N-ACYLPHOSPHATIDYLETHANOLAMINES: OCCURRENCE IN NATURE, STRUCTURE AND STEREOCHEMISTRY

R. ANEJA, J.S. CHADHA and J.A. KNAGGS
Unilever Research Laboratory Colworth /Welwyn,
The Frythe, Welwyn, Herts., England.

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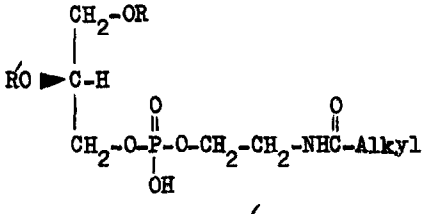
A new glycero-phospholipid constituent of soyabean "lecithin" has been isolated and shown to have the structure N-fatty acyl-O(1,2-difatty acyl-sn-glycero-3-phosphoryl)-ethanolamine.

Contrary to a recent claim (3), the occurrence of N-acylphosphatidyl-ethanolamines in natural lipids was demonstrated first by Bomstein (1) and later by Debuch and Wendt (2). In these independent studies (1,2,3) only the gross structure for this new member of the glycero-phospholipid class was established. No data were provided to distinguish between N-acyl-O-(1,2-diacyl-glycero-3-phosphoryl)-ethanolamine and the isomeric N-acyl-O-(1,3-diacyl-glycero-2-phosphoryl)-ethanolamine structures, or to define the stereochemistry of the substituents on the C-2 assymetric centre of the glycerol. We now present additional data and arguments which establish the basic stereochemical structure of this lipid class as N-acyl-O-(1,2-diacyl-sn-glycero-3-phosphoryl)-ethanolamine (I) (4).



(I)

The new Lipid from Soyabean "lecithin"

Our material was isolated (5) from the phosphatides of soyabean oil (commercial "lecithin"). It first attracted attention as a hitherto

unidentified phospholipid spot (blue colour with cold Zinzadze (6)reagent) on thin-layer chromatograms of commercial "lecithin" on silicagel G layers impregnated with triethylamine (7). The new lipid spot had an rf. (i) intermediate between phyto-sterol glucoside and phyto-sterolglucoside monoesters (ESG) (8) and (ii) identical with the rf. of an N-stearoyl-phosphatidylethanolamine (vide infra) which had been synthesised earlier in connection with another study.

The purified lipid, obtained as a triethylamine salt (5) was a light pale viscous oil, $[\alpha]_D^{22}$ + 4.7° (C,3 ethanol-free dry chloroform). Strong absorption bands in its I.R. spectrum (smear) at νmax 1724 cm^{-1} indicated an ester group, while bands at 1645 and 1527 cm^{-1} indicated an amide carbonyl. Both the I.R. evidence and the TLC evidence cited above, suggested that the new lipid was an N-acyl-phosphatidylethanolamine.

Degradation and Fatty acid Composition

In conformity with this conclusion, methanolysis of the pure lipid (lithium methoxide in ether-methanol) gave a (i) mixture of fatty acid methyl esters (Table I) and (ii) two other lipids,both more polar(TLC)than the natural lipid. The most polar of these two showed strong I.R, absorption (KBr disc) ν max 3333 cm^{-1} (OH), 1634 & 1527 cm^{-1} (CONH), 2915 & 1460 cm^{-1} (lipid CH_2). Similar methanolysis of the N-stearoyl derivative prepared from phosphatidylethanolamine (of bacterial origin, ex Koch Light) gave analogous results*.

Further acid hydrolysis (methanol-sulphuric acid) of the polar methanolysis product above liberated the fatty acid components from the amide link as methyl esters (Table I).

Synthetic analogues

In studies on the structure and stereochemistry of glycerophospho-lipids, it has become customary to employ degradation with phospholipase C(9)

* With both the natural and the synthetic sample, the accompanying comparatively less polar product is presumably a mono-deacylation product, i.e. a lyso compound.

to 1:2 or 1:3 diglycerides, in conjunction with lipolysis with phospholipase A to a fatty acid and a lysophospholipid. Since an N-stearoyl phosphatidylethanolamine has been reported (1) to be unaffected by phospholipase A, the following physico-chemical approach was employed in the present investigation.

