V. ON β-CHOLESTANOL, SOME OF ITS DERIVATIVES AND OXIDATION PRODUCTS.

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Gardner and Godden [1913] showed that coprostanone on oxidation by chromic acid yielded a small quantity of an acid, melting at 247°, identical with that obtained as a bye-product in the preparation of coprostanone from coprosterol. On oxidation by ammonium persulphate the coprostanone yielded as main products two isomeric substances of the formula $C_{27}H_{46}O_2$, melting respectively at 183–184° and 157–158°. These appeared to be lactones of a hydroxyacid or hydroxyacids of the formula $C_{27}H_{48}O_3$. It seemed possible that light might be thrown on the relationship of coprosterol to the various isomeric artificial bihydrocholesterols—ψ-coprosterol, β-cholestanol, etc.—by a study of their oxidation by means of ammonium persulphate. We therefore commenced our experiments with β-cholestanol but the work has been much delayed by the absence of one of us on more urgent duties.

In the meantime Windaus and his co-workers have very materially increased our knowledge of this group of alcohols. Uibrig and Windaus [1913, 1914, 1915] working on the four alcohols, (1) β-cholestanol, (2) ε-cholestanol, (3) coprosterol, and (4) ψ-coprosterol, showed that β-cholestanol and ε-cholestanol can be changed into one another by boiling in amyl alcohol solution with sodium amylate, and differ from one another only in the stereo-position of the hydroxyl group with regard to an asymmetric carbon atom, as in the case, for instance, of borneol and iso-borneol. A similar relationship obtains between coprosterol and ψ-coprosterol, as had been previously pointed out by Dorée and Gardner [1908]. β-Cholestanol and coprosterol, they maintain, differ from one another in the stereo-position of a hydrogen atom with regard to another asymmetric carbon atom, but they had not been able to change the one into the other. The relationship of ε-cholestanol to ψ-coprosterol is similar.

In a later paper Windaus [1916] gave an account of the reduction of

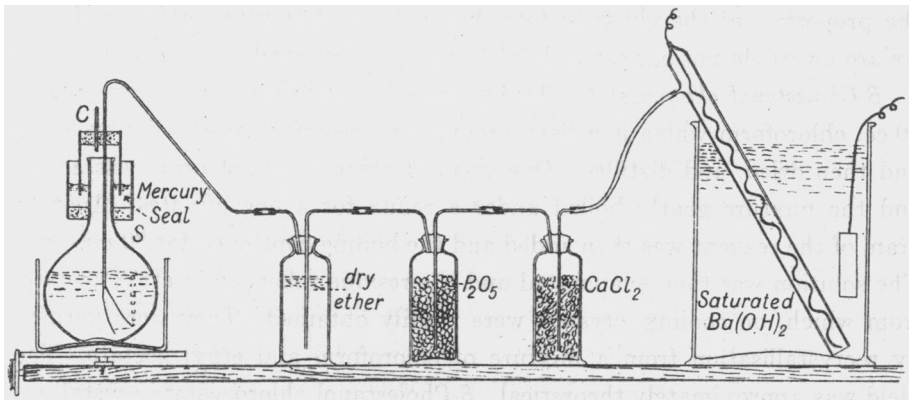
cholesterol by means of hydrogen at 200° in the presence of reduced nickel and described a new alcohol, γ-cholestanol, M.P. 146° and $[\alpha]_D^{18} + 29{\cdot}9°$. This he found to be really a conglomerate of β-cholestanol, ψ-coprosterol and ε-cholestanol in the sense of the partial racemate compounded of β-cholestanol and ψ-coprosterol. They crystallise together like a single compound and the product, γ-cholestanol, has a similar melting point and similar specific rotary power to β-cholestanol, the only difference being that γ-cholestanol crystallises from dilute alcohol in the anhydrous condition, whereas β-cholestanol crystallises with one molecule of water. The separation of the constituents of γ-cholestanol was effected by taking advantage of the fact that, whereas β-cholestanol and coprosterol are precipitable by digitonin, the isomers ψ-coprosterol and ε-cholestanol do not form insoluble compounds with this substance. The β-cholestanol was removed by means of digitonin and the isomerides partially separated by crystallisation from methyl alcohol. ε-Cholestanol separates first and the part which remains in the crude ψ-coprosterol is re-arranged into β-cholestanol by boiling the mixture with sodium and xylene. This β-cholestanol was again precipitated by digitonin and the pure ψ-coprosterol obtained. Finally, Windaus found that the ψ-coprosterol could be partly transformed into the isomeric coprosterol by heating with alcoholic sodium ethoxide at 180°. The coprosterol was separated from the mixture by precipitating with digitonin and recovered from the digitonide in the usual manner by boiling with xylene. From the provisional formulae proposed by Windaus we should expect the oxidation products of β-cholestanol and β-cholestanone to be very similar, if not identical, with those obtained by Godden and Gardner from coprosterol and coprostanone. The dicarboxylic acid, melting at 247°, obtained from coprosterol by Godden and Gardner and by J. Adamla [1911] from γ-cholestanol, we did not find among the oxidation products of either β-cholestanol or β-cholestanone.

