592. *An X-Ray Examination of Synthetic* (±)-α- *and* β-*Kephalins.*

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Long and side spacings are given for synthetic dilauroyl, dimyristoyl, dipalmitoyl, and distearoyl (±)-α- and β-kephalins (phosphatidylethanolamines). The data agree with an arrangement of double molecules lying across the reflecting planes at angles of ≏73° 20′ (α-compounds) and 55° 5′ (β-compounds). The side spacings readily distinguish between the (±)-α- and the β-isomer.

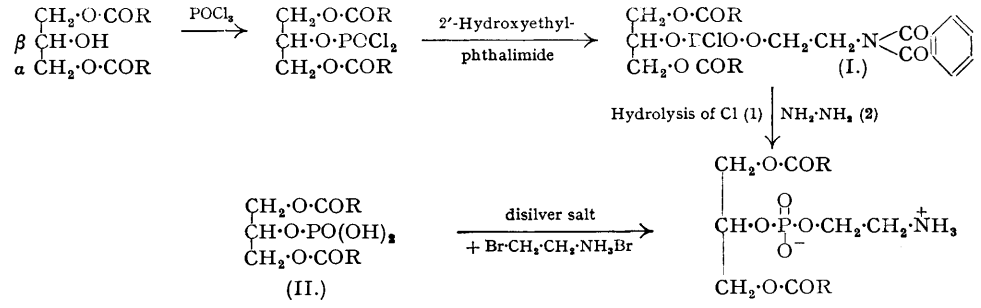
The present investigation was undertaken in order to obtained data to identify the natural kephalins, and also to establish the position of the phosphate group. Chromatographic work is in hand (cf. *J.*, 1951, 841) which we hope will shortly yield suitable natural specimens for comparison.

For the more fully investigated lecithin, it would now appear that the phosphate group is exclusively in the α-position of the glycerol molecule. α-Lecithin has long been known to exist, because of the isolation, from its products of hydrolysis, of optically active glycerophosphoric acid, which must, of course, be the α-acid. Similarly, the isolation of β-glycerophosphoric acid (Karrer and Salomon, *Helv. Chim. Acta*, 1926, **9**, 3) has been considered as proof of the existence of β-lecithin. Baer and Kates (*J. Biol. Chem.*, 1948, **175**, 79; 1950, **185**, 615) have, however, shown that acid and alkaline hydrolysis of α-lecithin give rise to both α- and β-glycerophosphoric acid, owing to the migration of the phosphate group. Consequently, the isolation of β-glycerophosphoric acid cannot be regarded as proof of the existence of β-lecithin. Further, they have shown (*J. Amer. Chem. Soc.*, 1948, **70**, 1394) that the *X*-ray diffraction patterns of synthetic (−)-α-dipalmitoyl-lecithin and natural dipalmitoyl-lecithin are identical.

The position with regard to kephalin is much less definite; from its close biological and chemical relationship to lecithin, it might also be expected to have the α-structure, a view which is supported by its reported optical activity (Levene and Rolf, *J. Biol. Chem.*, 1919, **40**, 1). This, however, could well be due to admixture with lecithin, with which it is invariably associated, or with sugars (Hutt *et al.*, *Nature*, 1950, **165**, 314). Folch (*J. Biol. Chem.*, 1942, **146**, 35), who claims to have prepared kephalin " in a relative state of purity for the first time," makes no reference to its optical activity and, unfortunately, also omits to indicate which glycerophosphoric acid it affords on hydrolysis. The recent statement by Rose (*J. Amer. Chem. Soc.*, 1947, **69**, 1384), that the long spacings of synthetic β-kephalin and natural kephalin are similar, would appear to be in favour of the β-structure but, as no details are given, it can be accepted only with reserve. With an *X*-ray comparison, one would expect either identity or non-identity, rather than the somewhat indefinite " similarity." It is surprising, too, that no mention is made of the side spacings, which are usually a prominent feature of the *X*-ray photographs.

