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SYNTHESIS OF THE NATURALLY OCCURRING PHYTANYL DIETHER ANALOGS OF PHOSPHATIDYL GLYCEROPHOSPHATE AND PHOSPHATIDYL GLYCEROL*

C. N. JOO** AND M. KATES
Division of Biosciences, National Research Council, Ottawa (Canada)
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SUMMARY

The diastereomers of the diphytanyl glycerol ether analogs of phosphatidyl glycerophosphate and phosphatidyl glycerol have been synthesized chemically and compared with the respective natural isomer isolated from the extremely halophilic bacterium, *Halobacterium cutirubrum*. The structure and configuration of the natural isomer of the phosphatidyl glycerophosphate was thus shown to be 2,3-di-*O*-(3′*R*,7′*R*, 11′*R*,15-tetramethylhexadecyl)-*sn*-glycero-1-phosphoryl-3″-*sn*-glycero-1″-phosphate (XVb); the natural phosphatidyl glycerol was shown to have the structure and configuration, 2,3-di-*O*-(3′*R*,7′*R*,11′*R*,15-tetramethylhexadecyl)-*sn*-glycero-1-phosphoryl-3″-*sn*-glycerol(XVIIIb). The implications of these findings on the biosynthetic mechanisms for these unusual phosphatides are discussed.

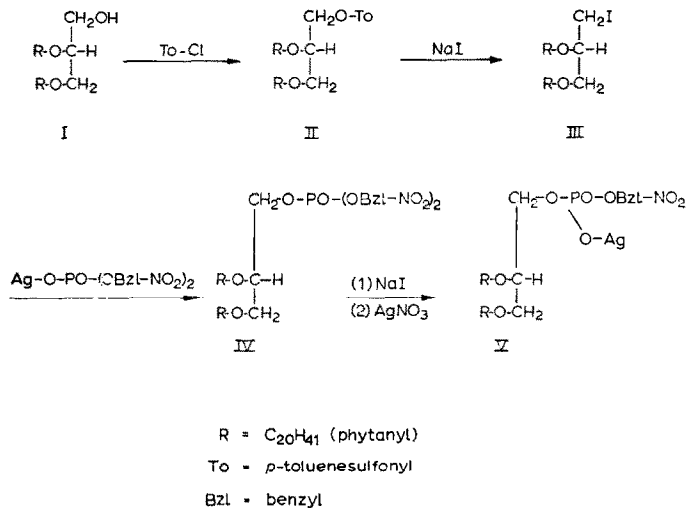
INTRODUCTION

The phospholipids of extremely halophilic bacteria are unique in containing ether-linked alkyl groups rather than fatty acid ester groups[1–7]. All of the phosphatides are derivatives of the glycerol diether 2,3-di-*O*-(3′,7′,11′,15-tetramethylhexadecyl)-*sn*-glycerol (di-*O*-phytanyl glycerol)[2,5,7], in which the phytanyl groups have the 3*R*, 7*R*, 11*R* configuration[8]. Two phosphatides have been identified on the basis of degradative studies: a major component, the glycerol diether analog of phosphatidyl glycerophosphate[2,5] (structure XV, Scheme III), and a minor component, the diether analog of phosphatidyl glycerol[1,4,6] (structure XVIII, Scheme III).

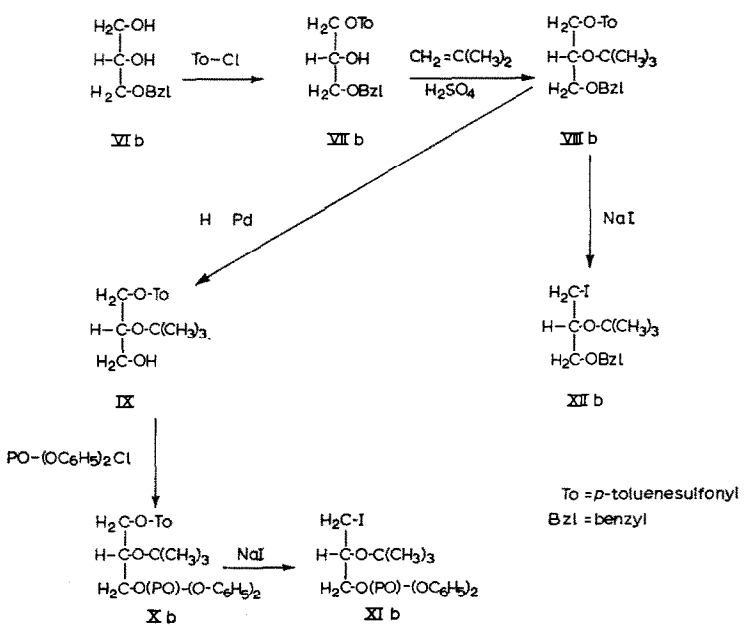
These phosphatides are unusual not only because they contain phytanyl ether groups, but also on account of the unusual L configuration of their glycerol diether moiety[7,9]. However, the configuration of the glycerophosphate or glycerol moiety in structures XV and XVIII, respectively, still remained to be determined. For this

* Issued as N.R.C. No. 10544.
** Present address: Department of Chemistry, University of Ottawa, Ottawa, Canada.

reason, and also to establish the structures XV and XVIII unambiguously, the synthesis of the two diastereomeric forms of each phosphatide structure was undertaken, and the synthetic products obtained were compared with the respective natural isomer isolated from *Halobacterium cutirubrum*. A preliminary account of this work has been presented previously[10].



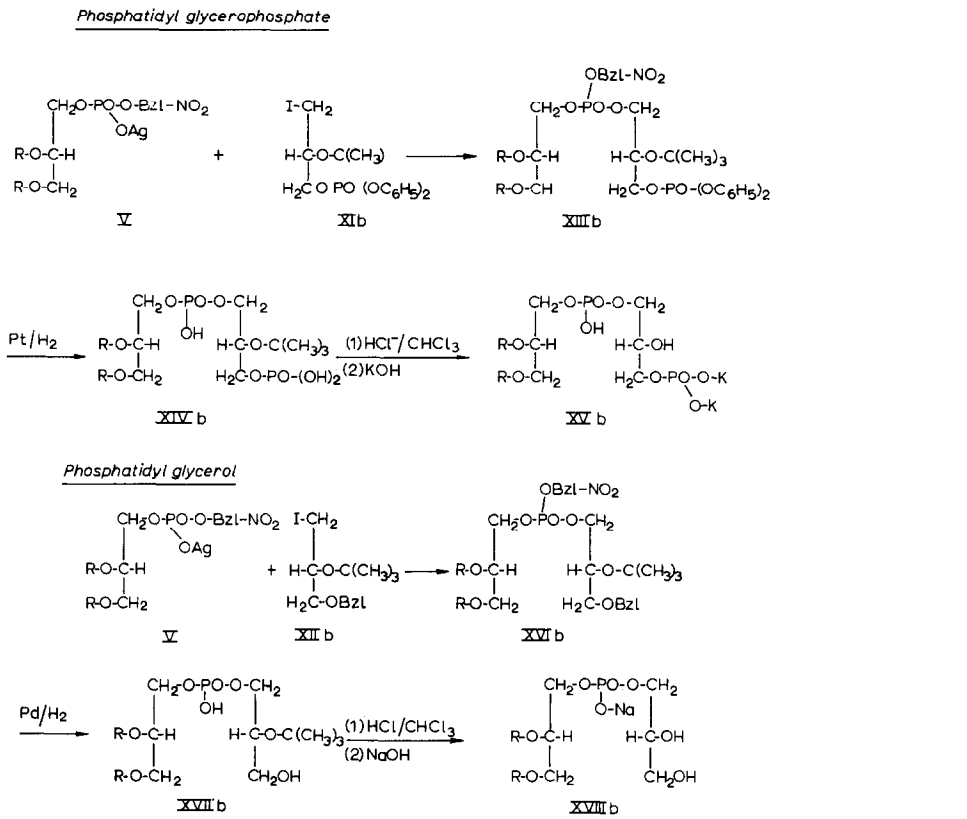
Scheme I. Synthesis of monosilver salt of 2,3-di-*O*-phytanyl-*sn*-glycerol-1-*p*-nitrobenzyl phosphate.



Scheme II. Synthesis of *X*-1-iodo-2-*tert.*-butyl-3-diphenylphosphoryl glycerol and *X*-1-iodo-2-*tert.*-butyl-3-benzyl glycerol. Structures are shown only for the enantiomeric series obtained from 1-*O*-benzyl-*sn*-glycerol (b-series); for the a-series, the starting material was 3-*O*-benzyl-*sn*-glycerol (VIa).

SYNTHETIC PROCEDURE

The general approach to the synthesis of the diether phosphatides (see Schemes I, II and III) involved the condensation of a suitably blocked monosilver salt of the phytanyl diether phosphatidic acid with a suitably substituted iodoglycerol derivative, followed by removal of the blocking groups by mild procedures; this approach had been used previously by VAN DEENEN and coworkers[11–13] for synthesis of *O*-aminoacylphosphatidyl glycerols.



Scheme III. Synthesis of 1-*sn*-phosphatidyl-3′-*sn*-glycero-1′-phosphate (phosphatidyl glycerophosphate) and 1-*sn*-phosphatidyl-3′-*sn*-glycerol (phosphatidyl glycerol).

Since the configuration of the phytanyl glycerol diether moiety of the phosphatides XV and XVIII had previously been established by synthesis[7,9], it was found convenient to use as starting material the 2,3-di-*O*-phytanyl-*sn*-glycerol (I) obtained directly by methanolysis of the total acetone-insoluble lipids of *H. cutirubrum*[5,9]. This diether was converted to the monosilver salt of 2,3-di-*O*-phytanyl-*sn*-glycerol-1-(*O*-*p*-nitrobenzyl)-phosphate (V) by reaction of the iodo diether (III) (prepared *via* the tosyl diether (II)) with silver di-*p*-nitrobenzyl phosphate[14], followed by replacement of one *p*-nitrobenzyl group in the product (IV) with Na^+ and finally with Ag^+ (see

Scheme I). The *p*-nitrobenzyl blocking group was used here instead of the benzyl group commonly used in this type of synthesis[11–13], since the monosilver salt of the diether monobenzyl phosphate was found not to be sufficiently reactive in the subsequent condensation with the substituted iodoglycerol.

