according to the procedure of Cummins, *et al.*,[13] to yield 1.2 g (58% of theory) of 3,4'-dihydroxyflavone (**16a**), mp 275–276° (lit.[20] mp 276°), and 670 mg (32% of theory) of **2-hydroxyphenylbenzofuran-3-carboxylic acid** (**17**), decomposing at 211°, $\nu_{C=O}$ 1685 cm^{-1} (lit.[13] mp 221°, $\nu_{C=O}$ 1686 cm^{-1}).

3,4'-Dihydroxy-3',5'-dialkylflavones (16b–d). General Procedure.—A solution of 0.90 mmole of 2',4-dihydroxy-3,5-dialkylchalcone (**8**) and 5 ml of 10% aqueous sodium hydroxide was cooled to ice-bath temperature in a 25-ml erlenmeyer flask. A 3% aqueous solution of hydrogen peroxide (3.4 ml, 3 mmoles) was added and the reaction mixture was allowed to warm to room temperature, let stand for 3 hr, and then poured into cold dilute hydrochloric acid. The resulting percipitate was suction filtered, water washed, dried, and recrystallized from methanol to give in good yields only the 3,4'-dihydroxy-3',5'-dialkylflavones (**16b–d**) (Table VI).

The AFO Oxidation of 2',4-Dihydroxy-3,5-di-*t*-butylchalcone (8e).—A solution of 500 mg (1.4 mmoles) of 2',4-dihydroxy-3,5-di-*t*-butylchalcone (**8e**), 10 ml of 10% aqueous sodium hydroxide, and 3 ml of methanol was heated until **8e** dissolved, then cooled to 0°. A solution of 3% aqueous hydrogen peroxide (6 ml, 5 mmoles) was added and the mixture was allowed to stand overnight at room temperature. The work-up according to the previously described procedure without recrystallization yielded 350 mg (quantitative) of crude **4-hydroxy-3,5-di-*t*-butylbenzoic acid** (**20**), pearl-colored flakes, mp 205–215° (lit.[21] mp 217–218°).

The infrared spectrum (CCl_4) contained a sharp band at 3620 (hindered phenolic); a strong sharp band at 1680 (aromatic acid C=O); and a sharp band at 1600 cm^{-1} (aromatic C=C). The nmr spectrum ($CDCl_3$) contained sharp singlets at 1.40 (18 H, alkyl CH), 5.63 (1 H, phenolic O—H), and 7.78 (2 H, aryl CH) ppm. The carboxylic acid proton could not be detected.

The mass spectrum of the crude product at high resolution and low voltage showed a molecular ion peak at *m/e* 250 corresponding to **20** and about 1% each of a peak at *m/e* 352 (**8e** or **9e**) and *m/e* 570.336 (unidentified).

Registry No.—**8a**, 13323-66-5; **8b**, 2631-01-8; **8c**, 14919-43-8; **8d**, 14929-97-6; **8e**, 14919-44-9; **9a**, 6515-37-3; **9b**, 2525-91-9; **9e**, 14919-46-1; **10**, 14919-47-2; **11**, 14919-48-3; **16a**, 14919-49-4; **16b**, 14919-50-7; **16c**, 14919-51-8; **16d**, 14919-52-9; **20**, 1421-49-4.

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The Structure of Plasmalogens. VIII. Preparation and Properties of Lysophosphatidal Choline[1a]

M. F. FROSOLONO,[1b] ALEMKA KISIC, AND MAURICE M. RAPPORT[1c]

Department of Biochemistry, Albert Einstein College of Medicine, Yeshiva University, Bronx, New York

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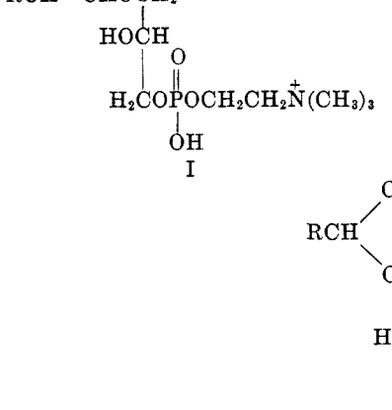
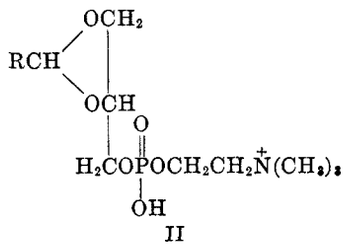
Preparations of crystalline lysophosphatidal choline were obtained in good yield from beef heart lecithin and physically characterized by means of the molar absorbancy index in chloroform at 6.02 μ and the optical rotation at various wavelengths in both methanol and chloroform solutions. The infrared absorption spectrum provides conclusive evidence that the choline plasmalogen isolated from sea anemones by Bergmann and Landowne was an α,β-unsaturated ether and not a cyclic acetal.

