A Study of Base-catalysed Opening of βγ-Epoxy-ketones

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The base-catalysed isomerization of typical steroidal βγ-epoxy-ketones to the corresponding γ-hydroxy-αβ-unsaturated ketones has been studied. A mechanism for the reaction is proposed, based on kinetic data including deuterium isotope effect studies. No neighbouring hydroxy-group participation was observed in the isomerization reaction.

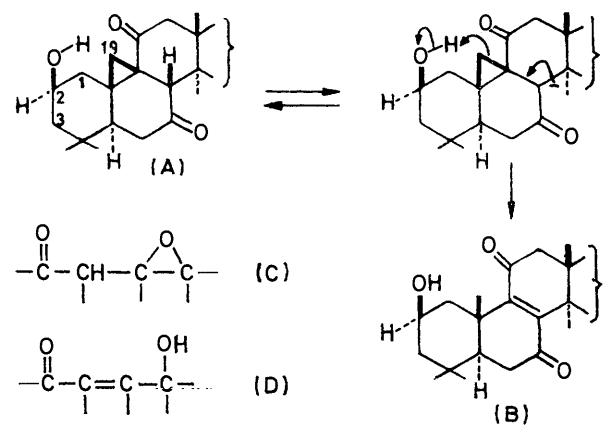
NEIGHBOURING group participation in 'cationic' reactions has been known for many years. It has been thoroughly investigated in a variety of systems and types of reaction, and the phenomenon is well understood.[1-3] A similar effect is also observed, in certain cases, in radical reactions.[1]

Participation of neighbouring groups in 'anionic' processes is not so common, however; only a few examples are known. Neighbouring hydroxy-group participation in the alkaline hydrolysis of esters has frequently been reported.[1,4,5] The hydroxy-group in these cases reacts as an electrophile by hydrogen bonding either to the carbonyl oxygen or to the ether oxygen atom. As a result, the attack by a hydroxide anion is facilitated and the transition state anion is stabilised. Nucleophilic participation by hydroxy-groups in ester hydrolysis is also known,[6] but this belongs to the class of 'cationic' reactions.

We recently reported[7] an unusual neighbouring hydroxy-group participation in the base-catalysed isomerization of βγ-cyclopropyl ketones. It was found that neither the 2α-hydroxy-isomer nor the 2-keto-derivative could be isomerized under the same basic conditions that smoothly isomerized the 2β-alcohol of part structure (A). This unexpected result was attributed to the 'participation of the (axial) 2β-hydroxy-group in protonating the incipient 19-methyl carbanion at the same time as the C(9)–C(19)-cyclopropane bond is breaking.'

The aim of the present investigation was to establish the generality of this phenomenon. In particular we were interested to see whether it exists in βγ-epoxy-ketones. Compounds of type (C) are known to undergo ready isomerization on treatment with alkali to the corresponding γ-hydroxy-αβ-unsaturated ketones (D).

Thus, steroidal 9α,11α-epoxy-7-ketones are isomerized to the 11α-hydroxy-Δ^8-7-ketones.[8] 5β,10β-Epoxy-3-keto-19-norsteroids give the 10β-hydroxy-Δ^4-3-ketones,[9] and steroidal 14β,15β-epoxy-17-ketones are isomerised to the 14β-hydroxy-Δ^{15}-17-ketones.[10,11] In all these cases the configuration of the C–O bond is retained. Other reactions, such as the conversion of 1-acetyl-4,5-epoxycyclohexene into acetophenone[12] and the rearrangement of phenylpropene oxide to give cinnamyl

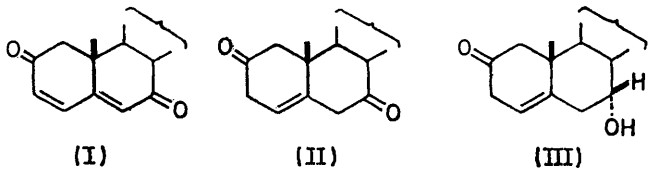


alcohol,[13] both of which are effected by sodamide, proceed by a similar type of mechanism. Thermal isomerization of βγ-epoxy-ketones is also known; thus 4α,5α-epoxycholestan-2-one is converted into 5α-hydroxycholest-3-en-2-one when heated to about 200 °C.[14]

Synthetic Aspects.—Cholesta-3,5-diene-2,7-dione (I) was chosen as a suitable starting material for the preparation of epoxy-steroids with oxygen substituents both at positions 2 and 7. This dienedione[15a] was obtained in 19% yield by oxidation of cholesta-3,5-dien-7-one with t-butyl chromate in refluxing carbon tetrachloride[15b] and showed the n.m.r. spectrum characteristic of these systems. Reduction of compound (I) with zinc and acetic acid in methanol at room temperature proceeded smoothly to give cholest-4-ene-2,7-dione (II). Reduction of the latter with sodium borohydride in methanol at 0 °C gave a mixture of which the main component (56%), isolated by t.l.c., was 7α-hydroxycholest-4-en-2-one (III) (see further below). A small

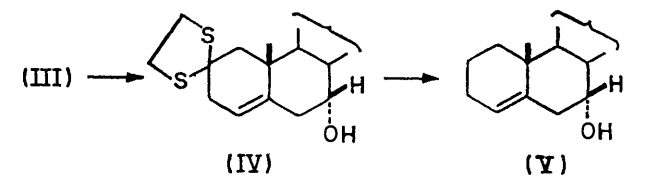
[1] B. Capon, *Quart. Rev.*, 1964, **18**, 45.
[2] L. Goodman, *Adv. Carbohydrate Chem.*, 1967, **22**, 109.
[3] W. P. Jencks, 'Catalysis in Chemistry and Enzymology,' McGraw-Hill, New York, 1969, p. 8.
[4] S. M. Kupchan, P. Slade, R. J. Young, and G. W. A. Milne, *Tetrahedron*, 1962, **18**, 499.
[5] H. B. Henbest and B. J. Lovell, *J. Chem. Soc.*, 1957, 1965.
[6] B. Capon, S. T. McDowell, and M. V. Raftery, *Chem. Comm.*, 1971, 389 and papers cited therein.
[7] D. H. R. Barton, C. F. Garbers, D. Giacopello, R. G. Harvey, J. Lessard, and D. R. Taylor, *J. Chem. Soc.* (*C*), 1969, 1050.
[8] C. Djerassi, E. Batres, M. Velasco, and G. Rosenkranz, *J. Amer. Chem. Soc.*, 1952, **74**, 1712.
[9] J. P. Ruelas, J. Iriarte, F. Kincl, and C. Djerassi, *J. Org. Chem.*, 1958, **23**, 1744.
[10] F. Sondheimer and S. Burstein, *Proc. Chem. Soc.*, 1959, 228.
[11] F. Sondheimer, S. Burstein, and R. Mechoulam, *J. Amer. Chem. Soc.*, 1960, **82**, 3209.
[12] E. A. Braude, E. R. H. Jones, F. Sondheimer, and J. B. Toogood, *J. Chem. Soc.*, 1949, 607.
[13] L. J. Haynes, I. Heilbron, E. R. H. Jones, and F. Sondheimer, *J. Chem. Soc.*, 1947, 1583.
[14] R. J. Conca and W. Bergmann, *J. Org. Chem.*, 1939, **4**, 29; 1953, **18**, 1104.
[15] (*a*) J. R. Hanson, personal communication; see also ref. 32; (*b*) K. Yasuda and H. Mori, *Chem. and Pharm. Bull.* (*Japan*), 1967, 179.

amount (<10%) of 2β-hydroxycholest-4-en-7-one was also formed. This product could not be separated by t.l.c. and its presence was detected only after treating the



(I) (II) (III)

crude mixture with hydrochloric acid in refluxing ethanol and isolating the corresponding conjugated ketone, 2β-hydroxycholest-5-en-7-one (see later). The n.m.r. spectrum of the hydroxy-ketone (III) indicated its structure. The chemical shift of the C-19 protons is known to be affected by both the nature and the location of substituents on rings A and B of the steroid.[16a] A 7-keto-group causes a downfield shift of 0·275 p.p.m. A 2-keto-group has an opposite effect and the magnitude of the shift is small (0·025 p.p.m.).[16a] The C-19 protons of cholest-4-ene-2,7-dione (II) resonate at τ 8·80, and those of the hydroxy-ketone (III) at τ 9·02. Thus it is the 7-keto-group of (II) which has been reduced. Moreover, the 2β-hydroxy-7-keto-isomer, which is the expected product if reduction occurs at the 2-keto-group,[17] should show an opposite effect; an additional downfield shift (0·250 p.p.m.) being caused by the 2β-hydroxy-group. In addition, the hydrogen atom geminal to the hydroxy-group in (III) gives rise to a multiplet of $W_{\frac{1}{2}}$ 7·0 Hz. This is a characteristic value for an equatorial proton.[18] Treatment of the hydroxy-ketone (III) either with acid (see before) or with base [19,20] did not give an αβ-unsaturated ketone. A chemical proof for the structure of (III) was finally obtained by the following transformations (Scheme 1). Treatment of (III) with ethane-



(III) (IV) (V)

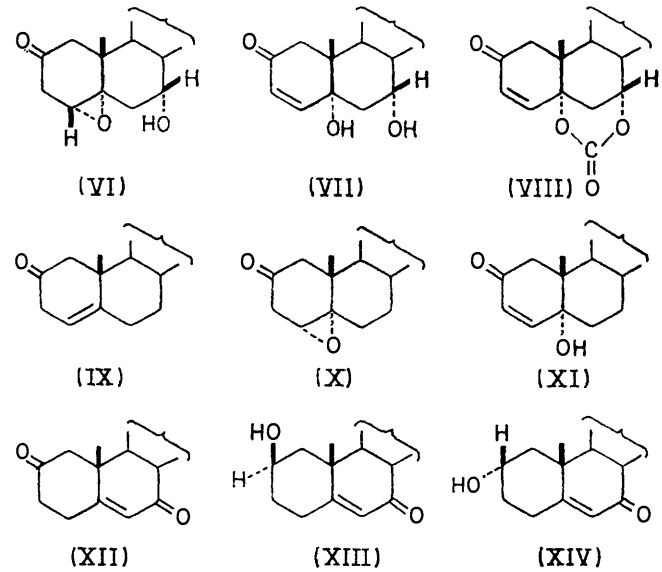
SCHEME 1

dithiol in acetic acid, with boron trifluoride as catalyst, gave the ethylene dithioacetal (IV). This was reduced with Raney nickel in refluxing ethanol to give epi-ψ-cholesterol [20] (V).

