205. *Steroids and the Walden Inversion. Part III.* (a) *Derivatives of 6-Ketocholestane;* (b) *A Direct Proof of the Stereochemical Orientation of the Hydroxyl Group in Cholesterol.*

By C. W. Shoppee.

On the assumption that the hydroxyl group in cholesterol and dihydrocholesterol is (β)-orientated (*i.e.*, projects above the general plane of the steroid nuclear ring system, as also do the angular methyl groups), the configurations of various C_3-substituted derivatives of cholestane (Part I; *J.*, 1946, 1138) and cholest-5-ene (Part II; *J.*, 1946, 1147) have been established. In Section (*a*) these results are applied to establish the configurations at C_3 of analogous derivatives of 6-ketocholestane and related compounds.

In Section (*b*), a direct proof of the (β)-orientation of the hydroxyl group in cholesterol is given; all configurations at C_3 assigned in Parts I and II, and in Section (*a*), thus become established independently of the previous assumption and are subject to qualification only in so far as future determination of the absolute configuration of some steroid centre of asymmetry may prove the actual stereochemical arrangement of the steroid nucleus to be the mirror-image of that at present accepted by convention.

In Part I (*J.*, 1946, 1138), the configurations of the epimeric 3-chlorocholestanes were established by reference to the assumed structure of dihydrocholesterol as 3(β)-hydroxycholestane, and in Part II (*J.*, 1946, 1147) the configuration of cholesteryl chloride as 3(β)-chlorocholest-5-ene was derived from its relationship to 3(β)-chlorocholestane. The consistent collation of a large body of data so achieved thus depends on the truth of the basic assumption that the hydroxyl group in cholesterol and dihydrocholesterol is (β)-orientated. In Section (*a*) consideration on the same basis is now extended to the stereochemical orientation at C_3 of derivatives of 6-ketocholestane and certain closely related compounds; in Section (*b*) a direct proof of the (β)-configuration of the hydroxyl group in cholesterol is given.

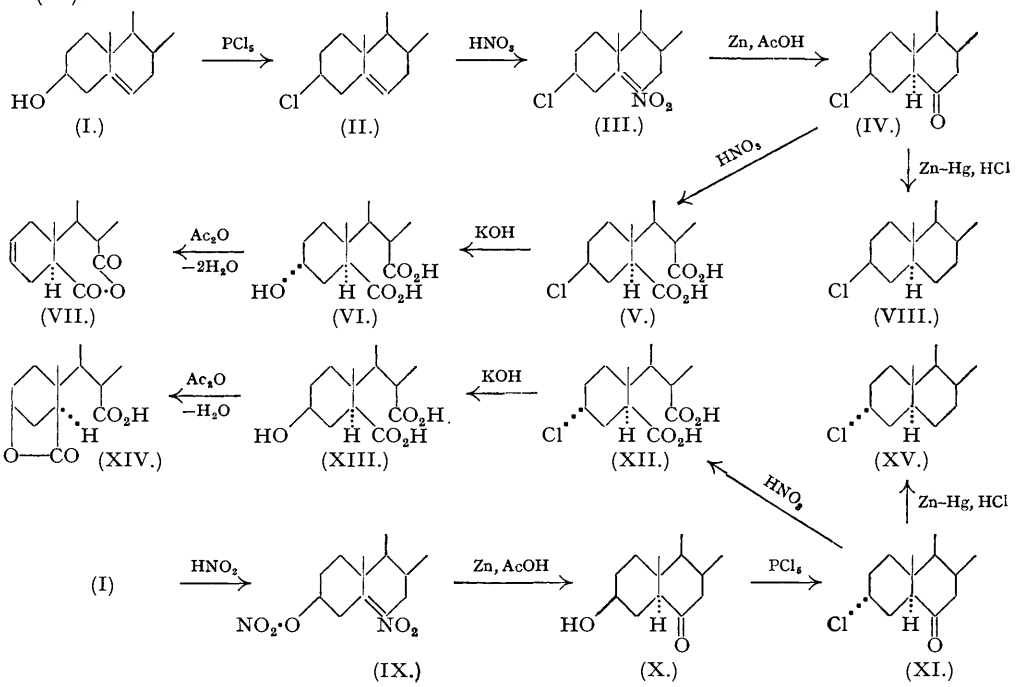
(a) *Derivatives of 6-Ketocholestane.*

In Part I (*loc. cit.*) it was shown that cholesterol (I) undergoes substitution with preservation of configuration to give cholesteryl chloride which is 3(β)-chlorocholest-5-ene (II). Nitration of (II) and reduction of the resulting 3(β)-chloro-6-nitrocholest-5-ene (III) (Mauthner and Suida, *Monatsh.*, 1903, **24**, 648; Windaus and Dalmer, *Ber.*, 1919, **52**, 162) gives the so-called "α"-chlorocholestan-6-one, m. p. 131°, which is therefore 3(β)-chlorocholestan-6-one (IV) * (cf. Ladenburg, Chakravorty, and Wallis, *J. Amer. Chem. Soc.*, 1939, **61**, 3483; Blunschy, Hardegger, and Simon, *Helv. Chim. Acta*, 1946, **29**, 199). The (β)-configuration so assigned is confirmed by Clemmensen reduction of (IV) to 3(β)-chlorocholestane (VIII) (Vanghelovici and Angelescu, *Bul. Soc. Chim. România*, 1935, **17**, 177), whilst membership of the cholestane series is proved by conversion into cholestan-6-one (Windaus and von Staden, *Ber.*, 1921, **54**, 1059) which by Clemmensen reduction gives cholestane (Windaus and Dalmer, *loc. cit.*). Windaus and von Staden (*loc. cit.*) by oxidation of (IV) with nitric acid obtained an "α"-chlorodicarboxylic acid, m. p. 263°, which can now be described as 3(β)-chlorocholestane-6||7-dicarboxylic acid (V). Windaus and von Staden (*loc. cit.*) found the acid (V) to be unaffected by treatment with 0·5N-potassium hydroxide at 50°, but to undergo hydrolysis with 4·5N-potassium hydroxide at 100° to give an "α"-hydroxydicarboxylic acid, m. p. 218°. We shall assume provisionally that hydrolysis is here accomplished by a simple bimolecular substitution

* The bromocholestanone, m. p. 123° (Windaus, *Ber.*, 1904, **37**, 2027; Blunschy, Hardegger, and Simon, *loc. cit.*), is similarly 3(β)-bromocholestan-6-one.

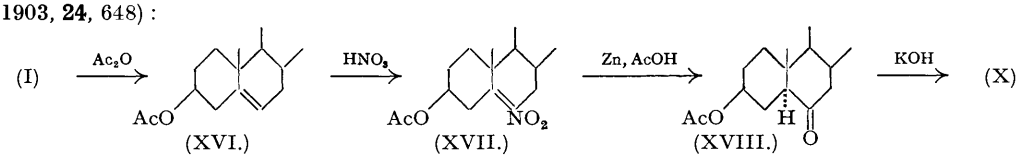
The ketone, m. p. 97°, obtained by Vanghelovici and Angelescu (*loc. cit.*) by treatment of 3(β)-chlorocholestan-6-one (IV) with sodium amalgam in boiling ethanol, described as "*iso*cholestan-6-one," but suggested to be the as yet unknown coprostan-6-one, is *i*-cholestan-6-one, m. p. 97° (Heilbron, Hodges, and Spring, *J.*, 1938, 759) and not cholestane-6-one, m. p. 98—99° (Windaus, *Ber.*, 1920, **53**, 488).

(mechanism S_N2) and so results in substantially complete inversion of configuration (see below); on this basis the hydroxy-acid is to be regarded as 3(α)-hydroxycholestane-6‖7-dicarboxylic acid (VI).



(I.) (II.) (III.) (IV.) (V.) (VI.) (VII.) (VIII.) (IX.) (X.) (XI.) (XII.) (XIII.) (XIV.) (XV.)

