121. *Phosphatides of the Human Brain.*

V. *The Isolation of Simple Kephalsins.*

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(Comm. by U. SUZUKI, M.I.A., Nov. 12, 1932.)

Bromination: The bromination of both α - and β -kephalins, which had been separated as described in the preceding paper, has been carried out in the same way. The kephalins were dissolved in ether and bromine was added drop by drop, stirring all the while and cooling thoroughly ($-5 \sim -7^\circ \text{C}$), until the smell and the colour of bromine remained persistently. The stirring continued for several hours and the flask was made to stand in an ice box for 24 hours. After that the solvent was evaporated off. The yield: from 4 g of α -kephalins, 5.53 g of the bromides; from 16 g of β -kephalins, 22.60 g of the bromides.

The separation: Both the bromides were treated with several solvents successively as shown briefly in the following scheme.

The hydrolysis: After each fraction had been separated it was purified and hydrolysed. The hydrolysis had been accomplished by boiling with 10% sulphuric acid for several hours (usually 8 hours) and the components were isolated and identified.

Fraction 1. The kephalin was insoluble in acetone and methyl alcohol but slightly soluble in absolute alcohol and easily soluble in ether, chloroform and benzene. M.P. $174-176^\circ \text{C}$. No bromine was

detectable. On hydrolysis, only stearic acid had been isolated (M.P. 68–68.5° C, C:75.86%, H:12.72% (calc. C:75.95, H:12.52)) in the yield of 99.15% of the amount calculated as distearyl- α -kephalin.

Fraction 2. The kephalin is insoluble in petroleum ether and methyl alcohol, with difficulty soluble in ether and alcohol but soluble in acetone, benzene and chloroform. M.P. 222–226° C. It contained Br to the extent of 51.04% (theory 50.11). On hydrolysis, dibromostearic acid (Br:36.24% (theory 36.18)) and the bromide (Br:66.82%) of a highly unsaturated acid were obtained in the yield of 26.50% (calc. 27.74) and 59.75% (calc. 60.95) respectively. The molar proportion of the two acids was 1.00:1.03.

Fraction 3. This kephalin (a semisolid substance) was insoluble in methyl alcohol but easily soluble in other organic solvents. It contained Br to the extent of 17.45% (calc. 17.70). After hydrolysis, stearic acid (M.P. 68–68.5° C, C:75.72%, H:12.61%) in the yield of 29.40% (theory 31.45) and dibromostearic acid (Br:36.27%) to the extent of 48.00% (theory 48.92) had been isolated and identified, the molar proportion of the acids being 1.00:1.04. This fraction could not be the equimolar mixture of the fractions 1 and 2 as the behaviours towards solvents were quite different.

Fraction 4. The kephalin is insoluble in acetone, methyl alcohol and cold alcohol. It is slightly soluble in warm alcohol and readily soluble in ether, chloroform and benzene. M.P. 247–249° C, Br:45.40% (calc. 44.57). On hydrolysis, stearic acid (M.P. 68–68.5° C, C:75.99%, H:12.97%) in the yield of 19.17% (theory 19.80) and the bromide of a highly unsaturated acid, which showed no sign of melting at 250° C, in the yield of 66.68% (calc. 67.66), were obtained. The Br content: 66.58% (65.79% as $C_{22}H_{36}O_2Br_3$). The molar proportion of the two acids was 1.00:1.01.

Fraction 5. It was insoluble in methyl alcohol but readily soluble in other common organic solvents. A semisolid substance. Br:17.96% (calc. 17.70). By hydrolysis, stearic acid (M.P. 68–68.5° C, C:75.39%, H:12.87%) and dibromostearic acid (Br:36.22%) were obtained. The yields of both the acids were 30.40% (calc. 31.45) and 47.62% (calc. 48.92) respectively. The molar proportion of the yields was, 1.00:1.00.