The substituted iodoglycerol used in the synthesis of the phosphatidyl glycerophosphate diastereomers was *X*-1-iodo-2-*tert.*-butyl-3-diphenylphosphoryl glycerol (XI), the two enantiomers of which (XIa and XIb) were synthesized from 3-*O*-benzyl-*sn*-glycerol (VIa) and 1-*O*-benzyl-*sn*-glycerol (VIb), respectively, as outlined in Scheme II. For synthesis of the diastereomers of phosphatidyl glycerol, the iodoglycerol used was *X*-1-iodo-2-*tert.*-butyl-3-benzyl glycerol (XII), the two enantiomers (XIIa and XIIb) being prepared also from 3-*O*- and 1-*O*-benzyl *sn*-glycerol, respectively (Scheme II).

Condensation of the silver salt of the diether mono-*p*-nitrobenzyl phosphate (V) with each enantiomer of XI or XII, gave the fully protected phosphotriester XIII or XVI, respectively (Scheme III). Removal of nitrobenzyl and phenyl groups from XIII, and nitrobenzyl and benzyl groups from XVI was effected by catalytic hydrogenolysis, and the *tert.*-butyl groups were removed by treatment with anhydrous HCl in chloroform[11–13]. The resulting diastereomeric free acids of XV and XVIII were neutralized, purified by preparative thin-layer chromatography, and finally converted to their potassium and sodium salts, respectively. These were then compared with corresponding salts of the natural isomers of phosphatidyl glycerophosphate and phosphatidyl glycerol isolated from *H. cutirubrum* and purified in the same way as the synthetic isomers.

RESULTS AND DISCUSSION

The two synthetic diastereomers of phosphatidyl glycerophosphate (XVa and XVb) and the natural isomer had the same elementary analyses which corresponded

TABLE I

PHYSICAL PROPERTIES OF DIASTEREOMERIC PHOSPHATIDYL GLYCEROPHOSPHATES AND PHOSPHATIDYL GLYCEROLS (PHYTANYL DIETHER ANALOGS)

Compound	$[\alpha]_D$ *in chloroform*			*Thin-layer chromatography* R_F *values**	
	Potassium-salt	*Sodium-salt*	*Barium-salt*	*Solvent 1*	*Solvent 2*
Phosphatidyl glycerophosphates					
Natural isomer	+1.89°	0.0°	−1.94°	0.20	0.63
1-*sn*-Phosphatidyl-3′-*sn*-glycero-1′-phosphate	+1.93°	0.0°	—	0.20	0.63
1-*sn*-Phosphatidyl-1′-*sn*-glycero-3′-phosphate	−2.24°	−1.8°	—	0.20	0.63
Phosphatidyl glycerols					
Natural isomer	—	+3.46°	—	0.56	0.47
1-*sn*-Phosphatidyl-3′-*sn*-glycerol	—	+3.43°	—	0.56	0.47
1-*sn*-Phosphatidyl-1′-*sn*-glycerol	—	−1.13°	—	0.56	0.47

* R_F values are the same for the sodium, potassium or barium salts of phosphoryl glycerophosphate. Solvent 1: chloroform–methanol–conc. ammonia (65:35:5, v/v/v); Solvent 2: chloroform–methanol–water (65:35:5, v/v/v).

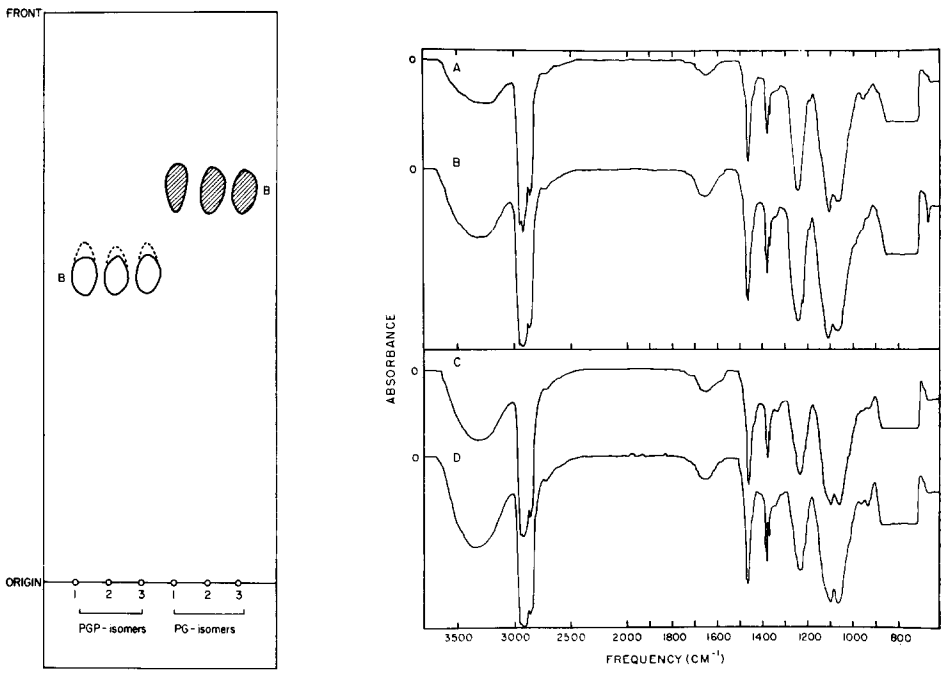


Fig. 1. Chromatogram of synthetic and natural isomers of the diphytanyl glycerol ether analogs of phosphatidyl glycerophosphate (PGP) and phosphatidyl glycerol (PG) on silicic acid-impregnated paper; solvent, diisobutyl ketone–acetic acid–water (40:25:5, v/v/v); stain, Rhodamine 6G (B, blue fluorescense under ultraviolet light), and periodate–Schiff stain[26] (hatched spots gave positive reaction); material applied: phosphatidyl glycerophosphate isomers: 1, natural isomer; 2, 1-*sn*-phosphatidyl-3'-*sn*-glycero-1'-phosphate (XVb); 3, 1-*sn*-phosphatidyl-1'-*sn*-glycero-3'-phosphate (XVa); phosphatidyl glycerol isomers: 1, natural isomer; 2,1-*sn*-phosphatidyl-3'-*sn*-glycerol (XVIIIb); 3,1-*sn*-phosphatidyl-1'-*sn*-glycerol (XVIIIa).

Fig. 2. Infrared spectra in carbon tetrachloride solution of (A) natural and (B) synthetic isomers of phosphatidyl glycerophosphate disodium salt (diphytanyl glyceryl ether analog); (C) natural and (D) synthetic isomers of phosphatidyl glycerol monosodium salt (diphytanyl glycerol ether analog).

to the dipotassium salt $C_{46}H_{94}O_{11}P_2K_2$. The synthetic and natural isomers also had identical mobilities and staining behavior on silica-impregnated paper in MARINETTI's[15] solvent system (Fig. 1), identical R_F values on thin-layer chromatograms in various solvents (Table I), and identical infrared spectra (Fig. 2). The main absorption bands in the infrared spectra are characteristic of OH groups (3300 cm^{-1}), bound water (1650 cm^{-1}), CH_2 and CH_3 groups (2960, 2920, 2870, 1465 cm^{-1}), isopropyl groups (1375–1365 cm^{-1}, doublet), P=O groups (1235 cm^{-1}, hydrogen-bonded), P–O$^-$ (ref. 16) and C–O–C ether groups (1110 cm^{-1}), and P–O–C groups (1060 cm^{-1}). These results show clearly that the natural phosphatide has the monomeric phosphatidyl glycerophosphate structure[5] and not the dimeric pyrophosphate structure proposed by FAURE, MARECHAL AND TROESTLER[3]. To establish the configuration of the glycerophosphate moiety, the optical rotation of the natural and synthetic diastereomers were compared. As shown in Table I, only the diastereomer synthesized from 1-diphenylphosphoryl-2-*tert.*-butyl-3-iodo-*sn*-glycerol (XIb) and having the configuration 1-*sn*-phosphatidyl-3'-*sn*-glycerol-1'-phosphate (XVb) had a specific rotation

(+1.9°, in chloroform for the potassium salt) identical with that of the natural isomer. It should be noted that the optical rotation of phosphatidyl glycerophosphate salt is strongly dependent on the nature of the cation; thus while the potassium salt of the natural isomer is dextrorotatory, the barium salt is laevorotatory and the sodium salt has no measurable rotation (Table I). A similar behaviour has been observed for salts of cardiolipin[12].

