation products are summarised in Table 1.

TABLE 1

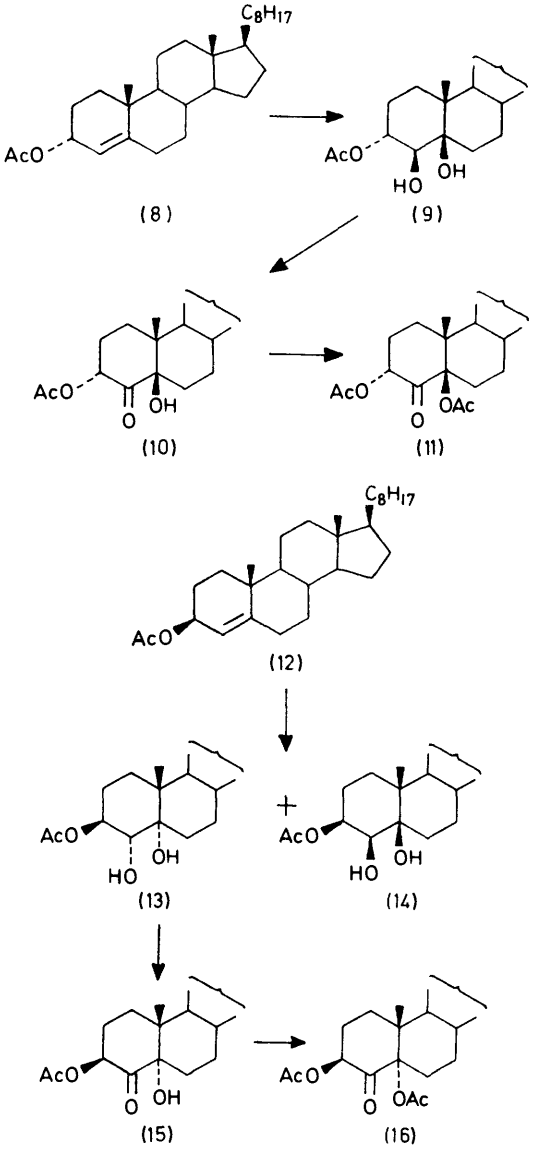
Isomer distribution in hydroxylations by osmium tetraoxide [a]

Olefin	4α,5α-Diol (%)	4β,5β-Diol (%)
Δ^4 (1)	24	76
3α-OAc-Δ^4 (8)	(2) [b]	98
3β-OAc-Δ^4 (12)	87	13
19-Nor-Δ^4 (17)	11	89
3α-OAc-19-nor-Δ^4 (22)	(<1) [b]	>99
3β-OAc-19-nor-Δ^4 (24)	77	23
2α-OAc-Δ^4 (29)	62	38
2β-OAc-Δ^4 (34)	8	92

[a] Relative yields of pure products isolated by chromatography. [b] Not characterised.

Spectroscopic data in support of the assignments are shown in Table 2. The hydroxylation products of 3α-(8) and 3β-acetoxycholest-4-ene (12) were further characterised by examination of the c.d. of the derived 4-ketones. Thus, oxidation of the 3α-acetoxy-4β,5β-diol (9) with chromium trioxide–pyridine gave the 4-ketone (10), in the n.m.r. spectrum of which the signal for the 3β-proton [δ 5.73 (q, *J* 12 and 7 Hz)] revealed that no isomerisation had occurred. A comparison of the c.d. spectra of methanolic solutions of (10) ($\Delta\varepsilon_{max.}$ −0.82 at 298 nm) and the derived 5-acetate (11) ($\Delta\varepsilon_{max.}$ +0.74 at 297 nm) showed that the dissignate influence of 5-acetylation is sufficiently strong to invert the sign of the Cotton effect. This phenomenon was also observed in analogues unsubstituted at C-3,[16] and may be regarded as diagnostic for a 5β-oxy-4-ketone system, since the equatorial 3α-acetoxy-group is not expected to contribute significantly to the Cotton effect. Similarly, successive oxidation and acid-catalysed acetylation of the 3β-acetoxy-4α,5α-diol (13) gave the 3β-acetoxy-5α-hydroxy- (15) and 3β,5α-diacetoxy-4-ketone (16), whose c.d. spectra in methanol [$\Delta\varepsilon_{max.}$ −0.97 at 298 nm (15); $\Delta\varepsilon_{max.}$ −2.46 at 290 nm (16)] are very similar to those [16] of 5-hydroxy- and 5-acetoxy-5α-cholestan-4-one, respectively.

In view of the powerful directing effect of a 3-acetoxy-group upon hydroxylation of cholest-4-enes, the study was extended to the related estr-4-enes in order to examine the role of the allylic 10β-methyl group. *cis*-Hydroxylation of 17β-acetoxyestr-4-ene (17) was reported [10] to give only one diol, of undetermined configuration. In this work, two products (18) and (19) were obtained (Table 1), whose lanthanoid-induced c.d.[17] and n.m.r. spectra (Table 2), supported the given assignments (*cf.* data for the cholestane-4,5-diols). The



3-acetoxyestr-4-enes (22) and (24) were prepared through reduction (lithium aluminium hydride) of estr-4-en-3-one [18] (20) to give a *ca.* 1 : 3 mixture of 3α- (21) and 3β-hydroxyestr-4-ene (23) (*cf.* reduction of 19-nortestosterone [19]), which were separated and acetylated. The hydroxylation products of (22) and (24) were unambiguously identified from n.m.r. data (Table 2).

