CXVIII. NOTE ON MONTIGNIE'S ISOMER OF CHOLESTEROL, AND THE EFFECT OF HEAT ON CHOLESTEROL.

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RECENTLY Montignie [1929] published a description of a new isomer of cholesterol, M.P. 137–137·5° and $[\alpha]_D - 19^\circ$. This isomer he obtained, along with cholesteryl cinnamate, M.P. 157–158°, and some unchanged cholesterol and cinnamic acid, by heating a mixture of two molecules of cinnamic acid and one molecule of cholesterol for $\frac{1}{2}$ hour at 200°. He regarded the substance as a new isomer on the ground that, on treatment with alcoholic potash, it only suffered a very small loss in weight, actually, if his figures be correct, 4·18 %, and that it yielded an acetate, M.P. 103°, a benzoate, M.P. 131–132°, and a dibromide, M.P. 76°. He also stated that the substance gave the Liebermann reaction a little more slowly than ordinary cholesterol.

Wishing to examine this isomer more closely in the hope that it might prove useful in some other investigations on which we are engaged, we repeated Montignie's work, but though we obtained similar results, we were unable to confirm his conclusion that he had in hand a new isomer of cholesterol.

EXPERIMENTAL.

The cholesterol used was obtained from gall-stones and was purified through the dibromide in the usual manner. It melted at 148·8° and had $[\alpha]_D = -39\cdot28^\circ$ in chloroform solution. It gave no reaction with Rosenheim's reagent, even after long standing.

10 g. of cholesterol were mixed with 8·08 g. of cinnamic acid and heated in a wide test-tube in a glycerol bath at 200° for $\frac{1}{2}$ hour. The product was poured into hot alcohol. The difficultly soluble portion, after cooling to room temperature, was filtered off (fraction A). The filtrate, on cooling in a freezing mixture of ice and salt, deposited a rich crop of white crystalline matter, which melted unsharply at 137–138° (fraction B). This was the new isomer described by Montignie. A number of preparations were made, and the fractions B all had approximately the same unsharp melting-point, but the yield varied apparently with the volume of alcohol used.

Fraction A was boiled with ethyl alcohol, and the undissolved portion, M.P. 157–157·5°, consisted of pure cholesteryl cinnamate. The alcoholic filtrate

on concentration yielded crops of crystals, melting respectively at 150–151°, 146–146·5°, 145° and 140°.

Fraction B. Various preparations on precipitation from alcoholic solution by digitonin gave the following results:

	Substance (g.)	Digitonin compound (g.)	% sterol
(1)	0·024	0·0980	99·24
	0·0236	0·0964	99·30
(2)	0·0198	0·0756	92·82
(3)	0·0334	0·1206	87·78
(4)	0·0236	0·0932	96·00

The ether washings of the digitonin compounds on evaporation gave a solid residue. This residue was boiled out with water and filtered. The filtrate was faintly acid and contained a small quantity of cinnamic acid. The insoluble portion was recrystallised from ethyl alcohol. It melted at 156°, and was cholesteryl cinnamate.

Liebermann's reaction. The reaction was carried out under rigidly the same conditions with chloroform solutions of specimen (1) and of pure cholesterol of the same concentration. We could detect no difference by eye either in the colours produced in the two cases, or in the rate at which they developed.

Acetyl derivative. The acetate was prepared in the usual manner by boiling with acetic anhydride. After recrystallisation from alcohol it melted at 113–114°. On mixing with pure cholesteryl acetate there was no lowering of melting-point.

Benzoyl derivative. The benzoate was prepared by treatment of fraction B in pyridine solution with benzoyl chloride. After recrystallisation from alcohol it melted at 145–146° to a turbid fluid which became clear at 172°. On cooling it showed the colour changes characteristic of cholesteryl benzoate. Mixed with pure cholesteryl benzoate it showed no lowering of melting-point.

Dibromide. A portion of fraction B on treatment by Windaus's method in ethereal solution with a solution of bromine in glacial acetic acid gave a crystallised dibromide, which after washing and drying melted at 115°. On the other hand the same sample of B, after recrystallisation from alcohol, gave a dibromide, m.p. 123°, which decomposed at 125–126°. This on mixing with a specimen of pure cholesterol dibromide gave no lowering of melting-point.

