685. *An X-Ray Examination of a Series of* (±)-1,2- *and* 1,3-*Di*-O-*acylglycerol Dihydrogen Phosphates* (α- *and* β-*Glycerophosphatidic Acids*).

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A series of long-chain (±)-1,2- and 1,3-di-*O*-acylglycerol dihydrogen phosphates (α- and β-glycerophosphatidic acids) has been prepared and the *X*-ray spacings have been determined.

The occurrence in Nature of phosphatidic acids was first noted by Chibnall and Channon,[1] during an investigation of the cytoplasma of cabbage and spinach leaves. Other workers [2-4] later isolated them from other plant sources and Hanahan and Chaikoff [5] showed them to be products of the enzymic breakdown of more complex phosphatides.

The first authentic synthesis of α-glycerophosphatidic acids was due to Baer,[6] who phosphorylated D-1,2-diglycerides with diphenyl phosphorochloridate and then removed the protecting phenyl groups by hydrogenolysis over Adams catalyst, to give the L-α-glycerophosphatidic acids.

Rose,[7] in a paper on the synthesis of phosphatidylethanolamine (cephalin), described the preparation of the quarter-quinolinium salt of 1,3-di-*O*-palmitoyl-β-glycerophosphatidic acid, from which he obtained the free acid. The 1,3-di-*O*-myristoyl salt was prepared by Hunter *et al.*[8] but the corresponding phosphatidic acid was not isolated.

Uhlenbroek and Verkade [9] prepared the DL-α-glycerophosphatidic acids and β-glycerophosphatidic acids by a method similar to Baer's [6] and emphasised the importance of using catalyst free from alkaline metals in the hydrogenolysis of the diphenyl esters; otherwise the acids were contaminated with sodium or potassium salts.

Hessel *et al.*[10] prepared dibenzyl esters by interaction of di-*O*-acylglycerol 1-iodide with silver dibenzyl phosphate and hydrogenolysed these in the presence of palladium to the DL-α-glycerophosphatidic acids. A similar reaction with silver diphenyl phosphate was reported by Baylis, Bevan, and Malkin.[11]

Baer and Maurukas,[12] using a method similar to that previously described by Baer,[6] but with a catalyst washed free from alkali metals repeated their preparations and obtained L-α-glycerophosphatidic acids with reproducible melting points; the melting points were not quoted in the paper by Baer.[6]

In the present work the α- and β-glycerophosphatidic acids have been prepared essentially by the methods of Uhlenbroek and Verkade.[9]

With α-glycerophosphatidic acids no difference in melting point was observed for α- and β-forms. This has previously been observed with the alkyl dihydrogen phosphates by Brown, Malkin, and Maliphant.[13] The single side spacing of 4·16 Å observed with the α-forms of the α- and β-glycerophosphatidic acids is typical of hexagonal or pseudo-hexagonal chain packing, whilst the more complicated set of side spacings of the tilted β-forms of the α- and β-glycerophosphatidic acids denote either orthorhombic or triclinic chain packing.

[1] Chibnall and Channon, *Biochem. J.*, 1927, **21**, 225, 233, 479, 1112.
[2] Smith and Chibnall, *Biochem. J.*, 1932, **26**, 1345.
[3] Jordan and Chibnall, *Ann. Bot.*, 1933, **185**, 163.
[4] Channon and Foster, *Biochem. J.*, 1934, **28**, 853.
[5] Hanahan and Chaikoff, *J. Biol. Chem.*, 1948, **172**, 191.
[6] Baer, *J. Biol. Chem.*, 1951, **189**, 235.
[7] Rose, *J. Amer. Chem. Soc.*, 1947, **69**, 1384.
[8] Hunter, Roberts, and Kester, *J. Amer. Chem. Soc.*, 1948, **70**, 3244.
[9] Uhlenbroek and Verkade, *Rec. Trav. Chim.*, 1953, **72**, 558.
[10] Hessel, Morton, Todd, and Verkade, *Rec. Trav. Chim.*, 1954, **73**, 150.
[11] Baylis, Bevan, and Malkin, *Chem. and Ind.*, 1955, 67.
[12] Baer and Maurukas, *J. Biol. Chem.*, 1955, **212**, 39.
[13] Brown, Malkin, and Maliphant, *J.*, 1955, 1584.

X-Ray Investigations.—This was carried out as described by Clarkson and Malkin,[13,14] by using Cu-K_α radiation ($\lambda = 1{\cdot}54$ Å). The results, as expected, are similar to those previously found for the glycerides and consist of an arrangement of double molecules lying vertically across (α-form) or inclined at an angle to (β-form) the reflecting planes.

