CXXVII.—*Cholestenone.*

By CHARLES DORÉE and JOHN ADDYMAN GARDNER.

CHOLESTEROL, which is an unsaturated secondary alcohol of the formula $C_{27}H_{46}O$, may be oxidised, under suitable conditions, to an unsaturated ketone, cholestenone, $C_{27}H_{44}O$ (Diels and Abderhalden, *Ber.*, 1904, **37**, 3092; Windaus, *Ber.*, 1906, **39**, 518). Although the $CH\cdot OH$ group of the cholesterol is converted to a carbonyl group in cholestenone, the behaviour of the two substances under the influence of reducing agents (*Ber.*, 1906, **39**, 884; 1908, **41**, 260) makes it doubtful whether their "carbon-skeleton" is identical in each case. In 1906 Windaus (*Ber.*, **39**, 2008) studied the oxidation of cholestenone, using neutral permanganate which, according to Tiemann and Semmler (*Ber.*, 1895, **28**, 1345), is less likely than other oxidising agents to produce intramolecular change. He obtained as the chief product a saturated keto-monocarboxylic acid, $C_{26}H_{42}O_3$, which contained one carbon atom less in the molecule than the parent substance, and which, on further oxidation, passed into a tricarboxylic acid, $C_{26}H_{42}O_6$.

These changes were, according to Windaus (*loc. cit.*), most simply explained by assuming that cholestenone contains a group, $CH:CH_2$, which is situated at the end of an open side-chain, so that the production of the acid may be represented by the following equation:

The tricarboxylic acid would then result by the rupture of the ring containing the carbonyl group thus:

The correctness of this explanation is based on the analyses of the

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ketonic acid and its derivatives, and their recognition as C_{26} compounds, a point which is somewhat difficult to settle by combustion alone; for example, Windaus found for the keto-carboxylic acid the figures $C = 77.18, 77.75$; $H = 11.14, 11.18$. These values agree equally well with the formula $C_{26}H_{42}O_3$, which requires $C = 77.55$, $H = 10.52$, and with $C_{25}H_{40}O_3$, which requires $C = 77.26$ and $H = 10.38$ per cent.

When the analyses of all the derivatives prepared are considered together, the C_{26} carbon content appears the more preferable, but as the modern view of the formula of cholesterol and the interpretation given to the many derivatives obtained from it by oxidation is based on the work referred to, it seemed to us desirable to submit the question to a further test.

Harries has shown (*Annalen*, 1906, **343**, 311) that unsaturated alcohols, when submitted to the action of ozone, combine with one molecule of ozone to form ozonides, whilst unsaturated ketones absorb four atoms of oxygen, namely, three at the ethylene linking and one at the carbonyl group. These ozonides on treatment with water decompose as follows:

aldehydes or ketones and hydrogen peroxide being formed. If Windaus's formula is correct, cholestenone should absorb four atoms of oxygen under the influence of ozone, and the ozonide formed should react with water in the following way:

These aldehydes would be at once oxidised by the hydrogen peroxide, and we should expect to find among the final products a C_{25} acid and probably either formic or carbonic acid derived from the form-aldehyde.

The action takes place broadly on the lines predicted, and we obtained as chief products the C_{26} ketonic acid described by Windaus, and carbon dioxide. Cholesterol, on similar treatment, formed an ozonide which also gave carbon dioxide on decomposition.

EXPERIMENTAL.

Ozonide of Cholestenone.—Ten grams of cholestenone were dissolved in 200 c.c. of dry chloroform, and a slow current of ozonised oxygen was bubbled through the solution until the ozone ceased to be absorbed and escaped freely. The chloroform was allowed to evaporate spontaneously, and the viscid oil left behind was dissolved in the

least quantity of ethyl acetate and poured into a large excess of light petroleum. A considerable proportion of the ozonide separated in a soft, resinous form, which was again dissolved and reprecipitated. The substance was placed in a vacuum desiccator and finally obtained as a colourless, glassy solid which was readily powdered. Two different preparations on analysis gave the following figures :

- I. 0.1767 gave 0.4224 CO₂ and 0.1458 H₂O. C = 65.29 ; H = 9.17.
 II. 0.1781 „ 0.4424 CO₂ „ 0.1459 H₂O. C = 67.75 ; H = 9.10.
       C₂₇H₄₄O·O₆ requires C = 67.50 ; H = 9.17.
       C₂₇H₄₄O·O₇ „ C = 65.40 ; H = 8.87 per cent.

These results seem to show that six or seven oxygen atoms are absorbed by the cholestenone molecule. We should have expected four atoms to be added in the case of a singly unsaturated ketone. It may be, however, that cholestenone contains a second ethylene linking which is not rendered apparent by the usual reagents.

Decomposition of the Ozonide by Water.—It was not, for subsequent experiments, found necessary to prepare the pure ozonide as above. Instead, the chloroform solution of the ozonide was mixed with about twenty times its volume of water and heated on the water-bath in a current of purified oxygen. The gases evolved were passed through measured quantities of standard baryta solution. The action was slow, the evolution of carbon dioxide continuing for many hours. The aqueous liquid remaining in the flask was milky, acid to litmus, and had a peculiar odour of orange oil which we have previously noticed in the course of some experiments on the oxidation of cholesterol with hydrogen peroxide. The solid product was left as a brown oil, which, on cooling, solidified to a brittle mass. This was dissolved in ether and the ethereal solution shaken with sodium carbonate to separate the acid products from unchanged cholestenone or other neutral substances.

