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Displacement Reactions of Cyclic Sulfites and Phosphates by Salts of Weak Acids Applicable to the Synthesis of Phospholipids and Other Natural Substances

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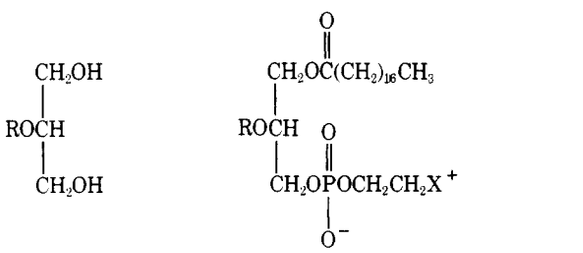
Six-membered ring cyclic sulfites **7a** derived from 2-*O*-benzylglycerol react in Me_2SO with salts of weak acids such as carboxylate and *p*-chlorophenoxide to yield 1-acyl- or 1-*O*-*p*-chlorophenoxy-2-*O*-benzylglycerols **8a**, **8b**, **8c**, and **8d** in good yields. In an analogous manner, the cyclic phenylphosphate **7b** reacts with carboxylate or phenylphosphate to yield the 1-acyl- or 1-phosphoryl-2-*O*-benzylglycerol 3-phosphates **8f** and **8h**. The potential applicability to the synthesis of glycerolphospholipids, e.g., the 2-arachidonoylphosphatidyldimethylethanolamine **2c**, and dinucleotides is demonstrated or discussed.

The structural feature present in glycerol (**1a**), i.e., the 1,3-diol system having at least one primary alcohol group, is present in natural products such as the glycerolphospholipids, sphingolipids, and also in carbohydrates that are found in oligosaccharides, glycolipids, and nucleotides. This paper describes a method which adds to other known and useful methods,[1] for the esterification and phosphorylation of the glycerol moiety.[2]

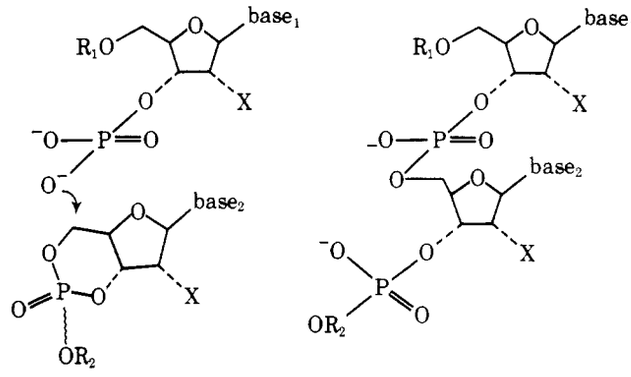
One of the purposes of this investigation was to facilitate the large-scale synthesis of glycerolphospholipids which would be esterified at C-1 and C-2 with a saturated and a polyunsaturated fatty acid, respectively. Such glycerolphospholipids play an important functional role in cell membranes in maximizing the activity of enzymes.[3] In addition, certain glycerolphospholipids, e.g., **2b**, esterified at C-2 with arachidonic acid, have been postulated as the immediate precursors of the fatty acids which are subsequently converted to prostaglandins.[4,5]



1 R	2 X	R
a H	a $N(CH_3)_3$	$CO(CH_2)_3(C{=}CCH_2)_4(CH_2)_3CH_3$
b CH_2Ph	b $NH(CH_3)_2$	H
	c $NH(CH_3)_2$	$CO(CH_2)_3(C{=}CCH_2)_4(CH_2)_3CH_3$

Another purpose of this study was to find a model reaction which may serve to indicate the potential that a nucleotide monophosphate ester may have in displacing a second cyclic 3′,5′-phenyltriphosphate ester to form a dinucleotide under relatively neutral conditions. This reaction which is under investigation may formally be represented by the general equations in Scheme I.

Scheme I



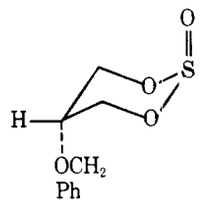
X = H or OH

R_1, R_2 = appropriate protecting group

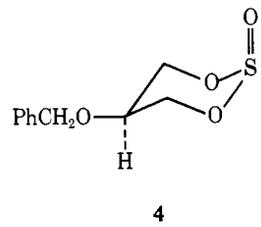
The result of this investigation is a new method for the selective acylation or phosphorylation of the 1,3-diol system, typified by glycerol (**1a**). The method involves a new ring opening reaction of cyclic esters, which are derived from sulfurous and phenylphosphoric acid and 2-*O*-benzylglycerol (**1b**), by salts of weak acids, such as carboxylic acids, *p*-chlorophenol, and phenylphosphoric acid.

Synthesis and Stereochemistry of Cyclic Sulfite and Phosphate Esters from 2-*O*-Benzylglycerol. For the purpose of this study, 2-*O*-benzylglycerol (**1b**), which possesses a suitably protected secondary hydroxyl group, was utilized to study the formation of the corresponding cyclic esters derived from sulfurous, phenylphosphoric, and sulfuric acid.

