452. *Steroids and Related Compounds. Part IX. Further Experiments on the Bromination of Oxidation Products of Cholestane-3β : 5α : 6β-triol.*

By B. Ellis and V. Petrow.

The processes of dehydrobromination and dehydration have been applied to 2-bromo-6-acetoxycholestan-5α-ol-3-one (III; R = Ac) and 2 : 2-dibromo-6-acetoxycholestan-5α-ol-3-one (V), but intermediates suitable for effecting the aromatisation of ring A have not been obtained.

With the object of effecting aromatisation of ring A of cholesterol, work was commenced in 1937 on the preparation of a 6-oxygenated cholesta-1 : 4-dien-3-one (I) from 6β-acetoxycholestan-5α-ol-3-one by the processes of bromination, dehydration, and dehydrobromination. The research had to be abandoned in 1938, the results obtained being reported in Part IV of this series (Ellis and Petrow, *J.*, 1939, 1078). The investigation has now been resumed, and we report some additional experiments.

Attempts to prepare (I) from (III; R = Ac) (Ellis and Petrow, *loc. cit.*) by removal of the elements of water and hydrogen bromide proved uniformly unsuccessful; *e.g.*, the compound was recovered unchanged after prolonged heating with acetic anhydride, after heating under reflux with 1·1 mols. of thionyl chloride in pyridine, and after treatment with dry hydrogen chloride in chloroform solution. Hot anhydrous formic acid led only to resinification. Dehydrobromination was next examined but, although reagents such as boiling collidine gave bromine-free products, these could not be induced to crystallise.

Experiments were therefore initiated on the preparation of (XVI) which possesses the structural features of cholestan-5α-ol-3 : 6-dione, a diketo-alcohol which is known to undergo ready dehydration (Pickard and Yates, *J.*, 1908, **93**, 1681). Oxidation of (III; R = H) offered the best potential route to this compound, but hydrolysis of (III; R = Ac) to the required alcohol proved unsuccessful. Reaction with alkali has already been shown to give the 2 : 5-oxide (Part IV, *loc. cit.*). Reaction with alcoholic hydrochloric acid has now been found to give cholest-4-en-3 : 6-dione (IV), formed, no doubt, through the intermediate formation of 2-bromocholestane-3 : 6-dione ⟶ 4-bromocholestane-3 : 6-dione ⟶ (IV) [cf. 6β-acetoxycholestan-5α-ol-3-one ⟶ cholestane-3 : 6-dione, Ellis and Petrow (*loc. cit.*); also (X) ⟶ (IV) (Experimental section)]. Attempts to prepare the required compound (XVI) by direct bromination of cholestan-5α-ol-3 : 6-dione (XIV) proved more encouraging. Treating (XIV) in chloroform–acetic acid solution with one molar equivalent of bromine gave a mixture of products from which unchanged (XIV), 4 : 7 : 7-tribromocholest-4-en-3 : 6-dione (XV) (Butenandt *et al.*, *Annalen*, 1937, **531**, 176), and a new monobromide, $C_{27}H_{43}O_3Br$, were isolated. The last compound differed from the 7-bromocholestan-5α-ol-3 : 6-dione of Sarett, Chakravorty, and Wallis (*J. Org. Chem.*, 1943, **8**, 405) and is regarded as 2-bromocholestan-5α-ol-3 : 6-dione (XVI). Its preparation gave erratic results and was only occasionally successful. This fact, coupled with the unsatisfactory yields obtained, precluded its further examination.

Accordingly, we turned our attention to the reactions of 2 : 2-dibromo-6β-acetoxycholestan-5α-ol-3-one, hoping thereby to obtain an intermediate suitable for conversion into (I).

Dibromination of (II) or monobromination of (III; R = Ac) is known to yield a dibromo-compound regarded as 2 : 2-dibromo-6β-acetoxycholestan-5α-ol-3-one (V) (Ellis and Petrow, *loc. cit.*). In striking contrast to its monobromo-analogue (III; R = Ac) (see above), (V) has now been found to undergo ready dehydration on simply heating it with acetic anhydride or acetic acid, to give an unsaturated compound formulated as 2 : 2-dibromo-6β-acetoxycholest-4-en-3-one (VI) on the basis of (*a*) its ultra-violet absorption spectrum, which shows a band at 250 mμ. compatible with its formulation as a dibromo-3-ketocholest-4-ene derivative, and (*b*) its conversion into a quinoxaline derivative.