On recrystallising fraction B from either methyl or ethyl alcohol, pure cholesterol was readily obtained.

It was also found possible by mixing pure cholesterol with small quantities of cinnamic acid and cholesteryl cinnamate, dissolving in alcohol, and freezing out the concentrated solution, to obtain a substance having all the properties of fraction B.

The mother-liquors obtained in the preparation of fractions A and B were evaporated to dryness. The dry residue was boiled out with water repeatedly and from the aqueous solution unchanged cinnamic acid, m.p. 133°, was obtained. The insoluble portion was easily separated by fractional crystallisa-

tion from alcohol into cholesteryl cinnamate and cholesterol. From these experiments we think it is clear that Montignie's substance was not an isomer of cholesterol, but an impure cholesterol, containing cinnamic acid and cholesteryl cinnamate.

On treatment, in chloroform solution, with Rosenheim's reagent (a saturated aqueous solution of trichloroacetic acid), none of the specimens of cholesterol, pure or impure, obtained in the above experiments gave any immediate coloration, but some of the specimens on long standing developed a pink colour. This was not due to any trace of allocholesterol, since on examining the solution with the spectroscope none of the bands of allocholesterol were observed. There was merely a slight general absorption. A specimen of allocholesterol, m.p. 117° and $[\alpha]_D + 4.14^{\circ}$, gave an absorption spectrum with a deep band at $4980 \text{ \AA.}$, and fainter bands at 5810 , 5610 and 5330 . On dilution the faint bands vanished, but the characteristic band 4980 persisted at a dilution fainter in colour than that given by the cholesterol specimens. In order to ascertain whether this pink colour may have been due to the effect of heat on cholesterol, a pure specimen of cholesterol was distilled in a high vacuum, others were heated at 185° *in vacuo* for many hours. The products after recrystallisation from alcohol melted at 148 – 148.5° and gave no colour with Rosenheim's reagent even on prolonged standing.

Specimens heated in open test-tubes at various temperatures from 160 to 200° , and for various lengths of time, after recrystallisation from alcohol melted at 148° , but with Rosenheim's reagent developed more or less rapidly a copper sulphate-blue colour, which was very persistent. The absorption spectrum was characterised by a well-marked band about 6600 , and in some cases a fainter band about 6068 . The results are given in the following table. The specimens of cholesterol used were obtained from either gall-stones or cod-liver oil, and were purified by the dibromide method and repeated recrystallisation from alcohol.

Source of cholesterol	Temp. of heating	Duration of heating	M.P. after recryst. from alcohol	Concentration of solution	Bands (middle)	
Cod-liver oil	160°	1 hour	148°	0.05 g. 5 cc. CHCl_3 2 cc. trichloroacetic acid	6600	6076 (faint)
	200°	1 „	148°	0.05 g. 5 cc. CHCl_3 2 cc. trichloroacetic acid	6682	—
	300°	$\frac{1}{2}$ „	148°	Not measured		
Gall-stones	180 – 185°	2 hours	148°	0.02 g. 5 cc. CHCl_3 3 cc. trichloroacetic acid	6607	6070 (faint)
	175°	20 „	148°	0.1 g. 5 cc. CHCl_3 3 cc. trichloroacetic acid	6600	6068 (faint)

Though the melting-point remained unchanged, the specific rotary power was depressed one or two degrees.

When cholesterol was heated at about 180° in a slow current of oxygen for 20 hours or more, a more profound change took place. An appreciable amount of CO_2 was produced and small quantities of acid substances distilled over. On

cooling, the melted mass set to a glass which did not crystallise on long standing. Though it contained a large proportion of unchanged cholesterol, this was difficult to crystallise from ethyl or methyl alcohol. A specimen recrystallised until it melted at 148° gave the blue colour with Rosenheim's reagent and its characteristic bands.

This reaction is under investigation and we hope to give a detailed account later.

REFERENCE.

Montignie (1929). *Bull Soc. Chim.* [4], **45/46**, 771.