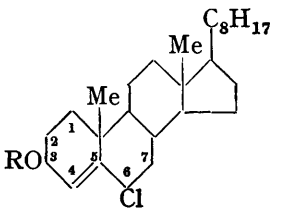
## 61. *The Conversion of 6-Chloro-3-benzoyloxy-$\Delta^4$-cholestene into $\Delta^{4:6}$-Cholestadienyl Benzoate.*

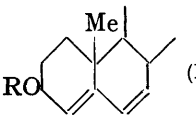
By F. S. Spring and G. Swain.

Treatment of 6-chloro-3-benzoyloxy-$\Delta^4$-cholestene (I, R = COPh) with silver nitrate in pyridine gives *$\Delta^{4:6}$-cholestadienyl benzoate* (II, R = COPh) (with other products). Similar treatment of cholesterol dibromide gives a mixture of $\Delta^4$-cholestenone, **3** : 6-diketo-$\Delta^4$-cholestene, and an alcohol, m. p. 119—120°, benzoylation of which gives $\Delta^{4:6}$-cholestadienyl benzoate; acetylation of the alcohol gives $\Delta^{4:6}$-cholestadienyl acetate. The esters of $\Delta^{4:6}$-cholestadienol are stable and characterised by a single intense absorption maximum at 2390 A. The parent alcohol, however, is not stable to alkali.

We have previously reported upon the dehalogenation of 6-chloro-3-benzoyloxy-$\Delta^4$-cholestene (I, R = COPh) (Spring and Swain, J., 1939, 1356) by means of potassium acetate in alcohol (*idem*, this vol., p. 83). In a further attempt to convert this substance into either $\Delta^{5:7}$- or $\Delta^{4:6}$-cholestadienyl benzoate, its behaviour with silver nitrate and pyridine (Dane, Wang, and Schulte, *Z. physiol. Chem.*, 1936, **245**, 80) has been examined. At room temperature, the reagent gave a mixture of a pyridinium salt, m. p. 158—159°, and the monobenzoate of *cis*-3 : 4-dihydroxy-$\Delta^5$-cholestene, m. p. 153—154°, identical with the product previously obtained by treatment of the 6-chloro-compound (I) with potassium acetate in alcohol. Treatment of (I, R = COPh) with the silver nitrate–pyridine reagent at 90°, on the other hand, gave a mixture of the pyridinium salt, m. p. 158—159°, the monobenzoate of the *cis*-diol, m. p. 153—154°, and a compound, m. p. 128—129°, which gave an intense yellow coloration with tetranitromethane in chloroform, a behaviour not observed with any of the other products obtained by dehalogenation of the 6-chloro-compound (I). The colour reactions, analysis and origin of the new compound, m. p. 128—129°, indicate that it is *$\Delta^{4:6}$-cholestadienyl benzoate* (II, R = COPh) and confirmation of this structure was found in its absorption spectrum, which exhibits a single maximum at 2390 A., $\varepsilon_{max.} = 33{,}000$.

(I.)

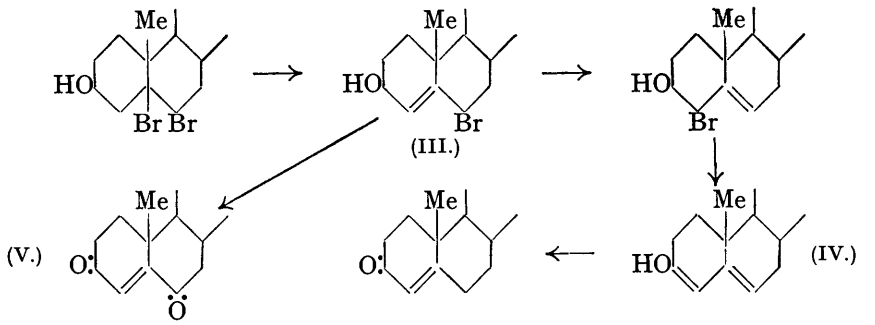



(II.)

Dane and Wang (*Z. physiol. Chem.*, 1937, **248**, 1) obtained an impure $\Delta^{4:6}$-cholestadienol (II, R = H) in small yield (*ca.* 4%) by treatment of cholesterol dibromide with silver nitrate in pyridine. This compound, m. p. 115—121°, gives an intense blue coloration with the antimony trichloride reagent; good analytical data could not be obtained for the free dienol. Dane and Wang ascribed the $\Delta^{4:6}$-structure to their product from a consideration of its origin, its colour reactions, and its absorption spectrum, but details of the latter are not given. In order to compare the $\Delta^{4:6}$-cholestadienol of Dane and Wang and its derivatives with the corresponding derivatives of $\Delta^{4:6}$-cholestadienol obtained from the 6-chloro-compound (I), the treatment of cholesterol dibromide with the silver

