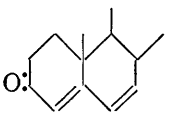
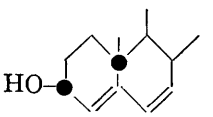
11. *The Preparation of* $\Delta^{4:6}$*-Cholestadien-3(β)-ol.*

By Vladimir A. Petrow.

A study of $\Delta^{4:6}$-cholestadien-3(β)-ol (II) has been undertaken in order to determine whether, in view of the mobility of the related 7 : 8-dehydrocholesterol ring system, it is converted into vitamin D_3 on irradiation with ultra-violet light.

The preparation of (II) has been claimed by Dane and Wang (*Z. physiol. Chem.*, 1937, **248**, 1; cf. Petrow, J., 1937, 1077), who obtained it by debromination of cholesterol dibromide, but it appears from the results described below that their product was largely contaminated with cholesterol. The desired $\Delta^{4:6}$-cholestadien-3(β)-ol has now been prepared essentially pure by the following unambiguous route (cf. Güntzel, *Ber.*, 1925, **58**, 1317): $\Delta^{4:6}$-Cholestadien-3-one (Dane, Wang, and Schulte, *Z. physiol. Chem.*, 1936, **245**,

(I.)   (II.)

80) (I) was reduced in *iso*propyl-alcoholic solution with the Meerwein–Ponndorf reagent. A portion of product on repeated crystallisation yielded the *addition complex*, m. p. 113°, of (II) and its epimeride (cf. Schoenheimer and Evans, *J. Biol. Chem.*, 1936, **114**, 567), which was found to contain equimolecular amounts of both isomerides by a digitonin estimation. The main bulk of the reduction product was treated with digitonin. The digitonide was partitioned in pyridine–ether (Schoenheimer and Dam, *Z. physiol. Chem.*, 1933, **215**, 59) and yielded $\Delta^{4:6}$-cholestadien-3(β)-ol (II), m. p. 127° (Dane and Wang, *loc. cit.*, give m. p. 115—121°), characterised by a digitonide, m. p. 224—227°, and an *acetate*, m. p. 79°. The compound gives a positive reaction with *p*-nitrobenzenediazonium chloride in acetic acid (Fieser and Campbell, *J. Amer. Chem. Soc.*, 1938, **60**, 159), and intense blue colours with arsenic and antimony trichlorides, trichloroacetic acid, chloral hydrate, and the mercury reagent (Rosenheim and Callow, *Biochem. J.*, 1931, **25**, 74). The Tortelli–Jaffé bromine colour reaction slowly develops on standing (cf. Westphal, *Ber.*, 1939, **72**, 1246).

The constitution assigned to (II) is based on the following evidence: (*a*) the presence of a conjugated system of double bonds spread over two rings follows from the ultra-violet absorption spectrum, which shows a band at 2390 A., $E^{1\%}_{1cm.} = 563$, and (*b*) the compound undergoes facile oxidation with the Oppenauer reagent (*Rec. Trav. chim.*, 1937, **56**, 137) to give the original ketone (I), characterised by the 2 : 4-*dinitrophenylhydrazone*, m. p. 232°. This reaction is shown only by αβ-unsaturated ketones, and it indicates that reduction of the carbonyl group of (I) has not been accompanied by a migration of the unsaturated linkages (cf. reduction of ergosterone; Heilbron, Kennedy, Spring, and Swain, J., 1938, 869; Windaus and Buchholz, *Ber.*, 1939, **72**, 597). Catalytic hydrogenation of 3(β)-acetoxy-$\Delta^{4:6}$-cholestadiene yielded cholestanyl acetate. The irradiation of $\Delta^{4:6}$-cholestadien-3(β)-ol (II) and of its acetate with ultra-violet light has been examined in collaboration with Mr. F. A. Robinson, M.Sc., F.I.C., and Glaxo Laboratories Ltd., but the products did not show vitamin-D activity.

Experimental.

Reduction of $\Delta^{4:6}$*-Cholestadien-3-one* (I).—A solution of 20 g. of $\Delta^{4:6}$-cholestadien-3-one (Dane, Wang, and Schulte, *loc. cit.*) and 20 g. of aluminium *iso*propoxide in 350 ml. of *iso*propyl alcohol was slowly distilled for 10 hours. The residue was taken up in 1500 ml. of ether, and 5% potassium hydroxide solution added with shaking until two homogeneous layers had been obtained. The ethereal layer was washed with water and dried over sodium sulphate, and the ether removed in a vacuum. The pale yellow residue was crystallised from aqueous acetone, yielding 11·5 g.

Addition Complex.—A portion of the reduction product was repeatedly crystallised from aqueous acetone and yielded the *addition complex* of (II) and its epimeride, m. p. 113° (Found:

C, 84·1; H, 11·3. $C_{27}H_{44}O$ requires C, 84·4; H, 11·4%). For the digitonin estimation 105 mg. of the addition complex were treated with 400 mg. of digitonin in 15 ml. of spirit, and the mixture refluxed for 15 minutes. After standing overnight, the digitonide was collected, washed with dry ether, and dried at 100°. Yield, 220 mg., corresponding to 51% of the β-epimeride.