Comparison with a synthetic analogue of the natural lipid forms the vital basis of this study. This was prepared by the treatment of O-(1,2-dimyristoyl-sn-glycero-3-phosphoryl)-ethanolamine (10) with stearic anhydride and triethylamine (11). The product, N-stearoyl-O-(1,2-dimyristoyl-sn-glycero-3-phosphoryl)-ethanolamine was purified by chromatography on SilicAR CC-4 and characterised as its mono-triethylammonium salt, $C_{57}H_{115}N_2O_9P$, m.p. 49°, $[\alpha]_D^{22}$ + 5.2° (C, 2.5 ethanol free dry chloroform), I.R. (nujol mull) νmax. 1724 cm^{-1} (C=O ester), and 1639, 1527 cm^{-1} (CONH). The 60MHz p.m.r. spectrum (in $CDCl_3$) of the product shows the differences from the spectrum of phosphatidylethanolamine (12) expected for the N-stearoyl derivative. Thus, the absorption due to $\overset{+}{N}H_3$ protons is absent. Surprisingly, the signal due to the CH_2-$\overset{+}{N}$, which occurs at ca. τ 6,7 in phosphatidylethanolamine, appears to have shifted upfield to ca. τ 6.9 (compare N-acetyl derivative below). The remaining signals are, of course, identical.

In view of the interest in the N-acetyl derivatives of phosphatidylethanolamine (2,5), an authentic sample was prepared by the action of acetic anhydride and pyridine on O-(1,2-dimyristoyl-sn-glycero-3-phosphoryl)-ethanolamine. The product, N-acetyl-O-(1,2-dimyristoyl-sn-glycero-3-phosphoryl)-ethanolamine, $C_{35}H_{68}NO_9P$, m.p. 93-95°, $[\alpha]_D^{22}$ + 5.7° (C, 1.0 $CHCl_3$), I.R. (KBr), νmax 1730 cm^{-1} (C=O ester), 1639 cm^{-1} and 1527 cm^{-1} (CONH); the 60 MHz p.m.r. spectrum was similar to that of the N-stearoyl derivative except that the CH_2-NCO signal occurs at τ 6.45. Further, the CH_2CO and the lipid chain CH_2 signals were less intense and there was a sharp 3H singlet at τ 7.91 for the acetyl CH_3.

TABLE I

Fatty acid composition of N-acyl phosphatidyl-ethanolamine of soyabean "lecithin"

Fatty acid	% Composition	
	O-acyl	N-acyl
16:0	34.6	64.8
18:0	4.2	8.8
18:1	9.8	16.9
18:2	47.7	9.3
18:3	2.8	0.2
18:4	0.9	-

TABLE II

Optical Rotation Data for N-acyl phosphatidylethanolamines (NEt_3 Salts)

	Natural (ex.soyabean "lecithin")	Synthetic (N-stearoyl-O,O,dimyristoyl-...)
$[\alpha]_D^{22}$	+4.7°(c,3.0)*	+5.2° (c,2.5)*
(MW)	1115	1003
MD	52.4	52.1

* In ethanol free dry chloroform.

Structure and Stereochemistry

The data presented above leave little doubt that the new lipid is an N-acyl-phosphatidylethanolamine. Now, by p.m.r. spectroscopy, it should be possible, in principle, to distinguish between a glycero-2-phosphoryl and a glycero-3-phosphoryl structure, but there are practical limitations with operations at 60 MHz (13). Nevertheless, a comparison of the p.m.r. spectrum of the synthetic and the natural N-acyl phosphatidylethanolamines was made. The natural material showed additional absorption signals at τ 7.9 (CH_2-C=), τ 7.22 (=C-CH_2-C=) and τ4.63 (CH=CH) (c.f. the fatty acid

composition, Table I) but the rest of the spectrum showed very little difference from the spectrum of the synthetic derivative N-stearoyl-phosphatidylethanolamine.

Finally, the optical rotation data for the N-acylphosphatidyl-ethanolamine (I) from soyabean are compared with that of the synthetic analogue N-Stearoyl-O(1,2-dimyristoyl-sn- glycero-3- phosphoryl) ethanolamine in Table II.

The agreement between the molar rotations for the two samples is excellent and proves that like the synthetic reference sample, the natural lipid also has the sn-glycero-3-phosphoryl structure (I).

Conclusion

Our studies were limited to the new lipid isolated from soyabean phosphatides and thus the conclusions are strictly valid for this material. However, as the structure is based on the almost ubiquitous sn-glycero-3-phosphoryl rather than on the very rare sn-glycero-1-phosphoryl (14) back-bone, it is reasonable to expect that the N-acyl P.Es.from other natural sources also belong to the sn-glycero-3-phosphoryl series. Further, in view of the earlier reports (15) describing the isolation of N-fatty acyl-ethanolamines from hydrolysates of phospholipid fractions from arachis (peanut) oil, egg yolk and vegetable lecithins, it would appear that N-acyl-phosphatidylethanolamines are widely distributed in nature. Finally, since the fatty acids involved in the amide linkage are very different from those involved in ester linkages, this new lipid class does not appear to be an artefact derived from the commonly occurring phosphatidylethanolamines.

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