XVIII. THE FATTY ACIDS OF PHRENOSIN AND KERASIN.¹

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(Received November 21st, 1935.)

INTRODUCTION.

THE constitution of the fatty acid components of the two cerebrosides phrenosin and kerasin has been the subject of many investigations since their first separation by Thudichum, and a review of the work of Thierfelder, Levene, Rosenheim, Klenk, *etc.* is given in the two monographs by MacLean and MacLean [1926] and Thierfelder and Klenk [1930], which cover the literature to the date of their respective publications. During the last five years, however, doubt has arisen as to the constitution of phrenosinic acid, and to appreciate the position it will be necessary to recapitulate briefly certain of the earlier researches, for it is out of them that the present dispute has arisen.

Thierfelder was the first to show that Thudichum's "neurostearic" acid was a hydroxy-acid of the apparent formula $C_{25}H_{60}O_3$, whilst Levene and Jacobs [1912] suggested that it was α -hydroxypentacosanoic acid from evidence based on its oxidation to what they then considered to be *n*-tetracosanoic acid (m.p. 81°). A synthesis of (racemic) α -hydroxy-*n*-pentacosanoic acid by Brigl [1915] however led to inconclusive results. Meanwhile Levene and West [1914] modified their former view, and claimed that the acid formed on oxidation was "lignoceric" acid $C_{24}H_{48}O_2$ with a branched chain and not the normal acid, so that phrenosinic acid itself had a branched chain. Additional evidence was furnished by Levene and Taylor [1922], who synthesised apparently identical samples of α -hydroxyisopentacosanoic acid from their "lignoceric" acid prepared from phrenosinic acid and the naturally occurring "lignoceric" acid of arachis (peanut) oil. In assessing the validity of this evidence it is to be remembered that the composite nature of "lignoceric" acid was not apparent at that time, so the fact that all the products made from it were complex mixtures was not realised.

The first suggestion that phrenosinic acid was not $C_{25}H_{60}O_3$ but $C_{24}H_{58}O_3$ was made by Klenk [1928, 1], who claimed that the main product of oxidation was *n*-tricosanoic acid, m.p. 78.5° and mol. wt. 354. Further, hydrogenation of α -hydroxynervonic acid, in which the α -position of the hydroxyl group had been

¹ The nomenclature used in this paper is that suggested by Posner and Gies [1906] and Rosenheim [1909; 1914], which was adopted by MacLean and MacLean [1926]; phrenosin = Thierfelder's "cerebron" and phrenosinic acid = Thudichum's "neurostearic" acid = Thierfelder's "cerebronic" acid. We are in agreement with the present view of Dr Rosenheim that since Thierfelder himself admitted before his death that his "cerebron" and Thudichum's phrenosin are one and the same substance [*cf.* Thierfelder and Klenk, 1930, p. 4] it seems only fair to Thudichum's memory that his nomenclature should be retained and the names "cerebron" and "cerebronic" acid be abandoned.

fixed by degradation, gave phrenosinic acid; the latter acid must therefore also be an α -hydroxy-acid, *i.e.* α -hydroxy-*n*-tetracosanoic acid. The view was disputed by Levene and Taylor [1928], who rigidly purified some earlier samples of phrenosin by repeated crystallisation from various solvents and obtained from the phrenosinic acid by oxidation a fatty acid, M.P. 79–80°, which had mol. wt. 367 and appeared to be “lignoceric” acid. A year later Taylor and Levene [1929] returned to the subject and reported that a new sample of phrenosinic acid, prepared from some phrenosin whose preparation was not described, had given on oxidation fatty acids of distinctly lower mol. wt. than before, and from them a tricosanoic acid, M.P. 77·8–78·6° mol. wt. 356, thought to be identical with that of Klenk, was prepared. Repeated fractional distillation of this acid, however, gave a low-boiling fraction, M.P. 74–75°, which had mol. wt. 341, corresponding to docosanoic acid, and higher-boiling fractions, one of which, M.P. 78·5–79·5°, had mol. wt. 367, corresponding to tetracosanoic (“lignoceric”) acid. They therefore suggested that phrenosinic acid was a mixture. Klenk and Diebold [1933] emphatically refuted this claim of Taylor and Levene. They again oxidised a large sample of phrenosinic acid, rigidly fractionated the resulting acids as esters in a still of the type designed by Jantzen and Tiedcke [1930] and claimed a yield of 85 % of *n*-tricosanoic acid, M.P. 79·5°, which gave no depression when melted with a sample of synthetic acid, M.P. 79·5–80°. The later publications of these authors [Levene and Heyman, 1933; Taylor and Levene, 1933; Levene and Yang, 1933; Klenk, 1934] merely emphasise their respective points of view.

Our experience in unravelling the constitution of the mixed fatty acids and alcohols of waxes [Chibnall *et al.* 1934] suggested to us that these conflicting views might well be reconciled if it be assumed that phrenosinic and “lignoceric” acids were not chemical entities, but were each of them a mixture of near homologues. Dr Rosenheim very kindly offered to place at our disposal for an investigation on these lines samples of highly purified phrenosin and kerasin prepared by him many years ago. The results were that the phrenosinic acid from the phrenosin was found to be a mixture of α -hydroxy-*n*-hexacosanoic and α -hydroxy-*n*-tetracosanoic acids, whilst the “lignoceric” acid from the kerasin, like the similar acid frequently isolated from other natural sources, is a mixture of *n*-hexacosanoic, *n*-tetracosanoic and *n*-docosanoic acids.

EXPERIMENTAL.

