164. *Studies in the Sterol Group. Part XXXII. The Bromination of 6-Ketocholestanyl Acetate.*

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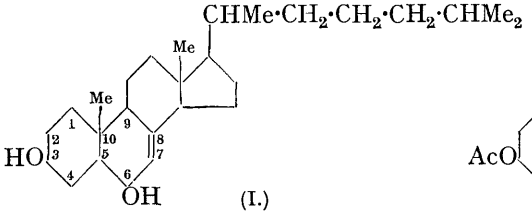
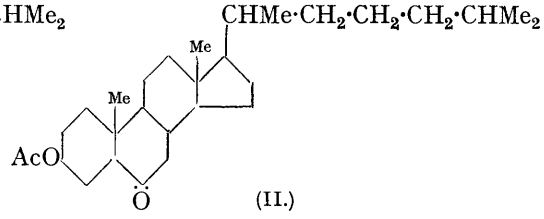
Of various alternative routes for the preparation of 7-dehydrocholesterol, one of the most promising appears to depend upon the accessibility of 3 : 6-dihydroxy-Δ^7-cholestene (I). With this general goal in view a study has been made of the bromination of 6-ketocholestanyl acetate (II).



(I.)



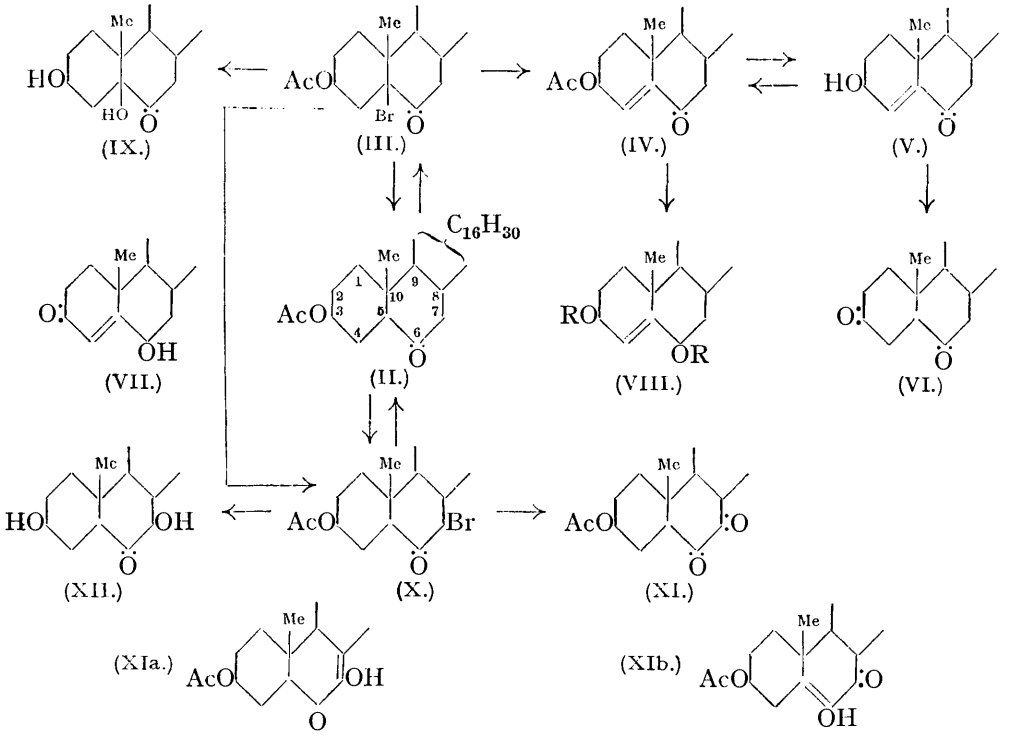
(II.)