(±)-1,2-*Di*-O-*acylglycerol* 3-(*Dihydrogen Phosphates*) (*α-Glycerophosphatidic Acids*).—These exist in two forms termed α and β in accordance with the terminology of the polymorphic forms of the glycerides.[13-15] The β-form, which is stable, is obtained by slow crystallisation from light petroleum (b. p. 40—60°) or ether; rapid crystallisation gives a mixture of α- and β-forms. The α-form is obtained from the melt or by crystallisation from a polar solvent (*e.g.*, acetone or alcohol). In the latter case it is associated with solvent which can only be removed in a high vacuum. The α- changes at room temperature to the β-form, the transformation taking several weeks. No difference in m. p. was observed for the two forms.

The long spacings of both forms are linear when plotted against carbon content and the intercepts at $c = 0$, which are a measure of the size of the end-groups, are 13 Å (α-form) and 9 Å (β-form). The results (Table 1) agree with a bimolecular structure with the long chains vertical to the reflecting planes (α-form) and titled at an angle of 59° across the reflecting planes (β-form).

TABLE 1.

Long and short spacings (Å) for α-glycerophosphatidic acids.

	α-Form		β-Form						
	Long spacing	Short spacing *	Long spacing	Short spacing					
Dilauroyl ...	44·0	4·14s	34·6	4·74vs	4·52m	4·22vs	3·94w	3·83m	3·68w	3·57m
Dimyristoyl	47·5	4·12s	39·2	4·70vs	4·46m	4·18vs	3·87m †	3·63m		
Dipalmitoyl	54·0	4·10s	43·6	4·64vs	4·48m	4·19vs	3·84m †	3·63m		
Distearoyl...	59·7	4·12s	48·1	4·69vs	4·49m	4·20vs	3·86m †	3·63m		

* The average single side spacing of 4·12 Å given by the α-forms,[14] is typical of hexagonal or pseudo-hexagonal chain packing.

† These lines are very thick and are almost certainly the unresolved lines given by the dilauroyl compound.

1,3-*Di*-O-*acylglycerol* 2-(*Dihydrogen Phosphates*) (*β-Glycerophosphatidic Acids*).—These compounds also exist in α- and β-modifications, which, however, melt at different temperatures (see Table 3), though in other respects the properties are similar to those already described.

TABLE 2.

Long and short spacings (Å) for β-glycerophosphatidic acids.

	α-Form		β-Form						
	Long spacing	Short spacing *	Long spacing	Short spacings					
Dilauroyl ...	—	—	36·3	5·16w	4·73vw	4·44vs	3·94vw	3·74vs	3·55m	3·34w
Dimyristoyl	45·3	4·15s	41·0	5·16w	4·82vw	4·45vs	3·84vw	3·61vs †		3·35w
Dipalmitoyl	50·1	4·22s	45·2	5·16w	4·78vw	4·38vs	3·94vw	3·72vs	3·57m	3·41w
Distearoyl...	55·8	4·12s	49·5	5·19w	4·73vw	4·41vs	3·89vw	3·70vs	3·54m	3·39w

* The average single side spacing of 4·16 Å given by the α-form,[14] is typical of hexagonal or pseudo-hexagonal chain packing.

† This line is very thick and is almost certainly the unresolved lines given by the other three acids.

The long spacings of both forms are linear when plotted against carbon content and the intercepts at $c = 0$ are 10 Å (α-form) and 10 Å (β-form). The results (Table 2) again agree with a bimolecular structure with the long chains vertical to the reflecting planes (α-form) and tilted at an angle of 57° across the reflecting planes (β-form).

[14] Clarkson and Malkin, *J.*, 1934, 666; Malkin, Shubagy, and Meara, *J.*, 1937 1409.
[15] Howe and Malkin, *J.*, 1951, 2663.

Experimental

1,2-*Di*-O-*palmitoylglycerol* 3-(*Diphenyl Phosphate*).—To 1,2-dipalmitin (5·7 g., 0·01 mole), dissolved in warm, dry pyridine (10 ml.), was added diphenyl phosphorochloridate (8 g., 0·03 mole). The solution was kept in a stoppered flask at 30—35° for 48 hr., then added to an ice-cold saturated aqueous sodium hydrogen carbonate (250 ml.). After 10 min., the solidified diphenyl ester was removed, washed with water, dissolved in warm acetic acid, and precipitated by addition of a little water. The ester was removed, dried *in vacuo* over sodium hydroxide pellets, and recrystallised from light petroleum (b. p. 40—60°) to yield the ester (6·7 g., 84%) (see Table 3).

Table 3.

α- and β-Glycerophosphatidic acids and their diphenyl esters.

