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Methods for the preparation of lysophosphatidylcholine

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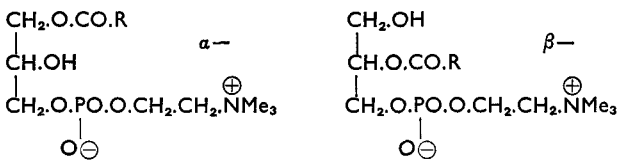
The surfactant and solubilizing properties of lysophosphatidylcholine (LPC) could have industrial uses if it became available in technical quantity and purity. The two most promising routes for obtaining it are isolation from cereal starches and mono-deacylation of egg phosphatidylcholine (PC). Baker's yeast autolysed by Letters's method yielded up to 0·3% of LPC. The phospholipase A activity of pancreatin powder gave 80% of the theoretical yield of LPC under optimum conditions; modifications designed to facilitate scale-up reduced the yield. Base-catalysed glycerolysis at 75° gave 23% of the theoretical yield of LPC, and a reaction between PC and ethanolamine up to 45%. The crude product from ethanolamine after acetone precipitation gave an emulsifier containing LPC, PC and long-chain *N*-2-hydroxyethylamide.

Lysophosphatidylcholine (LPC) has many properties (Robinson, 1961) that could be of use in pharmaceutical and food preparations. It forms clear, micellar solutions in water and in numerous organic solvents, it solubilizes enzymes and other proteins, it forms complexes with some proteins and polysaccharides, and it is a powerful surfactant and emulsifying agent. Unlike all industrial ionic surfactants except soap and phosphatidylcholine (PC) it is a normal metabolite (see *e.g.* Eisenberg, Stein & Stein, 1967). Until recently LPC was known mainly for its haemolytic effect, and its isolation from tissues was often attributed to autolysis or chemical degradation. Its relative abundance in intestinal contents (Nilsson & Borgström, 1967) and in foods such as the cereal starches (Acker & Schmitz, 1967; Wren & Merryfield, 1970) indicate that it must be a relatively harmless substance for oral administration. Unfortunately, it is much more costly than other surfactants.

The term LPC is used in this paper to denote one or both of the α- and β-isomers (1- and 2-acyl, respectively) in which the fatty acid composition is unspecified:



Either isomer, with a single acyl group, can be prepared in a high state of purity *via* costly synthetic intermediates (see *e.g.* Slotboom, Haas & van Deenen, 1967). A more attractive route to a technical grade of LPC would be through direct acylation of α-glycerylphosphorylcholine, but this is not readily available as a starting material.

Two other routes seem more feasible for LPC on a technical scale, namely isolation from cereal starches (Wren & Merryfield, 1970) and mono-deacylation of PC. Table 1 shows the most promising deacylation methods reported. In present work

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Table 1. *Published methods for the preparation of LPC by deacylation of PC.*

PC	Method	Yield, % of theoretical	Reference
Egg (purified)	Enzymic (phospholipase A activity in pancreatin)	60	Ansell & Hawthorne (1964)
	Controlled methanolysis	35–40	Marinetti (1962)
	$AlLiH_4$ in ether	?	Urakami & Yamaguchi (1963)
Soya (purified)	Enzymic (snake or bee venom)	51	Klenk & Debuch (1960, 1962)
Synthetic (dipalmitoyl)	Controlled methanolysis	$\geqslant$38	Pries (1965)

modifications of the pancreatin method were attempted, and also autolysis of baker's yeast (Letters, 1967), base-catalysed glycerolysis, and a novel use of ethanolamine.

MATERIALS AND METHODS

Analar solvents were used. Pancreatin powder was obtained from Hopkin and Williams. PC was isolated from fresh hen eggs (Wren & Merryfield, 1965) using 0·005% of the antioxidant butylated hydroxytoluene (Wren & Szczepanowska, 1964) in the solvents.

Solvents were removed in a rotary evaporator at $<50°$. Lipid fractions were identified by thin-layer chromatography and infrared spectrophotometry.

LPC was isolated from reaction products by chromatography on a silicic acid column (usually 20 g): product ($\geqslant$1 g) was developed with 200 ml each of 10% methanol, 50% methanol (to recover PC) and 100% methanol (to elute LPC). In some experiments 100 g of product was chromatographed on 750 g of silicic acid.