Bromination of this saturated keto-acetate with one mole of bromine either in cold acetic acid, or in a mixture of acetic acid and ether at 0° or 35°, yields in each case a monobromide, m. p. 162° (decomp.). This has been characterised as *5-bromo-6-ketocholestanyl acetate* (III), since on treatment with pyridine it gives *6-keto-3-acetoxy-Δ^4-cholestene* (IV), which shows the typical light-absorption properties of an $\alpha\beta$-unsaturated ketone. Hydrolysis of the unsaturated keto-acetate (IV) with boiling methyl-alcoholic potash yields 3 : 6-diketocholestane (VI), identified both by a comparison with an authentic specimen and by the preparation of the dioxime. The formation of the saturated diketone is clearly dependent upon an isomerisation of the intermediate unsaturated alcohol (V), and offers a striking similarity to a reaction described recently by Butenandt and Schramm (*Ber.*, 1936, **69**, 2289), who obtained 3 : 6-diketocholestane instead of the anticipated 6-hydroxy-3-keto-Δ^4-cholestene (VII). On the other hand, Dane, Wang, and Schulte (*Z. physiol. Chem.*, 1936, **245**, 81) have isolated the ketol (VII) (as the semicarbazone) by hydrolysis of its acetate with cold 0·1*N*-methyl-alcoholic potash. We have similarly found that hydrolysis of 6-keto-3-acetoxy-Δ^4-cholestene (IV) with cold 0·2*N*-methyl-alcoholic potash gives *3-hydroxy-6-keto-Δ^4-cholestene* (V), m. p. 151°, characterised as an $\alpha\beta$-unsaturated ketone by its absorption spectrum; acetylation of (V) gives 6-keto-3-acetoxy-Δ^4-cholestene (IV). That the mechanism proposed for the formation of 3 : 6-diketocholestane from (IV) is correct is demonstrated by the fact that 3-hydroxy-6-keto-Δ^4-cholestene (V), *when heated* with alcoholic potash, is isomerised to 3 : 6-diketocholestane (VI).

An attempt was made further to characterise the unsaturated keto-acetate (IV) by converting it into the known 3 : 6-diacetoxy-Δ^4-cholestene (VIII; R = Ac) (Westphalen, *Ber.*, 1915, **48**, 1064). Reduction of 6-keto-3-acetoxy-Δ^4-cholestene (IV) with aluminium *iso*propoxide yielded an unsaturated diol (VIII; R = H), which gave a *diacetate*, m. p. 154—155°, and a *dibenzoate* (VIII; R = COPh), m. p. 198—199°. Since the former is not identical with the diacetate, m. p. 127°, described by Westphalen, the two must differ in the orientation of the groups associated with C_6.

Hydrolysis of 5-bromo-6-ketocholestanyl acetate (III) with alcoholic potash gives

3 : 5-*dihydroxy*-6-*ketocholestane* (IX), m. p. 136° (*monobenzoate*, m. p. 170°). The presence of a tertiary hydroxyl group in (IX) is shown by its inability to form a dibenzoate, thus affording confirmation of the structure (III) allocated to the monobromide, m. p. 162°.



If the bromination of 6-ketocholestanyl acetate (II) be effected in acetic acid and ether at 35°, as previously described, and the reaction mixture then be heated under reflux for two hours, an isomeric monobromide, m. p. 145°, is produced to the exclusion of the compound, m. p. 162°. The bromination of (II) is dependent upon the presence of hydrogen bromide, since we find that the substitution reaction is completely inhibited by excess of potassium acetate and, further, that in many experiments the reaction can only be initiated by the addition of hydrogen bromide. Similar observations were made by Inhoffen (*Ber.*, 1936, **69**, 2141) in a study of the bromination of 3-keto-Δ^4-cholestene.

A survey of the conditions responsible for the preferential formation of the monobromides, m. p. 162° and 145°, has shown that the former is invariably the initial product and that, in the presence of hydrogen bromide, it is isomerised to the bromide, m. p. 145°. Confirmation of this mechanism was obtained in the observation that the monobromide, m. p. 162°, is converted into the lower-melting isomer on heating with hydrogen bromide in acetic acid. From this behaviour it was confidently anticipated that the two bromides would prove to be stereoisomers. With this in mind, unavailing attempts were made to convert the isomer, m. p. 145°, into 6-keto-3-acetoxy-Δ^4-cholestene (IV) by refluxing it either with pyridine, or with potassium acetate in alcoholic solution. Whereas treatment with silver nitrate and pyridine at room temperature or at 100° (Dane, Wang, and Schulte, *loc. cit.*) is similarly without effect, prolonged heating under reflux with this reagent gives a product, $C_{29}H_{46}O_4$, m. p. 156—157°, which has been characterised as 6 : 7-*diketocholestanyl acetate* (XI). Thus it produces an immediate green-violet coloration with ferric chloride and reacts with *o*-phenylenediamine, forming a *quinoxaline* derivative, m. p. 187°. 6 : 7-Diketocholestanyl acetate exhibits a broad band in the ultra-violet region of the spectrum with maximum at 2745 A.; it exists as the mono-enol modification (XIa or XIb), since the Zerewitinoff method indicates the presence of one active hydrogen atom. This extremely