When 2-*O*-benzylglycerol was heated with dimethyl sulfite it was converted to a mixture of diastereomeric cyclic sulfite esters which were separated by chromatography into the 1,3,2-dioxathiane 2-oxides **3** and **4**. A comparison of the NMR spectra of the isomers indicated that the predominant isomer and conformer, present as 67% of the mixture, was **3** in which the bulkier 5-benzyloxy group is in the axial position. In



3

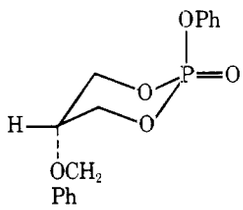


4

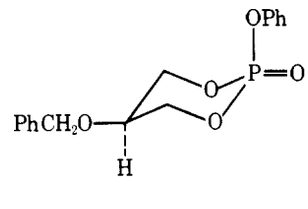
contrast to the values for the absorption frequencies in the NMR spectra for equatorial and axial hydrogen atoms in a cyclohexane ring, equatorial hydrogen atoms in 1,3,2-dioxathiane 2-oxides absorb at higher fields than their axial counterparts.[6] The C_5 hydrogen atom in the predominant isomer **3** exhibits a pentet centered at δ 3.46, which is at higher fields than its axial counterpart in **4**. The small coupling constant (J = 2 Hz) for the pentet of H_5e in **3** is that expected of an equatorial hydrogen coupled to the axial and equatorial hydrogens at C_4 and C_6. The results and assignment of configurations are also consistent with earlier observations that the S=O bond prefers the axial configuration in cyclic sulfite esters in the solution and the solid state, even when the 5 position of a 1,3,2-dioxathiane 2-oxide is occupied by a bulky substituent such as *tert*-butyl.[7]

Preparation of the cyclic sulfate ester from the corresponding sulfite by methods known to convert sulfites to cyclic sulfates,[8,9] e.g., by oxidation with peroxide or calcium permanganate, failed. For this reason, the reactions of the cyclic sulfate were not studied.

The phenylphosphates **5** and **6** were obtained as a mixture when 2-*O*-benzylglycerol was esterified with phenyl phosphodichloridate in pyridine. When the diastereomeric cyclic phosphates were separated by chromatography, the 5-benzyloxy-1,3-dioxa-2-phosphorinanes **5** and **6** were obtained in



5



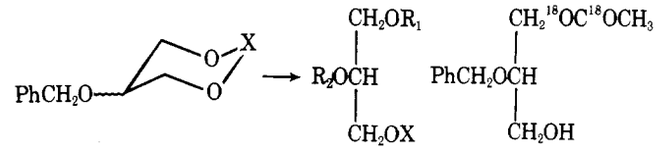
6

a ratio of 2:1, respectively. Their stereochemistry was assigned on the basis of spectral evidence. It has been established that in 2-oxo-2-phenoxy-1,3,2-dioxaphosphorinanes, unlike the sulfite (S=O) counterpart, the P=O bond is more stable in a conformation in which it is equatorial.[10,11] The NMR spectrum of the phosphate **5** parallels the spectrum of the analogous sulfite **3** and shows the absorption of the C-5 hydrogen at higher fields than its diastereomer. The shift (δ 3.60) to higher fields and the small coupling constants for the sextet (J = 1.5 Hz) are those expected of an equatorial proton in a six-membered ring cyclic phosphate having a chairlike conformation.[12]

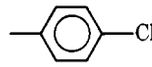
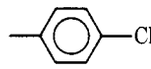
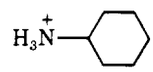
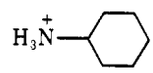
The derived cyclic sulfite and phosphate esters described above were then studied in regard to their nucleophilic displacement by salts of carboxylic acids and *p*-chlorophenoxide in order to achieve a reaction represented formally by **7** → **8**.

Reaction of Carboxylate and *p*-Chlorophenoxide with the Cyclic Sulfite Esters. The reaction of acyclic and cyclic aliphatic sulfites with nucleophiles such as hydroxide and alkoxide anions is known to take place with S–O bond cleavage.[13] On the other hand, nucleophiles such as tertiary amines, chloride and sulfide ions, and phosphines react with sulfites to give products of C–O cleavage.[8,9]

In our studies with carboxylate anions as nucleophiles, when the mixture of cyclic sulfite esters **7a** was heated in Me_2SO at