Melting points. The melting points recorded in this paper were obtained by the method described by Piper *et al.* [1931], in which the temperature of the bath is raised very slowly. Rapid heating according to the method of Levene [*cf.* Levene and Taylor, 1922] in which the temperature of the bath is raised 6–7° per min., gives a melting-point about 1° higher, *e.g.* a sample of phrenosinic acid which we record as melting at 102–102·5° would melt at 102·5–103·5° by Levene’s method.

1. *Phrenosinic acid.*

Preparation and general properties. The sample of phrenosin was of the same degree of purity as those described by Rosenheim [1914], and was reported by him to be practically free from phosphorus, to have the correct rotation and to show the absence of kerasin in the selenite plate test. It was hydrolysed in the usual way with 10 % methyl alcoholic H_2SO_4 , the phrenosinic acid separated as the ester and purified through the magnesium soap [Thierfelder, 1913; Klenk, 1927]. It melted at 101·3–101·5° and crystallised from alcohol in the well-known “cauliflower-like” masses described by Thudichum, which are made up of

irregular sphaero-crystals. Attempts to obtain crystals from warm acetone were unsuccessful, as the product gelled. X-ray analysis gave a very poor plate (Series No. B 390) showing three diffuse orders. Further purification was effected in the following way. The material (1.15 g.) was dissolved in pyridine (20 ml.), which was then poured into water. The aqueous solution was extracted twice with ether and then acidified with excess of hydrochloric acid. The precipitated phrenosinic acid was extracted with ether, the ethereal solution washed until free from mineral acid, filtered to remove a slight suspension of insoluble material and the solvent removed. The residue was crystallised from 30 ml. of alcohol at 0°, the product taken up in the same volume of warm acetone and filtered from a small insoluble residue. On cooling the acetone solution to 25° the phrenosinic acid separated in the usual "cauliflower-like" masses, which melted sharply at 102.3–102.6° and set in long needles at 101.8°. (Found: C, 75.54, 75.40; H, 12.47, 12.63 %; mol. wt. by titration 396. $C_{25}H_{50}O_3$ requires C, 75.30; H, 12.65 %, mol. wt. 398.4.) For a 6.01 % solution in pyridine the observed rotation was $\alpha_D^{25} + 0.20^\circ$ whence $[\alpha]_D^{25} = +3.33^\circ$. X-ray analysis (Series B 405) gave a better plate with 5 orders measuring 54 Å., but the lines were still diffuse.

Attempts to prepare larger crystals more suitable for X-ray analysis by using different solvents and conditions were not very successful, and the best photograph was finally obtained from material crystallised from 85 % ethyl alcohol. The spacings obtained (Series No. B 411) were 55.4 Å. and 50.9 Å., but in the absence at this stage in our investigation of data concerning the crystal spacings of α -hydroxy-acids we could make no suggestion of chain length and could only infer from the poor quality of the plate that the phrenosinic acid, if a chemical entity, was heavily contaminated with some other acid.

To effect further purification the acid was acetylated with acetyl chloride in the usual way, and the product, which was freely soluble in cold light petroleum, separated from that solvent on evaporation in clusters of microscopic broken needles which melted at 66–66.5°. (Found: C, 73.69, 73.61; H, 11.96, 11.79; OC.CH₃ 9.6. $C_{27}H_{52}O_4$ requires C, 73.55; H, 11.9; OC.CH₃ 9.7 %.) The acetyl value was determined by the micro-method of Kögl and Postowsky [1924]. It was extensively fractionated without change of m.p. and no evidence for the presence of "lignoceric" acid was obtained, showing that the phrenosinic acid was not contaminated with this acid. Acetylation therefore suggested that if phrenosinic acid were a mixture the components were all hydroxy-acids. The X-ray photograph of the acetyl derivative was extremely poor and uninterpretable.

Study of a series of synthetic i- α -hydroxy-n-fatty acids. Unlike Levene and Klenk, who have both handled relatively large amounts of phrenosinic acid during the course of their investigations, we had at our disposal only about 4 g. and were therefore obliged to proceed with very great caution. In our previous work on the constitution of the wax fatty acids success was only achieved after careful study of the physical properties and X-ray data of the whole series of higher *n*-fatty acids and we felt that some such study would amply repay us in the present case. Synthesis of α -hydroxy-acids of chain length from (say) C_{20} to C_{26} was however not feasible, and we were fortunate in being able to obtain from Dr N. K. Adam samples of the inactive acids from C_{13} to C_{18} prepared by Le Sueur [1905].¹ These products had been synthesised at a time when the criteria necessary for judging the purity of their *n*-fatty acid precursors were

¹ A sample of α -hydroxyheneicosanoic acid prepared by Le Sueur and Withers [1915], by an indirect method from erucic acid, gave a very poor photograph. The melting point (91–92°) was too low for the pure acid.

still ill-defined, and it is a great tribute to the experimental skill of the late Dr Le Sueur that they stand up so remarkably well to the exacting requirements of modern X-ray analysis, for all of them, after appropriate crystallisation from acetone, gave excellent X-ray photographs.