The formation of compound (III) as the main product in the sodium borohydride reduction of (II) indicates that the 7-keto-group is attacked more easily from the β- than from the α-side. This is in good agreement with the observed stereochemical course of sodium borohydride reduction of 7-keto-steroids.[17,20]

The structure of the hydroxy-ketone (III) was further confirmed as follows. 4α,5α-Epoxy-7α-hydroxycholestan-2-one (VI) was easily obtained in almost quantitative yield by treatment of compound (III) with monoperphthalic acid in ether at 0 °C. Epoxide (VI) was smoothly isomerized to 5α,7α-dihydroxycholest-3-en-2-one (VII) by chromatography on silica gel. A smooth isomerization also took place when the epoxide was treated with triethylamine in either methanol or ethanol at room temperature. The n.m.r. spectrum of the isomerisation product (VII) showed the characteristic AB quartet for the C-3 and C-4 protons. Treatment of the dihydroxy-compound (VII) with phosgene in chloroform–pyridine at 0 °C gave the corresponding cyclic carbonate (VIII). The formation of this carbonate proves the 5α,7α-diaxial relationship of the two hydroxy-groups. It, therefore, also proves the stereochemistry of the 4α,5α-epoxide ring in structure (VI).

In order to check the possibility of neighbouring hydroxy-group participation in the isomerization (VI) ⟶ (VII), another epoxy-ketone was needed for comparison. 4α,5α-Epoxycholestan-2-one was suitable and was synthesised by the following route. Cholest-4-en-2α-ol, prepared by the method of Fieser *et al.*,[21] was smoothly oxidised with Jones reagent to give cholest-4-en-2-one (IX). Treatment of the ketone (IX) with



(VI) (VII) (VIII) (IX) (X) (XI) (XII) (XIII) (XIV)

hydrochloric acid in refluxing ethanol slowly gave a product having $\lambda_{max.}$ 230 nm (lit.,[22] for cholest-3-en-2-one, 230 nm). This slow conjugation is probably the result of the relatively high internal strain in ring A of the product, caused by the 3,4-double bond.[23a]

[16] N. S. Bhacca and D. H. Williams, ' Applications of N.M.R. Spectroscopy in Organic Chemistry,' Holden-Day, San Francisco, 1964, (*a*) pp. 13—41; (*b*) p. 118; (*c*) p. 109; (*d*) p. 189.

[17] W. G. Dauben, E. J. Blanz, J. Jin, and R. Micheli, *J. Amer. Chem. Soc.*, 1956, **78**, 3752.

[18] L. M. Jackman and S. Sternhell, ' Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' Pergamon, London, 1969, p. 288.

[19] L. F. Fieser, *Org. Synth.*, 1955, **35**, 43.

[20] G. J. Kent and E. S. Wallis, *J. Org. Chem.*, 1959, **24**, 1235.

[21] L. F. Fieser and M. A. Romero, *J. Amer. Chem. Soc.*, 1953, **75**, 4716.

[22] C. Djerassi and T. Nakano, *Chem. and Ind.*, 1960, 1385.

[23] L. F. Fieser and M. Fieser, ' Steroids,' Reinhold, New York, 1959, (*a*) p. 276; (*b*) p. 177.

Epoxidation of the ketone (IX) with monoperphthalic acid in ether at 0 °C gave 4α,5α-epoxycholestan-2-one (X) in almost quantitative yield. As in the case of (VI), this epoxide (X) underwent a smooth isomerization when either chromatographed on silica gel or treated with triethylamine in ethanol at room temperature, to 5α-hydroxycholest-3-en-2-one (XI). Compounds (X) and (XI) had been prepared earlier, by a different route, by Bergmann *et al.*,[14] who also p ided chemical proof for the α-configuration of both ... epoxide ring in (X) and the 5-hydroxy-group in (XI). The physical constants reported for these compounds are in good agreement with our data.

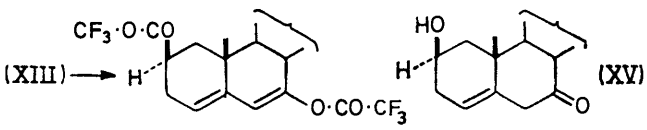
The resonance of the C-19 protons in the spectrum of (XI) occurs as a doublet (*J ca.* 0·7 Hz). The same phenomenon is observed in compounds (I), (VII), and (VIII). Usually the signal appears as a sharp singlet.[16a] There are, however, a few examples of 2-keto-steroids, such as 5α-androstan-2-one, in which the C-19 protons resonate as a doublet because they couple to the 1α-proton (through four σ-bonds).[16b] From the n.m.r. spectra of (I), (VII), (VIII), and (XI) it appears that the splitting into a doublet is a characteristic, in particular, of steroids containing a 3-en-2-one system.

When the 2-keto-epoxides (VI) and (X) had been prepared and their structures established, we looked for a synthetic route to the 7-keto-epoxides. For the preparation of the 2β-hydroxy-epoxides, 2β-hydroxycholest-4-en-7-one (XV) was required as a precursor. The sodium borohydride reduction of compound (II) had been shown previously to be an unsatisfactory route to this compound. Therefore, an alternative synthesis was devised. Treatment of cholest-4-ene-2,7-dione (II) with hydrochloric acid in refluxing ethanol gave cholest-5-ene-2,7-dione (XII), characterised by its u.v. and n.m.r. spectra. The reaction was clean; not a trace of the other possible conjugated isomer (cholest-3-ene-2,7-dione) was detected.

Reduction of the dione (XII) with sodium borohydride in methanol at 0 °C gave a mixture of two hydroxy-isomers. The i.r. spectra of both products showed only αβ-unsaturated ketone absorptions, which indicated that both were the corresponding C-2 alcohols. As expected,[17] 2β-hydroxycholest-5-en-7-one (XIII) was the major product (73%). The yield of the second isomer, 2α-hydroxycholest-5-en-7-one (XIV) was 26%. The configuration of the 2-hydroxy-group in both structures (XIII) and (XIV) was easily determined from their n.m.r. spectra. The 2α-proton in (XIII) resonates as a multiplet (τ 5·82) with $W_{\frac{1}{2}}$ 7·5 Hz, and the 2β-proton in (XIV) as a multiplet (τ 6·08) with $W_{\frac{1}{2}}$ 24 Hz. These values correspond to equatorial and axial orientations, respectively.[18] Moreover, the C-19 protons of (XIII) resonate at a lower field (τ 8·4) than the C-19 protons of (XIV) (τ 8·84), owing to the presence of the 2β-hydroxy-group.[16a]

[24] H. J. Ringold and S. K. Malhotra, *Tetrahedron Letters*, 1962, 669.
[25] D. Amar, V. Permutti, and Y. Mazur, *Tetrahedron*, 1969, **25**, 1717.

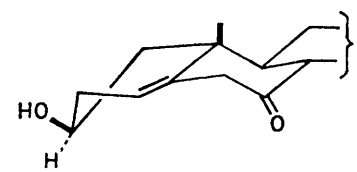
Deconjugation of compound (XIII) to give the corresponding βγ-unsaturated ketone (XV) was achieved by the following route (Scheme 2). Treatment of (XIII)



SCHEME 2

with trifluoroacetic anhydride gave a quantitative yield of 2β,7-bistrifluoroacetoxycholesta-4,6-diene, $\lambda_{max.}$ 229, 234, and 244sh nm indicating the heteroannular diene structure. The crude bistrifluoroacetate, when treated with triethylamine in methanol, gave 2β-hydroxycholest-4-en-7-one (XV) in 93% yield. In order to confirm the structure of (XV), it was treated with hydrochloric acid in refluxing ethanol to give back the αβ-unsaturated ketone (XIII) in high yield. Deconjugation of αβ-unsaturated ketones to the corresponding βγ-unsaturated isomers has been reported for some Δ^4-3-keto-steroids,[24,25] and the mechanism involving enol or enolate intermediates is well established.[26] Our method, which is similar to that of Mazur *et al.*,[25] who used trichloroacetic anhydride, seems to be more simple and convenient than the others.

