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The chemistry of the bile acids

Nobel Lecture, December 12, 1928

Scientific chemistry occupied itself with the constituent substances of the bile at an early stage. L. Gmelin, Thénard and also Berzelius already did work on the acids present in bile, and several publications of the Liebig Laboratory in Giessen dealt with this subject. But it was only in 1848 that A. Strecker succeeded in isolating from ox-gall, the two most wide-spread acids, *taurocholic acid* and *glycocholic acid*, two conjugated substances of cholic acid $C_{24}H_{40}0_5$, condensed amide-like with taurine and glycine at the carboxyl group.

In 1886 Mylius discovered desoxycholic acid $C_{24}H_{40}O_4$ as a further constituent of hydrolysed bile; this second acid was isolated in the laboratory of Hammarsten in its natural form conjugated with taurine and glycine, from non-saponified bile. To this prominent Swedish investigator also goes the credit for the biologically important finding that it is cholic acid which predominates in the biles of numerous vertebrate animals investigated; this finding characterized the general physiological importance of cholic acid.

A third monocarboxylic acid $C_{24}H_{40}0_3$, lithocholic acid, poorer in oxygen, was discovered in 1911 by Hans Fischer in ox-gall stones, and afterwards proved to be an integral constituent of ox and human bile.

A year before Mylius, in 1885, Latschinoff had isolated an acid very similar in composition to desoxycholic acid which he called "choleic acid". Later investigators took this as being an acid which is isomeric with desoxycholic acid, until in 1916 it could be shown that it consists of a remarkable addition product of desoxycholic acid and higher fatty acids (stearic acid, palmitic acid, oleic acid). This choleic acid contains 1 molecule of bound fatty acid per 8 molecules of desoxycholic acid, the fatty acid being bound so firmly that no dissociation into the constituents takes place during either salt formation or dissolution.

This observation prompted a comparison of the additive capacities of desoxycholic acid towards other substances, and the behaviour towards the series of fatty acids can be summarized by stating that these acids, right down to acetic acid, can unite in secondary-valence compounds of this type. Formic acid does not form "choleic acid", thus indicating that the chemical forces

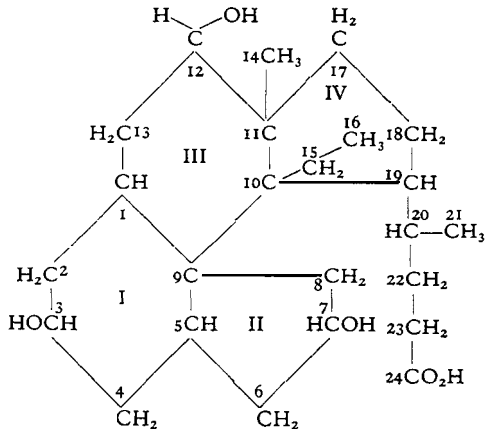
producing cohesion are to be found, not in the COOH groups, but in the saturated part of the fatty-acid molecule. H. Reinboldt has recently indicated that the choleic acids of the series being considered can be conceived as complex intercalation compounds of desoxycholic acid in the part of the fatty-acid molecule which has combining capacity. The fact that this capacity decreases with the number of carbon atoms in the fatty-acid molecule, indicates that one molecule of desoxycholic acid is held fast by the unit of each group $-CH_2 \cdot CH_2-$ or $-CH_2 \cdot CH_3$.

This intercalation capacity of desoxycholic acid is not restricted to the fatty acids. It is found in connection with all possible substances. Hydrocarbons, alcohols, esters, ethers, phenols, all unite with desoxycholic acid to form well-defined compounds containing as a rule only 2 molecules of desoxycholic acid. This generality of the addition capacity of desoxycholic acid has led to the use of the name "choleic acid" as a collective term.

The choleic acids containing neutral components insoluble in water, are absorbed by alkalies without decomposition taking place. On the other hand, the alkali salts of desoxycholic acid - and also those of cholic acid and of the conjugated cholic acid - will to a greater or a smaller extent absorb neutral substances insoluble in water. This applies, for example, in the case of fat, cholesterol, naphthalene, camphor, alkaloids such as strychnine, quinine, etc. A conclusion was drawn from this observation which is of importance with respect to the physiological function of the bile. It can be assumed that in the case of substances which areinsoluble in water, transitioninto soluble choleinates enables diffusion through the cells of the intestinal wall. This would apply in particular to the fats and to cholesterol during the course of normal metabolism, but this "choleic acid principle" has been confirmed in medicine in other cases also. Its therapeutic use has led to the technical production of camphorcholeic acid, "cadechol"; I mention this in particular here, because but for this practical aspect of the discovery in question the tests to determine the constitution of the bile acids would have soon reached an impasse. For it would have been impossible to master, simply with the technical resources of a scientific laboratory, the quantity of initial material then required.

