

Synthesis of sulfate esters of phosphatidylglycerol (diphytanyl ether analog)

A. J. Hancock¹ and M. Kates²

Department of Biochemistry, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5

Abstract Synthesis of 1-*sn*-phosphatidyl-3'-*sn*-glycero-1'-sulfate (phosphatidylglycero-1-sulfate) was achieved by mono-sulfation of 1-*sn*-phosphatidyl-3'-*sn*-glycerol (diphytanyl ether analog) with an equimolar amount of SO₃-pyridine complex at room temperature; with excess sulfation reagent at 60°C, the 1',2'-disulfate ester was obtained. The phosphatidylglycero-2-sulfate isomer was synthesized by an unambiguous route starting from the bacterial 2,3-di-*O*-phytanyl-*sn*-glycerol. The synthetic phosphatidylglycerosulfates were characterized by analytical, chromatographic, optical rotatory, and spectral (infrared and NMR) data and compared with the phosphatidylglycerosulfate isolated from *Halobacterium cutirubrum*.

Supplementary key words phosphosulfolipid · halophilic bacteria · 1-*sn*-phosphatidyl-3'-*sn*-glycero-1'-sulfate · 1-*sn*-phosphatidyl-1'-*sn*-glycero-2'-sulfate · 1-*sn*-phosphatidyl-3'-*sn*-glycero-1',2'-disulfate · 2-*O*-myristoyl-3-*O*-benzyl-*sn*-glycerol · sulfation of phosphatidylglycerol · 3'-*O*-benzyl phosphatidylglycerol · NMR spectra · infrared spectra · optical rotations · thin-layer chromatography

IN THE accompanying paper (1) we described the isolation and characterization of a new phosphosulfolipid in the extreme halophile, *Halobacterium cutirubrum*. Structural studies (1) indicated that this lipid was probably a

This work forms part of a Ph.D. thesis submitted by A. J. Hancock to the Department of Biochemistry, University of Ottawa, for the Ph.D. degree. Supported by grant A-5324 from the National Research Council of Canada.

Abbreviations: TLC, thin-layer chromatography; NMR, nuclear magnetic resonance; PG, phosphatidylglycerol; PGS, phosphatidylglycerosulfate; PG-1-S, 1-*sn*-phosphatidyl-3'-*sn*-glycero-1'-sulfate; PG-2-S, 1-*sn*-phosphatidyl-1'-*sn*-glycero-2'-sulfate; PG-1,2-di-S, 1-*sn*-phosphatidyl-3'-*sn*-glycero-1',2'-disulfate; TPS, 2,4,6-triisopropylbenzenesulfonyl chloride.

¹ Present address: Department of Biochemistry, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106.

² To whom requests for reprints should be addressed.

primary sulfate ester of *sn*-2,3-diphytanyl-glycero-1-phosphoryl-*sn*-3'-glycerol (phosphatidylglycerol, diether analog) (PG). Final proof of this structure was obtained by comparison (1) of the bacterial lipid with the synthetic *sn*-1- and *sn*-2-sulfate esters of PG. We present here, in detail, the procedures for synthesis of these compounds. The synthesis of the 1,2-disulfate ester of PG is also included for comparison. The procedures used are summarized in Schemes 1 and 2.

MATERIALS AND METHODS

The SO₃-pyridine complex and 2,4,6-triisopropylbenzenesulfonyl chloride (TPS) were products of Aldrich Chemical Co. (Milwaukee, Wis.).

Phosphorus was determined by the method of Allen (2), and sulfate by the barium chloranilate procedure of Spencer (3), as described in the accompanying paper (1).

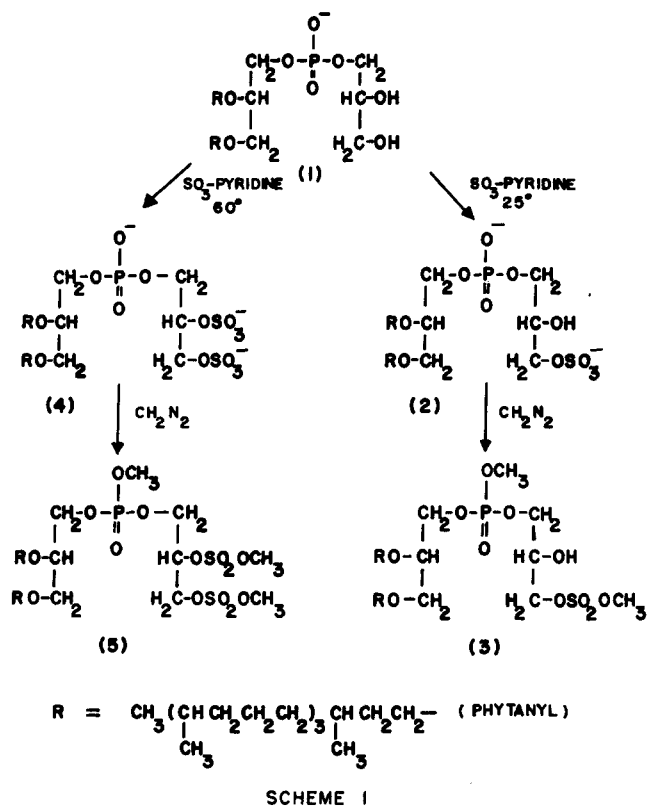
Optical rotations were measured in a Perkin-Elmer 141 automatic-readout polarimeter. Infrared spectra were taken in solution with a Beckman IR-10 spectrophotometer. 100-MHz NMR spectra were recorded for dilute solutions in [2H]chloroform with a Varian HA-100 NMR spectrometer equipped with a ³¹P-¹H spin decoupler. Chemical shifts are expressed as ppm(δ) relative to trimethylsilane taken as zero.

