150. *Enolic Derivatives of* 3 : 6-*Diketo*-Δ^4-*cholestene.*

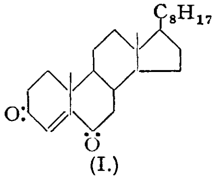
By Walter C. J. Ross.

3 : 6-*Dibenzoyloxy*-$\Delta^{2:4:6}$-*cholestatriene* and 3 : 6-*dibenzoyloxy*-$\Delta^{3:5:7}$-*cholestatriene* have been prepared from 3 : 6-diketo-Δ^4-cholestene, and some of their reactions have been studied. Benzoylation of the enol ether of 3 : 6-diketo-Δ^4-cholestene affords 3-*benzoyloxy*-6-*ethoxy*-$\Delta^{2:4:6}$-*cholestatriene.*

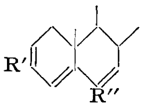
Enol esters of αβ-unsaturated ketones derived from sterols have been prepared by a number of workers (Heilbron, Kennedy, Spring, and Swain, *J.*, 1938, 869; Ruzicka and Fischer, *Helv. Chim. Acta*, 1936, **19**, 806; Inhoffen, *Ber.*, 1936, **69**, 2141; Westphal, *Ber.*, 1937, **70**, 2126). Westphal has shown that the enol esters derived from Δ^4-unsaturated 3-ketones have a $\Delta^{3:5}$-unsaturated structure. No enol esters of unsaturated 1 : 4-diketones appear to have been prepared in the sterol field.

It has been shown in these laboratories (unpublished results) that Δ^4-cholesten-3-one and Δ^4-androstene-3 : 17-dione readily yield enol benzoates when refluxed for a short time with benzoyl chloride, and it is now found that 3 : 6-diketo-Δ^4-cholestene (I) affords two different compounds according to the conditions employed.

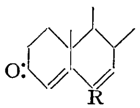
The lower-melting compound, formed in presence of pyridine, is the *dienol dibenzoate* of structure (II; R′ = R″ = OBz), since it can be hydrolysed with hot dilute acid or alkali to give the parent diketone (I). It exhibits an absorption maximum at 3070 A., ϵ = 18,200, which is entirely similar to that of *iso*ergostatrienone enol acetate (II; R′ = OAc, R″ = H, and with C_9H_{17} side-chain) which shows a maximum at 3040 A., ϵ = 16,600 (Heilbron *et al.*, *loc. cit.*). When a slight excess of alcoholic potassium hydroxide is added to a cold solution of the dibenzoate the optical rotatory power and the light absorption characteristics of the solution change. The solution remains laevorotatory, but the numerical value of the rotation falls slightly. The absorption maximum at 3070 A. is replaced by a double maximum at 2800 and 2900 A., ϵ = 14,500; during this change an



(I.)



(II.)

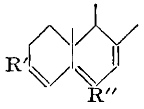


(III.)

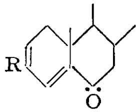
amount of alkali corresponding to the hydrolysis of one benzoate group is consumed. It is suggested that partial hydrolysis occurs with formation of the ester (III; R = OBz); 3-keto-$\Delta^{2:4}$-cholestadiene (III; R = H) exhibits an absorption maximum in the same region (at 2800 A., $\epsilon = 40,000$). Unfortunately, no crystalline material could be isolated from the solution after this mild hydrolysis.