facile oxidation of an α-bromo-ketone to an α-diketone is, we believe, due primarily to a *trans*-orientation of the halogen atom at C_7 relative to the hydrogen attached to C_8 and to the inherent reluctance to lose hydrogen bromide. The formation of an α-diketone from the bromide, m. p. 145°, can only be explained if the latter is *7-bromo-6-ketocholestanyl acetate* (X). This formulation has been confirmed by hydrolysis of the bromide to *3 : 7-dihydroxy-6-ketocholestane* (XII), m. p. 179°, which yields a *dibenzoate*, m. p. 169—170°, in contradistinction to the monobenzoate obtained from 3 : 5-dihydroxy-6-ketocholestane.

Butenandt and Wolff (*Ber.*, 1935, **68**, 2091) have shown that the orientation assumed by the halogen atom during the bromination of 3-ketocholane derivatives is dependent upon the stereochemical configuration of rings A and B; thus 3-ketocholestane gives a 2-bromo-substitution product, whereas 3-ketocoprostane is brominated exclusively at C_4. In marked contrast is the behaviour of 6-ketocholestanyl acetate, which can be brominated at either C_5 or C_7 according to the external conditions.

The metamorphoses of the various intermediates described in this paper are being further investigated together with the bromination of 7-ketocholestanyl acetate.

Experimental.

5-Bromo-6-ketocholestanyl Acetate (III).—(a) 6-Ketocholestanyl acetate (4·4 g.) in acetic acid (10 c.c.) and ether (50 c.c.) was treated with a solution of bromine in acetic acid (32 c.c.; 5%), added during 1 hour with stirring, the mixture being maintained at 0° throughout. Decolorisation proceeded rapidly, crystalline material separating after the addition of approximately half of the bromine solution. The ether was removed under reduced pressure, and the crystals collected and recrystallised from light petroleum (b. p. 60—80°), from which *5-bromo-6-ketocholestanyl acetate* (3·7 g.) separated in plates, m. p. 162° (decomp.; the m. p. varies with the rate of heating), $[\alpha]_D^{22°}$ — 133° ($l = 1$, $c = 2·3$ in chloroform). It is sparingly soluble in acetic acid and in light petroleum, but readily soluble in ether and in chloroform (Found : C, 66·3; H, 8·7. $C_{29}H_{47}O_3Br$ requires C, 66·5; H, 9·0%).

(b) 6-Ketocholestanyl acetate (1·0 g.) in ether (10 c.c.) was treated with a solution of bromine in chloroform (7·5 c.c.; 5%). The reaction, initiated by the addition of a drop of dilute hydrobromic acid, was complete in 10 minutes. The solvent mixture was removed in a stream of air, two crystallisations of the residue from acetic acid giving the monobromide, m. p. 162°.

7-Bromo-6-ketocholestanyl Acetate (X).—(a) 6-Ketocholestanyl acetate (5·5 g.) in acetic acid (12·5 c.c.) and ether (65 c.c.) was treated with a solution of bromine in acetic acid (40 c.c.; 5%) at 35°, the addition extending over 75 minutes. The mixture was then heated under reflux for 2 hours, the ether removed, and the solution set aside overnight at 15°. The hot solution was treated with water (5 c.c.) and the solid obtained on cooling was crystallised from aqueous acetic acid, *7-bromo-6-ketocholestanyl acetate* separating in lustrous plates, m. p. 144—145°, $[\alpha]_D^{22°}$ + 41° ($l = 1$, $c = 1·7$ in chloroform). From light petroleum the monobromide separates in hard prisms; it is moderately readily soluble in acetic acid and ethyl alcohol, very soluble in ether, chloroform and light petroleum (b. p. 60—80°), and in general considerably more soluble in the common organic solvents than the isomeric 5-bromo-6-ketocholestanyl acetate. It is moreover more stable than the latter, no decomposition occurring on heating to 220° (Found : C, 66·7; H, 8·6. $C_{29}H_{47}O_3Br$ requires C, 66·5; H, 9·0%).