EXPERIMENTAL PROCEDURES

SYNTHESIS OF PHOSPHATIDYLGLYCERO-1-SULFATE (PG-1-S) (Scheme 1)

Bacterial 2,3-diphytanyl-*sn*-glycero-1-phosphoryl-*sn*-3'-glycerol (PG) (1)

This lipid was isolated from total polar lipids of *H. cutirubrum* as a "mixed" (Na, Mg, NH₄) salt, as described in the accompanying paper (1).

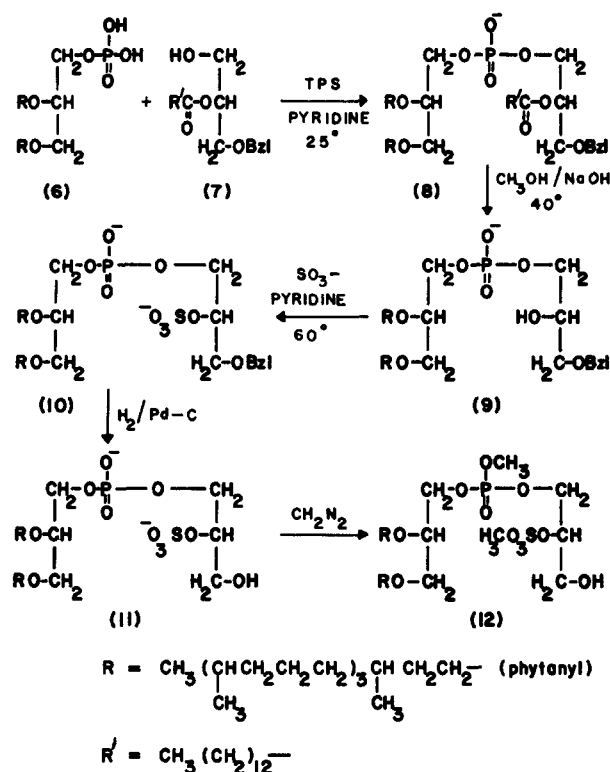


SCHEME 1

Synthesis of phosphatidylglycero-1-sulfate and phosphatidylglycero-1,2-disulfate (diphytanyl ether analogs).

2,3-Diphytanyl-*sn*-1-glycerophosphoryl-*sn*-3'-glycero-1'-sulfate (PG-1-S) (2)

A solution of "mixed" salts of PG (1; 42 mg, 52 μmoles) in 5 ml of anhydrous benzene was stirred with SO_3 -pyridine complex reagent (9.5 mg, 60 μmoles) at room temperature for 6 hr (see Scheme 1). The reaction mixture showed a major spot on TLC (R_F 0.38 in chloroform-methanol-90% acetic acid 30:4:20 [v/v/v]) corresponding to the monosulfated product and only traces of disulfated product (R_F 0.12). The mixture was cleared by centrifugation, concentrated in a nitrogen stream, and chromatographed on preparative silica gel H plates in chloroform-methanol-concentrated ammonium hydroxide 65:35:5 (v/v/v). The monosulfated product was eluted with 250 ml of chloroform-methanol-diethyl ether 1:1:1 (v/v/v) and the eluate was evaporated to dryness in the presence of benzene; the residue was dissolved in 10 ml of chloroform-methanol 1:1 (v/v), cleared by centrifugation, and diluted with 4.5 ml of 0.5 N aqueous HCl. After brief centrifugation of the biphasic system, the chloroform phase was neutralized with 0.2 N methanolic KOH, diluted with benzene, and concentrated to a small volume (ca. 0.5 ml), which was diluted with 10 vol of acetone. After several hours at 0°C , the precipitated potassium salt was centrifuged down, washed with cold acetone, and dried in vacuo to



SCHEME 2

Synthesis of phosphatidylglycero-2-sulfate (diphytanyl ether analog).

give 38 mg (39 μmoles , 75% yield) of chromatographically pure compound (2) (white powder). The synthetic product was indistinguishable on TLC from the bacterial PGS (Table 1) and gave analytical data expected for the

TABLE 1. Chromatographic mobilities of sulfated derivatives of phosphatidylglycerol

Compound	R_F Value in Solvent System:			
	1 ^a	2 ^a	3 ^a	4 ^b
Phosphatidylglycero-1-sulfate (bacterial)	0.30	0.33	0.00	0.41
Phosphatidylglycero-1-sulfate (synthetic) (2)	0.30	0.33	0.00	0.41
Phosphatidylglycero-2-sulfate (11)	0.33	0.39	0.00	0.44
Phosphatidyl-3-O-benzyl- <i>sn</i> -glycero-2-sulfate (10)	0.48	0.54	0.00	
Phosphatidylglycerol-1,2-disulfate (4)	0.10	0.12	0.00	0.15
Phosphatidylglycerol (1)	0.67	0.66	0.00	0.68
Phosphatidylglycero-1-sulfate, dimethyl ester (bacterial)			0.68	
Phosphatidylglycero-1-sulfate, dimethyl ester (synthetic) (3)			0.68	
Phosphatidylglycerol, monomethyl ester			0.52	

^a TLC on silica gel H in solvent systems: 1, chloroform-methanol-90% acetic acid 30:4:20 (v/v/v); 2, chloroform-methanol-conc ammonium hydroxide 65:35:5 (v/v/v); 3, chloroform-methanol-water 90:10:1 (v/v/v).

