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Friedrich Reinitzer[ab]
[a] Imperial Institute for Plant Physiology, German University, Prague [b] Imperial Institute for Plant Physiology, Germany University, Prague

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Contributions to the knowledge of cholesterol

by FRIEDRICH REINITZER
Assistant at the Imperial Institute for Plant Physiology at the German University, Prague

From the Imperial Institute for Plant Physiology of Professor Ad. Weiss at the Germany University, Prague

(Presented at the meeting of 3 May 1888)

A translation, from the German, of the original paper by Friedrich Reinitzer published in *Monatshefte für Chemie*, volume 9, pages 421–441, 1888 in which he describes his observations resulting in the discovery of liquid crystals. We are grateful to Granmont Incorporated of the Montedison Group for permission to publish their translation which has been revised slightly by Professors F. Schneider and H. Steyemeyer.

About $1\frac{1}{2}$ years ago, I reported the results of some studies [1] of a cholesterol occurring in the root of the carrot which had been given the name hydrocarotene by A. Husemann. At that time I stated that this compound, although not in the manner suggested by Husemann, was related to the red pigment of carrots (carotene) and through the latter, again, to the chlorophyll pigments. It was therefore of interest to investigate the nature of this substance more closely. However, since it is difficult to obtain in large quantities, but, on the other hand, since the cholesterols display a great mutual similarity of properties, I decided to undertake a preliminary study along this line, first with ordinary cholesterol which can easily be obtained in large quantities and whose nature is also completely unclarified. Then on the basis of the experience gained in this way, I intended to investigate the far more costly hydrocarotene more closely. I would like to communicate some of the results of this preliminary work in the following.

The cholesterol used for the experiments described here was obtained from the factory of H. Trommsdorff and was purified there by repeated treatment with alcoholic caustic potash. It had a melting point of 147·5°C (corr. = 148·5°). Ordinarily the melting point is reported at 145° to 146°. The value reported was, however, obtained even in the case of very slow heating. The thermometer used was completely accurate, divided into tenths of degrees. Wislicenus and Moldenhauer have reported the melting point to be 147° (*Annal. d. Chem.*, Vol. 146, p. 179).

Molecular weight of cholesterol

The molecular weight of cholesterol has not yet been established with the certainty that would be absolutely necessary in order to obtain a satisfactorily firm foundation for further studies of the nature of this substance. The presently most frequently used formula $C_{26}H_{44}O$ was first derived by Gerhardt according to Gmelin (*Handbuch d. org. Chemie*, Vol., 4, p. 2093) on the basis that this would bring it into best

agreement with the derivatives. Before then, many other formulas were proposed. Later Latschinoff and Walitzky attempted to prove that the formula $C_{25}H_{42}O$ would have greater probability for cholesterol. As evidence they cited in particular those facts which suggested a relationship between cholesterol and a pentaterpene. Hesse also gives preference to this formula, because the optical rotatory power of cholesterol is lower than that of phytosterol (*Lieb. Annal.*, Vol. 192, p. 175). Liebermann also ascribes a certain probability to Latschinoff's and Walitzky's statements (*Ber. d. d. chem. Gessell.*, Vol. 18, p. 1803). Recently T. Weyl [2] attempted, by determining the vapour density of the cholesterones and cholesterylenes derived from cholesterol to establish the molecular weight of cholesterol. The values obtained in this way, however, are unfortunately incapable of assuring complete certainty in this regard. This is because the above-mentioned hydrocarbons undergo dissociation and decompose into smaller molecules, to which Weyl assigns the formula $C_5H_8 = 1/5\ C_{25}H_{40}$, under the tacit assumption that they are similar and of the same size, from which then, naturally, the formula $C_{25}H_{42}O$ would follow for cholesterol. The vapour densities obtained, however, are far greater than the vapour density assignable to a compound of the formula C_5H_8 and it is therefore entirely possible that the hydrocarbons derived from cholesterol have a higher molecular weight than assumed, and that, in addition, the partial molecules formed during dissociation are not of the same size and so the average obtained exceeds the calculated vapour density. Therefore, as interesting and valuable as these studies are, they appear to me not to be sufficient to decide the question of the molecular weight of cholesterol. I, therefore, attempted by an exact investigation of the derivatives of cholesterol to determine its molecular weight. For this purpose I used, first of all, cholesteryl benzoate and attempted to determine the ratio in which benzoic acid and cholesterol are obtained by saponification of the same. The completely pure benzoate was obtained in boiling alcohol on the reflux condenser in solution, decomposed with an excess of normal alkali and back-titered with normal sulphuric acid. In this process, however, the determination of the end point of titration is very uncertain, and, moreover, the poor solubility of the benzoate very obstructive. It was therefore attempted to determine the quantity of cholesterol obtained by saponification of the benzoate. The saponified, cooled and solidified product was filtered off, washed with aqueous alcohol and then with hot water, dried and weighed. The filtrate was strongly concentrated, precipitated with water, and after washing out with boiling water, also dried and weighed. It was found, however, that the alkali benzoate, because of the unwettability of the cholesterol by water, was very difficult to remove completely from the cholesterol, because, on the other hand, a loss of cholesterol occurs due to the low solubility of cholesterol in aqueous solutions of alkali benzoate. Therefore, this procedure also had to be given up.