Although lysophosphatidal choline (I) has been used in a number of studies, namely, elucidation of the



I



II

α,β-unsaturated ether structure of plasmalogens,[2] inhibition of respiration of spermatozoa,[3] detection of microsomal lysoplasmalogenase,[4] lysis of erythrocytes,[3,5] and studies of the acyl transferase of liver[6] and erythrocytes,[7] details of the method of preparation of the crystalline compound have appeared only once.[3] In that report, the yield was poor. Crystalline lysophosphatidal choline (190 mg) was obtained from 5.2 g of beef heart lecithin. No physical characterization of the pure substance was presented. Lysophosphatidal choline is an important intermediate for several kinds of studies: comparison of its chemical properties with those of other α,β-unsaturated ethers and comparison of its biochemical properties with those of lysophosphatidyl choline (the acyl ester analog) and lysophosphatidalkyl choline (the saturated ether analog). It is the purpose of this paper to report a convenient method of preparation of lysophosphatidal choline in good yield and to provide a physical characterization of the product from its infrared absorption spectrum and its rotation in two solvents at a series of wavelengths. The characterization by infrared absorption has provided conclusive evidence that the choline plasmalogen isolated from sea anemones by Bergmann and Landowne (and reported in this journal[8]) was incorrectly designated by them to have the structure of a cyclic acetal of glycerol[2] and

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(8) W. Bergmann and R. A. Landowne, *J. Org. Chem.*, **23**, 1241 (1958).

had, indeed, the α,β-unsaturated ether structure of lysophosphatidal choline. This evidence eliminates the single remaining case in which an acetal structure has been reported for naturally occurring aldehydogenic phosphatides.

Results and Discussion

The starting material for the preparation of lysophosphatidal choline is beef heart lecithin, a lipid fraction that may be obtained conveniently either by chromatographing total beef heart lipids on alumina at 5° or from commercial suppliers (Sylvana Chemical Co., Millburn, N. J.). This fraction usually contains about 50% phosphatidal choline, 40% phosphatidyl choline, and small amounts of lysolecithin and sphingomyelin. When this material is saponified under mild conditions, the phosphatidyl choline and lysolecithin are converted to fatty acid and glycerylphosphorylcholine, the phosphatidal choline is converted to lysophosphatidal choline (I), and the sphingomyelin is unaffected. These substances can be readily separated by column chromatography on silicic acid. To obtain a good yield of lysophosphatidal choline requires that attention be paid to several details. The beef heart lecithin used as starting material should have a molar ratio of α,β-unsaturated ether to phosphorus that is not less than 0.45 and the content of sphingomyelin must be small (less than 10%) as judged by thin layer chromatography. Care must be exercised in neutralizing the alkaline reaction mixture to avoid a local excess of acid that may hydrolyze the labile enol ether group. Salts must be removed prior to column chromatography by a procedure that will restrict losses due to the water solubility of the lysophosphatide. And finally, chromatography on silicic acid must be carried out at low temperature (5°).

Lysophosphatidal choline, prepared by the method described, was chromatographically homogeneous on thin layer plates, showing that the preparations were uncontaminated by sphingomyelin; analyses for ester groups (Table I) showed the absence of lysophosphatidyl choline. The calculated percentage P for preparations with a ratio of palmitaldehyde to stearaldehyde lying between 6.5 and 15 (the values found here) is 6.39 to 6.43 for an unhydrated molecule and 6.15 to 6.19 for a hydrated molecule. The values found (Table I) are in agreement with these figures. Values for the molar ratio of aldehydogenic chains (based on *p*-nitrophenylhydrazone formation) to P range from 0.85 to 0.89 (Table I). The best recorded value for this ratio in natural products is 0.92.[2,9]

TABLE I
ANALYSIS OF LYSOPHOSPHATIDAL CHOLINE PREPARATIONS

Preparation	P, %	Aldehydogenic groups, μmole/mg of lipid	Aldehydogenic groups/P, molar ratio	Ester groups, μmole/mg	Molar absorbancy index (A_m) at 6.02 μ
1	6.17	1.75	0.88	0.00	173
2	6.27	1.72	0.85	0.00	178
3	6.20	1.72	0.86	0.00	174
4	6.31	1.81	0.89	0.00	198
5	6.32	1.75	0.86	0.00	178

(9) E. L. Gottfried and M. M. Rapport, *J. Biol. Chem.*, **237**, 329 (1962).

