160. *X-Ray Crystallographic Measurements on Phrenosinic (Cerebronic) Acid and its Oxidation Product.*

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The problem of the constitution of phrenosinic acid has recently been attacked by the methods of *X*-ray crystallography by Chibnall, Piper, and Williams (*Biochem. J.*, 1936, **30**, 105), whose results lead to the conclusion that this acid is a mixture of straight-chain α-hydroxy-acids, probably $C_{26}H_{52}O_3$, $C_{24}H_{48}O_3$, and even $C_{22}H_{44}O_3$, which accordingly on oxidation gives rise to a mixture of the *n*-fatty acids $C_{25}H_{50}O_2$, $C_{23}H_{46}O_2$, $C_{21}H_{42}O_2$. These conclusions agree with Levene and Taylor's experiments (*J. Biol. Chem.*, 1928, **80**, 227) in so far that the mean composition of the mixture of acids obtained on oxidation approximates closely to that of an acid $C_{24}H_{48}O_2$. On the other hand, the authors agree with Klenk and Diebold (*Z. physiol. Chem.*, 1933, **215**, 79) by confirming the presence in the oxidation product of acids containing an odd number of carbon atoms; they further suggest that the latter authors' method of fractionating their product may have led to the isolation of pure tricosanoic acid.

Through the kindness of Professor Robinson, it has been possible to examine both Klenk's preparation of cerebronic (phrenosinic) acid and the oxidation product, and to compare them with the synthetic acids $C_{24}H_{48}O_2$ and $C_{23}H_{46}O_2$ described by Ashton, Robinson, and Smith (this vol., p. 283). For better comparison, *X*-ray measurements were also made of the potassium salts of the last two acids and of the oxidation product. The main results obtained are in the table.

These measurements substantially confirm the conclusions reached by Chibnall, Piper, and Williams. The $d_{(001)}$ values here recorded for Klenk's preparation of the oxidation product are definitely lower than those found by the above authors (57·1, 52·0, for the lowest fractions), *i.e.*, are nearer to those of pure tricosanoic acid. Indeed, the *C*-polymorphic form of the oxidation product gives a *c* spacing almost identical with that attributed in the literature by extrapolation to tricosanoic acid itself (Francis, Piper, and Malkin, *Proc. Roy. Soc.*, 1930, **128**, 214).

It is, however, quite certain from the observations here recorded that Klenk's specimen is not pure tricosanoic acid, in spite of the process of purification to which it has been submitted. Pure tricosanoic acid proves not to crystallise in the normal *B*-form, and also cannot be maintained in the *C*-modification down to room temperature. Moreover, the molecular weight of Klenk's acid found by the *X*-ray method is higher than that required for pure $C_{23}H_{46}O_2$, being nearer to that for $C_{24}H_{48}O_2$, and the *c* spacing of its potassium salt is decidedly longer than that of pure tricosanoic acid. Taken together, the measurements indicate that Klenk's oxidation product is mainly tricosanoic acid, and that it is contaminated with an acid or acids of longer chain length, probably $C_{25}H_{50}O_2$ among others, as suggested by Chibnall, Piper, and Williams. Comparison with the figures given by these authors for binary mixtures of $C_{23}H_{46}O_2$ and $C_{25}H_{50}O_2$ suggests that the oxidation product contains at least traces of a third constituent.

It is interesting, further, that the *c* spacing measured on Klenk's phrenosinic acid is itself appreciably shorter than that recorded by Chibnall, Piper, and Williams on Dr. Rosenheim's preparation (55·4, 56·2 A.). This suggests that Klenk's preparation is more nearly pure $C_{24}H_{48}O_3$ and supports the contention raised by the above authors that the acid obtained from different sources may vary slightly in composition.

EXPERIMENTAL.