The β-cholestanone was less readily attacked by ammonium persulphate than coprostanone and the products obtained, though apparently lactones of the formula $C_{27}H_{46}O_2$, were different from those prepared from coprostanone and were more easily hydrolysed. They were very difficult to separate and the behaviour of the lower melting isomers suggested either that different isomers possessed the property of crystallising together like single substances, as Windaus has shown for different bihydrocholesterols, or that different isomers are readily re-arranged one into the other. None of the substances obtained was, however, identical with any of those got from coprosterol.

Experimental.

Preparation of β-cholestanol. The reduction of cholesterol to β-cholestanol was first carried out by Willstätter and Meyer [1908] by the use of hydrogen and platinum black in ethereal suspension. The exact technique of this method has been somewhat modified by us and with such good results that it may therefore be described. The dry pure ether used was prepared from the commercial product by treatment in the cold with about 10 % of its weight of a finely powdered mixture of dry potassium hydroxide and potassium permanganate in the proportion of about four parts of potash to one of permanganate. The mixture was occasionally shaken for a period of 24–28 hours and after settling the ether was decanted and distilled over phosphorus pentoxide.

The hydrogen, which it is essential should be pure and dry, was prepared electrolytically, the electrolyte employed being a saturated solution of barium hydroxide. The cathode was a silver coil and the anode a platinum coil. The general arrangement of the voltameter is shown in the accompanying diagram.



The Preparation of Dihydrocholesterol.

The hydrogen passed through a calcium chloride tower, through phosphorus pentoxide and then through dry ether. The latter served the purpose of arresting any traces of phosphorus pentoxide and of making up for loss of ether from the cholesterol solution by its own evaporation. The ethereal solution of cholesterol, which should be not more than three-fourths saturated, contained about 8 grams of platinum black to 400 cc. of the solution. The platinum black was prepared according to the method of Loew [1890].

It was found very necessary to keep the solution of cholesterol continually and vigorously shaken, in order that the heavy platinum black should be in

a continual state of suspension. We believe this suspension can be more completely established by the method shown in the figure. The flask containing the solution is kept in rapid rotation; the stirrer is fixed, but is composed of a bent, wide-mouthed tube, so arranged as to stir up the rotating ether. Such a movement can be readily obtained by mounting a flask in a glass dish by means of four corks and mounting the dish on a horizontal wheel rapidly rotated by means of a pulley coupled with a motor. Such a device was found completely to reduce 50 grams of cholesterol in 24 hours. The evaporation of the ether is avoided largely by the introduction of the ether wash bottle, reducing the outlet of the flask by the use of a mercury valve *S* and allowing the gas to escape by a capillary tube *C*. The completion of reduction is best ascertained by an estimation of the specific rotary power which is the reverse of that of cholesterol.

The rotation was found to be $[\alpha]_D^{18} + 28{\cdot}8°$, a value agreeing with that given by Willstätter and Meyer.

A number of esters were prepared at the beginning of our work with a view of comparing their properties with those of other isomers; we give here the properties of the chloroacetate, benzoate, and stearate which as far as we are aware do not appear to have been fully described.

β-Cholestanol chloroacetate. Two grams of β-cholestanol were dissolved in 20 cc. chloroform which had been previously freed from alcohol by washing, and then dried, and distilled. One gram of chloroacetyl chloride was added and the mixture gently boiled under a reflux for a few minutes. Another gram of the reagent was then added and the boiling continued for 30 minutes. The solution was then evaporated and the residue taken up in ethyl acetate, from which, on cooling, crystals were readily obtained. They were purified by recrystallisation from a mixture of chloroform and ethyl acetate. The yield was approximately theoretical. β-Cholestanol chloroacetate crystallises in glistening plates not unlike cholesterol in appearance and melts at 178–179°. It is readily soluble in ether and in chloroform but only slightly so in cold ethyl acetate.

Analysis: 0·2321 g.; 0·2210 H_2O; 0·6361 CO_2.

Found C = 74·15, H = 10·58.

Calculated for $C_{29}H_{49}ClO_2$ C = 74·92, H = 10·54.

