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ACID CATALYSED REACTIONS OF 5α-HYDROXY-STEROIDS—V*

SOME REACTIONS OF SUBSTITUTED CHOLEST-4-ENES

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Abstract—The four epimeric 3,6-diacetoxycholest-4-enes undergo equilibration at C-3, but not at C-6, in sulphuric acid (or HBF_4)–acetic acid–acetic anhydride solutions. The equilibrium ratios of epimers have been estimated. Under similar reactions conditions various 6β-substituted-3β-acetoxycholest-4-enes suffer acetolysis at C-6 with retention of configuration.

DURING investigation[1] of the reactions of 6β-substituted 5α-hydroxy steroids with sulphuric acid in acetic acid–acetic anhydride it became clear that the 6β-substituted 3β-acetoxy-cholest-4-enes formed in the reactions were susceptible to epimerization at C-3 and acetolysis at C-6 in the reaction medium. Collins and Hobbs[2] have demonstrated the lability of the bromine atom of 6α- and 6β-bromocholest-4-en-3-ones, either on chromatography over neutral or alkaline alumina, or on treatment with methanolic hydrogen chloride. In addition, treatment[3] of 3β-acetoxy-6α-bromocholest-4-ene with methanol–pyridine gave the 6β-methoxy derivative which on further reaction with methanolic hydrogen chloride was converted into 3β,6β-dimethoxycholest-4-ene.

Recently Snatzke has shown[4] that treatment of 3β-methoxy-5α-hydroxy-6β-acetoxycholestane (I) with $KHSO_4$–acetic anhydride at 50–55° gives, in addition to the expected rearranged product (II) and 3β-methoxy-6β-acetoxycholest-4-ene (IIIa), the 3β,6β- and 3α,6β-diacetoxycholest-4-enes (IIIb and IV). In addition, 3β-methoxy-6β-acetoxycholest-4-ene (IIIa) was partially converted by $KHSO_4$–acetic anhydride at 75° into the 3β,6β- and 3α,6β-diacetoxycholest-4-enes. (IIIb and IV).

We report reactions of 6-substituted-3β-acetoxycholest-4-enes with sulphuric acid–acetic acid–acetic anhydride at 30°. Under these conditions the 3β,6β-diacetate (IIIb) was partially epimerized to give the 3α,6β-diacetate (IV). The 3β- and 3α-epimers were isolated in the ratio ca. 3:1; no other products were detected. The pure 3α,6β-diacetate (IV) was converted under the same reaction conditions into a similar mixture of the C-3 epimers.

The inversion of configuration at C-3 is characterized by a large change in optical rotation (3β,6β-diacetate, $[\alpha]_D$ −15°; 3α,6β-diacetate, $[\alpha]_D$ + 130°). However, an attempt at determining the exact equilibrium ratio of the epimers under catalysis by sulphuric acid failed. No true equilibrium was attained as indicated in the Figure

* Part IV, M. J. Coppen, M. P. Hartshorn and D. N. Kirk, *J. Chem. Soc.*, in press.
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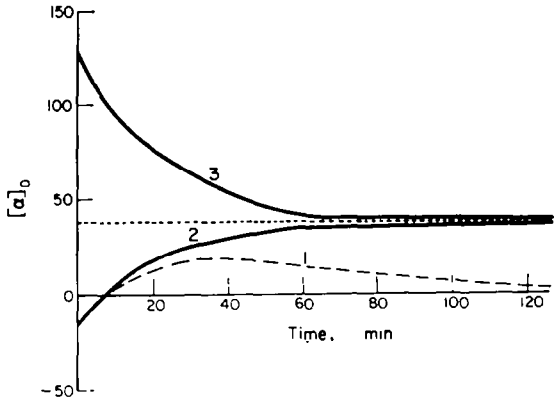
[1] J. W. Blunt, A. Fischer, M. P. Hartshorn, F. W. Jones, D. N. Kirk and S. W. Yoong, *Tetrahedron* **21**, 1567 (1965).
[2] D. J. Collins and J. J. Hobbs, *Austral. J. Chem.* 661 (1964).
[3] D. J. Collins and J. J. Hobbs, *Austral. J. Chem.* 677 (1964).
[4] G. Snatzke, *Liebig's Ann.* **686**, 167 (1965).



