S 37. *Lanosterol. Part VII. The Position of the Hydroxyl Group in the Lanosterol Molecule.*

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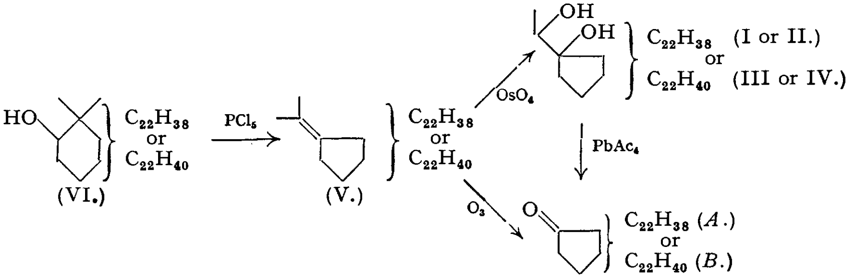
iso-" γ "-Lanostatriene and *iso*lanostadiene obtained by the dehydrating action of phosphorus pentachloride upon dihydroagnosterol (" γ "-lanosterol) and dihydrolanosterol respectively (cf. Part IV, *J.*, 1947, 1467), have been oxidised with osmium tetroxide and lead tetra-acetate to yield successively diols and ketones, together with high yields of acetone. The formation of acetone, also observed in the ozonolysis of the hydrocarbons, suggests the presence of an *iso*propylidene grouping in the molecule. The bearing of these results on the structure of lanosterol is discussed.

THE dehydration of alcohols of the lanosterol series to the corresponding hydrocarbons has recently been reported by us (cf. Part IV, *loc. cit.*), when the dehydrating action of both phosphorus pentachloride and phosphorus oxychloride was described. Dihydroagnosterol and dihydrolanosterol gave *iso*-" γ "-lanostatriene $C_{30}H_{48}$, and *iso*lanostadiene $C_{30}H_{50}$, repectively, and hydrocarbons isomeric with the above dehydration products were obtained with phosphorus oxychloride. We expressed the opinion, based on a consideration of several analogous cases in triterpene chemistry, that the phosphorus pentachloride dehydration presumably involved a retropinacolinic rearrangement accompanied by a contraction of the terminal ring A of lanosterol, and stated that experiments to confirm this view were in progress. In the meantime Ruzicka *et al.* (*Helv. Chim. Acta*, 1948, **31**, 818), following our method of preparation, have reported the results of oxidation experiments on the dehydration product from dihydrolanosterol, thus affording evidence in support of the reaction mechanism suggested above, and establishing at the same time the position of the secondary hydroxyl group in lanosterol. The continuation of our work had necessarily involved the study of the same series of reactions which were applied both to *iso*-" γ "-lanostatriene and *iso*lanostadiene. The results of our investigations, already briefly recorded elsewhere (*Nature*, 1949, **163**, 140), are summarised below.

iso-" γ "-Lanostatriene, when oxidised with osmium tetroxide in anhydrous ether, gave a mixture of two isomeric diols of formula $C_{30}H_{50}O_2$, which were separated by chromatographic analysis. Both *diol* (I) and *diol* (II) showed ultra-violet light absorption curves typical of members of the agnosterol series, with high-intensity maxima at 236, 245, and 251 mμ. Oxidation of both diols (I) and (II) with lead tetra-acetate in glacial acetic acid gave the same *ketone* (*A*), $C_{27}H_{42}O$, again exhibiting absorption maxima at the characteristic wave-lengths. At the same time acetone, isolated as the 2 : 4-dinitrophenylhydrazone, was formed in high yields. It was found advantageous in practice to isolate the ketone (*A*) and acetone from separate preparations: whilst only small quantities of the former substance were available after laborious purification from experiments in which the acetone had been removed by distillation, nearly theoretical yields of well-defined material resulted from experiments in which the longer heating had been omitted. The formation of the same ketone from diols (I) and (II) indicates that their isomerism is caused by a difference in the steric configuration of their hydroxyl groups. Application of the above reactions to *iso*lanostadiene gave analogous results. Oxidation of the hydrocarbon with osmium tetroxide gave two isomeric diols (III) and (IV), of formula $C_{30}H_{52}O_2$, each of which was subsequently converted into ketone (*B*), $C_{27}H_{44}O$. The properties and physical constants of the last three compounds were in essential agreement with those given by Ruzicka *et al.* (*loc. cit.*).