Our kephalins were prepared by Rose's method (*loc. cit.*), outlined below. We adopted Hunter, Roberts, and Kester's modification (*J. Amer. Chem. Soc.*, 1948, **70**, 3244), which consists in carrying through the synthesis to the end, without isolation of the intermediate phthalimide derivative (I), and which, by avoiding emulsion and crystallisation losses, results in a considerably increased yield. We found an equal improvement in yield when Rose's alternative method (using carbobenzyloxyethanolamine) was similarly carried through to the

end, but we preferred the phthalimide method because of the higher yield, and also because of the unavailability of phosphonium iodide, which Rose used for removing the carbobenzyloxy-group. It is noteworthy that, although Rose was unable to remove the latter group by catalytic hydrogenolysis, Baer, Marukas, and Russell (*Science*, 1951, **113**, 12) have now succeeded in doing so, from a series of diacyl α-glycerylphenylphosphorylcarbobenzyloxyethanolamines. This method, however, restricts the synthesis to saturated kephalins.



In agreement with Rose (*loc. cit.*), we found Kabashima's method (*Ber.*, 1938, **71**, 76) quite impracticable. This method consists in heating together bromoethylamine picrate and the monosilver salt of the phosphatidic acid (II) the latter being obtained by a Schotten–Baumann acylation of glycerophosphoric acid. We had no success with either stage of the reaction. A somewhat similar method, suggested by the work of Arnold (*Ber.*, 1940, **73**, 87), namely, heating the disilver salt of the phosphatidic acid (II) (made by direct phosphorylation of the diglyceride) with an alcoholic solution of bromoethylamine hydrobromide, gave an authentic specimen of kephalin, but in too low a yield for the method to be of any practical value.

X-*Ray Investigation.*—This was carried out as described in earlier papers (*J.*, 1934, 666; 1936, 1628), pressed layers and rods being used to give long and side spacings respectively. Exposures of one hour each side for the former, and of ½ hour for the latter are adequate.

TABLE I.

X-*Ray spacings* (A.) *of* (±)-α- *and* β-*kephalins* (1 : 2- *and* 1 : 3-*diacylphosphatidyl-ethanolamines, respectively*).

(±)-α-Kephalins	Long spacing	Side spacings *							
		w		w		vs	m		w
Dilauroyl	45·2	5·91		4·91		4·15	3·86		3·59
Dimyristoyl	49·9	5·96		4·94		4·18	3·87		3·6
Dipalmitoyl	55·3	5·93		4·92		4·16	3·84		3·59
Distearoyl	60·0	5·88		4·94		4·2	3·86		3·6
β-Kephalins		s	vs	m	m	w	m	m	m
Dilauroyl	35·9	5·65	4·72	4·31	4·17	3·89	3·70	3·55	3·42
Dimyristoyl	40·1	5·65	4·69	4·33	4·20	3·97	3·78	3·60	3·40
Dipalmitoyl	44·3	5·59	4·65	4·30	4·25	3·97	3·79	3·57	3·38
Distearoyl	48·3	5·59	4·64	4·33	4·23	3·89	3·79	3·63	3·38
(−)-α-Kephalin (Baer, Marukas, & Russell, *loc. cit.*)		Side spacings							
Dimyristoyl	5·84	5·12	4·69	4·09	3·82	2·99	2·42	2·25	—	1·97
	(0·3)	(0·3)	(0·5)	(1·0)	(0·7)	(0·2)	(0·1)	(0·1)	—	(0·3)
Dipalmitoyl	—	—	4·63	4·13	3·74	3·15	2·8	2·48	2·23	1·82
	—	—	(0·4)	(1·0)	(0·4)	(0·4)	(0·1)	(0·3)	(0·3)	(0·1)
Distearoyl	—	—	4·52	4·12	3·81	3·17	2·24	1·98	—	1·72
	—	—	(0·3)	(1·0)	(0·5)	(0·1)	(0·1)	(0·3)	—	(0·1)

Numbers in parentheses denote estimated intensity of lines.
* s = Strong, vs = very strong, m = moderate, w = weak.

The long spacings, plotted against the number of carbon atoms in the fatty acids, fall accurately on two widely separated straight lines, which give intercepts for no. of C = 0 of $\simeq$ 10 and 15 A. The data agree with an arrangement of double molecules lying at angles of $\simeq$ 73° 20′ (α) and 55° 5′ (β) across the reflecting planes (the angle between the zig-zag carbon atoms of

chains being assumed to be 116°). The side spacings readily distinguish between the α- and β-isomers (see Plate facing p. 2666, Figs. 13 and 14). As would be expected for a homologous series, the spacings and intensities for members of each group are closely similar. These and the long spacings should identify the kephalins with reasonable certainty.