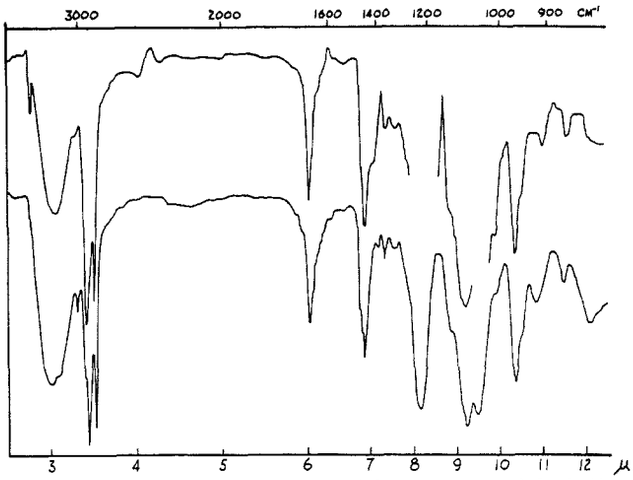

Figure 1.—Infrared spectrum of lysophosphatidal choline. Upper curve: 10 mg/ml in chloroform, cell path length, 1.0 mm. Regions of strong solvent absorption are omitted. Lower curve: thin film on sodium chloride plate.

The infrared absorption spectrum of lysophosphatidal choline in chloroform solution (Figure 1) shows a sharp band at 3600 cm^{-1} (2.78 μ) characteristic of an unassociated OH group,[10] a broad band at 3280 cm^{-1} (3.05 μ) corresponding to associated or bonded OH groups,[10] and a strong band at 1660 cm^{-1} (6.02 μ) which is characteristic for the asymmetric stretching of the unsaturated ether (1-alkenyl) group.[11] There is no absorption in the carbonyl region. The molar absorbancy index at 1660 cm^{-1} for five preparations (Table I) indicates that the average value of 180 provides a useful physical constant for characterization of lysophosphatidal choline.

Values for the specific rotation of four preparations of lysophosphatidal choline at wavelengths from 240 to 589 mμ show that the rotation is considerably higher in chloroform solution than in methanol (Table II). No deviations from plain negative ORD curves are seen at these wavelengths.

TABLE II
SPECIFIC ROTATION OF LYSOPHOSPHATIDAL CHOLINE PREPARATIONS AT VARIOUS WAVELENGTHS

			−[α] at 27°						
		Concn,	Wavelength, mμ						
Preparation	Solvent	%	240	260	300	340	400	500	589
1	CH_3OH	0.28	58.5	44.5	27.2	18.5	11.8	5.8	3.8
2		0.60	83.5	50.5	29.4	19.8	12.7	7.2	5.4
3		0.78	73.5	46.8	28.5	19.5	12.0	6.0	3.8
4		1.38	104.4	62.3	33.3	21.7	13.9	7.4	4.5
1	$CHCl_3$	0.28		110.0	70.5	47.8	30.5	18.7	14.8
2		0.63		121.5	76.5	52.3	31.7	18.3	12.5
3		0.80		132.5	81.0	53.5	32.5	18.8	13.8
4		1.38				47.8	31.2	18.5	13.5

In their study of the lipids of the sea anemone, *Anthopleura elegantissima*, Bergmann and Landowne reported[8] the isolation of an aldehydogenic phosphatide that was characterized as a cyclic acetal of glycerol from its infrared absorption spectrum, the presence of only one hydrocarbon chain per atom of phosphorus, and the very low degree of unsaturation (0.24 mole of double bond per atom of P). On the basis of this report, the chemical synthesis of this com-