(b) 5-Bromo-6-ketocholestanyl acetate (1·0 g.) in acetic acid (15 c.c.) was heated on the steam-bath for 15 minutes with a solution of hydrogen bromide in acetic acid (5 c.c.; 50%). The mixture showed a play of colour, passing through green to dark brown; after cooling, and the addition of water, the solution was seeded with the monobromide, m. p. 145°. The solid was collected and crystallised from dilute acetic acid, the 7-bromo-6-ketocholestanyl acetate (0·5 g.) obtained showing no depression in admixture with an authentic specimen. A solution of 5-bromo-6-ketocholestanyl acetate, treated in the same way but without the addition of hydrogen bromide, gave only the unchanged bromide.

Reduction of 7-Bromo- and 5-Bromo-6-ketocholestanyl Acetates.—A solution of each monobromide (1·0 g.) in ether was added to aluminium amalgam prepared from aluminium foil (5 g.) as described by Vogel (J., 1927, 594). The reaction mixture was treated with water from time to time during 36 hours, and the ether removed from the filtered liquid. After two crystallisations from methyl alcohol the residue gave 6-ketocholestanyl acetate, m. p. 127—128° either alone or in admixture with an authentic specimen.

6-Keto-3-acetoxy-Δ^4-cholestene (IV).—5-Bromo-6-ketocholestanyl acetate (11·2 g.) in dry pyridine (90 c.c.) was heated under reflux for 8 hours. The mixture was diluted with ice-cold

water, and the solid collected and washed with water. After two crystallisations from methyl alcohol (charcoal), 6-*keto*-3-*acetoxy*-Δ^4-*cholestene* (6·5 g.) separated in long hexagonal tables, m. p. 110°, $[\alpha]_D^{22°}$ − 50·5° (l = 1, c = 1·1 in chloroform) (Found: C, 78·7; H, 10·6. $C_{29}H_{46}O_3$ requires C, 78·7; H, 10·5%). It gives a yellow coloration with tetranitromethane in chloroform solution; this is unusual for an αβ-unsaturated ketone. It is sparingly soluble in methyl alcohol but readily in pyridine. Light absorption in alcohol: Maxima, (a) 2360 A., log ε = 3·8; (b) 3200 A., log ε = 1·95.

3 : 6-*Diketocholestane*.—A solution of 6-keto-3-acetoxy-Δ^4-cholestene (1·0 g.) in methyl-alcoholic potassium hydroxide (30 c.c.; 4%) was heated under reflux for 1 hour. The cold solution was diluted with water, and the solid collected and washed with water. After crystallisation from ethyl acetate–alcohol (charcoal), followed by recrystallisation from acetone, 3 : 6-diketocholestane (0·5 g.) was obtained in needles, m. p. 169° either alone or in admixture with an authentic specimen (Found: C, 80·6; H, 10·9. Calc. for $C_{27}H_{44}O_2$: C, 80·9; H, 11·1%). The dioxime, m. p. 202°, showed no depression in admixture with an authentic specimen.

3-*Hydroxy*-6-*keto*-Δ^4-*cholestene* (V).—A solution of 6-keto-3-acetoxy-Δ^4-cholestene (IV) (1 g.) in methyl-alcoholic potassium hydroxide (100 c.c.; 1%) was set aside for 43 hours at room temperature. The mixture was largely diluted with water and acidified with dilute hydrochloric acid, and the product isolated by means of ether. After a single crystallisation from methyl alcohol (charcoal) 3-*hydroxy*-6-*keto*-Δ^4-*cholestene* was obtained in short hard needles, m. p. 150—151°, $[\alpha]_D^{20°}$ − 13° (l = 1, c = 2·8 in chloroform) (Found: C, 81·0; H, 11·1. $C_{27}H_{44}O_2$ requires C, 80·9; H, 11·1%). It is very soluble in methyl alcohol but insoluble in light petroleum. Light absorption in alcohol: Maxima, (a) 2390 A., log ε = 3·8; (b) 3190 A., log ε = 1·93.