The higher-melting compound, formed with benzoyl chloride alone, is also a *dibenzoate,* as shown by analysis and the consumption of two equivalents of alkali on complete hydrolysis. It is formulated as 3 : 6-dibenzoyloxy-$\Delta^{3:5:7}$-cholestatriene (IV; R′ = R″ = OBz) and this structure is supported by light-absorption measurements. The substance exhibits an absorption maximum at 3160 A., $\epsilon = 20,200$, which is very similar to that of ergostatrienone enol acetate (IV; R′ = OAc, R″ = H, C_9H_{17} side-chain) which shows a maximum at 3165 A., $\epsilon = 21,400$ (Heilbron *et al., loc. cit.*), and that of $\Delta^{3:5:7:22}$-ergostatetrene (IV; R′ = R″ = H, C_9H_{17} side-chain), maximum at 3160 A., $\epsilon = 19,000$ (*idem, ibid.*). The triene system in structure (IV) is more heavily substituted than that in structure (II), and as would be expected the absorption maximum for the compound to which structure (IV) has been assigned occurs at a somewhat longer wave-length. As in the case of the dienol benzoates now described, the ergosterol derivative of structure (IV) has a higher melting point (146° as against 137°) than that of structure (II), and also a numerically greater value of the optical rotation (−143·5° as against −84·6°). 3 : 6-Dibenzoyloxy-$\Delta^{3:5:7}$-cholestatriene gives a positive Tortelli–Jaffé reaction as might be expected from the proposed formulation.

Complete hydrolysis of this dibenzoate with hot alcoholic potassium hydroxide yields a non-crystalline product, $[\alpha]_D^{20°}$ +48°, which exhibits an absorption maximum at 2560 A., $\epsilon = 3020$. The position of this maximum is consistent with the formation of a compound with the grouping O:C·C:C·C:O (cf. I) though the intensity is of a low order [3 : 6-diketo-Δ^4-cholestene (I) shows a maximum at 2520 A., $\epsilon = 14,400$]. The sign of the rotation indicates that the asymmetric centre at C_8 is involved in the hydrolysis mechanism. Treatment of 3 : 6-dibenzoyloxy-$\Delta^{3:5:7}$-cholestatriene with cold alcoholic potassium hydroxide—under conditions which cause complete hydrolysis of cholestenone enol benzoate—produces interesting changes in the optical rotatory power and of the light-absorption characteristics. The optical rotation soon changes from $[\alpha]_D^{20°}$ −85° to a positive value, finally becoming steady at +97°; the absorption maximum moves to 3100 A., $\epsilon = 7270$. During this treatment the solution develops a deep orange colour which gives rise to a yellow-green fluorescence on acidification with acetic acid. The hydrolysis of this dibenzoate is more difficult to interpret, particularly as no crystalline product was obtainable at either stage. One would expect the 3-benzoate group to be hydrolysed first with the formation of compound (VI): this explanation is, however, improbable since this substance would almost certainly be laevorotatory (compare 7-dehydrocholesterol, $[\alpha]_D^{20°}$ −113°) and would have an absorption maximum at the wave-lengths 2700—2800 A. It is now suggested that hydrolysis occurs at the 6-benzoate group and that it is accompanied by a rearrangement leading to the formation of the monobenzoate (V; R = OBz). In the conversion of structure (IV) into structure (V) two isomers (Va and Vb) could be formed which differ in the mode of locking of rings B and C; the existence of such a mixture could account for the fact that the hydrolysis products of this dibenzoate could not be crystallised.



(IV.)



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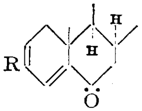
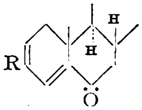
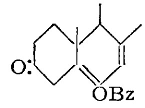


(Va.)



(Vb.)



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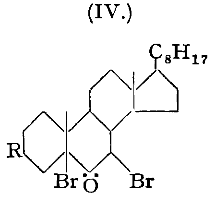

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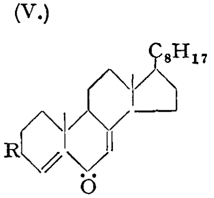

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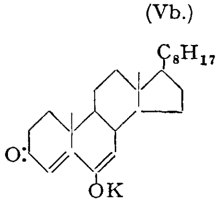
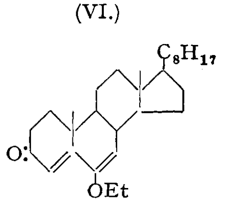


(IX.)



(X.)