I therefore proceeded to the analysis of a bromine derivative and selected for this purpose the bromoacetate. The acetate was chosen because it could be expected that it would change less easily during bromination than pure cholesterol, which was also confirmed by experience. Moreover, the acetate and its bromide are far more poorly soluble in alcohol than the non-acetylated cholesterol and therefore more easily purifiable. The bromination was carried out precisely in the manner reported by Wislicenus and Moldenhauer (*Lieb. Annal.*, Vol. 146, p. 178). The dry, completely pure cholesteryl acetate was dissolved in a little dry, very pure carbon disulphide and, by cooling with cold water, a solution of chlorine-free bromine in hydrogen sulphide added until a permanent yellow colouring occurred. To bring about this condition, 4 g of bromine had to be consumed by 10 g of the acetate. During the reaction, no

hydrogen bromide evolved. The liquid was evaporated at ordinary temperatures, at which time it turned dark red with partial decomposition and evolution of hydrogen bromide. The totally amorphous, yellow-coloured evaporation residue, was washed with cold water and dried over sulphuric acid under reduced pressure. Both procedures had to be repeated several times in order to remove the last traces of hydrogen bromide. The product was then dissolved in as little ether as possible and precipitated with alcohol. It precipitates out as splendid crystals and can easily be freed of the red mother liquor by washing with alcohol. By repeated recrystallization from ether/alcohol and washing with alcohol, the compound can be obtained completely colourless and with an invariable melting point. To determine the formula, two elemental analyses and two bromine determinations were performed on the substance dried over sulphuric acid under reduced pressure. To perform the latter, the compound was dissolved in ether/alcohol, this solution mixed with as much water as it could tolerate without clouding, and then decomposed with sodium amalgam. After evaporation, it was washed with boiling water and the bromine determined in the aqueous solution as silver bromide. The elemental analysis was performed with cupric oxide in a stream of oxygen in the presence of a silver wire and silver plate.

I. 0·5472 g of substance yielded 0·35065 g BrAg;
II. 0·3249 g substance yielded 0·7051 g CO_2 and 0·2446 g H_2O;
III. 0·3408 g substance yielded 0·2173 g BrAg;
IV. 0·3587 g substance yielded 0·7790 g CO_2 and 0·2618 g H_2O.

	Calculated for	
	$C_{26}H_{43}Br_2 \cdot C_2H_3O_2$	$C_{27}H_{45}Br_2 \cdot C_2H_3O_2$
C	58·53	59·18
H	8·03	8·18
Br	27·85	27·19
O	5·58	5·44

	Found				
	I	II	III	IV	Average
C	–	59·19	–	59·22	59·20
H	–	8·38	–	8·12	8·25
Br	27·26	–	27·13	–	27·19

From these volumes, it emerges unequivocally that the compound in question has the formula $C_{27}H_{45}Br_2 \cdot C_2H_3O_2$. From the previously reported quantity of bromine necessary for bromination and from the fact that no hydrogen bromide evolved at this time, it also follows that the substance is a bromine addition product, which also agrees with the bromine obtained by Wislicenus and Moldenhauer. Accordingly, therefore the formula of cholesterol must read $C_{27}H_{46}O$. This result is striking because it does not agree with previous assumptions. The question now arises of whether the latter can be brought into agreement with the analyses of the previously known derivatives. If one compares the result of analyses of cholesterol derivatives found by various observers with the percentage composition calculated on the assumption that cholesterol has one of the two formulas, $C_{26}H_{44}O$ or $C_{27}H_{46}O$, then one obtains the following: the analysis of cholesteryl chloride (Planer, *Lieb. Annal.*, Vol. 118, p. 25) and of sodium cholesterylate) Lindenmeyer, *Journ. f. pr. Chem.*, Vol. 90, p. 321) agree