Turning now to a discussion of the problem of the chemical structure of bile acids, I should say first that in view of the time at my disposal it will be impossible to give an exact account of the various stages of the decomposition which was used as the main means of determining the constitution. I will try to describe in rough outline the chemical methods by means of which we got some insight into the constitution of the large and in many ways monot-

onous molecule. The problem is not very attractive from the experimental viewpoint. There is no nitrogen, which adds interest and variety to the treatment of alkaloids. Only carbon, hydrogen and a little oxygen, all in the traditional combination, which does not lead us to expect any surprising results. The task would appear to be a long and unspeakably wearisome trek through an arid desert of structure. True, the wanderer in this apparently so unattractive region, finds friendly landscapes at all stages of his journey, and the large quantity of substances bringing him nearer his goal, accumulates around him like dear companions, although, clothed in the plain garment of colourlessness, they do not stand out either in their appearance or in their properties.



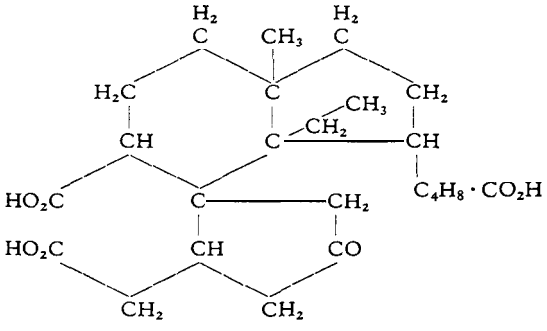
(I)

Cholic acid, $C_{24}H_{40}O_5$

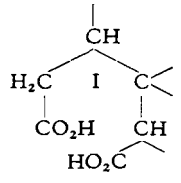
But the driving force, which steels the perseverance, lies in the problem itself. In order to get a comprehensive picture of the biological relationships in the wide field of chemically related natural substances which, in addition to the bile acids, includes the sterols, and very likely also the vegetable cardiac poisons from the group of saponins, the poisons from the skin secretions of toads, and probably also other important substances such as certain vitamins, we must above all be clear as to the fundamentals of their chemical structure.

My object, of briefly describing the extent to which the chemical nature of the bile-acid molecule has been determined, can best be achieved by basing my account on the formulae which so far appear to us to be the most likely ones. Formula (I) shows a saturated structure made up of four condensed

carbon rings and equipped with three branches. In a side chain is the carboxyl group determining the acid nature of the group. The three other oxygen atoms of the cholic acid are distributed in the molecule as alcoholic hydroxyl groups, in cholic acid they are located at the C-atoms 3, 7 and 12. In desoxycholic acid $Ca_4H_{40}O_4$ the OH-group at C_{12} is missing, and the single OH-group of lithocholic acid is bound at C_3. Windaus and I isolated a bile acid isomeric with desoxycholic acid $C_{24}H_{40}O_4$, at one and the same time, from the biles of various organisms, i.e. from that of the goose, of man and of the ox. This bile acid is called both chenodesoxycholic acid and anthropodesoxycholic acid. Though the two discoverers get on very well for the rest so far no agreement has been reached concerning a uniform nomenclature. In this acid the two alcoholic hydroxyl groups are at C_7 and C_{12}. Thus, it will be



(II)
Desoxybilianic acid, $C_{24}H_{36}O_7$



(III)
Iso-desoxybilianic acid, $C_{24}H_{36}O_7$

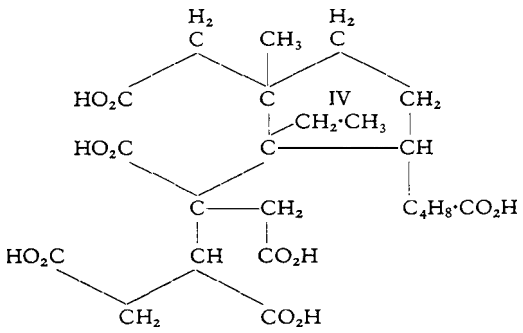
seen that the four most important bile acids are very closely related to each other, not only with respect to their fundamental structure, but also with respect to the distribution of their OH-groups.