Lanthanoid-induced c.d. spectra were recorded for all the diols prepared here (Table 2). It is noteworthy that the higher wavelength extrema of the 3α-acetoxy-4β,5β- and 3β-acetoxy-4α,5α-diols in the cholestane and estrane

[16] J. R. Bull and P. R. Enslin, *Tetrahedron*, 1970, **26**, 1525.
[17] J. Dillon and K. Nakanishi, *J. Amer. Chem. Soc.*, 1975, **97**, 5409, 5417, and references cited therein.
[18] K. J. Sax, R. H. Blank, R. H. Evans, L. I. Feldman, and C. E. Holmlund, *J. Org. Chem.*, 1964, **29**, 2351.
[19] J. A. Hartman, *J. Amer. Chem. Soc.*, 1955, **77**, 5151.

series [compounds (9), (13), (25), and (26)] are of opposite sign to those predicted by the chirality rule.[17] It has been recognised [17] that the rule may be invalidated by the presence of further functionality in the vicinity of planar (quasi-*trans*) form (I), to which β-face reagent approach should be sterically inhibited by the pseudo-axial 10β-methyl group. Since this is not the case, it is surprising that a pseudo-equatorial 3β-acetoxy-group

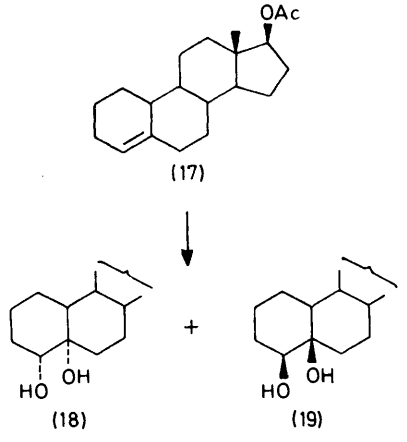
TABLE 2

N.m.r.[a] and Pr(dpm)$_3$-induced c.d.[b] spectral data of 4,5-diols

Diol	N.m.r. data	Δε	Predicted chirality
4α,5α- (2)	3.66 ($W_{\frac{1}{2}}$ 16 Hz. 4β-H)	+8.7 (314)	+
4β,5β- (4)	4.02 ($W_{\frac{1}{2}}$ 16 Hz. 4α-H)	−13.9 (315)	−
3α-OAc-4β,5β- (9)	3.93br (d, 9 Hz. 4α-H) 4.98 ($W_{\frac{1}{2}}$ 20 Hz, 3β-H)	+3.3 (312)	−
3β-OAc-4α,5α- (13)	3.58 (d. 9 Hz, 4β-H) 5.03br (q, 14 and 9 Hz, 3α-H)	−5.6 (312)	+
3β-OAc-4β,5β- (14)	3.99 (d. 3.5 Hz, 4α-H) 5.24 ($W_{\frac{1}{2}}$ 7 Hz, 3α-H)	−11.7 (317)	−
19-Nor-4α,5α- (18)	3.28 (q, 10 and 5 Hz, 4β-H)	+1.6 (313)	+
19-Nor-4β,5β- (19)	3.82 (q, 10 and 5 Hz, 4α-H)	−2.7 (315)	−
3α-OAc-19-nor-4β,5β- (25)	3.75 (d, 9.5 Hz, 4α-H) 5.0 (dq, 10. 9.5. and 5.5 Hz, 3β-H)	+2.5 (310)	−
3β-OAc-19-nor-4α,5α- (26)	3.22 (d, 9 Hz, 4β-H) 4.96 (sext, 9. 9. and 5 Hz, 3α-H)	−5.9 (310)	+
3β-OAc-19-nor-4β,5β- (27)	3.79 (d. 4 Hz, 4α-H) 5.26 ($W_{\frac{1}{2}}$ 8 Hz, 3α-H)	−6.1 (317)	−
2α-OAc-4α,5α- (30)	3.74 (q, 11 and 5 Hz, 4β-H) 4.98 (sept, 10, 10, 5, and 5 Hz, 2β-H)	+1.6 (315)	+
2α-OAc-4β,5β- (31)	4.28 (t, 8 Hz, 4α-H) 5.0br (t, 3 Hz. 2β-H)	−4.1 (314)	−
2β-OAc-4α,5α- (35)	3.92 (q, 9 and 7 Hz, 4β-H) 5.14 (t, 3 Hz, 2α-H)	+0.2 (315)	+
2β-OAc-4β,5β- (36)	4.1 (q. 12 and 4.5 Hz, 4α-H) 4.8 ($W_{\frac{1}{2}}$ 32 Hz, 2α-H)	−0.8 (312)	−

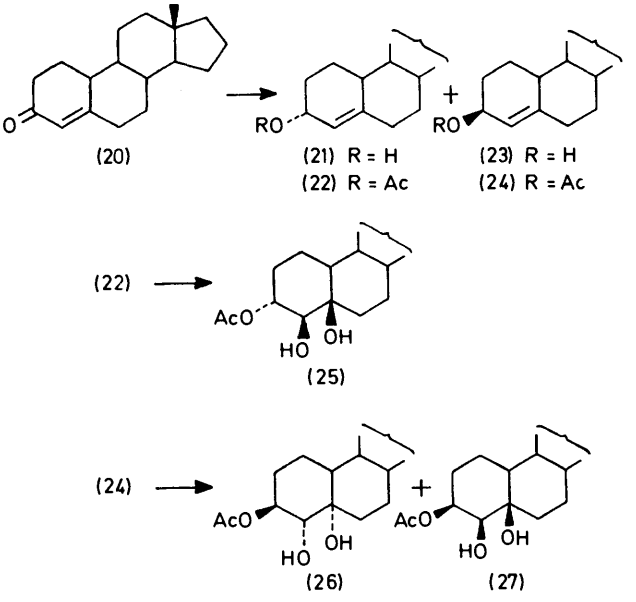
[a] For structurally significant proton signals, given as ' δ (width, or multiplicity and splitting. assignment).' [b] Spectra were determined with a JASCO J-20 instrument. Pr(dpm)$_3$ (*ca.* 0.7 mg) was added to a slight molar excess of each diol in dry carbon tetrachloride (10 ml), to give a solution *ca.* 1 × 10^{-4}M in Pr(dpm)$_3$. Spectra were recorded after 30—45 min. Δε Values are based upon the molar concentrations of Pr(dpm)$_3$ and are given for the longest wavelength extrema. Predicted chiralities are defined according to the reported [17] rule.

the α-glycol moiety. Consequently, the method cannot be used without supporting evidence in assigning the configuration of the 3-acetoxy-4,5-diols.



