Synthesis of *N*-Monomethyl- and *N,N*-Dimethylcephalins

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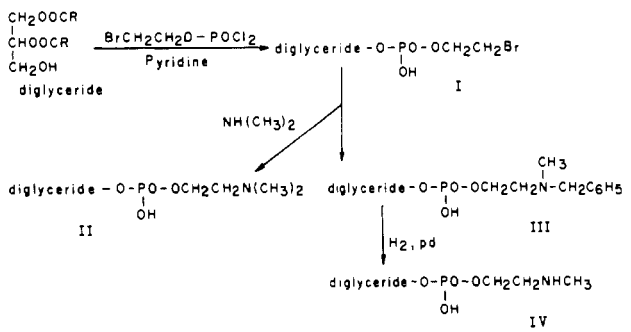
Monomethyl- and dimethylcephalins were synthesized from a common key intermediate, namely, a β-bromoethylphosphoryldiglyceride (I). Condensation of compound I with *N*-methylbenzylamine and debenzylation of the resulting product by catalytic hydrogenolysis led to the monomethyl derivative, while reaction of compound I with dimethylamine gave the dimethyl derivative. The maximum over-all yields were 20–25% and 30–35%, respectively.

The postulation that *N*-methyl- and *N,N*-dimethylcephalin are intermediates in the biosynthesis of lecithin has been substantiated recently by Bremer and Greenberg (1959, 1960, 1961; Bremer *et al.*, 1960). These authors have shown that both radioactive phospholipids are formed in the liver of the living rat and in rat liver homogenates in the presence of [methyl-^{14}C] methionine, and that lecithin formation is stimulated by the addition of dimethylcephalin.

Scheme I



While we were studying the synthetic approach to these biological intermediates as outlined in scheme I,[1] Baer and Pavanaram (1961a,b) reported both syntheses by two different routes. The synthesis of monomethylcephalin involved condensation of phenylphosphoryldichloride with a diglyceride and *N*-carbobenzoxy-*N*-methylethanolamine, and removal of the protective groups by catalytic hydrogenation. The dimethyl derivative was obtained by a nine-step synthesis starting from acetone glycerol.

In the chemical synthesis of phosphorylcholine derivatives the determining step is the phosphorylation reaction. In our synthetic work on the sphingomyelins (Shapiro *et al.*, 1958, 1959) we employed most advantageously β-chloroethylphosphoryldichloride as phosphorylating agent, in which the two-carbon unit served both as protecting group and as a basis for building up the choline moiety. Hirt *et al.* (1958) achieved a synthesis of lecithin by using the more reactive β-bromo analog. Baer and Pavanaram (1961b) reported an unsuccessful attempt to condense L-α-distearoylglycerylphosphorylethylene bromohydrin with dimethylamine. However, in view of the simplicity of scheme I it seemed worthwhile to study the reactions I → II and I → III in detail. After a series of experiments we found a proper method by which compounds II and III could be obtained in satisfactory yields. In the latter case the benzylated amine was used in preference to the less reactive monomethylamine. That the method is easily reproducible was shown by the synthesis of four monomethyl- and four dimethylcephalins.

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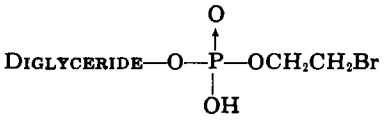
[1] A crude dimethylcephalin was prepared by this method in 1960 in Dr. Greenberg's laboratory by one of us (D. S.). See Bremer and Greenberg (1961).

Experimental Procedure

Materials.—D-α,β-Distearin was obtained from L-α-benzylglycerol ether according to the method of Baer and Kates (1950). It melted at 74–75° and had $[\alpha]_D^{26}$ −2.7° in chloroform (*c*, 10). D-α,β-Dipalmitin was prepared similarly; mp 68–69°; $[\alpha]_D^{25}$ −2.6° in chloroform (*c*, 10). The corresponding racemic compounds were synthesized according to Howe and Malkin (1951). β-Bromoethylphosphoryldichloride was prepared as described by Jean (1957); bp 76°/2 mm. *N*-Methylbenzylamine (bp 45–47°/2 mm; hydrochloride, mp 176–177°) was obtained in yields of about 70% either from *N*-benzylurethan (Basterfield *et al.*, 1926) or from *N*-methylbenzamide by reduction with lithium aluminum hydride (Wessely and Swoboda, 1951) in hot tetrahydrofuran. Chloroform was distilled over phosphorus pentoxide. Anhydrous pyridine was prepared by distilling over barium oxide (Baer *et al.*, 1959).

