Isolation of 1,3-distearoyl-glycero-2-phosphocholine (β-lecithin) from commercial 1,2-distearoyl-*sn*-glycero-3-phosphocholine

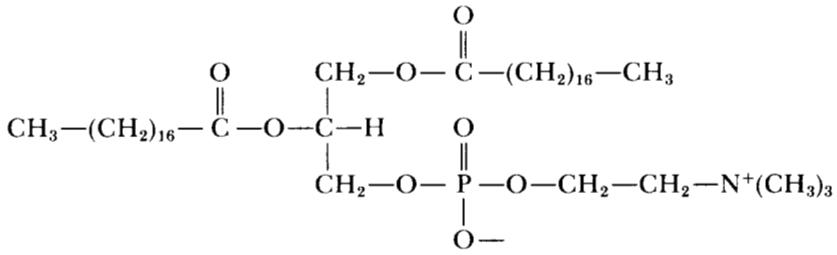
M. M. Ponpipom and R. L. Bugianesi

Merck Sharp & Dohme Research Laboratories, Rahway, NJ 07065

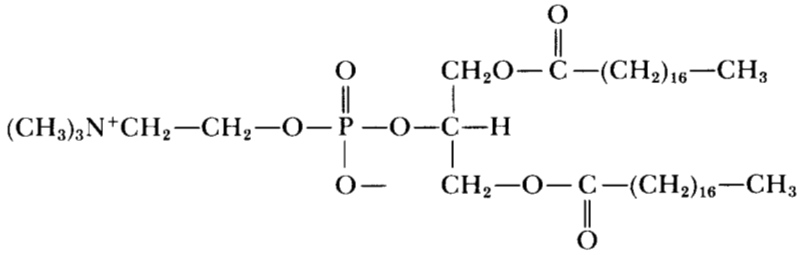
Summary Different batches of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC) had varying amounts of contaminants which appeared to affect systematic biological studies. This contaminant was separated by silica gel column chromatography followed by high performance liquid chromatography and identified as 1,3-distearoyl-glycero-2-phosphocholine (β-lecithin).—**M. M. Ponpipom and R. L. Bugianesi.** Isolation of 1,3-distearoyl-glycero-2-phosphocholine (β-lecithin) from commercial 1,2-distearoyl-*sn*-glycero-3-phosphocholine. *J. Lipid Res.* 1980. **21:** 136–139.

Supplementary key words preparative chromatography · phospholipids · β-lecithins

Liposomes have been used as carriers for delivering biologically active materials into cells (1–5). Lipid vesicles are formed when mixtures of phospholipid, cholesterol, and a charged amphiphile in varying molecular ratios are agitated or sonicated in an aqueous solution. Synthetic phospholipids such as 1,2-dioleoyl-, 1,2-dipalmitoyl-, and 1,2-distearoyl-*sn*-glycero-3-phosphocholine (DSPC, Compound 1) are often used to prepare liposomes for biological studies. These phospholipids are generally prepared from egg yolk lipids via *sn*-glycero-3-phosphocholine (6) by acylation with the desired acyl chloride (7) or fatty acid anhydrides (8, 9). Since most of these phospholipids are available commercially, they are often used as such without further purification. However, it should be stressed that most biochemical and physico-chemical studies of membrane constituents do require pure phospholipids. In this communication, we report the isolation and characterization of 1,3-distearoyl-glycero-2-phosphocholine (Compound 2), a contaminant of synthetic DSPC from a commercial source.[1]



1,2-Distearoyl-*sn*-glycero-3-phosphocholine (Compound 1)



1,3-Distearoyl-glycero-2-phosphocholine (Compound 2)

MATERIALS AND METHODS

Ten one-gram vials of DSPC (# P1138, from Sigma) were combined and chromatographed on a column of Silica gel 60 (500 g, 70–230 mesh ASTM, from E. Merck, Darmstadt, Germany) with chloroform–methanol–water 12:8:1 (v/v/v) as eluent. The DSPC obtained (7.9 g) gave a single spot by TLC (R_f 0.53) in chloroform–methanol–water 6:4:1 (v/v/v). The

Abbreviations: DSPC, 1,2-distearoyl-*sn*-glycero-3-phosphocholine; HPLC, high performance liquid chromatography; NMR, nuclear magnetic resonance spectroscopy; TLC, thin-layer chromatography.