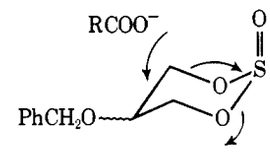


7 X
a S=O
b P=O(OPh)

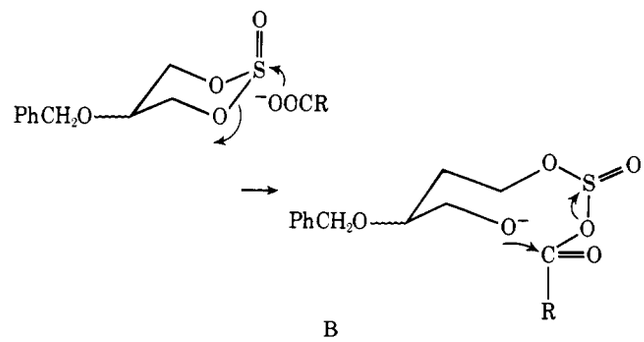
8	R_1	R_2	X
a	$COCH_3$	CH_2Ph	H
b	$COC_{15}H_{31}$	CH_2Ph	H
c	$COC_{17}H_{35}$	CH_2Ph	H
d		CH_2Ph	H
e		H	H
f	$COC_{17}H_{35}$	CH_2Ph	$P(OPh)O_2^-K^+$
g	$COC_{17}H_{35}$	CH_2Ph	$P(OPh)O_2^-Ag^+$
h	$P(OPh)O_2^-N^+(CH_3)_4$	CH_2Ph	$P(OPh)O_2^-N^+(CH_3)_4$
i	$P(OPh)O_2^-$	CH_2Ph	$P(OPh)O_2^-$
		
j	$COC_{17}H_{35}$	CH_2Ph	tosyl

130 °C with potassium acetate, palmitate, or stearate, the esters were converted in high yield to *rac*-1-acetyl-, 1-palmitoyl-, and 1-stearoyl-2-*O*-benzyl-*sn*-glycerol, **8a**, **8b**, and **8c**, respectively. No reaction occurred when the sulfites were heated with lithium stearate in THF solution.

Two mechanisms were considered for the ring opening of the cyclic sulfites. In the reaction with acetate ion, nucleophilic attack on the carbon atom could proceed with C–O cleavage and form an unstable sulfite as illustrated by mechanism A. Alternatively, attack by acetate could occur on the sulfur atom with S–O cleavage. An intermediate mixed anhydride would then form and internally acylate the resulting anion as represented by mechanism B.



A



B

In order to establish the mechanism of the reaction, isotopically labeled **8a** (**9**) was prepared from the sulfite **7a** using acetate in which both oxygen atoms were labeled with ^{18}O. The fragmentation patterns in the mass spectra of unlabeled and labeled acetate **8a** and **9** were studied, focusing on fragments resulting from the cleavage of the oxygen–acyl bond In the unlabeled acetate **8a** these are fragments *m/e* 181 and 43. In the product obtained with labeled acetate **9**, the fragment *m/e* 45 should be obtained regardless of which mechanism is op-

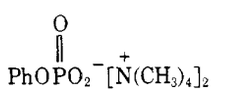
erating. However, the second fragment *m/e* 181 appears as fragment *m/e* 183 in the product only when mechanism A operates, i.e., when C–O bond cleavage occurs. This is true regardless of whether scrambling of the acetyl group occurs during the reaction or mass spectral analysis. The results show that the ratio of the mass peaks 183 and 181 in the labeled and unlabeled product is about 66:1. Thus, fission of the sulfite ester with carboxylate anion must occur predominantly by C–O cleavage, possibly with the assistance of the polar solvent Me_2SO.

In analogous manner to the reaction with carboxylate ion, *p*-chlorophenoxide ion reacted with the cyclic sulfites **7a** and gave **8d** in high yield.[14] When subjected to hydrogenolysis **8d** yielded chlorophenesin (**8e**),[15] a suppressor of humoral antibody response.

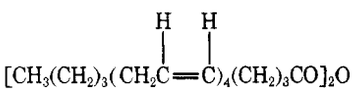
Reaction of Carboxylate and Phenylphosphate with the Cyclic Phosphate Esters 7b. Studies on the reaction of nucleophiles and cyclic phosphate triesters have shown that displacement with hydroxide or alkoxide occurs on the phosphorus atom.[16,17] Cyanide ion is known to attack the five-membered ring ethylene phosphate at the carbon atom with subsequent elimination of acrylonitrile.[18] Only recently has the displacement of a cyclic phosphate with trimethylamine been observed.[19] Nucleophilic displacement by carboxylate anion on cyclic phosphates has not been reported.

In our experiments when a mixture of 1,3-dioxa-2-phosphoranes **7b** was heated with potassium stearate in Me_2SO, the acyl and phosphoryl group were introduced in the desired positions in a single step to yield potassium 1-stearoyl-2-*O*-benzyl-3-phenylphosphorylglycerol (**8f**), isolated in 70% yield as the crystalline silver salt **8g**.

A second type of nucleophile which was prepared and utilized in a reaction with the cyclic phosphates **7b** was the bistetramethylammonium salt of phenylphosphoric acid (**10**). This substance was chosen because of its solubility in organic solvents. Also, being a salt of the weak acid, it would be expected to be a superior nucleophile when compared with a salt derived from diphenylphosphoric acid and, therefore, a more suitable model for the nucleophile in the reaction Scheme I. When the phosphate salt **10** was reacted with the cyclic phosphate **7b** in Me_2SO, a glycerol diphosphate was obtained which was isolated as the bistetramethylammonium glyceroldiphosphate **8h**. It was then converted by ion exchange chromatography to the cyclohexylammonium salt **8i**. A ^{13}C NMR spectrum of **8h** confirmed its symmetrical structure. A phosphorus analysis, ^{31}P NMR, and ^1H NMR spectra were consistent with the structural assignment for **8i**.