The two diastereomers of phosphatidyl glycerol (XVIIIa and XVIIIb) and the natural isomer had the same elementary analyses (corresponding to the sodium salt $C_{46}H_{94}O_8PNa$), mobility and staining behaviour on silica-impregnated paper (Fig. 1), R_F values on thin-layer chromatography in various solvents (Table I), and infrared spectra (Fig. 2). The latter contained strong absorption bands attributed to OH groups (3300 cm^{-1}), bound water (1650 cm^{-1}), CH_2 and CH_3 groups (2960, 2920, 2870, 1465, 1380–1370 cm^{-1}), P=O groups (1235 cm^{-1}), P–O$^-$ (ref. 16) and C–O–C ether groups (1100 cm^{-1}), and P–O–C groups (1055 cm^{-1}). The spectra of the phosphatidyl glycerols were in general similar to those of the phosphatidyl glycerophosphates, except that in the latter the P=O and P–O$^-$ bands were relatively more intense and the OH band less intense than in the spectra of the phosphatidyl glycerols (Table II). The relative intensities of the absorption bands (Table II) are consistent with the presence

TABLE II

RELATIVE INTENSITIES OF ABSORPTION BANDS IN INFRARED SPECTRA OF PHOSPHATIDYL GLYCEROPHOSPHATE AND PHOSPHATIDYL GLYCEROL

Compound	*Relative intensity of band**			
	OH (3300 cm^{-1})	*P=O (1235 cm^{-1})*	*P–O$^-$ (1110 cm^{-1})*	*P–O–C (1055 cm^{-1})*
Phosphatidyl glycerophosphate				
Natural isomer	0.34	1.5	2.2	1.9
Synthetic isomers	0.36	1.4	2.4	1.8
Phosphatidyl glycerol				
Natural isomer	0.48	0.8	1.4	1.4
Synthetic isomers	0.53	0.8	1.3	1.4

* Relative to the CH peak at 1465 cm^{-1}.

in the phosphatidyl glycerophosphate molecule of one OH and two P=O and P–O$^-$ groups, and the presence of two OH, one P=O and one P–O$^-$ group in the phosphatidyl glycerol molecule. When the optical rotations of the synthetic and natural isomers of phosphatidyl glycerol were compared (Table I), it was found that only the diastereomer synthesized from 1-benzyl-2-*tert.*-butyl-*sn*-glycerol (XIIb) and having the configuration 1-*sn*-phosphatidyl-3′-*sn*-glycerol (XVIIIb) had a rotation (+3.4° in chloroform, for the sodium salt) identical with that of the natural isomer.

These findings thus establish unambiguously the structure and configuration of the diether phosphatidyl glycerophosphate as 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycero-1′-phosphate (XVb), and of the diether phosphatidyl glycerol as 2,3-di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3′-*sn*-glycerol (XVIIIb). The unusual feature of these findings is that stereochemically the diether analog of the phosphatidyl glycerophosphate in extremely halophilic bacteria is the mirror image of the corresponding diester form found in rat liver[17] and *Escherichia coli*[18,19]; and the same

is also true of the diether analog of phosphatidyl glycerol compared to the diester form in spinach leaves[20] and non-halophilic bacteria [19,21,22].

Assuming the biosynthetic pathway for synthesis of the diether forms of phosphatidyl glycerophosphate and phosphatidyl glycerol is analogous to that for the diester forms established in *E. coli*[18,19], the pathway in halophiles would involve condensation of cytidine diphosphate diether with glycerophosphate to give phosphatidyl glycerophosphate, followed by dephosphorylation to phosphatidyl glycerol. To account for the unusual configuration of the glycerol and glycerophosphate moieties in the halophile phosphatides, the glycerophosphate stereomer utilized by the halophiles in the above pathway would be the *sn*-glycero-1-phosphate, rather than the usual *sn*-glycero-3-phosphate. In recent studies[23] to determine which stereomer of glycerophosphate is formed in *H. cutirubrum*, it was found that both the glycerokinase and the glycerophosphate dehydrogenase systems in this organism gave rise only to the normal *sn*-glycero-3-phosphate. Although this finding would appear to contraindicate the existence of the above biosynthetic pathway for synthesis of phosphatidyl glycerophosphate and phosphatidyl glycerol in extreme halophiles, there still remains the possibility that the halophiles might contain a separate enzyme system for synthesis of the *sn*-glycero-1-phosphate isomer, as has been found in various rat organs[24].

The other outstanding problem in the biosynthesis of the diether phosphatides is the biosynthesis of the diphytanyl glyceryl ether itself. If we assume, by analogy with the Kornberg–Kennedy pathway[25], that the diether phosphatidic acid is made by O-alkylation of glycerophosphate, then the latter would be the unusual *sn*-glycero-1-phosphate if no inversion of configuration occurs, but the normal *sn*-glycero-3-phosphate if inversion does occur. It is clear from these considerations that the extremely halophilic bacteria probably contain unusual biosynthetic mechanisms for synthesizing their unusual phosphatide components, and studies on these systems are in progress.

EXPERIMENTAL

Physical Methods

Infrared spectra were measured on thin films of oils or on their solutions in carbon tetrachloride, with a Perkin–Elmer Model 237B double-beam spectrometer (NaCl optics). Optical rotations were measured at 22° at 589 nm (sodium D line) in a Perkin–Elmer polarimeter, Model 141, with digital readout.

Chromatography

Rapid thin-layer chromatography for purity determinations and monitoring of columns was carried out on 7.5 cm × 2.5 cm microscope slides coated with plain silica gel (Research Specialties Co., Richmond, Calif.). The spots were visualized by spraying with 5% H_2SO_4 in ethanol followed by charring in a hot plate. The R_F values quoted are mean values of at least three independent determinations on microplates with an error of ± 0.05. For preparative separations of up to 100 mg of material, 20 cm × 20 cm glass plates with a 0.6–1 mm thick layer of plain silica gel (previously washed with chloroform–methanol, 1:1 (v/v)) were used. They were developed in rectangular jars lined with filter paper, and bands were visualized under ultraviolet light and by staining with iodine vapor; components were eluted with chloroform–methanol–ethyl ether (1:1:1, v/v/v).

Phosphatides were chromatographed on silicic acid-impregnated paper in diisobutyl ketone–acetic acid–water (40:25:5, v/v/v)[15], as described elsewhere[26]. The spots were visualized by staining with Rhodamine 6G and viewing the wet chromatograms under ultraviolet (366 nm) light. Vicinal-hydroxyl-containing compounds were detected by the periodate–Schiff staining technique[26].

Column chromatography was performed on Bio-Rad or Unisil silicic acid (200–325 mesh); the weight ratio of silicic acid to the substance applied was about 40:1. All solvents were reagent grade and were distilled before use. The boiling range of the light petroleum used was 30–60°.

Starting Materials

1,2-Isopropylidene-*sn*-glycerol ($[\alpha]_D^{24}+14.38°$, pure liquid), prepared according to the procedure of BAER[27], was converted to 3-*O*-benzyl-*sn*-glycerol (VIa) ($[\alpha]_D^{22}+5.96°$, pure liquid; $[\alpha]_D+3.2°$; *c*, 10 in chloroform) by a modification of the methods of SOWDEN AND FISCHER[28] and of HOWE AND MALKIN[29], as described elsewhere[30]. 1-*O*-Benzyl-*sn*-glycerol(VIb) ($\alpha_D-5.73°$, pure liquid) was prepared from 3-*O*-benzyl-*sn*-glycerol (VIa) as described by LANDS AND ZSCHOCKE[31], except that the final deacylation step was carried out in 2.5% methanolic HCl under reflux for 90 min.

2,3-Di-*O*-(3′*R*,7′*R*,11′*R*,15′-tetramethylhexadecyl)-*sn*-glycerol (natural di-*O*-phytanyl glycerol ether) (I) was obtained by hydrolysis of the lipids of *H. cutirubrum*, as described elsewhere[9].

Fractionation of total lipids of H. cutirubrum

1.02 g of total lipids extracted from *H. cutirubrum* as described previously[9] was dissolved in 3 ml of chloroform and diluted with 30 ml of acetone. After cooling, the precipitate was centrifuged and reprecipitated from 3 ml of chloroform by addition of 30 ml of acetone; yield, 907 mg (89%). The acetone-insoluble material was a mixture of the salts of phosphatidyl glycerol phosphate, phosphatidyl glycerol and glycolipid sulfate[32]. The mixture was separated by preparative thin-layer chromatography (50–100 mg per plate) in chloroform–methanol–conc. NH_4OH (65:35:5, v/v/v) into six fractions: Fraction 1 (R_F, 0.02; glycolipid sulfate); Fraction 2 (R_F, 0.1; glycolipid sulfate *plus* trace phosphatidyl glycerophosphate); Fraction 3 (R_F, 0.20; phosphatidyl glycerophosphate *plus* glycolipid sulfate); Fraction 4 (R_F, 0.44; unidentified); Fraction 5 (R_F, 0.58; phosphatidyl glycerol); Fraction 6 (R_F, 0.85 and 0.94; pigments and hydrocarbons). The fractions were eluted from the silic gel with chloroform–methanol–ether (1:1:1, v/v/v), and the extracts were diluted with benzene and brought to dryness *in vacuo*.

The major phosphatidyl glycerophosphate fraction (Fraction 3) was freed from glycolipid sulfate by preparative thin-layer chromatography in chloroform–methanol–water (80:20:2, v/v/v). (R_F of phosphatidyl glycerophosphate, 0.14; glycolipid sulfate, 0.02); the contaminating glycolipid sulfate was combined with glycolipid sulfate Fractions 1 and 2. All fractions were dissolved in a minimum of chloroform–benzene (1:1, v/v), freed from any silica by centrifugation, and precipitated by addition of 10 vol. of acetone. After cooling, the precipitates were centrifuged, washed with cold acetone and dried *in vacuo*; yields: glycolipid sulfate, 258 mg (28%) phosphatidyl glycerophosphate, 400 mg (44%); phosphatidyl glycerol, 35 mg (4%); Fraction 4, 6.4 mg (0.7%); Fraction 6, 18 mg (2%).

Sodium salt of natural phosphatidyl glycerophosphate

A solution of 300 mg of purified natural phosphatidyl glycerophosphate in 10 ml of chloroform and 10 ml of methanol was diluted with 9 ml of 1 M HCl. The mixture was centrifuged and the lower chloroform phase was diluted with an equal volume of benzene and evaporated under a stream of nitrogen. The remaining oily product was titrated in 3 ml of methanol with 3.35 ml of 0.18 M methanolic NaOH to the phenolphthalein end-point. The solution was then reduced in volume to 3 ml under a stream of nitrogen, diluted with 30 ml of acetone, and cooled overnight at 0°. The white precipitate was recovered by centrifugation and reprecipitated from methanol with acetone, yielding 294 mg of thin-layer chromatographic pure sodium salt of natural phosphatidyl glycerophosphate; $[\alpha]_D$ 0° (*c*, 7.35 in chloroform). The previously reported value of $[\alpha]_D$ $+2.3 \pm 0.3°$ for the sodium salt[5] was probably due to contamination with small amounts of the strongly dextrorotatory glycolipid sulfate ($[\alpha]_D$ +37°)[32].