(A) *Melting points.*

		Diacyl-α-glycerophosphatidic			Diacyl-β-glycerophosphatidic		
			(*b*) Acid			(*d*) Acid	
No.	Acyl	(*a*) Ph_2 ester	α-form	β-form	(*c*) Ph_2 ester	α-form	β-form
1.	Lauroyl	32—33 [1]	44—45 [1]	44—45 [1]	28—29 [1]	11—12 [1]	44—45 [1]
2.	Myristoyl	44; [1] L 38—39 [3]	54—55 [1]	54—55; [1, 2] L 61·5—62·5 [3]	36—37 [1]	35—36 [1]	55—56 [1]
3.	Palmitoyl	51—52; [1] L 47—48 [3]	62—63 [1]	62—63; [1] 62·5—63·5; [2] L 70—71 [3]	44; [1] 43—44 [2]	49—50 [1]	63—64; [1] 64—65; [2] 63 [4]
4.	Stearoyl	58; [1] 58—59; [2] L 54·5—55 [3]	70—71 [1]	70—71; [1, 2] L 75·5—76·5 [3]	51—52 [1, 2]	58—59 [1]	69—70; [1] 68·5—69·5 [2]

[1] Present work. [2] Uhlenbroek and Verkade; [9] Hessel, Morton, Todd, and Verkade.[10]

(B) *Analyses and yields.*

		Found (%) *					Calculated (%) *			
No.	Yield (%)	C	H	P	Equiv.	Formula	C	H	P	Equiv.
	α-Series: Ph_2 esters									
1a	71	68·1	9·0	4·5	—	$C_{39}H_{61}O_8P$	68·0	8·85	4·5	—
2a	78	69·0	9·1	4·45	—	$C_{43}H_{69}O_8P$	69·3	9·3	4·15	—
3a	84	70·6	9·65	3·7	—	$C_{47}H_{77}O_8P$	70·45	9·7	3·85	—
4a	90	71·25	10·15	3·5	—	$C_{51}H_{85}O_8P$	71·45	10·0	3·6	—
	α-Series: acids									
1b	72	60·3	10·25	5·65	263	$C_{27}H_{53}O_8P$	60·4	9·95	5·8	268·5
2b	80	62·5	10·7	5·0	295	$C_{31}H_{61}O_8P$	62·8	10·4	5·25	296·5
3b	89	65·0	10·3	4·6	331	$C_{35}H_{69}O_8P$	64·75	10·75	4·8	324·5
4b	96	66·2	11·1	3·95	355	$C_{39}H_{77}O_8P$	66·4	11·0	4·4	352·5
	β-Series: Ph_2 esters									
1c	72	68·2	9·0	4·35	—	$C_{39}H_{61}O_8P$	68·0	8·85	4·5	—
2c	79	69·1	9·2	4·0	—	$C_{43}H_{69}O_8P$	69·3	9·3	4·15	—
3c	85	70·3	9·4	3·5	—	$C_{47}H_{77}O_8P$	70·45	9·7	3·85	—
4c	87	71·2	9·9	3·3	—	$C_{51}H_{85}O_8P$	71·45	10·0	3·6	—
	β-Series: acids									
1d	84	60·25	10·35	5·5	267	$C_{27}H_{53}O_8P$	60·4	9·95	5·8	268·5
2d	80	62·7	10·35	5·0	290	$C_{31}H_{61}O_8P$	62·8	10·4	5·25	296·5
3d	85	64·65	10·75	4·8	322	$C_{35}H_{69}O_8P$	64·75	10·75	4·8	324·5
4d	90	66·35	11·1	4·55	349	$C_{39}H_{77}O_8P$	66·4	11·0	4·4	352·5

The 1,2- and 1,3-di-*O*-acylglycerol diphenyl phosphates listed in Table 3 were similarly prepared.

(±)-1,2-*Di*-O-*palmitoylglycerol* 3-(*Dihydrogen Phosphate*) (*Dipalmitoyl-α-glycerophosphatidic Acid*).—Platinum oxide (1·0 g.) was shaken in glacial acetic acid (10 ml.) in a hydrogenation flask in hydrogen until absorption ceased. The catalyst was then washed with 2N-hydrochloric acid, water, and finally glacial acetic acid by decantation. 1,2-Di-*O*-palmitoylglycerol 3-(diphenyl phosphate) (6·5 g.) in glacial acetic acid (50 ml.) was added and the whole was shaken in hydrogen until absorption ceased (6 hr.; uptake 1440 ml.; theor., 1455 ml.). The catalyst was removed and washed with a little chloroform. The solvents were removed *in vacuo* at

40—50°. The residue, crystallised from light petroleum (b. p. 40—60°) at 0°, yielded the acid ester (4·7 g., 89%) (see Table 3). Equivalent weights were determined by titration in dioxan with aqueous 0·1N-sodium hydroxide to phenolphthalein.

The acids listed in Table 3 were similarly prepared.

The L-forms of the diphenyl esters and of the α-acids differ in m. p. from the DL-forms.

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