[1] Different batches of DSPC had different amounts of contaminants which appeared to affect systematic in vivo tissue distribution studies.

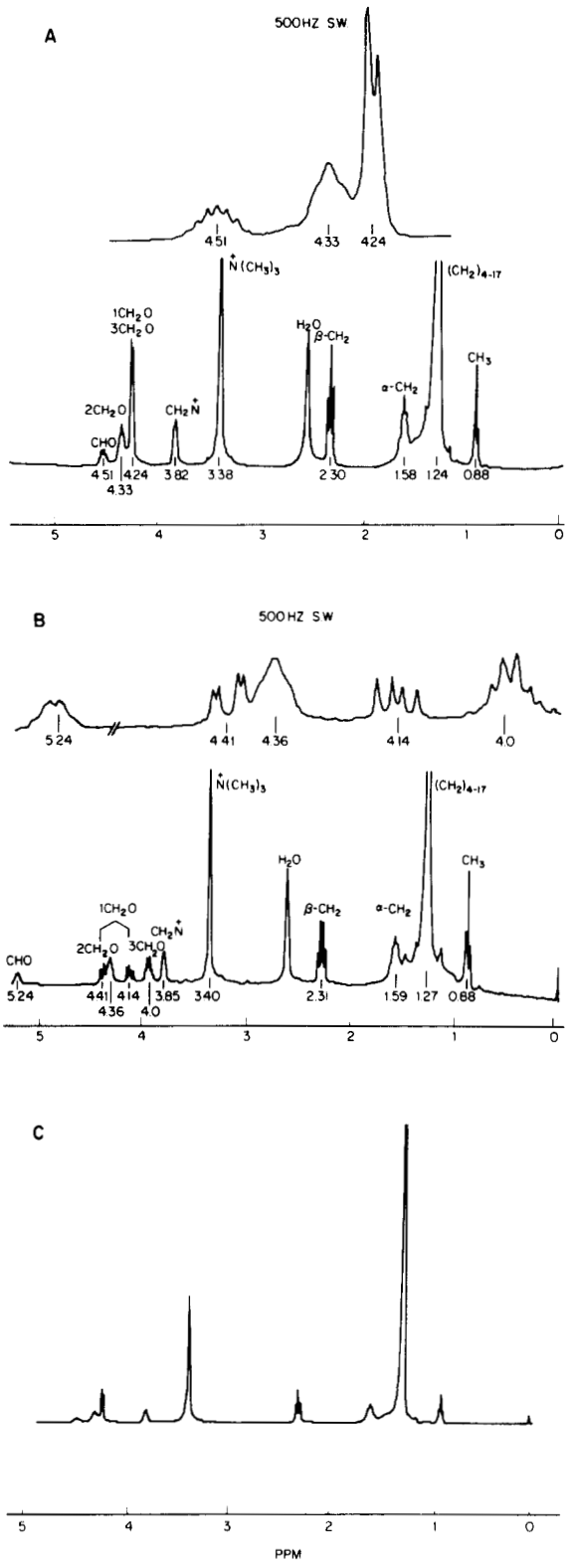


Fig. 1. A. Proton NMR spectrum of 1,3-distearoyl-glycero-2-phosphocholine (Compound 2) in $CDCl_3$ solution. B. Proton NMR spectrum of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (Compound 1) in $CDCl_3$ solution. C. Proton NMR spectrum of synthetic 1,3-distearoyl-glycero-2-phosphocholine (Compound 2) in $CDCl_3$ solution.

