CCXLVIII. ON THE MECHANISM OF THE FORMATION OF CHOLEIC ACIDS.

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It is well known since the work of Wieland and Sorge [1916] that desoxycholic acid may combine with a large number of organic substances acting as centres of co-ordination, so as to form molecular compounds, the so-called choleic acids. This phenomenon probably plays an important part in the metabolism of water-insoluble substances. Rheinboldt and co-workers [1927; 1929] and Sobotka and Goldberg [1932] have established a number of principles as to the arrangement of desoxycholic acid molecules round aliphatic mono- and di-carboxylic acids. In proportion to the length of the fatty acid chain the co-ordination numbers increase by certain intervals. The co-ordination number of acetic acid is 1, of propionic acid 3, of butyric to octanoic acid 4, of nonanoic to tetradecanoic acid 6 and of pentadecanoic and higher acids 8.

We have studied the influence exercised by changes in the chain structure of fatty acids upon their ability to act as co-ordination centres of choleic acids. A few similar experiments with lower fatty acids have been described by Sobotka and Goldberg [1932].

A systematic study of a series of isomeric fatty acids with branched chains would lead to a better understanding of the mechanism of the formation of choleic acids, a reaction which is rather unusual in organic chemistry. It also would be of importance in regard to the question of the absorption of fatty acids with branched carbon chains.

Since one of us (Abel) will not be able to participate in the further investigation of these problems, we are here describing the results obtained up to the present time. Further material will be communicated in a later paper.

Two ways exist of studying the composition of choleic acids. (1) The choleic acid is prepared by direct synthesis. The molecular composition of the compound is either calculated from the acid equivalent determined by titration or after removal of the desoxycholic acid in the form of its insoluble xylene compound the fatty acid component is estimated by titration [Wieland and Sorge, 1916]. (2) Determinations are made of the melting- and thawing-points of mixtures of the two components in different proportions. From the melting-thawing diagram thus obtained conclusions can be drawn as to the existence of a molecular compound in the particular case and as to its composition [Rheinboldt, 1925; Sobotka and Goldberg, 1932].

In the case of the fatty acids with branched chains examined by us we have almost always observed that the choleic acids formed were extremely labile, much more so than the choleic acids of the corresponding normal fatty acids, for which reason we have often had to use the melting-thawing diagram in order to establish the existence and molecular composition of co-ordination

compounds. Characteristic differences could be noted in the stability of choleic acids with α - and β -substituted fatty acids, the latter being more stable than the former. The fact that branched chain fatty acids as compared with normal fatty acids are less apt to form choleic acids and therefore are probably less easily absorbed perhaps explains the absence of such acids in nature.

The fatty acids that have hitherto been examined by us are summarised in Table I. A study of this table shows that in the case of the acids which we

Table I. *Formation of choleic acids with fatty acids.*

No. of exp.	Substance	Co-ordination number established by		Co-ordination number of corresponding normal fatty acid
		Crystallised choleic acid	Diagram	
1	α -Butylhexanoic acid	4	4	6
2	α -Ethyloctanoic acid	0	2	6
3	β -Methylnonanoic acid	6	—	6
4	α -Ethyldecanoic acid	4	—	6
5	α -Methylundecanoic acid	4	—	6
6	α -Ethylhexadecanoic acid	8	—	8
7	α -Dodecyltetradecanoic acid	0	4 and 8	8
8	Trichloroacetic acid	0	—	1
9	β -Iodopropionic acid	0	0	3
10	α -Bromopentanoic acid	0	0	4
11	α -Bromoisopentanoic acid	0	0	4

0 = no compound obtained.

— = not examined.

examined not only were the choleic acids formed relatively unstable but the co-ordination numbers were often lower than those of the straight chain isomerides. From this one may infer that the grouping within the carbon chain that is responsible for co-ordination has been changed. The methylene group in the α -position seems to be particularly important, as is shown by Exp. 3, when compared, for instance, with Exp. 5: while β -methylnonanoic acid has the normal co-ordination number 6, the co-ordination number of α -methylundecanoic acid containing two more methylene groups is only 4. On the other hand, the relation between the length of the main chain and that of the side-chain also seems to exert an important influence. The longer the main chain, as compared with the side-chain, the more similar is the behaviour of the fatty acid to that of the corresponding normal compound. On computing the relation between the two chains attached to the same carbon atom by dividing the molecular weight of the longer by that of the shorter chain one finds as quotients for Exps. 1–7 (see Table I): 1, 2.9, 6.3, 3.9, 8.5, 6.4, 1. With the sole exception of Exp. 5 all acids, the quotients of which exceed 6.3—that is where one chain preponderates—have a normal co-ordination number. The acids having smaller quotients show a diminution of their co-ordinative power.