The melting points, in agreement with Le Sueur's, are given in Table I, and as he observed, they lie on one smooth curve and show no odd-even alternation characteristic of the *n*-fatty acids. We have prepared all the acetyl derivatives

Table I. *Melting points and long crystal spacings of a series of i- α -hydroxy-n-fatty acids.*

No. of carbon atoms	M.P. (°C.)	Series no.	Crystal spacings in Å.			Acetyl derivative M.P. °C.	Difference between M.P. of acid and its acetyl derivative °C.
			Pressed (P) or melted (M) layer	A	B	C	
13	78	B 409	P	34.55	—	—	—
14	81.5–81.7	B 408	{P M}	— *38.7	33.7 33.75	31.8	56–56.5 25.2
15	84.5–84.7	B 396	{P M}	39.5 39.1	—	—	49.5–50 34.7
16	86.9–87.2	B 389	{P M}	— 42.3	38.0 38.05	—	63.2–63.5 23.7
17	89.3–89.6	B 395	P	44.6	—	—	59.3–59.5 30.1
18	91.5–91.7	B 394	{P M}	— —	42.25 42.3	39.0 40.2	70.1–70.3 21.4
Phrenosinic acid	—	B 411	{P M}	— —	55.4 56.2	50.8	66.5 36.5
70% C ₁₅ +30% C ₁₇	—	B 399	P	42.6	—	—	—
70% C ₁₆ +30% C ₁₈	—	B 400	P	—	39.7	37.6	54.5 25.5
40% C ₁₄ +40% C ₁₆ +20% C ₁₈	74.6	—	—	—	—	—	39.0 35.6

* Only first order observed.

and unexpectedly in this case there is a pronounced odd-even alternation in the M.P. curve. This is reflected in the figures given in the last column of Table I, showing the differences between the M.P. of the acids and their corresponding acetyl derivatives; that of the C₁₆ acid is 23.7°, of the C₁₇ acid 30.1° and of the C₁₈ acid 21.4°. Extrapolating from these results the C₂₄ and C₂₅ acids will melt at about 101° and 103°, and their acetyl derivatives about 83 and 85° respectively, giving a difference of 16–18°. (The synthetic C₂₅ acid of Brigl [1915] melted at 102–104°; no acetyl derivative was recorded.) These figures are in sharp contrast to those of phrenosinic acid and its acetyl derivative, with a difference of 36.5° as shown in Table I. It is true that this latter acid is optically active, so that a direct comparison is not warranted, yet the evidence certainly casts doubt on Klenk's contention that it is α -hydroxy-*n*-tetracosanoic acid, for in that case the optically active isomeride would melt at the most only 1–2° higher than the inactive acid, whilst its acetyl derivative would melt 17–18° lower. A series of mixed inactive acids and of the corresponding acetates was then prepared. The melting points of a few examples are recorded in Table I; these certainly lend support to the impression gained from the X-ray study of phrenosinic acid, that it is a mixture of hydroxy-acids. Before discussing the X-ray data given in Table I, which lead to a similar conclusion, it will be convenient to consider whether phrenosinic acid is a mixture which might contain β - or γ -hydroxy-acids.

Klenk [1927] showed that when a boiling alcoholic solution of magnesium acetate was added to a boiling alcoholic solution of phrenosinic acid the magnesium soap was immediately precipitated and he made use of this observation to separate this acid from "lignoceric" acid. The property is shared by the lower α -hydroxy-acids of Le Sueur, but γ -hydroxystearic acid [Clutterbuck, 1924], which has been tested, gives no insoluble magnesium soap under these conditions, so that if phrenosin on hydrolysis gives any γ -hydroxy-acid or its lactone these would, during the magnesium soap separation, pass into the "lignoceric" acid filtrate, for both of them would be soluble in boiling alcohol. The presence of a γ -hydroxy-acid or its lactone in phrenosinic acid can therefore be definitely excluded.

Brigl [1915] obtained the first direct evidence for the presence of an α -hydroxyl group in phrenosinic acid by preparing the chloralide; no yield was quoted however so that if the acid be a mixture the presence of a β -hydroxyl group is not thereby excluded. We have sought for evidence of the latter by very cautious oxidation. Under these conditions the α -hydroxy-acid should give the ($n-1$) aldehyde and its corresponding acid, whilst the β -hydroxy-acid should give the β -keto-acid, the methylketone and the ($n-2$) fatty acid. The oxidation gave 21.5% of neutral material, and a parallel oxidation of α -hydroxypalmitic acid gave a somewhat smaller yield. The material from the phrenosinic acid, on recrystallisation from alcohol, melted at 53–53.5°, and had all the properties of the lower (C_{13} – C_{17}) aldehydes described by Le Sueur [1905], *viz.*, it was readily soluble in all ordinary organic solvents in the cold, reduced permanganate slowly in the cold, gave a semicarbazone which crystallised in needles melting at 107–108° and was fairly readily polymerised in moist ether to give a product insoluble in cold ether and alcohol. Extrapolation of Le Sueur's data shows that n -tricosanaldehyde would melt at about 52–54° and its semicarbazone at about 109–110°, whereas the known data for methylnonadecanyleketone suggest that methylheneicosanyleketone would melt at about 67° and its semicarbazone at 130°. The evidence shows clearly that the hydroxy-group in phrenosinic acid is largely, if not entirely, in the α -position, in agreement with Klenk [1928, 1].

Since the series of α -hydroxy-acids given in Table I does not show alternation of m.p., the acids should adopt a crystalline modification characterised by a vertical molecule in the neighbourhood of the m.p. for both odd- and even-numbered chains [Malkin, 1931]. The odd-numbered acids examined were found to occur only in this A modification which was also found for α -hydroxypalmitic acid in a layer that had been very quickly cooled from the melt. A single, diffuse but measurable line for a similar layer of the α -hydroxymyristic acid gave a value suggesting that this modification was present, but we were unable to detect it with certainty in the case of α -hydroxystearic acid. A photograph taken with this acid on a heating stage at a temperature that kept it partly liquid did show a very weak long spacing, but the lines were too faint to measure with certainty. The modification can presumably exist only very near to the m.p. of this acid and must have a very small range of stability. Of the B and C forms observed for the even-chain acids B is the stable modification.