$\Delta^{4:6}$-*Cholestadien*-3(β)-*ol* (II).—9 G. of the reduction product in 900 ml. of absolute alcohol were treated with 15 g. of digitonin in 1000 ml. of 95% alcohol, and the mixture refluxed for a few minutes. After standing overnight at 0°, the precipitated digitonide was collected, thoroughly washed with dry ether, and dissolved in 300 ml. of warm pyridine, and a large bulk of dry ether added. After removal of the precipitated digitonin the ethereal filtrate was washed with water and dried over sodium sulphate, and the solvent removed in a vacuum at 65°. The solid residue on crystallisation from aqueous acetone yielded fine needles of $\Delta^{4:6}$-cholestadien-3(β)-ol, m. p. 126—127°, $[\alpha]_D^{20°}$ − 38·0°, $[\alpha]_{5461}^{20°}$ − 38·0° (*c*, 0·954 in chloroform; *l*, 4), α_{5461}/α_D = 1·00. The compound did not react with either selenium dioxide or mercuric acetate in boiling alcohol. It was very unstable to acids. Dane and Wang (*loc. cit.*) have described the *m*-dinitrobenzoate, m. p. 194°, but attempts to repeat the preparation were not successful. The m. p. given by these authors corresponds to that of cholesteryl *m*-dinitrobenzoate.

3(β)-*Acetoxy*-$\Delta^{4:6}$-*cholestadiene*.—This was prepared by treating 300 mg. of the alcohol in 5 ml. of pyridine with 2·5 ml. of redistilled acetic anhydride for 12 hours at room temperature. The product was poured into water and extracted with ether. The ethereal solution was washed with water and dried over sodium sulphate, and the ether removed in a vacuum. The residue on crystallisation from acetone–methyl alcohol yielded clusters of needles of 3(β)-*acetoxy*-$\Delta^{4:6}$-*cholestadiene*, m. p. 78—79° (Found: C, 81·5; H, 10·8. $C_{29}H_{46}O_2$ requires C, 81·6; H, 10·9%), $[\alpha]_D^{20°}$ − 71·6°, $[\alpha]_{5461}^{20°}$ − 61·2° (*c*, 0·960 in chloroform; *l*, 4), α_{5461}/α_D 0·835. Yield, 200 mg. The ultra-violet absorption spectrum in absolute alcohol showed a broad band at 2390 A., $E_{1\,cm.}^{1\%}$ = 615.

The acetate (200 mg.) was hydrogenated by shaking in glacial acetic acid solution, in the presence of hydrogen, with 200 mg. of palladium–charcoal. The product, isolated in the usual way, yielded cholestanyl acetate (30 mg.) on crystallisation from aqueous alcohol, m. p. 109°, not depressed in admixture with an authentic specimen.

Oppenauer Oxidation.—50 Mg. of $\Delta^{4:6}$-cholestadien-3(β)-ol in 1 ml. of acetone and 3 ml. of benzene were refluxed for 12 hours with 100 mg. of aluminium *tert.*-butoxide. Benzene (4 ml.) was added, and the solution washed with 2N-sulphuric acid, with water until neutral, and dried over sodium sulphate. The benzene was distilled off in a vacuum, and the residual oil taken into solution with 50 mg. of 2 : 4-dinitrophenylhydrazine in 5 ml. of alcohol. On addition of one drop of concentrated hydrochloric acid to the boiling solution, the 2 : 4-*dinitrophenylhydrazone* of $\Delta^{4:6}$-cholestadien-3-one was precipitated (70 mg.). On purification from chloroform–alcohol it formed dark red needles, m. p. 231—232° (Found: N, 10·2. $C_{33}H_{46}O_4N_4$ requires N, 10·0%), not depressed in admixture with a sample prepared from authentic $\Delta^{4:6}$-cholestadien-3-one (Dane, Wang, and Schulte, *loc. cit.*).

The " epi-fraction " of the reduction product was obtained from the alcoholic filtrate of the digitonide (above) by removal of the solvent in a vacuum at a slightly raised temperature. It formed a yellow oil, from which a crystalline product could not be isolated. As the ultra-violet absorption spectrum in *cyclo*hexane showed a band at 2740 A., $E_{1\,cm.}^{1\%}$ = 157, and a subsidiary band at 2380 A., $E_{1\,cm.}^{1\%}$ = 130, dehydration had apparently occurred in the course of its isolation with the formation of a cholestatriene.

The author is indebted to Dr. F. H. Carr, C.B.E., and the British Drug Houses Ltd. for assistance with the digitonin separation, and to Miss B. E. Stern, B.Sc., (Glaxo Laboratories Ltd.) for the ultra-violet absorption data. Acknowledgment is made for grants from the Royal Society and the Chemical Society.

QUEEN MARY COLLEGE, LONDON, E. 1. [*Received, October 9th,* 1939.]