better with the formula with 27 carbons. The analysis of the bromides (Wislicenus and Moldenhauer, op. cit.) and of the amines (Löbisch, *Ber. d. d. chem. Gesell.*, Vol. 5, p. 513) agree better with the formula with 26 carbons. The analyses of dinitrocholesterol, nitrocholesteryl chloride (Preis and Raymann, *Ber. d. d. chem. Gesell.*, Vol. 12, p. 224) and of bromocholesteryl chloride (Raymann, *Bull. de la Société Chim. de Paris*, Vol. 47, p. 898) permit an interpretation in favour of both formulas. The heptachlorocholesterol of Schwendler and Meissner (*Lieb. Annal.*, Vol. 59, p. 107) is probably not a uniform substance, since the analyses deviate from one another by up to 1 per cent; therefore, this substance cannot be taken into consideration here.

The so-called acetic acid cholesterol, a cholesterol with crystalline acetic acid (Hoppe-Seyler, *Journ. f. pr. Chem.*, Vol. 90, p. 331) displays an acetic acid content which would agree better with the formula with 27 carbons than any lower one, although here also the analyses deviate from one another by 1 per cent, thus significantly reducing their value for the present purpose. The studies published by Walitzky are unfortunately unavailable to me in original form, for which reason I was unable to include his analytic results in the comparison. Finally those derivatives which contain organic radicals consisting solely of the elements occurring in cholesterol are without significance for the present question because of the high molecular weight of the latter. It therefore appears that the probability of the correctness of both formulas is nearly equally great. Since there is presently no reason to doubt the correctness of the analyses or the purity of the substances, the only assumption that remains is that various homologous cholesterols occur in the animal body and were present in the compounds discussed here in more or less pure form. This assumption indeed becomes increasingly more probable upon closer examination. As is known, E. Schulze found, besides ordinary cholesterol, a second, isocholesterol, in lanolin, which clearly proves that mixtures of cholesterols can occur in one and the same animal. He also points out in a comment on a study performed with Barbieri (*Journ. f. pr. Chem.*, Vol. 25, p. 458) that the isocholesterol was probably not an isomer but rather a homologue of cholesterol. Hesse felt himself compelled for certain reasons to state that it was probably that the cholesterol to which he gives the formula $C_{25}H_{42}O$ occurs in the animal body mixed with phytosterol, to which he assigns the formula $C_{26}H_{44}O$ (*Lieb. Annal.*, Vol. 192, p. 175). It also follows from the properties of the presently known cholesterols that they very probably form two homologous series, as I attempted to confirm already at the conclusion of my report on hydrocarotene and carotene (op. cit. p. 729), and therefore, the discovery of two homologous cholesterols would not be surprising.

Incidentally, the data of various observers concerning the properties of cholesterol and its derivatives also deviate in various points from one another so significantly that in these cases isomerism should be assumed rather than homology. Thus, for example, the data of Walitzky concerning the behaviour of cholesterol with respect to sodium and of cholesteryl chloride with respect to alcoholic ammonia totally contradict the data of other observers. In this case, it is worthy of notice that Walitzky performed his studies exclusively with cholesterol extracted from the brain, while most of the other chemists used cholesterol produced from gallstones. It would therefore not be impossible for various cholesterols to occur in the different animal organs. This is all the more probable because Schulze and Barbieri have already demonstrated a similar behaviour for a plant, i.e. *Lupinus luteus* (op. cit.). Here it was found that in the cotyledons as well as in the unchanged seeds a cholesterol occurs which has greatest similarity to phytosterol or paracholesterol, while in the root and in the hypocotyl of

the germinating plants, a cholesterol of a much higher melting point and optical rotatory power so-called caulosterol, occurs. Schulze and Barbieri are of the opinion that ordinary cholesterol is still most certainly a single substance, since they obtained the same substance both from gallstones and from lanolin with the aid of the benzoate and since the benzoate always has uniform properties. However, if one considers how similar homologous substances are in their properties and how precisely their preparation with the aid of the more easily purified benzoate would have to lead to the same substances with greater certainty, then one can ascribe no very great value to this solitary observation.

It would now naturally require additional studies to establish whether the hypothesis advanced here corresponds to actual circumstances or whether it must be allowed to fall, and therefore, for the time being, I will refrain from a naming. At any rate, however, as a result, in studies with cholesterol it will be necessary to devote the greatest attention to the origin of the same and its uniformity. As regards the origin of my substance, it was obtained from gallstones. The origin of the latter is, however, unknown to me.