The hydroxy-ketone (XV) was resistant to monoperphthalic acid in ether at room temperature, but was slowly oxidised by *m*-chloroperbenzoic acid. This was a surprising result since the same 4,5-double bond in the hydroxy-ketone (III) readily underwent epoxidation with monoperphthalic acid even at 0 °C. It is known that neighbouring hydroxy-groups assist epoxidation reactions by co-ordination with the peroxy-acid.[27] Such assistance appears to occur in the case of (III), since (III) is epoxidized much faster than (IX) (see Experimental section for relative rates of epoxidation). Similar assistance could therefore be expected in the case of (XV). However, the results show that none occurs. Moreover, cholest-4-en-7-one is epoxidized faster than (XV) (see Experimental section). The reactivity of the 4,5-double bond in the various substrates towards epoxidation (see further below) appears



SCHEME 3

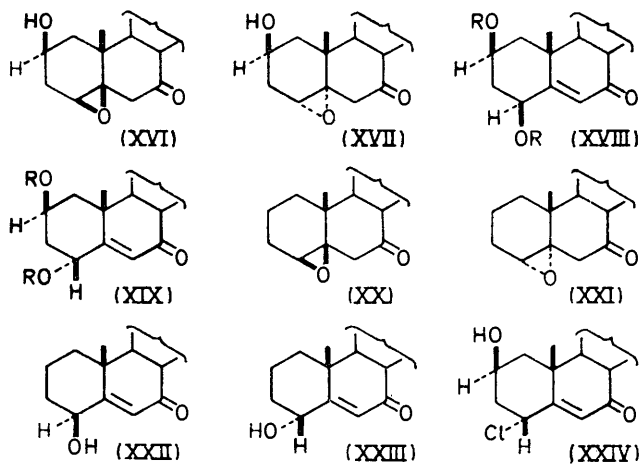
to be in the order: (III) > (IX) ≈ cholest-4-en-7-one > (XV). It is possible that ring A in (XV) assumes a different conformation (Scheme 3) from that of ring A

[26] S. K. Malhotra and H. J. Ringold, *J. Amer. Chem. Soc.*, 1964, **86**, 1997; 1965, **87**, 3228.
[27] H. B. Henbest and R. A. L. Wilson, *J. Chem. Soc.*, 1957, 1958.

in cholest-4-en-7-one, so that the 4,5-double bond in (XV) is sterically more hindered from the α-face and the 2β-hydroxy-group is not in a position to participate in the reaction to form the β-epoxide.

Although there is no direct evidence for this hypothesis, it is supported by the n.m.r. spectrum of compound (XV). The 2α-proton resonates as a multiplet with $W_{\frac{1}{2}}$ 15·0 Hz. This value is much higher than the normal value for equatorial protons [18] [*cf.* also (XIII)], and in fact comes in the region of values given for axial protons.[18] Additional support for the conformation of (XV) can be obtained by a comparison between the calculated [16a] and measured chemical shifts of the C-19 protons. Generally the effects of substituents on the chemical shift of the C-19 protons are additive, and deviations from additivity usually reflect a skeletal deformation.[16a] In the case of compound (XV) the measured chemical shift of the C-19 protons is τ 8·66 and the calculated value is τ 8·45. This is a much larger deviation than for any of the other compounds prepared in this work. For comparison, the chemical shifts (τ values) of the C-19 protons of other compounds, some of which have similar structures to (XV), are given (the value in parentheses is the calculated one): (II), 8·80 (8·72); (XII), 8·84 (8·85); (III), 9·02 (9·00); cholest-4-en-7-one, 8·79 (8·77); (IX), 9·03 (9·00); (XIII), 8·64 (8·58); and (XVIII), 8·44 (8·31).

Epoxidation of compound (XV) with *m*-chloroperbenzoic acid gave a mixture of two epoxy-isomers:



4β,5β-epoxy-2β-hydroxycholestan-7-one (XVI) (56%) and 4α,5α-epoxy-2β-hydroxycholestan-7-one (XVII) (17%). Both epoxides were isomerized smoothly by triethylamine in refluxing methanol to the corresponding γ-hydroxy-αβ-unsaturated ketones. Thus, (XVI) gave 2β,4β-dihydroxycholest-5-en-7-one (XVIII; R = H) (97%), and (XVII) gave 2β,4α-dihydroxycholest-5-en-7-one (XIX; R = H) in quantitative yield. The proof of structures (XVIII; R = H) and (XIX; R = H) will be discussed later.

For reasons already described, we were also interested in the preparation of the two 2-deoxy-analogues of epoxides (XVI) and (XVII). The readily available cholest-4-en-7-one [20] was epoxidized with *m*-chloroperbenzoic acid to give a mixture of 4β,5β-epoxycholestan-7-one (XX) (26%) and 4α,5α-epoxycholestan-7-one (XXI) (35%). These epoxides were isomerized smoothly by triethylamine in methanol to give 4β- and 4α-hydroxycholest-5-en-7-one [(XXII) and (XXIII) respectively; for proof of structures see later].

We have already discussed the different reactivities of compounds (III), (IX), and (XV) and cholest-4-en-7-one towards epoxidation. However, the epoxidation of (XV) and of cholest-4-en-7-one gives in each case a mixture of α- and β-epoxides, in contrast to the behaviour of (III) and (IX) which give exclusively the α-epoxide. The different epoxidizing reagents applied in each pair of substrates could hardly affect the stereochemical course of the reaction.[28] The phenomenon seems to be caused by the difference in the location of the keto-group in the two types of substrate.

The n.m.r. spectra of compounds (XVIII; R = H), (XXII), and (XXIII) provided conclusive evidence for their structures. To obtain information about the structure of compound (XIX; R = H), which was not sufficiently soluble in conventional n.m.r. solvents, we transformed it into the diacetate (XIX; R = Ac). Table 1 summarises the n.m.r. data for compounds

TABLE 1

N.m.r. data for steroidal hydroxy-enones

	τ Values			
	2-H	4-H	6-H	19-H_3
(XXII)	—	5·70 (m, $W_{\frac{1}{2}}$ 6·0 Hz)	4·30 (s)	8·62
(XXIII)	—	5·68 (m, $W_{\frac{1}{2}}$ 21·0 Hz)	3·91 (d, *J* 1·8 Hz)	8·82
(XVIII; R = H)	5·63 (m, $W_{\frac{1}{2}}$ 14·4 Hz)	5·63 (m, $W_{\frac{1}{2}}$ 14·4 Hz)	4·22 (s)	8·44
	a	*a*		
(XIX; (R = Ac)	4·72 (m, $W_{\frac{1}{2}}$ 8·7 Hz)	4·13 (ABXq, *J* 12·0 and 6·5 Hz)	4·17 (d, *J* 1·9 Hz)	8·62

[a] 2-H and 4-H signals overlap.

(XVIII; R = H), (XIX; R = Ac), (XXII), and (XXIII).

First, we discuss the spectra of the two isomers of 4-hydroxycholest-5-en-7-one. The $W_{\frac{1}{2}}$ value (6·0 Hz) corresponding to the C-4 proton resonance in the spectrum of (XXII) indicates that this proton is equatorial. The C-4 proton resonance in (XXIII), $W_{\frac{1}{2}}$ 21·0 Hz, is obviously that of an axial proton. The C-19 protons of (XXII) resonate at lower field than those of (XXIII), owing to the presence of the axial 4β-hydroxy-group.[16a] Additional evidence is provided by the resonance of the C-6 protons. Compound (XXII) shows a singlet at τ 4·30, whereas (XXIII) shows a doublet at τ 3·91 (*J* 1·8 Hz). A similar phenomenon is observed in the case of 6-substituted Δ^4-3-keto-steroids.[16c,d]

[28] L. F. Fieser and M. Fieser, ' Reagents for Organic Synthesis,' Wiley, New York, 1967, p. 136.

The n.m.r. data provide strong evidence for structures (XXII) and (XXIII). By using the same arguments, structures (XVIII; R = H) and (XIX; R = Ac) [and therefore also (XIX; R = H)] may be proven. There is also a correlation between the structures (XXII), (XXIII), (XVIII; R = H), and (XIX; R = H) and their specific optical rotations. The 4α-hydroxy-isomer in each pair of epimers is the more laevorotatory.[23b]

We wanted also to provide a further chemical proof for structure (XVIII; R = H) by forming the corresponding cyclic carbonate. However, when compound (XVIII; R = H) was treated with phosgene in pyridine–chloroform, a product containing chlorine was obtained in 76% yield. The n.m.r. spectrum of this compound shows that it has the structure (XXIV) (from similar arguments to the above). Hydrogenation of compound (XVIII; R = H) over platinum oxide to the corresponding saturated 2β,4β-dihydroxycholestan-7-one and treatment of this compound with phosgene did not give any cyclic carbonate either.

A cyclic carbonate was finally obtained as follows. Treatment of compound (XVIII; R = H) with ethyl chloroformate gave a mixture of two products from which the monoethyl carbonate (XVIII; R = CO_2Et, R = H) was separated. (There is no evidence as to whether the ethyl carbonate group is at C-2 or C-4.) Treatment of this compound with sodium hydride in refluxing benzene gave the required cyclic carbonate (XVIII; RR = C=O). Hydrolysis of the product gave back compound (XVIII; R = H). This finally established chemically the 2β,4β-diaxial relationship of the two hydroxy-groups.