These acids in the pure form give very good reflections. The orders are recorded up to the extreme angle of rock, and the intensity distribution differs characteristically from that of a n -acid. As usual the mixtures give poorer reflections and more diffuse lines, and frequently not enough orders of reflection are registered to establish the intensity distribution characteristic of the series. Phrenosinic acid gave a photograph characteristic of a mixture and since it crystallises in two forms it cannot be a mixture of odd-number acids. The mea-

sured spacings 55.4 Å. and 50.8 Å. agree tolerably well with the extrapolated data 55.5 Å. and 51.5 Å. for C_{24} hydroxy-acid, but not with the spacings for C_{23} or C_{25} which would be about 60 and 65 respectively. Thus the crystallographic measurements suggest a mixture of even-number hydroxy-acids of mean mol. wt. corresponding closely to C_{24} .

The fatty acids obtained by oxidation of phrenosinic acid. Two samples of the acid were oxidised, the subsequent treatment of the oxidation product differing in each case.

(1) 0.6 g. of phrenosinic acid was dissolved in 80 ml. of glacial acetic acid at 60° and treated at that temperature with 0.4 g. of CrO_3 in 20 ml. of the same solvent added in small amounts during the course of 15 min. Excess of water was then added and the precipitated fatty acid filtered off and thoroughly washed. It was then dissolved in 60 ml. of boiling alcohol and treated with a hot alcoholic solution of magnesium acetate to precipitate the major part of any unchanged phrenosinic acid. This was filtered off and the fatty acid remaining in the alcoholic filtrate recovered in the usual way. The product (0.45 g.) was then treated with boiling acetyl chloride for 2½ hours, the mixture poured into excess of chilled water and the precipitated material filtered off. This was dissolved in ether, well washed with water, the solvent removed and the residue crystallised from acetone at room temperature to free it from any acetylphrenosinic acid. The resulting material (0.35 g.) was then saponified with 5 % alcoholic NaOH for 1 hour to reconvert any acid anhydride formed in the previous operation, the sodium soap was well washed with ether and the acid recovered in the usual way. Two crystallisations from acetone at 37° gave fraction 1 (0.17 g.), M.P. 77.4–77.6°. The mother-liquors gave crystalline fractions with mol. wt. between 360 and 390, suggesting that the treatment with acetyl chloride had led to more extensive anhydride formation than was expected, and the products were discarded.

(2) Another 0.6 g. of phrenosinic acid was oxidised under the same conditions as before. Excess of water was then added and the precipitated fatty acid filtered off and thoroughly washed with water. The dry product (0.54 g.) was dissolved in ether, which was filtered to remove a small insoluble residue. An alcoholic solution of sodium ethoxide was then cautiously added until precipitation of the sodium soap was complete. This was centrifuged and washed repeatedly with ether; the fatty acid was recovered in the usual way and crystallised from acetone at 0°, M.P. 75.2–75.4°. This acid (0.43 g.) was then fractionated by the method of Klenk [1928, 1]. It was dissolved in 10 ml. of boiling methyl alcohol and treated successively with 0.3 ml., 0.7 ml. and 0.7 ml. of boiling methyl alcoholic lithium acetate, the precipitated soap in each case being filtered off hot. The first precipitate (27 mg.) was refluxed with 10 ml. of boiling methyl alcohol for 6 hours; solution was then complete, showing that very little unchanged phrenosinic acid was present. The recovered acid (10 mg.) melted at 75.4–75.6° (fraction 2). The second (0.18 g.) and the third (0.14 g.) samples of soap were converted into the free acids, which were crystallised from 10 ml. of acetone at 0° (0.15 g., M.P. 76.1–76.3°; 0.12 g., M.P. 75.6–75.8°). To obtain good crystals for X-ray analysis both acids were recrystallised slowly from acetone at 25° to give fraction 3 (95 mg.) and fraction 4 (80 mg.) respectively. The filtrate obtained after removal of the lithium soaps mentioned above gave no further precipitate on the addition of alcoholic lithium acetate, and on treatment gave 18 mg. of acid (fraction 5).

Fractions of fatty acids obtained by oxidation of phrenosinic acid.

Fraction	M.P.	C %	H %	Mol. wt. by titration
1	77.4-77.6	78.0	13.1	353
2	75.4-75.6	77.7	13.0	—
3	76.5-76.7	78.1	13.3	363
4	76.8-77.0	78.0	13.2	360
5	73.1-73.3	77.3	12.5	—

Fractions 1, 3 and 4, which represent the main bulk of the oxidised material in the two cases, have been further investigated in some detail. All three crystallised in fine plates which, under the microscope, were seen to consist of very small rhombs with sharp angles and straight cleavage edges, suggesting that they were either pure *n*-fatty acids or relatively simple mixtures.

X-ray analysis of the fatty acid fractions. From the mixed M.P. curves of Fig. 1 a list is made of all the mixtures which have melting-points close to that of the fraction to be analysed. From this list those mixtures which have mol. wt.

Fig. 1.

within ± 5 of that of the unknown mixture are transferred to the first column of Table II. The other columns contain the remaining relevant information. From an inspection of the table it is usually possible to find a binary mixture which is a good fit throughout. The spacings are correct to $\pm 0.5 \text{ \AA}$. Suggestions as to whether one is dealing with odd- or even-number mixtures are often obtained from an inspection of the intensity distribution in the photographs, for the even orders of reflection are usually absent from the pictures of the odd-number mixtures.