Distearoyl-DL-α-glycerylphosphorylethylene Bromohydrin.—A solution of 6.25 g (0.01 mole) of DL-distearin in 40 ml of dry chloroform and 2.4 ml (0.03 mole) of anhydrous pyridine was cooled to +5°. A part of the glyceride crystallized out. To the stirred suspension was added dropwise during a few minutes 2.7 ml (0.02 mole) of β-bromoethylphosphoryldichloride in the same volume of chloroform. Soon a clear solution was formed which was stirred at 5° for 1 hour and then at room temperature (20–25°) for another 6 hours. The cooled mixture was added in a thin stream to 240 ml of a vigorously stirred 5% solution of $Ba(OH)_2 \cdot 8H_2O$ containing a few drops of phenolphthalein. Stirring was continued for 45–60 minutes, the temperature being maintained at 10–12°. During this period the chloroform layer thickened and adhered to the walls of the flask. Its content was then cooled to 2–3°, the upper aqueous layer was decanted, and the separated semisolid mass was washed several times with cold water. Cold methanol (200 ml) was added and the mixture was left at 5° for 3 hours to complete precipitation. The product was filtered, washed with cold methanol, and dried over phosphorus pentoxide. It was then dissolved in 50 ml of dry chloroform, the solution was filtered and concentrated at reduced pressure to half its volume, and the barium salt was precipitated by slow addition of 100 ml of cold methanol. For further purification, the salt was dissolved in benzene, acetone was added, and the mixture was left for 6 hours at 5°. To obtain the free acid the

TABLE I



Diglyceride	Melting Point	$[\alpha]_D^{25}$	Calculated C	H	Br	P	Found C	H	Br	P
D-α,β-Distearin	71–73	+4.0°	60.65	9.93	9.84	3.82	60.63	10.00	9.54	3.56
DL-α,β-Distearin	72–73		60.65	9.93	9.84	3.82	60.34	9.85	9.86	3.71
D-α,β-Dipalmitin	62–63	+3.8°	58.20	9.60	10.57	4.09	58.45	9.80	10.24	3.85
DL-α,β-Dipalmitin	61–62		58.20	9.60	10.57	4.09	58.25	9.70	10.67	4.03

TABLE II

N,N-DIMETHYLCEPHALINS (II)

Diglyceride	Melting Point	$[\alpha]_D^{25}$	Calculated C	H	N	P	Found C	H	N	P
D-α,β-Distearin	168–169	+5.8°	66.54	11.17	1.80	3.99	66.35	11.28	1.71	3.68
DL-α,β-Distearin	160–162		66.54	11.17	1.80	3.99	66.40	11.24	1.76	4.06
D-α,β-Dipalmitin	164–165	+5.8°	65.06	10.92	1.94	4.30	64.96	11.04	2.05	4.13
DL-α,β-Dipalmitin	160–161		65.06	10.92	1.94	4.30	65.01	10.83	1.87	4.17

barium salt was dissolved in chloroform and the solution was shaken several times with 0.5 N H_2SO_4, cold methanol being added to facilitate separation. The organic layer was washed with water. The clear filtrate was evaporated in a rotary evaporator at 25°. The residue was dissolved in 15 ml of chloroform, the solution was filtered, and after addition of 50 ml of cold methanol it was allowed to stand at 8° for 4 hours. A second recrystallization gave the pure compound melting at 72–73°. The yields ranged from 50–60%.

Some preparations having the same melting point but showing a lower phosphorous content were recrystallized from dry *n*-hexane (20 ml/g). This procedure lowered the yield somewhat but gave a pure compound. The β-bromoethylphosphoryl esters of D-α,β-distearin, D-α,β-dipalmitin, and DL-α,β-dipalmitin were prepared analogously and in similar yields. Their properties are summarized in Table I.