^b Chromatography on silicic acid-impregnated paper in solvent system 4: diisobutylketone-acetic acid-water 40:25:5 (v/v/v).

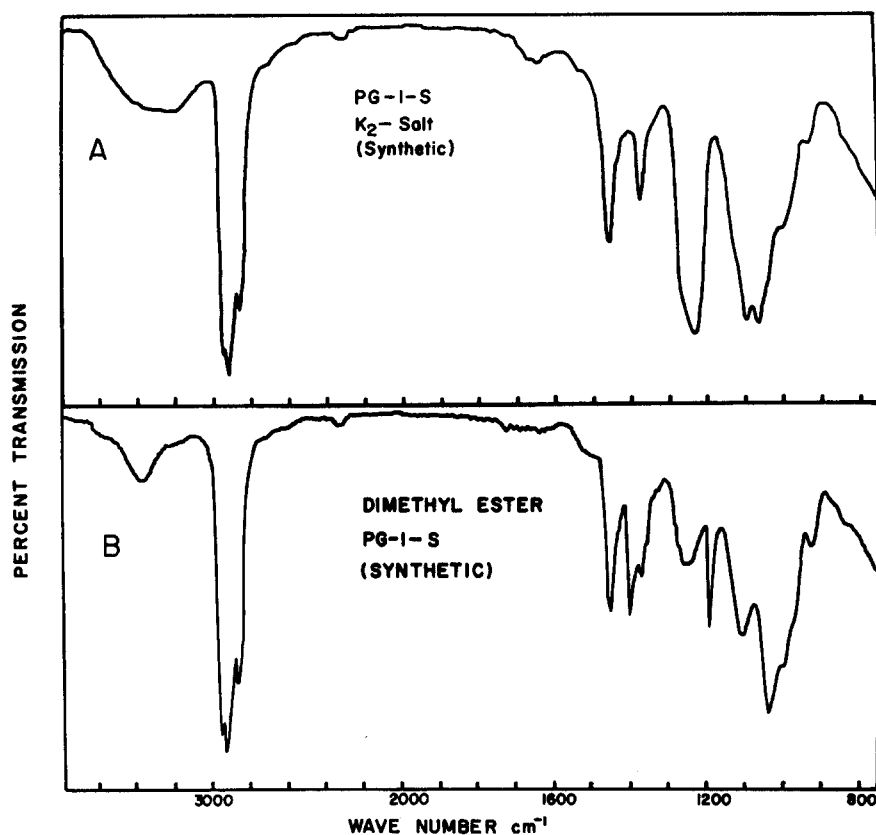


FIG. 1. Infrared spectra (CCl_4) of synthetic phosphatidylglycero-1-sulfate. A, dipotassium salt; B, dimethyl ester.

TABLE 2. Analytical data for synthetic phosphatidylglycerosulfates

Data	PG-1-S, K Salt		PG-2-S, K Salt		PG-1,2-di-S, K Salt	
	Found	Calcd ^a	Found	Calcd ^a	Found	Calcd ^b
C, %	56.58	56.29	56.13	56.29	49.96	50.24
H, %	9.96	9.76	9.21	9.76	8.03	8.62
P, %	3.30	3.16	3.28	3.16	2.86	2.82
S, %	3.42	3.27	3.52	3.27	5.61	5.83
K, %	8.71	7.96			11.0	10.7
S/P atomic ratio	1.00	1.00	1.04	1.00	1.90	2.00
K/S+P atomic ratio	1.05	1.00			1.05	1.00

^a Calculated for $\text{C}_{46}\text{H}_{98}\text{O}_{11}\text{PSK}_2 \cdot \text{H}_2\text{O}$ (981.47).

^b Calculated for $\text{C}_{46}\text{H}_{92}\text{O}_{14}\text{PS}_2\text{K}_2 \cdot \text{H}_2\text{O}$ (1099.6).

TABLE 3. Optical rotations^a for potassium salts of sulfated derivatives of phosphatidylglycerol

Compound ^b (K Salts)	589 nm	578 nm	546 nm	436 nm	365 nm
Phosphatidylglycero-1-sulfate (bacterial) ^c	+2.02°	+2.14°	+2.29°	+4.14°	+6.29°
1- <i>sn</i> -Phosphatidyl-3'- <i>sn</i> -glycero-1'-sulfate (synthetic)	+2.88°	+2.97°	+3.32°	+5.53°	+8.50°
1- <i>sn</i> -Phosphatidyl-3'- <i>sn</i> -glycero-1',2'-disulfate	+4.06°	+4.13°	+4.31°	+6.96°	+11.10°

^a In chloroform solution at 22°C.

^b All compounds are derivatives of 2,3-di-*O*-phytanyl-*sn*-glycero-1'-phosphoryl-*sn*-3'-glycerol. Data for synthetic 1-*sn*-phosphatidyl-3'-*sn*-glycero-2'-sulfate are not available.

^c Data from previous paper (1).