A somewhat similar change is recorded by Heilbron, Jackson, Jones, and Spring (*J.*, 1938, 102). Debromination of (VII; R = OAc) might be expected to give (VIII; R = OAc), but (V; R = OAc) is actually obtained. In our case hydrolysis of (IV; R′ = R″ = OBz) at the 6-benzoate group would be expected to give (VIII; R = OBz), but we suggest that (V; R = OBz) is actually formed. This structure accounts for the observed absorption maximum and for the sign of the rotation (compare V; R = OAc, Heilbron *et al., loc. cit.*, which has a maximum at 3170 A., $\epsilon = 6300$ and $[\alpha]_D^{20°}$ +27°). Dr. E. R. H. Jones informs the author that 6-keto-$\Delta^{2:4}$-cholestadiene (V; R = H) exhibits an absorption maximum at 3160 A., $\epsilon = 7500$ (unpublished work).

It had been hoped to prepare the mono-enol benzoates, which have been assumed to be intermediate hydrolysis products of the dibenzoates, by the following methods. 3 : 6-Diketo-Δ^4-cholestene forms a potassium compound (Windaus, *Ber.*, 1906, **39**, 2249) which has now been shown to have structure (IX) since it yields the known 6-enol ether (X) when heated with ethyl iodide. The structure of this ether is confirmed by the fact that it exhibits an absorption maximum at 2950 A. (Butenandt and Schramm, *Ber.*, 1936, **69**, 2289); the alternative structure (V; R = OEt) would be expected to show an absorption maximum at a longer wave-length. Treatment of the potassium compound with benzoyl chloride under the appropriate conditions should give the monobenzoate (III; R = OBz); it has, however, not been found possible to prepare the ester by the methods tried so far. Benzoylation of the enol ether in the presence of pyridine affords the expected 3-*benzoyloxy*-6-*ethoxy*-$\Delta^{2:4:6}$-*cholestatriene* (II; R′ = OBz, R″ = OEt), m. p. 126—127°, $[\alpha]_D^{20°}$ −48°, which exhibits an absorption maximum at 3080 A., ϵ = 13,200. This compound is re-converted into the enol ether on treatment with cold sodium methoxide. Mild acid hydrolysis of the compound would be expected to give the monobenzoate (V; R = OBz)—compare the conversion of testosterone enol ether into testosterone (Serini and Koster, *Ber.*, 1938, **71**, 1769). In practice, it has not been found possible to de-ethylate the compound without simultaneous elimination of the benzoate group.

When kept in solution 3 : 6-dibenzoyloxy-$\Delta^{3:5:7}$-cholestatriene undergoes interesting changes which can be followed by observing the optical rotation. The change in optical rotatory power is similar to that observed on partial hydrolysis; indeed, a very similar yellow-green fluorescence develops in the neutral or faintly acid solutions, and benzoic acid is liberated. As crystalline products could not be obtained in either case, it has not been possible to confirm the identity of the end products. The rotation change is considerably accelerated by exposure to light, is relatively unaffected by traces of acid, and is definitely retarded by a small amount of alkali.

EXPERIMENTAL.

M. ps. were determined in sealed capillaries and are uncorrected; unless otherwise stated light-absorption measurements were made in chloroform–alcohol (1 : 1) solution; rotations were determined in chloroform solution.

Difficulty has been experienced in the preparation of Δ^4-cholestene-3 : 6-dione by direct oxidation of cholesterol. The yields obtained by the methods described in the literature have been uniformly low. The oxidation of cholesterol by chromic acid in acetic acid solution has been studied and the following method has been found to give consistent results. Advantage has been taken of the fact that the diketone can be extracted from ether solution by concentrated alkali (Windaus, *loc. cit.*) to develop an improved method of isolating the substance from the oxidation mixture.

