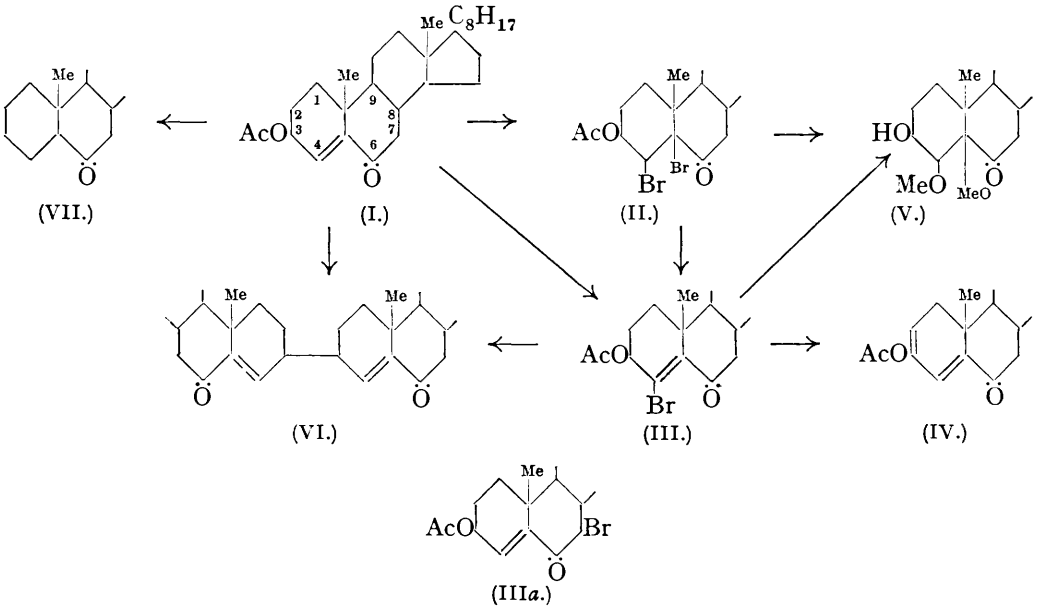
264. *Studies in the Sterol Group. Part XXXVIII. The Bromination of 6-Keto-3-acetoxy-Δ⁴-cholestene.*

By H. Jackson and E. R. H. Jones.

4 : 5-Dibromo-6-ketocholestanyl acetate and 4-bromo-6-keto-3-acetoxy-Δ⁴-cholestene have been prepared and characterised. Catalytic reduction of 6-keto-3-acetoxy-Δ⁴-cholestene yields 6-ketocholestane.

Bromination of 6-keto-3-acetoxy-Δ⁴-cholestene (I) (Heilbron, Jones, and Spring, J., 1937, 801) with two moles of bromine yields the unstable 4 : 5-*dibromo*-6-*ketocholestanyl acetate* (II), m. p. 81—82° (decomp.), the constitution of which is readily established by its quantitative conversion with potassium iodide in acetone (Schoenheimer, *J. Biol. Chem.*, 1935, **110**, 461) into the original unsaturated ketone (I). The ultra-violet absorption spectrum of the dibromide is similar to that of 5-bromo-6-ketocholestanyl acetate (Barr, Heilbron, Jones, and Spring, this vol., p. 335), the ketone band being displaced to longer wave-lengths by the α-halogen atom.