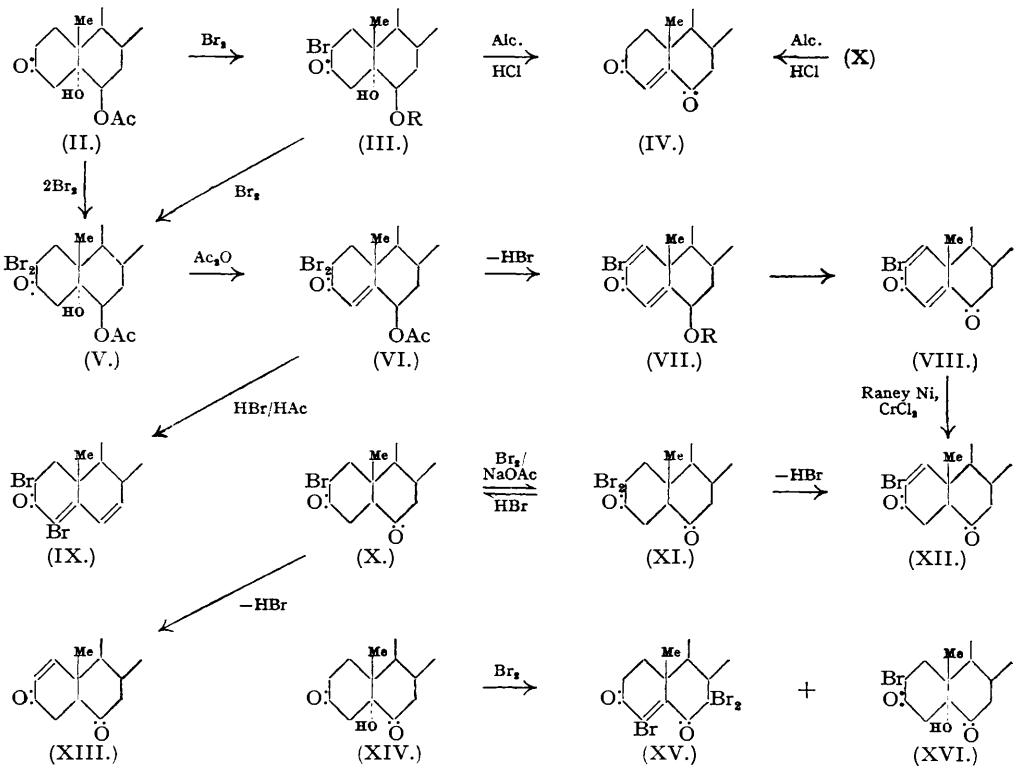
Although the dibromide (V) was sparingly soluble in acetic acid and separated from solution during preparation, it slowly redissolved when the reaction mixture was kept at room temperature for several hours. The solution now contained a new dibromo-ketone, $C_{27}H_{40}OBr_2$, also obtained by treating 2 : 2-dibromo-6β-acetoxycholest-4-en-3-one (VI) with hydrobromic acid–acetic acid and evidently formed from it by loss of the elements of acetic acid.

The new dibromo-ketone failed to liberate iodine from sodium iodide in benzene–alcoholic solution on warming, or to form a quinoxaline derivative with *o*-phenylenediamine. These observations exclude a 2 : 2-dibromo-formulation. Its ultra-violet absorption spectrum revealed a maximum at 292·5 mμ. and a minimum at 235·0 mμ., and this indicated a cholesta-4 : 6-dien-3-one containing substituent bromine atoms (see Jackson and Jones, *J.*, 1940, 569). The constitution of a 2 : 4-dibromocholesta-4 : 6-dien-3-one (IX) has therfore been assigned to this compound. Similar transformations of 2 : 2-dibromo-3-keto-steroids of the 5-*allo*-series into the corresponding 2 : 4-dibromoketones under the influence of hydrobromic acid–acetic acid have previously been reported (see Djerassi and Scholz, *J. Amer. Chem. Soc.*, 1947, **69**, 2404), and the elimination of the acetoxy-grouping from (VI) is paralleled by the conversion of 3β-acetoxycholest-5-en-7-one into cholesta-3 : 5-dien-7-one (Jackson and Jones, *loc. cit.*). 6β-Acetoxycholest-4-en-3-one (Part IV, *loc. cit.*), in contrast, gives only the saturated cholestane-3 : 6-dione on treatment with hydrobromic acid.