A comparison of the hydroxylation results (Table 1) reveals that they cannot be explained only in terms of steric hindrance to reagent approach. If this were so, a marked preference for α-attack would obtain in the unsubstituted cases. Despite the relative flexibility of ring A in Δ^4-steroids, it is recognised [20,21] that the lowest energy conformation of cholest-4-ene (1) is the mono-

exerts such a powerful inhibitory effect upon β-face attack in (12). However, in accepting that a hitherto



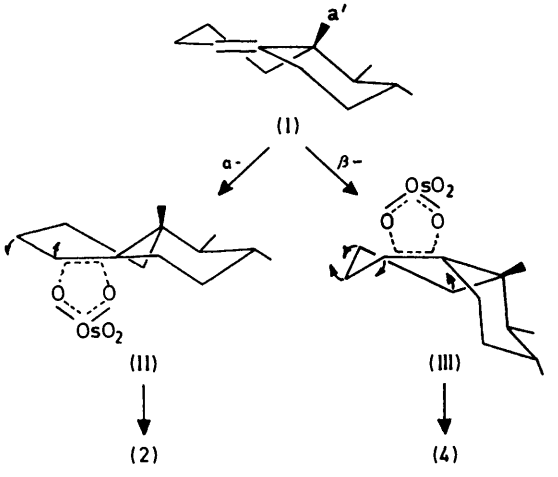
undefined factor is responsible for the cholest-4-ene (1) result, the greater β-face stereoselectivity displayed by

[20] R. Bucourt. *Topics Stereochem.*, 1974, **8**, 159, and references cited therein.

[21] J. Bordner. S. G. Levine, Y. Mazur, and L. R. Morrow, *Cryst. Struct. Comm.*, 1973. **2**. 59.

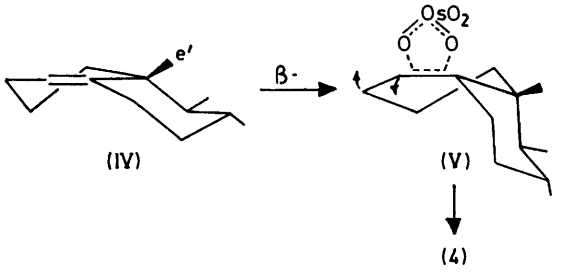
the 3α-acetoxy-compound (8) can then be rationalised in steric terms.

An examination of the conformational changes experienced by cholest-4-ene (1) during synchronous bond formation at C-4 and C-5 reveals that α-attack upon the monoplanar conformer (I) should proceed *via* a relatively low-energy state (II) having a pre-chair environment about C-5; subsequent rotation of the 3,4-bond (as depicted by arrows) leads to the chair form of the 4α,5α-product (2). However, β-attack upon (I) will generate a pre-twist environment about C-5, and the higher-energy intermediate (III) must then undergo ring inversion in order to give the chair form of the 4β,5β-product (4). This suggests that the conformer



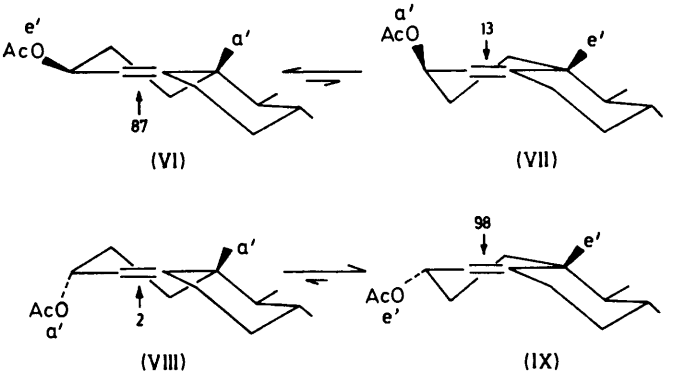
leading to products of α-attack may be represented by (I), but that another conformer should be sought to explain β-face attack.

Deformations of ring A in Δ^4-3-ketones have been demonstrated by *X*-ray analysis;[22] the presence of 2β-substitution is an extreme case which gives rise to conformations approaching the inverted monoplanar (quasi-*cis*) form.[23] Although such conformations are energetically unfavourable in testosterone or 19-nortestosterone, calculations have shown[24] that the modest energy difference between idealised quasi-*trans* and quasi-*cis* forms allows a small equilibrium population of the latter. Dreiding models of a Δ^4-steroid skeleton show that the analogous quasi-*cis* form of cholest-4-ene (1) would approximate closely to a 1,2-diplanar conformer (IV). While it is inconceivable that a significant ground state population of (IV) is present in solution, such a conformer would account for β-face hydroxylation of cholest-4-ene (1) since the resultant pre-chair environment about C-5 in the primary reagent–reactant complex (V) would facilitate low energy transformation to the 4β,5β-product. The analogy with catalytic hydrogenation[14] suggests that the interaction between the incipient C(5)–O bond and the 7α- and 9α-protons in (II) is energetically more demanding than α-face steric compression in (V) and, as a consequence, reagent approach



to (I) is less efficient than to (IV), despite the preponderance of the former conformer in solution. Accordingly, the Curtin–Hammett principle[25] may be invoked to reconcile the inconsistency between ground state reactant conformations and the product distribution.