Hammarsten was the first to show that the three OH-groups of cholic acid are of secondary-alcoholic nature, and already in 1881 he succeeded in converting cholic acid into dehydrocholic acid which contains six H-atoms less, through oxidation with chromium trioxide. All other bile acids behave analogously.

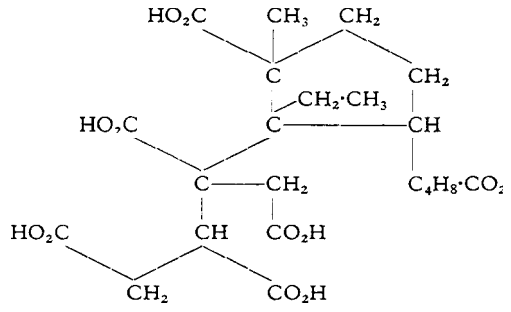
The first break-through into the molecule was achieved proceeding from desoxycholic acid, which can be opened up at the first ring by nitric acid via the stage of diketo acid. Here the two isomeric desoxybilianic acids form (Formulae (II) and (III)). The size of the ring was determined on the basis of Blanc's principle, according to which on thermal decomposition dicarboxylic

acids with 1,5-arrangement of the two CO_2H-groups turn into cyclic anhydrides, while those with 1,6- or 1,7-arrangement losing CO_2 and H_2O turn into cyclic ketones. The cyclic ketone produced in this way from desoxybilianic acid, was opened up into a hexacarboxylic acid via various intermediate products, in which only a single ring was retained and which was thus called solanellic acid (Formula (IV)). Through the thermal decomposition of this acid a new pentanone ring was produced from the split-up third ring, the further splitting-up of which led to biloidanic acid (V) $C_{22}H_{32}O_{12}$.

In this acid the paths of the decomposition from desoxycholic acid and cholic acid converged. Bilianic acid, the oxidation product of cholic acid corresponding to the desoxybilianic acid mentioned, can also be decomposed to form biloidanic acid via various intermediate products such as cilianic acid,



(IV)
Solanellic acid, $C_{23}H_{34}O_{12}$

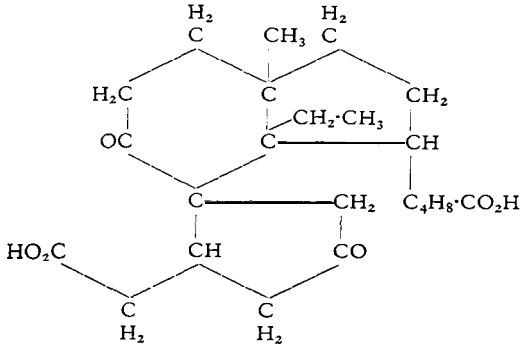


(V)
Biloidanic acid, $C_{22}H_{32}O_{12}$

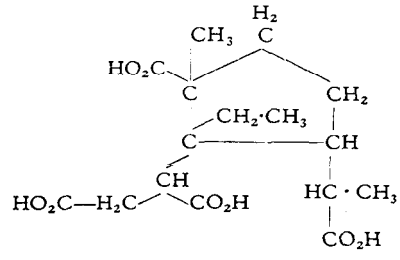
ciloidanic acid, which were explained with the help of the chemists M. Schenck and W. Borsche. But all attempts to penetrate into the last ring of the molecule proceeding from biloidanic acid, were unsuccessful.

As seen above, in the two experiments described only two carbon atoms were clearly removed from the large molecule. The principle followed up till then, that of proceeding step by step, had to be abandoned for the object of the investigation to be attained within a reasonable time. This measure was justified by the fact that the structure of the molecule this side of the unknown fourth ring was known in all its details.