In the case of fraction 1 only one of the possible comparison mixtures (85% C₂₃ + 15% C₂₅) has two spacings which differ by about $5.0 \text{ \AA}$, but the M.P. of this

Table II. *Melting points and long crystal spacings of the fatty acid fractions obtained by oxidation of phrenosinic acid, together with certain standards for comparison.*

comparison.			Mixed m.p. of fraction with comparison mixture (1 : 1)	Long crystal spacings in Å.		Remarks	
Substance	Mol. wt.	m.p. (°C.)		Series no.	Pressed Melted		
Fraction 1	353	77.4-77.6	—	B 402	57.1 52.0	No even orders	
90% C ₂₃ +10% C ₂₅	357.2	77.8-78.0	77.5-77.7	B 412	55.7 52.1	"	
85% C ₂₃ +15% C ₂₅	358.6	76.9-77.1	77.0-77.2	B 438	57.1 52.1	"	
35% C ₂₂ +65% C ₂₄	358.5	77.3-77.6	77.0-77.3	Extra- polated	57.5 54.0	Even orders would appear	
20% C ₂₂ +80% C ₂₃	351.6	77.5-77.7	77.2-77.4	B 428	53.3 51.3	Even orders appear	
10% C ₂₁ +90% C ₂₃	353	77.7-78.0	77.5-77.7	B 430	53.3 51.6	No even orders	
Fraction 3	363	76.5-76.7	—	B 441	59.2- 52.6	52.3 —	"
80% C ₂₃ +20% C ₂₅	360	76.3-76.5	76.4-76.6	B 413	60.9- 57.45	57.1 —	"
Fraction 3a	—	77.0-77.2	—	B 447	57.6- 53	51.7	"
85% C ₂₃ +15% C ₂₅	358.5	76.9-77.1	70.0-77.2	B 438	57.1	52.1	"
Fraction 5	360	76.8-77.0	—	B 445	57.3	52.4	"
85% C ₂₃ +15% C ₂₅	358.6	76.9-77.1	76.8-77.0	B 438	57.1	52.1	"
40% C ₂₂ +60% C ₂₄	357.2	77.0-77.2	76.4-76.6	Extra- polated	57	54	Even orders would appear

mixture is a little low. The mixed M.P. however is between the two respective values, and we suggest that the mixture (87 % C₂₃ + 13 % C₂₅) melting at 77.4° and having spacings about 56.5 Å. and 52 Å. is a rather better fit, though the upper spacing is definitely too low. It will be noticed that the spacings only correspond at all well with those of mixed odd-number acids, and that for a range of mixtures of these acids containing 0-30 % of the longer component the steep rise of the B curve allows a very accurate assessment of the proportions of a simple binary mixture. It seems probable therefore that there is slight contamination of this fraction by a third acid, probably C₂₁, and that the proportions of the main bulk of the mixture are about 87 % C₂₃ + 13 % C₂₅.

Only one synthetic binary mixture corresponds at all for both melting point and mol. wt. with fraction 3. Both the fraction and the mixture have the long A spacing, but their behaviour on melting differs. The appearance of the low spacing (52 Å.) on melting corresponds to less than 20 % of the longer chain, whilst the A spacing suggests at least 20% of this chain, and this anomaly might quite well be due to contamination. Recrystallisation gave fraction 3a, and though there was not enough material to allow a redetermination of the mol. wt. it will be seen that there is good agreement for the rest of the data with the mixture 85 % C₂₃ + 15 % C₂₅. We believe therefore that fraction 3 is a mixture 83 % C₂₃ + 17 % C₂₅ with a trace of C₂₁.

Table III. *Melting points and long crystal spacings of mixtures of n-docosanoic and n-tetracosanoic acids.*

Percentage composition		M.P. (°C.)	Spacings in Å.		
C ₂₂	C ₂₄		Series no.	Pressed layer B	Melted layer C
100	0	80-80.2	—	53.5	48.3
90	10	78.2-78.4	B 422	54.4, 50.2	49.2
80	20	74.9-75	B 423	53.5	49.17
70	30	74.5-74.6	B 420	56.3	53.8
60	40	75.4-75.5	B 424	56.5	53.6
50	50	75.5-75.7	B 421	56.3	54.5
0	100	84-84.2	—	57.7	52.6

The spacing data for fraction 5 limit the choice of the two comparison mixtures to 85 % C_{23} + 15 % C_{25} and the fit throughout is good.

It will be noticed that certain odd-even chain mixtures in Table IV have spacings which agree well with those found for the fractions discussed above, but all such mixtures are definitely ruled out by their m.p. and mol. wt.

Reduction of phrenosinic acid. We have repeated the experiments of Klenk [1928, 2], but with only 0.37 g. of phrenosinic acid available the results were inconclusive. Much neutral material was obtained and the acidic products (0.14 g.) on fractionation gave a small amount of acid, m.p. 78.0–78.5°, which, when viewed under the microscope, was seen to consist of masses of broken leaflets having no recognisable cleavage angles or edges. As one would expect, the X-ray photograph was too poor for interpretation.

The constitution of phrenosinic acid. Reviewing briefly the evidence given above we are able to state that the phrenosinic acid obtained from the sample of phrenosin prepared by Dr Rosenheim was definitely a mixture of even-number α -hydroxy-acids of mean mol. wt. corresponding closely to C_{24} , which on oxidation gave a mixture of odd-number n -fatty acids, the main bulk of which was definitely identified as 85 % C_{23} + 15 % C_{25} . We feel justified therefore in drawing the conclusion that the phrenosinic acid mixture consisted in very large part of α -hydroxy- n -tetracosanoic acid (85 %) and α -hydroxy- n -hexacosanoic acid (15 %). Our findings are thus in substantial agreement with those of Klenk, and we have no doubt that by fractional distillation of the methyl esters of the oxidised acids he obtained n -tricosanoic acid in good yield. We differ from him only on the composite nature of the phrenosinic acid, a point which we feel needs further discussion, because the components will vary somewhat according to the degree of purification to which the parent phrenosin has been subjected, and therein we believe lies the origin of the discrepancies between his results and Levene's.