The highly unsaturated acid of fractions 2 and 4: Various controversial views are debated as to the nature of the highly unsaturated acid of kephalins. MacArthur and Burton¹⁾ considered the acid to be

1) J. Am. Chem. Soc. **38**, 2 (1916), 1375.

really clupanodonic acid of C_{18} which forms an octabromide. Levene and Rolf¹⁾ claimed that they found arachidonic acid in the brain kephalin. Klenk²⁾ could find no arachidic but behenic acid among the hydrogenated products of the acids of kephalins. Page and Rudy³⁾ isolated 2 acids which form α - and β -octabromobehenic acid.

We compare the data of these authors with those of the present writers :

Author	M.P.	Br(%)
MacArthur & Burton	Darkens at 220° but does not melt	67.7–69.4
Levene & Rolf	Decomposes at above 250°	67.42
Klenk	Does not melt at 260°	66.7
Page and Rudy	α melts at above 250° with decomposition β at 163–164°	67.32–67.50
Nishimoto & Suzuki	Fr. 2 Does not decompose at 250° Fr. 4 Does not decompose at 250°	66.82 66.58

It is obvious from the above table, that the acid we obtained in fractions 2 and 4 is the substance in question. The acid was debrominated and hydrogenated following Suzuki and Inoue's⁴⁾ method. As the result we obtained behenic acid which melted at 80–81°C and the M.P. did not alter by mixing with behenic acid obtained by hydrogenation of erucic acid and clupanodonic acid of sardine oil. Now the question arises whether or not the unsaturated acid is clupanodonic acid, as we know, so far, no other acids than clupanodonic which have the chain of 22 carbons and 4 or 5 unsaturation centers. The theoretical bromine contents of 3 acids which may be suspected are

		Br(%)
(a) Octabromobehenic acid	$C_{22}H_{36}O_2Br_8$	65.79
(b) Octabromoarachidic acid	$C_{20}H_{32}O_2Br_8$	67.75
(c) Decabromobehenic acid	$C_{22}H_{34}O_2Br_{10}$	70.75
(from clupanodonic acid)		

and the acid we obtained contained 66.58–66.82% of Br, namely, the numerical values come between those which are given by (a) and (b). But the probability of the unsaturated acid being arachidonic acid is excluded as the acid gives behenic acid instead of arachidic acid when it is hydrogenated.

And again the bromide of the acid behaved different with (c)

1) J. Biol. Chem. **54** (1922), 91.

2) Z. physiol. Chem. **195** (1930), 217.

3) ibid. **205** (1932), 115.

4) Proc. **6** (1930), 266.

towards solvents. While decabromobehenic acid from clupanodonic acid is pretty soluble in alcohol and benzene and specially readily soluble in hot benzene, the bromides we obtained was insoluble in ether, alcohol and benzene, cold or hot, in this connection resembling rather octabromoarachidic acid. Moreover, it was observed that the two bromoacids behaved quite differently when they were heated in the same bath and on both the sides of the same thermometer. While the bromide from clupanodonic acid melted at 216°C with decomposition, with the bromide of the new acid, only the darkening of the colour has been observed at above 250°C . As the most probable case, therefore, we consider that the acid is an acid of 22 carbons which form octabromides, preferably docosatetrenoic acid. We cannot account for the fact, however, how the analytical value of Br we obtained deviates from the calculated value in comparison with other cases. This anomalous result is due, presumably, to the difficulty of recrystallization as no suitable solvents were found.

Our results conform, thus, with those obtained by Klenk and Page and Rudy in the case of α -octabromide. As to the β -octabromide of Page and Rudy, we cannot say much but, we consider, it is not improbable that more than one product may be obtained from the same acid by steric isomerization which we often encounter during the handling of unsaturated fatty acid.

It is claimed, some times, that it is exceedingly difficult to get phosphorus free fatty acids from kephalin¹⁾ but in no case of this study have we experienced such difficulties.

To write the constitutions of the kephalins we isolated in the form of common usage.

1) Maclean: Lecithin etc. (1918), p. 44.