LV. ON THE OXIDATION OF COPROSTEROL AND COPROSTANONE.

PART I.

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Dorée and Gardner [1908] showed that coprosterol, $C_{27}H_{48}O$, was readily oxidised on treatment with the theoretical quantity of chromic acid with the production of the corresponding ketone, coprostanone, $C_{27}H_{46}O$. This coprostanone crystallised in glistening leaves which, under the microscope, appeared to consist of thin plates, generally square, with one or all of the corners slightly truncated. It melted at $62-63^{\circ}$ to a clear liquid. It yielded an amorphous semicarbazone, melting at 192° , and an amorphous oxime, melting at 71° . On treatment with phenylhydrazine it behaved in an abnormal manner and yielded a crystalline derivative, melting at 192° , which was subsequently shown by Dorée [1909] to be coprosteryl-carbazole, formed from the coprostanone-phenylhydrazone with elimination of ammonia. As the yield of pure coprostanone in the above preparation was only 60%, it appeared desirable to submit the reaction to further investigation.

Oxidation of coprosterol by chromic acid. To make sure of oxidising the whole of the coprosterol, a considerable excess of chromic acid was used—three times the amount taken by Dorée and Gardner.

10 g. coprosterol dissolved in 130 cc. of glacial acetic acid, and heated to 70° , were treated in the course of 1 hour with a solution of 8.5 g. chromic anhydride in a little dilute acetic acid, the temperature being maintained by the heat of the reaction. After adding the chromic acid, the liquid was heated on the water bath for one hour, and then diluted with water. The bulk of the acetic acid was next neutralised with sodium carbonate, and the still strongly acid liquor was thoroughly extracted with ether. The ethereal solution was now thoroughly extracted with dilute caustic soda, and on evaporating the ether nearly pure coprostanone was obtained in a yield in all experiments of about 70 per cent.

The caustic soda extracts on acidification with hydrochloric acid gave a white crystalline precipitate of an acid mixed with an oily substance. The oil was got rid of by washing with petroleum ether, in which the crystals were insoluble. During the extraction of the ethereal solution of the oxidation product with caustic soda a small quantity of an insoluble basic chromium salt also separated, and this on treatment with hot hydrochloric acid yielded a further quantity of the crystalline acid. This acid is soluble in ether, but only difficultly soluble in benzene and in chloroform. It is sparingly soluble in hot acetone and somewhat more readily in boiling ethyl acetate or alcohol. It can be readily purified by recrystallisation from a mixture of ether and acetone or from ethyl acetate. It usually separates as a fine powder, and under the microscope appears in the form of well-defined square rods. It melts at 247° to a clear liquid, without any decomposition. For analysis the substance was dried in vacuo. It proved rather difficult to burn.

(1) 0.2054 g.; 0.5604 CO_2 ; 0.1934 H_2O .

(2) 0.2433 g.; 0.6628 CO_2 ; 0.2358 H_2O .

Found (1) C=74.41; (2) 74.30. Calc. for $\text{C}_{27}\text{H}_{44}\text{O}_4$, C=74.94; $\text{C}_{27}\text{H}_{46}\text{O}_4$, C=74.59.
H=10.46; 10.76. H=10.26; H=10.67.

In other combustions in which the carbon came low, the hydrogen was 10.38, 10.23 and 10.63.

Sodium salt. On titration with caustic soda in alcoholic solution 0.6304 g. required 14.43 cc. N/5 NaOH. With another specimen 0.3134 g. required 7.24 cc. N/5 NaOH. Mol. wt. found (1) 436.8, (2) 432.8. Calc. for $\text{C}_{27}\text{H}_{46}\text{O}_4$, 434.

Ammonium salt. The acid dissolved in strong ammonia giving a soapy solution. The bulk of the free ammonia was boiled off, and on cooling the liquid set to a perfectly clear stiff jelly. The jelly dried up in vacuo, with gradual evolution of ammonia, to a white amorphous mass, which dissolved in hot water giving a slightly opalescent soapy solution. For analysis the substance, dried finally at 100° , was decomposed by excess of standard acid, filtered and the acid titrated back with soda. 0.3157 g. was decomposed with 25 cc. N/5 sulphuric acid and the excess of acid required 42.5 cc. soda. 25 cc. N/5 acid = 49.2 soda; percentage of N=3.19. Calc. for $\text{C}_{27}\text{H}_{46}\text{O}_4\text{N}$ =3.1%.