Specific Rotation Changes during Equilibration of 3β,6β- (IIIb) and 3α,6β-Diacetoxycholest-4-enes (IV).

(curve 1) and the total steroid recoverable diminished as the reaction time was prolonged. Slow sulphonation is believed to account for this loss of neutral steroid. Use of hydrofluoroboric acid instead of sulphuric acid as the catalyst allowed a steady final reading of the specific rotation of the solution to be attained, starting either from the 3β- (curve 2) or 3α-epimer (curve 3). The equilibrium rotation corresponds to a 3β-:3α- ratio of 1·8:1. The rate of the sulphuric acid catalysed epimerization process was greater than that for hydrofluoroboric acid, the latter being used at 10X the concentration of sulphuric acid to achieve a similar rate of reaction.

The apparent selectivity of epimerization of both 3β,6β- and 3β,6α-diacetoxycholest-4-enes, with no change in stereochemistry at C_6, raises questions of mechanistic interpretation. It is hoped that work now in progress will provide a solution to the problem. 3β,6α-Diacetoxy-5α-hydroxycholestane (V) has been reported[4] to give 3β,6α-diacetoxycholest-4-ene (VI) on treatment with $KHSO_4$–acetic anhydride at 75°. Reaction of the 3β,6α-diacetoxy-5α-hydroxy- compound (V) with sulphuric acid–acetic acid–acetic anhydride at 20° was followed polarimetrically. The crude product, isolated when a constant rotation for the reaction mixture was attained, was shown

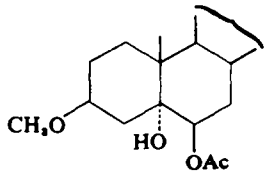
TABLE 1. NMR SPECTRA* OF 3,6-DISUBSTITUTED CHOLEST-4-ENES: C-4, C-18 AND C-19 PROTON RESONANCES. (τ-VALUES).

Compound	4-H[a]	$C^{19}H_3$	$C^{18}H_3$
3β,6α-Diacetate (VI)	4·58(4)	8·89	9·31
3β,6β-Diacetate (IIIb)	4·41(3)	8·83	9·27
3α,6α-Diacetate (VII)	4·41(5)	8·95	9·32
3α,6β-Diacetate (IV)	4·25(6·5)	8·90	9·27
3β-OAc, 6β-F (IIId)	4·43(5·5)	8·82[b]	9·29
3β-OAc, 6β-Cl (IIIe)	4·45(4·5)	8·63	9·25

* Determined at 60 Mc in $CDCl_3$ with $CHCl_3$ and $(CH_3)_4Si$ as internal standards.

[a] Signal appeared as doublet or broad singlet. Figure in parentheses gives width at half-height in c/s.

[b] Doublet, J = 2 c/s, due to spin-spin coupling with 6β-F.



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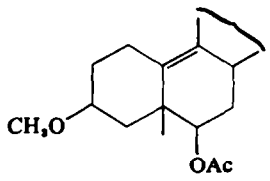

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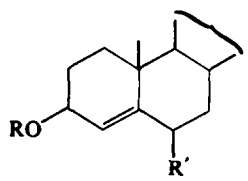

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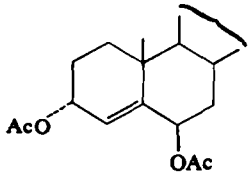

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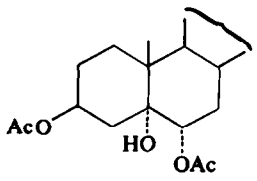
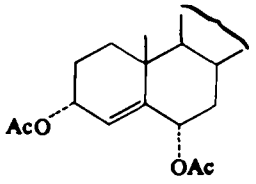
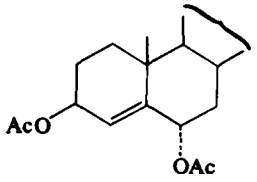


