80. *The Kephalins of Soy Bean.*

By Bunsuke SUZUKI and Uzo NISHIMOTO.

Biochemical Laboratory, Faculty of Agriculture, Kyoto Imperial University.

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Pressed soy bean flakes were extracted with boiling alcohol and the alcoholic extract was cooled spontaneously when the larger part of oils had been separated in a layer. Dark colored semi solid matters obtained by evaporating the alcoholic layer, were used as the starting material. (The authors wish to thank Mr. S. Sera and Dr. M. Sato of the Central Institute of the South Manchuria Railway Co. who provided the material in a large quantity). This was dried by mixing with anhydrous calcium sulfate. The dried material was treated briefly as follows :

The yield:—0.56% of the starting material.

The analysis:—Amino-N (Van Slyke): 1.76%, Total-N: 1.82%, P: 3.52%, Amino-N/Total-N: 0.967, N/P: 1.

The hydrolysis:—25 g of the kephalins were hydrolysed with a 3% sulfuric acid solution. The fatty acids floated, when cooled, on the surface of the hydrolysate, were gathered on a filter paper. Lead soaps of the acids were formed in an alcoholic solution. The well washed and dried lead soaps were treated with ether. The unsaturated acids obtained from ether soluble lead soaps were brominated in the usual way using petroleum ether as the solvent. Two bromo-acids were formed; the one was soluble in ether and the other insoluble while both were insoluble in petroleum ether and soluble in benzene. Tetrabromostearic acid (M.P.: 114–115° C, Br: 53.22% (calc. 53.28)) and hexabromostearic acid (M.P.: 176–177° C, Br: 63.60% (calc. 63.32)) were identified as the components of the mixture and no other substance. The saturated acid obtained from the ether insoluble lead soap was stearic acid (M.P.: 69° C, C: 75.59% (calc. 75.95), H: 12.97% (calc. 12.75)).

The mother liquor of the fatty acids was made alkaline by adding a slight excess of Ba(OH)_2 and sulfuric acid and Ba(OH)_2 were removed as Ba-sulfate and carbonate. The filtrate was concentrated and Ba-glycerophosphate was obtained by adding absolute alcohol to the solution. The Ba-glycerophosphate was dissolved in a small amount of water and an excess of a $\text{Ba(NO}_3)_2$ solution was added whereupon Ba-glycerophosphate precipitated as a double salt with $\text{Ba(NO}_3)_2$ almost quantitatively (ca. 99%) and no trace of the α -glycerophosphate was found. $(\text{CH}_2\text{OH} \cdot \text{CH}(\text{OPO}_3\text{Ba})\text{CH}_2\text{OH})_2\text{Ba(NO}_3)_2$, P: 6.97% (calc. 7.08), Ba: 46.77% (calc. 47.02).

From the mother liquor of Ba-glycerophosphate colamine was separated as the picrate. M.P.: 158–159° C, C: 32.92% (calc. 33.09), H: 3.32% (calc. 3.47).

In the above analysis, we found only β -glycerophosphoric acid and

no trace of α -form. This fact made the separation of each individual kephalin quite simple.

From 25 g kephalin, about 16 g of fatty acids among which 7.8 g, that is almost half the amount, was stearic acid, the remainder being linoleic and linolenic acid in equimolecular proportions.

In our case β -glycerophosphoric acid is excluded so that our preparation must be either one or the other of the mixtures, that is: Dilinoleo- β -kephalin + Dilinolenio- β -kephalin + $2 \times$ Distearo- β -kephalin or Linoleo-stearo- β -kephalin + Linolenio-stearo- β -kephalin or Linoleo-linolenio- β -kephalin + $2 \times$ Distearo- β -kephalin.

To see which might be the case, the original kephalin preparation was brominated in petroleum ether and it was divided into two fractions, soluble and insoluble in the solvent.

The fraction insoluble in petroleum ether:—The original preparation was easily soluble in petroleum ether so that if distearo-kephalin is contained in the preparation it is excluded from this fraction. The yield of the fraction is 7 g out 10 g of non brominated kephalin mixtures. N: 1.14%, P: 2.53%, Br: 39.26%; calc. as hexabromostearyl-stearyl- β -kephalin, N: 1.12%, P: 2.38%, Br: 38.57%. By hydrolysing 4 g of the brominated kephalin, stearic acid (M.P.: 68–69°C and the M.P. did not alter by mixing pure stearic acid) and hexabromostearic acid (M.P.: 177–178°C, Br: 63.34%, calc. 63.32) were obtained in the yield of 0.8 g and 1.8 g respectively in pure state (the combined yield before the separation: 3.0 g) by using petroleum ether, the former being soluble in the solvent while the latter is insoluble. The molar proportions of stearic acid and linolenic acid is 0.8:1.1. Here again we could find no trace of α -glycerophosphoric acid.

The fraction soluble in petroleum ether:—In this fraction, also, the mixing of distearyl-kephalin is excluded. The original preparation is insoluble in acetone while the brominated kephalin is easily soluble in the solvent so that, by treating with acetone, distearyl-kephalin would have been obtained if it had been present. The original preparation is easily soluble, moreover, in petroleum ether and when it is in solution it is not recovered unless the solvent is driven off while the brominated kephalin may be recovered by concentrating the solution. If the preparation contained distearyl-kephalin, therefore, it must remain in the mother liquor and it was found that it contained a mere trace of bromo-kephalin.

The analytical data are shown as, N: 1.31%, P: 2.91, Br: 30.06%, calc. as tetrabromostearyl-stearyl- β -kephalin, N: 1.14%, P: 2.48%,

Br : 29.11%. Out of 4 g of the kephalin 3 g of fatty acids were obtained. The mixture of the acid was treated with petroleum ether stearic acid going into the solution while tetrabromostearic acid remained insoluble. The yield of each acid in a pure state were 0.7 g and 1.2 g respectively which correspond to the molar proportion of 1.0 : 1.1.

From 4 g of the brominated kephalin, glycerophosphoric acid was recovered as Ba-salt in the yield of 0.81 g (theory : 1.09) and, from that amount of the Ba-salt, 0.92 g (theory : 1.15) of Ba- β -glycerophosphate, as the double salt with $\text{Ba}(\text{NO}_3)_2$, has been recovered. The solubility of the double salt in water is about 1%. Now the volume of the solution was 20 cc so that 0.2 g must be added to the yield of 0.92 g, thus, making the yield almost theoretical. This ascertains that our preparation did not contain α -glycerophosphoric acid.

From above considerations, we can assign the following constitutions to two β -kephalins we separated from soy bean

Linoleo-stearo- β -kephalin.

(The tetrabromide is soluble in petroleum ether.)

Linoleno-stearo- β -kephalin.

(The hexabromide is insoluble in petroleum ether.)

The authors do not go so far as to claim that these are the only kephalins contained in soy bean, because there are fears of losing α -kephalins somewhere in the course of the separation. This anticipation is not groundless as B. Suzuki and Y. Yokoyama were able to separate the lecithins of soy bean into α - and β - series by using suitable solvents. The results of the experiment will be published later.