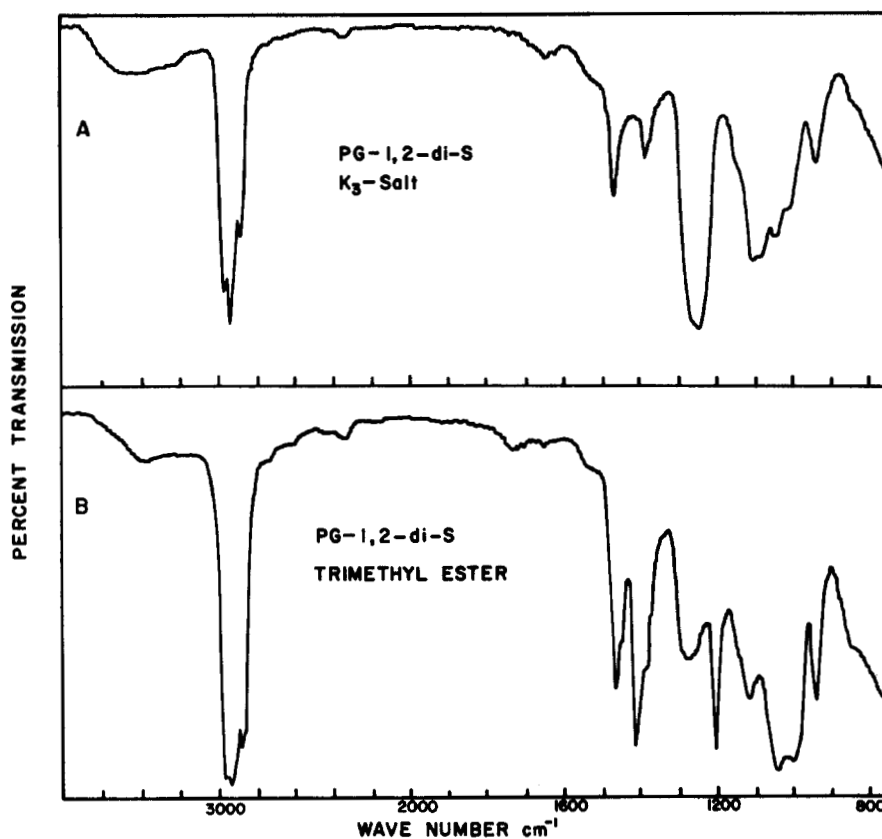


FIG. 2. Infrared spectra (CCl_4) of synthetic phosphatidylglycero-1,2-disulfate. *A*, tripotassium salt; *B*, trimethyl ester.

potassium salt of PGS (Table 2). Its optical rotations are given in Table 3 and its infrared spectrum is shown in Fig. 1*A*.

Dimethyl ester of synthetic PG-1-S (3)

Synthetic PG-1-S (8 mg) was converted to its dimethyl ester with diazomethane as described for the bacterial PGS (1). The R_F of the dimethyl ester is given in Table 1, and its infrared and NMR spectra are shown in Figs. 1*B* and 4*A*, respectively.

2,3-Di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-3'-*sn*-glycero-1',2'-disulfate (PG-1,2-di-S) (4)

A solution of "mixed" salts of PG (1) (25 mg, 30 μmoles) in 5 ml of anhydrous benzene was stirred with sulfur trioxide-pyridine reagent (16 mg, 0.10 mmole) at 60°C for 1 hr (see Scheme 1). The mixture was centrifuged, the supernatant solution was concentrated in a nitrogen stream, and the product was chromatographed on preparative TLC plates in chloroform-methanol-concentrated ammonium hydroxide 65:35:5 (v/v/v) to remove traces of monosulfated product (R_F 0.12 and 0.33 for PG-1,2-di-S and PG-1-S, respectively). The disulfate was eluted from the silica and converted to its tripotassium salt as described for the monosulfated derivative. The precipitate was washed with cold acetone and dried

in vacuo to give 18 mg (17 μmoles , 57% yield) of chromatographically pure white powder. The tripotassium salt of PG-1,2-di-S had the mobilities in various solvents given in Table 1; its analytical data, optical rotation values, and infrared spectrum are given in Tables 2 and 3 and Fig. 2*A*, respectively.

Trimethyl ester of PG-1,2-di-S (5)

PG-1,2-di-S (6 mg) was converted to its methyl ester with diazomethane as described for PG-1-S. The infrared spectrum of the trimethyl ester is shown in Fig. 2*B* and the NMR spectrum in Fig. 4*B*.

SYNTHESIS OF PHOSPHATIDYLGLYCERO-2-SULFATE (PG-2-S) (Scheme 2)

2,3-Di-*O*-phytanyl-*sn*-glycerophosphoric acid (6)

This compound was prepared by phosphorylation of the natural diphytanyl glycerol ether (2,3-di-*O*-phytanyl-*sn*-glycerol [4]) as described by Kates et al. (5). The potassium salt had $[\alpha]_D^{25} = +1.75^\circ$ (3.1 g/dl in chloroform); reported $[\alpha]_D^{25} = +1.78^\circ$ (5).

Analysis: $\text{C}_{43}\text{H}_{87}\text{O}_6\text{PK}_2 \cdot \text{H}_2\text{O}$ (827.32);