Analysis. Calc. for $C_{46}H_{94}O_{11}P_2Na_2 \cdot H_2O$ (948.6): C, 58.2; H, 10.1; P, 6.54; Na, 4.85. Found (for sample dried at room temperature): C, 58.52; H, 9.5; P, 6.44; Na, 5.0.

The infrared spectrum of the natural phosphatidyl glycerol phosphate (sodium salt) is shown in Fig. 2, and its chromatographic properties are given in Table I and Fig. 1.

Barium salt of natural phosphatidyl glycerophosphate

To a solution of 147 mg of sodium salt of phosphatidyl glycerophosphate in 10 ml of methanol was added dropwise a 20% $BaCl_2$ aqueous solution (0.25 ml) until no further precipitation occurred. After cooling in an ice-bath, the precipitate was centrifuged, washed twice with methanol, and dried *in vacuo*; yield, 155 mg of thin-layer chromatographic pure barium salt; $[\alpha]_D$ −1.9° (*c*, 7.75 in chloroform).

Analysis. Calc. for $C_{46}H_{94}O_{11}P_2Ba_{\frac{1}{2}}$ (1089):C, 50.6; H, 8.54; P, 5.69; Ba, 18.9. Found: P, 5.61; Ba, 18.4.

Potassium salt of natural phosphatidyl glycerophosphate

A solution of 50 mg of barium salt of phosphatidyl glycerolphosphate in 10 ml of chloroform and 10 ml of methanol was diluted with 9 ml of 0.5 M HCl. The mixture was centrifuged and the chloroform phase was diluted with benzene and evaporated under a stream of the nitrogen. The residue was titrated in 2 ml of methanol with 0.6 ml of 0.157 M methanolic KOH to the phenolphthalein end-point. The solution was concentrated to 1 ml, diluted with 10 ml of acetone, and cooled at 0°. The precipitate was centrifuged, washed with a small volume of cold acetone, and reprecipitated from 0.5 ml of chloroform by addition of 3 ml of acetone; yield, 42.3 mg of thin-layer chromatographic pure potassium salt of phosphatidyl glycerophosphate; $[\alpha]_D$ +1.89° (*c*, 4.23 in chloroform); reported[3] $[\alpha]_D$ +2.5°.

Analysis. Calc. for $C_{46}H_{94}O_{11}P_2K_2$ (962.8): C, 57.40; H, 9.85; P, 6.45; K, 8.13; K: P atomic ratio 1.0. Found: C, 57.11; H, 10.02; P, 6.43; K, 8.07; K/P atomic ratio, 1.0.

Sodium salt of natural phosphatidyl glycerol

To a solution of 30 mg of purified natural phosphatidyl glycerol in 2 ml of

chloroform and 2 ml of methanol was added 1.8 ml of 0.5 M HCl. The mixture was centrifuged, and the chloroform phase was concentrated under a nitrogen stream. The oily residue was titrated in 0.5 ml of methanol with 0.21 ml of 0.18 M methanolic NaOH to the phenolphthalein end-point. The solution was concentrated to 0.5 ml, cleared by centrifugation, diluted with 5 ml of acetone, and cooled at 0°. The precipitate was centrifuged, and reprecipitated from 0.3 ml of chloroform by addition of 2 ml of acetone; yield, 26.5 mg of thin-layer chromatographic pure sodium salt of phosphatidyl glycerol; $[\alpha]_D$ +3.46° (*c*, 1.79 in chloroform); reported[4] for the potassium salt, $[\alpha]_D$ +2.6°.

Analysis. Calc. for $C_{46}H_{94}O_8PNa$ (829.6): C, 66.7; H, 11.41; P, 3.74; Na, 2.77. Found: C, 66.89; H, 11.0; P, 3.67; Na, 2.53.

The infrared spectrum of the sodium salt of phosphatidyl glycerol is shown in Fig. 2, and its chromatographic properties are given in Table I.

2,3-Di-O-(3'R,7'R,11'R,15'-tetramethylhexadecyl)-1-O-p-toluenesulfonyl-sn-glycerol (II)

A solution of 0.70 g (3.7 mmoles) of *p*-toluenesulfonyl chloride in 4 ml of dry pyridine was added dropwise to 1.32 g (2.02 mmoles) of purified natural phytanyl glycerol diether (I) in 8 ml of dry pyridine at 0° (ice-bath). After 24 h at 20° the mixture was diluted with 50 ml of 10% sulfuric acid and extracted with three 50-ml portions of ethyl ether. The combined ether extracts were washed several times with saturated sodium bicarbonate solution, followed by water until neutral, and evaporated under reduced pressure; the oily product was dried *in vacuo*, yielding 1.51 g (92.5%) of thin-layer chromatographic pure compound II; R_F, 0.73 in chloroform–ether (20:1, v/v); $[\alpha]_D$ +2.91° (*c*, 1.72 in chloroform).

Analysis. Calc. for $C_{50}H_{94}O_5S$ (807.3); C, 74.38; H, 11.73. Fo und:C, 74.56; H, 11.50.

Compound II showed strong absorption bands in the infrared characteristic of the tosyl group (1600, 1375, 1190, 1180, 1100, 980, 830, 815, 670 cm^{-1}), of ether groups (1118 cm^{-1}) and of CH_2 and CH_3 groups (2960, 2920, 2870, 1465 cm^{-1}); no OH absorption was present.

1-Iodo-2,3-di-O-(3'R,7'R,11'R-15'-tetramethylhexadecyl-sn-glycerol (III)

A solution of 1.42 g (1.75 mmoles) of compound II and 0.9 g (6 mmoles) of anhydrous sodium iodide in 30 ml of dry acetone was refluxed for 27 h. After centrifugation to remove the acetone-insoluble sodium tosylate, the supernatant was concentrated under reduced pressure. The residue was dissolved in ethyl ether and the solution was cleared by centrifugation and washed twice with 5% sodium thiosulfate solution followed by water to remove any free iodine. The solvent was evaporated under reduced pressure and the product was dried *in vacuo* yielding 1.32 g (98.4%) of thin-layer chromatographic pure iodo diether (III); $[\alpha]_D$ +3.2° (*c*, 3.4 in chloroform); R_F, 0.79 in chloroform.

Analysis. Calc. for $C_{43}H_{87}IO_2$ (763.0): C, 67.68; H, 11.49. Found: C, 68.10; H, 11.20.

Compound (III) showed no absorption in the infrared for OH or tosyl groups but had strong absorption bands for phytanyl groups (2960, 2920, 2870, 1465, 1375–1365, 735 cm^{-1}) and ether groups (1118 cm^{-1}).

2,3-Di-O-phytanyl-1-O-di-p-nitrobenzylphosphoryl-sn-glycerol (IV)

A mixture of 1.93 g (2.52 mmoles) of the iodo diether (III) and 2.4 g (5.05 mmoles) of silver di-*p*-nitrobenzyl phosphate[14] in 50 ml of xylene was heated under reflux with magnetic stirring for 17 h. After removal of xylene under reduced pressure, the dark brown oily residue was dissolved in ethyl ether and the solution was centrifuged to remove silver salts, and concentrated under reduced pressure. The crude product (2.57 g) was purified by chromatography on a silicic acid column (100 g) using the following eluting solvents and collecting 250-ml fractions: light petroleum (Fraction 1); light petroleum–benzene (1:1, v/v) (Fractions 2–17); light petroleum –benzene (1:2, v/v) (Fraction 18); benzene (Fractions 19–21); benzene–chloroform, (3:2, v/v) (Fraction 22); benzene–chloroform (1:1, v/v) (Fractions 23–27); chloroform (Fractions 28–33); and ethyl ether–chloroform (1:1, v/v) (Fraction 34). The desired product appeared in Fractions 28–33, but Fractions 31–33 (0.251 g) were contaminated with a slower moving impurity. The pure Fractions 28–30 were combined and brought to dryness *in vacuo* yielding 1.3 g (55.2%) of thin-layer chromatographic pure compound IV; R_F, 0.56 in chloroform–ether (3:1, v/v); $[\alpha]_D$ +5.33° (*c*, 2.63 in chloroform).

Analysis. Calc. for $C_{59}H_{99}O_{11}PN_2$ (1003.4): C, 68.22; H, 9.94; P, 3.09. Found: C, 69.00; H, 10.20; P, 3.02.

Compound IV showed no OH absorption in the infrared, but had strong absorption bands for phytanyl groups (2960, 2920, 2870, 1465, 1375–1365 cm^{-1}), nitrobenzyl groups (1610, 1525, 1350, 900, 865, 765, 740 cm^{-1}), phosphate group (1280, 1040, 1020 cm^{-1}) and C–O–C ether groups (1110 cm^{-1}).

Silver salt of 2,3-di-O-phytanyl-1-O-p-nitrobenzylphosphoryl-sn-glycerol (V)

A solution of 1.2 g (1.2 mmoles) of compound IV and 1.2 g (8 mmoles) of anhydrous sodium iodide in 40 ml of dry acetone was heated under reflux with magnetic stirring for 23 h. After cooling the reaction mixture, the acetone phase was carefully decanted and the oily residue was dissolved in 2 ml of chloroform and precipitated by addition of 20 ml of acetone. After cooling, the oily precipitate was centrifuged, washed with a small volume of cold acetone and dried *in vacuo*; yield, 0.93 g (88%) of thin-layer chromatographic pure sodium salt of compound V; $[\alpha]_D$ +5.1° (*c*, 8.5 in chloroform); R_F, 0.39 in chloroform–methanol (9:1, v/v).

Analysis. Calc. for $C_{50}H_{93}O_8PNNa$ (890.3); C, 67.90; H, 10.53; P, 3.48. Found: C, 67.42; H, 10.30; P, 3.46.