In the following, some previously unknown or only imprecisely known properties of several derivatives of cholesterol will be reported, in which cholesterol, naturally, must be assigned the formula $C_{27}H_{46}O$.

1. *Cholesteryl acetate* $C_{27}H_{45} \cdot C_2H_3O_2$

Löbisch (*Ber. d. d. chem. Gesellsch.*, Vol. 5, p. 513) prepared this substance with the aid of acetyl chloride and reported its melting point at 92°. I used acetic acid anhydride for the preparation. 10 g of cholesterol were lightly boiled with 7 g of acetic acid anhydride for 1 hour on the reflux condenser, carefully washed with water and recrystallized initially from ether, then from ether/alcohol, until the substance displayed an unchanged melting point. The latter was found to be 114–114·4°C (corr. 114·3–114·7). Raymann, who prepared the substance some time ago in almost the same way, found 113° (*Bull. de la Sociéte Chim. de Paris*, Vol. 47, p. 898). Mr. Hofrath v. Zepharovich kindly consented to study the compound crystallographically and reported the following to me concerning it:

"Crystal system monosymmetrical.

$$a:b:c = 1{\cdot}8446:1:1{\cdot}7283. \ \beta = 73°38'$$

Cross-stretched narrow tablets or needles, predominantly bounded by {001} *oP*, {100} $\infty P\infty$, {$\bar{1}$01}$P\infty$ and {110} ∞P: subordinately {101} $\infty P\infty$, {001}$P\infty$, {111} $-P$ and {112} $-$ 1/2*P*. Twins along {001}.

	Calculated	Measured	*Z*
(001): (100)	= 73°38′	73°43′	13
(001): ($\bar{1}$01)	= 50°41·3′	50°40′	12
(110): (100)	= 60°32′	60°32′	10
(110): (001)	= 82°2′	82°2′	15
(111): (100)	= 57°32·3′	57°32′	2
(111): (110)	= 25°51·6′	25°42′	2
(112): (111)	= 16°22·3′	16°20′	3

The main extinctions lie parallel to the edge between (001) and (100) and perpendicular to it; no optic axes are seen through these planes. The small dimensions of the crystals prevented a further optic investigation."

During the cooling process of the molten cholesteryl acetate a peculiar, very splendid colour phenomenon occurs before solidification (not after it as reported by Raymann). The phenomenon can already be observed in a wide capillary tube, as is used to determine the melting point. However it can be observed much better if the substance is melted on an object glass covered with a cover glass, one then sees, when viewed in reflected light, in one place a strong emerald green colour appears, which rapidly spreads over the entire sample, then becomes blue–green, in places also deep blue, then changes to yellow–green, yellow, orange–red, and finally bright red. From the coldest places, the sample then hardens into spherocrystals which, spreading quite rapidly, suppress the colour phenomenon at which time the colour simultaneously turns pale. In transmitted light, the phenomenon takes place in the supplementary colours which, however, are unusually pale and scarcely perceptible. Similar colour phenomena appear to occur in several cholesterol derivatives. Thus, Planar (op. cit.) reports that cholesteryl chloride displays a violet colour during cooling from the melt which vanishes again upon solidifying. Raymann (op. cit.) reports similar observations on the same substance. Löbisch (op. cit.) reports that cholesterylamine when melted displays a bluish-violet 'fluorescence' and also mentions the occurrence of the same phenomenon in the case of cholesteryl chloride. I myself observed a similar phenomenon in cholesteryl benzoate (see below), and Latschinoff reports for the silver salt of cholestenic acid, which is formed by oxidation of cholesterol, that it turns steel blue when melted, which fact is probably to be explained in the same way. An accompanying phenomenon occurring in cholesteryl benzoate, to be described below, as well as the perceptible changes observed under the microscope during the occurrence of the colour phenomenon suggested to me that perhaps physical isomerism was present here, and therefore I requested Professor O. Lehmann in Aachen, who is probably presently the most familiar with these phenomena, to make a more detailed investigation of the acetate and benzoate along this line. He was kind enough to perform the investigation and indeed found that trimorphism was present in both compounds. The cause of the colour phenomenon, however, has not yet been satisfactorily explained. It is only known that it is closely related to the precipitation and redissolution of a presently still completely enigmatic substance. Whether this substance formed and disappears as a result of a physical or chemical change cannot be decided at present. In the following, the most important results of the studies by Professor Lehmann on cholesteryl acetate will be presented. (He will publish them in detail later.)