*Distearoyl-*DL*-α-glycerylphosphoryl(N,N-dimethyl)-ethanolamine.*—Attempts to condense the amines with the barium salt of compound I under a variety of conditions were not successful; however, the reaction with free acid proceeded satisfactorily. Time and amine concentration seem to be important factors. Into a thoroughly dried thick-walled tube was placed a solution of 1 g of compound I in 4 ml of dry toluene, and 3 ml of a 10% solution (w/v) of dimethylamine in toluene was added, care being taken to exclude moisture during this operation. The tube was sealed and heated at 60–65° for 6 hours. The mixture was then cooled, the crystals of dimethylamine hydrobromide (mp 234–235°) were filtered off, and the filtrate was evaporated *in vacuo*. Dry benzene was added and evaporated again. The residue, which was void of halogen (Beilstein test), was dissolved in 5 ml of dry chloroform, 15 ml of dry methanol was added slowly, and the solution was left for 8 hours at 8°. A product (730 mg) melting at 153–155° with previous softening at 95° was obtained. To assure that the compound is not the amine salt, a chloroform solution was shaken with hydrochloric acid. The recovered product showed no change in its nitrogen content. After a second recrystallization from the same solvent mixture the material was finally purified by a slow crystallization from 50 ml of absolute ethanol at 8° (Baer and Pavanaram, 1961b). The vacuum-dried dimethylcephalin (610 mg, 63%) slightly sintered at about 135° and melted at 161–162°. Passing the chloroform-methanol solution (4:1) through a column of silicic acid did not raise the melting point.

By the same procedure and in similar yields were prepared the analogous dimethylcephalins deriving from D-α,β-distearin, D-α,β-dipalmitin, and DL-α,β-dipalmitin (Table II).

*Distearoyl-*DL*-α-glycerylphosphoryl(N-benzyl-N-methyl)-ethanolamine.*—One gram of compound I was allowed to react as described above with 1.5 ml of *N*-methylbenzylamine at 60–65° for 40 hours. The cooled mixture was filtered from the deposited crystals and the toluene was removed *in vacuo* at 25°. The oily residue was dissolved in chloroform and the solution was shaken several times with 0.5 N hydrochloric acid and washed with water. To the separated chloroform layer were added 15 ml of methanol and 5 ml of distilled water, and the emulsion was stirred vigorously with 2.5 g each of IR 45 and IRC 50 for 2 hours. The resins were filtered off and the clear filtrate was concentrated in a rotary evaporator. The residue was redissolved in 6 ml of chloroform, the solution was filtered, and 20 ml of cold methanol was added in small portions. Precipitation was completed by cooling at 5° for 3 hours. A second crystallization gave 440 mg (42%) of a product melting at 130–132°.

Anal. Calcd. for $C_{49}H_{90}O_8NP$ (852.19): C, 69.06; H, 10.65; N, 1.64; P, 3.64. Found: C, 69.19; H, 10.67; N, 1.63; P, 3.34.

*Dipalmitoyl-*DL*-glycerylphosphoryl(N-benzyl-N-methyl)-ethanolamine.*—This was prepared similarly in 38% yield; mp 125–127°.

Anal. Calcd. for $C_{45}H_{82}O_8NP$ (796.09): C, 67.89; H, 10.38; N, 1.76; P, 3.89. Found: C, 67.65; H, 10.50; N, 1.55; P, 3.60.

To avoid losses by crystallization the aminated derivatives of D-distearin and D-dipalmitin were not purified. The crude products were debenzylated directly as described for the racemic compounds.

*Distearoyl-*DL*-α-glycerylphosphoryl(N-methyl)ethanolamine.*—The *N*-benzyl derivative (500 mg) was dissolved with slight warming in 60 ml of glacial acetic acid and the solution was shaken for 4 hours with hydrogen in the presence of 0.5 g Pd black at an initial pressure of 50 psi. The mixture was warmed to 40–45° and the clear solution was evaporated *in vacuo*. The residue was dissolved in 5 ml of dry chloroform, the

TABLE III
N-METHYLCEPHALINS (IV)