When cholesterol (I) is treated with nitric acid at − 15° (cf. Heilbron, Jackson, Jones, and Spring, *J.*, 1938, 104) it is converted into 6-nitrocholesteryl nitrate (IX) which by treatment with zinc and acetic acid and subsequent hydrolysis gives cholestan-3(β)-ol-6-one (X) (Windaus, *Ber.*, 1903, **36**, 3752; cf. Windaus and von Staden, *Ber.*, 1921, **54**, 1059). The incidental esterification of the hydroxyl group in the nitration of cholesterol and subsequent removal of the nitro-group by hydrogenolysis or hydrolysis cannot affect the orientation of the oxygen atom attached to C_3.' Moreover, a conversion of (I) into (X), during which the hydroxyl group is preserved intact, can also be effected; cholesterol (I) as cholesteryl acetate (XVI) by nitration in the presence of sodium nitrite gives 6-nitrocholesteryl acetate (XVII), which by reduction yields 3(β)-acetoxycholestan-6-one (XVIII), hydrolysed to (X) (Mauthner and Suida, *Monatsh.*, 1903, **24**, 648):



(XVI.) (XVII.) (XVIII.)

That the hydroxyl group in (X) is actually (β)-orientated is proved by Wolff–Kishner reduction to cholestan-3(β)-ol in almost quantitative yield. Membership of the cholestane series is proved by oxidation of (X) with chromium trioxide to cholestane-3 : 6-dione (Windaus, *Ber.*, 1903, **36**, 3752) reducible to cholestane (Windaus, *Ber.*, 1917, **50**, 133).

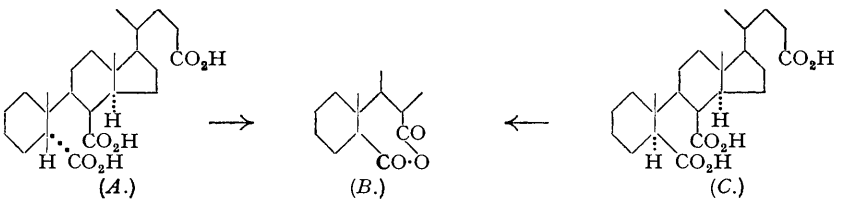
In Part I (*loc. cit.*) it was shown that in saturated derivatives of cholestane replacement of a hydroxyl group at C_3 by chlorine using phosphorus pentachloride leads to inversion of configuration; if, owing to the heavy damping imposed by passage through two saturated carbon atoms, the polar effect at C_3 of the carbonyl group at C_6 in (X) may be regarded as very small, then treatment of (X) with one molecule * of phosphorus pentachloride should lead to

* The enolic form of (X) does not appear to be involved; for although this as the entity undergoing substitution would lead to retention of configuration at C_3 because of the influence of the $C_{5:6}$-double bond, then up to 50% yield of the as yet unknown 3(β) : 6-dichlorocholest-5-ene should appear in the reaction product.

substitution with practically complete inversion of configuration. The so-called " β "-chlorocholestan-6-one, m. p. 181°, so prepared (Windaus and Stein, *Ber.*, 1904, **37**, 3699; Windaus and von Staden, *loc. cit.*) is accordingly 3(α)-chlorocholestan-6-one (XI); structurally, it differs from (IV) only in respect of the configuration of the chlorine atom at C_3, since both (IV) and (XI) by treatment with boiling quinoline give cholest-2-en-6-one (Blunschy, Hardegger, and Simon, *loc. cit.*), whilst Windaus and von Staden also state (*Ber.*, 1921, **54**, 1060) that both (IV) and (XI) yield cholestan-6-one by treatment unspecified. Finally, Clemmensen reduction of (XI) gives 3(α)-chlorocholestane (XV). Oxidation of (XI) with nitric acid (Windaus and Stein, *loc. cit.*) furnishes a " β "-chlorodicarboxylic acid, m. p. 243°, which is clearly 3(α)-chlorocholestane-6||7-dicarboxylic acid (XII); this acid is readily hydrolysed by brief treatment with 0·5N-potassium hydroxide at 50° to a " β "-hydroxydicarboxylic acid, m. p. 242°. We shall again provisionally assume that hydrolysis occurs by a simple bimolecular substitution (S_N2) and so proceeds with substantially complete inversion to give 3(β)-hydroxycholestane-6||7-dicarboxylic acid (XIII).

The two hydroxy-acids (VI) and (XIII) differ only in configuration at C_3, because by oxidation with chromium trioxide they afford the same 3-ketocholestane-6||7-dicarboxylic acid (Windaus and Stein, Windaus and von Staden, *locc. cit.*).

The hydroxy-acid (VI) by treatment with hot acetic anhydride and vacuum distillation gives the unsaturated anhydride (VII); * this is a neutral substance, non-extractable from its



(*A*.) (*B*.) (*C*.)

ethereal solution with 2N-sodium carbonate, and non-titratable with cold 0·01N-sodium hydroxide. The hydroxy-acid (XIII) was originally reported by Windaus and Stein (*loc. cit.*, p. 3705) to pass by extended treatment with hot acetyl chloride into an anhydro-compound, m. p. 212—214°, which these authors state " wohl sicher als Anhydrid und nicht als Lactonsäure anzusehen ist ", because it appeared in that part of the reaction product which was insoluble in sodium carbonate. The subsequent investigation of Lettré (*Ber.*, 1935, **68**, 766), now confirmed, shows that the acid (XIII) by brief treatment with hot acetic anhydride readily gives an anhydro-compound, m. p. 214°, which is the lactonic acid (XIV); it is now found that this cannot be extracted from its ethereal solution with sodium carbonate, titrates as a monobasic acid with cold 0·01N-sodium hydroxide, and reacts with diazomethane to give the lactonic *methyl* ester. The formation of a lactonic acid from the acid (XIII) only is consistent with its formulation as a 3(β)-hydroxy-acid in which both the hydroxyl group at C_3 and the carboxyl group attached to C_5 lie on the same side of, and by convention above, the general plane of the original nuclear ring *A*.

Examination of models mechanically so constructed as to permit free rotation about the bond-axes (for the loan of which the author is much indebted to Mr. W. A. Wightman) shows that if the *cyclo*hexane ring, formerly ring *A* of the steroid nucleus, is a chair-form, then, because the chair is completely rigid, the hydroxyl and carboxyl groups, situated as they are on non-adjacent carbon atoms, cannot interact to yield a γ-lactone with C–C and C–O bonds of normal length (cf. Fig. 1). If, however, the *cyclo*hexane ring is a boat-form, which is highly mobile, an arrangement can readily be achieved which appears to render normal γ-lactone formation not only possible but almost inevitable (cf. Fig. 2). Since it has been shown that the chair ⟶ boat transformation in *cyclo*hexane is a relatively facile process (Part I), such a transformation may, indeed must, be regarded as preceding lactone formation. It remains to be added that the models confirm that an (α)-orientated hydroxyl group at C_3 cannot interact

* The statement that 3(α)-hydroxycholestane-6||7-dicarboxylic acid (VI) does not form a lactone (Sobotka, " The Chemistry of the Sterids ", Baltimore, 1938, p. 61; Strain, Gilman's " Organic Chemistry ", New York, 1943, Vol. II, p. 1376) appears to be without experimental foundation apart from the evidence now given in this paper. The non-formation of a pyro-ketone here may be compared with the case of cholane-6||7 : 24-tricarboxylic acid (thilobilianic acid) (*A*) (Wieland and Dane, *Z. physiol. Chem.*, 1932, **210**, 268) and 5-*allo*cholane 6||7 : 24-tricarboxylic acid (*allo*thilobilianic acid) (*C*) (*idem*, *ibid.*, 1932, **212**, 41) which by pyrolysis at 290° yield the same anhydride (*B*), with inversion at C_5 in one case, and not the expected pyro-ketone.

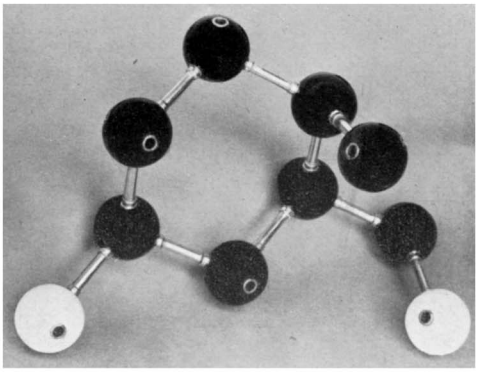


FIG. 1.
The acid (XIII) *as a chair-form.*

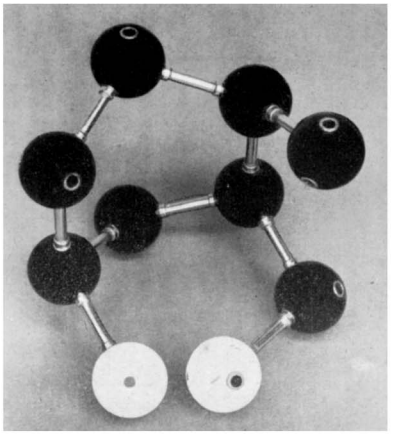


FIG. 2.
The acid (XIII) *as a boat-form.*

(*The white spheres represent the oxygen atoms of the hydroxyl group at* C_3 *and the hydroxyl group of the carboxyl group attached to* C_5.)