Treatment of (I) with one mole of bromine proceeds with evolution of hydrogen bromide and gives 4-*bromo*-6-*keto*-3-*acetoxy*-Δ⁴-*cholestene* (III), m. p. 115—116°, which exhibits the typical light-absorption properties of an αβ-unsaturated ketone. It can also be obtained by heating an ethereal solution of the dibromide (II) for two minutes or by heating it under reflux with potassium acetate in acetic acid. By treatment with pyridine, the monobromide (III) is converted almost quantitatively into 6-keto-3-acetoxy-Δ$^{2:4}$-cholestadiene (IV), previously obtained from 5 : 7- and 5′ : 7-dibromo-6-ketocholestanyl acetates (Heilbron, Jackson, Jones, and Spring, this vol., p. 102). Under the same conditions the dibromide (II) yields neither the monobromide (III) nor the doubly unsaturated ketone (IV), 6-keto-3-acetoxy-Δ⁴-cholestene (I) being the only product isolated (cf. Butenandt, Schramm, and Kudszus, *Annalen*, 1937, **531**, 192).



With sodium methoxide in methyl alcohol, the dibromide (II) gives a crystalline product $C_{29}H_{50}O_4$, m. p. 149—150°, which contains two methoxy-groups, forms a *monobenzoate*, m. p. 129—130°, is stable to cold methyl-alcoholic potassium hydroxide, and exhibits no intense absorption in the ultra-violet region of the spectrum above 2200 A. This evidence suggests the constitution 3-*hydroxy*-6-*keto*-4 : 5-*dimethoxycholestane* (V) for

the compound, the mechanism of its formation from the dibromide being immediately apparent. Hydrolysis of the monobromide (III) with potassium hydroxide in alcohol yields non-crystallisable gums giving intense colorations with alcoholic ferric chloride, but the dimethyl ether (V) is obtained from (III) by the action of sodium methoxide in methyl alcohol. The formation of (V) in this case, although it can be represented as an addition of methyl alcohol to the ethylenic linkage of an intermediate monomethyl ether, is not readily explicable, but it further emphasises the close relationship between the bromides (II) and (III). It was at first considered that the monobromide (III) might be represented by the structure (III*a*), but its reactions precluded this possibility.

An attempt to regenerate the unsaturated ketone (I) from the monobromide (III) by reduction with zinc dust in acetic acid or alcoholic solution gave 3 : 3′-*bis*-(6-*keto*-Δ^4-*cholestenyl*) (VI), m. p. 257—258°, unaffected by alkaline hydrolysis, and exhibiting the typical ultra-violet absorption of an αβ-unsaturated ketone. The isolation of the same product under identical experimental conditions from 6-keto-3-acetoxy-Δ^4-cholestene (I) indicates its mode of formation and confirms its constitution.

In an attempt to prepare 6-ketocoprostanyl acetate, a substance of considerable interest for stereochemical studies, the catalytic reduction of 6-keto-3-acetoxy-Δ^4-cholestene has been studied. Unfortunately, reduction of the ethylenic linkage proceeds with loss of acetic acid, 6-ketocholestane (VII) (Windaus, *Ber.*, 1920, **53**, 489) being formed in the presence of hydrogen and palladium rather than the saturated diketone corresponding to (VI).

Experimental.

4 : 5-*Dibromo*-6-*ketocholestanyl Acetate* (II).—Solutions of 6-keto-3-acetoxy-Δ^4-cholestene (1·0 g.) in acetic acid (10 c.c.) and bromine in acetic acid (14·8 c.c.; 5%; 2 mols.) were rapidly mixed at 18°. 4 : 5-Dibromo-6-ketocholestanyl acetate (1·0 g.) separated immediately, and was recrystallised by dissolving it in the minimum of cold ether, diluting with an equal volume of acetic acid, and adding water to cloudiness; the *dibromide* separated in plates, m. p. 81—82° (decomp.). It is stable in air but readily decomposes in warm solution (Found: C, 57·7; H, 7·4. $C_{29}H_{46}O_3Br_2$ requires C, 57·5; H, 7·7%). *Light absorption in alcohol*: Inflexion, 3100 A., log ε = 2·0.