Phrenosinic Acid (Klenk).—The acid supplied by Klenk was too finely crystalline to allow direct single-crystal measurements to be obtained. Attempts were therefore made to recrystallise it from acetone and from benzene, but in both cases gel formation occurred, as described by Chibnall, Piper, and Williams. From benzene the effect was particularly striking, the whole solution after gentle warming setting immediately to a jelly on cooling.

By melting and recrystallisation under a heat gradient, sheets of phrenosinic acid could be obtained which appeared under the microscope to be single crystals. They showed a good extinction and in convergent light what appeared to be a rather poor positive biaxial figure slightly inclined to the plane of the sheet (001). The *X*-ray examination proved, however, that these crystals were invariably twins on (001) of two probably triclinic crystals with *a* axes inclined at an angle of about 19°. All the *X*-ray reflections were very much blurred, as would be expected from an impure substance, and accurate measurements were therefore impossible.

Oxidation Product of Phrenosinic Acid (Klenk).—By recrystallisation from acetone, this acid was obtained in very good, small, rhomb-shaped plates, showing the forms {001} (dominating) and {110} (Fig. 1). Through (001) a positive biaxial figure was observed with a very small optic axial angle, α along *b*, γ much inclined to (001). The unit cell dimensions (see table) show that the *B*-polymorphic type of fatty acid structure is present as measured by Muller for stearic acid, where $a = 5{\cdot}55$, $b = 7{\cdot}38$, and $c \sin \beta = 43{\cdot}76$ A. (*Proc. Roy. Soc.*, 1927, *A*, **114**, 535).

The density of the crystals was determined by flotation in very dilute zinc sulphate solutions, the centrifuge being used to hasten equilibrium. The value obtained was 1·037 ± 0·003, and this, combined with the cell dimensions, gives as the " molecular weight " of the acid present 364 ± 6 (Calc.: for $C_{24}H_{48}O_2$, 368; for $C_{23}H_{46}O_2$, 354). In the case of a mixture, the measurement might lose significance owing to variation among individual crystals. Such variation was not observed on the two different crystals examined.

FIG. 1.

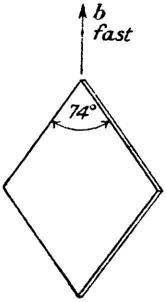
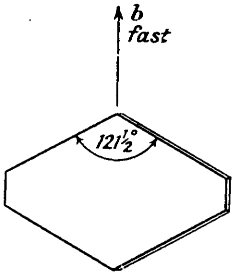


FIG. 2.



On melting and recooling, a second polymorphic modification could be obtained in sheets, which were approximately single crystals. A flake broken off gave from oscillation photographs about *a* the typical *C*-type spacings recorded in the table.

Tricosanoic Acid, $C_{23}H_{46}O_2$ (Ashton, Robinson, and Smith).—By slow cooling from solution in acetone, crystals of tricosanoic acid were obtained as large irregular flakes up to 2 mm. long. These proved to be triclinic, and therefore not to belong to either of the normal *B*- and *C*-types, though the axes may be chosen to show the relation of the structure with both of these. The direction of the fast extinction in the plane of the plate (001) lies along [010], which is also the direction along which the crystals are generally elongated; but the (010) plane most similar in intensity to (010) of the *B*-type is at angle of 73° to this direction. The cell dimensions given in the table show the close relation between this structure and the *B*-type. Further, in this cell *h*0*l* reflections do not appear for *h* odd.

On melting and then recooling these crystals, the *C*-modification first appears, but this is transformed exceedingly rapidly as the temperature falls, so that it was never possible to make *X*-ray or optical measurements on this variety at ordinary temperatures. In this, the behaviour of pure tricosanoic acid differs markedly from that of the oxidation product of phrenosinic acid, on which measurements of the *C*-form are easily obtainable. Such stabilisation of a metastable modification is, however, often produced by quite small traces of impurity (cf. picrates of the Diels hydrocarbon, Gamble, Kon, and Saunders, J., 1935, 644).