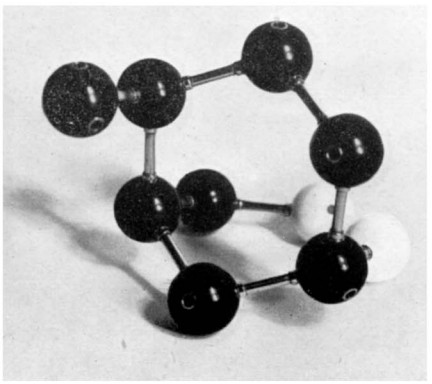


FIG. 3.
The acid (XX) *as a chair-form.*

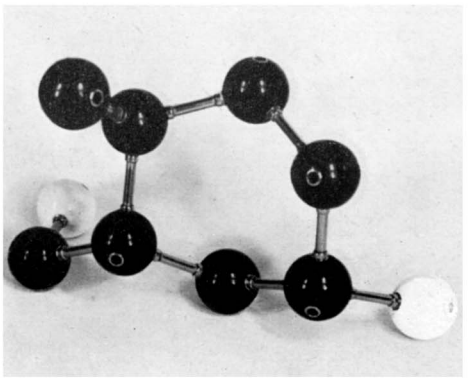


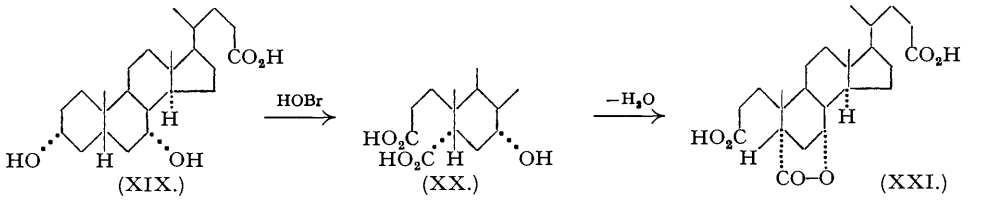
FIG. 4.
The acid (XX) *as a boat-form.*

(*The white spheres represent the oxygen atoms of the hydroxyl group at* C_5 *and the hydroxyl group of the carboxyl group attached to* C_7.)

[*To face p.* 1034

with a (β)-orientated carboxyl group attached to C_5, as in the 3(α)-hydroxy-acid (VI), either in the chair- or the boat-form, to give a γ-lactone.

The ready conversion of the 7(α)-hydroxytricarboxylic acid (XX), derived from chenodeoxycholic acid (XIX) by oxidation with hypobromite, into the lactonic acid (XXI) (Windaus and v. Schoor, *Z. physiol. Chem.*, 1926, **157**, 181; cf. Borsche and Franck, *Ber.*, 1926,



(XIX.) (XX.) (XXI.)

59, 1748) represents an analogous but stereochemically inverse case. Both the hydroxyl group at C_7 and the carboxyl group attached to C_5 are (μ)-orientated, *i.e.*, they lie on the same side of, and by convention below, the general plane of the original nuclear ring *B*; if the *cyclo*hexane ring bearing them is a chair-form (cf. Bastiansen and Hassell, *Nature*, 1946, **157**, 765) then production of a γ-lactone with bonds of normal lengths appears to be a natural consequence (cf. Fig. 3). If the *cyclo*hexane ring is, however, a boat-form [cf. the structure for coprostane proposed by Ruzicka, Furter, and Thomann (*Helv. Chim. Acta*, 1933, **16**, 331)], then owing to incorporation in another rigid ring system (ring *C must* be a chair-form), γ-lactone formation appears to be impossible (cf. Fig. 4).

The reaction sequence (I $\xrightarrow{\text{IX}}$ XIV) has previously been considered by Lettré (*loc. cit.*) on the assumption that cholesterol is a 3(β)-hydroxy-compound, but without the knowledge, provided by this series of papers, of the configuration of the intermediates. Conversely, other authors (Miescher and Fischer,* *Helv. Chim. Acta*, 1938, **21**, 336, especially 352; Sobotka, " The Chemistry of the Sterids ", pp. 60, 61; Bergmann,† *Chem. and Ind.*, 1939, **58**, 512) have attempted to derive from the (β)-configuration of the hydroxyl group in (XIII), but in the absence of knowledge of the stereochemical orientation at C_3 of the various intermediates in the transformation of (I) into (V) and (I) into (XII), a proof of the proposal made by Ruzicka (Ruzicka, Furter, and Thomann, *Helv. Chim. Acta*, 1933, **16**, 327; Ruzicka, Brüngger, Meyer, and Eichenberger, *ibid.*, 1934, **17**, 1407) on the basis of the Auwers–Skita rule that the hydroxyl group in cholesterol is (β)-orientated. As Ruzicka himself has pointed out (Ruzicka, Goldberg, Meyer, Brüngger, and Eichenberger, *ibid.*, p. 1395; Ruzicka, Goldberg, and Wirz, *ibid.*, 1935, **18**, 61) use of the Auwers–Skita rule involves an element of uncertainty; ‡ moreover, there is a further element of uncertainty in regard to the stereochemical course of the replacement of chlorine by hydroxyl in the hydrolysis of the chloro-acids (V) and (XII). If both these reactions proceeded with preservation of configuration the conclusion would be that the hydroxyl group in cholesterol is (α)-orientated; or, conversely, that all the transformations discussed above, and in Parts I and II, should and could consistently be re-formulated on the basis that cholesterol is an (α)-hydroxy-compound. On this account it is necessary here more closely to examine the validity or otherwise of the provisional assumption, made above, that alkaline hydrolysis of the epimeric chloro-acids (V) and (XII) proceeds by a simple bimolecular mechanism (S_N2) with substantially complete inversion of configuration.

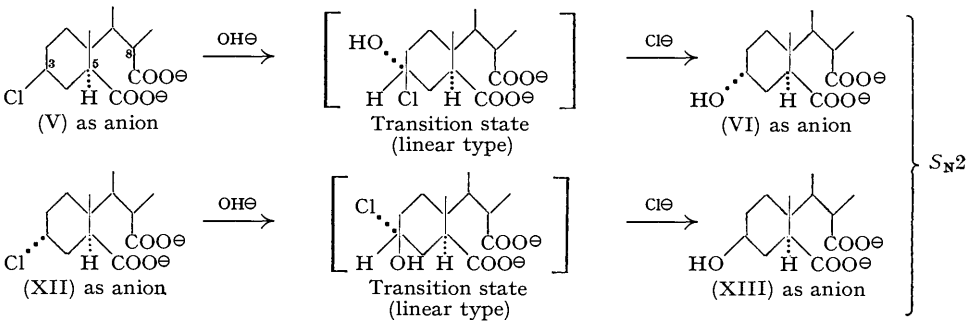
The system involved in these reactions is analogous to that present in 3-chlorobutane-1-carboxylic acid. Alkaline hydrolysis of (±)-3-bromobutane-1-carboxylic acid at 15° leads

* Miescher and Fischer say: " Dass zweimalige *Walden*'sche Umkehrung durch Ersatz von Hydroxyl in (X) durch Chlor und durch Rückwandlung von Chlor in (XII) in Hydroxyl eintritt, und damit die sterische Lage der Hydroxylgruppe erhalten bleibt, wird durch neuere Befunde in der Sterinreihe von Ruzicka (*Helv. Chim. Acta*, 1935, **18**, 998; 1936, **19**, 1407) und Marker (*J. Amer. Chem. Soc.*, 1935, **57**, 2358; 1936, **58**, 481) *erwiesen* ". This is incorrect, for none of these papers *proves* the stereochemical configuration at C_3 of the compounds in question; use of the verb *erwiesen* is unwarranted, and it may be remarked that Miescher and Fischer conclude the paragraph containing the above quotation by the words " so wäre doch noch eine Ergänzung des Beweismaterials erwünscht ".

† Contrary to the statement of Bergmann, the *absolute* configuration of any centre of asymmetry in the steroid nucleus has not yet been determined.