Conversion into 6-*keto*-3-*acetoxy*-Δ^4-*cholestene* (I). (*a*) The dibromo-acetate (0·5 g.) was added to a solution of potassium iodide (1 g.) in boiling acetone (15 c.c.), and the mixture boiled for 1 minute, whereupon potassium bromide separated. The solution was rendered cloudy with water, and on cooling, 6-keto-3-acetoxy-Δ^4-cholestene crystallised out in quantitative yield, m. p. 110°, unaltered on admixture with an authentic specimen.

(*b*) A solution of the dibromo-acetate (0·5 g.) in anhydrous pyridine (7 c.c.) was heated under reflux for 2½ hours. Precipitation with water yielded an oil which solidified, and after treatment with charcoal, several crystallisations from methyl alcohol furnished 6-keto-3-acetoxy-Δ^4-cholestene, m. p. 108°, not depressed on admixture with an authentic specimen.

4-*Bromo*-6-*keto*-3-*acetoxy*-Δ^4-*cholestene* (III).—(*a*) To 6-keto-3-acetoxy-Δ^4-cholestene (4·4 g.) in ether (25 c.c.) at 18° a solution of bromine in acetic acid (40 c.c.; 4%; 1·0 mol.) was added dropwise, with stirring, during 10 minutes; decolorisation proceeded rapidly with evolution of hydrogen bromide. The gum, isolated with ether, crystallised on boiling with methyl alcohol–acetone, and the solid (5·0 g.) m. p. 105°, on recrystallisation from methyl alcohol yielded 4-*bromo*-6-*keto*-3-*acetoxy*-Δ^4-*cholestene* in large plates, m. p. 115—116° (Found: C, 66·6; H, 8·4. $C_{29}H_{45}O_3Br$ requires C, 66·7; H, 8·4%). *Light absorption in alcohol*: Maxima, (*a*) 2450 A., log ε = 3·9; (*b*) 3350 A., log ε = 2·3. The monobromide did not react with potassium acetate in acetic acid during one hour at 100°, with potassium acetate in boiling alcohol during 12 hours, or with silver nitrate in pyridine at 20° during 48 hours.

(*b*) A solution of 4 : 5-dibromo-6-ketocholestanyl acetate (400 mg.) and fused potassium acetate (1·0 g.) in acetic acid (15 c.c.) was heated under reflux for 50 mins. The semi-solid residue precipitated by addition of water, on crystallisation from aqueous alcohol, gave 4-bromo-6-keto-3-acetoxy-Δ^4-cholestene, m. p. 115—116° (100 mg.), identical with the specimen prepared by method (*a*).

(*c*) On boiling a solution of the dibromo-acetate (1·0 g.) in dry ether (25 c.c.) a vigorous evolution of hydrogen bromide occurred. Addition of acetic acid and precipitation with water gave an oil, which crystallised from alcohol–acetone, giving 4-bromo-6-keto-3-acetoxy-Δ^4-cholestene (300 mg.), m. p. 114—115°, not depressed by admixture with an authentic specimen.

6-Keto-3-acetoxy-$\Delta^{2:4}$*-cholestadiene* (IV).—4-Bromo-6-keto-3-acetoxy-Δ^4-cholestene (100 mg.) in anhydrous pyridine (5 c.c.) was heated under reflux for 7 hours. Crystallisation was effected by careful addition of water, and after two recrystallisations from methyl alcohol, 6-keto-3-acetoxy-$\Delta^{2:4}$-cholestadiene (30 mg.) separated in pale yellow needles, m. p. 139—140°, identified by mixed m. p. with authentic specimen.