In these terms, the steric role of a 3-acetoxy-group will be secondary to that of influencing the equilibrium between the monoplanar and 1,2-diplanar conformers in solution. Thus, a 3β-acetoxy-group will anchor the conformer (VI) favouring α-attack and, in adopting a pseudo-axial orientation in the higher energy 1,2-diplanar conformer (VII), will also hinder β-attack. Similarly, a 3α-acetoxy-group will destabilise (VIII) while sterically suppressing α-attack, and the conformer (IX) will dominate the reaction outcome. The results obtained in the 19-nor series (Table 1) suggest that the absence of the 10β-methyl group shifts the conformational equilibria more in favour of the quasi-*cis* forms. This is compatible with the finding[24] that the energy difference between quasi-*trans* and quasi-*cis* conformers is lower in 19-nortestosterone than in testosterone.



Hydroxylation of the isomeric 2-acetoxycholest-4-enes was also studied, since it was expected that the steric and conformational roles of the 2-acetoxy-group could be differentiated, and thereby provide further evidence for the influence of ring A conformation in determining isomer distribution. If steric hindrance

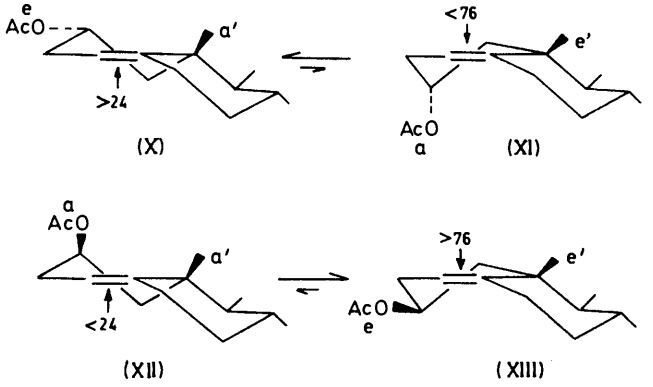
[22] C. Romers. C. Altona, H. J. C. Jacobs. and R. A. G. de Graaff in Chem. Soc. Specialist Periodical Report, ' Terpenoids and Steroids,' vol. 4, 1974. pp. 548—558.

[23] W. L. Duax. C. Eger, S. Pokrywiecki, and Y. Osawa. *J. Medicin. Chem.*. 1971. **14**, 295; Y. Osawa and J. O. Gardner, *J. Org. Chem.*, 1971, **36**. 3246.

[24] R. Bucourt, N. C. Cohen, and G. Lemoine. *Bull. Soc. chim. France*, 1975. 903.

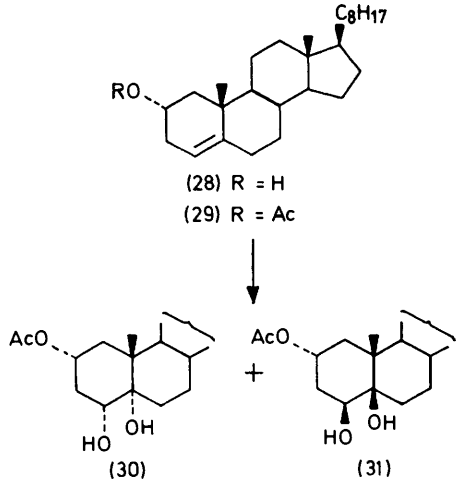
[25] E. L. Eliel, ' Stereochemistry of Carbon Compounds,' McGraw-Hill. New York. 1962. pp. 237—239.

by a proximate substituent were dominant during osmylation of the 4,5-double bond, then the reaction of the 2α-acetoxy-compound (29) would lead to an isomer distribution similar to that from cholest-4-ene (1), since



the equatorial 2α-substituent cannot interfere with reagent approach. If, however, the 2α-acetoxy-group serves as a conformational anchor stabilising the monoplanar conformer (X) at the expense of (XI) (in which the group is axial), this would render the reagent–reactant complex of the latter of higher energy than that derived from cholest-4-ene (1). Consequently, less β-attack should occur in (29) than in (1).

Similarly, steric considerations alone suggest that β-attack upon the 2β-acetoxy-compound (34) would be strongly inhibited if the ground state conformation is quasi-*trans* (XII). However, the analogy with 2β-substituted Δ^4-3-ketones,[23] and spectroscopic evidence regarding the conformation of 2β-hydroxy-Δ^4-compounds,[26,27] supports the probability that (34) prefers

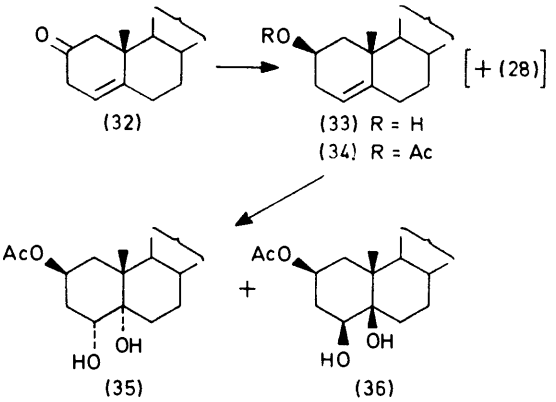


the 1,2-diplanar form (XIII), even in the ground state. If it is correctly contended that such a conformer is a precondition for preferred β-face attack, then a greater proportion of the corresponding 4β,5β-diol should be obtained from (34) than from (1).

[26] D. H. R. Barton and Y. Houminer. *J.C.S. Perkin I.* 1972. 919.

2α-Hydroxycholest-4-ene (28) was prepared as described,[28] and the derived acetate (29) was treated with osmium tetraoxide to give a mixture of two diols, the major component of which (Table 1) was shown (Table 2) to be the 2α-acetoxy-4α,5α-diol (30).