Acetone precipitation was effected by adding just enough chloroform to the product to make it pourable and then stirring into acetone at 5°. The precipitate was recovered by centrifuging and washed with a little acetone.

RESULTS AND DISCUSSION

When purified egg PC was subjected to the full pancreatin method (Ansell & Hawthorne, 1964) LPC was isolated in 80% of theoretical yield by silicic acid chromatography. When the concentration of PC was raised, when pancreatin powder was used without the removal of insoluble matter, or when impure sources of PC (egg yolk, whole egg or commercial soya PC) were used, the yield fell to less than 50% of theoretical.

Baker's yeast, after autolysis for 20 h while mixed with an equal weight of acetone–water (7:3, v/v), and subsequent extraction with *n*-butanol (Letters, 1967), gave up to 0·3% of LPC. No extra LPC could be obtained by adding egg yolk or purified egg lecithin at the beginning of the autolysis period.

Base-catalysed glycerolysis is applied to triglyceride fats in the production of monoglycerides; if applied to PCs (Mattikow, 1942) at moderate temperature it should give a useful emulsifying mixture containing LPC and monoglyceride. When purified egg PC was heated at 75° for 7 days *in vacuo* with one-quarter of its weight of 2% NaOH in glycerol, the product yielded: LPC (23% of theoretical) 15%;

PC 28%; mono-, di-, triglycerides 45%. When commercial soya PC was used the reaction was slower, but it could be speeded by using ethanolamine as catalyst in place of NaOH.

Fatty acids (Roe, Scanlan & Swern, 1949) and triglycerides (Roe, Stutzman & others, 1952; Naudet, Sambuc & others, 1952) react with ethanolamine to give long-chain *N*-2-hydroxyethylamides (ethanolamides). PC has now been found to react similarly, and under suitable conditions can be converted to PC and ethanolamide. The latter is a non-ionic surfactant and, like LPC, occurs naturally in animal tissues (Bachur, Masek & others, 1965), in bean leaves, wheat and soya beans (Wren & Merryfield, 1965) and in peas (Quarles, Clarke & Dawson, 1968). No toxic effect has been found in any of several biological tests made with ethanolamides (Macht & D'Alelio, 1936; Merck, 1958; Coburn & Rich, 1960; Coburn, 1961). Hence the reaction products of PC and ethanolamine merit consideration for use in pharmaceutical and food preparations.

Table 2. *Yields of LPC obtained from PC and ethanolamine under various conditions (expressed as % of starting material; theoretical yield, 66%).*

Conditions	Molar ratio, PC: ethanolamine	Egg PC (purified)	Egg PC (BDH)	Soya PC (commercial)
Stirred under N_2 for 30–50 min at 140°	1:1	17, 22	18	4
	1:3	30		
	1:6		22	
Refluxed with chloroform (1:1, w/v) for 3 h	1:1	9	18	
	1:2		20	
	1:3	24, 28	22	
Kept over silica gel at room temp. for 18 h (or 2–3 days*)	1:1	20, 23*		4
	1:2	25*		6
	1:3	30		

Table 2 shows the yields of LPC obtained under various conditions and isolated quantitatively by silicic acid chromatography. Acetone precipitation of the reaction products gave 'technical-quality' emulsifiers: the composition of the product obtained at 140° from purified egg PC was: LPC 40%; PC 49%; ethanolamide 11%. Traces of ethanolamine contaminating these preparations were removed *in vacuo* over P_2O_5; however, preparations from soya PC retained an unpleasant odour derived from the starting material.

Table 3. *Fatty acid compositions of egg PC and LPC made from it.*

	C_{16}	$C_{16:1}$	C_{18}	$C_{18:1}$	$C_{18:2}$
PC (total)	34·0	2·2	17·6	31·6	14·4
PC (α-position*)	61		25	9·5	2·4
PC (β-position*)	4·8	1·7	2·4	59	26
LPC (enzymic, pancreatin)	67·7	2·3	24·1	4·6	
LPC (ethanolamine, 140°)	36·4	6·1	18·5	30·2	8·7
LPC (ethanolamine, chloroform)	33·0	1·6	15·6	31·3	17·7

* Menzel & Olcott (1964).

Fatty acid compositions (Table 3) show that, unlike the enzyme but like other basic catalysts (Marinetti, 1962), ethanolamine deacylates the PC molecule non-specifically.

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