Lignoceric Acid, $C_{24}H_{48}O_2$ (Ashton, Robinson, and Smith).—Lignoceric acid crystallised from acetone in small rhomb-shaped plates showing the forms {001} (dominating), {100} (very small), and {110} (Fig. 2). The plane of the optic axes is (010), and γ is much inclined to the *c* face. The (200) reflection is very strong. Oscillation photographs were taken about *a*. This crystal modification is evidently the *C*-variety similar to that described by Brill and Meyer (*Z. Krist.*, 1928, **67**, 570) for lauric acid in which $a = 9{\cdot}76$, $b = 4{\cdot}98$, $c = 36{\cdot}9$, and $\beta = 48°\ 6'$. It is interesting that in this case the *C*- and not the *B*-variety has separated directly from solution (cf. Francis, Piper, and Malkin, *loc. cit.*).

Potassium Salts of Lignoceric and Tricosanoic Acids and of the Oxidation Product of Phrenosinic Acid.—The potassium salts were prepared by warming equivalent quantities of the acid and of potassium hydroxide together in alcoholic solution. With $C_{23}H_{46}O_2$, the acid potassium salt separated first, as proved by the *X*-ray measurements. This was subsequently converted into the normal salt.

The salt from the oxidation product of phrenosinic acid consisted of very poor, cloudy, minute crystals matted together so that they appeared to form plates. It was only possible to obtain a photograph to give the *c* spacing from a sample pressed on to a cover slip. The normal potassium salt of tricosanoic acid was photographed similarly for comparison.

The acid potassium salt of tricosanoic acid formed small, clear, lath-shaped crystals showing the (001) face with very small faces {100}, {210}, and elongated along [010]. The extinction is straight, the fast direction being along the needle axis. The *X*-ray measurements indicated the presence of an orthorhombic structure of some complication with the double layer of molecules probably placed lengthwise along *c*. Oscillation photographs were taken about the *a* and the *b* axis.

Substance.	Space group.	*n*.	*a*, A.	*b*, A.	*c*, A.	β.	d_{001}, A.	d_{010}, A.
Phrenosinic acid	$P1$	4	6·1	—	—	—	53·9	8·1
Oxidn. product of phrenosinic acid	*B* $P2_1/a$	4	5·57 ± 0·03	7·41 ± 0·03	—	—	56·50 ± 0·10	—
	C $P2_1/a$	4	9·0	5·0	65·8	128°	51·8	—
$C_{23}H_{46}O_2$	$P1$ or $P\bar{1}$	4	5·5	8·8	—	γ 73	53·1 (53·4)	7·5
$C_{24}H_{48}O_2$	*C* $P2_1/a$	4	9·0	4·97	66·5	127·5	52·8 (52·8)	—
K salt of oxidn. product	—	—	—	—	—	—	52·6	—
$C_{23}H_{45}O_2K$..................	—	—	—	—	—	—	51·7 (51·8)	—
$C_{23}H_{45}O_2K,C_{23}H_{46}O_2$ ...	*Cmmb* ?	16	18·1	8·5	63·3	—	63·3 (63·25)	—
$C_{24}H_{47}O_2K$..................	$P1$ or $P\bar{1}$	2	5·6	4·2	65·4	124	54·2 (54·45)	—

Except where otherwise stated the measurements are not accurate to more than ±2%. Figures in parentheses are given for comparison from papers by Piper (J., 1929, 234) and Francis, Piper, and Malkin (*loc. cit.*).

The normal potassium salt of lignoceric acid also formed sufficiently good single crystals for measurement. These were very curious, curved, irregular plates, tending to be elongated along [110]. Through the plane of the plate (001) an optic eye is visible, part of a positive biaxial figure. The plane of the optic axes is (010), γ is much inclined to the *c* face. The crystals are probably triclinic, but can be given pseudomonoclinic axes. Oscillation photographs were taken about the *a* and the *b* axis.

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