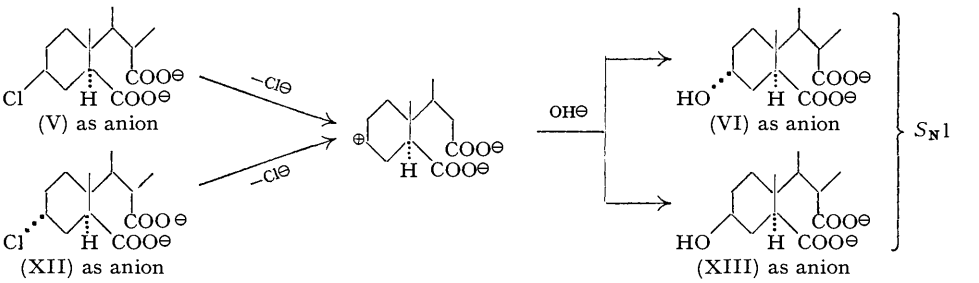
‡ The Auwers–Skita rule states that catalytic hydrogenation of a carbonyl group in an acidic medium usually gives *cis*-forms, but in a neutral medium leads to *trans*-forms. It is uncertain whether the C_5–H bond or the C_5–C_6 bond should be considered in applying the rule, and choice of the latter alternative would lead to configurations at C_3 opposite to those conventionally attributed to dihydrocholesterol, coprostanol, and their epimerides.

directly to γ-valerolactone (Fittig and Messerschmidt, *Annalen*, 1881, **208**, 94; Fittig and Fränkel, *ibid.*, 1889, **255**, 30), but, were data available as to the steric course of hydrolysis for the



(+)- and (−)-enantiomorphs, it seems improbable that they could contribute to the elucidation of our case involving the acids (V) and (XII), in which inclusion of the system in a six-membered ring limits the relative spatial positions of the chlorine atom at C_3 and the carboxylate-ion group attached to C_5. Since polar effects are known to suffer heavy damping by transmission through saturated carbon atoms, the effect so propagated of the carboxylate-ion group attached to C_5 (and likewise, *a fortiori*, of the carboxylate-ion group at C_8) on the steric course of substitution at C_3 should be small. The electrostatic field of the carboxylate-ion groups may well increase the activation energy of the transition state by several kg.-cals./mole by virtue of the extra work required for the approach of the attacking hydroxyl ion; nevertheless, it seems probable in view of the influence of the geometrical character of the steroid nucleus upon the form of the transition state that the substitution of Cl by OH in (V) and (XII) occurs by mechanism S_N2 with substantially complete inversion to give respectively the anions of the acids (VI) and (XIII).

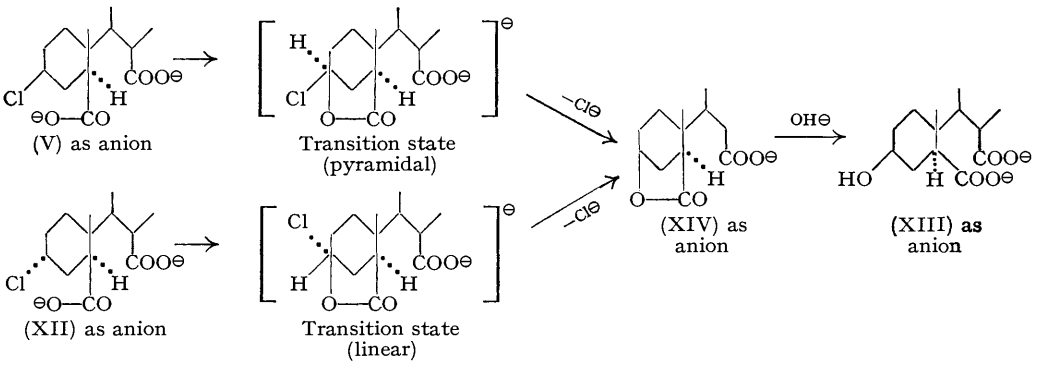
An alternative possibility, however, is that hydrolysis of the chloro-acids (V) and (XII) proceeds by mechanism S_N1, although it seems improbable that the electron-repulsion of the carboxylate-ion groups, transmitted through the chain of saturated carbon atoms, could afford effective facilitation of such a process.



If the carboxylate-ion group attached to C_5 does not intervene to influence the steric course of the reaction undergone by the intermediate ion, then the stereochemical result must be local but very nearly complete racemisation with production of very nearly equal quantities of the 3-epimeric hydroxy-acids (VI) and (XIII). If the carboxylate-ion group attached to C_5 does intervene to preserve configuration at C_3, then, as a consequence of the electrostatically maintained pyramidal configuration of the intermediate ion, the common product must be (VI) and not (XIII). It was found by Windaus *et al.* (*locc. cit.*), and is now confirmed, that hydrolysis of the chloro-acids (V) and (XII) furnishes high yields of individual epimeric hydroxy-acids; we therefore conclude that hydrolysis does not occur by mechanism S_N1. This conclusion is substantiated by the circumstance that hydrolysis is brought about in the presence of considerable concentrations of hydroxyl ions, and it is known that mechanism S_N2 increases in importance, relative to a possible competitive mechanism S_N1 facilitated by electron-repelling substituents, as the hydroxyl-ion concentration increases (cf. Dostrovsky, Hughes, and Ingold, *J.*, 1946, 187).

The polar effect of the carboxylate-ion group attached to C_5 might, however, become effective

in another way, the carbon atom (C_3), bearing the chlorine atom, being attacked directly to give rise to an intramolecular modification of the bimolecular mechanism.



In this case, the molecular geometry of the chloro-acid (V) determines the form of the transition state and would lead inevitably to the pyramidal type, which corresponds with retention of configuration and would furnish the anion of the lactonic acid (XIV), which by subsequent acyl–oxygen fission (corresponding to ordinary alkaline ester hydrolysis), which cannot cause inversion, would give the hydroxy-acid (XIII).

It may be noted that the formation of such a pyramidal transition state is rendered intrinsically unlikely because the exclusion principle shows the required energy of activation will be prohibitively high; further, the electrostatic factor arising from the mutual repulsion of the dipole field in the C–Cl link and the anionic charge of the carboxylate-ion group must also impede the formation of a pyramidal transition state, $C\begin{smallmatrix}O-(\delta-)\\Cl\ (\delta-)\end{smallmatrix}$. Application of an analogous intramolecular mechanism to the chloro-acid (XII) would produce the anion of the *same* lactonic acid (XIV), because here the molecular geometry of the chloro-acid (XII) again determines the form of the transition state but leads inevitably to the linear type, the consequence of which is inversion of configuration. Since it is established experimentally that hydrolysis of the chloro-acids (V) and (XII) furnishes high yields of individual epimeric hydroxy-acids, the foregoing intramolecular mechanism is excluded.

We conclude therefore that it is highly probable that the conversion of the chloro-acids (V) and (XII) proceeds by mechanism S_N2 with substantially complete inversion of configuration, and, with Miescher and Fischer, Sobotka, and Bergmann, that the hydroxyl-group in cholesterol is (β)-orientated.

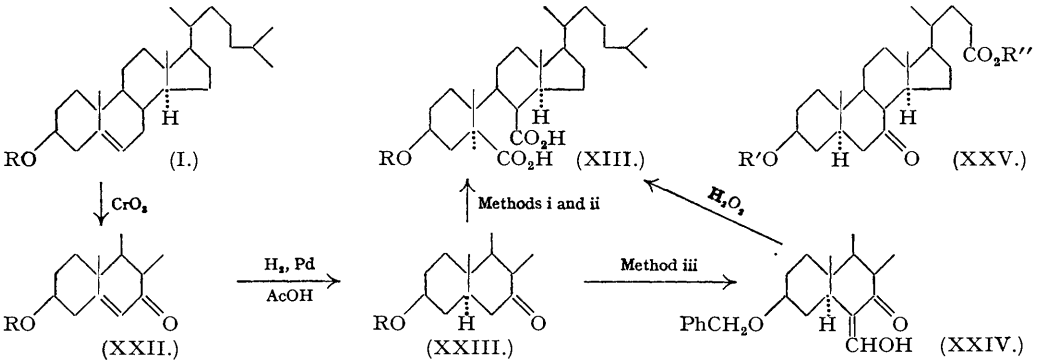
(b) *A Direct Proof of the Stereochemical Orientation of the Hydroxyl Group in Cholesterol.*

The foregoing analysis shows that it is highly probable that the (β)-configuration of the hydroxyl group of the hydroxy-acid (XIII) can justifiably be related to the configuration of the hydroxyl group in cholesterol (I) in the light of knowledge now adduced as to the configuration at C_3 of the various intermediates. There remains, however, an element of uncertainty in regard to the steric course of the conversion of the chloro-acids (V) and (XII) into the hydroxy-acids (VI) and (XIII). In view of the importance of the matter and this defect in the argument, it seemed desirable to evolve a flawless proof of the stereochemical orientation of the hydroxyl group in cholesterol.