First modification. Is obtained by crystallization from solvents. Monosymmetrical. The crystal forms are those described above, measured by Professor v. Zepharovich. When heated the crystals become cloudy before melting by breaking up into a heap of crystals of the third modification.

Second modification. Produced by a rapid cooling of the melt in the form of spherocrystals. If xylene is added to them, single crystals can be obtained. The latter form thin, large flakes of the monosymmetrical system, have a rhombic outline and an apical angle of about 63°. When heated the crystals of this modification also become cloudy by transforming into a heap of crystals of the third modification.

Third modification. Produced by heating the first and second modifications as well as by slow cooling the molten substance. They could not be obtained in definable crystals. The simplest and most regular forms were elongated rectangles with symmetrical extinction.

From these results it follows that the first modification is enantiotropic to the third, the second monotropic to the third. The first modification is most stable at ordinary temperatures, the third at higher temperatures. The second, however, was incapable under any circumstances of causing the other two to transform into their own natural state.

Professor Lehmann's study of the colour phenomenon has shown that it is produced by the precipitation of a substance whose structure resembles an aggregate of spherocrystals, as polygonal areas can be recognized, each of which displays a black cross between crossed nicols. Upon closer study, however, one sees that this substance consists of drops which acquire a jagged outline due to very fine crystals perceptible only at strong magnifications. In other words, the substance is quite liquid, and the shape of the drops can usually be changed by moving the cover glass. If the finest distribution and most uniform mixing possible of the precipitated substance with the remaining liquid is brought about by shaking movements, the brightness and beauty of the colour phenomenon is significantly enhanced. The colour-producing substance also displays a strong rotation of the plane of polarization of light which varies with temperature and which varies in intensity of the individual colours and is directed toward the right at higher temperatures and to the left at lower temperatures. If the colour phenomenon vanishes upon further cooling and gives way to crystallization, then the precipitated substance redissolves by suddenly being set into peculiar motion and gradually disappears.

The nature of the colour-producing substance has not been determined to date. No impurities can be present, because the phenomenon occurs in different cholesterol derivatives and I have also already observed it in a derivative of hydrocarotene.

Cholesteryl acetate decomposes when heated above the melting point with yellow and brown coloration and evolution of pungent burnt-smelling vapours. A small portion of the substance sublimates without decomposing at this time. However, I was unable to demonstrate the splitting-off of acetic acid, as reported by Raymann. I also cannot confirm his statement that the acetate is decomposed by water. Even after prolonged boiling with water, I could not demonstrate decomposition. The acetate when partially decomposed by heating has the peculiarity that it is brought into a state by rapid cooling in which it displays the above-mentioned colour phenomenon, permanently, at ordinary temperature.

2. *Bromocholesteryl acetate* $C_{27}H_{45}Br_2 \cdot C_2H_3O_2$

The synthesis of this substance has already been described earlier. It is poorly soluble in alcohol, readily soluble in ether. It is obtained from ether/alcohol upon very slow evaporation as very thin, 1-2 cm long, highly lustrous tablets. The compound is dimorphous. The two modifications were formed under essentially the same conditions upon crystallization from ether/alcohol. The first three forms obtained by recrystallization of the same substance were asymmetrical, the last formed crystals, however, monosymmetrical. Mr. Hofrath v. Zepharovich was so kind as to undertake the crystallographic study and reports to me the following results:

"A. Monosymmetrical form

$$a:b:c = 1{\cdot}3283:1:2{\cdot}5346.\ \beta = 82°9'$$

Orthodiagonally elongated, thin tablets with predominant $\{001\}\ oP$, $\{100\}\ \infty P\infty$, $\{\bar{1}11\}P$ and subordinated $\{011\}P\infty$, $\{101\}\ -P\infty$, $\{\bar{1}01\}P\infty$, $\{\bar{1}12\}\frac{1}{2}P$.

	Calculated	Measured	Z
(001):(100) =	–	82° 9′	20
(011):(001) =	68° 17′	68° 17′	6
(011):(100) =	87° 6′	87° 5′	4
($\bar{1}$11):(001) =	–	76° 45·6′	13
($\bar{1}$11):($\bar{1}$00) =	–	56° 43·3′	9
($\bar{1}$12):(001) =	61° 2′	61° 10′	7
($\bar{1}$12):($\bar{1}$00) =	62° 55·5′	62° 47·5′	3

Cleavability completely along {001}. Main vibration directions parallel and perpendicular to (001 : 100). Plane of optical axes parallel to {010}.