β-Cholestanol benzoate. 1·5 grams of β-cholestanol were dissolved in 15 cc. of pure dry pyridine and 2·5 cc. of benzoyl chloride added. The mixture was allowed to stand overnight, poured into about 50 cc. of water and the precipitated benzoate filtered off and washed. It was crystallised from

a mixture of benzene and acetone and finally from ethyl acetate and was thus obtained in the form of small plates. The yield was satisfactory. It is only slightly soluble in cold alcohol and not very soluble in hot. It is fairly soluble in ether, readily in benzene, and slightly so in acetone. It melts at 155° and during the melting process displays an extraordinarily brilliant fluorescence. On gently heating in a melting point tube it assumes at about 138–139° a reddish tinge, and at 140° begins to soften and run together to an opaque opalescent mass of a red tinge with flashes of green. At 145° it is still opaque and the play of colours becomes more intense, red and emerald green predominating according to the point of view. The fluorescence becomes brighter as the temperature rises, until at 155° the colours suddenly vanish and the liquid becomes clear. On cooling, the liquid becomes opaque again at 155° and the colour display appears again in the reverse order. The colours are not shown in transmitted light. The colour display may be very strikingly demonstrated by heating between two microscope slides. Owing to this colour display the exact melting point could not be determined.

In chloroform solution the rotation was found to be $[\alpha]_D^{23} + 23{\cdot}3°$.

Analysis: 0·2222 g.; 0·21 H_2O; 0·6725 CO_2.

Found C = 82·54, H = 10·50.

Calculated for $C_{34}H_{52}O_2$, C = 82·92, H = 10·57.

β-Cholestanol stearate. An attempt to obtain this substance by a process similar to that employed for the preparation of the benzoate was not successful, only a very small percentage yield being obtained. A good yield was obtained by adding to 1 gram of the β-cholestanol about twice its weight of stearyl chloride, the whole being in chloroform solution. The solution was left to stand in a closed flask for a day, then boiled gently for half-an-hour and finally evaporated to dryness. This stearate was almost insoluble in alcohol, but soluble in a mixture of alcohol and chloroform, from which it readily crystallised in soft flaky crystals. It melts at 100° and is readily soluble in ether. The rotation in ether solution was $[\alpha]_D^{20} + 18{\cdot}4°$.

Analysis: 0·1599 g.; 0·1869 H_2O; 0·4856 CO_2.

Found C = 82·82, H = 12·99.

Calculated for $C_{45}H_{82}O_2$, C = 82·57, H = 12·54.

Oxidation of β-cholestanol. Diels and Abderhalden [1906] first showed that β-cholestanol is readily oxidised to a ketone, β-cholestanone, by the action of chromic acid in acetic solution. This ketone melts at 128–129°, whereas coprostanone melts at 62–63°. In the preparation of coprostanone from coprosterol Godden and Gardner found that the yield never exceeded

70 %, and the ketone was accompanied by considerable quantities of a dibasic acid, $C_{27}H_{46}O_4$ melting at 247°, and smaller amounts of a non-crystalline acid.

In our experiments on the preparation of β-cholestanone the yield was from 80–90 %. The glacial acetic solution of the oxidation product was diluted with water and extracted with ether. On shaking the washed ethereal solution of the ketone with dilute aqueous potash a trace of a crystalline acid was obtained on acidifying the alkaline extract. The amount of this acid appeared to be slightly increased by conducting the oxidation in more gentle fashion. We did not obtain it in sufficient quantity for further investigation, but it was quite different from the above mentioned dibasic acid, M.P. 247°, obtained from coprosterol.

Oxidation of β-cholestanone by ammonium persulphate. This oxidation was carried out in a manner somewhat similar to that described by Godden and Gardner in the case of coprostanone, but took place less readily. Four grams of the ketone were dissolved in 150 cc. of glacial acetic acid and 4 grams of ammonium persulphate in 8 cc. of water were added. The mixture was placed on a water bath for an hour and left over-night at a temperature of about 80°. The next day another 4 grams of the persulphate were added in small amounts and the mixture allowed to stand for 4 days at 80°. On cooling crystals separated and were filtered off. The filtrate was then diluted and extracted with ether. This extract was added to an ethereal solution of the crystals already separated and the whole extracted by shaking with a solution of sodium carbonate. This extract on acidification yielded a trace only of an oil. The ethereal solution was then further extracted with 10 cc. potash. The ether solution thus extracted was found to contain a small amount of unchanged ketone. Several oxidations were carried out in this way. The potash extract on acidification with hydrochloric acid yielded a flocculent precipitate, soluble in alcohol, from which it crystallised in clots of fine needles. These were recrystallised from ethyl acetate and melted at about 180°. On dissolving the crystals in ether and extracting with potash it was found that now very little of the substance was removed. On evaporating the ether and recrystallising the residue from alcohol crystals melting round 180° were again obtained. This behaviour recalls the properties of the lactones from coprostanone described by Godden and Gardner. The crystals were readily soluble in ether, alcohol, or ethyl acetate, and when allowed to cool after melting in a capillary tube showed an opalescent appearance and after 30 seconds the contents of the tube suddenly gave a slightly

audible crackle. This phenomenon, by remelting, could be repeated indefinitely.