Silver salt. This was prepared by precipitating a neutral solution of the sodium salt with silver nitrate. The white precipitate was well washed with water, in which it was quite insoluble, and dried at 100° .

(1) 0.298 gave on ignition 0.0998 g. of silver.

(2) 0.1467 ,, ,, 0.0489 ,, ,,

Mol. wt. of acid found (1) 430.6. Calc. for $\text{C}_{27}\text{H}_{46}\text{O}_4$, 434.
(2) 433.8.

From the analysis of these salts and the combustions of the acid itself, the acid appears to be a dibasic acid of the formula $C_{27}H_{46}O_4$.

The oil formed along with this acid and dissolved away by petroleum ether as described above, would not crystallise on long standing. It was an acid and formed an insoluble barium salt on precipitating the solution in ammonia with barium chloride. This substance has not yet been further investigated.

Oxidation of coprostanone by chromic acid. In order to ascertain whether the acid $C_{27}H_{46}O_4$ was a bye-product in the preparation of coprostanone, or formed by the further oxidation of the coprostanone by the excess of chromic acid, one gram of coprostanone was dissolved in glacial acetic acid and heated to 70° with 0.35 gram of chromic anhydride dissolved in dilute acetic acid until all the red colour had gone. It was then heated to boiling, cooled and poured into water. The turbid liquid was extracted with ether, and on evaporating the ether a green oil containing chromium was obtained. This was freed from chromium by warming with hydrochloric acid on the water bath. The liquid was then extracted with ether, the ethereal solution evaporated and the brown oil left taken up with petroleum ether. The petroleum was allowed to evaporate spontaneously and left a brown oil, which on long standing in a desiccator deposited some crystalline matter. The oil was dissolved away by petroleum ether, and the crystalline matter recrystallised from hot ethyl acetate. It melted at 247° and was identical with the acid described above. The yield was however small. The oil dissolved by the petroleum was acid in character and formed an insoluble barium salt.

Oxidation of coprostanone by ammonium persulphate. As the yield of acid $C_{27}H_{46}O_4$ was small by both methods of preparation, we thought it desirable to try other oxidising agents and ultimately selected ammonium persulphate. This reagent is known to act on cyclic ketones [Baeyer and Villiger, 1899] breaking the ring and changing them to hydroxy-acids or their lactones; and we hoped in this way to break the ring of the coprostanone at the CO group and obtain a hydroxy-acid which might on further oxidation give the dibasic acid $C_{27}H_{46}O_4$. 10 g. coprostanone were dissolved in 375 cc. of glacial acetic acid, and to the solution were added 10 g. ammonium persulphate, dissolved in the least possible amount of water and diluted with an equal volume of glacial acetic acid. The mixture was then heated on a water bath and another 10 g. of powdered ammonium persulphate gradually added. The heating was continued for four hours, during which gas was slowly evolved. This gas was mainly oxygen from the persulphate, but contained some carbon dioxide. After standing several days the liquid was

diluted with an equal volume of water and thoroughly extracted with ether. The ethereal solution was repeatedly shaken with water to get rid of the bulk of the dissolved acetic acid, and then extracted with 10 % caustic soda solution several times, until a sample of the alkaline extract no longer gave a precipitate on acidification. The soda solution on shaking with the ethereal extract turned dark brownish red in colour. The various caustic soda extracts were mixed and on standing overnight bulky gelatinous clots of a colourless sodium salt separated out (A). These were somewhat difficult to filter, but it was found possible to syphon off the greater portion of the mother liquor. The mother liquors and filtrates, which were now pale yellow, on acidification with hydrochloric acid gave an amorphous precipitate of an acid substance (B). The ethereal solution, freed from acid matter, gave on evaporation a small quantity of a neutral substance (C).

Examination of (A). The clots were readily soluble in hot water giving a clear soapy solution, from which they separated on cooling, but more readily if the solution was made strongly alkaline. On acidifying the aqueous solution with hydrochloric acid a crystalline precipitate was thrown down which, in the preparation described, amounted to about 65 % of the weight of the coprostanone taken. In different preparations, however, the relative amounts of (A), (B) and (C) varied considerably with the conditions of reaction.