B. Asymmetrical form

The elements resemble those of A, but for the calculation a sufficient number of precise determinations is missing. The combinations of the rhomboid tablets, often similar to the monosymmetrical and interpreted as $\{001\} \cdot \{100\} \cdot \{\bar{1}11\} \cdot \{11\bar{1}\}$, also possess similar edge angles, as the following comparison shows:

	A	*Z*	*B*	*Z*
(001):(100) =	82° 9′	20	81° 17′	25
($\bar{1}$11):(001) =	76° 46′	13	77° 57′	9
($\bar{1}$11):($\bar{1}$00) =	56° 43′	9	56° 35′	5
($\bar{1}$12):(001) =	61° 10′	7	60° 18′	4
($\bar{1}$12):($\bar{1}$00) =	62° 47·5′	3	64° 57′	4

Cleavability along {001} and {010}. On one table, I found (010):(100) = 88° 47′ and (010):(001) = 96° 56′, on two others (010):(001) = 91° 43′ and 91° 57′. In optical behaviour, no difference is noted between the geometrically similar forms A and B."

The substance is somewhat photosensitive. In diffuse daylight, it becomes yellow after about 3–4 weeks, later reddish-yellow to brown. At this time hydrogen bromide evolves. The discoloured substance is amorphous. Upon exclusion of light, the compound is completely stable. The melting point is not quite the same for the two physically isomeric forms. The monosymmetrical form melts at 117·6°C (corr. = 118·0), the asymmetrical form at 115·4°C (corr. = 115·8). Upon melting, the substance usually turns weakly yellowish. After cooling, it remains glass-like and can no longer be caused to crystallize. Apparently a slight decomposition occurs.

If sodium amalgam is allowed to act on the compound in an ether solution, again one obtains cholesterol; however, besides the latter, yet another substance appears to have been formed. After the removal of the sodium by transformation into sodium chloride and repeated treatment with ether, a small quantity of cholesterol can be obtained by fractional crystallization in the form of colourless crystals in addition to a larger quantity of a yellow, amorphous substance. The cholesterol thus obtained

melted at 146·5° but was still not completely pure, because the molten product had a weak yellow colour. Wislicenus and Moldenhauer also obtained cholesterol, with a melting point of 147° (op. cit), from bromocholesterol by means of sodium amalgam.

Since the formation of a cholesterol derivative with two new hydroxyl groups is expected by reaction of an alkali with the bromoacetate, this reaction was carried out. Aqueous caustic potash acts only very slowly and incompletely; it must therefore be used in alcoholic form. At this time a yellow substance was obtained which was poorly soluble in alcohol, readily soluble in ether, which could not be crystallized and therefore could not be studied further. It forms a very viscous sticky product which after prolonged standing becomes covered with a hard brittle pulverizable layer. If its chloroform solution is mixed with sulphuric acid, the sulphuric acid layer turns blood-red with green fluorescence, while the chloroform layer assumes only a very pale pink-red colouring. It therefore behaves differently from cholesterol.

3. *Cholesteryl benzoate* $C_{27}H_{45} \cdot C_7H_5O_2$

This substance was first synthesized by Berthelot (*Ann, de chim.* [3], Vol. 56, p. 54) by heating with benzoic acid, then by Schulze (*Journ. f. pr. chem.* [2], Vol. 7, p. 170) by heating with benzoic acid anhydride. I used the latter procedure in a somewhat simpler version. 10 g of anhydrous cholesterol were heated with 12 g of benzoic acid anhydride for about $1\frac{1}{2}$ hours in an open flask in the sulphuric acid bath to 150–160°C. The transformation into the ester is then almost complete and only very little remains unreacted. One also never obtains a brownish coloured melt, such as obtained by Schulze. If the heating is performed for only $\frac{1}{2}$ hour to about 130–140°, then about 60 per cent of the cholesterol escapes the reaction. The solidified melt was extracted twice with boiling methyl alcohol and the residue repeatedly recrystallized from ether/alcohol. The shape of the crystals and the solubility relationships agree precisely with the data of Schulze. The most beautiful crystals are obtained by slow evaporation of a solution in ether mixed with as much alcohol as it can tolerate without clouding.