calculated: P, 3.74

found: P, 3.80

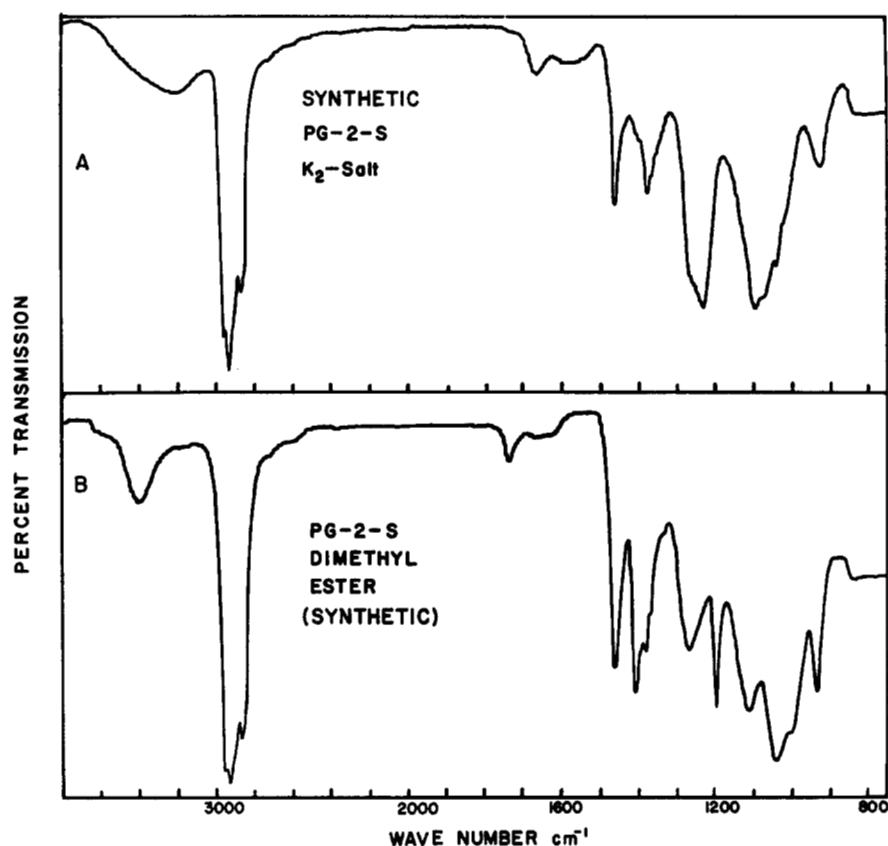


FIG. 3. Infrared spectra (CCl_4) of synthetic phosphatidylglycero-2-sulfate. A, dipotassium salt; B, dimethyl ester.

The potassium salt was converted to the free acid form as described elsewhere (5).

2-O-Myristoyl-3-O-benzyl-*sn*-glycerol (7)

This substance was prepared as described for the stearoyl compound by De Haas and van Deenen (6) by monoacylation of 3-O-benzyl-*sn*-glycerol (7); it was separated from the 3-O-myristoyl isomer by preparative TLC using sodium acetate-impregnated plates to prevent acyl migration. Examination of the product by NMR as described previously (6) showed that it contained only a trace of the 3-O-myristoyl isomer.

2,3-Di-O-phytanyl-*sn*-glycero-1-phosphoryl-1'-(2'-O-myristoyl-3'-O-benzyl)-*sn*-glycerol (8)

The dried free acid form of 2,3-di-O-phytanyl-*sn*-glycerophosphate (6), prepared from the potassium salt (120 mg, 0.16 mmole), was mixed with a solution of 2-O-myristoyl-3-O-benzyl-*sn*-glycerol (7) (125 mg, 0.32 mmole) in 10 ml of anhydrous pyridine, and the mixture was brought to dryness under reduced pressure. The residue was dried in vacuo over phosphorus pentoxide for 12 hr and then stirred with 20 ml of anhydrous pyridine and dry TPS (194 mg, 0.64 mmole) at room temperature for 8 hr (Scheme 2). The mixture was then cooled on ice, stirred with 1 ml of water for 30 min, and

evaporated to dryness under reduced pressure. Trituration of the residual oil with cold diethyl ether precipitated triisopropylbenzenesulfonic acid, which was removed by centrifugation and washed twice with diethyl ether. The combined ethereal supernates were washed with water, 0.5 N hydrochloric acid, again with water, and brought to dryness under reduced pressure in the presence of benzene.

The residual oil on TLC in chloroform-methanol-concentrated ammonium hydroxide (80:20:2, v/v/v) showed a major phosphate-positive spot with R_F 0.70. The crude product was purified by preparative TLC first in chloroform-diethyl ether 3:1 (v/v) (R_F 0.00) then in chloroform-methanol-concentrated ammonium hydroxide 90:10:1 (v/v/v) (R_F 0.30) in the same direction. The product was eluted with chloroform-methanol-diethyl ether 1:1:1 (v/v/v), converted to the potassium salt, and precipitated by acetone, as described for PG δ (1). The oily precipitate was washed with cold acetone and dried in vacuo; yield, 137 mg (0.2 mmole, 75%) of chromatographically pure product (8) having $[\alpha]_D^{22} = +2.90^\circ$ (4.1 g/dl in chloroform).

Analysis: $\text{C}_{67}\text{H}_{126}\text{O}_9\text{PK}$ (1145.8); calculated: P, 2.70
found: P, 2.66