To a solution of 0.93 g (1.05 mmoles) of the sodium salt of compound V in 60 ml of hot acetone, was added a solution of 255 mg (1.5 mmoles) of silver nitrate in 15 ml of acetone–water (2:1, v/v). The mixture was cooled at 0° overnight, and the oily precipitate was centrifuged, washed with a small volume of cold acetone and dried *in vacuo*; yield, 980 mg (96%) of thin-layer chromatographic pure silver salt (V); $[\alpha]_D$ +2.8° (*c*, 1.8 in chloroform); R_F, 0.39 in chloroform–methanol (9:1, v/v).

Analysis. Calc. for $C_{50}H_{93}O_8PNAg$ (975.2); C, 61.58; H, 9.61; P, 3.18. Found: C, 61.28; H, 9.35; P, 3.15.

The silver salt of compound V had a similar infrared spectrum to that of IV, except that the P=O band was shifted to 1200 cm^{-1} and a band at 1100 cm^{-1}, attributed to an ionized P–O^- (ref. 16) was present.

1-O-p-Toluenesulfonyl-3-O-benzyl-sn-glycerol (VIIa)

A cold solution of 4.92 g (25.8 mmoles) of *p*-toluenesulfonyl chloride in 10 ml of anhydrous pyridine was added dropwise with stirring at 0° during a period of 100 min to a solution of 4.7 g (25.8 mmoles) of 3-*O*-benzyl-*sn*-glycerol (VIa) in 20 ml of anhydrous pyridine. The mixture was allowed to stand at room temperature overnight, then diluted with ice and 40 ml of 10% sulfuric acid, and extracted with several portions of ethyl ether. The combined ether extracts were washed with 10% sulfuric acid and with water, diluted with benzene, and concentrated under reduced pressure. The residual product (8.0 g) was shown by thin-layer chromatography to be mainly a mixture of mono- and ditosyl benzyl glycerol; it was chromatographed on a column of 70 g of silicic acid with the following solvents (120-ml fractions): benzene–chloroform (1:1, v/v) (Fractions 1 and 2); benzene–chloroform (1:2, v/v) (Fractions 3 and 4); chloroform (Fractions 5–13). The desired 1-monotosyl compound VIIa appeared in Fractions 5–12 but Fractions 5–7 were contaminated with the faster moving ditosyl derivative. The pure Fractions 8–12 were combined and concentrated *in vacuo* to yield 4.0 g of thin-layer chromatographic pure compound VIIa. Fractions 5–7 were combined (3.12 g) and rechromatographed on a column of 40 g of silicic acid as described above, yielding a further 1.92 g of pure compound VIIa; total yield, 5.92 g (68.2%); $[\alpha]_D$ +7.05° (*c*, 6.4 in chloroform); R_F, 0.30 in chloroform–ether (9:1, v/v).

Analysis. Calc. for $C_{17}H_{20}O_5S$ (336.4): C, 60.71; H, 6.19. Found: C, 60.74; H, 5.99.

3-O-p-Toluenesulfonyl-1-O-benzyl-sn-glycerol (VIIb)

540 mg (3.37 mmoles) of 1-*O*-benzyl-*sn*-glycerol (VIb) were reacted with 643 mg (3.37 mmoles) of *p*-toluenesulfonyl chloride in anhydrous pyridine as described above for its enantiomer. The crude product (900 mg) was fractionated by preparative thin-layer chromatography using chloroform–ethyl ether (9:1, v/v) as solvent, yielding 790 mg (69.7%) of thin-layer chromatographic pure compound VIIb; $[\alpha]_D$ −6.7° (*c*, 13.4, in chloroform); reported[13] $[\alpha]_D$ −6.07°.

Compounds VIIa and VIIb had identical infrared spectra which showed bands for OH (3480 cm^{-1}), aromatic rings (3085, 3050, 3025, 1600, 1500, 980, 940, 840, 820, 740, 700, 670 cm^{-1}), sulfonyl group (1365, 1190, 1180 cm^{-1}), C–O–C ether (1100 cm^{-1}), and secondary alcoholic C–O groups (1080 cm^{-1}).

1-O-p-Toluenesulfonyl-2-O-tert.-butyl-3-O-benzyl-sn-glycerol (VIIIa)

Gaseous isobutylene (8.5 g, 150 mmoles) was bubbled into an ice-cold solution of 4.0 g (12 mmoles) of compound VIIa in 20 ml of dichloromethane. A suspension of 0.2 ml of conc. sulfuric acid in 10 ml of dichloromethane was added, and the mixture was shaken and left at room temperature for 5 days. The product was isolated as described by BONSEN, DE HAAS AND VAN DEENEN[11], and purified on a column of 50 g of silicic acid with the following eluting solvents (100-ml fractions): light petroleum–benzene (1:2, v/v) (Fraction 1); benzene (Fractions 2–6); benzene–chloroform (1:1, v/v) (Fractions 7 and 8); chloroform (Fractions 9–18). The desired compound (VIIIa) appeared in Fractions 2–15, which were combined and concentrated *in vacuo* to give 3.4 g (73%) of thin-layer chromatographic pure compound VIIIa; $[\alpha]_D$ +6.28° (*c*, 4.95 in chloroform); R_F, 0.68 in chloroform–ether (9:1, v/v).

Analysis. Calc. for $C_{21}H_{28}O_5S$ (392.5); C, 64.27; H, 7.20. Found: C, 64.60; H, 7.52.

3-O-p-Toluenesulfonyl-2-O-tert.-butyl-1-O-benzyl-sn-glycerol (VIIIb)

Compound VIIb (790 mg, 2.35 mmoles) was reacted with isobutylene (4 g) in methylene chloride (15 ml) in the presence of 0.1 ml of concentrated sulfuric acid as described for its enantiomer. The product was purified by chromatography on a column of 17 g of silicic acid with the solvents given above; yield 754 mg (82%); $[\alpha]_D$ −6.37° (*c*, 3.8 in chloroform); reported[13] $[\alpha]_D$ −6.17°.

Analysis. Calc. for $C_{21}H_{28}O_5S$ (392.5): C, 64.27; H, 7.20. Found: C, 64.39; H, 7.25.

Isomers VIIIa and VIIIb had identical infrared spectra showing absorption bands for aromatic rings (3085, 3055, 3025, 1600, 1495, 975, 955, 850, 835, 820, 740, 700, 670 cm^{-1}), sulfonyl group (1365, 1190, 1180), C–O–C ether (1100 cm^{-1}), and $-C(CH_3)_3$ (2975, 2920, 2860, 1455, 1390, 1255, 1230 cm^{-1}); OH absorption was absent.

1-O-p-Toluenesulfonyl-2-O-tert.-butyl-sn-glycerol (IXa)

Compound VIIIa (1.3 g) was debenzylated by hydrogenolysis in abs. ethanol (35 ml) with freshly prepared palladium–charcoal catalyst[33] (from 500 mg of palladium chloride) at room temperature and atmospheric pressure. The reaction was complete after 30 min, when the catalyst was removed by centrifugation and washed twice with ethanol and once with chloroform; combined supernates were concentrated under reduced pressure, yielding 0.82 g (90%) of thin-layer chromatographic pure compound IXa; R_F, 0.27 in chloroform–ethyl ether (9:1, v/v); $[\alpha]_D$ −28.5° (*c*, 2.7 in chloroform).

Analysis. Calc. for $C_{14}H_{22}O_5S$ (302.4): C, 55.62; H, 7.34. Found: C, 55.42; H, 7.60.

3-O-p-Toluenesulfonyl-2-O-tert.-butyl-sn-glycerol (IXb)

Compound VIIIb (754 mg; 1.9 mmoles) was debenzylated as described for compound VIIIa; yield, 522 mg (90%) of thin-layer chromatographic pure compound IXb; R_F, 0.27 in chloroform–ethyl ether (9:1, v/v); $[\alpha]_D$ +27.7° (*c*, 1.95 in chloroform).

Isomers IXa and IXb had the same infrared spectrum showing absorption bands for OH (3450 cm^{-1}), tosyl (1600, 1500, 1365, 1190, 1180, 975, 955, 850, 820, 670 cm^{-1}), $-C(CH_3)_3$ (2980, 2940, 2880, 1460, 1390 cm^{-1}), C–O–C ether (1100 cm^{-1}), and primary alcoholic C–O (1055 cm^{-1}) groups.

1-O-p-Toluenesulfonyl-2-O-tert.-butyl-3-O-diphenylphosphoryl-sn-glycerol (Xa)

A solution of 0.77 g (2.55 mmoles) of compound IXa and 0.92 g (3.4 mmoles) of diphenylphosphoryl chloride in 5 ml of anhydrous pyridine was kept at room temperature for 24 h. After addition of water (25 ml) the mixture was extracted with ethyl ether. The extract was washed with 0.25 M HCl followed by water and concentrated under reduced pressure. The residual colorless oil (1.29 g) was fractionated on a silicic acid column (20 g) using the following solvents: benzene, 30-ml fractions (Fractions 1 and 2); chloroform, 30-ml fractions (Fractions 3 and 4); chloroform–ether, 50-ml fractions (Fractions 5 and 6); ethyl ether, 50 ml for each fraction (Fractions 7 and 8). The desired compound Xa appeared in Fractions 4 and 5 but Fraction 5 was contaminated with a slower moving substance. Fraction 4 was dried *in vacuo* and yielded 1.17 g (86%) of thin-layer chromatographic pure compound Xa; R_F, 0.64 in chloroform–ether (9:1, v/v); $[\alpha]_D$ +1.77° (*c*, 4.7 in chloroform).

Analysis. Calc. for $C_{26}H_{31}O_8SP$ (534.5): C, 58.47; H, 5.85; P, 5.80. Found: 58.50; H, 5.92; P, 5.81.