Monodehydrobromination of (VI) was readily accomplished with boiling collidine, a 2-bromo-6β-acetoxycholesta-1 : 4-dien-3-one (VII; R = Ac) being obtained in excellent yield. Attempts to convert this compound into (I), however, proved unsuccessful. Thus it was unaffected by chromous chloride in acetone (see Julian *et al.*, *J. Amer. Chem. Soc.*, 1945, **67**, 1728) or by zinc (Inhoffen and Zuchlsdorf, *Ber.*, 1943, **76**, 233) or Raney nickel in boiling alcohol.

Hydrolysis of (VII; R = Ac) with methanolic potash, followed by oxidation, gave 2-bromocholesta-1 : 4-dien-3 : 6-dione (VIII), but this dione, too, was recovered unchanged after

prolonged treatment with zinc dust in alcohol. Raney nickel or chromous chloride, however, led to partial reduction with formation of a bromocholesten-3 : 6-dione, m. p. 221°, identical



with a compound (m. p. 204—207°) obtained by Sarett and co-workers (*loc. cit.*) by the action of boiling pyridine on 2 : 2-dibromocholestan-3 : 6-dione (XI) and assigned by them the constitution of a 2-bromocholest-4-en-3 : 6-dione. We are unable to accept this formulation on the following evidence : (i) the ultra-violet absorption spectrum reveals a maximum at 257 mμ. characteristic of 2-bromocholest-1-en-3-ones which are now known to form an exception to Woodward's rule (*J. Amer. Chem. Soc.*, 1941, **63**, 1123; 1942, **64**, 76) (see Djerassi and Scholz, *loc. cit.*); (ii) the compound is recovered unchanged after prolonged heating with collidine, a behaviour hardly consistent with a 2-bromo-Δ^4-structure. Accordingly, we assign the constitution of a 2-bromocholest-1-ene-3 : 6-dione (XII) to this product. Cholest-4-ene-3 : 6-dione (IV), it may be added, likewise readily undergoes reduction to cholestane-3 : 6-dione on treatment with the chromous chloride reagent, a reaction which furnishes a convenient route to this saturated diketone.

Attempts to reduce (XII) with zinc in alcohol proved unsuccessful, a somewhat surprising result, since Djerassi and Scholz (*loc. cit.*) have achieved smooth conversion of the analogous 2-bromo-3-ketoandrost-1-en-17α-yl 17-hexahydrobenzoate into the corresponding androst-1-en-3-one derivative under similar experimental conditions. These authors describe the conversion of the same hexahydrobenzoate into the 4-bromo-Δ^1-ketone by treatment with hydrobromic acid–acetic acid. Similar isomerisation of (XII) would clearly furnish a key intermediate for the preparation of (I), but the compound failed to react in the required way and was recovered unchanged.

Lastly, the action of hydrobromic acid–acetic acid on (XI) was examined when it was hoped to effect conversion into the corresponding 2 : 4-dibromo-derivative. Unfortunately, only reduction occurred to give a 2-bromocholestane-3 : 6-dione (X). The position of the bromine atom in this compound followed from the observation that dehydrobromination gave a new αβ-unsaturated ketone, $\lambda_{max.} = 228$ mμ., not identical with (IV), which we believe to be cholest-1-ene-3 : 6-dione (XIII).

Experimental.

(M. p.s are uncorrected. Microanalyses are by Drs. Weiler and Strauss, Oxford. Rotations were measured in chloroform solution in a 2-dm. tube unless otherwise stated. Absorption spectra, measured in *iso*propyl-alcoholic solution, were kindly determined by Dr. R. E. Stuckey and Mr. P. Stross, B.Sc., Analytical Department, The British Drug Houses Ltd.)

Acid Hydrolysis of 2-Bromo-6β-acetoxycholestan-5α-ol-3-one.—Finely powdered (III; R = Ac) (2 g.) in boiling ethanol was treated with concentrated hydrochloric acid (2·0 ml.), added under reflux. After 5 hours the product, cholest-4-ene-3 : 6-dione (IV), was precipitated with water; it formed faintly yellow plates (from aqueous ethanol), m. p. 122—123° (Found : C, 81·2; H, 10·3. Calc. for $C_{27}H_{42}O_2$: C, 81·3; H, 10·6%), not depressed in admixture with an authentic specimen.