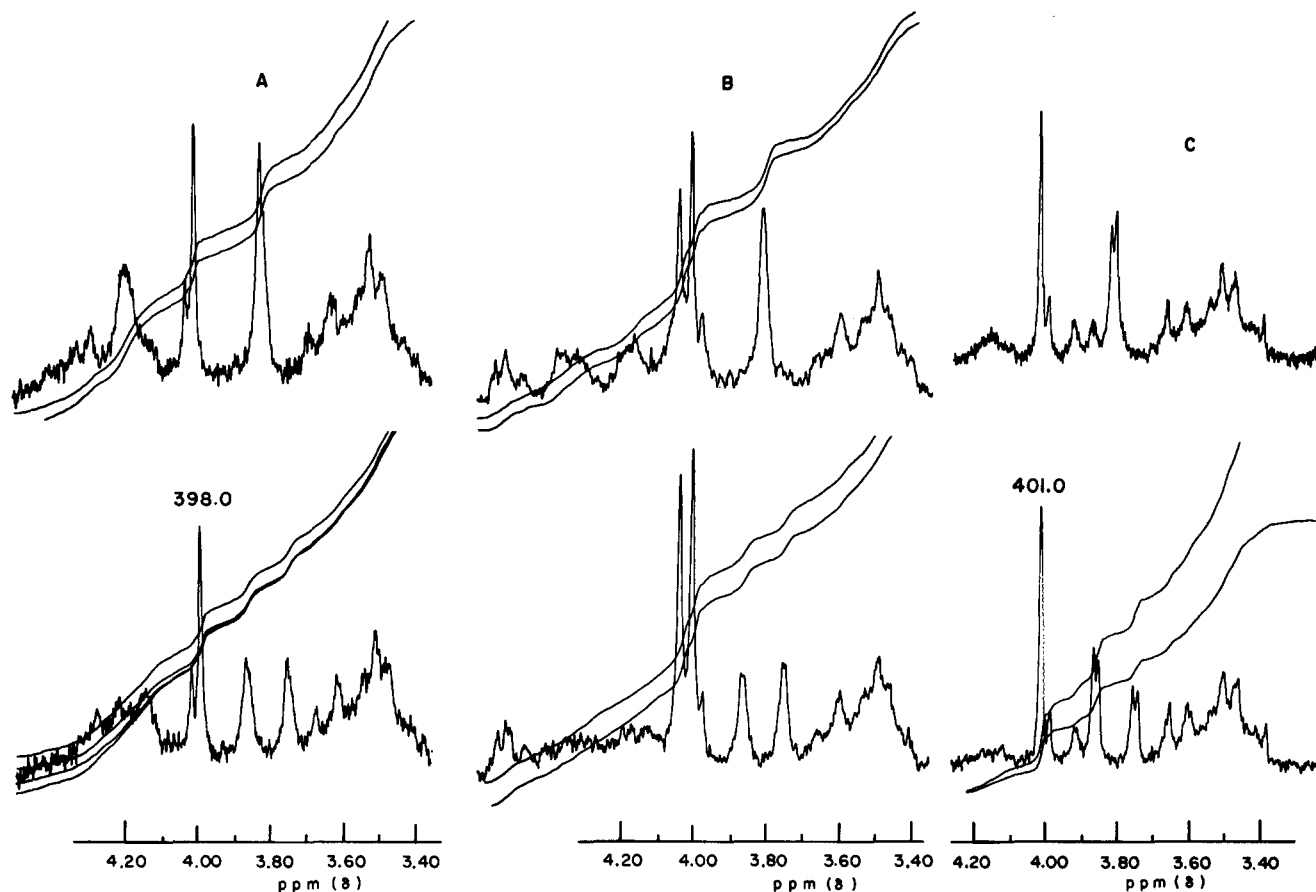


FIG. 4. NMR spectra (expanded downfield region; sweep width, 250 Hz) in ^2H chloroform of: *A*, synthetic phosphatidylglycero-1-sulfate dimethyl ester; *B*, phosphatidylglycero-1,2-disulfate, trimethyl ester; and *C*, phosphatidylglycero-2-sulfate. Top spectra are ^{31}P - ^1H decoupled; bottom spectra are normal.

The infrared spectrum (liquid film) of compound (**8**) showed absorption for the following groups: ester carbonyl (1740 cm^{-1}), aromatic (3025 , 2065 , 3095 , 1495 , 690 cm^{-1}), phytanyl (2850 , 2930 , 2960 , 1455 , 1375 – 1365 [doublet] cm^{-1}), myristoyl (CH_2) $_n$ (730 cm^{-1}), bonded $\text{P}=\text{O}$ (1240 cm^{-1}), $\text{C}-\text{O}-\text{C}$, $\text{P}-\text{O}^-$ (1100 cm^{-1}), and $\text{P}-\text{O}-\text{C}$ (1055 cm^{-1}). Hydroxyl absorption was absent.

2,3-Di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-1'-(3'-*O*-benzyl)-*sn*-glycerol (3-*O*-benzyl-PG) (**9**)

The 2-*O*-myristoyl derivative (**8**) was deacylated by a modification of the procedure described elsewhere (**8**), as follows: a solution of (**8**) (potassium salt; 137 mg, 0.12 mmole) in 2.0 ml of chloroform-methanol 2:3 was treated with 2.0 ml of 0.2 *N* methanolic NaOH at 40°C . Deacylation was complete after 40 min (R_f values of [**8**] and deacylated product [**9**] were 0.70 and 0.48, respectively, in chloroform-methanol-concentrated ammonium hydroxide 80:20:2 [v/v/v]); 3.2 ml of chloroform, 0.8 ml of methanol, and 3.6 ml of 0.5 *N* aqueous HCl were then added successively to the cooled reaction

mixture. The biphasic system was briefly centrifuged, and the chloroform phase was neutralized with 0.2 *N* methanolic KOH and concentrated to a small volume. The residual oil was diluted with acetone, and the precipitated phospholipid product (**9**) was centrifuged down, washed with cold acetone to remove traces of methyl myristate, and dried in vacuo; yield, 89 mg of oily product (0.095 mmole, 79%). An analytical sample was obtained by preparative TLC in chloroform-methanol-concentrated ammonium hydroxide 80:20:2 (v/v/v), followed by reconversion to the potassium salt as described above. It had $[\alpha]_D^{22} = +1.0^\circ$ (2.43 g/dl in chloroform).