nitrate reagent was reinvestigated.* In our hands a large amount of ether-insoluble pyridinium complex was formed together with an ether-soluble resin, from which on long standing a small amount of solid separated; trituration of the resin with light petroleum as described by Dane and Wang did not prove a satisfactory method for the isolation of the dienol. The resin was successfully resolved into its components by solution in light petroleum and filtration through a column of aluminium oxide. The least heavily adsorbed fraction readily gave Δ⁴-cholestenone, which formed 50—60% of the ether-soluble resin. The formation of Δ⁴-cholestenone from cholesterol dibromide may be formulated as follows :




Alternative mechanisms are (*a*) the intermediate formation of Δ⁴·⁶-cholestadienol and its subsequent isomerisation to Δ⁴-cholestenone [*via* the enol-form (IV)] and (*b*) oxidation to 5 : 6-dibromo-3-ketocholestane, followed by debromination and isomerisation to Δ⁴-cholestenone.

The second component of the ether-soluble resin was obtained by continued washing of the chromatogram with light petroleum and was identified as 3 : 6-diketo-Δ⁴-cholestene (V), m. p. 121—122°. The formation of this diketone is probably to be attributed to the intermediate formation of 6-bromo-Δ⁴-cholestenol (III) and its subsequent oxidation, a reaction bearing a marked resemblance to the conversion of 7-bromo-6-ketocholestanyl acetate and 6-bromo-7-ketocholestanyl acetate into 6 : 7-diketocholestanyl acetate by means of the same reagent (Heilbron, Jones, and Spring, J., 1937, 801; Barr, Heilbron, Jones, and Spring, J., 1938, 334).

The most heavily adsorbed fraction of the ether-soluble resin was obtained in needles, m. p. 119—120°; this substance gives an intense blue coloration with the antimony trichloride reagent and a deep yellow coloration with tetranitromethane. It exhibits absorption maxima at 2395 A. and 2850 A.; repeated crystallisation did not achieve any alteration in the location or intensities of these maxima. This fraction contains a large proportion of Δ⁴·⁶-cholestadienol, since on benzoylation it readily gives Δ⁴·⁶-cholestadienyl benzoate, m. p. 128—129°, identical with the ester obtained by treatment of 6-chloro-3-benzoyloxy-Δ⁴-cholestene (I, R = COPh) with silver nitrate and pyridine, and exhibiting a *single* absorption maximum at 2390 A. ($\varepsilon_{max.}$ = 33,000). Likewise, acetylation of the fraction, m. p. 119—120°, gives Δ⁴·⁶-cholestadienyl acetate, m. p. 77—78°, $[\alpha]_D$ −67°, exhibiting a single absorption maximum at 2390 A., $\varepsilon_{max.}$ = 22,000, data in good agreement with those recorded by Petrow (J., 1940, 66) for the acetate (m. p. 78—79°; $[\alpha]_D$ −71·6°; $\lambda_{max.}$ 2390 A., $\varepsilon_{max.}$ ≏ 26,000) of Δ⁴·⁶-cholestadienol prepared by the reduction of Δ⁴·⁶-cholestadienone. Δ⁴·⁶·²²-Ergostatrienyl acetate, which contains the same major chromophor as Δ⁴·⁶-cholestadienyl acetate, exhibits an absorption maximum at 2400 A., $\varepsilon_{max.}$ ≏ 27,000 (Güntzel, *Ber.*, 1939, **72**, 1318).

Hydrolysis of Δ⁴·⁶-cholestadienyl acetate at 20° gave a product, m. p. 116—117°, $[\alpha]_D$ −34·9°, which exhibits a single absorption maximum at 2380 A., $\varepsilon_{max.}$ = 14,000. Hydrolysis of Δ⁴·⁶-cholestadienyl benzoate with boiling methyl-alcoholic potassium hydroxide, on the other hand, gave a product, m. p. 124—125°, which exhibits light absorption maxima at 2395 A., $\varepsilon_{max.}$ = 14,000, and at 2840 A., $\varepsilon_{max.}$ = 9000. Δ⁴·⁶-

* The experiments described in this paper were completed in October, 1939, prior to the appearance of a paper on Δ⁴·⁶-cholestadienol by Petrow (J., 1940, 66).