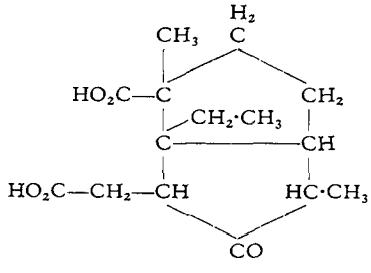
On oxidation of the pyrodesoxybilianic acid with permanganate, a diketodicarboxylic acid (VI) had been isolated as an intermediate product; this was turned into a tetracarboxylic acid $C_{16}H_{24}O_8$ by means of nitric-sulphuric acid in a smooth reaction. Here seven carbon atoms are broken off, including, for reasons to do with the closed part of the molecule, two from the side



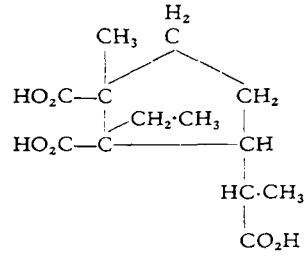
(VI)
Diketo-dicarboxylic acid, $C_{23}H_{34}O_6$



(VII)
Tetracarboxylic acid, $C_{16}H_{24}O_8$



(VIII)
Keto-dicarboxylic acid, $C_{15}H_{22}O_5$

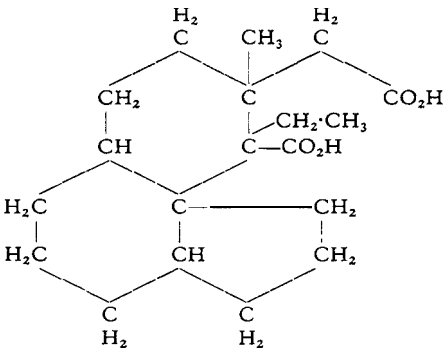


(IX)
Tricarboxylic acid, $C_{13}H_{20}O_6$

chain, which is thus shortened by two C-atoms. Formula (VII) is proved for this tetracarboxylic acid, because on thermal decomposition, it turns into a keto-dicarboxylic acid $C_{15}H_{22}O_5$ (VIII) unfortunately the yield is very low), and this can then be turned into a tricarboxylic acid $C_{13}H_{20}O_6$ (IX).

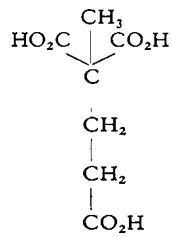
Since week-long operations yield only 5 g of this acid from 1 kg of desoxycholic acid, in determining the constitution the attack was transferred to another point of the molecule, viz., the side chain, about which some knowledge was already available from the work of Windaus on cholesterol. We were able to assume that a methylated C-atom is located in the γ-arrangement to the COOH group. This side of the molecule had also to be opened by the oxidation decomposition method. Proceeding from the ester of cholanic acid, $C_{24}H_{40}O_2$, the parent substance of the whole group, we turned this by means of the Grignard reaction into diphenylated carbinol, which was then submitted to oxidation by chromic acid. In this way one C-atom after another was split off. The fourth split away with the third, indicating that it was replaced by a CH_3-group. Proceeding from the acid $C_{20}H_{32}O_2$, the monot-

ony of this procedure underwent a welcome change. On oxidizing the corresponding tertiary carbinol, we came across a dicarboxylic acid $C_{10}H_{30}O_4$, etiobilianic acid (X). Thus the opening of the fourth ring was achieved and the number of links in it was found by thermal decomposition according to Blanc, which gave an anhydride and not a cyclic ketone. The fourth ring was thus recognized to be a fifthring, and from the formation of the etiobilianic acid it could be concluded that in addition to the C-atom carrying the side chain, there is a methylene group. The relationships between the side chain and ring II (lactone formation with the OH-group at C_7, condensation with the CO-group of the dehydro acids) indicate that the position of the side chain is as shown in the formulae.



(X)

Etiobilianic acid, $C_{19}H_{30}O_4$



(XI)

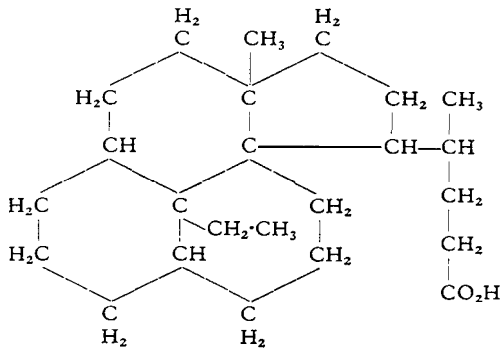
α-Methylglutaric-α-carboxylic acid, $C_7H_{10}O_6$