Further evidence for the presence of the *iso*propylidene grouping in *iso*-" γ "-lanostatriene and *iso*lanostadiene was obtained by ozonolysis. When treated with ozonised oxygen in anhydrous media, the hydrocarbons afforded ozonides which were decomposed by water to yield acetone, the ketone (*A*) or (*B*), and small quantities of unidentified acidic products.



The formation of acetone, (1) when the diols obtained by osmium tetroxide oxidation of *iso*-"γ"-lanostatriene and *iso*lanostadiene are further oxidised with lead tetra-acetate, and (2) by the direct ozonolysis of the hydrocarbons, indicates the presence of a terminal *iso*propylidene grouping in these hydrocarbons. The pinacolinic rearrangement occurring when triterpenoid alcohols are dehydrated with phosphorus pentachloride (Ruzicka *et al.*, *Helv. Chim. Acta*, 1945, **28**, 767, 942, 1628) thus takes place also in the analogous formation of *iso*-"γ"-lanostatriene and *iso*lanostadiene. As previously shown by Ruzicka (*loc. cit.*) this rearrangement presupposes the location of the hydroxyl group of the original alcohol in a terminal ring in a position adjacent to the *gem.*-dimethyl groups. The structure of the terminal hydroxyl-bearing ring of dihydroagnosterol and dihydrolanosterol, and hence lanosterol itself, can therefore be represented by the partial formula (VI).

EXPERIMENTAL.

Melting points are uncorrected. Optical rotations were determined in chloroform solution at 19°. The authors are indebted to Dr. E. A. Braude for the ultra-violet absorption measurements.

iso-"γ"-Lanostatriene, prepared as described in Part IV (*loc. cit.*) and purified by several crystallisations from chloroform–methanol, formed colourless lustrous elongated plates, m. p. 136—137° (Found: C, 88·18; H, 11·80. Calc. for $C_{30}H_{48}$: C, 88·23; H, 11·76); $[\alpha]_D$ +38·7°; +40·4° (*c*, 0·606; 0·540). The $[\alpha]_D$ value was erroneously reported in Part IV as 11°.

Diol (I) *and Diol* (II).—A solution of *iso*-"γ"-lanostatriene (2·5 g.; 1 mol.) in anhydrous ether (100 ml.) was treated with osmium tetroxide (1·8 g.; 1·1 mols.) and the brown liquid left at room-temperature for 6 days. After removal of the solvent, the residue was taken up in benzene (40 ml.) and refluxed for 3 hours with a solution of mannitol (8 g.) and potassium hydroxide (8 g.) in water–ethanol (20 and 40 ml. respectively), the two phases of the mixture being mixed by occasional shaking. The liquid was quickly evaporated to small volume (40 ml.) under reduced pressure, the residue poured into water (200 ml.), and the product isolated by extraction with benzene. The washed benzene extracts were distilled to small volume (40 ml.), the solution diluted with light petroleum (b. p. 60—80°; 200 ml.), and the products absorbed on alumina (100 g., P. Spence, grade H).

Elution of the column with benzene–light petroleum (b. p. 60—80°) (1 : 1; 5 × 500 ml.) and removal of the solvent gave a solid residue, which was recrystallised several times from acetone, with addition of a few drops of water, and yielded lustrous plates of *diol* (I), m. p. 142—143° (Found: C, 80·97; H, 11·47. $C_{30}H_{50}O_2$ requires C, 81·45; H, 11·31%); $[\alpha]_D$ +46·5° (*c*, 0·493). Ultra-violet absorption spectrum (in *cyclo*hexane): maxima at 236, 244, and 251 mμ ($E^{1\%}_{1\,\text{cm.}}$ = 395, 440, and 330 respectively).