This crystalline matter was fractionally crystallised from various solvents —alcohol, acetone, ether and methyl alcohol. A fraction separated from alcohol in clots of microscopic needles. This, after repeated recrystallisation from alcohol and from acetone melted sharply at 201–202°.

Analysis: 0·1034 g.; 0·10655 H_2O; 0·30585 CO_2.

Found C = 80·67, H = 11·45.

Calculated for $C_{27}H_{46}O_2$, C = 80·52, H = 11·50.

It was not attacked by acetic anhydride and had no ketonic properties. It was not hydrolysed by shaking the ethereal solution with potash, strong or dilute, but on boiling with alcoholic soda it yielded a salt soluble in water. On acidification the substance was re-formed and after recrystallisation from alcohol melted at 201–202°. It was presumably therefore a lactone of an acid $C_{27}H_{48}O_3$.

In addition to this lactone a small quantity of a body melting at 184–186° was obtained, the solubility of which was of the same order. This crystallised from alcohol in thin plates or needles very similar in appearance to the substance of the same melting point obtained by Godden and Gardner from coprostanone. It was not, however, identical, as on mixing the two substances the product melted between 160° and 170°. The amount of the pure substance was too small for combustion or further examination.

The crystalline matter left after the separation of these two bodies consisted of small needles and had the appearance of a single substance. Its behaviour on melting however showed that this was not the case, as it gradually softened through a considerable range of temperature. After repeated fractionation the following fractions were obtained:

A. A very small fraction melting at 98–100°. On combustion this was found to contain 80·5 % of carbon and 12·2 % of hydrogen. It may possibly be a low-melting lactone $C_{27}H_{46}O_2$, but the quantity was too small for further investigation.

B. Fine matted needles, which on heating began to shrink together at 144°, softened and melted at 152–153° and ran to a clear liquid at 157–158°.

C. Indeterminate crystalline matter. On heating it began to shrink together at 120° and melted at 130° to a turbid liquid, which became clear at 138–139°. This behaviour was shown again on cooling and re-heating.

D. Small needle-like crystals. It began to soften at 143°, softened at 150° and ran between 159° and 161° to a clear liquid. On cooling long needles

began to form in the clear liquid at 158–159°, but the whole mass did not become opaque until a much lower temperature. On re-heating it became clear again at 150°, but the needles did not vanish until 159–161°. Further recrystallisation did not alter the melting point materially.

Analysis:

(1) 0·1005 g.; 0·1013 H_2O; 0·2946 CO_2.

(2) 0·0894 g.; 0·0915 H_2O; 0·2633 CO_2.

		C	H
Found	(1)	79·94	11·20
	(2)	80·32	11·50
Calculated for $C_{27}H_{46}O_2$,		80·52	11·50.

This was the main fraction. It was different in properties from the substance of similar melting point obtained from coprostanone. On boiling with weak alcoholic soda it yielded a sodium salt which dissolved in water to a soapy solution. On acidification the substance was not re-formed again, but a pasty precipitate was obtained. We had not sufficient for further examination of this acid. The substance was evidently not pure, but consisted mainly of a lactone or mixture of lactones, of the above formula.

Fraction C was analysed.

0·1119 g.; 0·1134 H_2O; 0·3366 CO_2.

Found C = 82·04, H = 11·26.

It was thought that perhaps this might contain some unchanged ketone, or even unchanged β-cholestanol, but mixed meltings did not seem to bear this out.

Fraction B was also a mixture and on combustion was found to contain 81·7 % of carbon and 11·44 % of hydrogen.

It was thought that if these intermediate fractions consisted of mixtures of high and low melting lactones it might be possible to destroy the lower melting ones by further oxidation with chromic acid, as Godden and Gardner had found that this was the case with similar mixtures from coprostanone. The results were negative and crop C gave a product of more or less unchanged melting point.

It would appear from these results that β-cholestanone on oxidation with persulphate yields a mixture of lactones of the formula $C_{27}H_{46}O_2$, probably two of high melting point and one or more of lower melting point, but as far as we were able to ascertain they were different from those obtained from coprostanone.

We hope later on to be able to give a more complete account of these various substances.

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