Kinetic Studies.—The relative ease of isomerization of the βγ-epoxy-ketones to the corresponding γ-hydroxy-αβ-unsaturated ketones was studied by measuring the reaction rates. Isomerizations were carried out in ethanol containing equal amounts of triethylamine and triethylamine hydrochloride. In each case the reaction obeyed the kinetic equation $-d[epoxide]/dt = k[epoxide][triethylamine]$. Thus, it is pseudo-first order in the epoxide, since the triethylamine is not consumed and its concentration remains constant. Table 2 summarises the rate constants obtained.

TABLE 2

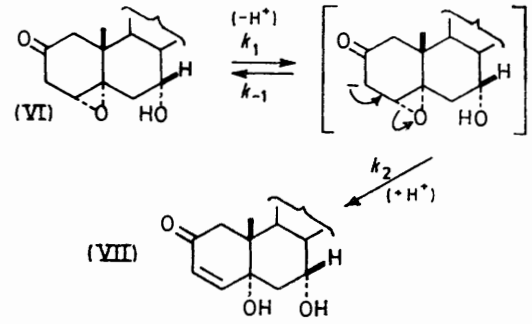
Rate constants for the triethylamine-catalysed isomerization of βγ-epoxy-ketones in ethanol at 30·0 °C

	Epoxide	10^4k/l s^{-1} mol^{-1}	
2-Keto-	4α,5α-epoxide (X)	3190, 3140 [a]	Average k_H/k_D = 3·00
	7α-hydroxy-4α,5α-epoxide (VI)	3450, 3580 [a]	
	3,3-dideuterio-7α-hydroxy-4α,5α-epoxide (VIa)	1260, 1080 [a]	
7-Keto-	4β,5β-epoxide (XX)	9·50	
	2β-hydroxy-4β,5β-epoxide (XVI)	2·96	
	4α,5α-epoxide (XXI)	1·83	
	2β-hydroxy-4α,5α-epoxide (XVII)	5·66	

[a] Two measurements with two different base concentrations.

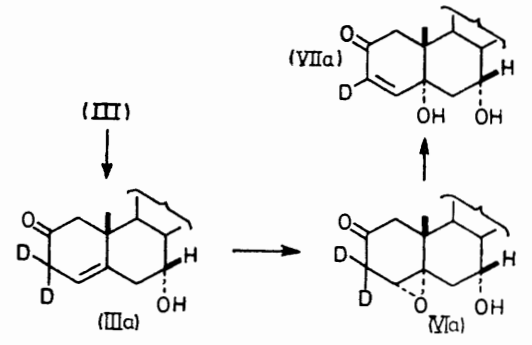
We expected that any neighbouring hydroxy-group participation would be reflected by rate enhancement. However, comparison of the rate constants for (X) and (VI), and for (XX) and (XVI) shows that no anchimeric assistance is operative [enhanced rates are expected only in (VI) and (XVI), where the hydroxylic hydrogen atom is within bonding distance of the epoxide oxygen]. We decided therefore to investigate the mechanism of the isomerization more thoroughly in order to find out the reasons for this behaviour.

A reasonable mechanism for the isomerization is shown in Scheme 4, for the case of (VI).



SCHEME 4

The isomerization reactions of compounds (VI), (X), and (XXI), when carried out in absolute ethan[^{2}H]ol, gave in each case no incorporation of deuterium either into the product or into the starting material. This indicated that each epoxide molecule which gives the anionic intermediate rapidly collapses to the open form without being reprotonated to give back the starting material ($k_{-1} \ll k_2$). This suggested that the formation of the anion might be the rate-determining step, and we decided to check whether there was any deuterium isotope effect in this reaction. For this purpose, 3,3-dideuterio-4α,5α-epoxy-7α-hydroxycholestan-2-one (VIa) was prepared as follows. Treatment of compound (III) (M^+ 416) with triethylamine in ethan[^{2}H]ol, deuterium oxide, and tetrahydrofuran gave material (IIIa) (M^+ 418)



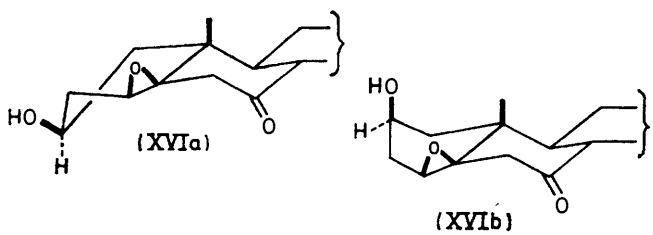
which contained not less than 70% of [$^2H_2$]-product and about 20% of [$^2H_1$]. Compound (IIIa) was epoxidised as previously to give (VIa) without any loss of deuterium. To confirm the structure of (VIa) [and also that of (IIIa)] with respect to the location of the deuterium, it was isomerized with triethylamine in ethanol to give material (VIIa) (M^+ 417) whose n.m.r. spectrum

showed instead of the AB quartet for the C-3 and C-4 protons [as in (VII)], a singlet for the C-4 proton at τ 3·47 [the C-4 proton in (VII) shows τ 3·49]. This indicates that the two deuterium atoms in (VIa) are both located at C-3. The incorporation of deuterium only at C-3 is expected, since this position is activated both by the 2-keto-group and by the 4,5-double bond. It also fits the observed course of enolization of 2-keto-steroids, which proceeds unilaterally towards C-3.[22] Furthermore it demonstrates that the Δ^4-2-keto-steroid can form the enolate and exchange deuterium much faster than it conjugates.

Comparison between the rate constants of isomerization for (VI) and (VIa) indicated a large isotope effect: $k_H/k_D = 3{\cdot}00$. Owing to the small amounts of non- and mono-deuteriated species in (VIa) this is a minimum figure but it clearly establishes that the loss of a C-3 proton is involved in the rate-determining step. This large isotope effect may indicate that the anion in Scheme 4 is not a discrete intermediate in the reaction and that a concerted mechanism may be operating. Such a mechanism, a limiting form of Scheme 4, has been proposed for several β-elimination reactions in which a large isotope effect is found.[29]

The absence of neighbouring hydroxy-group participation in the case of (VI) can now be discussed in terms of the mechanisms shown. If Scheme 4 represents the reaction mechanism and the anion is a discrete intermediate, the reaction rate is determined only by k_1 and therefore any expected assistance by the neighbouring 7α-hydroxy-group is involved in a non-rate-determining step and therefore cannot be detected. In the case of the βγ-cyclopropyl ketone (see before), the breakage of the cyclopropyl C–C bond is probably the rate-determining step and so the neighbouring hydroxy-group assistance is observed.[7]

The isomerization of the 7-keto-epoxides probably proceeds by a similar mechanism to that for the 2-keto-epoxides. In the case of (XVI) the n.m.r. signal of the 2α-proton, having a $W_{\frac{1}{2}}$ value of 26 Hz, shows that this proton is axial.[18] Therefore the conformation of the molecule must be the one shown in (XVIa) and not



(XVIa) (XVIb)

(XVIb). The 2β-hydroxy-group in (XVIa) is not in a position to allow hydrogen bonding with the 4β,5β-epoxide, whereas in conformation (XVIb), which must be readily available from (XVIa), as well as in the product (XVIII; R = H), there is a possibility of strong hydrogen bonding between the hydroxy- and the epoxy-groups or the two hydroxy-groups, respectively.

Table 2 shows that there is no observable effect of neighbouring hydroxy-group participation on the rate of epoxide opening. It also shows that 2-keto-epoxides are opened much faster than 7-keto-epoxides. This difference can also be explained by the proposed mechanism, since a 6,7-enol double bond is more strained, and hence more difficult to form, than a 2,3-enol double bond.[23b] Also the C-3 protons are less sterically hindered than the C-6 protons.

EXPERIMENTAL

All m.p.s were determined with a Kofler hot-stage apparatus. I.r. spectra were recorded with Unicam SP 200 and Perkin-Elmer 254 spectrophotometers. U.v. spectra were recorded for solutions in ethanol with a Unicam SP 800 spectrophotometer. N.m.r. spectra were taken for solutions in deuteriochloroform with tetramethylsilane as internal standard, with a Varian T 60 or HA-100 spectrometer; $W_{\frac{1}{2}}$ refers to the width of a band at half height. Optical rotations were determined for solutions in chloroform with a Perkin-Elmer model 141 polarimeter. Mass spectra were recorded on an A.E.I. MS9 spectrometer. Both qualitative and preparative t.l.c. was carried out on silica gel G. Plates were eluted with light petroleum (b.p. 40—60°) containing 10—20% acetone. Unless otherwise stated, the course of each reaction was followed by t.l.c. To develop the spots, plates were sprayed with 6N-sulphuric acid and heated. Anhydrous sodium sulphate was used for drying solutions.