10



11

Synthesis of the 2-Arachidonoylglycerolphospholipid 2c. Compounds **8b** and **8c** were demonstrated by others[2,20,22] to be useful intermediates in the synthesis of glycerolphospholipids, possessing a saturated fatty acyl group at C-1 and polyunsaturated fatty acyl group at C-2. The 1-stearoyl-2-*O*-benzylglycerol (**8c**) was converted by way of the monotosylate **8j** to the lysophosphatidyldimethylethanolamine (**2b**), and arachidonic acid was converted to its anhydride **11** with dicyclohexylcarbodiimide in carbon tetrachloride. Reaction of **2b** with **11** in the presence of pyridine and a small amount of 4-dimethylaminopyridine, to facilitate the acylation, yielded the *rac*-2-arachidonoylglycerolphospholipid **2c**.

Experimental Section

Microanalyses were performed by Mr. Emmanuel Zielinski and associates, and spectra were run by Mr. John Damascus and associates of Searle Laboratories.

TLC runs were on 7.6-cm microscope slides covered with a 0.25-mm thickness of Woelm F silica, with a magnesium aluminum silicate binder. Visualization of spots was by phosphomolybdic acid, 5% in EtOH (w/v), followed by heat. Column chromatography used Mallinckrodt SilicAR CC-4 or CC-7 silicic acid. The weight ratio of adsorbent to material was 100:1. Materials were applied as benzene solutions and, unless indicated otherwise, eluted with benzene containing increasing amounts of EtOAc.

NMR spectra were taken on a Varian A-60A, T-60, or XL-100. All spectra are 60 MHz unless specified otherwise. Location of peaks are in parts per million using Me_4Si as an internal standard. The ^{31}P NMR spectrum was obtained through the courtesy of J. N. Shoolery of Varian Associates.

The mass spectral analyses were performed by J. Hribar and W. Aksamit using an AEI MS-30 mass spectrometer (70 eV, 4 kV accelerating volts) by direct insertion probe (source 220 °C).

Dimethyl sulfoxide (distilled in glass) was obtained from the Burdick-Jackson Laboratories, Inc. Melting points were taken on a Fisher-Johns hot-stage apparatus and are uncorrected. Hydrogenations were done by Mr. M. Scaros and Ms. J. Serauskas and chromatography by B. Smith and R. Nicholson and their associates at Searle Laboratories.

2β-Oxo-5α-benzyloxy-1,3-dioxa-2-thiane (3) and 2β-Oxo-5β-benzyloxy-1,3-dioxa-2-thiane (4) or 7a. A mixture of 50 g (0.29 mol) of 2-*O*-benzylglycerol[21] and 32.5 g (0.33 mol) of dimethyl sulfite was heated so that the temperature of the mixture was brought up slowly to 128 °C over a period of 3 h. The reaction was followed by using TLC plates and elution of the plates with ethyl acetate–benzene (30:70). The mixture was heated for an additional 4 h at 128 °C and then distilled slowly to yield product **7a**, bp 148–152 °C (0.7 mm), which was a mixture of two isomers, in average yield of 40.5 g (61%). An NMR spectrum of the product indicated a ratio of **3** and **4** to be 2:1. The mixture of diastereomers (530 mg) in ethyl acetate–benzene (3:7) was separated by dry column chromatography on CC-7 silica gel (150 g) equilibrated with 12 mL of water on a 3.7 cm (width) column and developed with 200 mL of ethyl acetate–benzene (3:7). The column was divided into four fractions of silica gel after sampling by TLC. The first fraction was extracted with 125 mL of methylene chloride and yielded 118 mg of **3**: δ 3.46 (pentet, $J_{5e,4e} \sim J_{5e,6e} \sim J_{5e,4a} \sim J_{5e,6a}$ = 2 Hz, 1 H, C_{5e} H), 3.93 (J_{gem} = −12.5 Hz, broad doublets, $J_{4e,5e} \sim J_{6e,5e} \sim J_{4a,5e} \sim J_{6a,5e}$ = 1.5 Hz, 2 H, $C_{4e,6e}$ H), 4.91 (J_{gem} = −12.5 Hz, broad doublets, $J_{4a,5e} \sim J_{6a,5e} \sim J_{4e,5e} \sim J_{6e,5e}$ = 1.5 Hz, 2 H, $C_{4a,6a}$ H), 4.67 (s, 2, OCH_2), and 7.34 (s, 5, Ph).

Anal. Calcd for $C_{10}H_{12}O_4S$: C, 52.67; H, 5.27. Found: C, 52.97; H, 5.25. The fourth fraction was extracted with 125 mL of methylene chloride and yielded 16 mg of **4**: δ 3.80–4.10 (m, 3, C_{5e} H and $C_{4e,6e}$ H), 4.35–4.75 (m, 2, C_{5a} H, $C_{4a,6a}$ H), 4.58 (s, 2, OCH_2), and 7.32 (s, 5, C_6H_5); the C_{5a} H in **4** was too difficult to resolve and its absorption band was found overlapping, at higher fields than the C_{5e} H in **3**, with bands for the C_4 and C_6 hydrogen atoms.