Analysis: $\text{C}_{65}\text{H}_{100}\text{O}_8\text{PK}$ (935.41);

calculated: C, 68.05; H, 10.78; P, 3.31
found: C, 67.85; H, 10.21; P, 3.43

The infrared spectrum closely resembled that of the myristoyl derivative (**8**) except for the absence of carbonyl and (CH_2) $_n$ absorption (1740 and 730 cm^{-1} , respectively) and the presence of hydroxyl absorption (3280 cm^{-1} , broad).

2,3-Di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-1'-*sn*-glycero-3'-*O*-benzyl-2'-sulfate (10)

A solution of compound (9) (potassium salt; 85 mg, 0.091 mmole) in 15 ml of anhydrous benzene was stirred at 60°C for 3 hr with SO₃-pyridine complex reagent (30 mg, 0.19 mmole). The cooled mixture was cleared by centrifugation, the residue was washed with benzene, and the combined supernatant solutions were concentrated to a small volume under reduced pressure. The crude product was purified by preparative TLC in chloroform-methanol-concentrated ammonium hydroxide 80:20:2 (v/v/v) (*R_F* 0.18), and the desired product was eluted from the silica and converted to the potassium salt form as described for compound (9); yield of chromatographically pure compound (10) (white powder) was 75 mg (0.071 mmole, 75%); [α]_D²⁵ = -2.38° (2.10 g/dl in chloroform).

Analysis: C₅₃H₉₉O₁₀PSK₂·H₂O (1055.6);
calculated: C, 60.30; H, 9.64; P, 2.93; S, 3.04;
S/P atomic ratio, 1.00
found: C, 60.91; H, 9.24; P, 2.95; S, 3.23;
S/P atomic ratio, 1.06

The infrared spectrum resembled that of compound (9) except for the relatively more intense absorption band centered at 1240 cm⁻¹ (P=O, and S=O asymmetric stretch) and the sulfate bands at 1045 cm⁻¹ (shoulder, symmetric stretch S=O), 940 cm⁻¹ (C—O—S), and 840 cm⁻¹ (C—O—S, weak); hydroxyl absorption was absent.

Dimethyl ester of 3-*O*-benzyl-PG-2-sulfate

The dimethyl ester was prepared as described for PG-1-S. The infrared spectrum showed sulfate absorption at 1405 and 1195 cm⁻¹ and at 935 cm⁻¹ (intense, C—O—S, secondary sulfate). Hydroxyl absorption was absent. NMR assignments: phytanyl (78 H), 0.75–1.78 δ ; P—OCH₃, doublet 3.72, 3.83 δ (which collapsed to singlet 3.78 δ on ³¹P-¹H spin decoupling); S—OCH₃, singlet 3.94 δ ; —OCH₂C₆H₅, 4.57; —OCH₂C₆H₅, 7.30 δ .

2,3-Di-*O*-phytanyl-*sn*-glycero-1-phosphoryl-1'-*sn*-glycero-2'-sulfate (PG-2-S)

3-*O*-Benzyl phosphatidylglycerosulfate (10) (potassium salt; 40 mg, 0.38 mmole) was hydrogenolyzed with palladium-charcoal catalyst (4) in 6 ml of chloroform-methanol 1:1 (v/v) at room temperature and pressure. After 30 min, the catalyst was removed by centrifugation and washed with 4 ml of chloroform-methanol 1:1 (v/v); the combined supernatant solutions were immediately diluted with 4.5 ml of 0.1 N aqueous HCl, and the biphasic system was briefly centrifuged. The chloroform phase was neutralized with 0.2 N methanolic KOH, and the potassium salt of the PG-2-S (11) was isolated

as described for compound (10). Yield, 35 mg (94%); [α]_D²⁵ = -6.08° (1.65 g/dl in chloroform).

Analytical data for PG-2-S potassium salt are given in Table 2. The PG-2-S migrated as a single spot in three solvent systems (see Table 1); in each system the PG-2-S had slightly higher mobilities than the bacterial PG-1-S. The infrared spectrum of PG-2-S potassium salt is shown in Fig. 3A.

Dimethyl ester of PG-2-S (12)

The ester was prepared as described for the PG-1-S. The infrared and NMR spectra are shown in Figs. 3B and 4C, respectively.

Bacterial PGS

The "natural" form of PGS was isolated from the lipids of *H. cutirubrum* as described in the accompanying paper (1).

RESULTS AND DISCUSSION

The synthesis of the PG-1-sulfate was carried out by direct sulfation of the pure bacterial phosphatidylglycerol (1) (diphytanyl ether analog) (Scheme 1), using an equimolar amount of SO₃-pyridine complex at room temperature. Under these conditions, monosulfation occurred almost exclusively (90–95%); the resulting PGS (2) was isolated as the dipotassium salt in good yield (ca. 75%) in a chromatographically (Table 1) and analytically (Table 2) pure state. The infrared spectrum of the dipotassium salt, as well as of the dimethyl ester (3) (Fig. 1), showed absorption at 980 cm⁻¹ (shoulder), indicative of a primary sulfate, and only weak absorption at 935 cm⁻¹, attributable to a secondary sulfate (9). The major S—OCH₃ signal at δ 3.98 in the NMR spectrum of the methyl ester (Fig. 4A) could therefore be assigned to a primary S—OCH₃ group and the minor S—OCH₃ signal at δ 4.01 to a secondary sulfate group. It appeared then that monosulfation of PG occurred predominantly at position 1 and only to a limited extent (ca. 8%) at position 2.