The alkaline liquors were acidified when the acids separated in a thick clot. This, after drying, was extracted with light petroleum, which removed some oily, non-crystalline material and left the main product of the reaction in the form of a white powder, the yield being 25 per cent. of the original cholestenone. It was purified by recrystallisation from acetone and from a mixture of light petroleum and benzene, and was obtained from the latter solvent in leaves which melted at 155° :

- 0.2179 gave 0.6154 CO₂ and 0.2082 H₂O. C = 77.02 ; H = 10.62.
       C₂₆H₄₂O₃ requires C = 77.55 ; H = 10.52 per cent.

The quantity of carbon dioxide evolved in these experiments was always very much less than would correspond with the oxidation of a terminal CH₂ group to carbon dioxide. Thus 0.6745 of pure ozonide

gave 0.00649 of carbon dioxide, or about 1 per cent., whilst theory requires 9.8 per cent. In other experiments also the quantity obtained was about one-tenth part of that theoretically required. It seemed likely that this was due to the preferential oxidation of the C_{26} aldehydic substance, presumably formed, by the hydrogen peroxide. To test this we decomposed the crude product obtained by ozonising 10 grams of cholestenone, in the presence of very dilute hydrogen peroxide and sulphuric acid. The yield of carbon dioxide now obtained agreed very well with the explanation above suggested.

0.268 Gram of carbon dioxide was obtained together with 2.30 grams of the acid $C_{26}H_{42}O_3$. The quantity of carbon dioxide theoretically produced with this quantity of acid is 0.245 gram. The neutral products of the reaction contained a considerable quantity of unchanged cholestenone.

Identification of the Acid $C_{26}H_{42}O_3$.—The acid obtained by us agrees in all its properties with that obtained by Windaus by the oxidation of cholestenone with permanganate. On titration:

0.2571 required 6.37 c.c. *N*/10 sodium hydroxide. Equivalent = 403.
 $C_{26}H_{42}O_3$, monobasic, requires 402.

The *oxime* was prepared according to the directions given by Windaus, and was obtained from ethyl acetate in needles which melted and decomposed at 191° :

0.1794 gave 0.4928 CO_2 and 0.1802 H_2O . $C = 74.86$; $H = 11.16$.
 $C_{26}H_{43}O_3N$ requires $C = 74.75$; $H = 10.33$ per cent.

This acid formed a beautifully crystalline *potassium* salt.

On oxidising the keto-acid with potassium hypobromite, an acid was obtained which after crystallisation from benzene sintered at 127° and melted at 130° with gas evolution. It was deliquescent. On titration:

0.2240 required 14.0 c.c. *N*/10 sodium hydroxide. Equivalent = 160.
 $C_{26}H_{42}O_6$, tribasic, requires 150.

The keto-acid therefore on further oxidation gives the tricarboxylic acid $C_{26}H_{42}O_6$.

In all these particulars our acid corresponded with the ketomono-carboxylic acid described by Windaus.

Action of Ozone on Cholesterol.—Ten grams of anhydrous cholesterol were ozonised in chloroform solution as described above. The product was purified by solution in ethyl acetate and precipitation with light petroleum. It dried in a vacuum to a perfectly transparent glass which readily powdered. It had all the properties of an ozonide. When heated on platinum it enflamed, and it liberated iodine from potassium iodide solution:

0.1796 gave 0.4896 CO₂ and 0.1687 H₂O. C = 74.35, [View Article Online](#) H = 10.44.

C₂₇H₄₆O·O₃ requires C = 74.66; H = 10.59 per cent.

It thus appears that the ozonide is formed by the addition of three atoms of oxygen at the ethylene linking of the alcohol in the normal manner.

Rotation.—0.9647 gram made up to 25 c.c. with chloroform gave $\alpha + 1.12^\circ$ in a 2-dm. tube; hence $[\alpha]_D^{20} + 14.51^\circ$. The rotation of cholesterol is $[\alpha]_D^{20} - 36^\circ$.

On treatment with water in a manner similar to that described under cholestenone, the aqueous distillate had an odour of orange oil, was acid, and after neutralisation and evaporation gave a little solid matter which strongly reduced ammoniacal silver solution and probably consisted of barium formate. In the absorption vessels containing the baryta a copious precipitate of barium carbonate was formed, which was collected and identified in the usual way. The water remaining in the flask was milky. It was acid, and gave the magenta test for aldehydes. The solid left was a brown resin, which dissolved in alkalis and gave precipitates with salts of the heavy metals. We have, however, not as yet been able to separate any of the constituents in a state of purity, but we are carrying out other experiments on derivatives of cholesterol which we hope will throw some light on these compounds.

Conclusions.

These experiments on cholestenone fully confirm the hypothesis of Windaus that this ketone contains an unsaturated side-chain with the double link in a vinyl group. If the relationship of cholestenone to cholesterol is that of a simple ketone to its secondary alcohol, then the latter substance must contain a similar unsaturated side-chain. This is confirmed by our observation that cholesterol forms an ozonide, which on decomposition with water yields carbon dioxide. Direct experimental proof of this point has hitherto been lacking if we except the somewhat complex reduction of cholestenone to cholesterol through an isomeric β -cholesterol, recently carried out by Diels and Linn (*Ber.*, 1908, **41**, 260).

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PHYSIOLOGICAL LABORATORY,
UNIVERSITY OF LONDON.