Anal. Calcd for $C_{10}H_{12}O_4S$: C, 52.67; H, 5.27. Found: C, 53.01; H, 5.44.

Attempts at oxidation of cyclic sulfite ester **7a** with calcium permanganate in acetic acid–chloroform (1:1), with 30% aqueous hydrogen peroxide in acetone, or with chromium trioxide–sulfuric acid yielded mostly starting material as evidenced by TLC.

2α-Oxo-2β-phenoxy-5α-benzyloxy-1,3-dioxa-2-phosphorane (5) and 2α-Oxo-2β-phenoxy-5β-benzyloxy-1,3-dioxa-2-phosphorane (6) or 7b. To a solution of 11.25 g (61.8 mmol) of 2-*O*-benzylglycerol,[21] 75 mL of acetone (AR grade which was distilled from molecular sieves), and 7.6 g of pyridine, which was cooled in an ice bath, was added in 25 min a solution of 12.7 g of dichlorophenyl phosphate. A precipitate of pyridine hydrochloride appeared and the mixture stood at room temperature for 18 h. A TLC on silica gel developed with ethyl acetate–benzene (30:70) indicated that the reaction was complete. The mixture was filtered and the collected solid was washed with acetone. The filtrate was distilled to dryness under reduced pressure and the residue was dissolved in ethyl acetate–benzene. The solution was washed with three 150-mL portions of water, dried over magnesium sulfate, and distilled to dryness. Recrystallization of the crude product **7b** by removal of the ethyl acetate by distillation at low temperatures and pressure gave 5.8 g (45%) of **5**. Crystallization of the product from ethyl acetate gave pure **5**: mp 120–123 °C; δ 3.60 (sextet, $J_{5e,4e} \sim J_{5e,6e} \sim J_{5e,4a} \sim J_{5e,6a} \sim J_{5ep}$ = 2 Hz, 1 H, C_{5e} H), 4.33, measured from center of band (J_{gem} = −12, $J_{4e,5e} \sim J_{6e,5e} \sim J_{4a,5e} \sim J_{6a,5e}$ = 1.5 Hz, 2 H, $C_{4e,6e}$ H), 4.72, measured from center of band (J_{gem} = −12, $J_{4e,5e} \sim J_{6e,5e} \sim J_{4a,5e} \sim J_{6a,5e}$ = 1.5 Hz, 2 H, $C_{4a,6a}$ H), 4.67 (s, 2, OCH_2), and 7.30, 7.37 (2 s, 10, 2 C_6H_5).

Anal. Calcd for $C_{16}H_{17}O_5P$: C, 60.00; H, 5.35. Found: C, 59.96; H, 5.37.

The mother liquor from the recrystallization of **5**, which was rich in **6**, was evaporated to dryness. The 2.5 g of the mixture was dissolved in ethyl acetate–benzene (1:7) and separated by dry column chromatography on CC-7 silica gel (150 g) equilibrated with 12 mL of water and developed with 150 mL of ethyl acetate–benzene (1:7). The column was divided into six parts. The third fraction containing **6** was extracted with ethyl acetate and methylene chloride to yield 818 mg of product **6**, mp 40–45 °C: δ 3.91–4.67 (m, 5, C_{5a} H, C_4 and C_6H_2), 4.50 (s, OCH_2), and 7.30, 7.34 (2 s, 10, 2 C_6H_5). The C_{5a} H in **6** was too difficult to resolve and its absorption band was found overlapping, at higher fields than the C_{5e} H in **3**, with bands for the C_4 and C_5 hydrogen atoms.

Anal. Calcd for $C_{16}H_{17}O_5P$: C, 60.00; H, 5.35. Found: C, 59.95; H, 5.37.

***rac*-1-Acetyl-2-*O*-benzyl-*sn*-glycerol (8a).** When 73.8 g (0.32 mol) of **7a** was reacted with 32 g (0.326 mol) of anhydrous potassium acetate in Me_2SO at temperatures of 132–145 °C according to the procedure outlined for the preparation of **8b**, 67 g of crude acetate **8a** was obtained containing traces of sulfite ester or 2-*O*-benzylglycerol. The substance was purified by column chromatography on CC-7 silica gel and elution with methylene chloride–hexane (1:1) with increasing amounts of ethyl acetate. The pure acetate was obtained when the column was eluted with ethyl acetate–methylene chloride–hexane (15:42.5:42.5): IR 3600 (OH) and 1745 cm^{-1} (Ac); δ (D_2O exchanged) 2.07 (s, 3, $COCH_3$), 3.65–3.78, 4.20–4.28 (m, 5 H, $-OCH_2CHCH_2O-$), 4.67 (s, 2, OCH_2), and 7.35 (s, 5 H, $-C_6H_5$); mass spectrum *m/e* 224 (M^+), 183/181 (M − 43/M − 45) (16:1), 165 (M − acetoxy), 167 (no peak), and 43/45 ($CH_3CO/CH_3C^{18}O$) (11:1).