In order to give such a proof, it is necessary to preserve intact the vital hydroxyl group during conversion of cholesterol (I; R = H) into the hydroxy-acid (XIII; R = H), and three methods, involving protection of the hydroxyl group by suitable acylation or alkylation, were envisaged. All three methods possess common initial stages: (*a*) the well-established oxidation of cholesterol esters (I) to the 7-ketocholesteryl compounds (XXII), (*b*) hydrogenation of the latter to the corresponding derivatives of 7-ketocholestane (XXIII).

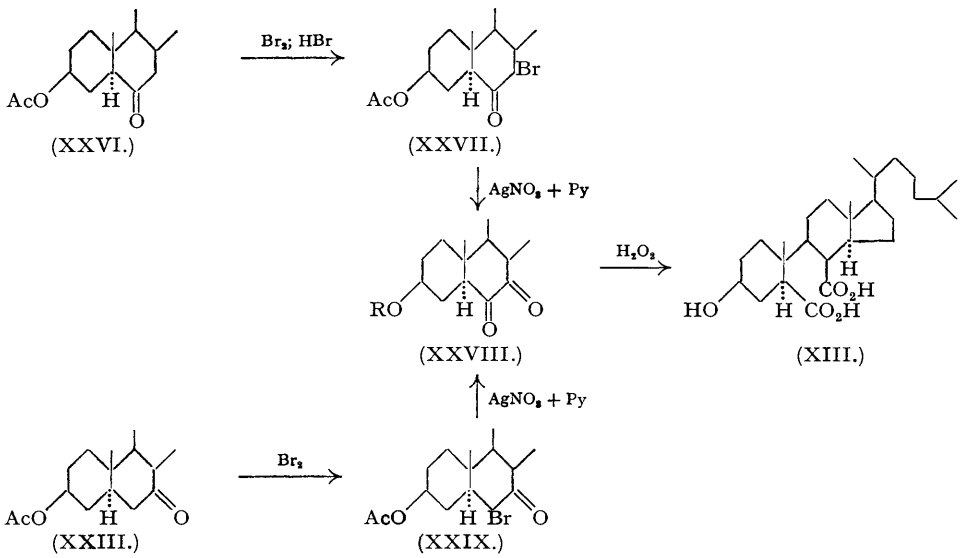
Method (i) (with Dr. W. C. J. Ross). The direct oxidation with chromium trioxide in acetic acid of 7-ketocholestanyl acetate (XXIII; R = Ac) and chromatographic analysis of the methyl esters of the resulting acetoxy-acids led to the isolation as the sole crystalline product of *methyl 7-keto-3*(β)*-acetoxy-5-allocholanate*, m. p. 133° (XXV; R′ = Ac, R″ = Me), hydrolysed to 3(β)*-hydroxy-7-keto-5-allocholanic acid*, m. p. 193° (XXV; R′ = R″ = H). The subsequent discovery that methyl 3(β)-acetoxycholestane-6||7-dicarboxylate (dimethyl ester of XIII;

R = Ac) is non-crystalline led later to the alkaline hydrolysis of the individual non-crystallisable fractions, but from none of these could the acid (XIII; R = H) be isolated.



Method (ii) (with Dr. N. GOUGH). This involved the oxidation of 7-ketocholestanyl benzoate (XXIII; R = Bz) with potassium hypoiodite (cf. Wettstein, Fritzsche, Hunziger, and Miescher, *Helv. Chim. Acta*, 1941, **24**, 332E; Heer and Miescher, *ibid.*, 1945, **28**, 156); it was hoped that the greater resistance to hydrolysis of benzoyloxy- as compared with acetoxy-groups (cf. Ruzicka, Wettstein, and Kagi, *ibid.*, 1935, **18**, 1478; Shoppee, *J.*, 1946, 1134) might permit extensive oxidative fission of ring *B* between C_6 and C_7 before hydrolysis of the protecting benzoyl group occurred. This hope proved to be unfounded, because large quantities of benzoic were readily isolated from the acidic fraction of the reaction product; the residual acidic material proved intractable and was probably a complex mixture containing cholestane-2||3- and -3||4-dicarboxylic acids among other products.

Method (iii). As originally envisaged, this involved conversion of 7-keto-3(β)-benzyloxycholestane (XXIII; R = CH_2Ph) by condensation with ethyl formate into the 7-keto-6-hydroxymethylene compound (XXIV), with subsequent fission of ring *B* with alkaline hydrogen peroxide to the benzyloxy-acid (XIII; R = CH_2Ph) (cf. Billeter and Miescher, *Helv. Chim. Acta*, 1946, **29**, 859) and final hydrogenolysis of the protecting benzyl group to the hydroxy-acid (XIII; R = H) (cf. Heer and Miescher, *ibid.*, 1945, **28**, 156). It was, however, discovered that a compound, even more suitable than (XXIV) and readily available, had already been described by Heilbron, Jones, and Spring (*J.*, 1937, 801); these authors obtained from 6-ketocholestanyl



acetate (XXVI) [Formula XVIII of Section (*a*)] by bromination, isomerisation of the first-formed 5-bromo-derivative to 7-bromo-6-ketocholestanyl acetate (XXVII) and oxidation of this

with silver nitrate in pyridine, the acetoxy-diketone (XXVIII; R = Ac). And in fact an oxidation with hydrogen peroxide of the hydroxy-diketone (XXVIII; R = H) was actually carried out by Dr. E. R. H. Jones in 1937 (private communication, see below) with a view to establishing the positions at C_6 and C_7 of the two carbonyl groups in (XXVIII). The acid resulting from this oxidation could not be satisfactorily identified; it melted at 225—226°, and although it failed to depress the m. p. (239—240°) of a genuine specimen of the hydroxy-acid (XIII), it gave unsatisfactory results on analysis (Found: § C, 70·0, 70·4; H, 9·6, 9·5. Calc. for $C_{27}H_{46}O_5$: C, 71·96; H, 10·29%). The matter was not further pursued because in 1938 the structure of the acetoxy-diketone (XXVIII; R = Ac) was secured by its preparation from 7-ketocholestanyl acetate by Barr, Heilbron, Jones, and Spring (*J.*, 1938, 334). This preparation has been repeated; 7-ketocholestanyl acetate (XXIII) was converted into the 6" β "-bromo-derivative, m. p. 143° (XXIX), which by oxidation with silver nitrate in hot pyridine readily gave the acetoxy-diketone (XXVIII; R = Ac), which was also obtained from a specimen of the 7-bromo-6-keto-compound (XXVII) kindly supplied by Dr. Jones. The acetoxy-diketone (XXVIII; R = Ac) was hydrolysed with dilute methanolic potassium hydroxide at 20° to the *hydroxy-diketone* (XXVIII; R = H), which gave a green colour with alcoholic ferric chloride solution, and this was oxidised with alkaline hydrogen peroxide.