Our own results leave no doubt that phrenosin, like lecithin, kephalin and (as we show later) kersin, is a mixture, the components of which differ only in the chain length of the fatty acid. As Dr Rosenheim's sample of phrenosin was exhaustively freed from kersin and then recrystallised 4 times from pyridine, it would naturally represent a phrenosin rich in the more insoluble (*i.e.* longer-chain acid) components. Levene and Taylor's earlier sample had also been rigidly purified, and the acids obtained on oxidation of the phrenosinic acid had a slightly higher mol. wt. (367) than ours. But their later sample, which they required in large bulk, was clearly not so well purified, for the oxidised acids in this case had a definitely lower mol. wt., and in our opinion show clearly the presence of n -heneicosanoic as well as n -tricosanoic and n -pentacosanoic acids. The lower-boiling fraction, m.p. 74–75°, mol. wt. 341, which they suggested was docosanoic acid, is clearly about 40 % C_{21} + 60 % C_{23} (m.p. 73.5, mol. wt. 343) and their higher-melting fraction, m.p. 79.5–80°, mol. wt. 367, which they suggested was "lignoceric" acid, is clearly about 40 % C_{23} + 60 % C_{25} (m.p. 77.8°, mol. wt. 371) in both instances allowance being made for the difference between Levene's and our own methods of taking melting-points. There is no doubt therefore that brain contains at least three phrenosins, whose acidic components are α -hydroxy- n -docosanoic, α -hydroxy- n -tetracosanoic and α -hydroxy- n -hexacosanoic acids respectively and that following Klenk's [1928, 2] suggestion, they are the α -oxidation products of the corresponding kersins.

2. *Thudichum's neurostearic acid.*

Through the kindness of Dr Rosenheim and Sir Henry Dale we have been able to examine a sample of Thudichum's original preparation of neurostearic acid, which is now preserved together with many other of his preparations at the National Institute for Medical Research [cf. Rosenheim, 1930]. The label on the specimen bottle bore the inscription "Neurostearic Acid, m.p. 85.5°; C=75.88%, H=12.85%, O=11.27%", showing that it was the identical specimen described by Thudichum [1879].

We found that the mol. wt. by titration, which Thudichum did not determine, was 381 and that for a 6.8% solution in pyridine the observed rotation was $\alpha_D^{18} + 0.06^\circ$, whence $[\alpha]_D^{18} = +0.9^\circ$. These values suggest that the preparation contained approximately 25-30% of phrenosinic acid, the remainder being "lignoceric" acid. 0.5 g. in 10 ml. of boiling methyl alcohol was treated with 1 ml. of 10% lithium acetate in the same solvent and the precipitated soap filtered hot (0.25 g.). This was suspended in 20 ml. of methyl alcohol, which was then boiled under reflux for 7 hours. The insoluble residue was filtered hot (0.12 g.) and the free acid recovered (0.11 g. m.p. 89-90°). The treatment with alcoholic lithium acetate (0.25 ml.) was then repeated, giving 60 mg. of fairly pure phrenosinic acid, m.p. 97.0-97.5°. (Found: C, 75.6; H, 12.7%; mol. wt. by titration 394.) For a 2.4% solution in pyridine the observed rotation was $\alpha_D^{18} + 0.1^\circ$, whence $[\alpha]_D^{18} = +3.7^\circ$.

3. "Lignoceric" acid from kerasin.

The acid generally referred to as "lignoceric" acid, which has mol. wt. corresponding to $C_{24}H_{48}O_2$ and m.p. constant at 80-81° under the usual conditions of purification, is the constituent fatty acid of kerasin and sphingomyelin and can be isolated from many naturally occurring fats and waxes. Meyer *et al.* [1913] were the first to show that it was not identical with synthetic *n*-tetracosanoic acid, which melted at 84.5°, and it became customary to regard the lower-melting acids as an isotetracosanoic acid with a branched chain. Brigl and Fuchs [1922] however examined the acid (m.p. 80-81°) prepared from beechwood tar, and isolated from it *n*-tetracosanoic acid, m.p. 84.5°, identical with the synthetic acid, and an isomeride, m.p. 74.5°, which they called *isolignoceric* acid. Levene *et al.* [1924], applying Brigl and Fuchs's methods to the lignoceric acid from arachis (peanut) oil and from kerasin, were unable to isolate any acid melting higher than 80.5-81° and reiterated the former view that lignoceric acid was not identical with *n*-tetracosanoic acid. Klenk [1927] however, who obtained the acid, m.p. 80-81°, from kerasin and fractionated it with methyl alcoholic lithium acetate, isolated a product, m.p. 83-84°, which was identified as *n*-tetracosanoic acid. More recently Jantzen and Tiedcke [1930] have fractionated the higher-melting fatty acids from arachis (peanut) oil in a new type of still and have obtained *n*-eicosanoic acid, *n*-docosanoic *n*-tetracosanoic acid (in largest amount) together with a residue of longer-chain acids. The purity of the fractions was checked against synthetic products, and no evidence for the presence of "*iso*-behenic" or "lignoceric" acid was obtained. Further, the crystal spacings of the two acids of Brigl and Fuchs have been examined [Francis *et al.* 1930]. That melting at 84.5° is definitely *n*-tetracosanoic acid, whilst the *isolignoceric* acid, m.p. 74.5°, showed no evidence of possessing a branched chain and had spacings of 57.2 Å. when pressed and of 52.52 Å. when melted, values which are similar to those of 50% C_{23} + 50% C_{24} , a mixture which melts at 79.8° and has spacings of 57.5 Å. and 52.0 Å. It was suggested on this evidence that C_{23} and C_{24} were probably the main components of this mixture, but recent experience has led us

to believe that mixtures of odd- and even-number acids do not occur in nature. It is probable that the *isolignoceric* acid was not a binary mixture but a more complicated mixture of the even-number acids, C_{22} , C_{24} , C_{26} , though we have not sufficient comparative data to suggest a percentage composition. Taken as a whole the composite nature of "lignoceric" acid appears to us to be definitely proven and in the present research we have sought to do no more than suggest the possible composition of the acid from kersin.