***rac*-1-Di^{18}-*O*-Acetyl-2-*O*-benzyl-*sn*-glycerol (9).** To a solution of 250 mg (3.9 mmol) of [^{18}O]acetic acid (90%, Bio-Rad Laboratories) in 25 mL of anhydrous toluene was added 438 mg (3.9 mmol) of potassium *tert*-butoxide. The mixture was heated at reflux for 1 h and then distilled to dryness at 120 °C under reduced pressure. Me_2SO (15 mL) was added to the potassium acetate in an atmosphere of nitrogen and the mixture was heated to 104 °C. Then 890 mg (3.9 mmol) of sulfite ester was added and the mixture was stirred at 130–135 °C for 3.5 h. The Me_2SO was removed by distillation and the residue was extracted with methylene chloride. The mixture was filtered and the filtrate was distilled to dryness. The crude product was purified by dry column chromatography on CC-7 silica gel (150 g) equilibrated with 12 mL of water for 16 h and developed with 200 mL of ethyl acetate–benzene (3:17). The second of four fractions upon extraction with ethyl acetate and removal of solvent yielded 125 mg of pure **9**. An NMR ($CDCl_3$) was identical with that of **8a**; mass spectrum *m/e* 183/181 (M − 43/M − 45) (1:4.1), 165 (M − acetoxy) 167 (no peak), and 43/45 ($CH_3CO/CH_3C^{18}O$) (1:4.5).

***rac*-1-Palmitoyl-2-*O*-benzyl-*sn*-glycerol (8b).** Potassium palmitate was prepared by adding 33.7 g (0.3 mol) of potassium *tert*-butoxide to 76.9 g (0.3 mol) of palmitic acid in 3.5 L of dry toluene in a drybox. The mixture was heated at reflux for 1 h in an atmosphere of nitrogen and then concentrated by distillation to 2 L in 2 h. The potassium palmitate, which was collected by filtration, washed with toluene, and dried in an atmosphere of nitrogen, weighed 83.3 g (93%).

A mixture of 83.3 g of potassium palmitate and 1250 mL of Me_2SO was heated to 105 °C in an atmosphere of nitrogen and then 63.7 g (0.28 mol) of the unseparated mixture **7a** was added in 15 min. The potassium palmitate dissolved as the reaction proceeded. The mixture was heated at 130 °C for 4–6 h, and then concentrated by distillation at 80 °C (<1 mm) to remove the Me_2SO. The residue was dissolved in 4 L of methylene chloride. Some insoluble material was removed in a slow filtration through a layer of Supercel. The methylene chloride solution was washed with three 400-mL portions of water, dried over a mixture of sodium and magnesium sulfate, filtered through a layer of Supercel, and distilled to dryness. The crude product, which weighed 102.2 g (86%), was purified by crystallization from 500 mL of methanol cooled in a dry ice–isopropyl alcohol bath for 1 h. The product was collected in an insulated glass sintered funnel and then heated at 40 °C under reduced pressure to remove residual methanol. The product **8b**[20] remained solid when stored at 0 °C and was analytically pure: δ 0.83–0.90, 125 [m, 35, $(CH_2)_{14}CH_3$], 2.20–2.47 (m, CH_2CO, OH), 3.48–3.55 and 4.18–4.27 (m, 5, OCH_2CHCH_2O), 4.65, 4.67 (d, 2, OCH_2Ph), and 7.37 (s, 5, C_6H_5).

Anal. Calcd for $C_{26}H_{44}O_4$: C, 74.24; H, 10.54. Found: C, 74.20; H, 10.31.

***rac*-1-Stearoyl-2-*O*-benzyl-*sn*-glycerol (8c).** When 63.2 g (0.277 mol) of **7a** was treated with 87.2 g (0.3 mol) of potassium stearate in 1.2 L of Me_2SO according to the procedure outlined for the preparation of **8b**, 121 g (100%) of crude **8c** and 104.4 g of pure **8c**[20] were obtained after crystallization from very cold methanol (−20 to −60 °C). The product **8c** was converted with excess *p*-toluenesulfonyl chloride in pyridine at 0 °C to its tosylate **8j**. The NMR spectra of **8c** and **8j**, like **8b**, were consistent with the assigned structures reported previously.[20,22,24]

***rac*-1-*O*-*p*-Chlorophenyl-2-*O*-benzylglycerol (8d).** A solution of 4.1 g (0.018 mol) of the cyclic sulfite **7a**, 50 mL of Me_2SO, and 3.25 g (0.0194 mol) of the potassium salt of *p*-chlorophenol was heated with stirring in an atmosphere of nitrogen at 127–130 °C for 1 h. The mixture was concentrated by distillation under reduced pressure (0.1 mm) and then added to 250 mL of an ice–water mixture. The aqueous mixture was extracted with two 100-mL portions of methylene chloride. The methylene chloride solution was washed with 30 mL of water and 30 mL of 3% aqueous sodium bicarbonate, dried over sodium sulfate, and distilled to dryness. The crude product weighed 4.6 g. It was purified by low-pressure column chromatography utilizing 250 g of Woelm silica gel under 200 psi pressure and a flow rate of 10 mL/min. Elution with ethyl acetate–benzene (3:17) gave fractions which when combined yielded 2.28 g of analytically pure **8d**: δ 3.7–4.1 (m, 5, OCH_2CHCH_2O), 4.70 (s, 2, OCH_2Ph), 6.71–7.2 (m, 4 H, $-C_6H_4Cl$), and 7.33 (s, 5 H, C_6H_5).