Bromination of Cholestan-5α-ol-3 : 6-dione (XIV).—(XIV) (2·08 g.) in chloroform (100 ml.) and acetic acid (10 ml.) at 28° was treated with bromine (880 mg., 1·1 mols.) in acetic acid (10 ml.), absorption being complete within 1 minute. After immediate addition of excess of sodium acetate solution, the non-aqueous layer was removed, washed, and dried, and the solvent distilled off. The residue, in acetone (30 ml.), was cooled strongly and fraction A, m. p. 180° (decomp.) (400 mg.), separated and was removed. Cautious dilution of the filtrate gave fraction B, m. p. 200° (decomp.) (400 mg.), followed by fraction C (600 mg.) (a gum which slowly crystallised). Purification of fraction A from acetic acid gave unchanged starting material. Crystallisation of fraction B from aqueous acetone and alcohol gave 2-*bromocholestan-5α-ol-3 : 6-dione* in leafy plates, m. p. 238° (decomp.), $[\alpha]_D^{21}$ −22·6° (*c*, 1·89; *l*, 1) (Found : C, 65·4; H, 8·6; Br, 15·5. $C_{27}H_{43}O_3Br$ requires C, 65·5; H, 8·7; Br, 16·1%). Fraction C, after purification from aqueous acetone, gave thin needles of 4 : 7 : 7-tribromocholest-4-ene-3 : 6-dione, m. p. 195° (decomp.), $[\alpha]_D^{22}$ +15·2° (*c*, 1·41) (Found : C, 51·1; H, 6·4. Calc. for $C_{27}H_{39}O_2Br_3$: C, 51·0; H, 6·2%), not depressed in admixture with an authentic specimen (Butenandt *et al.*, *Annalen*, 1937, **531**, 176).

Bromination in the presence of excess of sodium acetate gave a complex mixture of products from which only unchanged material could be isolated.

2 : 2-*Dibromo-6β-acetoxycholest-4-en-3-one* (VI).—When 2 : 2-dibromo-6-acetoxycholestan-5α-ol-3-one (V) was heated under reflux with acetic anhydride (8·5 vols.) for 2½ hours, or acetic acid for 1 hour, 2 : 2-*dibromo-6β-acetoxycholest-4-en-3-one* was obtained, hard needles (from aqueous acetone), m. p. 154—155°, $[\alpha]_D^{21}$ −10·8° (*c*, 1·5) (Found : C, 58·5; H, 7·1. $C_{29}H_{44}O_3Br_2$ requires C, 58·0; H, 7·3%); yield 50%. Light absorption : $E_{1\,cm.}^{1\%}$ (250 mμ.) = 157. The *quinoxaline* derivative, prepared by heating the dibromo-ketone (1·0 g.) and *o*-phenylenediamine (1 g.) in absolute ethanol (25 ml.) under reflux for 6 hours, formed thin plates (from aqueous acetone), m. p. 204° (Found : N, 5·6. $C_{35}H_{48}O_2N_2$ requires N, 5·3%).

2 : 4-*Dibromocholesta-4 : 6-dien-3-one* (IX).—(*a*) 6-Acetoxycholestan-5α-ol-3-one (2·3 g.) in acetic acid (50 ml.) was treated with bromine (1·6 g.) in acetic acid (9 ml.). Rapid decolorisation occurred, followed by separation of (V), which slowly dissolved, dissolution being complete in 18 hours. Precipitation with water and extraction with ether gave 2 : 4-*dibromocholesta-4 : 6-dien-3-one*, flat needles (from methanol–acetone), m. p. 178° (decomp.), $[\alpha]_D^{20}$ +37·8° (*c*, 2·38; *l*, 1) (Found : C, 60·2, 59·9; H, 7·0, 7·3. $C_{27}H_{40}OBr_2$ requires C, 60·0; H, 7·5%). The absorption spectrum showed a maximum at 292·5 mμ. ($E_{1\,cm.}^{1\%}$ = 270) and a minimum at 235 mμ. ($E_{1\,cm.}^{1\%}$ = 134). (*b*) 2 : 2-Dibromo-6-acetoxycholestan-5α-ol-3-one (1·0 g.) in glacial acetic acid (30 ml.) containing hydrobromic acid (1 ml. of 48%) was heated under reflux for 3 minutes. Precipitation with water gave (IX), identified by m. p. and mixed m. p. (*c*) Treatment of 2 : 2-dibromo-6-acetoxycholest-4-en-3-one (VI) with hydrobromic acid–acetic acid as in (*b*) likewise gave (IX). The compound occasionally separated from aqueous acetone in a polymorphic form, m. p. 154° (decomp.). A mixture of the two forms softened at 154° and melted with decomposition at 178°.