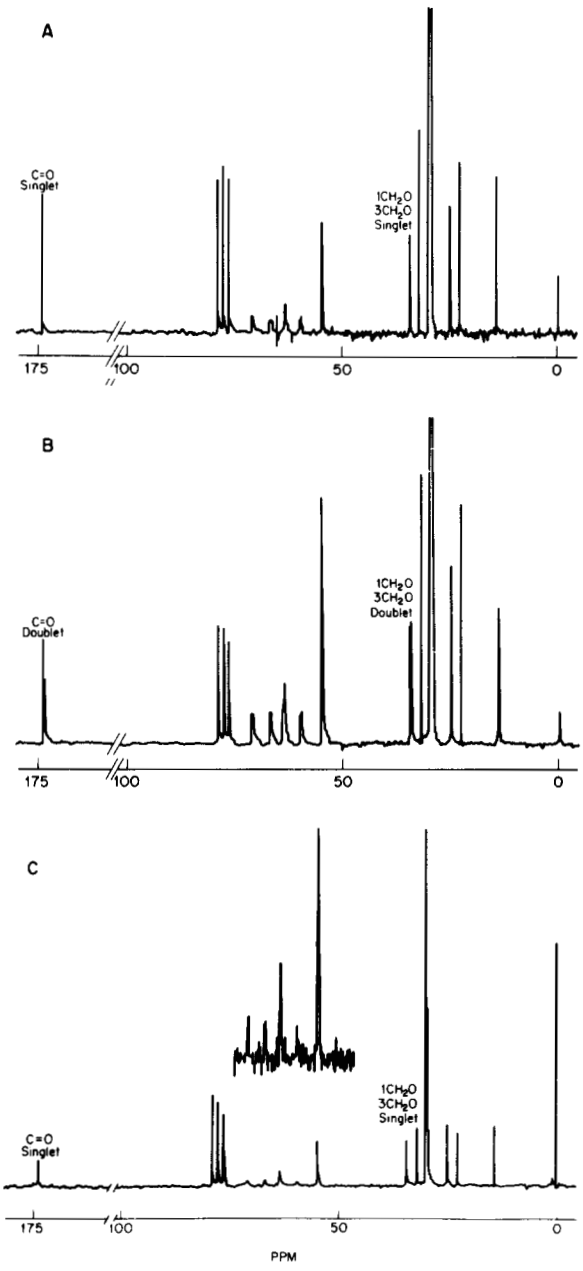


Fig. 2. A. ^{13}C-NMR spectrum of 1,3-distearoyl-glycero-2-phosphocholine (Compound 2) in $CDCl_3$ solution. B. ^{13}C-NMR spectrum of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (Compound 1) in $CDCl_3$ solution. C. ^{13}C-NMR spectrum of synthetic 1,3-distearoyl-glycero-2-phosphocholine (Compound 2) in $CDCl_3$ solution.

forerunning fractions (2 g) (containing two spots) were separated by means of PrepPak™ 500/Silica on a Waters Associates Prep LC/System 500 at 250 ml/min using chloroform–methanol–water 12:8:1 (v/v/v) as a liquid phase. The column was first conditioned with water–methanol 1:9 (v/v). The first two fractions (100 mg) which contained about 20% of a more mobile component were analyzed by HPLC (10) (Waters)

TABLE 1. Proton chemical shifts of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (Compound 1) and 1,3-distearoyl-glycero-2-phosphocholine (Compound 2)

Compound	Choline			Glycerol			Methylene			Methyl 18
	Me_3N^+	CH_2N^+	CH_2O	$1CH_2O$	CHO	$3CH_2O$	2	3	4–17	
1	3.40	3.85	4.36	4.14[a] 4.41[d]	5.24	4.0	2.31[b]	1.59	1.27	0.88[c]
2	3.38	3.82	4.33	4.24[e]	4.51	4.24[e]	2.30[c]	1.58	1.24	0.88[c]

[a] J 7.0, 12.0 Hz (d, d).
[b] Jvic 7.0 Hz (q).
[c] Jvic 7.0 Hz (t).
[d] J 2.5, 12.0 Hz (d, d).
[e] J 5.0 Hz (d).
The NMR spectra were measured at 300 MHz in $CDCl_3$ using a Varian SC300 spectrometer. Chemical shifts were expressed in ppm downfield from internal TMS.

using 3/8 in × 4 ft LC Porasil type A silica gel (37–75 microns). The solvent and conditioning systems were the same as above.