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pound with a C_{16} hydrocarbon chain (1,2-O-hexadecylideneglyceryl-3-phosphorylcholine) was recently described.[12] However, in their paper Bergmann and Landowne characterized their compound as one which "rapidly gave a positive Schiff's aldehyde test." This behavior is unexpected for cyclic glyceryl acetals[13] but is a well-recognized feature of α,β-unsaturated ethers.[14] The infrared absorption spectrum of this aldehydogenic lipid of Bergmann and Landowne shows a band of medium strength at about 5.75 μ which was not assigned by them.[8] Rather, the infrared spectrum is described as "notably void of a carbonyl band." As a result of private correspondence with both Dr. Bergmann[15] and Dr. Landowne,[16] we have been informed that the published spectrum is in error and should be shifted about 0.3 μ to the right with regard to the abscissa scale. This would place the unassigned band close enough to the band characteristic of the α,β-unsaturated ether (6.02 μ) to make the presence of this structural feature unambiguous and would require the assignment of the lysophosphatidal choline structure to the sea anemone phosphatide. No band at this wavelength was found in the synthetic acetal choline phosphatide.[12] Thus, the sole observation which is not accounted for by the unsaturated ether structure is the failure of Bergmann and Landowne's compound to add bromine in the analysis for unsaturation. Since unsaturated ethers are exceedingly labile to acid hydrolysis with loss of the unsaturation, inadvertent hydrolysis of the sample by an acidic solvent prior to addition of the bromine reagent would have precluded detection of this structural feature. In the absence of further evidence, it must be concluded that cyclic acetal structures do not contribute significantly to the aldehydogenic property associated with naturally occurring lipids.

Experimental Section

Solvents.—All organic solvents were redistilled before use except for the spectral grade chloroform used for optical measurements.

Analytical Methods.—Lipid weights were determined after drying *in vacuo* over P_2O_5 for 24 hr at room temperature. Phosphorus was determined by a modification of the method of Beveridge and Johnson[17] after digestion with perchloric acid. Aldehydogenic lipids were measured by the *p*-nitrophenylhydrazone method,[18] and unsaturated ethers were measured according to the method of Norton.[19] Thin layer chromatography was carried out as described earlier.[5] Ester groups were determined according to Rapport and Alonzo.[20]

Infrared Spectra.—These were determined with a Perkin-Elmer double grating instrument (Model 237), in chloroform solution, with a sodium chloride cell of a 1.082-mm light path.

Optical Rotations.—These were determined with a Cary spectropolarimeter (Model 60) in solution in chloroform or methanol, with a cell of a 1-cm light path.

Beef Heart Lecithin.—This purified phosphatide fraction, prepared according to Pangborn,[21] was obtained either commercially (Sylvana Chemical Co., Millburn, N. J.) or by column chromatography on alumina at 5°.[22]

Lysophosphatidal Choline.—To 1.0 g of beef heart lecithin dissolved in 444 ml of methanol at 37° was added 56 ml of 0.895 *N* KOH in 95% methanol. The solution was stirred mechanically for 30 min in a bath at 37°. After cooling to −60°, 9.9 ml of 4.83 *N* HCl was added (95% neutralization). Then 1000 ml of chloroform and 290 ml of water were added, the mixture was well mixed, and the phases were allowed to separate at 4° (12 hr).[23] The upper (aqueous) phase was removed and the lower phase was taken to dryness *in vacuo*. The residue was dissolved in 50 ml of chloroform–methanol (1:1 v/v) and contained 55% of the phosphorus in the starting material. The lipid was subjected to chromatography at 4° on a column of 100 g of silicic acid (Unisil, 200–325 mesh, activated by heating for 12 hr at 125°). An initial fraction of 250 ml of chloroform–methanol (1:1) was collected, then a second fraction of 1000 ml of chloroform–methanol (1:3) was taken followed by 40 successive fractions of 50 ml each of methanol. The latter were monitored by thin layer chromatography and those containing lysophosphatidal choline (fractions 10 to 27) were combined and evaporated to dryness *in vacuo* to give 275 mg of crude product having *P* 6.33%. The product was recrystallized by dissolving 264 mg in 0.4 ml of ethanol with slight warming, adding 4.0 ml of hexane, centrifuging to remove a small quantity of insoluble material, and then adding 6.0 ml of hexane. After 2 hr at room temperature and 12 hr at 4°, the crystals were collected by centrifugation to give 200 mg of product that was chromatographically homogeneous when 100 μg was examined by thin layer chromatography. Analysis is shown in Table I, preparation 1. The yield based on the phosphatidal choline content of the beef heart lecithin was 75%.

Acknowledgment.—We wish to thank Miss Mary Marsh for the analyses of aldehydogenic chains as their dimethyl acetals by gas–liquid partition chromatography.

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