V

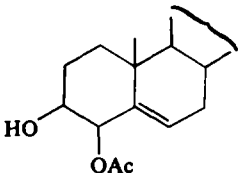
a R,—CH_3; R′,—OAc
b R,—Ac; R′,—OAc
c R,—Ac; R′,—OCH_3
d R,—Ac; R′,—F
e R,—Ac; R′,—Cl
f R,—Ac; R′,—I



VII



VI



VIII

to consist largely of two compounds, the 3β,6α-(VI) and 3α,6α-diacetoxycholest-4-enes (VII) obtained in 52% and 40% yields respectively. The structure of the novel 3α,6α-epimer (VII) was inferred from its NMR spectrum (Table 1) and its very high positive specific rotation, as required by the Mills' rule. It was apparent that the 3,6α-epimers undergo equilibration in a similar manner to the 3,6β-epimers. A polarimetric estimate of the position of equilibrium gave the 3β,6α-:3α,6α- ratio as 0·94:1 although the rate of attainment of equilibrium was faster (15X) than for the 3,6β-epimers. Either sulphuric or hydrofluoroboric acid led to stable equilibrium rotations for the 6α-acetoxy compounds. The above results may be rationalized in terms of the rapid and virtually complete conversion of the 3β,6α-diacetoxy-5α-hydroxycholestane (V) into the Δ^4-3β,6α-diacetate (VI; in accord with Snatzke's observations[4]) followed by the slower equilibration at C-3 under the acidic conditions.

We have also examined the acetolysis of various 6β-substituted-3β-acetoxycholest-4-enes. The reaction of the 6β-methoxy compound (IIIc) with sulphuric acid–acetic acid–acetic anhydride was followed until the maximum positive value ([α]D +29°) was obtained for the mixture. Chromatographic separation of the products gave starting material (IIIc; 46%) and 3β,6β-(IIIb) and 3α,6β-diacetoxycholest-4-enes (IV; 28% and 12% respectively).

Similar reactions were carried out using the 6β-fluoro and 6β-chloro derivatives.

In the case of the 6β-fluoro compound (IIId; prepared by dehydration of 3β-acetoxy-6β-fluoro-5α-hydroxycholestane with thionyl chloride–pyridine at 0°) the maximum positive rotation of the reaction mixture was reached in a similar time to that of the 3β,6β-diacetoxycholest-4-ene (IIIb). The products (separated on alumina) at that time were 3β,6β-(IIIb) and 3α,6β-diacetoxycholest-4-enes (IV; 46% and 20% respectively) and a mixture (30%) of more polar products which were not identified. The rate of attainment of the maximum specific rotation for reaction mixtures using 3β-acetoxy-6β-chlorocholest-4-ene (IIIe) was slower (5X) than for the 6β-fluoro (IIId) and 6β-acetoxy (IIIb) compounds. The IR spectrum, TLC on silica, and qualitative analysis of the crude product for chlorine showed it to contain a considerable proportion of the starting material and no hydroxyl group containing substance. However, chromatography on deactivated alumina afforded 3β,6β-(IIIb) and 3α,6β-diacetoxycholest-4-enes (IV; 20% and 9% respectively) and three more polar products. The first two of these to be eluted (total ca. 20%) were incompletely separated and were not identified, but were shown (IR spectra) to be hydroxy-acetoxy compounds. The remaining product (40%) was identified as 4β-acetoxycholest-5-en-3β-ol[5] (VIII). The latter compound was subsequently obtained in high yield by passing the 6β-chloro-Δ^4-compound (IIIe) through a column of alumina. It seems probable that the two minor polar components were derived by reaction of 3α-acetoxy-6β-chlorolest-4-ene with alumina. The significantly faster replacement of fluorine (by acetate) for the 6β-fluoro-Δ^4- compound (IIId) compared with the analogous reaction of the 6β-chloro-Δ^4- compound (IIIe) is consistent with the known susceptibility[6] of reactions of alkyl fluorides to acid catalysis.