2β-Hydroxycholest-4-ene (33) was prepared by hydride reduction of cholest-4-en-2-one [26] (32). It has been reported [27] that the 2β-hydroxy-compound (33) is the major product from reduction of (32) with lithium tri-t-butoxyaluminium hydride in tetrahydrofuran. In our hands this procedure gave a less favourable mixture (*ca.* 60 : 40) than reduction with sodium borohydride in methanol at 10 °C. The resultant mixture (*ca.* 75 : 25) was separated by repeated chromatography on silica gel, and the major product (33) was converted into the 2β-acetoxy-compound (34). The n.m.r. signals for the 2α-proton in (33) ($W_{\frac{1}{2}}$ 19 Hz) and (34) (m, $W_{\frac{1}{2}}$ 20 Hz) are compatible with a ground state 1,2-diplanar conformation (XIII) for both derivatives.[26,27] The major product of hydroxylation of (34) was identified (Table 2) as the 2β-acetoxy-4β,5β-diol (36).



The hydroxylation results obtained for (29) and (34) are consistent with the hypothesis that conformer (X) favours α-attack whereas conformer (XIII) favours β-attack. Taken together, the results reported here demonstrate that the trend in stereoselectivity accords with that predicted by the conformation of least energy of the reagent–reactant complex. Although the steric bulk of a proximate substituent may have some influence upon the stereochemical outcome, we prefer to interpret its major role as that of a conformational anchor, whose perturbing influence upon the equilibrium favouring β-attack in cholest-4-ene (1) dictates the isomer distribution.

EXPERIMENTAL

M.p.s were determined with a Kofler hot-stage apparatus. Spectra were recorded as follows: i.r., Perkin-Elmer 237 instrument, solvent chloroform; n.m.r., Varian HA100 instrument, solvent deuteriochloroform with tetramethylsilane as internal standard; mass spectra, A.E.I. MS9 instrument. Optical rotations were determined for solutions in chloroform at 24 °C with a Perkin-Elmer 241

[27] V. Černý, M. Buděšinský. and F. Sǒrm, *Coll. Czech. Chem. Comm.*, 1973. **38**. 565.

[28] T. Koga and M. Tomoeda. *Tetrahedron*, 1970. **26**. 1043.

polarimeter, and c.d. spectra were recorded with a JASCO J-20 instrument. Silica gel refers to Kieselgel 60 (Merck).

General Procedure for Hydroxylations.—Osmium tetraoxide (1.05 mol) was added to a solution (2.5%) of the olefin (1 mol) in pyridine. After 48 h at 25 °C, aqueous sodium disulphite (10%; 5 mol) was added and the mixture was stirred for 1 h, then extracted with benzene or chloroform. The combined extracts were washed with aqueous sodium chloride. dried, and evaporated *in vacuo*.

Hydroxylation of Cholest-4-ene (1).—The hydroxylation product of cholest-4-ene (1) (1.45 g) was adsorbed on silica gel (150 g) and eluted with ethyl acetate–benzene (1 : 1) to give the 4α,5α-diol (2) (0.339 g), m.p. 138—140° (plates from aqueous methanol), $[\alpha]_D$ +19° (*c* 0.6), δ 0.66 (13β-CH_3), 0.94 (10β-CH_3), and 3.66br (1 H, $W_{\frac{1}{2}}$ 16 Hz. 4β-H) (lit.,[1] m.p. 139—140°, $[\alpha]_D$ +13.1°). Further elution with the same solvent gave the 4β,5β-diol (4) (1.076 g), m.p. 139—140° (needles from methanol), $[\alpha]_D$ +27° (*c* 0.7), δ 0.66 (13β-CH_3), 0.96 (10β-CH_3), and 4.02br (1 H, $W_{\frac{1}{2}}$ 16 Hz, 4α-H) (lit.,[1] m.p. 135—136°, $[\alpha]_D$ +24.8°).

Treatment of the diol (2) with acetic anhydride–pyridine at 25 °C gave the 4α-acetate (3), m.p. 150—152°, $[\alpha]_D$ +35° (*c* 0.7), δ 0.65 (13β-CH_3), 0.98 (10β-CH_3), 2.05 (OAc), and 4.98 (1 H, t, *J* 8 Hz, 4β-H) (lit.,[6] m.p. 149°. $[\alpha]_D$ +35°); similar treatment of the diol (4) gave the 4β-acetate (5), m.p. 115—117° (rods) or 131—133° (needles) (from chloroform–methanol; both crystalline modifications were mutually interconvertible), $[\alpha]_D$ +23° (*c* 1.1), δ 0.65 (13β-CH_3), 0.97 (10β-CH_3), 2.06 (OAc), and 5.38 (1 H, t, *J* 8 Hz, 4α-H) (lit.,[13] m.p. 115—117°, $[\alpha]_D$ +31°).

Oxidation of the Cholestane-4,5-diols.—(*a*) The 4α,5α-diol (2) (0.05 g) in pyridine (1.5 ml) was added to stirred chromium trioxide (0.1 g) in pyridine (1 ml) at 0 °C. The mixture was kept at 25 °C for 16 h, then poured into aqueous sodium hydrogen carbonate and ice. The product was extracted with benzene and chromatographed on silica gel (5 g) with ethyl acetate–benzene (1 : 19) to give 5-hydroxy-5α-cholestan-4-one (6) (0.026 g), m.p. 158—161° (from chloroform–methanol), $[\alpha]_D$ +58° (*c* 1.2) (lit.,[6] m.p. 159°, $[\alpha]_D$ +55°).

(*b*) Oxidation of the 4β,5β-diol (4) (0.1 g) as in the previous experiment gave, after chromatography, the 5β-hydroxy-4-ketone (7) (0.048 g), m.p. 175—178° (from chloroform–methanol), $[\alpha]_D$ +6° (*c* 0.8) (lit.,[1] m.p. 173—175°, $[\alpha]_D$ +18.3°).