If we assume that the third ring is attached to the two C-atoms 10 and 11 of ring IV, then only three C-atoms are now unaffected by the structural investigation. We can make a positive statement about one of these C-atoms. On oxidation of the diketo-dicarboxylic acid $C_{23}H_{34}0_6$ mentioned above, in addition to the tetracarboxylic acid $C_{16}H_{24}0_8$ a small amount of a malonic acid was isolated, which is closely related to α-methylglutaric acid. It has the Formula (XI). The methyl group found here cannot be identical with that which we encountered in the side chain; it must be attached to C_{II}, which is the only way of explaining the occurrence of a malonic acid. But we are still unable to decide whether the other three C-atoms of the tricarboxylic acid $C_7H_{10}O_6$ come from ring III or ring IV. Thus, the formula for bile acid corresponding to the present status of our knowledge is still hypothetical, in so far as the location of the last two carbon atoms has not been determined very precisely. At present there are simply indications that the proposed ar-

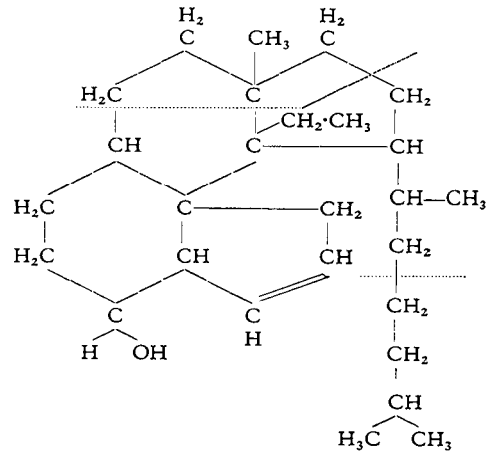
rangement with an ethyl group at C_{10}, has advantages over one with an ethyl group at C_{18} or a division into two methyl groups at the points mentioned.

But the most recent investigations, which I cannot go into in detail here, introduce Formula (XII) into the range of the discussion.

This, then, characterizes in rough terms the status of our knowledge of the chemical constitution of the bile-acid molecule. Despite the unsuccessful attempts of physiologists at producing experimentally the transformation of cholesterol into cholic acid in the animal organism, it must be assumed that the vegetable sterols provided by food are transformed by the cell according



(XII)
Cholanic acid, $C_{24}H_{40}O_2$



(XIII)
Cholesterol, $C_{27}H_{46}O$

to the system of the decomposition reaction carried out by Windaus on pseudocholestane. When we attempt to get a picture of the mechanism by which the plant cell builds up the sterols, reference to the formula of cholesterol (XIII) shows that its molecule consists of three isoprene groups and a residue of twelve rectilinearly-bound carbon atoms. Since the plant can easily convert carbohydrates into fats, it may perhaps be assumed that an intermediate stage of this transformation is employed for condensation with the terpene-type component. The rings are formed either through intramolecular dehydrations or through splitting off water.

Relationships with natural amino acids are found in the existence of the bile acids conjugated with glycine and taurine. They are even more pronounced in the poison of native toads, which is without doubt clearly related

to the bile acids. In this, the so-called bufotoxin, we find a molecule of suberic acid esterified by an OH-group of the fundamental structure $C_{24}H_{34}O_5$; the other carboxyl of the suberic acid is bound like an amide with arginine. A second hydroxyl carries the acetyl group. If the chain of the suberylarginine is split-off hydrolytically, the strong cardiac poison bufotalin remains, the acetic ester of a trioxy-lactone $C_{24}H_{34}O_5$. It can be assumed that here the typical carboxyl group of the bile-acid molecule has been lactonized to give the tertiary y-arrangement. No accurate check has yet been carried out on the structural association between bile acids and toad poison. Here the difficulties of preparing the material are particularly great.

When the last puzzles of our constitution problems have been solved, we can expect as usual the synthesis of the compounds. In cholanic acid there are seven asymmetric carbon atoms, and in cholic acid there are ten; as yet no procedure has been developed for the experimental production of the peculiar bonding of the numerous rings. While I acknowledge that it is the duty of the organic analyst to carry out syntheses such as have been carried out so excellently in the investigation of the coloured components of the colouring matter of blood, I must decidedly repudiate this in the field being discussed. I am fully conscious, however, of the duty to follow the course that has been embarked upon right to the end.