3-O-p-Toluenesulfonyl-2-O-tert.-butyl-1-O-diphenylphosphoryl-sn-glycerol (Xb)

160 mg (0.53 mmole) of compound IXb was phosphorylated with 0.54 g (2 mmoles) of diphenylphosphoryl chloride in 5 ml of anhydrous pyridine and the product was isolated as described for compound Xa. The product (256 mg) was purified by preparative thin-layer chromatography in chloroform as solvent; yield, 210 mg (74.2%); R_F, 0.26 in chloroform; 0.64 in chloroform–ethyl ether (9:1, v/v); $[\alpha]_D$ $-1.67°$ (*c*, 4.2 in chloroform).

Compounds Xa and Xb had identical infrared spectra which showed absorption bands for P–O–C (aromatic) (1195 cm^{-1}), P–O–C (aliphatic) (1030 cm^{-1}), P=O (1295 cm^{-1}), sulfonyl (1370, 1180 and 1100 cm^{-1}), aromatic (3090, 3060, 3040, 1600, 1590, 1490, 960, 835, 820, 780, 690, 670 cm^{-1}), *tert.*-butyl (2975, 2930, 2880, 1390, 1375, 1225 cm^{-1}), and C–O–C ether (1110 cm^{-1}) groups.

1-Iodo-2-O-tert.-butyl-3-O-diphenylphosphoryl-sn-glycerol (XIa)

Compound Xa (480 mg, 0.90 mmole) was converted to the iodo derivative (XIa) by reaction with sodium iodide (400 mg) in boiling anhydrous acetone (15 ml). After centrifugation of the sodium *p*-toluenesulfonate, the supernatant was evaporated to dryness under reduced pressure. The oily residue was dissolved in ethyl ether, and the solution was washed with 5% sodium thiosulfate followed by water. The solvent was removed under reduced pressure, yielding 350 mg of crude product which was purified by preparative thin-layer chromatography in chloroform; yield, 220 mg (41%) of thin-layer chromatographic pure iodide XIa; $[\alpha]_D$ $+4.3°$ (*c*, 7.01 in chloroform); R_F, 0.32 in chloroform.

Analysis. Calc. for $C_{19}H_{24}O_5PI$ (490.3); C, 46.54; H, 4.93; P, 6.30. Found: C, 47.38; H, 5.10; P, 6.23.

3-Iodo-2-O-tert.-butyl-1-O-diphenylphosphoryl-sn-glycerol (XIb)

Compound Xb (735 mg; 1.37 mmoles) was converted to the iodo derivative (XIb) as described for compound Xa, and the product obtained (844 mg) was purified by preparative thin-layer chromatography in chloroform; yield 518 mg (77%) of thin-layer chromatographic pure iodide; R_F, 0.30 in chloroform; $[\alpha]_D$ $-4.3°$ (*c*, 5.2 in chloroform).

The iodo derivatives XIa and XIb had identical infrared spectra which showed absorption bands for aromatic rings (3060, 3040, 1590, 1490, 960, 780, 690 cm^{-1}), *tert.*-butyl group (1455, 1390, 1370 and 1220 cm^{-1}), and phosphate group (1295, 1195, 1070, 1035, 1025 cm^{-1}); bands for sulfonyl groups were absent.

1-Iodo-2-O-tert.-butyl-3-O-benzyl-sn-glycerol (XIIa)

Compound VIIIa (0.5 g; 1.3 mmoles) was reacted with sodium iodide (1.9 g; 13 mmoles) in anhydrous acetone under reflux for 42 h. Following centrifugation, the acetone phase was concentrated under reduced pressure. The residual oil was dissolved in ethyl ether and the solution was washed with 5% sodium thiosulfate solution and with water, and concentrated under reduced pressure to dryness; yield, 0.45 g (97%) of thin-layer chromatographic pure iodo compound XIIa; R_F, 0.69 in chloroform–ethyl ether (20:1, v/v); $[\alpha]_D$ $+3.8°$ (*c*, 4.3 in chloroform).

Analysis. Calc. for $C_{14}H_{21}P_2I$ (364.2): C, 46.17; H, 5.81. Found: C, 47.50; H, 5.95.

3-Iodo-2-O-tert.-butyl-1-O-benzyl-sn-glycerol (XIIb)

Compound XIIb was prepared as described for its enantiomer (XIIa) by reaction of the tosyl compound VIIIb (178 mg; 0.45 mmole) with sodium iodide (1 g) in boiling acetone (15 ml); yield, 130 mg (79%) of thin-layer chromatographic pure iodo compound XIIb; R_F, 0.57 in chloroform and 0.70 in chloroform–ether (20:1, v/v); $[\alpha]_D$ −4.3° (*c*, 5.2 in chloroform).

The enantiomers XIIa and XIIb had identical infrared spectra which showed prominent bands for *tert.*-butyl (2980, 2940, 2865, 1455, 1390, 1370, 1235 cm^{-1}), benzyl (3090, 3070, 3030, 1500, 905, 735, 695 cm^{-1}), and C–O–C (1130, 1110 cm^{-1}) groups, but bands for sulfonyl groups were absent.

2,3-Di-O-phytanyl-sn-glycero-1-nitrobenzylphosphoryl-1'-glycero-2'-O-tert.-butyl-3'-diphenylphosphate (XIIIa)

A mixture of the silver salt V (740 mg; 0.76 mmole) and iodo compound XIa (570 mg; 1.16 mmoles) in 50 ml of dry benzene was heated under reflux with stirring for 24 h. After removal of silver salts by centrifugation, the benzene supernatant was concentrated under reduced pressure and the residue was purified by chromatography on a silicic acid column (100 g) eluted with the following solvents: benzene, 100 ml (Fraction 1); benzene–chloroform (1:1, v/v), 250-ml fractions (Fractions 2–11); chloroform, 250-ml fractions (Fractions 12–19). The desired product (XIIIa) appeared in Fractions 10–15 but Fractions 10 and 11 were contaminated with a fast moving impurity. The latter fractions were then purified by preparative thin-layer chromatography in chloroform–ether (3:1, v/v) and combined with Fractions 12–15, yielding 900 mg (96%) of thin-layer chromatographic pure compound XIIIa; R_F, 0.55 in chloroform–ether (3:1, v/v); $[\alpha]_D$ +4.3° (*c*, 8.2 in chloroform).

Analysis. Calc. for $C_{69}H_{117}O_{13}P_2N$ (1230.6): C, 67.34; H, 9.58; P, 5.03. Found: C, 67.00; H, 9.79; P, 5.09.

2,3-Di-O-phytanyl-sn-glycero-1-nitrobenzylphosphoryl-3'-glycero-2'-O-tert.-butyl-1'-diphenylphosphate (XIIIb)

After reaction of the silver salt V (270 mg; 0.277 mmole) with the iodo compound XIb (220 mg; 0.45 mmole) in 15 ml of dry benzene under reflux for 24 h, the product XIIIb was isolated as described for its diastereomer XIIIa and purified by preparative thin-layer chromatography in chloroform–ethyl ether (9:1, v/v) as solvent; yield, 263 mg (77%) of thin-layer chromatographic pure compound XIIIb; R_F, 0.55 in chloroform–ether (3:1, v/v); 0.24 in chloroform–ether (9:1, v/v); $[\alpha]_D$ + 2.09° (*c*, 5.86 in chloroform).

Analysis. Calc. for $C_{69}H_{117}O_{13}P_2N$ (1230.6): C, 67.34; H, 9.58; P, 5.03. Found: C, 68.02; H, 9.49; P, 5.07.

The diastereomers XIIIa and XIIIb had identical infrared spectra showing prominent bands for phytanyl (2960, 2920, 2860, 1465, 1380, 1370 cm^{-1}), aromatic (3070, 3040, 1610–1600–1590 (triplet), 1490, 1190, 960, 865, 780, 760, 740, 690 cm^{-1}), nitro (1525, 1350 cm^{-1}), phosphate (1285, 1030, 1015 cm^{-1}), *tert.*-butyl (1390, 1370, 1225 cm^{-1}) and C–O–C ether (1110 cm^{-1}) groups.

2,3-Di-O-phytanyl-sn-glycero-1-phosphoryl-(1'-sn-glycero-2'-O-tert.-butyl-3'-phosphate) (XIVa)

210 mg (0.17 mmole) of compound XIIIa were subjected to hydrogenolysis in 10 ml of abs. ethanol with 150 mg of $PtO_2 \cdot H_2O$ at 22° and atmospheric pressure. After 50 min the uptake of hydrogen was complete (116 ml; calc., 90 ml); the catalyst was removed by centrifugation and the ethanol phase was concentrated to dryness under reduced pressure. The oily residue was dissolved in 4 ml of chloroform and 4 ml of methanol, and the solution was diluted with 3.6 ml of 1 M HCl. The chloroform phase (diluted with benzene) was then evaporated under nitrogen, and the oily residue (free acid) was titrated in 0.5 ml of methanol with 0.18 M methanolic NaOH (2.0 ml; calc., 1.9 ml) to the phenolphthalein end-point. After centrifugation to remove some insoluble material, the methanol supernatant was concentrated to 1 ml under a nitrogen stream, diluted with 1.5 ml of acetone, and cooled; the precipitate was removed by centrifugation, and the clear supernatant was further diluted with 8.5 ml of acetone, and cooled. The desired sodium salt of XIVa was collected by centrifugation, washed with cold acetone and dried in vacuo; yield, 100 mg (60%) of thin-layer chromatographic pure product XIVa; $[\alpha]_D$ −4.1° (*c*, 1.35 in methanol–chloroform (2:1, v/v)); R_F, 0.48 in chloroform–methanol–water (80:20:2, v/v/v).

Analysis. Calc. for $C_{50}H_{102}O_{11}P_2Na_2$ (986.8): P, 6.29. Found: P, 6.17.