Windaus and Stein (*Ber.*, 1904, **37**, 3705) observed that the hydroxy-acid (XIII) retained 1 mol. of water after drying in a vacuum (Found: * C, 69·61; H, 10·55. Calc.† for $C_{27}H_{46}O_5,H_2O$: C, 69·19; H, 10·32%), but obtained excellent figures after drying at 110° for an unspecified period and at an unspecified pressure (Found: * C, 72·01; H, 10·41. Calc.† for $C_{27}H_{46}O_5$: C, 71·96; H, 10·29%). Since it is known that steroids with free hydroxyl groups may retain water with great tenacity, it appeared possible that the low m. p. and unsatisfactory analyses obtained by Dr. Jones and recorded above might be due to this cause. The crystalline acid resulting from the oxidation of the hydroxydiketone (XXVIII; R = H) was dissolved in a little anhydrous dioxan and dried by repeated azeotropic distillation with benzene; the acid, so obtained, crystallised from acetone–pentane in aggregates of felted needles, m. p. 241—242°, either alone or admixed with a genuine specimen of 3(β)-hydroxycholestane-6‖7-dicarboxylic acid (XIII); this was prepared by the method of Windaus and Stein (*Ber.*, 1904, **37**, 3704), subjected to thorough drying as described above, and crystallised from acetone–pentane to give aggregates of felted needles, m. p. 241—242°. The acid obtained by Dr. Jones in 1937, and kindly made available by him, was originally crystallised repeatedly (following Windaus and Stein, *loc. cit.*) from aqueous acetone. It formed flat plates which became opaque at 130—140°, and melted at 226—228°; by thorough drying as described above and crystallisation from acetone–pentane, there were obtained characteristic aggregates of felted needles, m. p. 241—242°, alone or on admixture with the other specimens of the same m. p. For further identification the specimens of the hydroxy-acid (XIII) obtained by the foregoing degradation and by the method of Windaus and Stein (*loc. cit.*) were converted by brief treatment with boiling acetic anhydride into the lactonic acid (XIV) (Lettré, *loc. cit.*); both preparations had m. p. 214—215° and gave no depression on admixture. The dimethyl ester and acetoxy-dimethyl ester of the two specimens of the acid (XIII) were prepared for further comparison, but could not be induced to crystallise.

The absolute configuration of no single steroid centre of asymmetry has yet been determined, and it may be that the actual stereochemical arrangement of the cholesterol molecule will prove to be the mirror-image of that accepted by convention today. Subject only to this reservation, it can now be stated that the hydroxyl group situated at C_3 in cholesterol is (β)-orientated.

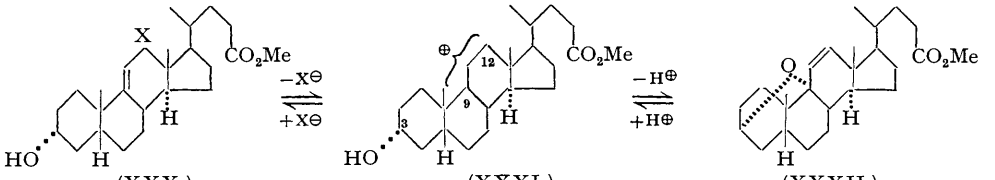
A similar statement can be made in respect of " β "-sitosterol, stigmasterol, and ergosterol since these substances have been converted into 3(β)-acetoxynor*allo*cholanic acid (Wieland, Dane, and Martius, *Z. physiol. Chem.*, 1933, **215**, 15; Fernholz and Chakravorty, *Ber.*, 1934, **67**, 2021), and in respect of *all* 3-hydroxy-steroids which, directly or indirectly, have been correlated with cholesterol in regard to configuration at C_3, *e.g.*, the cortical substances A, D, J, K, L, N, O, P, R, and V (Reichstein and Shoppee, " Vitamins and Hormones ", 1943, I, 345), the cardiac aglycones digitoxigenin (Hunziker and Reichstein, *Helv. Chim. Acta*, 1945, **28**, 1472), gitoxigenin (K. Meyer, *ibid.*, 1946, **29**, 718, 1580), and scillarene-A (Stoll and Renz, *ibid.*, 1941, **24**, 1380), and the steroid sapogenins, sarsasapogenin, smilagenin, tigogenin, *neo*tigogenin, chlorogenin, and diosgenin (Shoppee, *Ann. Rev. Biochem.*, 1942, XI, 103).

Since this work was commenced, an indirect proof of the (β)-orientation of the hydroxyl

* Windaus and Stein's analytical figures have been recalculated using modern atomic weights.

† Windaus and Stein based their calculated figures on the cholesterol formula, $C_{27}H_{44}O$, in use in 1904, and so give the molecular formula of the hydroxy-acid (XIII) as $C_{27}H_{44}O_5$.

group in cholesterol has been given by Kendall *et al.* (*J. Biol. Chem.*, 1946, **164**, 569). These workers show that deoxycholic acid can be converted into methyl 12-halogeno-3(α)-hydroxychol-9(11)-enate (XXX); this is stable in anhydrous non-polar solvents, but by treatment in chloroform solution with water at 20° is converted almost quantitatively *via* the mesomeric ion (XXXI) into the Δ^{11}-3 : 9-oxide (XXXII), which with hydrochloric or hydrobromic acid regenerates (XXX).



X = Cl or Br

Since ether-formation does not involve inversion of configuration (Kenyon and McNicol, *J.*, 1935, **123**, 14; Hughes, Ingold, and Masterman, *J.*, 1937, 1197), and since the formation of an oxide bridge between C_3 and C_9 in the presence of the (β)-orientated angular methyl group at C_{10} can *only* occur if the oxide is a 3(α) : 9(α)-oxide, as is demonstrable by use of models,* the hydroxyl group in (XXX) and hence in deoxycholic acid must be (α)-orientated. Because the configuration of the hydroxyl group at C_3 in the bile acids is known to be the opposite of that in cholesterol, it follows by exclusion that the hydroxyl group in cholesterol is (β)-orientated.

Experimental.

All m. p.s were determined thermo-electrically on a Kofler block and are therefore corrected; limit of error ± 2°. All solvents for chromatographic analyses were specially purified and rigorously dried; for drying ethereal extracts, brief treatment with sodium sulphate was used.

Section (a).

3(β)-*Hydroxycholestane from* 3(β)-*Hydroxycholestane*-6-*one* (X).—The hydroxy-ketone (X) (m. p. 144°; 200 mg.), 100% hydrazine hydrate (0·5 c.c.), and a solution of sodium (200 mg.) in ethanol (5 c.c.) were heated at 170—175° for 6 hours. The product was poured into sufficient 2N-hydrochloric acid, the greater part of the ethanol removed under reduced pressure, and the precipitate filtered off. The dry precipitate (190 mg.) was dissolved in benzene (1 c.c.) and introduced on to a column of aluminium oxide (Spence, activity I—II, 6 g.) prepared in pentane. After elution with pentane, benzene, and ether, which removed insignificant amounts of oil, elution with acetone–ether (1 : 1) gave pure 3(β)-hydroxycholestane (161 mg.), plates from methanol, m. p. 125° with transition to needles, m. p. 141°; the same behaviour on melting was observed by admixture with a genuine specimen. Further elution of the column with acetone gave only traces of material, but use of acetone–chloroform–methanol (1 : 1 : 1) gave a little gum (20 mg.).

3(α)-*Chlorocholestane* (XV) *from* 3(α)-*Chlorocholestan*-6-*one* (XI).—3(α)-Chlorocholestan-6-one was prepared from the hydroxy-ketone (X) by treatment with phosphorus pentachloride according to Windaus and Stein (*loc. cit.*) as modified by Ruzicka *et al.* (*Helv. Chim. Acta*, 1934, **17**, 1389), and crystallised from acetic acid. The chloro-ketone (m. p. 181°; 200 mg.) was suspended in a mixture of acetic acid (2 c.c.) and concentrated hydrochloric acid (2 c.c.); amalgamated zinc wool (1 g.) was added, a slow stream of hydrogen chloride passed into the flask through a tube terminating just above the surface of the mixed acids, and the mixture heated under reflux until the original crystals became converted into semi-solid droplets. The cooled liquid was decanted from residual zinc, and excess of hydrogen chloride removed under reduced pressure; the zinc was well washed with pentane, and the pentane washings used to extract the aqueous liquid. The pentane extract was washed with water, 2N-sodium carbonate, and again with water, dried, and evaporated. The product gave a yellow colour with tetranitromethane, and to remove unsaturated by-products was treated with excess of 1% chromium trioxide solution in acetic acid on the steam-bath for 0·5 hour. After working up in the usual manner, the product was dissolved in pentane and filtered through a column of aluminium oxide (Spence, 5 g.) prepared in pentane. Evaporation of the filtrate gave an oil which crystallised by inoculation; repeated recrystallisation from acetone finally gave a poor yield of 3(α)-chlorocholestane, m. p. 100—102°, which did not depress the m. p. of a genuine specimen.

3(α)-*Hydroxycholestane*-6||7-*dicarboxylic Acid* (VI).—This was prepared by the method of Windaus

* Mr. Wightman's models show that a 3(α) : 9(α)-oxide with normal bond lengths for the C–O–C linkage is readily formed if rings *A* and *B* of the coprostane nucleus are both boat-forms; this is not so in the case of the modification, based on a structure proposed for *cis*-decalin by Bastiansen and Hassell (*Nature*, 1946, **157**, 765), in which despite *cis*-union both rings *A* and *B* are chair-forms, but again becomes the case when ring *A* of this structure becomes a boat-form with ends at C_3 and C_{10}, and ring *B* remains a chair-form (cf. Ruzicka, Furter, and Goldberg, *Helv. Chem. Acta*, 1938, **21**, 509, Plate II, Fig. 3*a*).