Cholestadienyl esters have not been hydrolysed to a substance with the properties expected of $\Delta^{4:6}$-cholestadienol; in the case of the hydrolysis product, m. p. 116—117° (" cold " hydrolysis), although it exhibits a single absorption maximum at 2380 A., the low intensity of this absorption compared with that of the parent $\Delta^{4:6}$-cholestadienyl acetate indicates that it is not pure $\Delta^{4:6}$-cholestadienol. The appearance of the absorption maximum at 2840 A., during the hydrolysis of $\Delta^{4:6}$-cholestadienyl benzoate (" hot " hydrolysis), may be due to partial dehydration to a cholestatriene. It is clear that $\Delta^{4:6}$-cholestadienol is not stable to alkali; Petrow (*loc. cit.*) has observed that the dienol is not stable to acids.

## Experimental.

*Treatment of 6-Chloro-3-benzoyloxy-$\Delta^4$-cholestene with Silver Nitrate in Pyridine.*—The chloro-compound (10 g.) was heated on the steam-bath for 2 hours with silver nitrate (20 g.) and dry pyridine (400 c.c.). The cooled solution was poured into water (1500 c.c.) and extracted with ether. The extract was washed with water and then with hydrochloric acid (5%), which caused the separation of a pyridinium complex (2·5 g.) in silky needles. The complex crystallised from chloroform–light petroleum in felted needles, m. p. 158—159° (Found: N, 2·85; Cl, 4·8%). The ethereal solution was washed with water, and the dried (sodium sulphate) solution evaporated. The residual semi-crystalline mass (6·6 g.) was fractionated from ethyl acetate–methyl alcohol (2 : 1); the top crop, when recrystallised from the same solvent, gave the mono-benzoate of *cis*-3 : 4-dihydroxy-$\Delta^5$-cholestene (1·1 g.) in blades, m. p. 153—154°, undepressed by the specimen described by Spring and Swain (1941, *loc. cit.*); it was further characterised by hydrolysis, the *cis*-3 : 4-diol being obtained, m. p. 176°, undepressed by an authentic specimen. Concentration of the mother-liquor from the top crop gave a more soluble fraction, which after several recrystallisations from ethyl acetate–methyl alcohol (2 : 1) gave *$\Delta^{4:6}$-cholestadienyl benzoate* (0·6 g.) in small prisms, m. p. 128—129°, $[\alpha]_D^{21°}$ −81° ($l = 1$, $c = 1·7$ in chloroform) (Found: C, 83·45; H, 10·0. $C_{34}H_{48}O_2$ requires C, 83·6; H, 9·9%). The benzoate gives an intense yellow coloration with tetranitromethane in chloroform and an intense blue coloration with the antimony trichloride reagent.

*Treatment of Cholesterol Dibromide with Silver Nitrate in Pyridine.*—Freshly prepared, dry cholesterol dibromide (48 g.) was added to a solution of silver nitrate (110 g.) in pyridine (600 c.c.) at room temperature. When solution was complete, the mixture was set aside for 5 days with exclusion of light. Ether (approx. 2 l.) was added to the solution until no further precipitation of silver salts and pyridinium compounds occurred. The separated solids were removed, and the ethereal solution washed with dilute hydrochloric acid (5%) and aqueous sodium carbonate (5%). Removal of the solvent from the dried (sodium sulphate) extract yielded a brown resin (15 g.), which was dissolved in light petroleum (b. p. 40—60°; 100 c.c.), and the solution filtered through a column of aluminium oxide (1" diam., 10" long). The chromatogram was developed by washing with solvents, and the following fractions collected:

| Fraction. | Solvent. | Residue after removal of solvent. | Colour with antimony trichloride. |
|---|---|---|---|
| I | Light petroleum (100 c.c.) | Cryst. solid (5·1 g.) | Yellow-brown |
| II | ,, ,, (200 c.c.) | ,, ,, (0·95 g.) | ,, |
| III | ,, ,, (300 c.c.) | ,, ,, (0·40 g.) | Yellow |
| IV | ,, ,, (600 c.c.) | Part. cryst. (0·55 g.) | Green-yellow |
| V | Benzene–light petroleum (1 : 3) (100 c.c.) | ,, ,, (0·05 g.) | Green |
| VI | ,, ,, ,, (1 : 3) (600 c.c.) | Resinous solid (1·55 g.) | Green-blue |
| VII | Benzene (200 c.c.) | ,, ,, (0·7 g.) | Blue |

*$\Delta^4$-Cholestenone.*—Recrystallisation of fractions I and II from acetone–methyl alcohol (2 : 1) gave $\Delta^4$-cholestenone in prismatic needles, m. p. 80—81°, undepressed by an authentic specimen. The semicarbazone separated from 95% acetic acid in needles, m. p. 223—224° (decomp.), either alone or when mixed with an authentic specimen.