Sulfation of PG at 60°C with an excess of SO₃-pyridine complex (Scheme 1) gave the chromatographically (Table 1) and analytically (Table 2) pure PG-1,2-disulfate (4). Its infrared spectrum (Fig. 2) showed both primary and secondary sulfate C—O—S bands (995 and 940 cm⁻¹, respectively), and its NMR spectrum (Fig. 4B) had two well-resolved S—OCH₃ signals of equal intensity at δ 4.01 ppm. These data indicated that discrete S—OCH₃ signals (differing by 3 Hz) in the NMR spectrum are in fact given by primary and secondary sulfate esters, the assignments for these groups being most likely the upfield and downfield signals, respectively.

To establish the assignments for primary and secondary monosulfate S—OCH₃ signals in the NMR spectrum unequivocally, the synthesis of the phosphatidylglycero-2-sulfate was carried out by the unambiguous route shown in Scheme 2. In this procedure, bacterial 2,3-di-*O*-phytanyl-*sn*-glycerol ether was converted (5) to diphytanyl ether phosphatidic acid (6), which was readily condensed in the presence of TPS in dry pyridine (10) with 2-*O*-myristoyl-3-*O*-benzyl-*sn*-glycerol (7) to give the acyl PG benzyl ether (8). The latter was deacylated in warm methanolic NaOH, and the benzylated product (9) was sulfated with SO₃-pyridine complex in dry benzene at 60°C. Debenzylation of the resulting compound (10) was readily achieved by hydrogenolysis over palladium-charcoal catalyst, and the synthetic PG-2-S³ (11) was isolated as its potassium salt or converted to its methyl ester (12). The chromatographically (Table 1) and analytically (Table 2) pure product obtained showed only the secondary sulfate absorption band (935 cm⁻¹) in the infrared (Fig. 3) and gave a single S—OCH₃ NMR signal at δ 4.01 ppm (Fig. 4C). The upfield S—OCH₃ signal (δ 3.98 ppm, Fig. 4) may be assigned unambiguously to the primary sulfate ester group, and the synthetic monosulfated PG (2) must therefore be the PG-1-S isomer. Since only the synthetic PG-1-S isomer was found to be identical with the PGS isolated from *H. cutirubrum* (Tables 1–3 and Figs. 1 and 4; see Ref. 1) the natural compound must have the structure 1-*sn*-phosphatidyl-3'-*sn*-glycero-1'-sulfate (diphytanyl ether analog) (see Fig. 4, Ref. 1).

³ This compound has the configuration *sn*-1-phosphatidyl-1'-*sn*-glycero-2'-sulfate. The *sn*-1-phosphatidyl-3'-*sn*-glycero-2'-sulfate isomer would have been more appropriate, but its synthesis required the 1-*O*-benzyl-*sn*-glycerol (11) as starting material, which was less readily accessible than the 3-*O*-benzyl isomer (7) used here. NMR and IR spectral assignments for the secondary sulfate are, however, independent of the configuration of the sulfated glycerol moiety.

The authors are indebted to Mr. F. McClusky for the NMR spectra, and to Mrs. E. Szabo and Mr. G. Ben-Tchavtchavdze for the figures. Elemental analyses were performed by A. Bernhardt, Mikroanalytisches Laboratorium, Elbach über Engelskirchen, West Germany. The support of this work by a grant (A-5324) from National Research Council of Canada is gratefully acknowledged.

Manuscript received 24 October 1972; accepted 27 February 1973.

REFERENCES

- Hancock, A. J., and M. Kates. 1973. Structure determination of the phosphatidylglycerosulfate (diether analog) from *Halobacterium cutirubrum*. *J. Lipid Res.* **14**: 422–429.
- Allen, R. J. L. 1940. The estimation of phosphorus. *Biochem. J.* **34**: 858–865.
- Spencer, B. 1960. The ultramicro determination of inorganic sulfate. *Biochem. J.* **75**: 435–440.
- Joo, C. N., T. Shier, and M. Kates. 1968. Characterization and synthesis of mono- and diphytanyl ethers of glycerol. *J. Lipid Res.* **9**: 782–788.
- Kates, M., C. E. Park, B. Palameta, and C. N. Joo. 1971. Synthesis of diphytanyl ether analogues of phosphatidic acid and cytidine diphosphate diglyceride. *Can. J. Biochem.* **49**: 275–281.
- De Haas, G. H., and L. L. M. van Deenen. 1965. Structural identification of isomeric lysolecithins. *Biochim. Biophys. Acta.* **106**: 315–325.
- Kates, M., T. H. Chan, and N. Z. Stanacev. 1963. Aliphatic diether analogs of glyceride-derived lipids. I. Synthesis of *D*- α,β -dialkyl glyceryl ethers. *Biochemistry.* **2**: 394–397.
- Marshall, M. O., and M. Kates. 1972. Biosynthesis of phosphatidylglycerol by cell-free preparations from spinach leaves. *Biochim. Biophys. Acta.* **260**: 558–570.
- Haines, T. H. 1971. The chemistry of the sulfolipids. *Progr. Chem. Fats Other Lipids.* **11**(3): 299–345.
- Aneja, R., and A. P. Davies. 1970. The synthesis of a spin-labelled glycerophospholipid. *Chem. Phys. Lipids.* **4**: 60–71.
- Gigg, J., and R. Gigg. 1967. 1-*O*-Benzyl-L-glycerol and *D*-(glycerol 1,2-carbonate). *J. Chem. Soc. C.* 1865–1866.