Cholesterol (100 g.) was dissolved in warm acetic acid (4 l.) and the solution was cooled to 25° with vigorous stirring; this gave a fine suspension of crystals. A mixture of chromic acid (50 g.), water (50 c.c.), and acetic acid (500 c.c.) was added during ½ hour with stirring. The temperature during the addition was maintained at 23—25° by cooling. The mixture was next stirred for 2 hours at 20°, by which time the separated cholesterol had passed back into solution. After being diluted with six times its volume of water, the mixture was extracted with ether (total, 2 l.). The extract was washed twice with dilute sulphuric acid, once with water, and then with sufficient 10N-sodium hydroxide to render the mixture almost neutral. The aqueous layer was then run off and the ether layer was well shaken with excess of 10N-sodium hydroxide which extracted the acidic material and the diketone, a deep yellow sludge being formed. The supernatant ethereal layer was poured off and the aqueous layer was washed with fresh ether; it was then diluted with ten times its volume of water and re-extracted with ether. The dried ethereal layer gave a solid product (30 g.) on evaporation; this, after recrystallisation from methanol, had m. p. 122—123°. A twice recrystallised specimen had m. p. 124—125°, $[\alpha]_D^{20°}$ −38° (c = 0·54).

If the temperature is kept below 20° during the addition of the oxidising mixture a 5—10% yield of cholestane-3 : 6-dione-5-ol is obtained while the yield of the above diketone falls to 10—15%. The former compound can easily be separated from the mixture because of its relative insolubility—it remains suspended in the initial ether extract and can be filtered off. It has been converted into the unsaturated diketone by heating for a short time just above the m. p. or by heating with potassium hydrogen sulphate, but a more convenient method is to suspend it in twenty times its volume of benzene and to reflux the mixture with excess of phosphoric oxide for ½ hour. The benzene solution is then percolated through a short column of alumina in order to remove any unchanged material: the evaporated eluate gives the required diketone, m. p. 122°.

3 : 6-*Diketo*-Δ^4-*cholestene*-6-*enol Ether.*—A solution of the potassium compound of 3 : 6-diketo-Δ^4-cholestene was prepared by adding the theoretical amount of N/10-alcoholic potassium hydroxide to a hot solution of the compound in ten times its volume of alcohol. The solution became deep green and appeared red by transmitted light. Excess of ethyl iodide was added and the mixture was refluxed until it was neutral to moist litmus paper. During this heating the solution first changed to a light orange and then to an intense crimson colour. On dilution with water a red gum separated; this solidified on rubbing with methanol. After one crystallisation from methanol, followed by a washing with light petroleum (which removed most of the adsorbed red colour), the product had m. p. 161—163°. A mixture with a specimen, m. p. 166°, prepared according to the directions of Windaus (*Ber.*, 1907, **40**, 257) had m. p. 162—164°. A purified specimen of the ether made by Windaus's method had $[\alpha]_D^{20°}$ +2°. It gives a yellow coloration with a solution of antimony trichloride in chloroform.

3-*Benzoyloxy*-6-*ethoxy*-$\Delta^{2:4:6}$-*cholestatriene.*—The enol ether (1 g.) was refluxed for 20 minutes with a mixture of pyridine (5 c.c.) and benzoyl chloride (5 c.c.). The product was poured into a large excess of water and agitated until the benzoyl chloride had been hydrolysed. The water was then poured off the gum and methanol was added; in a short time the mass had become solid, and the mixture was then boiled and cooled, and the solid filtered off. The product was dissolved in ether and methanol was added; on boiling off the ether the *compound* separated in small prisms (0·8 g.), m. p. 126—127°, $[\alpha]_D^{20°}$ −48° (c = 0·80) (Found : C, 81·1; H, 9·4. $C_{36}H_{50}O_3$ requires C, 81·5; H, 9·5%). Light absorption : maximum at 3080 A., ϵ = 13,200. The substance gives a yellow coloration with a solution of antimony trichloride in chloroform; a yellow-orange colour in the Liebermann–Burchard test; and a faint yellow coloration when its solution in chloroform–alcohol is treated with one drop of 10N-sodium hydroxide.