Mr. Hofrath v. Zepharovich, who was kind enough to analyse these crystals reported the following to me concerning them:

"Crystal system tetragonal.

$$a:c = 1:0{\cdot}9045.$$

Quadratic tablets with plane $\{001\}oP$ and very narrow, usually horizontally striated side faces of $\{111\}P$, $\{443\}\ \frac{4}{3}P$, $\{221\}2P$ and $\{441\}4P$.

	Calculated	Measured	Z
$(111):(001)$	= 51° 59′	52° 25′	7
$(443):(001)$	= 59° 37′	59° 44′	17
$(443):(44\bar{3})$	= 75° 10·6′	75° 12′	2
$(221):(001)$	= 68° 39′	68° 43′	17
$(221):(2\bar{2}1)$	= 82° 23′	82° 24′	5
$(441):(001)$	= 78° 76·5′	78° 35′	7

The small crystals were found to be uniaxially negative in the conoscope."

With respect to the melting point, a significant deviation from Schulze's data was noted. He found it to be 150–151°. However, despite continued careful purification

I was able to find only 145·5° (corr. 146·6°). However, it struck me that the substance, in this case, melted not into a clear transparent but always into a cloudy only translucent liquid, which I initially considered to be a sign of impurities, although both microscopic and crystallographic examinations of the compound revealed no signs of nonuniformity. Upon closer examination, it was then also noted that when heated to higher temperatures, the clouding suddenly vanishes. This happens at 178·5° (corr. 180·6). At the same time I found that the substance heated thus highly displayed, upon cooling, colour phenomena quite similar to those already described for the acetate. This remarkable phenomenon of the presence of two melting points, if one may express it thus, and the appearance of the colour phenomenon were primarily what made me think that here and in the case of the acetate, physical isomerism must be present, for which reason I requested Professor Lehmann in Aachen to make a closer investigation of these circumstances. The most important result of his studies of the benzoate, briefly summarized, are the following.

Cholesteryl benzoate, like the acetate, can occur in three modifications.

First modification. Is obtained by crystallization from solvents and forms the above-described tetragonal crystals. It melts far higher than the other two modifications. When heated the crystals remain clear, and therefore do not transform into another modification.

Second modification. This is formed upon the solidification and rapid cooling of the molten substance. It melts a little but not much lower than the third modification and forms flat needles or narrow flakes of the rhombic system.

Third modification. Formed by slow cooling of the molten substance, and by heating the rapidly solidified substance (second modification) until it almost melts. It melts a little higher than the second modification and forms thin, wide leaves with a nearly square outline and symmetrical extinction.

The three modifications are in the relationship of monotropy to one another.

The colour phenomenon which occurs upon cooling of the molten substance takes place somewhat differently from that of the acetate.

When the clear-molten compound is cooled, in one place a deep violet-blue colour appears which spreads rapidly over the entire substance and vanishes again almost equally rapidly, a uniform clouding appearing in its place. The substance then remains clouded but liquid for some time; upon further cooling, then for the second time the same colour phenomenon appears, and as the same progresses, a crystallisation of the substance occurs after it and with it also a simultaneous disappearance of the colour phenomenon. If the molten layer of the benzoate is at least 2–3 mm thick, then besides the violet-blue colour, all other colours reported for the acetate also appear. The colour-producing substance here also causes the clouding. It precipitates out in drops as does the acetate, in which crystals are found and it dissolves again shortly before solidification. The process of precipitation and dissolution is accompanied by the colour phenomenon, while in the intervening time, only simple clouding is induced. The colour-producing substance, as in the case of the acetate, also displays chromatic polarization, except that this is not as strong as there, and not so many colours occur during it. The other details observable under the microscope and the explanation of the phenomenon cannot be reported at this time, because the studies in this regard have not yet been completed.

Whether the deviating statement of Schulze regarding the melting point was caused by the clouding occurring during melting, which indeed makes observation very difficult and erroneous, or whether Schulze perhaps was studying a different cholesterol, cannot be decided from the available data.

Incidentally, it might be mentioned here that I, once I became aware of the colour phenomenon, have now also found it in hydrocarotene. In this case, however, only the benzoate displayed it, while the acetate is free from it, a fact which can be utilized to facilitate distinguishing these two cholesterols. The hydrocarotyl benzoate also displays two melting points, quite like the corresponding compound of cholesterol, which I also previously overlooked.

Cholesteryl benzoate when boiled with water does not decompose at all, decomposes very poorly and insignificantly with aqueous caustic potash and decomposes rather quickly with alcoholic caustic potash, especially upon the addition of ether. When heated above the melting point, it decomposes partially, at which time benzoic acid fumes off and condenses in colder regions, sublimating in part without decomposing.