Hydroxylation of Cholest-4-en-3α-yl Acetate (8).—Hydroxylation of compound (8) (0.178 g) and chromatography of the product on silica gel (27 g) with ethyl acetate–benzene (1 : 1) gave a minor product (0.003 g) (M^+ 462), followed by 5β-*cholestane*-3α,4β,5-*triol* 3-*acetate* (9) (0.155 g), m.p. 175—177° (from chloroform–methanol), $[\alpha]_D$ +24° (*c* 1.0), δ 0.65 (13β-CH_3), 0.96 (10β-CH_3), 2.08 (OAc), 3.93br (1 H. d, *J* 9 Hz, 4α-H), and 4.98br (1 H, $W_{\frac{1}{2}}$ 20 Hz, 3β-H) (Found: C, 75.2; H, 10.6%; M^+, 462. $C_{29}H_{50}O_4$ requires C, 75.3; H, 10.9%; *M*, 462).

3α-*Acetoxy*-5-*hydroxy*-5β-*cholestan*-4-*one* (10).—Treatment of the 3α-acetoxy-4β,5β-diol (9) (0.14 g) with chromium trioxide (0.25 g) in pyridine (6 ml) at 25 °C for 16 h, followed by chromatography of the product on silica gel (15 g) with ethyl acetate–benzene (1 : 9), afforded the 4-*ketone* (10) (0.08 g), m.p. 200—205° (from acetone), $[\alpha]_D$ +64° (*c* 1.5), δ 0.65 (13β-CH_3), 1.01 (10β-CH_3), 2.12 (OAc), and 5.73 (1 H. q, *J* 12 and 7 Hz, 3β-H) (Found: C, 75.6; H, 10.3%; M^+, 460. $C_{29}H_{48}O_4$ requires C, 75.6; H, 10.5%; *M*, 460).

3α,5-*Diacetoxy*-5β-*cholestan*-4-*one* (11).—Compound (10) (0.03 g) was treated with toluene-*p*-sulphonic acid (0.005 g) in acetic anhydride (1 ml) at 25 °C for 30 h. Water was added and the solid product was filtered off and adsorbed on silica gel (6 g). Elution with ethyl acetate–benzene (1 : 9) afforded unidentified material (0.007 g) followed by the *diacetoxy-ketone* (11) (0.021 g), m.p. 161—163° (from acetone–methanol), $[\alpha]_D$ +49° (*c* 1.2), $\nu_{max.}$ 1 730br cm^{-1} (Found: C, 74.2; H, 10.0%; M^+, 502. $C_{31}H_{50}O_5$ requires C, 74.1; H, 10.0%: *M*, 502).

Hydroxylation of Cholest-4-en-3β-yl Acetate (12).—The hydroxylation product of cholest-4-en-3β-yl acetate (12) (0.504 g) was adsorbed on silica gel (70 g). Elution with ethyl acetate–benzene (1 : 1) gave starting material (0.098 g) followed by 5α-*cholestane*-3β,4α,5-*triol* 3-*acetate* (13) (0.345 g), m.p. 177—180° (from chloroform–methanol), $[\alpha]_D$ +22° (*c* 0.6), δ 0.66 (13β-CH_3), 0.98 (10β-CH_3), 2.08 (OAc), 3.58 (1 H, d, *J* 9 Hz, 4β-H), and 5.03br (1 H, q, *J* 14 and 9 Hz, 3α-H) (Found: C, 75.6; H, 11.1%; M^+, 462). Further elution with the same solvent afforded 5β-*cholestane*-3β,4β,5-*triol* 3-*acetate* (14) (0.052 g), m.p. 142—146° (from acetone–methanol), $[\alpha]_D$ +55° (*c* 0.8), δ 0.66 (13β-CH_3), 1.01 (10β-CH_3), 2.11 (OAc), 3.99 (1 H, d after D_2O exch., *J* 3.5 Hz, 4α-H), and 5.24br (1 H, $W_{\frac{1}{2}}$ 7 Hz, 3α-H) (Found: C, 75.4; H, 10.8%; M^+, 462).

3β-*Acetoxy*-5-*hydroxy*-5α-*cholestan*-4-*one* (15).—Treatment of the 3β-acetoxy-4α,5α-diol (13) (0.2 g) with chromium trioxide (0.3 g) in pyridine (8 ml) at 25 °C for 16 h gave a product which was adsorbed on silica gel (20 g). Elution with ethyl acetate–benzene (1 : 9) afforded the 4-ketone (15) (0.084 g), m.p. 192—195° (from chloroform–methanol), $[\alpha]_D$ +5° (*c* 1.0), δ 0.65 (13β-CH_3), 0.79 (10β-CH_3), 2.13 (OAc), and 5.92 (1 H, q, *J* 12 and 7 Hz, 3α-H) (lit.,[29] m.p. 178—181°, $[\alpha]_D$ +9°).

3β,5-*Diacetoxy*-5α-*cholestan*-4-*one* (16).—Treatment of compound (15) (0.05 g) with toluene-*p*-sulphonic acid in acetic anhydride, as in a previous experiment, followed by chromatography on silica gel. afforded the *diacetoxy-ketone* (16) (0.032 g). m.p. 173—177° (from acetone–methanol), $[\alpha]_D$ +10° (*c* 0.3). $\nu_{max.}$ 1 730br cm^{-1} (Found: 74.0; H, 10.0%: M^+, 502).