Further elution of the alumina column with benzene (3 × 500 ml.) and benzene–chloroform (1 : 1; 500 ml.) resulted in a product which gave, on crystallisation from aqueous acetone, the microcrystalline *diol* (II), $C_{30}H_{50}O_2$, m. p. 128—130° (Found: C, 80·68; H, 11·31%); $[\alpha]_D$ +54·3° (*c*, 0·774). Ultra-violet absorption spectrum: maxima at 235, 245, and 251 mμ ($E^{1\%}_{1\,\text{cm.}}$ = 390, 435, and 330 respectively). Exhaustive elution of the column with chloroform gave a small quantity of a yellow oil.

Ketone (*A*).—Diol (I) (1·6 g.; 1 mol.) was dissolved in chloroform (5 ml.) and glacial acetic acid (50 ml.), and treated with lead tetra-acetate (3·2 g.; 2 mols.). The resulting yellow liquid was left overnight at room temperature, briefly heated to 60°, and poured into water (400 ml.), and the product exhaustively extracted with ether. The combined ethereal extracts were washed until neutral with sodium hydroxide solution and water, the ether distilled off, and the crude product purified by filtration of its benzene solution through alumina (15 g.). The solid residue obtained after removal of the solvent gave, on two crystallisations from chloroform–aqueous methanol, *ketone* (*A*) as a silky mass of lustrous needles, m. p. 85—87° (Found: C, 84·51; H, 10·59. $C_{27}H_{42}O$ requires C, 84·82; H, 10·99%); $[\alpha]_D$ +301° (*c*, 0·540). Ultra-violet absorption spectrum (in *cyclo*hexane): Maxima at 236, 242, and 251 mμ ($E^{1\%}_{1\,\text{cm.}}$ = 360, 360, and 360 respectively).

Diol (II), when oxidised with lead tetra-acetate by the same procedure, gave the identical ketone *A*, m. p. 85—86°, $[\alpha]_D$ +296° (*c*, 0·608).

Ketone (*A*) *semicarbazone*. A solution of ketone (*A*) (1 g.) and anhydrous sodium acetate (1 g.) in ethanol (150 ml.) was treated with semicarbazide hydrochloride (0·6 g.) in water (1 ml.) and refluxed for 2 hours. After distillation to small bulk the residual liquid was poured into water (100 ml.), and the product extracted with benzene and purified by filtration through alumina (10 g.). Two crystallisations from aqueous ethanol gave the *semicarbazone* as a white microcrystalline powder, m. p. 264° (decomp.) (Found : N, 9·23. $C_{28}H_{45}ON_3$ requires N, 9·57%).

Isolation of acetone. In separate experiments, diol (I) and diol (II) (1 g.; 1 mol.), contained in closed 50 ml.-distilling flasks, were oxidised with lead tetra-acetate (2 g.; 2 mols.). After 6 hours at room temperature, water (15 ml.) was added, and the mixture partly distilled, two fractions of distillate (20 ml. each) being collected (residue, *X*). The first fraction was treated with a solution of 2 : 4-dinitrophenylhydrazine (0·5 g.) in pure methanol (20 ml.) and sulphuric acid (1 ml.); after 24 hours at room temperature, well-defined flat orange blades of acetone 2 : 4-dinitrophenylhydrazone, m. p. 124—126°, were obtained (dry weight, 250 mg.), giving no depression of m. p. with authentic material. The orange filtrate gave, on dilution with water, extraction with ether, and crystallisation from methanol, a further crop (85 mg.). The second fraction of the distillate gave on similar treatment only traces of orange needles, m. p. 122—125°. The residue (*X*), when worked up in the usual manner, gave a product from which small yields of ketone (*A*), m. p. 85—87°, were isolated after chromatographic purification.