Isomerisation of 3-*Hydroxy*-6-*keto*-Δ^4-*cholestene* (V) *to* 3 : 6-*Diketocholestane* (VI).—3-Hydroxy-6-keto-Δ^4-cholestene (100 mg.) in methyl-alcoholic potassium hydroxide (5 c.c.; 10%) was heated under reflux for 75 minutes. The solution was diluted with water, and the solid collected and washed with water. The crude product in methyl alcohol was decolorised with charcoal and, after removal of the solvent, taken up in acetone. Refrigeration effected the separation of a small quantity of a resinous solid. The decanted liquor was concentrated, and on cooling, 3 : 6-diketocholestane (20 mg.) separated which after one crystallisation from acetone showed no depression in m. p. in admixture with an authentic specimen.

3 : 6-*Diacetoxy*-Δ^4-*cholestene* (VIII, R = Ac).—Freshly distilled aluminium *iso*propoxide (5 g.) was added to a solution of 6-keto-3-acetoxy-Δ^4-cholestene in dry *iso*propyl alcohol (40 c.c.), and the mixture heated under reflux for 18 hours. The solvent was removed, the residue set aside for 50 minutes at 17° with a solution of methyl-alcoholic potassium hydroxide (50 c.c.; 6%), and the mixture largely diluted with water and extracted with ether. After drying (sodium sulphate), the solvent was removed, and the residual gum triturated with acetone to give a solid (1·1 g.), which separated from ethyl acetate in a micro-crystalline form, m. p. 178—179°. With the antimony trichloride reagent the diol gives an immediate pink coloration, changing to blue on standing. The diol (0·2 g.) was heated under reflux for 30 minutes with acetic anhydride (5 c.c.) and sodium acetate. Water was added to the hot solution, and the solid collected and washed with water. After two crystallisations from methyl alcohol 3 : 6-*diacetoxy*-Δ^4-*cholestene* separated in fine needles, which softened at 152°, m. p. 154—155°, clearing completely at 157°; $[\alpha]_D^{22°}$ + 24·8° (l = 1, c = 1·0 in chloroform) (Found: C, 76·6; H, 10·5. $C_{31}H_{50}O_4$ requires C, 76·5; H, 10·4%). With the antimony trichloride reagent it slowly gives a sapphire-blue coloration.

3 : 6-*Dibenzoyloxy*-Δ^4-*cholestene* (VIII, R = COPh).—The diol (0·4 g.), m. p. 178—179°, in pyridine (1·8 c.c.) was set aside at room temperature for 18 hours with benzoyl chloride (0·6 c.c.). The addition of water precipitated a gum, which was triturated with methyl alcohol. Two crystallisations of the crude solid from chloroform–acetone gave the *dibenzoate* in small rectangular plates, m. p. 198—199°, clearing completely at 208°; $[\alpha]_D^{22°}$ + 83·9° (l = 1, c = 1·8 in chloroform) (Found: C, 80·3; H, 8·7. $C_{41}H_{54}O_4$ requires C, 80·4; H, 8·9%). The dibenzoate is sparingly soluble in methyl alcohol and acetone, and very soluble in chloroform; with the antimony trichloride reagent it slowly gives a sapphire-blue coloration.

3 : 5-*Dihydroxy*-6-*ketocholestane* (IX).—5-Bromo-6-ketocholestanyl acetate (1·0 g.) was heated under reflux with methyl-alcoholic potash (30 c.c.; 10%) for 2 hours. After dilution with water the resinous product was isolated by means of ether and taken up in hot methyl alcohol, and the solution cooled. The clear solution was decanted from the separated gum, diluted with water, and set aside at 0°; a yellow solid (0·6 g.) separated which after two crystallisations from methyl alcohol (charcoal) gave 3 : 5-dihydroxy-6-ketocholestane in rectangular tables, softening at 128°, m. p. 138°, $[\alpha]_D^{22°}$ + 29·3° (l = 1, c = 2·2 in chloroform). It is spar-

ingly soluble in methyl alcohol but very soluble in chloroform and pyridine. The diol separates with solvent of crystallisation which cannot be completely removed even after prolonged desiccation. The *monobenzoate*, prepared by the action of benzoyl chloride (0·25 c.c.) on the diol (150 mg.) in excess of pyridine at room temperature for 15 hours, separates from methyl alcohol–acetone either in long fine needles or in soft plates, the two forms being interconvertible; m. p. 170°, $[\alpha]_D^{20°}$ + 23·0° (l = 1, c = 0·8 in chloroform). It is very sparingly soluble in methyl alcohol but readily soluble in acetone (Found: C, 78·3; H, 9·7. $C_{34}H_{50}O_4$ requires C, 78·1; H, 9·7%).