With the halogenated fatty acids which we examined we did not observe any formation of choleic acids at all. This may be due to the introduction of a relatively very heavy constituent into the chain. In halogenated fatty acids however where the halogen does not outweigh the carbon chain normal co-ordination numbers are observed. Dibromobehenic acid $C_{22}H_{42}O_2Br_2$ was found by Rheinboldt *et al.* [1927] to have the co-ordination number 8.

The fact that relatively small changes in the molecule of a chemical compound may have a far-reaching influence upon its co-ordinative power is reminiscent of certain immuno-chemical phenomena [*cf.* Landsteiner, 1933].

EXPERIMENTAL.

A. *Material.*

The halogenated acids which we examined were purified preparations of Schering-Kahlbaum. The fatty acids were all prepared by synthesis.

(1) *α -Butyl-n-hexanoic acid* [Levene and Cretcher, 1918], B.P. 108–110°/2 mm. was prepared from ethyl dibutylmalonate, B.P. 111–114°/3 mm., which was converted into dibutylmalonic acid, M.P. 164–165° (corr.).

(2) *α -Ethyl-n-octanoic acid* [Dox, 1925], B.P. 135–137°/3 mm. was prepared from ethyl ethyl-hexylmalonate, B.P. 113–115°/3 mm. [Dox, 1924].

(3) *β -Methyl-n-nonanoic acid* [Levene and Taylor, 1922], B.P. 115–120°/3 mm. was prepared from ethyl 1-methylheptylmalonate, B.P. 131–133°/3 mm.

(4) *α -Ethyl-n-decanoic acid* [von Braun and Teuffert, 1929], B.P. 151–152°/3 mm. was prepared from ethyl ethyloctylmalonate, B.P. 142–143°/4 mm.

(5) *α -Methyl-n-undecanoic acid.* To a solution of 0.66 g. Na in 25 ml. of absolute alcohol 6.0 g. of ethyl methylmalonate and 7.3 g. of freshly purified nonyl iodide (B.P. 75°/4 mm.) were added. After boiling the mixture for 8 hours the alcohol was partly removed by distillation and the residue diluted with acidified water and extracted with ether. The ethereal solution was washed with dilute thiosulphate solution and water, dried and concentrated. The remaining oil was distilled *in vacuo*. 5.6 g. of ethyl methyl-nonylmalonate (64 % of the theoretical yield) were obtained as a colourless oil, B.P. 147–148°/5 mm. (Found: C, 67.75; H, 10.45 %. $C_{17}H_{32}O_4$ requires C, 67.94; H, 10.74 %.) The ester was saponified according to the method of Adams *et al.* [1928]. Methyl-nonylmalonic acid was obtained by crystallisation from benzene in the form of small plates (3.2 g.), M.P. 94–95°. (Found: C, 63.62; H, 9.54 %. $C_{18}H_{34}O_4$ requires C, 63.87; H, 9.90 %.) By heating *in vacuo* CO_2 was eliminated and α -methylundecanoic acid was obtained as an oil which solidified on cooling. The acid had B.P. 125–127°/6 mm. (Found: C, 72.07; H, 11.89 %. Mol. wt. by titration 198.5. $C_{12}H_{24}O_2$ requires C, 71.94; H, 12.09 %. Mol. wt. 200.2.)

(6) The syntheses of *α -ethyl-n-hexadecanoic acid* and *α -n-dodecyl-n-tetradecanoic acid* have already been described [Chargaff, 1932].

B. *Choleic acids.*

In order to prepare the choleic acids the two components were dissolved in a small quantity of hot alcohol. In all cases where stable choleic acids were obtainable, crystals separated on cooling and were then recrystallised from 2 to 4 times their weight of absolute alcohol. The desoxycholic acid used was a pure preparation of Riedel-de Haën which melted at 174°.

The ratio fatty acid : desoxycholic acid was either determined by the xylene or xylene-calcium method [Wieland and Sorge, 1916; Rheinboldt *et al.*, 1927] or by titration of the acid equivalents of the choleic acids with alcoholic KOH and α -naphtholphthalein as indicator.

The thawing-melting diagrams were made according to the indications of Rheinboldt [1925] and Sobotka and Goldberg [1932]. In the case of liquid substances the mixtures were prepared by vigorous shaking of the melted components contained in closed thin-walled tubes in an oil-bath at 195°. After cooling the contents were thoroughly ground.

(1) *α -Butyl-n-hexanoic acid-tetracholeic acid.* 0.29 g. of α -butyl-n-hexanoic acid and 4 g. of desoxycholic acid, dissolved in 6 ml. of hot absolute alcohol, gave, after four recrystallisations from a little alcohol, 1.5 g. of fine plates which melted at 147–148°. (Found: acid equivalent 350.4, 348.2. $[C_{24}H_{40}O_4]_4 \cdot [C_{10}H_{20}O_2]$ requires acid equivalent 348.3.)