Hydroxylation of Estr-4-en-17β-yl Acetate (17).—Estr-4-en-17β-yl acetate [30] (17) (0.31 g) [m.p. 80—82° (from aqueous methanol), $[\alpha]_D$ +43° (*c* 0.7); prepared by acetylation of the corresponding hydroxy-compound [31]], was hydroxylated and the product was adsorbed on silica gel (50 g). Elution with ethyl acetate–benzene (1 : 1) afforded 5α-*estrane*-4α,5,17β-*triol* 17-*acetate* (18) (0.034 g), m.p. 124—126° (plates from aqueous acetone; depending upon the rate of heating these plates sometimes underwent phase changes at *ca.* 125—126° and 146° to give a crystalline modification, m.p. 157—158°), $[\alpha]_D$ +5° (*c* 0.6), δ 0.81 (13β-CH_3), 2.04 (OAc), 3.28 (1 H, q, *J* 10 and 5 Hz, 4β-H), and 4.6 (1 H, t, *J* 8 Hz, 17α-H) (Found: C, 71.2; H, 9.5%; M^+, 336. $C_{20}H_{32}O_4$ requires C, 71.4; H, 9.6%; *M*, 336). Further elution with the same solvent afforded 5β-estrane-4β,5,17β-triol 17-acetate (19) (0.27 g), m.p. 197—199° (from aqueous acetone), $[\alpha]_D$ +11° (*c* 1.0), δ 0.8 (13β-CH_3), 2.04 (OAc), 3.82br (1 H, m simplifying to q, *J* 10 and 5 Hz, upon addition of D_2O, 4α-H), and 4.61 (1 H, t, *J* 8 Hz,

[29] S. Julia and J.-P. Lavaux, *Bull. Soc. chim. France*. 1963, 1238.

[30] D. P. Strike, D. Herbst, and H. Smith, *J. Medicin. Chem.*, 1967, **10**. 446.

[31] M. S. deWinter, C. M. Siegmann, and S. A. Szpilfogel, *Chem. and Ind.*, 1959, 905.

17α-H) (Found: C, 71.1; H, 9.7%; M^+, 336) (lit.,[9] m.p. 201—203°, $[\alpha]_D$ +14°).

Reduction of Estr-4-en-3-one (20).—Lithium aluminium hydride (0.5 g) was added in portions to a stirred solution of estr-4-en-3-one [18] (20) (3.2 g) in ether (60 ml) at 25 °C. After 1 h the excess of reagent was destroyed and the product was isolated and adsorbed on silica gel (350 g). Elution with ethyl acetate–benzene (1 : 4) gave *estr-4-en-3α-ol* (21) (0.656 g), m.p. 96—98° (from aqueous acetone), $[\alpha]_D$ +103° (*c* 1.0), δ 0.75 (13β-CH_3), 4.12br (1 H, $W_{\frac{1}{2}}$ 10 Hz, 3β-H), and 5.54br (1 H, d, *J* 4 Hz, 4-H) (Found: C, 83.25; H, 10.9%; M^+, 260. $C_{18}H_{28}O$ requires C, 83.0; H, 10.8%; *M*, 260). Further elution with the same solvent gave mixed fractions (0.38 g), followed by *estr-4-en-3β-ol* (23) (2.05 g), m.p. 100—101° (from aqueous acetone; phase change at 55—65°), $[\alpha]_D$ +20° (*c* 0.7), δ 0.75 (13β-CH_3), 4.16br (1 H, $W_{\frac{1}{2}}$ 18 Hz, 3α-H), and 5.38br (1 H, $W_{\frac{1}{2}}$ 4.5 Hz, 4-H) (Found: C, 82.8; H, 10.9%; M^+, 260).

Treatment of the 3α-ol (21) with acetic anhydride–pyridine at 25 °C gave *estr-4-en-3α-yl acetate* (22), m.p. 77—78° (from acetone–methanol), $[\alpha]_D$ +192° (*c* 1.2), δ 0.74 (13β-CH_3), 2.02 (OAc), 5.16br (1 H, $W_{\frac{1}{2}}$ 10 Hz, 3β-H), and 5.5br (1 H, d, *J* 4 Hz, 4-H) (Found: C, 79.4; H, 10.2%; M^+, 302. $C_{20}H_{30}O_2$ requires C, 79.4; H, 10.0%; *M*, 302). Similar treatment of the 3β-ol (23) gave *estr-4-en-3β-yl acetate* (24) as an oil, $[\alpha]_D$ +11° (*c* 0.6), δ 0.74 (13β-CH_3), 2.04 (OAc), 5.25br (1 H, $W_{\frac{1}{2}}$ 22 Hz, 3α-H), and 5.32br (1 H, $W_{\frac{1}{2}}$ 5 Hz, 4-H) (Found: M^+, 302; combustion analyses were unsatisfactory owing to decomposition).

Hydroxylation of Estr-4-en-3α-yl Acetate (22).—The hydroxylation product of estr-4-en-3α-yl acetate (22) (0.475 g) was adsorbed on silica gel (50 g). Elution with ethyl acetate–benzene (1 : 1) gave a trace of crystalline material (*ca.* 0.001 g) (M^+ 336) followed by *5β-estrane-3α,4β,5-triol 3-acetate* (25) (0.457 g), double m.p. 123—125° and 137—139° (from aqueous acetone), $[\alpha]_D$ +6° (*c* 1.0), δ 0.71 (13β-CH_3), 2.08 (OAc), 3.75 (1 H, d, *J* 9.5 Hz, 4α-H), and 5.0 (1 H, dq, *J* 10, 9.5, and 5.5 Hz, 3β-H) (Found: C, 71.3; H, 9.6%; M^+, 336).

Hydroxylation of Estr-4-en-3β-yl Acetate (24).—Hydroxylation of compound (24) (1 g) and chromatography of the product on silica gel (100 g) with ethyl acetate–benzene (2 : 3) gave *5α-estrane-3β,4α,5-triol 3-acetate* (26) (0.65 g), m.p. 189—194° (from acetone), $[\alpha]_D$ +12° (*c* 0.8), δ 0.72 (13β-CH_3), 2.1 (OAc), 3.22 (1 H, d. *J* 9 Hz, 4β-H), and 4.96 (1 H, sext, *J* 9,9, and 5 Hz, 3α-H) (Found: C, 71.1; H, 9.7%; M^+, 336), and *5β-estrane-3β,4β,5-triol 3-acetate* (27) (0.125 g), m.p. 190—193° (from acetone), $[\alpha]_D$ +47° (*c* 0.6), δ 0.71 (13β-CH_3), 2.12 (OAc), 3.79 (1 H, d, *J* 4 Hz. 4α-H), and 5.26br (1 H, $W_{\frac{1}{2}}$ 8 Hz, 3α-H) (Found: C, 71.3; H, 9.9%; M^+. 336). Further elution with the same solvent afforded more (27) (0.132 g) contaminated with traces of unidentified material (t.l.c.).