Cholesta-3,5-diene-2,7-dione (I).—To cholesta-3,5-diene-7-one,[30] m.p. 111° (10 g) in dry carbon tetrachloride (350 ml) was added a standard solution (70 ml) of t-butyl chromate in carbon tetrachloride.[31] The solution was refluxed for 22 h, then filtered while still warm through Celite. The heavy brown precipitate was washed with warm methylene chloride until the filtrate remained uncoloured. The combined yellow organic solution was filtered through silica gel MFC, then washed with water and dried. The solvent was removed under reduced pressure to give a yellow solid (2·6 g). The dienedione was purified by column chromatography on silica gel MFC, with 19 : 1 light petroleum (b.p. 40—60°)–acetone as eluant. Recrystallisation from methanol gave *cholesta-3,5-diene-2,7-dione* (I) as yellow needles (2·0 g, 19%), m.p. 154—156°, $[\alpha]_D$ −220° (*c* 1·00), $\nu_{max.}$ (Nujol) 1679, 1667, 1620, and 1580 cm^{-1}, $\lambda_{max.}$ 286·5 nm (ε 25,100), τ 8·76 (d, *J ca.* 0·7 Hz, 19-H_3), 4·08 (s, H-6), and 3·48 (ABq, τ_4 3·06, τ_3 3·90, J_{AB} 9·7 Hz, H-4 and H-3) (Found: C, 81·85; H, 10·1. $C_{27}H_{40}O_2$ requires C, 81·75; H, 10·15%). Unchanged dienone (0·5 g) was recovered. Attempts to improve the yield by changing the conditions (temperature, amount of t-butyl chromate, and solvent) gave no better result. There was always some loss of organic material as tar. The dienedione itself decomposes slowly under the oxidation conditions.

Cholest-4-ene-2,7-dione (II).—Cholesta-3,5-diene-2,7-dione (500 mg) was dissolved in hot methanol (250 ml) and the solution was cooled to room temperature. Zinc dust (2·5 g)

[29] K. B. Wiberg, *Chem. Rev.*, 1955, **55**, 713.
[30] A. Nickon and J. F. Bagli, *J. Amer. Chem. Soc.*, 1961, **83**, 1498.
[31] K. Heusler and A. Wettstein, *Helv. Chim. Acta*, 1952, **35**, 289.

and glacial acetic acid (2·5 ml) were added. The mixture was stirred for 15 min, then filtered and poured into water. The product was extracted with ether. The solution was washed with water, aqueous sodium hydrogen carbonate, and water again, then dried and evaporated under vacuum to give needles (500 mg). Recrystallisation from acetone–water gave the pure enedione (480 mg, 96%), m.p. 157—159° (lit.,[32] 158—160°), $[\alpha]_D^{21}$ +61° (*c* 0·50), $\nu_{max.}$ (Nujol) 1710 cm^{-1}, τ 8·80 (s, 19-H_3) and 4·72 (m, H-4) (Found: C, 81·2; H, 10·55. Calc. for $C_{27}H_{42}O_2$: C, 81·35; H, 10·6%).

Reduction of Cholest-4-ene-2,7-dione (II) *with Sodium Borohydride.*—The steroid (500 mg) was dissolved in warm methanol (200 ml) and the solution was cooled to 0 °C. A slight excess of sodium borohydride (*ca.* 1·1 equiv.) was added and the solution was stirred at 0 °C for 1 h. After dilution with water, the product was extracted with methylene chloride. The solution was washed with water, dried, and evaporated under reduced pressure. The residue (490 mg) was separated by t.l.c. to give pure 7α-*hydroxycholest-4-en-2-one* (III) (281 mg, 56%), needles, m.p. 160—161° (from acetone–water), $[\alpha]_D^{26}$ +93° (*c* 0·52), $\nu_{max.}$ (Nujol) 3520 and 1710 cm^{-1}, τ 9·02 (s, 19-H_3), 6·20 (m, $W_{\frac{1}{2}}$ 7·0 Hz, H-7β), and 4·68 (m, H-4) (Found: C, 80·9; H, 11·05. $C_{27}H_{44}O_2$ requires C, 80·95; H, 11·05%). A small amount (<10%) of 2β-hydroxycholest-4-en-7-one (XIII) is also formed. The rest of the material is mainly a mixture of dihydroxy-compounds (no carbonyl absorption in the i.r.).

Epi-ψ-cholesterol (V) *from* 7α-*Hydroxycholest-4-en-2-one* (III).—(a) *Ethylene dithioacetal* (IV) *of* 7α-*hydroxycholest-4-en-2-one.* To a solution of the steroid (III) (110 mg) in glacial acetic acid (2 ml) was added ethanedithiol (0·1 ml) and boron trifluoride–ether (2 drops). After 2 h at room temperature, the solution was cooled to 0 °C and poured into cold 4N-sodium hydroxide. The product was extracted with ether and the ether solution washed several times with water, dried, and evaporated under reduced pressure. The dithioacetal (IV) was separated by t.l.c. as an oil (60 mg, 46%), $\nu_{max.}$ (CCl_4) 3580 cm^{-1}, τ 8·81 (s, 19-H_3), 6·69 (4H, s, S·[CH_2]$_2$·S), 6·21 (m, $W_{\frac{1}{2}}$ 8·5 Hz, H-7β), and 4·53 (m, H-4).

(b) *Reduction of the dithioacetal* (IV) *with Raney nickel.* The dithioacetal (60 mg) in absolute ethanol (15 ml) was refluxed with an excess of Raney nickel for 20 h. The cooled mixture was filtered and evaporated under reduced pressure. Separation by t.l.c. gave pure epi-ψ-cholesterol (V) (35 mg, 72%). Recrystallisation from acetone–water gave needles, m.p. 86—87°, $[\alpha]_D$ +46° (*c* 0·30) (lit.,[20] m.p. 85—86°, $[\alpha]_D$ +44·5°), $\nu_{max.}$ (Nujol) 3500 cm^{-1}, τ 9·01 (s, 19-H_3), 6·32 (m, $W_{\frac{1}{2}}$ 8·0 Hz, H-7β), and 4·64 (m, H-4) (Found: C, 83·85; H, 11·9. Calc. for $C_{27}H_{46}O$: C, 83·85; H, 12·0%), identical with authentic epi-ψ-cholesterol (t.l.c., mixed m.p. $[\alpha]_D$, and i.r.).

4α,5α-*Epoxy*-7α-*hydroxycholestan-2-one* (VI).—7α-Hydroxycholest-4-en-2-one (III) (100 mg) in ether (8 ml) was treated at 0 °C with a slight excess of monoperphthalic acid and left at 0 °C for 1 h. More ether (50 ml) was added and the solution was filtered. The filtrate was washed with aqueous 3% sodium hydrogen carbonate (2 × 30 ml) and water, dried, and evaporated under reduced pressure to give almost pure *epoxide* (96 mg, 92%), m.p. 129—136°. Recrystallisation from a small amount of ether gave needles, m.p. 134—136°, $[\alpha]_D^{28}$ +118° (*c* 0·27), $\nu_{max.}$ (CCl_4) 3590 and 1715 cm^{-1}, τ 8·98 (s, 19-H_3), at 6·98 (m, $W_{\frac{1}{2}}$ 6·0 Hz, H-4β), and 6·11 (m, $W_{\frac{1}{2}}$ 6·0 Hz, H-7β) (Found: C, 77·9; H, 10·65. $C_{27}H_{44}O_3$ requires C, 77·85; H, 10·65%).

Isomerization of the Epoxide (VI).—The epoxy-steroid (VI) (90 mg) in a few drops of benzene was placed on the origin of a silica gel G thin-layer plate and left for 1 h. The plate was then eluted and the product which showed u.v. activity was separated as pure 5α,7α-*dihydroxycholest-3-en-2-one* (VII) (87 mg, 97%), needles, m.p. 219—222° (decomp.) (from acetone–water), $[\alpha]_D$ +37° (*c* 0·25), $\nu_{max.}$ (CCl_4) 3470, 3380 (broad peak, unchanged on further dilution), and 1665 cm^{-1}, $\lambda_{max.}$ 219 nm (ε 8600), τ 9·03 (d, *J ca.* 0·7 Hz, 19-H_3), 6·01 (m, $W_{\frac{1}{2}}$ 8·5 Hz, H-7β), and 4·32 (ABq, τ_4 3·49, τ_3 4·16 J_{AB} 9·8 Hz, H-4 and H-3) (Found: C, 77·7; H, 10·6. $C_{27}H_{44}O_3$ requires C, 77·85; H, 10·65%).

Cyclic Carbonate (VIII) *of* 5α,7β-*Dihydroxycholest-3-en-2-one* (VII).—The dihydroxy-steroid (VII) (40 mg) in chloroform (9 ml) and pyridine (1 ml) was cooled to 0 °C, and phosgene was passed through the solution for 1 h. The mixture was left overnight at 0 °C under anhydrous conditions. Excess of phosgene was then removed by bubbling nitrogen through the solution, which was then poured into ice-cold aqueous sodium hydrogen carbonate. The product was extracted with ether and the ether solution washed with dilute hydrochloric acid, then with water, and dried. The ether was removed under vacuum. T.l.c. gave the pure *cyclic carbonate* (VIII) (25 mg, 59%). Recrystallisation from anhydrous methanol gave plates, m.p. 184—185°, $[\alpha]_D^{28}$ −69° (*c* 0·18), $\nu_{max.}$ (CCl_4) 1760 and 1690 cm^{-1}, $\lambda_{max.}$ 216 nm (ε 10,100), τ 8·95 (d, *J ca.* 0·7 Hz, 19-H_3), 5·52 (m, $W_{\frac{1}{2}}$ 6·7 Hz, H-7β), and 3·75 (ABq, τ_4 3·58, τ_3 3·93, J_{AB} 10·2 Hz, H-4 and H-3), M^+ 442 (Found: C, 76·0; H, 9·6. $C_{28}H_{42}O_4$ requires C, 76·0; H, 9·55%).