Action of Hydrobromic Acid–Acetic Acid on 6β-Acetoxycholest-4-en-3-one.—When the ketone (500 mg.) in acetic acid (15 ml.)–hydrobromic acid (0·7 ml. of 48%) was left at 37° for 18 hours and the carmine-coloured solution was diluted with water, cholestane-3 : 6-dione (200 mg.) was obtained, identified after crystallisation from aqueous acetone by m. p. and mixed m. p. with an authentic specimen.

2-*Bromo-6-acetoxycholesta-1 : 4-dien-3-one* (VII; R = Ac).—When 2 : 2-dibromo-6-acetoxycholest-4-en-3-one (19·7 g.) was heated under reflux with collidine (100 ml.) for 7 minutes, 2-*bromo-6-acetoxycholesta-1 : 4-dien-3-one* was obtained, hard prismatic needles from aqueous acetone, m. p. 123—124°, $[\alpha]_D^{23}$ −32·3° (*c*, 1·15) (Found : C, 66·9; H, 8·3. $C_{29}H_{43}O_3Br$ requires C, 67·1; H, 8·3%); yield 82%. Light absorption : $E_{1\,cm.}^{1\%}$ (253 mμ.) = 248.

2-*Bromocholesta-1 : 4-dien-6β-ol-3-one* (VII; R = H).—A solution of potassium hydroxide (1·4 g.) in 95% methanol (25 ml.) was added rapidly to a boiling solution of the foregoing compound (11·7 g.) in methanol (200 ml.). Separation of crystalline material began almost immediately and this was collected when the mixture was cold, giving 2-*bromocholesta-1 : 4-dien-6β-ol-3-one*, large shining plates (from aqueous acetone), m. p. 240°, $[\alpha]_D^{23}$ −37·8° (*c*, 1·06) (Found : C, 68·1; H, 8·7. $C_{27}H_{41}O_2Br$ requires C, 67·9; H, 8·6%); yield 90%. Light absorption : $E_{1\,cm.}^{1\%}$ (255 mμ.) = 305. Acetylation gave (VII; R = Ac), m. p. 123—124°.

2-*Bromocholesta-1 : 4-diene-3 : 6-dione* (VIII).—Chromic acid (600 mg.) in acetic acid (10 ml. of 90%) was added to a stirred suspension of the finely powdered foregoing compound (2·0 g.) in acetic acid (60 ml.) at 75°. After 7 minutes, excess of brine was added, and the precipitated solids were collected and crystallised from aqueous acetone, giving 2-*bromocholesta-1 : 4-diene-3 : 6-dione* in leafy plates, m. p. 182°, $[\alpha]_D^{23}$ −139° (*c*, 1·74) (Found : C, 68·1; H, 8·1. $C_{27}H_{39}O_2Br$ requires C, 68·2; H, 8·2%); yield 60%. The absorption spectrum showed a maximum at 255 mμ. ($E_{1\,cm.}^{1\%}$ = 234) and a minimum at

232 mμ. ($E^{1\%}_{1\,cm.}$ = 139). The dione was recovered unchanged after being refluxed with zinc dust in alcohol for 10 hours.

2-Bromocholest-1-ene-3 : 6-dione (XII).—(*a*) Alcoholic chromous chloride solution (10 ml.) (Conant and Cutler, *J. Amer. Chem. Soc.*, 1926, **48**, 1023) was added, under carbon dioxide, to 2-bromocholesta-1 : 4-diene-3 : 6-dione (500 mg.) in acetone (30 ml.), and the mixture kept for 45 minutes at room temperature. The product, isolated with ether, was fractionated from aqueous acetone, giving *form A* of 2-bromocholest-1-ene-3 : 6-dione in silvery platelets, m. p. 221°, $[\alpha]_D^{19}$ −61° (*c*, 1·64; *l*, 0·5) (Found: C, 67·6; H, 8·5. $C_{27}H_{41}O_2Br$ requires C, 67·9; H, 8·7%); yield 30%. Careful dilution of the mother-liquors gave *form B*, fine needles, m. p. 160—162°, $[\alpha]_D^{21}$ −63° (*c*, 1·1; *l*, 1) (Found: C, 68·1; H, 8·5%); yield 50%. Forms A and B gave identical absorption spectra: $E^{1\%}_{1\,cm.}$ (257 mμ.) = 156.