The close similarity between these product mixtures and the by-products obtained from the Westphalen rearrangements of the corresponding 6β-substituted-5α-hydroxy compounds[1] can be explained if the primary reactions of the 5α-hydroxy compounds are (a) rearrangement (b) dehydration leading to Δ^4-derivatives and (c) acetylation at C-5. The Δ^4-derivatives undergo the further reactions described above. The relative importance of these three primary reactions is determined by the C-6 substituent. The extent of the dehydration leading to Δ^4-compounds is in the order 6βF < 6β-OCH_3 6β-Cl < 6β-Br 6β-OAc < 6β-I 6α-OAc. The rate of acetolysis of the 6β-iodo-Δ^4-compound (IIIf), presumably formed transitorily in >25% yield on treatment of the 5α-hydroxy-6β-iodo compound with sulphuric acid–acetic acid–acetic anhydride, could not be studied. Attempted dehydration of the iodohydrin with thionyl chloride–pyridine gave cholesteryl acetate in high yield. The reaction presumably involves loss of I^+, possibly assisted by nucleophilic attack by a pyridine molecule: an analogous reaction was postulated to account for the formation of cholesteryl acetate in significant yield during the iodohydrin-sulphuric acid–acetic acid–acetic anhydride[1] reaction.

EXPERIMENTAL

Rotations were measured for $CHCl_3$ solutions at room temp. IR spectra were recorded for CS_2 solutions. Alumina used for chromatography was Peter Spence, Grade H, deactivated by addition of 5% of 10% acetic acid. Light petroleum refers to the fraction of b.p. 50–70°.

[5] V. Petrow, O. Rosenheim and W. W. Starling, *J. Chem. Soc.* 135 (1943).
[6] A. Streitweiser, *Chem Rev.* **56**, 622 (1956).

Equilibration of 3,6-diacetoxycholest-4-enes

The specific rotations of acetic acid–acetic anhydride (4:1) solutions of the steroid (1%) and HBF_4 (0·4%) were measured at 30°.

	Initial $[\alpha]_D$	Final $[\alpha]_D$	Time for half reaction
3β,6β-Diacetate (IIIb)	−15°	+37°	14–15 min
3α,6β-Diacetate (IV)	+130°	+36°	
Equilibrium ratio 3β-:3α-, 1·8:1 (ΔF 0·35 Kcal mol^{-1} at 30°)			
3β,6α-Diacetate (VI)	+13°	+84°	< 1 min
3α,6α-Diacetate (VIII)	+153°	+85°	
Equilibrium ratio 3β-:3α-, 0·94:1 (ΔF ~ 0·03 Kcal mol^{-1} at 30°).			

Preparative reactions of Δ⁴-diacetates

(a) *3β,6α-Diacetoxycholest-4-ene* (VI). A solution of steroid (330 mg) in acetic acid (28 ml) and acetic anhydride (7 ml) containing H_2SO_4 (100%; 14 mg) was kept at 20° for 2 hr. Isolation by means of $CHCl_3$ gave a crude product (310 mg), from which VI (122 mg) and VII (121 mg) were separated by chromatography on alumina.

(b) *3β,6β-Diacetoxycholest-4-ene* (IIIb). A solution of steroid (5 g) in acetic acid (200 ml) and acetic anhydride (50 ml) containing H_2SO_4 (100%; 100 mg) was kept at 20° for 2 hr. Isolation by means of $CHCl_3$ gave a crude product from which IIIb (3 g) and IV (1 g), m.p. and mixed m.p. 105–106°, $[\alpha]_D$ +136° (*c* 1·0) (Lit.[7] value, m.p. 102·5–103·5° $[\alpha]_D$ +117°) were separated by chromatography.