2,3-Di-O-phytanyl-sn-glycero-1-phosphoryl-(1'-sn-glycero-2'-O-tert.-butyl-3'-phosphate) (XIVb)

Compound XIIIb (295 mg; 0.24 mmole) was freed of *p*-nitrobenzyl and phenyl groups by hydrogenolysis in abs. ethanol (10 ml) with platinum catalyst (170 mg of $PtO_2 \cdot H_2O$), and the product (XIVb) was isolated as the sodium salt, as described for preparation of the diastereomer XIVa; yield of thin-layer chromatographic pure XIVb, 140 mg (60%); $[\alpha]_D$ −2.72° (*c*, 2.6 in methanol–chloroform (2:1, v/v); R_F, 0.50 in chloroform–methanol–water (80:20:2, v/v/v).

Analysis. Calc. for $C_{50}H_{102}O_{11}P_2Na_2$ (986.8): P, 6.29. Found: P, 6.31.

Diastereomers XIVa and XIVb had identical infrared spectra; the main absorption bands were due to CH_2 and CH_3 (2960, 2920, 2820, 1465 cm^{-1}), *tert.*-butyl (1390, 1370 cm^{-1}), bonded P=O (1230 cm^{-1}), P–O⁻ and C–O–C ether (1110 cm^{-1}), and P–O–C (1075 cm^{-1}) groups; only weak OH absorption was present.

2,3-Di-O-(3'R,7'R,11'R,15'-tetramethylhexadecyl)-sn-glycero-1-phosphoryl-(1''-sn-glycero-3''-phosphate) (XVa)

To an ice-cold solution of 100 mg (0.1 mmole) of the *tert.*-butyl compound XIVa, in 2 ml of anhydrous chloroform (freshly distilled over P_2O_5) was added 20 ml of an ice-cold 0.48 M solution of HCl in anhydrous chloroform (prepared by bubbling gaseous HCl into dry chloroform). The mixture was kept in an ice-bath for 90 min and then diluted with 22 ml of methanol and 19.8 ml of water and centrifuged briefly. The chloroform phase was diluted with benzene and concentrated under a stream of nitrogen, and the oily residue was titrated (phenolphthalein end-point) in methanol (1.0 ml) with 0.18 M methanolic NaOH (1.1 ml). The crude sodium salt (XVa) was purified by preparative thin-layer chromatography to remove unreacted compound XIVa using chloroform–methanol–conc. ammonia (65:35:5, v/v/v) as solvent. The desired compound XVa was extracted with 100 ml of methanol–chloroform–ether

(1:1:1, v/v/v) from the silica gel and converted to the free acid by diluting its solution in 4 ml chloroform and 4 ml of methanol with 3.6 ml of 1 M HCl. After centrifugation, the chloroform phase was concentrated under nitrogen and the oily product was titrated in 0.5 ml of methanol with 0.85 ml of 0.16 M methanolic KOH to the phenolphthalein end-point. The solution was concentrated to 1 ml under a stream of nitrogen, cleared by centrifugation, diluted with 9 ml of acetone and cooled. The white precipitate was centrifuged, washed with cold acetone and reprecipitated from 0.5 ml of chloroform with 2.5 ml of acetone; yield of potassium salt XVa, dried *in vacuo*, 45 mg (46%); $[\alpha]_D$ −2.24° (*c*, 4.5 in chloroform); R_F, 0.20 in chloroform–methanol–conc. ammonia (65:35:5, v/v/v); 0.60 in chloroform–methanol–water (65:35:5, v/v/v).

Analysis. Calc. for $C_{46}H_{94}O_{11}P_2K_2$ (962.8): P, 6.45; K, 8.13. Found: P, 6.46; K, 8.00.

The sodium salt of XVa was prepared as described for the natural product and had the same thin-layer chromatographic mobilities as the corresponding potassium salt; its specific rotation, however, was $[\alpha]_D$ −1.8° (*c*, 3.7 in chloroform).

Analysis. Calc. for $C_{46}H_{94}O_{11}P_2Na_2$ (930.6): C, 59.35, H, 10.18; P, 6.66; Na, 4.94. Found: C, 58.91; H, 10.00; P, 6.43; Na, 4.73.

2,3-Di-O-(3'R,7'R,11'R1,5-tetramethylhexadecyl)-sn-glycero-1-phosphoryl-(3''-sn-glycero-1''-phosphate) (XVb)

To a solution of 80 mg of *tert.*-butyl derivative XIVb in 1.5 ml of dry chloroform was added 12.5 ml of cold 0.5 M HCl in dry chloroform. After 70 min in an ice-bath, the product XVb was isolated, purified by preparative thin-layer chromatography and converted to the potassium salt as described for the diastereomer XVa; yield, 37.5 mg (48%); R_F, 0.2 in chloroform–methanol–conc. ammonia (65:35:5, v/v/v); 0.60 in chloroform–methanol–water (65:35:5, v/v/v); $[\alpha]_D$ +1.93° (*c*, 2.75 in chloroform).

Analysis. Calc. for $C_{46}H_{94}O_{11}P_2K_2$ (962.8): C, 57.40; H, 9.85; P, 6.45; K, 8.13. Found: C, 57.11; H, 10.23: P, 6.43; K, 8.07.

The sodium salt of XVb was prepared as described for the natural product; R_F values identical with those of potassium salt (Table II); $[\alpha]_D$ 0° (*c*, 4.0 in chloroform).

Analysis. Calc. for $C_{46}H_{94}O_{11}P_2Na_2$ (930.6): C, 59.35; H, 10.18; P, 6.66; Na, 4.94. Found: C, 59.12; H. 10.60; P, 6.64; Na, 5.28.

The potassium and sodium salts of both diastereomers XVa and XVb had the same infrared spectra, which were identical to that of the natural compound (Fig. 2, Table II).

2,3-Di-O-phytanyl-sn-glycero-1-p-nitrobenzylphosphoryl-1'-(2'-O-tert.-butyl-3'-benzyl)-sn-glycerol (XIVa)

A mixture of 740 mg (0.76 mmole) of the silver salt V and 540 mg (1.48 mmoles) of 1-iodo-2-*O-tert.*-butyl-3-*O*-benzyl-*sn*-glycerol (XIIa) in 50 ml of dry benzene was refluxed with stirring for 18 hours. Following centrifugation to remove silver salts, the supernatant was concentrated under reduced pressure and the residue was purified by chromatography on a silicic acid column (100 g) eluted with the following solvents: benzene, 100 ml (Fraction 1); benzene–chloroform (1:1, v/v), 250 ml for each fraction (Fractions 2–12); and chloroform, 250 ml for each fraction (Fractions

13–16). The desired product appeared in Fractions 11–14 which were combined and concentrated under reduced pressure; yield 743 mg (91.3%) of thin-layer chromatographic pure compound XVIa; $[\alpha]_D$ +8.33° (*c*, 2.76 in chloroform); R_F, 0.29 in chloroform–ether (10:1, v/v); 0.73 in chloroform–ether (3:1, v/v).

Analysis. Calc. for $C_{64}H_{114}O_9PN$ (1072.5): C, 71.67; H, 10.72; P, 2.89. Found: C, 71.34; H, 10.97; P, 2.84.

2,3-Di-O-phytanyl-sn-glycero-1-p-nitrobenzylphosphoryl-3'-(2'-O-tert.-butyl-1'-O-benzyl)-sn-glycerol (XVIb)

129.8 mg (0.357 mmole) of compound XIIb was reacted with 348 mg (0.357 mmole) of silver salt V in 15 ml of dry benzene under reflux with stirring for 4 h. The product XVIb was purified by preparative thin-layer chromatography in chloroform–ethyl ether (10:1, v/v) as solvent; R_F, 0.29 in chloroform–ethyl ether (10:1, v/v); yield, 300 mg (79%) of thin-layer chromatographic pure compound XVIb; $[\alpha]_D$ +0.93° (*c*, 3.0 in chloroform).

Analysis. Calc. for $C_{64}H_{114}O_9PN$ (1072.54): C, 71.67; H, 10.72; P. 2.89. Found: 71.81; H, 10.90; P, 3.04.

Diasteriomers XVIa and XVIb had identical infrared spectra which showed prominent bands for phytanyl (2960, 2920, 2860, 1465, 1380, 1370 cm^{-1}), aromatic (3090, 3070, 3030, 1615, 1500, 1195, 865, 760, 735, 700 cm^{-1}), *tert.*-butyl (1455, 1390, 1370, 1230 cm^{-1}), nitro (1530, 1350 cm^{-1}), phosphate (1285, 1035, 1015 cm^{-1}) and C–O–C ether (1110 cm^{-1}) groups; OH absorption was absent.

2,3-Di-O-phytanyl-sn-glycero-1-phosphoryl-1'-(2'-O-tert.-butyl)-sn-glycerol (XVIIa)

740 mg (0.69 mmole) of blocked phosphatidyl glycerol diastereomer (XVIa) were hydrogenolyzed in 25 ml of abs. ethanol with palladium–charcoal catalyst (freshly prepared from 750 mg of palladium chloride according to HESSEL *et al.*[33]) at atmospheric pressure and room temperature. Hydrogen uptake was complete (170 ml; calc., 181 ml) within 40 min. After centrifugation to remove the catalyst, the ethanol phase was concentrated under reduced pressure, and the residue was dissolved in 10 ml of chloroform and 10 ml of methanol and diluted with 9 ml of 1 M HCl. The chloroform phase was evaporated to dryness under a stream of nitrogen and the oily residue (free acid) was titrated in 2 ml of methanol with 2.4 ml of 0.25 M methanolic NaOH (phenolphthalein end-point). After centrifugation, the solution was reduced to 2 ml under a stream of nitrogen, diluted with 20 ml of acetone and cooled. The precipitate was centrifuged and reprecipitated from 1 ml of methanol (solution cleared by centrifugation) with 10 ml of acetone, as above. The sodium salt of XVIIa was collected by centrifugation, washed with cold acetone and dried *in vacuo*; yield, 389 mg (63%) of thin-layer chromatographic pure product; R_F, 0.76 in chloroform–methanol–water (65:35:5, v/v/v); $[\alpha]_D$ +2.8° (*c*, 1.5 in chloroform).