RESULTS AND DISCUSSION

The more mobile component (5 mg, R_f 0.59) was isolated and shown by TLC and NMR (**Figs. 1**A and **2**A) to be identical to synthetic 1,3-distearoyl-glycero-2-phosphocholine (11, 12) (Figs. 1C and 2C) which was prepared in good yield from 2-bromoethyl dichlorophosphate and 1,3-distearoyl glycerol (13). This material was crystallized from butanone, mp 73–75°C (to liquid crystal) and 231–232°C (to isotropic liquid) (Anal. calculated for $C_{44}H_{88}NPO_8.0.5\ H_2O$: C, 66.13; H, 11.23; N, 1.75; P, 3.88. Found: C, 65.96; H, 11.27; N, 1.89; P, 3.97); by differential thermal analysis there was a main endothermic transition at 68.5°C, and a shallow endotherm at 100°C. It readily formed liposomes and had a transition temperature of 51°C as compared to 55°C for DSPC.

The peak assignments of proton and carbon NMR were made with the aid of literature references (14–16) and are summarized in **Tables 1** and **2,** respectively. The structure of 1,3-distearoyl-glycero-2-phosphocholine is readily established from its NMR spectra (see Figs. 1A and 1B). Since the two fatty acyl chains are equivalent, the CH_2OCO glycerol protons absorb as a doublet (J 5.0 Hz) at δ 4.24 ppm in contrast to the two doublets of doublets at δ 4.14 and 4.41 for DSPC. This finding is also consistent with the ^{13}C proton noise decoupled spectra (see Figs. 2A and 2B). The two α-carbon and carbonyl nuclei both absorb as singlets at δ 34.2 and 173.6, respectively, as compared to the pairs at δ 34.3 and 34.2 for the α-carbon nuclei and δ 173.5 and 173.1 for the carbonyl nuclei in DSPC.

1,3-Distearoyl-glycero-2-phosphocholine was probably formed from the migration of the phosphoryl group from the 3-carbon atom (17–19) during acid or base hydrolysis of egg yolk lecithin followed by acylation with stearic anhydride or stearoyl chloride. Although 1,3-diacyl-*sn*-glycero-2-phosphocholines have not been found so far as naturally occurring constituents, many of these so-called β-lecithins, which have proven to be useful for the elucidation

TABLE 2. Carbon chemical shifts of 1,2-distearoyl-*sn*-glycero-3-phosphocholine (Compound 1) and 1,3-distearoyl-glycero-2-phosphocholine (Compound 2)

Compound	C = 0	Choline			Glycerol			Methylene					Methyl 18
		Me_3N^+	CH_2N^+	CH_2O	$1CH_2O$	CHO	$3CH_2O$	2	16	4–15	3	17	
1	173.5 173.1	54.3	66.4 66.2	59.4 59.3	63.1	70.5[a]	63.4	34.3 34.2	31.9	29.7 29.4 29.2	24.9	22.7	14.1
2	173.6	54.5	66.7 66.6	59.3	63.1	70.3	63.1	34.2	31.9	29.8 29.4 29.2	24.9	22.7	14.1

[a] ^{3}Jc-p 5.0 Hz (d).
The NMR spectra were measured at 25.2 MHz in $CDCl_3$ using a Varian XL-100 spectrometer. Chemical shifts were expressed in ppm downfield from internal TMS.

of the specificity of phospholipase A_2, have been synthesized (20).

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