Dehydration of 3β,6α-Diacetoxy-5α-hydroxycholestane (V)

A solution of steroid (500 mg) in acetic acid (40 ml) and acetic anhydride (10 ml) containing H_2SO_4 (100%; 10 mg) was kept at 30° for 1 hr. Isolation by means of $CHCl_3$ gave a crude product (486 mg). Crystallization from pentane gave VI (174 mg), m.p. 162–163°, $[\alpha]_D$ +26° (*c* 1·0); Lit.[8] value m.p. 166–167°, $[\alpha]_D$ +20°. The residue from the crystallization was adsorbed onto alumina (10 g). Elution with light petroleum–benzene (50:1) gave further VI (65 mg). Elution with light petroleum–benzene mixtures (50:1 to 8:1) gave fractions (total ca. 35 mg) shown (IR spectrum and $[\alpha]_D$) to consist mainly of VII contaminated with VI. Finally, elution with light petroleum–benzene (8:1) gave the *3α,6α-diacetate* (VII; 199 mg) as prisms (from MeOH), m.p. 108–109°, $[\alpha]_D$ +158° (*c* 1·03), ν_{max} 1740–1730 and 1244–1230 cm^{-1} (broad maxima, OAc), 1656 cm^{-1} (C=C). (Found: C, 76·5; H, 10·6. $C_{31}H_{50}O_4$ requires: C, 76·5; H, 10·35%.)

Acetolysis of 6β-substituted-3β-acetoxycholest-4-enes

A solution of the steroid (0·02 M) and H_2SO_4 (0·008 M) in acetic acid (66%), acetic anhydride (16%) and CCl_4 (18%) was kept at 30° until the maximum positive specific rotation for the solution was attained.

	Initial $[\alpha]_D$	Final $[\alpha]_D$	Relative rates
6β-F (IIId)	−27°	+15°	1
6β-Cl (IIIe)	−71°	+6°	5
6β-OAc(IIIb)	−15°	+18°	~1

Acetolysis of 6β-methoxy-3β-acetoxycholest-4-ene (IIIc)

A solution of steroid (188 mg) in acetic acid (8 ml) and acetic anhydride (2 ml) containing H_2SO_4 (100%; 10 mg) were kept at 30° for 1·75 hr. Isolation by means of benzene–ether (1:1) gave a crude

[7] Y. Urushibara and K. Mori, *Bull. Chem. Soc. Japan* **31**, 1068 (1958).
[8] V. Prelog and E. Tagmann, *Helv. Chim. Acta* **27**, 1867 (1944).

Product analysis

	3β,6β-diacetate (IIIb)	3α,6β-diacetate (IV)	Others
6β-F (IIId)	46%	20%	ca. 30% unidentified
6β-Cl (IIIe)	20%	9%	40% VIII 25% unidentified hydroxyacetates.

product (175 mg) which was adsorbed onto alumina (25 g). Elution with light petroleum–benzene (20:1) gave starting material (IIIc; 86 mg; 46%). Light petroleum–benzene (10:1) and (4:1) eluted IIIb (54 mg; 28%) and IV (23 mg; 12%) respectively.

Attempted dehydration of 3β-acetoxy-5α-hydroxy-6β-iodocholestane

Thionyl chloride (1·5 ml) was added dropwise to a solution of the steroid (1·5 g) in pyridine (25 ml) at 0° and the solution kept at 0° for 20 min. Isolation by means of pentane and crystallization of the crude product from EtOH gave cholesteryl acetate (899 mg), m.p. 114–115°, $[\alpha]_D$ −43° (*c* 1·0).

3β-Acetoxy-6β-flourocholest-4-ene (IIId)

A solution of the 5α-hydroxy-6β-fluoro compound (2 g) in pyridine (20 ml) containing $SOCl_2$ (2 ml) was kept at 0° for 30 min. Isolation by means of pentane and crystallization of the crude product from MeOH gave the *6β-fluoro-Δ⁴-compound* (IIId; 1·2 g) as needles, m.p. 69–70°, $[\alpha]_D$ −35° (*c* 1·0), ν_{max} 1730 and 1240 cm^{-1} (OAc). (Found: C, 77·8; H, 10·9; F, 4·3. $C_{29}H_{47}O_2F$ requires: C, 78·0; H, 10·6; F, 4·25%.)

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