6 : 7-*Diketocholestanyl Acetate* (XI).—A solution of 7-bromo-6-ketocholestanyl acetate (1·0 g.) and silver nitrate (2 g.) in pyridine (20 c.c.) was heated under reflux for 5 hours. Ether was added to the cold solution, and the mixture washed with dilute sulphuric acid and water. After drying and evaporation a red-brown gum was obtained, which was taken up in hot alcohol (charcoal). The solid (160 mg.) separating on cooling was recrystallised from methyl alcohol to give **6 : 7-*diketocholestanyl acetate*** in fine hard needles, m. p. 156—157°, $[\alpha]_D^{20°}$ − 108° (l = 1, c = 1·0 in chloroform) (Found: C, 76·0; H, 9·8. $C_{29}H_{46}O_4$ requires C, 75·9; H, 10·1%). It is freely soluble in dilute aqueous-alcoholic sodium hydroxide to give a yellow solution, from which it is precipitated by acidification. With alcoholic ferric chloride, an immediate green-violet coloration is produced. Active hydrogen determination (Zerewitinoff): The diketone (8·12 mg.) evolved 0·48 c.c. of methane at 18° and 757 mm., corresponding to 1·1 atoms of active hydrogen per mole. Light absorption in alcohol: Maximum, 2745 A., log ε = 4·03.

Quinoxaline Derivative of **6 : 7-*Diketocholestanyl Acetate*.**—The diketone (100 mg.) was fused with *o*-phenylenediamine (100 mg.) for 40 minutes at 140—150°. The dark brown mass solidified on cooling; it was crystallised from ethyl acetate, and the product (35 mg.) recrystallised from acetone, from which the *quinoxaline* derivative separated in colourless hair-like needles, m. p. 186—187° (Found: C, 79·4; H, 9·7. $C_{35}H_{50}O_2N_2$ requires C, 79·2; H, 9·4%).

3 : 7-*Dihydroxy*-6-*ketocholestane* (XII).—A solution of 7-bromo-6-ketocholestanyl acetate (2·3 g.) in methyl-alcoholic potassium hydroxide (70 c.c.; 10%) was heated under reflux for 2 hours. After dilution with water the resinous product was isolated by means of ether and dissolved in methyl alcohol. On cooling and decantation from resin, a mass of yellow needles (1·1 g.) separated, which after two crystallisations from methyl alcohol (charcoal) formed lustrous plates, m. p. 179°, $[\alpha]_D^{22°}$ + 31·4° (l = 1, c = 1·0 in chloroform). **3 : 7-*Dihydroxy*-6-*ketocholestane*** is sparingly soluble in methyl alcohol, soluble in acetone, and very soluble in chloroform and pyridine (For analysis the specimen was dried in a vacuum at 100° for 8 hours. Found: C, 77·2; H, 11·0. $C_{27}H_{46}O_3$ requires C, 77·4; H, 11·1%). The keto-diol sublimes unchanged in needles at 220°/10^{-3} mm., whereas the isomeric **3 : 5**-dihydroxy-6-ketocholestane suffers profound decomposition under similar conditions. The *dibenzoate*, prepared by treatment of the keto-diol (120 mg.) in excess of pyridine with benzoyl chloride (0·17 c.c.) for 20 hours at room temperature, separates from methyl alcohol–acetone in long hard needles, m. p. 169—170°, $[\alpha]_D^{20°}$ + 62·0° (l = 1, c = 1·0 in chloroform). It is sparingly soluble in methyl alcohol but very soluble in acetone (Found: C, 78·7; H, 8·7. $C_{41}H_{54}O_5$ requires C, 78·5; H, 8·7%).

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