A small quantity of the clotted matter was filtered on the pump, washed with cold water to get rid of excess of alkali and further purified by means of alcohol. It was obtained as a white powder perfectly soluble in hot water. For analysis it was dissolved in water and precipitated by a measured quantity of standard acid. The precipitate was filtered, well washed and the filtrates titrated with alkali.

0.2488 g. was found to contain sodium equivalent to 6.1 cc. N/10 caustic soda; percentage sodium = 5.64. Calc. for $C_{27}H_{44}O_3Na$ = 5.21.

The crystalline precipitate obtained on acidifying the aqueous solution of this sodium salt can be separated into two substances by fractional crystallisation from ethyl acetate or better from alcohol. The less soluble substance, present in small quantity, crystallised in thin plates and after several crystallisations melted at 183–184°. The more soluble substance, constituting the bulk of the material, crystallised in minute needles and melted at 157–158°.

Substance melting at 157–158°. This body was easily soluble in ethyl acetate, acetone and benzene, but somewhat less so in alcohol. It was sparingly soluble in petroleum ether, but more soluble in the fraction of

petroleum boiling at 156–195°. On combustion the following results were obtained :—

- (1) 0.1549; 0.4568 CO₂; 0.1595 H₂O.
- (2) 0.1571; 0.4625 „; 0.1617 „
- (3) 0.1640; 0.4834 „; 0.1680 „
- (4) 0.2557; 0.7503 „; 0.2627 „

	I	II	III	IV	Calc. for C ₂₇ H ₄₆ O ₃
C	80.42	80.29	80.40	80.02	C 80.52
H	11.44	11.43	11.38	11.41	H 11.50

Combustion does not however throw much light on the number of carbon atoms in the molecule, as decrease of the molecular weight by CH₂ makes very little difference in the percentage composition thus—

			C	H
C ₂₇ H ₄₆ O ₂	...	...	80.52	11.50
C ₂₆ H ₄₄ O ₂	...	...	80.34	11.42
C ₂₅ H ₄₂ O ₂	...	...	80.14	11.31
C ₂₄ H ₄₀ O ₂	...	...	79.93	11.19

The molecular weight was therefore determined by depression of the freezing point of benzene :—

- (1) 0.2975 g. in 20 cc. benzene of sp. gr. 0.883, depression of freezing point 3.115° to 2.915°.
- (2) 0.5993 g. in 15 cc. benzene of sp. gr. 0.883, depression of freezing point 3.115° to 2.565°.

Molecular weight found	I 412.7.	Calc. for C ₂₇ H ₄₆ O ₂ , 402.37.
	II 403.1.	

The substance did not go into solution on boiling for a short time with an aqueous solution of caustic soda, nor on shaking a solution in ether with either 10, 20 or 30 % caustic soda, though as originally obtained in the acetic acid solution it did so quite readily. It apparently contained no OH group, as it was quite unaffected by prolonged boiling with either acetic anhydride or acetyl chloride, and on pouring into water and extracting with ether it was recovered unchanged in both cases. It was probably a very stable lactone of a hydroxy-acid of the formula C₂₇H₄₈O₃.

Examination of (C). The neutral substance left in the ether after extraction with caustic soda, can be readily purified by recrystallisation from alcohol, or ethyl acetate, or petroleum ether. It melted at 183–184° and was identical with the substance of the same melting point obtained along with the lactone melting at 157–158°. This was proved by a mixed melting point. It crystallised in long thin plates, or glistening leaves, which under the microscope appeared to be rectangular plates, some square, some long. It proved difficult to burn and on combustion gave the following results :—

- (1) 0.1197; 0.3514 CO₂; 0.1235 H₂O.
 (2) 0.1720; 0.5052 „ ; 0.1672 „
 (3) 0.1455; 0.4264 „ ; —
 (4) 0.1580; 0.4615 „ ; 0.1594 „

	I	II	III	IV
C	80.06	80.11	79.92	79.66
H	11.46	10.80	—	11.21

0.8365 g. in 15 cc. benzene of sp. gr. 0.883, depression of freezing point 0.76°. Molecular weight found, 407; calc. for C₂₇H₄₆O₂, 402.37. The substance was not affected by shaking in ethereal solution with either 10, 20 or 30% caustic soda solution.