4.0 g. of kersin, of the same degree of purity as that described by Rosenheim [1914; 1916], when treated according to his directions gave 1.35 g. of "lignoceric" acid. After several crystallisations from acetone at room temperature this melted at 80.3–80.5°. (Found: C, 78.4, H, 13.1 %, mol. wt. by titration 369. $C_{24}H_{48}O_2$ requires C, 78.2, H, 13.1 %, mol. wt. 368.4.) These values are in general agreement with those of other workers. The crystal spacings (B 446) were 59.1 Å. pressed and 52.45 Å. melted, the plate having 7 sharp orders with the second well developed. The acid is definitely not a mixture of odd-number acids, and if a binary number of even-number acids, the m.p. and spacings suggest approximately 20 % C_{22} + 80 % C_{24} . But the observed mol. wt. is too high for such a mixture and, being equal to that of the C_{24} acid, suggests a ternary mixture in which this acid is accompanied by small and approximately equal amounts of C_{22} and C_{26} . We accordingly prepared two such mixtures (1) 10 % C_{22} + 80 % C_{24} + 10 % C_{26} . The m.p. was 80.8–81° and the crystal spacings were (B 431) 59.1 Å. pressed and 53.2 Å. melted, the plate being comparable in all respects with that of the "lignoceric" acid (B 446). (2) 15 % C_{22} + 70 % C_{24} + 15 % C_{26} . The m.p. was 79.1–79.3° and the X-ray photograph (B 432) showed only 5 diffuse orders giving a C spacing in both the pressed (55.7 Å.) and melted (54.8 Å.) layers. The mixed m.p. of mixture (1) with the "lignoceric" acid was 80.5–80.7, and in our opinion the former represents very closely the composition of the latter. At least three kersins therefore are present in brain, containing *n*-docosanoic, *n*-tetracosanoic and *n*-hexacosanoic acids as their acidic components respectively, and although we have no direct evidence on the point we feel that the same conclusion applies to sphingomyelin, which also contains "lignoceric" acid, m.p. 80–81°.

Many different mixtures of the *n*-acids, C_{20} , C_{22} , C_{24} , C_{26} and perhaps C_{28} can have m.p. 80–81° and mol. wt. about 368 and such undoubtedly occur widespread in nature and have hitherto all been referred to as "lignoceric" acid. But the pure C_{24} acid, m.p. 84.5°, has always been referred to by former workers as *n*-tetracosanoic acid, and it seems to us that the continued use of the name "lignoceric" acid for the product m.p. 80–81° tends to obscure its composite and varying nature with the result that unjustifiable conclusions as to its metabolism, based on the assumption that its molecule contains 24 (6×4) carbon atoms are still being drawn [cf. Klenk, 1928, 2]. We therefore recommend that the name "lignoceric" acid be abandoned, and that henceforth such acids be referred to simply as "mixed fatty acids m.p. 80–81°" [cf. "melissic" acid, *etc.*; Chibnall *et al.* 1934]. As long-chain α -hydroxy-acids occur, according to our present knowledge, only in cerebrosides, there seems less objection to the retention of the name "phrenosinic" acid.

4. *Melting points and long crystal spacings of certain mixtures of odd- and even-number n-fatty acids.*

The following series of mixtures of *n*-fatty acids have been prepared and examined during the course of the present research, and are to be regarded as an extension of the work of Piper *et al.* [1934]; the X-ray data include the spacings

observed for both pressed and melted layers. The series covers all possible binary combinations of the acids C_{21} – C_{25} , and the M.P. data, which call for no special comment, are given in Tables III and IV and graphically in Fig. 1.

Long crystal spacings of mixtures of n-docosanoic and n-tetracosanoic acids. Measurements of a series of mixed even-number *n*-fatty acids, C_{28} and C_{30} , have been given [Piper *et al.* 1931] and we now give some further measurements of a few mixtures of C_{22} and C_{24} acids. The latter are slightly more comprehensive in that they show the spacings in both B and C forms throughout the range of proportions investigated.

The crystal forms and transitions for pure odd-number n-acids and their mixtures. The odd-number acids appear in four modifications A, B, C, D [de Boer, 1927]. The A form has a vertical chain and has not been observed in pure acids containing more than 17 carbon atoms; B, C and D are characterised by increasing tilts of the chains. For chain lengths of 19 or more carbon atoms the B form is stable and transforms reversibly to C at a temperature a few degrees below the M.P. The B form is therefore usually adopted by crystals obtained from solution or from the melt, though sometimes the C form is stabilised by rapid cooling or by the presence of small amounts (up to 15 %) of a homologous impurity. We have not observed the D form for chain lengths greater than 15 carbon atoms.

The binary mixtures of odd-number acids give results which are numerically consistent. The spacings depend much less on the treatment of the specimen than is the case for the mixtures of even-number acids, and do allow one to fix the proportions of a binary mixture with a reasonable degree of certainty. The measurements are plotted in Fig. 2 and allow some straightforward generalisations.