Cholest-4-en-2α-yl Acetate (29).—Treatment of the alcohol [28] (28) with acetic anhydride–pyridine at 25 °C afforded the *acetate* (29), m.p. 62—65° (from acetone–methanol), $[\alpha]_D$ +19° (*c* 0.3), δ 0.66 (13β-CH_3), 1.08 (10β-CH_3), 2.0 (OAc), 4.94br (1 H, $W_{\frac{1}{2}}$ *ca.* 23 Hz, 2β-H), and 5.1br (1 H, d, *J* 5 Hz, 4-H) (Found: C, 81.4; H, 11.4%; M^+, 428. $C_{29}H_{48}O_2$ requires C, 81.25; H, 11.3%; *M*, 428).

Hydroxylation of Cholest-4-en-2α-yl Acetate (29).—The hydroxylation product of cholest-4-en-2α-yl acetate (29) (0.99 g) was adsorbed on silica gel (100 g). Elution with ethyl acetate–benzene (2 : 3) afforded *5α-cholestane-2α,4α,5-triol 2-acetate* (30) (0.609 g), m.p. 145—148° (from acetone–hexane), $[\alpha]_D$ +2° (*c* 0.3), δ 0.65 (13β-CH_3), 0.91 (10β-CH_3), 2.03 (OAc), 3.74 (1 H, q, *J* 11 and 5 Hz, 4β-H), and 4.98 (1 H, sept, *J* 10, 10, 5, and 5 Hz, 2β-H) (Found: C, 75.4; H, 10.9%; M^+, 462). Further elution with the same solvent gave *5β-cholestane-2α,4β,5-triol 2-acetate* (31) (0.37 g), m.p. 141—143° (from acetone–methanol), $[\alpha]_D$ +8° (*c* 0.4), δ 0.66 (13β-CH_3), 0.96 (10β-CH_3), 2.04 (OAc), 4.28 (1 H, t, *J* 8 Hz, 4α-H), and 5.0br (1 H, t, *J* 3 Hz, 2β-H) (Found: C, 75.0; H, 11.1%; M^+, 462).

Reduction of Cholest-4-en-2-one (32).—Sodium borohydride (0.3 g) was added in portions to a stirred solution of cholest-4-en-2-one [26] (32) (1.609 g) in methanol (200 ml) at 10 °C. After 30 min at 10 °C the reaction was quenched by addition of aqueous ammonium chloride, and the product (1.57 g) was extracted with dichloromethane and adsorbed on silica gel (220 g). Elution with ethyl acetate–benzene (1 : 9) afforded cholest-4-en-2β-ol (33) (0.962 g), m.p. 115—117° (from aqueous acetone), $[\alpha]_D$ −5° (*c* 0.3), δ 0.66 (13β-CH_3), 1.09 (10β-CH_3), 3.94br (1 H, $W_{\frac{1}{2}}$ 19 Hz, 2α-H), and 5.16br (1 H, t, *J* 3.5 Hz, 4-H) (Found: C, 84.2; H, 12.2%; M^+, 386. $C_{27}H_{46}O$ requires C, 83.9; H, 12.0%; *M*, 386) (lit.,[27] m.p. 105—106°, $[\alpha]_D$ ±0°). Further elution gave mixed fractions (0.452 g), followed by cholest-4-en-2α-ol (28) (0.131 g), m.p. and mixed m.p. 138—141°. Repeated chromatography of the mixed fractions (*ca.* 1 : 1 mixture by t.l.c.) gave more pure (33) and (28).

Treatment of the alcohol (33) with acetic anhydride–pyridine at 25 °C gave the *2β-acetate* (34) as an oil, $[\alpha]_D$ +8° (*c* 0.3), δ 0.66 (13β-CH_3), 1.06 (10β-CH_3), 1.99 (OAc), 5.02 (1 H, quint, '*J*' 4 × 5 Hz, 2α-H), and 5.17br (1 H, t, *J* 3.5 Hz, 4-H) (Found: M^+, 428).

Hydroxylation of Cholest-4-en-2β-yl Acetate (34).—The hydroxylation product of cholest-4-en-2β-yl acetate (34) (0.68 g) was adsorbed on silica gel (80 g). Elution with ethyl acetate–benzene (3 : 7) afforded *5β-cholestane-2β,4β,5-triol 2-acetate* (36) (0.61 g), m.p. 74—79° (from pentane containing a trace of pyridine), $[\alpha]_D$ +17° (*c* 0.3), δ 0.66 (13β-CH_3), 1.01 (10β-CH_3), 2.02 (OAc), 4.1 (1 H, q, *J* 12 and 4.5 Hz, 4α-H), and 4.8 (1 H, m, $W_{\frac{1}{2}}$ 32 Hz, 2α-H) (Found: C, 75.5; H, 10.5%; M^+, 462). Further elution with the same solvent afforded *5α-cholestane-2β,4α,5-triol 2-acetate* (35) (0.053 g), m.p. 179—182° (from methanol), $[\alpha]_D$ +21° (*c* 0.5), δ 0.64 (13β-CH_3), 1.05 (10β-CH_3), 2.0 (OAc), 3.92 (1 H, q, *J* 9 and 7 Hz, 4β-H), and 5.14br (1 H, t, *J* 3 Hz, 2α-H) (Found: C, 75.3; H, 11.1%; M^+, 462).

[6/1463 *Received*, 26*th July*, 1976]