

# On the Spontaneous Decomposition of Lecithin.

By

Shichizo Kato and Osamu Shinoda.

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It is well known that to get lecithin in a pure state, special care must be taken in the preparation from animal or plant materials, employing pure reagents and protecting it from oxidation, and also in its preservation, avoiding the action of air, light and moisture as far as possible.<sup>1</sup>

When lecithin is treated with mineral acids<sup>2</sup>, it yields choline. According to E. Fourneau and Piettre<sup>3</sup>, however, unsaturated fatty acids in lecithin were removed by alcoholysis.

The present research was, therefore, undertaken to see what chemical changes would occur when lecithin was kept in a reagent bottle at ordinary temperature for a long time.

The material used for this experiment consisted of two samples, one a dark plastic mass containing a white crystalline substance, and the other with the same appearance but lacking the crystalline substance, and both were marked "extra pure lecithin from eggs, made by E. Merck, Germany."

The former sample was secured through the courtesy of Prof. I. Matsuo, of our Medical College, and was bought in Germany eight years ago, and kept in his laboratory until 1921; and the latter was bought in 1922, in Kyoto.

The first sample, on analysis, after drying to constant weight in vacuo, gave the following results:

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<sup>1</sup> H. Maclean: *Lecithin and Allied Substances*, 35-36 (1918).

<sup>2</sup> G. Maruzzi: *Zs. Physiol. Chem.*, **55**, 352 (1908).

<sup>3</sup> *Bull. Soc. Chim.*, (4); **11**, 805 (1912).

0.1355 grm. of the substance gave 0.2918 grm. CO<sub>2</sub> and 0.1409 grm. H<sub>2</sub>O.

0.1313 grm. of the substance gave 0.0037 grm. N<sub>2</sub> (Kjeldahl's method).

0.1700 grm. of the substance gave 0.0219 grm. Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>.

C=59.61 ; H=11.80 ; N=7.82 ; P=3.58.

$$\frac{\text{NH}_2\text{-N}}{\text{total N}} = \frac{57}{100} ; \text{N : P} = 1 : 0.6.$$

As to the lecithin from Merck, J. Bang has already stated in the following words<sup>1</sup>; "Es enthielt 2.05% N und 3.35% P. Die richtige Formel ist C<sub>43</sub>H<sub>80</sub>NPO<sub>9</sub> mit 1.35% N und 3.9% P. Mercks Preparat ist demnach sehr unrein und zu wissenschaftlichen Zwecken vollkommen unbrauchbar."

The so-called pure lecithin from Merck analysed by the authors, seems impure when our analytical figures compared with those of the lecithin prepared by Stern and Thierfelder<sup>2</sup> from egg yolk, under special precautions :

C=64.63 ; H=10.96 ; N=2.08 ; P=3.97 ; P : N=1 : 1.16.

Our second sample, however, showing the following analytical results, strongly resembles in its elementary composition Stern and Thierfelder's sample :

0.0909 grm. of the substance gave 0.2129 grm. CO<sub>2</sub> and 0.0879 grm. H<sub>2</sub>O.

0.1420 grm. of the substance gave 0.0182 grm. Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>.

0.0949 grm. of the substance gave 0.0022 grm. N<sub>2</sub> (by Kjeldahl's method).

C=64.00 ; H=10.74 ; N=2.32 ; P=3.59 ; N : P=1.1 ; 1 ;

$$\frac{\text{NH}_2\text{-N}}{\text{total N}} = \frac{32}{100}.$$

20 grm. of the first sample, dissolved in ether and concentrated to a small volume, and poured into dry acetone to precipitate the lecithin. The brown-colored precipitate, separated from the solution, dried under reduced pressure and redissolved in ether. The precipitation of the lecithin from the ether solution with acetone, was repeated three times and the final product was analysed after drying in vacuo. It amounted to 8 grm.

0.1951 grm. of the substance gave 0.3639 grm. CO<sub>2</sub> and 0.1800 grm. H<sub>2</sub>O.

0.1276 grm. of the substance gave 0.0242 grm. Mg<sub>2</sub>P<sub>2</sub>O<sub>7</sub>.

0.1044 grm. of the substance gave 0.0016 grm. N<sub>2</sub> (Kjeldahl's method).

<sup>1</sup> Chem. u. Biochem. d. Lipoide, 50 (1911).

<sup>2</sup> Z. Physiol. Chem., 53, 379 (1907).

C=50.88 ; H=10.35 ; N=1.53 ; P=5.28 ; N : P=1 : 1.4 ;

$$\frac{\text{NH}_2\text{-N}}{\text{total N}} = \frac{39}{100}.$$

The purified sample, as seen by the foregoing experimental results, contains less carbon and more phosphorus than the true lecithin, and this discrepancy between fact and theory indicates that some of the fatty acids have been removed from the lecithin molecule.