Cholest-4-en-2-one (IX).—2α-Hydroxycholest-4-ene [21] (m.p. 133—135°; 100 mg) in acetone (20 ml) was treated at room temperature with standard Jones reagent (0·15 ml).[33] After 10 min the solution was poured into water and the product was extracted with ether. The ether solution was washed with water, aqueous sodium hydrogen carbonate, and water again. Evaporation under reduced pressure and separation by t.l.c. gave *cholest-4-en-2-one* (IX) (83 mg, 83%), needles, m.p. 103—104° (from acetone–water), $[\alpha]_D^{24}$ +126°, $\nu_{max.}$ (Nujol) 1720 cm^{-1}, τ 9·03 (s, 19-H_3), and 4·79 (m, H-4) (Found: C, 84·35; H, 11·5. $C_{27}H_{44}O$ requires C, 84·3; H, 11·55%). In the synthetic route to 2α-hydroxycholest-4-ene, following the method of Fieser *et al.*,[21] some modifications and observations were made. These are summarised.

(*a*) The yield of 2α-acetoxycholest-4-en-3-one obtained from the 6β-bromocholest-4-en-3-one by refluxing in acetic acid and potassium acetate was increased to 54% by adding 10% (v/v) of acetic anhydride to the reaction mixture, and continuing refluxing for 2·5 h. (*b*) The reported [21] $[\alpha]_D$ value for 2α-hydroxycholest-4-en-3-one ethylene dithioacetal is +30°. We obtained a value of +121° (*c* 0·68), and other constants were similar to the reported values, *e.g.* m.p. and i.r. To eliminate any error, our hydroxy-compound was reacetylated in pyridine and acetic anhydride, to give 2α-acetoxycholest-4-en-3-one ethylene dithioacetal, which was identical with an authentic sample (t.l.c., $[\alpha]_D$, i.r. spectrum, and mixed m.p.). Rehydrolysis of the acetate gave again a hydroxy-compound with the same $[\alpha]_D$ value as before. (*c*) Desulphurization of 2α-hydroxycholest-4-en-3-one ethylene dithioacetal with Raney

[32] J. R. Hanson and E. Premuzic, *J. Chem. Soc.* (*C*), 1969, 1201.
[33] K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 1946, 39.

nickel gave 2α-hydroxycholest-4-ene and a second compound (13%), identical with authentic cholest-4-en-2-one, (t.l.c. $[\alpha]_D$, i.r. spectrum, and mixed m.p.).

4α,5α-Epoxycholestan-2-one (X).—Cholest-4-en-2-one (IX) (120 mg) in ether (10 ml) was treated at 0 °C with a slight excess of monoperphthalic acid and the solution was left at 0 °C for 40 h. Work-up as in the case of (VI) gave almost pure α-epoxide (X) (110 mg, 88%). Recrystallisation from ethanol–ether gave needles, m.p. 172—174°, $[\alpha]_D^{28}$ +134° (*c* 0·40) (lit.,[14] m.p. 172°, $[\alpha]_D^{25}$ +141°), $\nu_{max.}$ (Nujol) 1708 cm^{-1}, τ 8·97 (s, 19-H_3), and 6·97 (m, $W_{\frac{1}{2}}$ 5 Hz, H-4β) (Found: C, 80·75; H, 11·0. Calc. for $C_{27}H_{44}O_2$: C, 80·95; H, 11·05%).

Isomerization of the Epoxide (X).—The epoxy-steroid (X) (90 mg) was isomerized on a plate of silica gel G as in the case of epoxide (VI). Recrystallisation from ethanol–water gave flat needles (86 mg, 96%) of 5α-hydroxycholest-3-en-2-one (XI), m.p. 173—174° (decomp.), $[\alpha]_D^{28}$ +35° (*c* 0·33) (lit.,[14] m.p. 173·5—174°, $[\alpha]_D^{26}$ +36°), $\nu_{max.}$ ($CHCl_3$) 3620 and 1678 cm^{-1}, $\lambda_{max.}$ 223 nm (ε 7600), τ 9·00 (d, *J ca.* 0·7 Hz, 19-H_3) and 3·78 (ABq, τ_4 3·44, τ_3 4·12, J_{AB} 9·8 Hz, H-4 and H-3) (Found: C, 80·7; H, 10·95. Calc. for $C_{27}H_{44}O_2$: C, 80·95; H, 11·05%).

Attempt to Conjugate the Enones (III) *and* (IX).—(i) *7α-Hydroxycholest-4-en-2-one* (III). (a) *With acid.* The steroid (50 mg) in ethanol (20 ml) and 6N-hydrochloric acid (3 drops) was refluxed for 30 min. T.l.c. indicated no reaction. Additional refluxing for 5 h gave many products but not a trace of a u.v.-active compound ($\lambda_{max.}$ 230 nm).

(b) *With base.* The steroid (100 mg) in t-butyl alcohol (30 ml) under argon at room temperature was treated with potassium t-butoxide (30 mg). After 30 min, t.l.c. indicated no reaction. The solution was left overnight at room temperature. T.l.c. indicated the formation of many products. After work-up, none of the products showed the expected u.v. absorption.

(ii) *Cholest-4-en-2-one* (IX). *With acid.* The steroid (60 mg) in ethanol (10 ml) and 6N-hydrochloric acid (3 drops) was refluxed for 2 h. T.l.c. indicated the formation of one product (*ca.* 50%, estimated from t.l.c.), $\lambda_{max.}$ 230 nm. The rest of the material was unchanged ketone (IX).

Conjugation of Cholest-4-ene-2,7-dione (II).—Cholest-4-ene-2,7-dione (II) (500 mg) in ethanol (150 ml) and 6N-hydrochloric acid (0·5 ml) was heated on a steam-bath for 30 min. After dilution with water, the product was extracted with methylene chloride. The solution was washed with aqueous sodium hydrogen carbonate and water, and then dried and evaporated in vacuum. T.l.c. indicated the presence of one product only. Recrystallisation from acetone–water gave *cholest-5-ene-2,7-dione* (XII) as needles (450 mg, 90%), m.p. 170—171°, $[\alpha]_D$ −101° (*c* 0·16), $\nu_{max.}$ (Nujol) 1705 and 1670 cm^{-1}, $\lambda_{max.}$ 233 nm (ε 13,500), τ 8·84 (s, 19-H_3) and 4·23 (s, H-6) (Found: C, 81·25; H, 10·6. $C_{27}H_{42}O_2$ requires C, 81·35; H, 10·6%).

Reduction of Cholest-5-ene-2,7-dione (XII) *with Sodium Borohydride.*—The steroid (300 mg) in methanol (100 ml) was treated at 0 °C with a slight excess (*ca.* 1·1 equiv.) of sodium borohydride. After 3 h at 0 °C the solution was poured into water and products were extracted with methylene chloride. The organic layer was washed with water, dried, and evaporated under vacuum. T.l.c. gave 2β-*hydroxycholest-5-en-7-one* (XIII) (220 mg, 73%). Recrystallisation from light petroleum (b.p. 40—60°)–ether gave needles, m.p. 197—198°, $[\alpha]_D^{25}$ −118° (*c* 0·17), $\nu_{max.}$ (Nujol) 3430 and 1650 cm^{-1}, $\lambda_{max.}$ 239 nm (ε 13,500), τ 8·64 (s, 19-H_3), 5·82 (m, $W_{\frac{1}{2}}$ 7·5 Hz, H-2α), and 4·37 (d, *J ca.* 1·5 Hz, H-6) (Found: C, 80·95; H, 11·05. $C_{27}H_{44}O_2$ requires C, 80·95; H, 11·05%). Also isolated was 2α-*hydroxycholest-5-en-7-one* (XIV) (80 mg, 26%), needles, m.p. 175—176° [from light petroleum (b.p. 40—60°)–ether], $[\alpha]_D^{20}$ −112° (*c* 0·41), $\nu_{max.}$ (Nujol) 3310 and 1675 cm^{-1}, $\lambda_{max.}$ 235 nm (ε 12,700), τ 8·84 (s, 19-H_3), 6·08 (m, $W_{\frac{1}{2}}$ 24·0 Hz, H-2β), and 4·38 (d, *J* 0·8 Hz, H-6) (Found: C, 80·9; H, 10·85. $C_{27}H_{44}O_2$ requires C, 80·95; H, 11·05%).

Deconjugation of 2β-*Hydroxycholest-5-en-7-one* (XIII).—The steroid (200 mg) was dissolved with shaking (5 min) in trifluoroacetic anhydride (3 ml) and left at room temperature for 4 h. The solvent was then evaporated off under reduced pressure to give 2β,7-bistrifluoroacetoxycholesta-4,6-diene, $\nu_{max.}$ (Nujol) 1790 cm^{-1}, and no hydroxy-absorption, $\lambda_{max.}$ (cyclohexane) 229, 234, and 244sh nm. The crude product was dissolved in methanol (15 ml) and treated with triethylamine (0·2 ml) at room temperature. After 30 min the solution was poured into water and the product extracted with ether. The ether layer was washed with water, dilute hydrochloric acid, water, aqueous sodium hydrogen carbonate, and water again, dried, and evaporated under vacuum. The residue gave pure 2β-*hydroxycholest-4-en-7-one* (XV) as needles (185 mg, 93%), m.p. 167—169° (from acetone–water), $[\alpha]_D^{25}$ −75° (*c* 0·26), $\nu_{max.}$ ($CHCl_3$) 3600 and 1715 cm^{-1}, τ 8·66 (s, 19-H_3), 6·06 (m, $W_{\frac{1}{2}}$ 15·0 Hz, H-2α), and 4·76 (m, H-4) (Found: C, 80·75; H, 10·95. $C_{27}H_{44}O_2$ requires C, 80·95; H, 11·05%).