The thawing-melting diagram of mixtures of desoxycholic and butylhexanoic acids is reproduced in Fig. 1. At 9.7 % of butylhexanoic acid both curves show a maximum

Fig. 1.

Fig. 2.

Fig. 1. System α -butyl-*n*-hexanoic acid + desoxycholic acid.

Fig. 2. System α -ethyl-*n*-octanoic acid + desoxycholic acid.

Fig. 3.

Fig. 4.

Fig. 3. System α -*n*-dodecyl-*n*-tetradecanoic acid + desoxycholic acid.

Fig. 4. System β -iodopropionic acid + desoxycholic acid.

corresponding to a compound of the co-ordination number 4 (required: 9.8 % $C_{10}H_{20}O_2$) and m.p. 150°.

(2) *α-Ethyl-n-octanoic acid-dicholeic acid*. A crystalline choleic acid could not be obtained. As Fig. 2 shows, there exists a molecular compound $[C_{24}H_{40}O_4]_2 \cdot [C_{10}H_{20}O_2]$ of m.p. 154° at 18.0 % ethyloctanoic acid (required: 17.9 %).

(3) *β-Methyl-n-nonanoic acid-hexacholeic acid*. 0.29 g. fatty acid and 4 g. desoxycholic acid in 10 ml. absolute alcohol gave 2.5 g. of a choleic acid which on recrystallisation from alcohol was obtained in beautiful platelets of m.p. 170.5°. For this compound, which was much more stable than most of the choleic acids examined, a molecular ratio of 1 : 6 was found. (Found: acid equivalent 358.3, 358.4, fatty acid (xylene-calcium method) 6.96, 7.03 %. $[C_{24}H_{40}O_4]_6 \cdot [C_{10}H_{20}O_2]$ requires acid equivalent 360.8, fatty acid 6.79 %.)

(4) *α-Ethyl-n-decanoic acid-tetracholeic acid*. From 0.4 g. fatty acid and 2.8 g. desoxycholic acid in 12 ml. absolute alcohol a crystalline choleic acid was obtained which after two recrystallisations had m.p. 176°. The co-ordination number was 4. (Found: acid equivalent 355.0, 355.4. Required by $[C_{24}H_{40}O_4]_4 \cdot [C_{12}H_{24}O_2]$ acid equivalent 353.9.)

(5) *α-Methyl-n-undecanoic acid-tetracholeic acid*. From 0.2 g. fatty acid and 3.1 g. desoxycholic acid in 10 ml. absolute alcohol crystals were obtained which had m.p. 179° and the molecular ratio 1 : 4. (Found: acid equivalent 354.3, 353.2. $[C_{24}H_{40}O_4]_4 \cdot [C_{12}H_{24}O_2]$ requires acid equivalent 353.9.)

(6) *α-Ethyl-n-hexadecanoic acid-octacholeic acid*. From 0.5 g. fatty acid and 5 g. desoxycholic acid in 20 ml. absolute alcohol after three recrystallisations 2 g. of small plates were obtained which had m.p. 178–179°. The choleic acid showed the normal ratio 1 : 8. (Found: fatty acid (xylene-calcium method) 8.31, 8.18 %. Required by $[C_{24}H_{40}O_4]_8 \cdot [C_{18}H_{36}O_2]$ fatty acid 8.31 %.)

(7) The synthesis of a series of hexacosanoic acids with branched carbon chains has been described by one of us [Chargaff, 1932]. The experiments undertaken with a view to prepare the choleic acids of this series led to a number of high-melting optically active crystalline products to which however no rational co-ordination numbers could be assigned in most of the cases. This anomaly may have the following causes: (i) The fatty acid component being only sparingly soluble in alcohol, recrystallisations from alcohol bring about the total decomposition of the molecular compound and the pure fatty acid crystallises. (ii) The high-molecular fatty acids with branched chains apparently form choleic acids in multiple proportions. The thawing-melting diagram of *α*-dodecyltetradecanoic and desoxycholic acids (Fig. 3), and particularly the thawing-point curve, demonstrates the existence of two choleic acids with the molecular ratios 1 : 4 and 1 : 8. The formation of two different choleic acids by the same substance has already been described for *d*-camphor [Rheinboldt, König and Flume, 1929], for *n*-butyric acid and for ethyl butyrate [Sobotka and Goldberg, 1932].

(8) With *halogenated lower fatty acids* we could not obtain any choleic acids at all, nor did the thawing-melting diagrams reveal the existence of co-ordination compounds. As an example the diagram obtained with *β*-iodopropionic acid is shown in Fig. 4.

SUMMARY.

A number of fatty acids with branched carbon chains and of halogenated fatty acids were studied in regard to their ability to form choleic acids. In comparison with the choleic acids of normal fatty acids essentially different results were obtained, since the co-ordinative power of fatty acids is considerably changed by the introduction of substituent groups. These phenomena are discussed with regard to the choleic acid problem.

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