Hydrolysis of the above Ester.—The ester (100 mg.) was shaken overnight with sodium methoxide (10 c.c., 3%) in methyl alcohol. The mixture deposited fine prismatic needles, m. p. 162—163°, not depressed by admixture with the enol ether of 3 : 6-diketo-Δ^4-cholestene.

3 : 6-*Dibenzoyloxy*-$\Delta^{2:4:6}$-*cholestatriene.*—3 : 6-Diketo-Δ^4-cholestene (2 g.) was heated under reflux for ½ hour with pyridine (10 c.c.) and benzoyl chloride (10 c.c.). The product was worked up as before and the dibenzoate was recrystallised from ether–methanol, also as described above. The yield of *dibenzoate*, m. p. 178—179°, was 1·75 g. After a chromatographic purification by percolating a solution in benzene through a column of activated alumina and evaporating the colourless eluate the substance crystallised from ether–methanol in flattened needles, m. p. 179—180°, $[\alpha]_D^{20°}$ −32° (c = 0·80) (Found : C, 81·5, 80·9; H, 8·4, 8·5. $C_{41}H_{50}O_4$ requires C, 81·2; H, 8·3%). Light absorption : maximum at 3070 A., ϵ = 18,200. The substance gives an eosin-like coloration with a solution of antimony trichloride in chloroform; a pale green colour in the Liebermann–Burchard test; a yellow coloration when its solution in chloroform–alcohol is treated with one drop of 10N-sodium hydroxide; and a negative Tortelli–Jaffé reaction.

3 : 6-*Dibenzoyloxy*-$\Delta^{3:5:7}$-*cholestatriene.*—3 : 6-Diketo-Δ^4-cholestene (3 g.) was heated under reflux for ½ hour with

benzoyl chloride (10 c.c.). Most of the excess of benzoyl chloride was removed by distillation at 1—2 mm.; the *product* then partly solidified. The crude material was ground under light petroleum (b. p. 40—60°) and the solid (1·5 g.) was filtered off and washed with cold acetone. After a chromatographic purification as above, it crystallised from acetone in colourless flattened needles, m. p. 205—208°, $[\alpha]_D^{20^\circ}$ -85° ($c = 1{\cdot}15$) (Found : C, 80·8, 80·9; H, 8·2, 8·0. $C_{41}H_{50}O_4$ requires C, 81·2; H, 8·3%). Light absorption : maximum at 3160 A., $\epsilon = 20{,}200$; maximum at 3160 A., $\epsilon = 18{,}800$ (in *cyclo*hexane solution). The substance gives an intense eosin-like coloration with a chloroform solution of antimony trichloride; the following rapidly changing sequence of colours in the Liebermann–Burchard test: pink with yellow fluorescence, green with yellow fluorescence, deep green, deep blue; a deep orange coloration when its solution in chloroform–alcohol is treated with one drop of 10N-sodium hydroxide; and a positive reaction (brown-green layer at the interface) in the Tortelli–Jaffé test.

Hydrolysis of 3 : 6-*Dibenzoyloxy*-$\Delta^{2:4:6}$-*cholestatriene*.—(1) The dibenzoate (200 mg.) was heated under reflux with N/10-alcoholic potassium hydroxide (10 c.c.) for one hour : titration of the excess of alkali indicated that 6·7 c.c. had been used (theory for the complete hydrolysis of two benzoate groups is 6·6 c.c.). The mixture was then heated to boiling and diluted with water until a permanent precipitate formed. This was quickly filtered off and the filtrate was re-heated and allowed to cool slowly. Large plates were deposited and these after recrystallisation from methanol had m. p. 122—123°, unaltered by admixture with an authentic specimen of 3 : 6-diketo-Δ^4-cholestene.