Added, May 24th, 1948.—See also the discussion by Barton (this vol., p. 340).

and von Staden (*loc. cit.*); the crude acid obtained by hydrolysis of 3(β)-chlorocholestane-6‖7-dicarboxylic acid (V) (m. p. 265°; 350 mg.) was dried by dissolution in a little anhydrous dioxan and repeated azeotropic distillation with benzene, and the residue (m. p. 215—220°; 293 mg.), obtained by final evaporation in a vacuum, recrystallised from dioxan to give clusters of small prisms, transformed at 200—205° into needles, m. p. 218—221°.

Cholest-2 (or ?3)-ene-6‖7-dicarboxylic Anhydride (VII).—The hydroxy-acid (VI) (50 mg.) was heated with pure acetic anhydride (1 c.c.) under reflux for 0·5 hour; after removal of the reagent under reduced pressure, the residual oil was taken up in ether, and the ethereal solution washed with sodium hydrogen carbonate, 2N-sodium carbonate, and water; acidification of the alkaline washings gave no turbidity. The ethereal solution was dried, evaporated, and kept for some hours at 50° in a high vacuum (finally 0·001 mm.); the viscous oil so obtained gave no colour with tetranitromethane in chloroform solution, but could not be induced to crystallise. Analysis of the oil gave figures which suggest it to consist essentially of 3(α)-hydroxycholestane-6‖7-dicarboxylic anhydride rather than the corresponding 3(α)-acetoxy-compound (Found: C, 75·47; H, 9·57. Calc. for $C_{27}H_{44}O_4$: C, 74·96; H, 10·25%. Calc. for $C_{29}H_{46}O_5$: C, 73·22; H, 9·74%). By distillation in a molecular flask at 160—180° (bath temp.)/0·001 mm., there was obtained an oil, giving a strong yellow colour with tetranitromethane in chloroform, which crystallised when moistened with methanol and kept at 0°; recrystallisation from methanol gave *cholest-2 (or ?3)-ene-6‖7-dicarboxylic anhydride* as prisms, m. p. 134° (Found: C, 77·78, 77·76; H, 10·43, 10·11. $C_{27}H_{42}O_3$ requires C, 78·21; H, 10·21%). The anhydride neither discharged the colour nor evolved gas when treated with an ethereal solution of diazomethane and was recovered unchanged.

3(β)-Hydroxycholestane-6‖7-dicarboxylic Acid (XIII).—This was prepared by the procedure of Windaus and Stein (*loc. cit.*); the crude hydroxy-acid, obtained by hydrolysis of 3(α)-chlorocholestane-6‖7-dicarboxylic acid (m. p. 243°; 350 mg.), was completely dried by solution in a little anhydrous dioxan and repeated azeotropic distillation with dry benzene, and the residue (295 mg.), obtained by final evaporation in a vacuum, recrystallised from acetone–pentane, from which it separated in circular aggregates of felted needles, m. p. 241—242° (gas evolution). The *dimethyl* ester was prepared by dissolving the acid (50 mg.) in the minimum quantity of anhydrous dioxan and treating the solution with ethereal diazomethane at 0° for 0·25 hour; the yellow solution was poured into ice-cold 2N-hydrochloric acid, and the ethereal layer washed with 2N-sodium carbonate, then water, dried, and evaporated. The resulting oil could not be induced to crystallise; it was distilled in a molecular flask at 140—160° (bath temp.)/0·001 mm., but thereafter still resisted all attempts to induce crystallisation (Found: C, 72·48; H, 10·43. $C_{29}H_{50}O_5$ requires C, 72·75; H, 10·53%). The *3(β)-acetoxy-dimethyl* ester was prepared by esterifying the acid (50 mg.) as above, and dissolving the product in pyridine (0·30 c.c.) and adding acetic anhydride (0·25 c.c.). After 16 hours at 20°, excess of the reagents was removed under reduced pressure at 30°, and the product subjected to the usual washing of the ethereal solution with 2N-hydrochloric acid, 2N-sodium carbonate, and water, drying, and evaporation. The resultant oil was distilled in a molecular flask at 130—150° (bath temp.)/0·001 mm., but could not be induced to crystallise (Found: C, 71·72; H, 9·82. $C_{31}H_{52}O_6$ requires C, 71·50; H, 10·06%).

3(β)-Hydroxycholestane-6‖7-dicarboxylic Acid 6 ⟶ 3-Lactone (XIV).—The hydroxy-acid (XIII) (50 mg.) was heated with pure acetic anhydride for 0·5 hour (cf. Lettré, *loc. cit.*); the product, obtained by complete evaporation in a vacuum, was dissolved in ether, and the ethereal solution washed thrice with 2N-sodium carbonate. Acidification of these alkaline extracts produced no turbidity, and subsequent extraction with ether failed to give a significant quantity of material. The foregoing ethereal solution was washed with water, dried, and evaporated to yield a colourless oil (37 mg.), which tended to separate from ether–pentane as a gel, but crystallised readily when rubbed with a trace of acetone. Recrystallisation from ether–pentane or methanol gave the lactonic acid as needles, m. p. 214—215°, resolidifying to a mass of needles at 210°. When dissolved in a little pure acetone, the substance titrated as a mono-basic acid with 0·01N-sodium hydroxide [Found: *M* (monobasic), 428. Calc. for $C_{27}H_{44}O_4$: *M*, 432·6]. The lactonic acid, when dissolved in ether, discharged the yellow colour of ethereal diazomethane with a brisk evolution of gas; after addition of excess of diazomethane and standing at 0° for 0·25 hour, the mixture was worked up in the usual manner to give the lactonic *methyl* ester as a colourless oil which could not be induced to crystallise before or after distillation at 150—160°/0·001 mm. (Found: C, 75·12; H, 10·36. $C_{28}H_{46}O_4$ requires C, 75·30; H, 10·36%). The ester crystallised after being kept for some months; to remove traces of colour and dust-particles, the material (40 mg.) was chromatographed over aluminium oxide (1·2 g.; Merck–Brockmann) in pentane, and the ester eluted with benzene–pentane (1 : 4, 3 × 4 c.c. and 1 : 3, 3 × 4 c.c.). These fractions were united and the ester twice crystallised from methanol to give fine, rectangular prisms (11 mg.), m. p. 104—105° (Found, after grinding and drying at 60°/0·01 mm.: C, 75·46; H, 10·38%). Another crystal-form from methanol, stout prisms, m. p. 96—97°, with transformation to long prismatic needles, m. p. 104°, was also observed.

An attempt to prepare the lactonic acid (XIV) using acetyl chloride for 3 hours (cf. Windaus and Stein, *loc. cit.*) gave a very small quantity of material non-extractable from ethereal solution with 2N-sodium carbonate; this crystallised from ether–pentane in flat plates, m. p. 188—190°, but was not further investigated. The bulk of material was precipitated by acidification of the alkaline extracts, and appeared to consist of unaltered hydroxy-acid (XIII).

Section (b).

Method (i) (with Dr. W. C. J. Ross).—*Methyl 7-keto-3(β)-acetoxy-5-allocholanate* (XXV; R′ = Ac, R″ = Me). Hydrogenation of 7-keto-3(β)-acetoxycholest-5-ene (XXII) with palladium in acetic acid (cf. Windaus and Kirchner, *Ber.*, 1920, **53**, 614; Barr, Heilbron, Jones, and Spring, *loc. cit.*) gave 7-keto-3(β)-acetoxycholestane (XXIII; R = Ac), m. p. 142—143°. This ketone (2·0 g.) was treated with a 2·7% solution of chromium trioxide in 90% acetic acid for 1 hour on the stream-bath; the cooled solution was diluted with sufficient water, extracted with ether, and the ethereal solution separated into neutral and acidic fractions by use of 2N-sodium carbonate. The acidic material (160 mg.), which solidified by

rubbing with methanol, was esterified with ethereal diazomethane at 0°, and the product acetylated by treatment with pyridine–acetic anhydride at 20° for 16 hours. The acetoxy-methyl esters (165 mg.) to obtained were dissolved in 0·5 c.c. of benzene and introduced on to a column of aluminium oxide (Merck–Brockmann, activity III—IV, 5 g.) prepared in pentane (25 c.c.). The column was eluted by the " durchlauf Methode " using eluates of 25 c.c. as shown in the table, the m. p.s recorded in which are subsequent to recrystallisation from methanol.