Upon stronger heating it becomes yellow and then solidifies partially glass-like after cooling. In this state the colour phenomenon can be permanently preserved in it at ordinary temperatures by rapid cooling, as in the case of the acetate.

4. *Sodium cholesterylate* $C_{27}H_{45}O \cdot Na$

O. Lindenmeyer obtained the compound above by reacting sodium with cholesterol dissolved in purified petroleum with the evolution of hydrogen (*Journ. f. pr. Chem.*, Vol. 90, p. 321). Walitzky reports that sodium does not evolve hydrogen from anhydrous cholesterol (Beilstein, *Handbuch d. org. Chem.*, first edition, Vol. 2, p. 1376). Although Lindenmeyer expressly states having used completely anhydrous cholesterol and purified petroleum over sodium, it was nevertheless necessary to provide clarity on the subject by repeating the test. The preparation took place, with minor deviations, according to the data of Lindenmeyer. 10 g of cholesterol which had been completely dehydrated by drying at 100° was dissolved in petroleum ether. The latter had been previously dehydrated and purified by standing and distillation over calcium chloride and then over sodium. Then sodium which had been purified by remelting under petroleum and cut into paper-thin flakes under petroleum ether was added in a small excess. Hydrogen begins to evolve immediately and the pieces of sodium become coated with a white crust, quite as Lindenmeyer describes. The very fine dispersion of the sodium is very important for the greatest possible acceleration of the reaction. At ordinary temperatures, the latter otherwise takes place quite slowly. On the other hand, if the heating is performed on the water bath using a reflux condenser, then the reaction is completed in about 2–3 days with the quantity of cholesterol reported above, as may be recognized from the fact that the sodium pieces are no longer coated with a white crust but remain completely clean. The liquid is then a thick white slurry. This slurry was filtered off, washed with a small amount of petroleum ether and then freed of sodium by dissolving in anhydrous chloroform. Dissolution takes place so rapidly that the sodium has scarcely any effect on the chloroform. When the solution is allowed to evaporate spontaneously or when evaporated on the water bath, one obtains the compound with all properties already described by Lindenmeyer. Regarding the latter one should add at most that the substance is also a little soluble in petroleum ether.

The compound obtained was used to test whether it could not be transformed into an acid by treatment with carbon dioxide.

However, when introduced into the chloroform solution, only sodium carbonate precipitated out, while pure cholesterol remained in solution.

5. *Nitrocholesterol*

If a hot saturated solution of cholesterol in glacial acetic acid is treated with fuming nitric acid (specific gravity 1·54) at boiling heat as long as strong evolution of red vapours occurs. Then the liquid poured into cold water, and after washing with water and drying under vacuum one obtains a solid, dark-red-yellow, in the pulverized state yellow, odourless and tasteless nitro compound which so far has resisted attempts at crystallization.

The latter is insoluble in water but dissolves in aqueous ammonia and aqueous caustic alkali very readily to form a dark red bitter-tasting liquid which reacts neutrally in the saturated state and yields yellow or red–brown precipitates with the water-soluble salts of the alkaline earth and most metals. This substance is apparently the same as is formed in the Schiff reaction for cholesterol.

It is readily soluble in alcohol, very readily soluble in ether, chloroform, benzene and glacial acetic acid. The alkaline solution can be very easily oxidized by potassium permanganate, but I have not yet succeeded in obtaining a detectable oxidation product.

When heated on the platinum plate, the substance burns rapidly and easily but without any real deflagration. It melts at 93 to 94°C with strong foaming, indicating decomposition.

Finally, permit me to express my deepest thanks to all those who supported me in carrying out the present work, including: Professor Dr. Weiss for providing the materials and for relieving the burden of other work in the experiment, Professor Dr. F. W. Gintl for kindly permitting the use of the facilities of his laboratory, Mr Hofrath von Zepharovich for helpfully carrying out the crystal measurements and Professor Dr. O. Lehmann for carrying out the microphysical studies.

References

[1] *Sitzgsber. d. kais. Akad. d. Wissensch.*, Vol. 94, p. 719.

[2] *Arch f. Anat. und Physiol., physiolog. Abthlg.*, 1886, p. 180 (*Proceedings of the Physiological Society at Berlin*, Session of 30 October 1885), extracted in *Ber. d. d. chem. Gesellsch.*, 1886, abstracts, p. 618.