(2) The dibenzoate (200 mg.) was dissolved in chloroform (12·5 c.c.) and N/10-alcoholic potassium hydroxide (7·5 c.c.) was added. Finally the volume of the solution was made up to 25 c.c. with alcohol and the mixture was left at room temperature. The rotation which originally corresponded to $[\alpha]_D^{20^\circ}$ -32° became -16° after 24 hours and had not changed further after 48 hours (both these values being calculated on the weight of dibenzoate used). A solution similarly treated showed the following light absorption changes :

Hours after addition of alkali.	Maxima at		Intensity $\epsilon =$	
0	2300 A.	3070 A.	48,400	18,200
2	2300 A.	2800—2900 A.	35,000	15,100
20	2300 A.	2800—2900 A.	34,000	14,500

The absorption maximum at 2300 A. is due to the benzoyl groups; the intensity of this maximum falls because potassium benzoate slowly crystallises from the solution. No other crystalline material could be isolated from the mixture.

Hydrolysis of 3 : 6-*Dibenzoyloxy*-$\Delta^{3:5:7}$-*cholestatriene*.—(1) The ester (200 mg.) was hydrolysed as described above; 6·0 c.c. of alkali were consumed. The product, which could not be crystallised even after a chromatographic resolution, had $[\alpha]_D^{20^\circ}$ $+48^\circ$ ($c = 0{\cdot}80$) and exhibited an absorption maximum at 2560 A., $\epsilon = 3020$. The ester was recovered unchanged after heating with excess of sodium carbonate or bicarbonate, or after heating with an alcoholic solution of hydrogen chloride for one hour.

(2) When the ester was treated with alcoholic potassium hydroxide in the cold under the conditions described above the rotation changed from -85° to $+97^\circ$. The solution had to be acidified with acetic acid before the rotation was taken since it had become of such a deep red colour that it was opaque to sodium light. After acidification it showed a yellow-green fluorescence. The absorption spectrum changes during this alkali treatment were as follows :

Hours after addition of alkali.	Maximum at		Intensity $\epsilon =$	
0	2300 A.	3160 A.	37,600	20,200
2	2300 A.	3100 A.	34,600	9,700
20	2300 A.	3100 A.	34,600	7,270
After acidification	2300 A.	3080 A.	34,600	7,270

Once again no crystalline material could be isolated despite repeated attempts.

Rotation Changes in Solutions of the Dibenzoates.—A solution of the dibenzoate (II; R′ = R″ = OBz), m. p. 179—180°, appeared to undergo no change of rotatory power on keeping in light, but when a solution of the higher-melting isomeride (IV) was exposed to diffused daylight in a stoppered flask the following changes were observed (all values are calculated on the weight of dibenzoate taken). The solution which was in B.P. chloroform had $c = 0{\cdot}76$.

Time (hours)	0	17	41	65	89	233	329	377	425
$[\alpha]_D^{20^\circ}$	−83°	−57°	−26°	−3°	+16°	+64°	+92°	+102°	+103°

Assuming that the change from $[\alpha]_D^{20^\circ}$ -83 to $+103^\circ$ represents the complete action, the following table shows the amount of action after 100 hours under varying conditions. In each experiment 25 c.c. of solution ($c = 1{\cdot}0$ in B.P. chloroform) were used.

Conditions.	% Action in 100 hours.
Kept in darkness in neutral solution	7·5
Kept in diffused daylight in neutral solution	46·5
Kept in diffused daylight with 2 drops of acetic acid added	48·5
Kept in diffused daylight with 2 drops of N/10-hydrochloric acid added	53·5
Kept in diffused daylight with 2 drops N/10-sodium hydroxide added	27·0

The action is accompanied by hydrolysis since benzoic acid can be extracted from the solution by shaking with a little sodium carbonate solution. During the above changes the solution assumes a yellow-green fluorescence.

The author's thanks are due to Professor G. A. R. Kon for his interest in this work, to Dr. E. A. Braude for measuring the absorption spectra, and to Organon Laboratories, Ltd., for permission to publish the results.

Research Department, Organon Laboratories, Ltd., London, W.C.2. [*Received, February 11th*, 1946.]