In Table I, we also give, for comparison, spacings calculated from the results of Baer, Marukas, and Russell (*loc. cit.*) for three synthetic (−)-α-kephalins. These are characterised by three strong lines of approximately 4·6, 4·1, and 3·8 A. (the strong shorter spacings are second orders of these). It is difficult to say how far the stereochemical difference affects the crystallographic structure in these compounds, but it is of interest that the 4·1 and 3·8 spacings are very close to our strongest spacings for the (±)-α-kephalin, and that the 4·6 spacing is also very close to the strongest line of our β-kephalin. Whilst these facts are barely sufficient to warrant the deduction that β-kephalin is present in the above compounds, this possibility cannot be overlooked with all synthetic α-kephalins, owing to the facile transformation of 1 : 2-diglycerides, the starting material, into 1 : 3-diglycerides.

Experimental.

1 : 3-*Diglycerides.*—These were prepared by the acid chloride method (Malkin, Shurbagy, and Meara, *J.*, 1937, 1409); we do not recommend the method of direct acylation of glycerol used by Rose (*loc. cit.*), which we find leads to low-melting products containing triglycerides.

1 : 2-*Diglycerides.*—These were prepared as described by Howe and Malkin (preceding paper).

2′-*Hydroxyethylphthalimide* (Rose, *loc. cit.*).—28 G. of freshly distilled ethanolamine were added to 59·2 g. of phthalic anhydride. When the heat of reaction had subsided, the mixture was heated at 150° for 30 minutes, and after cooling to 90° it was poured into 800 c.c. of water. The crystalline product was filtered off on cooling, and recrystallised from water; it had m. p. 130·5° (Rose gives 126—127°).

Solvents and Reagents.—The chloroform used was freed from alcohol with concentrated sulphuric acid, dried ($CaCl_2$; P_2O_5), and distilled, shortly before use. Pyridine was dried (KOH) and fractionated. Phosphorus oxychloride was always fractionated before use. The fatty acids used were Kahlbaum's "K," or highly purified acids, kindly supplied by Messrs. Price's (Bromborough) Ltd. Where necessary, these were purified by fractionation of the ethyl esters.

α- *and* β-*Kephalins.*—These were made essentially by the same method, except that, following Hunter *et al.* (*loc. cit.*), 2 mol. proportions of 2′-hydroxyethylphthalimide were used for β-kephalins. This method is described in detail only for (±)-α-dimyristoylkephalin; details should be followed closely. Particulars of differences in the amounts of solvent used in individual cases are given in Table II, which gives also yields and m. p.s.

Table II.

Kephalin derivative	Diglyceride dissolved in c.c. of $CHCl_3$	Glycol monomethyl ether, c.c.	Yield, %, based on diglyceride	M. p.	Recorded m. p.
β-Dilauroyl-	50	100	52	208°	—
β-Dimyristoyl-	50	100	47	207	173—174° [1]
β-Dipalmitoyl-	70	200	57	206	192—193, 195—198 [2]
β-Distearoyl-	120	400	76	198	—
(±)-α-Dilauroyl-.....	50	100	55	210	
(±)-α-Dimyristoyl-	50	100	74	207 [3]	
(±)-α-Dipalmitoyl-	70	200	72	198 [3]	
(±)-α-Distearoyl- ...	120	400	80	196 [3]	

[1] Hunter *et al.* (*loc. cit.*). [2] Rose (*loc. cit.*). [3] Baer, Marukas, and Russell (*loc. cit.*) find all three (−)-α-kephalins to melt in the neighbourhood of 175°.

(±)-α-*Dimyristoylkephalin.* In a 250-c.c. three-necked flask, fitted with a mercury-sealed, mechanically driven Hershberg stirrer, dropping-funnel, and calcium chloride tube (all apparatus previously oven-dried) were placed 10 c.c. of chloroform (alcohol-free; freshly distilled) and 1·535 g. (0·01 mole) of phosphorus oxychloride. The flask was surrounded by an ice-bath and 4·5 c.c. of dry, distilled pyridine were added dropwise to the mixture with vigorous stirring. When all the pyridine had been added, the flask was surrounded by a bath kept at 10—15°, and 5·12 g. of αβ-dimyristin (0·01 mole), dissolved in 50 c.c. of alcohol-free chloroform and 0·5 c.c. of dry pyridine, were added dropwise to the vigorously stirred mixture during 1 hour. The mixture was then stirred for 30 minutes with the bath at 25° and for a further 30 minutes with the bath at 45°.