(*b*) Form A, m. p. 221°, was obtained when (VIII) (500 mg.) was heated under reflux for 18 hours with Raney nickel (*ca.* 500 mg.) in ethanol (7 ml.).

(*c*) 2 : 2-Dibromocholestane-3 : 6-dione (1·1 g.) was heated under reflux with collidine (5 ml.) for 1·5 minutes, and collidine hydrobromide (403 mg. ≡ 1·0 atom bromine) removed after addition of ether. The ethereal solution was washed with dilute hydrochloric acid, etc., and the dark product chromatographically purified on alumina (B.D.H. for chromatographic analysis). The column (12 × 1·4 cm.) was prepared in light petroleum (b. p. 40—60°). A crystalline fraction (200 mg.) was obtained by elution with benzene–light petroleum (4 : 1). Its purification gave form A, m. p. 220—221°, alone or in admixture with an authentic specimen. The same product was obtained when pyridine was employed in place of collidine as described by Sarett *et al.* (*loc. cit.*).

The compound (XII) was recovered unchanged after (*a*) 18 hours' heating under reflux with zinc dust in ethanol, and (*b*) treatment with hydrobromic acid–acetic acid for 24 hours at room temperature.

Reduction of Cholest-4-ene-3 : 6-dione.—The compound (400 mg.) in acetone (10 ml.) was treated with chromous chloride (10 ml.) under carbon dioxide. After 10 minutes the crystalline solids (250 mg.) were collected and purified from aqueous ethanol, giving cholestane-3 : 6-dione, m. p. 169—170°, alone or in admixture with an authentic specimen.

2-Bromocholestane-3 : 6-dione (X).—A suspension of 2 : 2-dibromocholestane-3 : 6-dione (5·25 g.) in chloroform (40 ml.) and acetic acid (75 ml.) containing hydrobromic acid (2 ml. of 48%) was shaken for 4 hours. The product, isolated with ether, was crystallised from aqueous acetone, giving *2-bromocholestane-3 : 6-dione* in needles, $[\alpha]_D^{20}$ −10·4° (*c*, 1·5) (Found: C, 67·5; H, 8·8. $C_{27}H_{43}O_2Br$ requires C, 67·6; H, 9·0%); yield 20%. The m. p. (decomp.) varied from sample to sample and with the rate of heating, decomposition usually occurring at 140—180°. The absorption spectra of different preparations revealed the presence of variable small amounts of an impurity absorbing at 252 mμ. Reduction of (X) with chromous chloride furnished cholestane-3 : 6-dione in good yield, and refluxing it with alcoholic hydrochloric acid gave cholest-4-en-3 : 6-dione.

2 : 2-Dibromocholestane-3 : 6-dione (XI).—The foregoing compound (1·1 g.) in acetic acid (20 ml.) and chloroform (5 ml.) was treated with bromine (400 mg., 1·1 mols.) in acetic acid (5 ml.) containing sodium acetate (300 mg.). After 6 hours at room temperature the product (350 mg.) was collected and crystallised from chloroform–ethyl acetate, giving 2 : 2-dibromocholestane-3 : 6-dione in micro-needles which decomposed between 175° and 195°; $[\alpha]_D^{20}$ +68·3° (*c*, 1·31) (Found: C, 57·9; H, 8·1. Calc. for $C_{27}H_{41}O_2Br$: C, 58·1; H, 7·6%). Sarett *et al.* (*loc. cit.*) give a decomposition temperature of 175—195° and $[\alpha]_D^{24}$ +65°.

Cholest-1-ene-3 : 6-dione (XIII).—2-Bromocholestane-3 : 6-dione (1·1 g.) was heated under reflux with collidine (8 ml.) for 15 minutes. After addition of ether, collidine hydrobromide and a *compound*, small plates (from acetic acid), m. p. >300° (Found: N, 2·0. $C_{35}H_{52}O_2NBr$ requires N, 2·3%), were removed, leaving *cholest-1-ene-3 : 6-dione*, plates (from aqueous ethanol), m. p. 161° (Found: C, 79·7; H, 10·1. $C_{27}H_{42}O_2,\frac{1}{2}H_2O$ requires C, 79·6; H, 10·6%). Light absorption: $E^{1\%}_{1\,cm.}$ (228 mμ.) = 200.

The authors thank the Directors of The British Drug Houses Ltd. for permission to publish this work.

Research Laboratories,
The British Drug Houses Ltd., London, N.1. [*Received, April 27th*, 1950.]