Anal. Calcd for $C_{16}H_{17}ClO_3$: C, 65.64; H, 5.85. Found: C, 65.32; H, 5.83.

1-*O*-*p*-Chlorophenylglycerol (8e). A mixture of 2.58 g of **8d**, 140 mL of benzene, 260 mg of 10% Pd/C, and 0.5 mL of a saturated solution of HCl in isopropyl alcohol was shaken in an atmosphere of hydrogen (atmospheric pressure). After 8.5 h an additional 260 mg of catalyst was added and the hydrogenation was continued for 1.5 h. The mixture was diluted with 100 mL of benzene and separated by filtration. The benzene solution was washed with aqueous sodium bicarbonate solution and water, dried over sodium sulfate, and evaporated to dryness. The crude product weighed 1.58 g. It was purified by column chromatography on Woelm silica gel (250 g) under 200 psi pressure and a flow rate of 10 mL/min. Elution with ethyl acetate gave fractions which yielded 501 mg of crude **8e**. Crystallization of the crude product from hexane yielded 365 mg of **8e**, mp 80–81 °C.

Anal. Calcd for $C_9H_{11}ClO_3$: C, 53.34; H, 5.47. Found: C, 53.41; H, 5.41.

Silver *rac*-1-Stearoyl-2-*O*-benzyl-*sn*-glycerol 3-Phenylphosphate (8g). A crude mixture of 1.38 g (4.3 mmol) of cyclic phosphates **7b** in 25 mL of Me_2SO was added dropwise with stirring to a mixture of 1.49 g of potassium stearate and 40 mL of Me_2SO maintained at 124 °C. The mixture was heated at 130 °C for 2 h and then concentrated by distillation at 80 °C (0.3 mm) to remove Me_2SO. The residue, a yellow-brown solid, was dissolved in ether. A small amount of remaining precipitate was removed by filtration and the filtrate was evaporated to dryness. An NMR of the product, which weighed 2.3 g, was consistent with the structure of **8f**: δ 0.831–1.33 [m, 35, $(CH_2)_{16}CH_3$], 1.93–2.13 (m, CH_2CO), 3.67–4.16 (m, 5, OCH_2CHCH_2O), 4.43 (s, 2, $PhCH_2O$), and 7.07–7.28 (m, 10, 2 C_6H_5). The salt was dissolved in 137 mL of acetone and the slightly hazy solution was filtered and warmed to about 50 °C. To this solution, protected from light, were added a warm solution of 663 mg (3.9 mmol) of silver nitrate, 19 mL of water, and 38 mL of acetone. A precipitate appeared which on further warming to 53 °C dissolved. The solution upon cooling in an ice–salt mixture yielded colorless crystals, 2.3 g (74%), of analytically pure **8g**.

Anal. Calcd for $C_{34}H_{52}O_7AgP$: C, 57.43; H, 7.37. Found: C, 57.31; H, 7.37.

Tetramethylammonium and Dicyclohexylammonium Salts of 2-*O*-Benzylglycerol 1,3-Bisphenylphosphate (8h and 8i). The bistetramethylammonium salt of phenylphosphoric acid was prepared in the following way. To a cold solution of 500 mg (2.87 mmol) of phenylphosphoric acid[23] in 3 mL of distilled water was added a solution of 2.1 mL of 2.69 N tetramethylammonium hydroxide in methanol. The solution was evaporated to dryness at 35 °C (0.1 mm) to yield 918 mg of the amorphous salt **10**, which was used without further purification. When 1 equiv of base was used to neutralize the phenylphosphoric acid, a crystalline monoammonium salt was obtained. The NMR spectrum of the monoammonium salt exhibited maxima which indicated the presence of one tetramethylammonium group for each phenyl group.