Diglyceride	Melting Point	$[\alpha]_D^{25}$	Calculated				Found			
			C	H	N	P	C	H	N	P
D-α,β-Distearin	178–180	+7.1°	66.19	11.11	1.84	4.06	66.05	11.11	1.86	3.98
DL-α,β-Distearin	171–172		66.19	11.11	1.84	4.06	66.02	11.20	1.53	3.86
D-α,β-Dipalmitin	177–178	+8.0°	64.65	10.85	1.99	4.39	64.61	10.79	2.10	4.22
DL-α,β-Dipalmitin	170–171		64.65	10.85	1.99	4.39	64.49	10.84	2.04	4.24

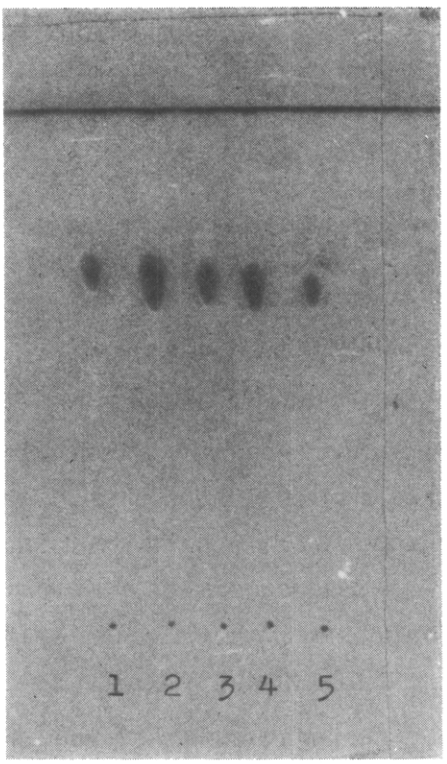


FIG. 1.—Thin-layer chromatogram of methylated and nonsubstituted cephalins on silica gel G plates. Solvent, chloroform-methanol-water, 65:25:4. Detecting spray, ammonium molybdate–perchloric acid. Compounds: (1) dipalmitoyl-L-α-glycerylphosphorylethanolamine; (2) distearoyl-L-α-glycerylphosphoryl(*N*-methyl)ethanolamine; (3) dipalmitoyl-L-α-glycerylphosphoryl(*N*-methyl)ethanolamine; (4) distearoyl-L-α-glycerylphosphoryl(*N*,*N*-dimethyl)ethanolamine; (5) dipalmitoyl-L-α-glycerylphosphoryl-(*N*,*N*-dimethyl)ethanolamine.

solution was filtered, 15 ml methanol was added slowly, and the mixture was kept at 5° for 8 hours. The precipitate was recrystallized once more from chloroform and methanol. The dried product weighed 275 mg (62%) and melted at 171–172° with slight sintering at about 140°. A sample of 200 mg was dissolved in 3 ml of a mixture of chloroform and methanol (4:1), and the solution was passed through a column of 10 g silicic acid. Elution with the same solvent mixture (four fractions of 100 ml) gave homogenous material with unchanged melting point.

Hydrogenolysis of the DL-dipalmitin intermediate yielded the corresponding *N*-methylcephalin in 55%. Debenzylation of the crude oily products resulting from the amination of 1 g of the respective optically active β-bromoethylphosphates gave 400–425 mg of the pure monomethylcephalins.

Chromatography.—All monomethyl- and dimethylcephalins were shown to be chromatographically homogenous compounds. The lipids were run on silicic acid–impregnated paper along with synthetic phosphatidylethanolamine as reference substance. Following the procedure of Marinetti and Stotz (1956), we used as solvent system a mixture of *n*-butyl ether, glacial acetic acid, chloroform, and water (40:35:6:5), and a 0.001% aqueous solution of Rhodamine B as spray reagent. Each lipid gave only a single fluorescent spot in ultraviolet light, the mobility of the methylated derivatives being similar to that of the nonsubstituted cephalin. The purity was further confirmed by a thin-layer chromatogram (Wagner *et al.*, 1961) as shown in Figure 1.

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CORRECTION

In the paper by K. Murray in Volume 3, No. 1, January, 1964, on page 12, left-hand column, line 15 should read: ". . . observed in the histone hydrolysates was ϵ-*N*-methyl. . . ."