3-Hydroxy-6-keto-4 : 5-dimethoxycholestane (V).—(*a*) A solution of sodium methoxide in methyl alcohol (12·5 c.c.; 10%) was added to one of 4-bromo-6-keto-3-acetoxy-Δ^4-cholestene (1·1 g.) in methyl alcohol (75 c.c.) and the mixture set aside at 20° for 18 hours. Addition of dilute acetic acid (60 c.c.; 20%) and scratching produced a flocculent solid which, after being washed with water was crystallised from aqueous methyl alcohol; *3-hydroxy-6-keto-4 : 5-dimethoxycholestane* (225 mg.) separated as a felt of fine needles, m. p. 149—150°. It sublimes without change at 155°/10^{-3} mm., but the m. p. gradually falls on keeping (Found: C, 75·6; H, 10·5; OMe, 12·3. $C_{29}H_{50}O_4$ requires C, 75·3; H, 10·9; OMe, 13·6%). The *monobenzoate*, prepared with benzoyl chloride in pyridine at room temperature, separated from methyl alcohol in octagonal tablets, softening at 126°, m. p. 129—130° (Found: C, 76·7; H, 9·8. $C_{36}H_{54}O_5$ requires C, 76·3; H, 9·6%).

(*b*) A solution of 4 : 5-dibromo-6-ketocholestanyl acetate (500 mg.) in methyl alcohol (25 c.c.) was treated with a solution of sodium methoxide in methyl alcohol (10 c.c.; 5%) and set aside for 20 hours at 20°. The yellow solution was acidified with dilute acetic acid (10%), and a flocculent precipitate separated. Crystallisation from aqueous methyl alcohol gave 3-hydroxy-6-keto-4 : 5-dimethoxycholestane, m. p. 146—147°, not depressed by admixture with a specimen prepared by method (*a*).

3 : 3′-Bis-(6-keto-Δ^4*-cholestenyl)* (VI).—(*a*) 4-Bromo-6-keto-3-acetoxy-Δ^4-cholestene (900 mg.) in acetic acid (25 c.c.) was heated at 100° for 15 minutes with zinc dust (4 g.). The cooled solution was filtered, diluted with water, and the precipitated oil isolated with ether. The solution of the oil in methyl alcohol gradually deposited crystals (150 mg.) which, after two recrystallisations from methyl alcohol–chloroform, yielded the *compound* (VI) in lustrous plates, m. p. 257—258° [Found: C, 84·6; H, 11·1; *M*, 730. $C_{54}H_{86}O_2$ requires C, 84·5; H, 11·3%; *M* (Rast), 766]. *Light absorption in alcohol*: Maxima, (*a*) 2440 A., log ε = 3·6; (*b*) 3140 A., log ε = 1·7.

(*b*) A solution of 4-bromo-6-keto-3-acetoxy-Δ^4-cholestene (200 mg.) in absolute alcohol was added to zinc dust (2 g., treated with ammonium chloride and dried), and the mixture heated under reflux for 1½ hours. The hot solution was filtered, and the compound which separated on cooling (20 mg.), m. p. 257—258°, was identical (mixed m. p.) with that prepared by method (*a*).

(*c*) A hot solution of 6-keto-3-acetoxy-Δ^4-cholestene (0·9 g.) in acetic acid (25 c.c.) was treated with zinc dust (4 g.) at 100° for 40 mins. The oil isolated by ether gave 200 mg. of the same compound as above; m. p. and mixed m. p. 256—258°.

6-Ketocholestane (VII).—A solution of 6-keto-3-acetoxy-Δ^4-cholestene (1·5 g.) in ether (100 c.c.) was shaken with palladium-black (200 mg.) and hydrogen for one hour, 1 mol. of the gas being absorbed. The catalyst was removed, and evaporation of the solvent yielded a gum, smelling strongly of acetic acid, which crystallised in contact with methyl alcohol. Several recrystallisations from the latter solvent gave 6-ketocholestane in clusters of fine needles, m. p. 97—98°, not depressed in admixture with an authentic specimen (Found: C, 83·8; H, 11·9. Calc. for $C_{27}H_{46}O$: C, 83·8; H, 12·0%).

We are indebted to Professor I. M. Heilbron, D.S.O., F.R.S., and Dr. F. S. Spring for their interest and encouragement, and we thank the Rockefeller Foundation for a grant.

THE UNIVERSITY, MANCHESTER. [*Received, August 3rd,* 1938.]