In order to calculate the yield of acetone obtained under the conditions of the above experiment, a mixture of acetone (0·135 g., corresponding to 1 g. of diol), chloroform (5 ml.), and glacial acetic acid (50 ml.) was slowly distilled, and two fractions of distillate (20 ml. each) collected. Addition of a solution of 2 : 4-dinitrophenylhydrazine sulphate (quantities as above) to each fraction, and isolation of the product as previously described, gave a total of 0·45 g. of acetone 2 : 4-dinitrophenylhydrazone, thus indicating a 74% yield of acetone in the lead tetra-acetate oxidation of the diol. Absence of acetone in the solvent used was confirmed by leaving a solution of 2 : 4-dinitrophenylhydrazine in methanol–sulphuric acid for 48 hours, by which time no solid had been formed.

Diol (III) *and Diol* (IV).—A solution of *iso*lanostadiene (3·2 g.) in anhydrous ether (120 ml.) was treated at room temperature with osmium tetroxide (2·3; 1·1 mols.) for 5 days, and the crude product isolated as previously described. Chromatographic separation of the material absorbed on an alumina column (120 g.; Spence, grade H) using benzene–light petroleum (b. p. 60—80°) (1 : 1 ; 5 × 500 ml.) and benzene (4 × 500 ml.) gave two series of eluates, from which diol (III), m. p. 144—145°, $[\alpha]_D$ +54° (*c*, 0·723) (Found : C, 80·78; H, 11·56. Calc. for $C_{30}H_{52}O_2$: C, 81·08; H, 11·71%), and diol (IV), m. p. 117—119°, $[\alpha]_D$ +28° (*c*, 438) (Found : C, 81·25; H, 11·60%), were isolated by crystallisation from aqueous acetone.

Ketone (*B*).—To a solution of diol (III) (1 g.; 1 mol.) in chloroform (5 ml.) and glacial acetic acid (40 ml.), lead tetra-acetate (2 g.; 2 mols.) was added, the solution left for 6 hours at room temperature, and the product isolated as previously described. Crystallisation from chloroform–aqueous methanol gave long silky needles of ketone (*B*), m. p. 81—83°, $[\alpha]_D$ +196° (*c*, 0·480) (Found : C, 84·21; H, 11·23. Calc. for $C_{27}H_{44}O$: C, 84·38; H, 11·46%). No absorption in the near-ultra-violet.

In a separate experiment, acetone was isolated in 68% yield, as previously described, as the 2 : 4-dinitrophenylhydrazone, m. p. 125—126°.

Ketones A and B. A solution of *iso*-"γ"-lanostatriene (2 g.) in carbon tetrachloride (60 ml.) was treated with excess of ozonised oxygen at 0° for 2 hours. After removal of the solvent under reduced pressure, the residual oily ozonide was decomposed by refluxing it with water (150 ml.) for 1 hour, and the resulting reaction mixture was partly distilled, two fractions of distillate (40 ml. each) being collected. (residue, *Y*). Treatment with a solution of 2 : 4-dinitrophenylhydrazine (0·8 g.) in water (10 ml.)–sulphuric acid (1 ml.) caused deposition, after several hours, of an orange solid (320 mg.), which gave, on crystallisation from aqueous methanol, acetone 2 : 4-dinitrophenylhydrazone, m. p. 125—126°, not depressed by admixture with authentic material. The residues *Y* were diluted with water and extracted with ether, and the ethereal extracts were washed with sodium hydroxide and water until neutral. The solvent was removed, and the benzene solution of the residue purified by filtration through alumina (12 g.). Final crystallisation from chloroform–aqueous methanol gave small yields of ketone (*A*), m. p. 84—86° (Found : C, 84·41; H, 10·62%). Acidification of the alkaline washing liquids gave small quantities of an acidic product which was not further investigated.

Ozonolysis of *iso*lanostadiene as described above gave acetone (isolated as the 2 : 4-dinitrophenylhydrazone) and ketone (*B*), m. p. 81—82°.

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