*3 : 6-Diketo-$\Delta^4$-cholestene.*—Fraction III separated from light petroleum in slightly yellow needles, m. p. 105—110°. After two recrystallisations from the same solvent this gave 3 : 6-diketo-$\Delta^4$-cholestene, m. p. 121—122°, undepressed by an authentic specimen (Found: C, 81·3; H, 10·5. Calc. for $C_{27}H_{42}O_2$: C, 81·4; H, 10·55%). Fraction IV proved to be mainly 3 : 6-diketo-$\Delta^4$-cholestene, but the purification of the diketone from this fraction was much more difficult to achieve than in the case of fraction III; fraction V was not examined.

*$\Delta^{4:6}$-Cholestadienyl Acetate.*—The aluminium oxide was divided into four sections, A, B, C, and D (lengths 1", 2½", 3", and 3½" commencing from the top). Section A was not examined;

the remaining three sections were eluted with acetone; removal of the solvent yielded the following fractions :

| Fraction. | Residue (after removal of acetone). | Colour with antimony trichloride. | Colour with tetranitromethane. |
|---|---|---|---|
| B | Resin (0·75 g.) | Intense blue | Intense yellow |
| C | Oily solid (1·2 g.) | ,, ,, | ,, ,, |
| D | ,, ,, (2·2 g.) | ,, ,, | ,, ,, |

Fractions VI and VII, B, C, and D were combined and crystallised thrice from methyl alcohol to give a compound in small needles, m. p. 119—120°, $[\alpha]_D^{20°}$ −27·4° ($l = 1$, $c = 0·8$ in chloroform). Analysis gave a persistently low carbon value (Found : C, 83·3; H, 11·7. Calc. for $C_{27}H_{44}O$ : C, 84·4; H, 11·5%). It gives an intense blue coloration with antimony trichloride and an intense yellow coloration with tetranitromethane in chloroform. *Light absorption in alcohol*: Maxima (*a*) 2395 A., $\varepsilon_{max.} = 12{,}500$; (*b*) 2850 A., $\varepsilon_{max.} = 12{,}000$. Acetylation of this compound (0·2 g.) was effected by refluxing for 45 minutes with pyridine (1·5 c.c.) and acetic anhydride (1·5 c.c.). The solution was diluted with water, and the product isolated by means of ether. Removal of the ether and crystallisation of the residue from ethyl acetate–methyl alcohol (1 : 1) gave $\Delta^{4:6}$-cholestadienyl acetate in needles, m. p. 77—78°, $[\alpha]_D^{20°}$ −67° ($l = 1$, $c = 1·0$ in chloroform); it gave an intense blue solution with antimony trichloride in chloroform and a deep yellow solution with tetranitromethane in chloroform. A mixture of the dienyl acetate with $\Delta^4$-cholestenone enol-acetate (m. p. 79°) had m. p. 74° (Found : C, 81·7; H, 11·0. Calc. for $C_{29}H_{46}O_2$ : C, 81·7; H, 10·8%).

*$\Delta^{4:6}$-Cholestadienyl Benzoate.*—The product, m. p. 119—120°, described above (0·3 g.) was heated on the steam-bath for 45 minutes with pyridine (2 c.c.) and benzoyl chloride (2 c.c.). The mixture was poured into aqueous sodium bicarbonate and extracted with ether; the product was thrice crystallised from ethyl acetate–methyl alcohol (2 : 1), from which $\Delta^{4:6}$-cholestadienyl benzoate separated in small prisms, $[\alpha]_D^{17°}$ −75° ($l = 1$, $c = 1·2$ in chloroform), m. p. 128—129°, undepressed by the specimen obtained from 6-chloro-3-benzoyloxy-$\Delta^4$-cholestene.

*Hydrolysis of $\Delta^{4:6}$-Cholestadienyl Acetate.*—The acetate (1·0 g.) in absolute methylated spirit (50 c.c.) was treated with alcoholic potassium hydroxide (50 c.c.; 2%) solution at room temperature, and the mixture set aside for 70 hours. Water (250 c.c.) was added, and after standing for 1 hour at 0°, the crystalline precipitate was collected, washed with water, and recrystallised from methyl alcohol, from which the product separated in plates, m. p. 116—117°, $[\alpha]_D^{18°}$ −34·9° ($l = 1$, $c = 0·8$ in chloroform). Analysis again gave a low carbon value.

*Hydrolysis of $\Delta^{4:6}$-Cholestadienyl Benzoate.*—The benzoate (0·35 g.) was refluxed with methyl-alcoholic potassium hydroxide solution (20 c.c.; 3%) for 2 hours. The product was crystallised from methyl alcohol to give an alcohol, m. p. 124—125°, which gave an intense blue coloration with the antimony trichloride reagent and an intense yellow solution with tetranitromethane in chloroform. The carbon value was again low. The product became slightly yellow on exposure to light and air, and after three weeks the m. p. was 80—85°.

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