The bath-temperature was then reduced to 10—15° and 1·91 g. (0·01 mole) of 2′-hydroxyethylphthalimide, dissolved in 70 c.c. of alcohol-free chloroform, were added to the vigorously stirred mixture during one hour. The mixture was then stirred for 30 minutes with the bath at 30°, followed by a further 30 minutes with the bath at 40°. The bath-temperature was then reduced to 10—15° and a few drops of water (*ca.* 0·2 c.c.) were added to the reaction mixture, which was then stirred for 30 minutes. The solvent was then removed under reduced pressure, by use of a water-pump and, finally, a mechanical vacuum pump and a temperature of $<40°$. (It is particularly important to remove all solvent and so avoid troublesome emulsions.)

The residue was triturated with N-hydrochloric acid at 0—10° and filtered, the precipitate being well washed with cold water (<15°) until free from mineral acid. The precipitate (dried on filter) was dissolved in 100 c.c. of neutral glycol monomethyl ether, a mechanical stirrer and a temperature of <40° being used. When dissolution was complete, 20 c.c. of 0·5N-sodium hydroxide solution were added dropwise to the vigorously stirred mixture, the addition taking about 1 hour. When the addition was complete, 1 g. (0·01 mole) of 50% w/w hydrazine hydrate solution was added to the mechanically stirred mixture, the temperature of which was gradually raised until dissolution was complete (the solution usually contains a small amount of suspended material, which is insoluble until the temperature is raised). Thereafter, the mixture was gently refluxed for 1 hour and then kept overnight. The precipitated material was filtered off and washed with a little neutral glycol monomethyl ether. Further impurities were removed by three extractions with 100 c.c. of boiling ether and finally with 200 c.c. of boiling ether; the yield was 4·7 g., and the m. p. 207° (74% based on 1 : 2-dimyristin).

α- and β-Kephalins may be crystallised from alcohol, but for *X*-ray work slow crystallisation from tetrahydrofuran is preferable. On crystallisation from alcohol on a microscope slide, between crossed nicols, brilliant dark crossed spherulites are observed. The analytical results were as follows: *β-Dilauroylkephalin* [Found: C, 59·6; H, 10·2; P, 5·2; N, 2·4%; equiv. (titration in EtOH; thymolphthalein), 570. $C_{29}H_{58}O_8NP$ requires C, 60·0; H, 10·05; P, 5·35; N, 2·4%; equiv., 579·7]. *β*-Dimyristoylkephalin (Found: C, 62·3; H, 10·4; P, 4·6; N, 1·9%; equiv., 626. Calc. for $C_{33}H_{66}O_8NP$: C, 62·4; H, 10·4; P, 4·9; N, 2·2%; equiv., 635). *β*-Dipalmitoylkephalin (Found: C, 63·9; H, 10·8; P, 4·2; N, 2·0%; equiv., 684. Calc. for $C_{37}H_{74}O_8NP$: C, 64·25; H, 10·7; P, 4·5; N, 2·0%; equiv., 692). *β-Distearoylkephalin* (Found: C, 65·35; H, 10·7; P, 4·0; N, 1·9%; equiv., 737. $C_{41}H_{82}O_8NP$ requires C, 65·9; H, 11·0; P, 4·15; N, 1·9%; equiv., 748). (±)-*α-Dilauroylkephalin* (Found: C, 59·8; H, 10·05; P, 5·0; N, 2·7%; equiv., 570. $C_{29}H_{58}O_8NP$ requires C, 60·0; H, 10·05; P, 5·35; N, 2·4%; equiv., 580). (±)-*α-Dimyristoylkephalin* (Found: C, 62·2; H, 10·3; P, 4·6; N, 2·5%; equiv., 627. $C_{33}H_{66}O_8NP$ requires C, 62·4; H, 10·4; P, 4·9; N, 2·2%; equiv., 636). (±)-*α-Dipalmitoylkephalin* (Found: C, 64·1; H, 10·55; P, 4·1; N, 2·1%; equiv., 687. $C_{37}H_{74}O_8NP$ requires C, 64·25; H, 10·7; P, 4·5; N, 2·0%; equiv., 692). (±)-*α-Distearoylkephalin* (Found: C, 65·3; H, 10·6; P, 3·85; N, 2·0%; equiv., 741. $C_{41}H_{82}O_8NP$ requires C, 65·9; H, 11·0; P, 4·15; N, 1·9%; equiv., 748).

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