The analyses threw no light on the relationship of the substance to the lactone melting at 157–158°. The molecular weight determination perhaps suggests that it is a stable lactone isomeric with the 157–158° body. It may however be a lactone of lower molecular weight produced by further oxidation, or possibly some derivative of a ketonic or diketonic nature. To test this 0.95 g. of the lactone (M.P. 156°) was dissolved in glacial acetic acid and 5.7 cc. of 10% chromic acid solution added. The mixture was kept at 70° for half an hour, and then the temperature was raised to the boiling point for a few minutes. The liquid was then poured into cold water and the precipitated matter filtered off. The filtrate was extracted with ether and a further quantity of solid obtained, which was added to the precipitate. This precipitate was crystallised from alcohol from which it separated in needles and plates, melting at 169–171°. This weighed 0.38 g. or 40% of the weight of the original substance. After several recrystallisations it melted at 183–184°. This was shown by a mixed melting point determination to be identical with the substance melting at 183–184° obtained from (C). The lactone (M.P. 157–158°) appears therefore to yield this neutral substance melting at 183–184° on oxidation. The latter body itself on similar treatment did not appear to be further oxidised, and was recovered unchanged. It did not appear to have ketonic properties for the following reasons: (1) sodium amalgam had no effect on the solution of the substance in moist ether in presence of sodium bicarbonate; (2) in attempts to prepare a semicarbazone in the usual manner, the substance was recovered unchanged; (3) on heating 1 mol. of the substance in alcoholic solution with 6 mols. of hydroxylamine hydrochloride and the equivalent amount of dry sodium acetate for 20 hours, the substance was recovered quantitatively and unchanged. On repeating experiment (3) however using caustic soda instead of sodium acetate, needle-shaped crystals were obtained on evaporating the alcohol. These melted at 268–274°. This was not however an oxime as it contained only a trace of

nitrogen—under 1%. The same or a very similar substance, melting at 264–286°, was also obtained on substituting phenylhydrazine hydrochloride for the hydroxylamine in the above experiment. This contained about 0.9% of nitrogen. We had not sufficient material for the further investigation of these substances. It would seem probable however from these results that the substance of M.P. 183–184° is a stable lactone either isomeric with or containing less carbon than the lactone of M.P. 157–158°.

Examination of substance (B). The acid precipitated on acidifying the alkaline solution was a light brown amorphous resin-like substance, readily soluble in alkalis giving a soapy solution. It was easily soluble in the common organic solvents, but would not crystallise. From petroleum ether it dried up to a brittle resin. It was dissolved in ammonia and reprecipitated by hydrochloric acid several times, and well washed. After drying it melted at 88° to a stiff paste and decomposed at 114°. The yield was only small.

Barium salts. The resinous acid was dissolved in ammonia, the excess of alkali exactly neutralised by hydrochloric acid and the solution precipitated with baryta water. The precipitate, after being well washed with water, dried to a light brown powder. This was readily soluble in chloroform, and when the solvent was allowed to evaporate spontaneously separated on the sides of the beaker in light brown brittle flakes. On analysis—

- (1) 0.1367; 0.0327 BaSO₄.
 (2) 0.2173; 0.0494 „
 (3) 0.3541; 0.8468 CO₂; 0.3047 H₂O.

	Ba	C	H
I	14.11	—	—
II	13.50	—	—
III	—	65.22	9.56
Calc. for C ₂₇ H ₄₅ O ₄ Ba _{1/2}	13.68	64.53	9.03
Calc. for C ₆₄ H ₉₅ O ₇ Ba	13.83	65.25	9.64

The filtrate obtained after precipitating with baryta in the preparation of the above salt was acidified with hydrochloric acid and the small amount of amorphous resin precipitated was filtered and well washed. It was then dissolved in hot baryta water and the excess of baryta precipitated by carbon dioxide. The milky liquid was well boiled and the barium carbonate filtered off. The filtrate was evaporated to small bulk and left in a desiccator. Crystals gradually separated, which under the microscope appeared to be small plates. There was only sufficient for a barium estimation after drying at 100°.

0.1424; 0.0590 BaSO₄; Ba found=24.39; Calc. for C₂₇H₄₄O₄Ba=24.12; C₆₄H₉₃O₇Ba₂=24.35.

We hope shortly to be in a position to give a further account of the reactions of these various substances.

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