Fraction.	Eluant.	Eluate.
1—3	Pentane	Traces of oil
4	Benzene–pentane (1 : 10)	Much oil
5—7	,, ,, (1 : 5)	Some oil
8, 9	,, ,, (1 : 2·5)	,,
10	,, ,, (1 : 1)	Oil, inoculated with Fr. 13 but did not cryst.
11	,, ,, (1 : 1)	Crystallised spontaneously, m. p. 115—125°
12	,, ,, (1 : 1)	,, ,, m. p. 118—125
13	Benzene	,, ,, m. p. 130—133
14	,,	Cryst. by inoculation with Fr. 13, m. p. 131—134
15	,,	,, ,, ,, ,, m. p. 128—132
16, 17	,,	Traces of cryst. material, m. p. 120—126
18	Ether–benzene (1 : 1)	Little oil, failed to cryst. by inoculation
19	Ether	Trace of oil
20	,,	—

Fractions 11—17 were united (61 mg.) and recrystallised from methanol to give *methyl* 7-*keto*-3(β)-*acetoxy*-5-allo*cholanate* (XXV; R′ = Ac, R″ = Me) (46 mg.) as prisms, m. p. 130—133° (Found,* after drying at 60°/0·01 mm.: C, 72·80; H, 9·92. $C_{27}H_{42}O_5$ requires C, 72·61; H, 9·50%). Hydrolysis of the ester with hot 4% methanolic potassium hydroxide (≡3 mols.), removal of methanol after saturation with carbon dioxide, and acidification yielded 3(β)-*hydroxy*-7-*keto*-5-allo*cholanic acid*, prisms from ether–pentane, m. p. 193° (Found,* after drying for 6 days at 75—80°/0·01 mm.: C, 73·74; H, 9·85. $C_{24}H_{38}O_4$ requires C, 73·80; H, 9·81%).

Subsequently, when it was found that methyl 3(β)-acetoxycholestane-6‖7-dicarboxylate was non-crystalline, Fractions 4—10 were subjected individually to alkaline hydrolysis; attempts to crystallise the various precipitates of acids obtained by acidification of the hydrolysates were uniformly unsuccessful.

Method (ii) (with Dr. N. Gough).—7-Keto-3(β)-benzoyloxycholestane (XXIII; R = Bz) (m. p. 159—160°, 4 g.) was dissolved in dioxan, and, to the mechanically stirred solution at 20°, solutions of iodine (7·3 g.) in methanol (90 c.c.) and potassium hydroxide (10 g.) in water (15 c.c.) plus methanol (35 c.c.) were added dropwise and simultaneously during 1·5 hours, iodine being always present in excess. After 0·5 hour at room temperature, the volume was reduced to about ½ under diminished pressure, and the solution diluted and extracted thrice with ether. The ethereal extract was shaken twice with N-sodium carbonate, and the united alkaline extracts acidified and re-extracted with ether; this extract, after being washed with sodium thiosulphate solution and water, was dried and evaporated. The residue by crystallisation from acetone–pentane gave benzoic acid, m. p. 120—121° (700 mg. = 73% hydrolysis of the starting material); the mother liquor by evaporation gave an intractable gum, which contained further small quantities of benzoic acid, but from which no other crystalline material could be isolated.

Method (iii). 3(β)-*Hydroxy*-6 : 7-*diketocholestane* (XXVIII; R = H).—7-Keto-3(β)-acetoxycholestane (XXIII; R = Ac), m. p. 142—143°, was converted *via* 6″ α″-bromo-7-keto-3(β)-acetoxycholestane (as XXIX), m. p. 175°, into 6″ β″-bromo-7-keto-3(β)-acetoxycholestane (as XXIX), m. p. 143°, and the latter bromide (1 g.) oxidised with a boiling 10% solution of silver nitrate in pyridine (20 c.c.) for 2 hours to give 6 : 7-diketo-3(β)-acetoxycholestane (XXVIII; R = Ac), m. p. 156—157°, according to the procedure of Barr, Heilbron, Jones, and Spring (*loc. cit.*). The diketone so obtained was hydrolysed according to the following directions most kindly supplied by Dr. E. R. H. Jones: the acetoxy-diketone (400 mg.) was treated with 2% methanolic potassium hydroxide solution (30 c.c.) at 20° for 20 hours, and the resulting solution diluted with sufficient water, acidified with 2N-hydrochloric acid, and extracted with ether. The ethereal extract was washed with sodium hydrogen carbonate, then water, dried, evaporated, and the solid residue crystallised twice from methanol to give 3(β)-*hydroxy*-6 : 7-*diketo-cholestane* as fine needles, m. p. 152—153° (Dr. Jones records m. p. 151·5—152·5°), giving an olive-green colour with ethanolic ferric chloride, which appear to contain 1 mol. of methanol (Found, § after drying at 100° in a vacuum for 8 hours: C, 75·3; H, 10·85. $C_{27}H_{44}O_3$,MeOH requires C, 75·0; H, 10·8%).

3(β)-*Hydroxycholestane*-6‖7-*dicarboxylic acid* (XIII).—The hydroxy-diketone (XXVIII; R = H) (200 mg.) was dissolved in hot ethanol (25 c.c.), and 30% hydrogen peroxide (0·6 c.c.) added, followed by 2N-potassium hydroxide (1·25 c.c.) (cf. Butenandt and Schramm, *Ber.*, 1936, **68**, 2298). The mixture was warmed on the steam-bath for 10 minutes, and kept at 20° for 16 hours. The colourless solution, after removal of ethanol under reduced pressure at 20°, was diluted and extracted with ether; the aqueous layer, after removal of dissolved ether under reduced pressure, was acidified with 2N-hydrochloric acid, and the precipitate filtered off and dried in a vacuum desiccator. The dry precipitate was dissolved in a little anhydrous dioxan (freshly distilled over sodium) and repeatedly evaporated under reduced pressure with successive amounts of dry benzene. The residue was recrystallised from acetone–pentane to give 3(β)-hydroxycholestane-6‖7-dicarboxylic acid (40 mg.) as aggregates of felted needles, m. p. 241—242° (gas evolution), undepressed by a genuine specimen of m. p. 241—242°, prepared by the method of Windaus and Stein (*loc. cit.*) (Found,* after drying at 100° for 3 hours without loss of weight in a weighing bottle: C, 71·63; H, 10·41. Calc. for $C_{27}H_{46}O_5$: C, 71·96; H, 10·30%). For further identification, the hydroxy-acid was converted into the lactonic acid (XIV); the hydroxy-acid (20 mg.) was heated with acetic anhydride for 0·5 hour and worked up as previously, and the lactonic acid, which

again was not extracted from ethereal solution with 2N-sodium carbonate, crystallised from ether–pentane; two crystalline forms were obtained—needles and clusters of prisms—both melting at 213—215° and re-solidifying to a mass of needles on cooling to 210°. The mixed m. p. with a genuine specimen of m. p. 214—215° showed no depression (m. p. 213—215°), whilst titration with 0·01N-potassium hydroxide confirmed the monobasic nature of the substance [Found: *M* (monobasic), 434. Calc. for $C_{27}H_{44}O_4$: *M*, 432·6], which also reacted vigorously with an ethereal solution of diazomethane.

The microanalyses (*) were performed in the micro-analytical laboratory of the Eidg. Techn. Hochschule, Zürich, through the kindness of Prof. L. Ruzicka, For. Mem. R.S.; the microanalyses (§) were carried out in 1937—1938 by the late Mr. Boston in the micro-analytical laboratory of Prof. Sir Ian Heilbron at the University of Manchester; other micro-analyses are by Dr. Weiler and Dr. Strauss of Oxford. The photographs (Figs. 1—4) were taken by Mr. Blackledge and Mr. J. Hainsworth at the University of Leeds.

The author wishes to record his gratitude to Dr. E. R. H. Jones for the gift of specimens and for making available his original laboratory note-books, and gratefully to acknowledge the support of the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund, and the Anna Fuller Fund.

THE CHESTER BEATTY RESEARCH INSTITUTE,
THE ROYAL CANCER HOSPITAL (FREE), UNIVERSITY OF LONDON. [*Received, June 4th*, 1947.]