Fig. 2. Crystal spacings, $C_{22} + C_{24}$ acids.

(1) When A and C spacings appear they lie in two straight lines joining the spacings of the pure acids. This is what would be expected on any simple theory, but has not been observed in any other mixtures of aliphatic compounds with which we have made measurements.

(2) The B spacings lie on a smooth curve which rises well above the line joining the spacings of the pure acids. Thus, when both B and C spacings occur there is in general a much greater numerical difference between them than for mixtures of two even-number acids. A B spacing lying on this curve has with a single exception been observed for every pair of odd-number acids examined.

(3) For proportions of 0 to 15 % of the longer-chain acid we obtained a B spacing from a pressed and C from a melted layer, but no A spacing.

Table IV. *Melting-points and long crystal spacings of certain odd-number and odd-even-number binary mixtures of n-fatty acids.*

Percentage composition		M.P. °C.	Series no.	Crystal spacings in Å.			
C ₂₃	C ₂₅			Pressed layer (P) or melted layer (M)	A	B	C
100	0	79.4-79.6	—	—	60*	83.5	51.8*
90	10	77.8-78.0	B 412	{ P M	— —	55.7 —	— 52.1
85	15	76.9-77.1	B 438	{ P M	— —	57.1 —	— 52.1
80	20	76.1-76.3	B 413	{ P M	60.95 —	57.45 57.1	— —
70	30	76.0-76.3	B 414	{ P M	61.6 —	58.1 58.4	— —
50	50	77.0-77.2	B 415	{ P M	63 —	59.4 —	— 54.1
40	60	77.7-77.8	B 440	{ P M	— —	59.9 59.2	— —
30	70	78.7-78.8	B 439	{ P M	— —	59.5 59.6	— —
20	80	80.2-80.4	B 434	{ P M	— —	58.7 59.3	— —
10	90	81.8-82.0	B 433	{ P M	— —	57.5 —	— 55.6
0	100	83.2-83.4	—	—	65*	57.8	56.2*
C ₂₅	C ₂₇						
50	50	80.9-81.1	B 398	P	67	64.25	—
70	30	80.5-80.7	B 397	P	66.34	—	—
C ₂₁	C ₂₃						
10	90	77.7-78.0	B 430	{ P M	— —	53.25 —	— 51.65
C ₂₃	C ₂₄						
90	10	79.0-79.2	B 417	{ P M	— —	57.1 57.0	54 51.8
80	20	78.8-79.0	B 416	{ P M	62.3 —	56.6 57.2	— 52.4
20	80	82.0-82.2	B 437	{ P M	— —	58.0 —	52.8 —
10	90	83.0-83.2	B 436	{ P M	— —	57.65 —	52.4 52.6
C ₂₂	C ₂₃						
90	10	78.6-78.8	B 425	{ P M	— —	52.8 —	48.1 49.0
80	20	77.6-77.8	B 426	{ P M	— —	54.0 —	49.0 —
70	30	76.4-76.6	—	P	—	53.3	—
20	80	77.5-77.7	B 428	{ P M	— —	53.3 —	— 51.25
10	90	78.1-78.3	B 427	P	—	52.5	—

* Extrapolated.

(4) For proportions of 20 % to 50 % of the longer-chain acid both A and B spacings appeared for the pressed and a B spacing for the melted layer. The equimolar mixture was an exception and had a C spacing for the melted layer.

(5) Proportions of 60 % to 80 % of the longer-chain acid gave only a B spacing for both pressed and melted layers.

(6) 90 % or more of the longer-chain acid gives results similar to those for the 0-15 % mixtures.

The measurements are recorded in Table IV and are shown graphically for the mixtures of *n*-tricosanoic and *n*-pentacosanoic acids in Fig. 2.

Additional assistance in distinguishing between odd- and even-numbered chains of the series of *n*-acids is given by a slight but perceptible difference in the intensity distribution, for the even orders are definitely weaker in the odd-number series. We have found that if the ninth order of reflection is visible then if the mixture is of even-number acids, the second order will appear, but if the mixture is of odd-number acids there is frequently no observable trace of any even order. But the intensity distribution in mixtures is variable, and evidence of this nature is suggestive and corroboratory rather than definitive.

Photographs have been taken of several odd-even binary mixtures, and the spacings are recorded in Table IV. The behaviour of such mixtures is complicated and has not been reduced to a set of rules, but where we have obtained spacings agreeing numerically with those of mixtures from natural sources we have always found that there is no accord in the m.p. and mol. wt. measurements. Since the behaviour of the oxidised acids from phrenosinic acid strongly suggests that they are mainly composed of two acids, we consider that there is no evidence to support a suggestion of a mixture of odd- and even-number acids.

SUMMARY.

Brain contains at least three phrenosins whose acidic components are α -hydroxy-*n*-docosanoic, α -hydroxy-*n*-tetracosanoic and α -hydroxy-*n*-hexacosanoic acids respectively. Phrenosinic (cerebronic) acid is a mixture of these acids. Brain also contains at least three kerasins, whose acidic components are *n*-docosanoic, *n*-tetracosanoic and *n*-hexacosanoic acids respectively. Evidence that "lignoceric" acid is a mixture is summarised and the suggestion made that the name is misleading and should be abandoned.

Several binary mixtures of *n*-fatty acids of chain length from C_{21} to C_{25} have been prepared, and their melting points and crystal spacings are recorded.

In conclusion we should like to record our grateful thanks to Dr Rosenheim for providing us with samples of highly purified brain cerebrosides and for the interest he has taken in this work. Our thanks are also due to Prof. Francis and Dr Malkin for kindly providing us with samples of pure synthetic higher *n*-fatty acids.

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