An attempt to deconjugate the title compound (XIII) with potassium t-butoxide[24] gave almost quantitative recovery of the starting material. To confirm the structure of the non-conjugated compound (XV), it was reconjugated as follows. The steroid (XV) (10 mg) in ethanol (2 ml) was treated with 6N-hydrochloric acid (2 drops) and the solution was heated on a steam-bath for 20 min. The mixture was then worked up as in the case of (XII) to give 2β-hydroxycholest-5-en-7-one (9 mg), identical with an authentic sample (t.l.c., u.v. and i.r. spectra, and mixed m.p.).

Epoxidation of 2β-*Hydroxycholest-4-en-7-one* (XV).—The steroid (300 mg) in ether (40 ml) was treated *m*-chloroperbenzoic acid (150 mg) and the solution was left at room temperature for 72 h. It was then washed with aqueous sodium hydrogen carbonate, dilute aqueous sodium sulphite, and water, dried, and evaporated under reduced pressure. T.l.c. gave 4β,5β-*epoxy*-2β-*hydroxycholestan*-7-*one* (XVI) (176 mg, 56%), needles, m.p. 114—116° (from acetone–water), $[\alpha]_D$ −44° (*c* 0·32), $\nu_{max.}$ ($CHCl_3$) 3580 and 1710 cm^{-1}, τ 8·72 (s, 19-H_3), 6·99 (d, *J* 4·1 Hz, H-4α), and 6·33 (m, $W_{\frac{1}{2}}$ 26 Hz, H-2α) (Found: C, 77·6; H, 10·5. $C_{27}H_{44}O_3$ requires C, 77·85; H, 10·65%).

Also formed was 4α,5α-*epoxy*-2β-*hydroxycholestan*-7-*one* (XVII) (53 mg, 17%), needles, m.p. 152—153° (from methanol–water), $[\alpha]_D^{20}$ +8° (*c* 0·14), $\nu_{max.}$ ($CHCl_3$) 3580 and 1710 cm^{-1}, τ 8·48 (s, 19-H_3), 7·02 (d, *J* 2·4 Hz, H-4β), and 5·94 (m, $W_{\frac{1}{2}}$ 13·2 Hz, H-2α) (Found: C, 77·6; H, 10·5. $C_{27}H_{44}O_3$ requires C, 77·85; H, 10·65%).

Treatment of the title compound with a large excess of monoperphthalic acid in ether gave no reaction.

Isomerization of the Epoxides (XVI) *and* (XVII).—(a) *Epoxide* (XVI). The steroid (40 mg) in methanol (5 ml) and triethylamine (5 drops) was heated on a steam-bath for 2 h. The solvent was removed under reduced pressure. The product was separated by t.l.c. to give 2β,4β-*dihydroxycholest-5-en-7-one* (XVIII; R = H) (39 mg, 97%), needles, m.p. 184—186° (from acetone–water), $[\alpha]_D^{26}$ −65° (*c* 0·15),

$\nu_{max.}$ ($CHCl_3$) 3550 and 1670 cm^{-1}, $\lambda_{max.}$ 233 nm (ε 10,700), τ 8·44 (s, 19-H_3), 5·63 (overlapping, $W_{\frac{1}{2}}$ 14·4 Hz, H-2α and H-4α), and 4·22 (s, H-6) (Found: C, 77·7; H, 10·55. $C_{27}H_{44}O_3$ requires C, 77·85; H, 10·65%).

(*b*) *Epoxide* (XVII). The steroid (50 mg) in methanol (5 ml) and triethylamine (5 drops) was heated on a steam-bath for 2 h. Work-up as before and separation by t.l.c. gave 2β,4α-*dihydroxycholest-5-en-7-one* (XIX; R = H) (50 mg, 100%), needles, m.p. 237—240° (decomp.) (from methanol), $[\alpha]_D^{20}$ −103° (*c* 0·18), $\nu_{max.}$ ($CHCl_3$) 3590 and 1665 cm^{-1}, $\lambda_{max.}$ 239 nm (ε 12,400), too insoluble in all conventional solvents for an n.m.r. spectrum to be obtained (Found: C, 77·6; H, 10·55. $C_{27}H_{44}O_3$ requires C, 77·85; H, 10·65%).

2β,4α-*Diacetoxycholest-5-en-7-one* (XIX; R = Ac).—The dihydroxy-steroid (XIX; R = H) (25 mg) in pyridine (3 ml) and acetic anhydride (0·5 ml) was left at room temperature overnight. The solution was poured into water and the product was extracted with ether. The ether layer was washed with dilute hydrochloric acid and water, dried, and evaporated under vacuum. The *diacetate* was separated by t.l.c. (26 mg, 86%). Recrystallisation from methanol–water gave plates, m.p. 158—160°, $[\alpha]_D$ −60° (*c* 0·19), $\nu_{max.}$ (Nujol) 1735, 1675, and 1245 cm^{-1}, $\lambda_{max.}$ 232 nm (ε 12,200), τ 8·62 (s, 19-H_3), 4·72 (m, $W_{\frac{1}{2}}$ 8·7 Hz, H-2α), 4·17 (d, *J* 1·9 Hz, H-6), 4·13 (ABX quartet $J_{4\beta,3\beta}$ 6·5 Hz, H-4β), and 7·90 and 7·88 (both s, Ac) (Found: C, 74·3; H, 9·8. $C_{31}H_{48}O_5$ requires C, 74·35; H, 9·65%).

Cyclic Carbonate (XVIII; RR = C=O) *of* 2β,4β-*Dihydroxycholest-5-en-7-one* (XVIII; R = H).—The dihydroxy-steroid (50 mg) in dry ether (15 ml) and dry pyridine (15 ml) was treated with excess (×10) of ethyl chloroformate. The mixture was left at room temperature for 16 h. After dilution with water, the products were extracted with ether. The ether solution was washed with dilute hydrochloric acid and water, then dried and evaporated under reduced pressure. The residue [$\nu_{max.}$ ($CHCl_3$) 3580, 1740, and 1675 cm^{-1}], which was a mixture of a hydroxy-monoethyl carbonate and a diethyl dicarbonate was separated by t.l.c. to give the hydroxy-monoethyl carbonate (24 mg) as an oil, $\lambda_{max.}$ ($CHCl_3$) 3580, 1740, 1675, and 1280 cm^{-1}. The oil in dry benzene (25 ml) was treated with excess of sodium hydride; the mixture was refluxed for 1 h, cooled to room temperature, and filtered. The filtrate was washed with water, dried, and evaporated under vacuum. T.l.c. gave the pure *cyclic carbonate* (XVIII; RR = C=O) (19 mg, 36% based on the dihydroxy-compound), needles, m.p. 268—271° (decomp.) (from ethanol), $[\alpha]_D^{23}$ −12° (*c* 0·42), $\nu_{max.}$ ($CHCl_3$) 1745, 1675, and 1120 cm^{-1}, $\lambda_{max.}$ 226 nm (ε 10,800), τ 8·59 (s, 19-H_3), 5·03 (overlapping multiplet, $W_{\frac{1}{2}}$ 10·2 Hz, H-2α and H-2β), and 4·14 (s, H-6), M^+ 442 (Found: C, 75·85; H, 9·45. $C_{28}H_{42}O_4$ requires C, 76·0; H, 9·55%).

To confirm the structure of the cyclic carbonate it was hydrolysed as follows. The carbonate (5 mg) in methanol (5 ml) and water (2 ml) was treated with sodium carbonate (10 mg). The solution was refluxed for 1 h, then the methanol was removed under vacuum. The product was extracted with ether and the ether solution was washed with water, dried, and evaporated under vacuum. T.l.c. gave the dihydroxy-compound (XVIII; R = H) (4 mg), identical with the authentic sample (t.l.c. and mixed m.p.).

Treatment of 2β,4β-*Dihydroxycholest-5-en-7-one* (XVIII; R = H) *with Phosgene*.—The steroid (25 mg) in chloroform (5 ml) and triethylamine (3 ml) was treated at −20 °C with excess (×15) of a solution of phosgene in chloroform. A yellow precipitate was formed; after 2 h more chloroform (25 ml) was added and the solution was poured into water. The chloroform layer was washed with water, dilute hydrochloric acid, and water again, then dried and evaporated under vacuum. T.l.c. gave 4α-*chloro*-2β-*hydroxycholest-5-en-7-one* (XXIV) (19 mg, 76%), needles, m.p. 197—199° (decomp.) (from acetone–water), $[\alpha]_D$ −45° (*c* 0·40), $\nu_{max.}$ ($CHCl_3$) 3600 and 1670 cm^{-1}, $\lambda_{max.}$ 233 nm (ε 11,800), τ 8·61 (s, 19-H_3), 5·77 (m, $W_{\frac{1}{2}}$ 11·0 Hz, H-2α), 4·91 (m, $W_{\frac{1}{2}}$ 22 Hz, H-4β), and 3·78 (d, *J* 1·8 Hz, H-6). The compound gave a positive reaction to a test for chlorine (Found: M^+, 434·2949. $C_{27}H_{43}ClO_2$ requires *M*, 434·2951).