Analysis. Calc. for $C_{50}H_{102}O_8$ PNa (885.8): P, 3.50. Found: P, 3.48.

2,3-Di-O-phytanyl-sn-glycero-1-phosphoryl-3'-(2'-O-tert.-butyl)-sn-glycerol (XVIIb)

The fully blocked diastereomer XVIb (140 mg; 0.14 mmole) was freed of *p*-nitrobenzyl and benzyl groups by hydrogenolysis in abs. ethanol (10 ml) with palladium–charcoal catalyst (prepared from 150 mg palladium chloride[33]), and the product XVIIb was isolated in form of the sodium salt as described for the diastereo-

mer XVIIa; yield 70 mg (61%) of thin-layer chromatographic pure product; R_F, 0.75 in chloroform–methanol–water (65:35:5, v/v/v).

Analysis. Calc. for $C_{50}H_{102}O_8PNa$ (885.8): P, 3.50. Found: P, 3.25.

Diasteriomers XVIIa and XVIIb had identical spectra showing prominent absorption bands for OH (3300 cm^{-1}), phytanyl (2960, 2920, 2870, 1465, 1380, 1370), *tert.*-butyl (1450 (sh), 1390 (sh), 1370, 1200 cm^{-1}) phosphate (1235, 1100, 1070 cm^{-1}) and ether C–O–C (1100 cm^{-1}) groups.

2,3-Di-O-(3'R',7'R,11'R,15'-tetramethylhexadecyl)-sn-glycero-1-phosphoryl-1''-sn-glycerol (XVIIIa)

A stream of dry HCl gas was passed through a solution of the sodium salt of the *tert.*-butyl compound XVIIa (129 mg; 0.146 mmole) in anhydrous chloroform (12 ml) at 0° for 60 min. The mixture was diluted with 12 ml of methanol and 10.8 ml of water, and centrifuged briefly. The chloroform phase (diluted with benzene) was concentrated under a stream of nitrogen, and the residue was titrated in 2 ml of methanol with 0.6 ml of 0.33 M methanolic NH_4OH. The crude salt of XVIIIa was purified by preparative thin-layer chromatography in chloroform–methanol–conc. ammonia (65:35:5, v/v/v) as solvent; yield, 59 mg of thin-layer chromatographic pure ammonium salt of XVIIIa; R_F, 0.56 in chloroform–methanol–ammonia (65:35:5, v/v/v); 0.47 in chloroform–methanol–water (65:35:5,v/v/v). The ammonium salt was then converted to the free acid by diluting its solution in 20 ml of chloroform–methanol (1:1, v/v) with 9 ml of 0.5 M HCl. After centrifugation, the chloroform phase was concentrated under a stream of nitrogen, and the residual free acid was titrated in 2 ml of methanol–chloroform (1:1, v/v) with 0.3 ml of 0.25 M methanolic NaOH solution (phenolphthalein end-point). The mixture was concentrated to dryness under nitrogen and the residue redissolved in 1 ml of methanol–chloroform (1:1, v/v); the solution was cleared by centrifugation, diluted with 10 ml of acetone, and cooled. The precipitate was centrifuged, washed with cold acetone and reprecipitated from 1 ml of methanol with 10 ml of acetone; 49.5 mg (41%) of thin-layer chromatographic pure sodium salt of XVIIIa; $[\alpha]_D$ −1.13° (*c*, 2.47 in chloroform).

Analysis. Calc. for $C_{46}H_{94}O_8P\,Na\cdot H_2O$ (847.6): C, 65.3°; H, 11.71; P, 3.66. Found: C, 64.30; H, 10.86; P, 3.65.

2,3-Di-O-(3'R,7'R,11'R-15'-tetramethylhexadecyl)-sn-glycerol-1-phosphoryl-3''-sn-glycerol (XVIIIb)

300 mg (0.28 mmole) of blocked isomer XVIb was hydrogenolyzed in 15 ml of abs. ethanol with palladium–charcoal catalyst (prepared from 300 mg of palladium chloride) at room temperature for 30 min (uptake of hydrogen, 76 ml; calc., 69). After removal of the catalyst by centrifugation the ethanol supernatant was concentrated under reduced pressure. The residue was dissolved in 5 ml of ethyl ether, and the solution centrifuged to remove the last traces of catalyst, and brought to dryness under a stream of nitrogen; yield of thin-layer chromatographic pure free acid XVIIb, 234 mg; R_F, 0.76 in chloroform–methanol–water (65:35:5, v/v/v).

Compound XVIIb was freed of its *tert.*-butyl group by passing a stream of dry HCl gas through a solution of the free acid XVIIb (234 mg; 0.26 mmole) in anhydrous chloroform (15 ml) at 0° for 50 min. The mixture was diluted with 15 ml of methanol and 13.5 ml of water, and centrifuged. The chloroform phase was concentrated to

2 ml, neutralized with 3 ml of 0.33 M methanolic NaOH and concentrated to dryness. The crude product XVIIIb was purified by preparative thin-layer chromatography in chloroform–methanol–conc. ammonia (65:35:5, v/v/v) as solvent. The purified ammonium salt of XVIIIb was converted to the sodium salt, as described for the corresponding diastereomer XVIIIa, and the sodium salt was precipitated twice from its solution in 1 ml of methanol by addition of 10 ml of acetone. Yield of thin-layer chromatographic pure sodium salt of XVIIIb, 92 mg (45%); $[\alpha]_D$ +3.43° (*c*, 2.36 in chloroform).

Analysis. Calc. for $C_{46}H_{94}O_8P\,Na\cdot H_2O$ (847.6): C, 65.30, H, 11.71; P, 3.66; Na, 2.72. Found: C, 65.70, H, 12.00; P, 3.63; Na, 2.95.

The diastereomers XVIIIa and XVIIIb had the same infrared spectra which were identical to that of the natural compound (Fig. 2, Table II).

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REFERENCES

1 S. N. SEHGAL, M. KATES AND N. E. GIBBONS, *Can. J. Biochem. Physiol.*, 40 (1962) 69.
2 M. KATES, P. S. SASTRY AND L. S. YENGOYAN, *Biochim. Biophys. Acta*, 70 (1963) 705.
3 M. FAURE, J. MARECHAL AND J. TROESTLER, *Compt. Rend.*, 257 (1963) 2187.
4 M. FAURE, J. MARECHAL AND J. TROESTLER, *Compt. Rend.*, 259 (1964) 941.
5 M. KATES, L. S. YENGOYAN AND P. S. SASTRY, *Biochim. Biophys. Acta*, 98 (1965) 252.
6 M. KATES, B. PALAMETA, C. N. JOO, D. J. KUSHNER AND N. E. GIBBONS, *Biochemistry*, 5 (1966) 4092.
7 M. KATES, B. PALAMETA AND L. S. YENGOYAN, *Biochemistry*, 4 (1965) 1595.
8 M. KATES, C. N. JOO, B. PALAMETA AND T. SHIER, *Biochemistry*, 6 (1967) 3329.
9 C. N. JOO, M. KATES AND T. SHIER, *J. Lipid Res.*, 9 (1968) 782.
10 C. N. JOO AND M. KATES, *Biochim. Biophys. Acta*, 152 (1968) 800.
11 P. P. M. BONSEN, G. H. DE HAAS AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 106 (1965) 93.
12 G. H. DE HAAS, P. P. M. BONSEN, AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 116 (1966) 114.
13 P. P. M. BONSEN AND L. L. M. VAN DEENEN, *Biochemistry*, 6 (1967) 1114.
14 L. ZERVAS AND I. DILARIS, *Chem. Ber.*, 89 (1956) 925.
15 G. V. MARINETTI, J. ERBLAND AND J. KOCHEN, *Federation Proc.*, 16 (1957) 837.
16 M. B. ABRAMSON, W. T. NORTON AND R. KATZMAN, *J. Biol. Chem.*, 240 (1965) 2389.
17 J. Y. KIYASU, R. A. PIERINGER, H. PAULUS AND E. P. KENNEDY, *J. Biol. Chem.*, 238 (1963) 2293.
18 Y.-Y. CHANG AND E. P. KENNEDY, *J. Lipid Res.*, 8 (1967) 447.
19 Y.-Y. CHANG AND E. P. KENNEDY, *J. Lipid Res.*, 8 (1967) 456.
20 F. HAVERKATE AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 106 (1965) 78.
21 F. HAVERKATE AND L. L. M. VAN DEENEN, *Biochim. Biophys. Acta*, 84 (1964) 106.
22 J. KANFER AND E. P. KENNEDY, *J. Biol. Chem.* 239 (1964) 1720.
23 M. K. WASSEF, J. SARNER AND M. KATES, *Can. J. Biochem.*, in the press.
24 M. R. STETTEN AND D. ROUNBEHLER, *J. Biol. Chem.*, 243 (1968) 1823.
25 A. KORNBERG AND W. E. PRICER, JR., *J. Biol. Chem.* 204 (1953) 345.
26 M. KATES, in G. V. MARINETTI, *Lipid Chromatographic Analysis*, Vol. 1, Marcel Dekker, New York, 1967, p. 1.
27 E. BAER, *Biochem. Prep.*, 2 (1952) 31.
28 J. C. SOWDEN AND H. O. L. FISCHER, *J. Am. Chem. Soc.*, 63 (1941) 3244.
29 R. J. HOWE AND T. MALKIN, *J. Chem. Soc.*, (1951) 2662.
30 M. KATES, T. H. CHAN AND N. Z. STANACEV, *Biochemistry*, 2 (1963) 394.
31 W. E. M. LANDS AND A. ZSCHOCKE, *J. Lipid Res.*, 6 (1965) 324.
32 M. KATES, B. PALAMETA, M. B. PERRY AND G. A. ADAMS, *Biochim. Biophys. Acta*, 137 (1967) 213.
33 L. W. HESSEL, I. D. MORTON, A. R. TODD AND P. E. VERKADE, *Rec. Trav. Chim.*, 73 (1954) 150.