In order to discover the nature of the fatty acids remaining in the glycerin-phosphoric fragment of lecithin, 7 grm. of the purified substance was hydrolysed with 10% alcoholic soda solution, and the sodium salts isolated from the reaction products were converted into lead salts which are insoluble in ether.

The free acids obtained from the lead salt, assumed to consist of stearic and palmitic acids, were induced to separate from each other by means of the fractional crystallization of their magnesium salts in 98% alcohol solution according to the direction of Heinzl<sup>1</sup>.

It did not, however, yield the desired result, the melting point of the nine fractions ranging from 52 to 57°.

The acetone solution separated from the insoluble lecithin-like compound described above, was concentrated under reduced pressure to a thick syrup, amounting to 3 grm., and dissolved in absolute ether. On passing dry ammonia gas in the ethereal solution, a white precipitate formed separate from the solution, and one part of the ammonium salt was converted into calcium salt, and the remainder into lead salt, and both salt were analysed :

0.124 grm. of the calcium salt gave 0.2887 grm. CO<sub>2</sub>, 0.1252 grm. H<sub>2</sub>O and 0.0041 grm. ash.

C=63.37 ; H=11.27 ; Ash=2.45.

The free acid obtained from the calcium salt, melting at 52-53°.

0.1027 grm. of the substance gave 0.2718 grm. CO<sub>2</sub> and 0.1190 grm. H<sub>2</sub>O,

C=72.19 ; H=12.96.

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<sup>1</sup> J. prak. Chem., 66, 12 (1855).

The solution of 0.1021 gm. requires 3.9 c.c. N/10 NaOH solution for neutralization,

whence M.W.=270.

The other sample of the free acid prepared from the lead salt, gave on analysis the following results :

0.1082 gm. substance gave 0.2767 gm. CO<sub>2</sub> and 0.1188 gm. H<sub>2</sub>O.

C=73.13 ; H=12.88 ; M.W.=272 ; M.P. 52-53.°

To our surprise, the acids isolated as a cleavage product of lecithin so far as we had studied their elementary compositions, molecular weights, melting points, and chemical properties, were also found to consist of a mixture of stearic and palmitic acids.

The second sample purified by fractional precipitation seven times as usual, was found to contain the following composition by analysis :

0.1129 gm. substance gave 0.1009 gm. H<sub>2</sub>O, 0.2616 gm. CO<sub>2</sub> and 0.0099 gm. ash.

0.125 gm. gave ammonia by Kjeldahl, method which requires 1.91 c.c. N/10 HCl to neutralise.

0.4851 gm. substance gave 0.1194 gm. Agl.

C=63.25 ; H=9.94 ; Ash=8.75 ; N=2.14 ;

$$\frac{\text{NH}_2\text{-N}}{\text{total N}} = \frac{16}{100} ; \quad \text{Glycerin}=9.66.$$

As seen in the foregoing results, lecithin, on keeping, suffers spontaneous decomposition and the fatty acids were gradually separated from the glycerin phosphoric acid part, and the products resulting were poor in carbon and hydrogen but rich in phosphorus.

The acetone solution supposed to contain the fatty acids which broke off from the lecithin molecule, was concentrated to a syrup under reduced pressure, the fatty acids were extracted with ether, and dry ammonia gas passed in the ethereal solution, whereby the ammonium salt was precipitated which was then transformed into lead salt as usual.

The lead salts, thus obtained, differing from those of the oleic and linoleic acids in their solubility in ether, were assumed to be saturated compounds.

The molecular weight of the mixed acids showed 265 which corresponds to the mean value of that for stearic and palmitic acids.

The mixture was subjected in 98% alcohol solution to a fractional precipitation with acetate of magnesium. After repeating the precipitation 17 times, it was divided into 7 portions, and each portion washed with alcohol and ether and then decomposed with hydrochloric acid. The free acid of each fraction was examined as to its melting point and molecular weight and the results were compared with those of Heinz<sup>1</sup> in his investigation of the mixture of stearic and palmitic acids.

Fraction	gm.	M.P.	M.W.
1.	0.1	63—64°	.....
2.	0.15	56°	282.
3.	0.15	54°	.....
4.	0.2	semisolid	.....
5.	0.4	61°	.....
6.	0.2	57—58°	272.
7.	0.35	semisolid	.....

According to Heinz's table, our less soluble fraction melting at 63–64°, will be considered as a mixture of 70% stearic acid and 30% palmitic acid, on the contrary, the more soluble fraction melting at 61° predominates in palmitic acid (95%).

### Summary.

1. Pure ovo-*lecithin* from Merck was analysed, and was confirmed to be a mixture of true *lecithin* and *cephalin*.
2. While ovo-*lecithin* keeps at ordinary temperature for a long time, the fatty acids were gradually separated from the glycerine-phosphoric acid part, and a product resulted which was rich in phosphorus and nitrogen but poor in carbon and hydrogen.

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<sup>1</sup> *Loc. cit.*