2β,4β-*Dihydroxycholestan-7-one*.— 2β,4β-Dihydroxycholest-5-en-7-one (XVIII) (40 mg) in absolute ethanol (8 ml) was hydrogenated at room temperature over platinum oxide (10 mg). The progress of the reaction was followed by the disappearance of the absorption at 234 nm. After 2 h the solution was centrifuged and decanted, and the ethanol was removed under vacuum. T.l.c. gave 2β,4β-*dihydroxycholestan-7-one* (21 mg, 52%), needles, m.p. 194—195° (from ethanol–water), $[\alpha]_D^{28}$ −21° (*c* 0·13), $\nu_{max.}$ ($CHCl_3$) 3590, 3530, and 1705 cm^{-1}, τ 8·44 (s, 19-H_3), 6·12 (m, $W_{\frac{1}{2}}$ 9·5 Hz, H-4α), and 5·68 (m, $W_{\frac{1}{2}}$ 11·4 Hz, H-2α), M^+ 418 (Found: C, 77·25; H, 10·85. $C_{27}H_{46}O_3$ requires C, 77·45; H, 11·05%).

Epoxidation of Cholest-4-en-7-one.—The steroid [20] (m.p. 98—99°; 200 mg) in methylene dichloride (10 ml) was treated at room temperature with *m*-chloroperbenzoic acid (100 mg). After 4 h, the solution was washed with aqueous sodium hydrogen carbonate, dilute sodium sulphite solution, and water, then dried and evaporated under vacuum. T.l.c. gave 4β,5β-*epoxycholestan-7-one* (XX) (54 mg, 26%). The compound failed to crystallize from a variety of solvents although t.l.c. and n.m.r. evidence indicated high purity. It could be isomerized quantitatively to the open form (see later), and showed $[\alpha]_D^{28}$ −38° (*c* 0·34), $\nu_{max.}$ ($CHCl_3$) 1708 cm^{-1}, τ 8·79 (s, 19-H_3), and 7·03 (d, *J* 4·8 Hz, H-4α) (Found: C, 80·85; H, 11·1. $C_{27}H_{44}O_2$ requires C, 80·95; H, 11·05%). Also separated was 4α,5α-*epoxycholestan-7-one* (XXI) (73 mg, 35%), needles, m.p. 119—122° (from ethanol–water), $[\alpha]_D^{25}$ +16° (*c* 0·41), $\nu_{max.}$ (Nujol) 1710 cm^{-1}, τ 8·70 (s, 19-H_3) and 7·12 (d, *J* 2·6 Hz, H-4β) (Found: C, 81·1; H, 11·15. $C_{27}H_{44}O_2$ requires C, 80·95; H, 11·05%).

Isomerization of Epoxides (XX) *and* (XXI). (a) *Epoxide* (XX). The steroid (30 mg) in methanol (5 ml) and triethylamine (0·5 ml) was left at room temperature for 3 h. The solvent was removed under vacuum. T.l.c. gave pure 4β-*hydroxycholest-5-en-7-one* (XXII) (29 mg, 97%), needles, m.p. 150—152° (from acetone–water), $[\alpha]_D^{28}$ −81° (*c* 0·22), $\nu_{max.}$ ($CHCl_3$) 3590 and 1670 cm^{-1}, $\lambda_{max.}$ 234 nm (ε 9900), τ 8·62 (s, 19-H_3), 5·70 (m, $W_{\frac{1}{2}}$ 6·0 Hz, H-4α), and 4·30 (s, H-6) (Found: C, 80·8; H, 10·9. $C_{27}H_{44}O_2$ requires C, 80·95; H, 11·05%).

(b) *Epoxide* (XXI). The steroid (40 mg) in methanol (5 ml) and triethylamine (0·5 ml) was heated on a steam-bath for 3 h. The solvent was removed under vacuum. T.l.c. gave pure 4α-*hydroxycholest-5-en-7-one* (XXIII) (35 mg, 88%), needles, m.p. 162—164° (from acetone–water), $[\alpha]_D^{26}$ −128° (*c* 0·55), $\nu_{max.}$ ($CHCl_3$) 3580 and 1670 cm^{-1}, $\lambda_{max.}$ 238 nm (ε 12,300), τ 8·82 (s, 19-H_3), 5·68 (m, $W_{\frac{1}{2}}$ 21 Hz, H-4β), and 3·91 (d, *J* 1·8 Hz, H-6) (Found: C, 80·8; H, 10·9. $C_{27}H_{44}O_2$ requires C. 80·95; H, 11·05%).

3,3-*Dideuterio*-7α-*hydroxycholest*-4-*en*-2-*one* (IIIa).— A solution of steroid (III) (50 mg) in dry tetrahydrofuran (1 ml), ethan[^{2}H]ol (3 ml) and deuterium oxide (1 ml) was

deoxygenated by passing argon through it for 20 min. Triethylamine (0·05 ml) was added and the solution was sealed under argon and left for 24 h at room temperature. The solvent was then evaporated off under vacuum. The whole treatment was then repeated. The mass spectrum (after isotropic exchange with ethanol—OH for O^2H) showed M^+ 418. Comparison with the mass spectrum of (III) (M^+ 416) indicated that (IIIa) contained at least 70% of [$^2H_2$]- and about 20% of [$^2H_1$]-material. T.l.c. indicated small amounts of impurities. However, attempted purification by chromatography on silica gel resulted in complete loss of deuterium. Hence the compound was used without purification.

3,3-*Dideuterio*-4α,5α-*epoxy*-7α-*hydroxycholestan*-2-*one* (VIa).—The crude material (IIIa) (50 mg) was epoxidized with monoperphthalic acid in dry ether as previously. Five recrystallisations from small amounts of ether gave 4 mg of the pure epoxide, m.p. 133—135 °C, M^+ 418. Comparison with the mass spectrum of (VI) (M^+ 416) indicated that (VIa) contains at least 65% of [$^2H_2$]- and about 25% of [$^2H_1$]-material.

3-*Deuterio*-5α,7α-*dihydroxycholest*-3-*en*-2-*one* (VIIa).—The crude compound (VIa) (35 mg) was isomerized as before and the product was purified by t.l.c. (yield 25 mg). Recrystallisation from acetone–water gave needles, m.p. 219—220°, τ 3·47 (s, H-4); absorptions of H-7β and 19-H_3 identical with those of (VII); M^+ 417. Comparison with the mass spectrum of compound (VII) (M^+ 416) showed that (VIIa) contained at least 70% of [$^2H_1$]-material. The presence of about 30% of non-deuteriated component was shown by the n.m.r. spectrum (both by integration and by the appearance of a weak AB quartet for the C-3 and C-4 protons).

Deuterium Exchange Experiments.—(i) *Checking incorporation into the product.* In each case the epoxy-steroid (3 mg) in absolute ethanol[^2H]ol (99% deuterium) (0·5—1·0 ml) was treated with triethylamine (0·01—0·1 ml) and the reaction was allowed to proceed to completion at room temperature. The solvent was evaporated off under vacuum and the residue (after isotopic exchange—OH for O^2H) was examined by mass spectrometry.

(ii) *Checking incorporation into the starting material.* The reaction was carried out as in (i) but the process was stopped at about 30% completion (followed by t.l.c.) and the solvent was evaporated off under vacuum at 0 °C. The mixture of the starting material and product was examined by mass spectrometry (after isotopic exchange —OH for O^2H). In the case of compounds (X) and (VI), where the reaction is relatively fast, a smaller amount of triethylamine (0·1 mg) was used and the reaction was followed by u.v. spectroscopy.

Rate Measurements.—Reactions were carried out in a buffer solution of equal amounts of triethylamine and triethylamine hydrochloride in absolute ethanol at 30·0 °C. A Unicam SP 1800 u.v. spectrophotometer attached to a chart recorder was used.

In the case of the 2-keto-epoxides (VI) and (X), a low concentration of base (2×10^{-4} or 8×10^{-4} mol l^{-1}) was sufficient to effect the transformation at a suitable rate, and it was possible to follow the progress of the reaction by carrying it out in a u.v. spectrophotometer cell and obtaining the kinetic curve directly from the recorder. 7-Keto-epoxides (XVI), (XVII), (XX), and (XXI), because of their low reactivity, required a much larger concentration of base ($1·6 \times 10^{-1}$ mol l^{-1}). This precluded the use of direct measurement since triethylamine absorbs slightly at 215—230 nm. The progress of the reaction was observed as follows: at intervals of 0·5—1·0 h samples were taken, cooled to 0 °C, and evaporated to dryness under vacuum at 0 °C. Their absorption was then measured in ethanol (triethylamine hydrochloride does not show any u.v. absorption at 210—240 nm even in high concentration).

In each experiment, a straight line was obtained by plotting ($E - E_t$) *vs.* time, where E is the absorption when the reaction is complete and E_t is the absorption at time t. Rate constants were calculated from the half-lives of the reactions. In the case of compound (VIa) a straight line was obtained only after 35% completion. (The reaction proceeds faster in its initial stage because of the non-deuteriated component in the starting material.)

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