A solution of 860 mg (2.68 mmol) of **10** and 780 mg (2.44 mmol) of cyclic phosphates **7b** in 25 mL of Me_2SO was heated at 125–130 °C for 1.8 h. The Me_2SO was removed at 75–80 °C (0.1–0.3 mm). The residue was extracted with four portions of 30 mL of ether and then dissolved in 25 mL of distilled water. A small amount of precipitate (37 mg) which remained was the cyclic phosphate ester. The aqueous solution containing the product was distilled to dryness at 45 °C (0.1 mm) to yield 990 mg of the crude product **8h**: ^{1}H NMR (Me_2SO) δ 3.10

[s, 24 $^{+}N(CH_3)_4$], 4.50 (s, 2, OCH_2Ph), and 7.10–7.27 (m, 15, 3 C_6H_5); ^{13}C NMR [D_2O–dioxane (67:41)] decoupled, δ 56.1 (CH_3), 65.7 (OCH_2), 72.7 (OCH_2Ar), 77.8 (OCH), 120.8, 121 (C-2, 6 of PhO), 1.8 (C-4 of PhO), 128.8, 129, 129.2 (C-2, 4, 6 of Ph), 130.5 (C-3 of Ph), 138.2 (C-1 of Ph), and 152.9 (C-1 of PhO).

Anal. Calcd for $C_{30}H_{46}N_2O_9P_2$: H, 7.24; N, 4.37. Found: H, 7.49; N, 4.25.[22]

A 3-mL sample of Dowex 50 W-X8 (1.7 mg/mL resin bed, 20–50 mesh) hydrogen form was exchanged with cyclohexylamine and then washed with distilled water. A solution of 500 mg of **8h**, obtained above, in 5 mL of water was passed through a column of the cyclohexylamine treated resin. The column was eluted with an additional amount of water to collect the salt **8i**. The aqueous fractions containing the product were collected and evaporated to dryness under reduced pressure. Crystallization of the residue from methanol and acetone yielded crystals, mp 174–178 °C: R_f 0.26 [NaOAc–EtOH–H_2O (0.05:1:1)]; ^1H NMR δ 1.13–2.18 [m, 20, $2(CH_2)_5$], 3.78–4.20 (m, 5, OCH_2CHCH_2O), 4.62 (s, 2, OCH_2Ph), and 7.07–7.35 (m, 15, 3 C_6H_5); ^{31}P NMR (D_2O) (H_3PO_4, δ 56.46) δ 54.94 (1400 transients with proton decoupling), a partially resolved triplet was found when decoupler was turned off.

Anal. Calcd for $C_{34}H_{50}N_2O_9P_2$: C, 58.95; H, 7.28; N, 4.04; P, 8.95. Found: C, 56.60; H, 7.38; N, 4.30; P, 8.79.[24]

***rac*-1-Stearoyl-2-arachidonoyl-*sn*-glycerol-3-phosphoryl-(*N*,*N*-dimethyl)ethanolamine (2c).** Arachidonic acid anhydride was prepared in the following way. A solution of 10.7 g (35 mmol) of arachidonic acid (90% purity from Hormel Institute) in 200 mL of dry carbon tetrachloride was added, with stirring over a period of 5 min and in an atmosphere of nitrogen, to a solution of 3.72 g (18 mmol) of dicyclohexylcarbodiimide in 110 mL of carbon tetrachloride. After stirring in the dark for 6 h, the mixture was filtered through a Celite filter pad. The filtrate was distilled to dryness under reduced pressure at 50 °C. The oil 11 which remained weighed 10.7 g [R_f 0.89, MeOH–$CHCl_3$ (1:19)] and was utilized without further purification.

A mixture of 3.90 g (8.1 mmol) of the lysolipid **2b**, prepared from **8j** by the method of Van Deenan,[22,24] 10.7 g (17 mmol) of freshly prepared 11, 29 mL of pyridine (distilled over CaH), and 290 mg of 4-dimethylaminopyridine was stirred at 23 °C for 18 h. A TLC indicated that the reaction was complete. The solution was poured into 250 mL of toluene and concentrated by distillation to remove the pyridine. The concentrated solution was evaporated to dryness at 50 °C (0.3 mm). The crude product weighed 16.1 g. It was dissolved in methanol–chloroform (1:19) and purified by chromatography on 1400 g of CC-7 silica gel. Elution with methanol–chloroform (1:19) gave a forefraction which was discarded. Elution with methanol–chloroform (1:9) yielded **2c** weighing 4.38 g (70%); TLC R_f 0.47 on CC-7 silica gel; 95 $CHCl_3$: 35 MeOH:6 H_2O δ 0.80–1.02 (t, 6, 2 CH_3), 1.30 [s, broad $(CH_2)_{13}$, $(CH_2)_4$, $(CH_2)_3$], 1.67–2.41 (m, 10, $C{=}CCH_2$, 2 CH_2CO), 4.43–4.64, [m, s, 12, $(C{=}CCH_2C{=}C)_3$, $N(CH_3)_2$], 3.20–3.33 (m, hidden with D_2O, NCH_2), 3.97–4.43 (m, 7, OCH_2CHCH_2O, OCH_2O), and 5.33, 5.41, 5.50 (m, 8, 4 cis CH=CH).

Anal. Calcd for $C_{45}H_{82}NO_8P$: H, 10.24; N, 1.82. Found: H, 10.03; N, 1.66.[24]

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Sesquiterpene Lactones of *Eupatorium perfoliatum*[1,2]

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Structures of four new sesquiterpene lactones isolated from *Eupatorium perfoliatum* L. were determined by a combination of chemical and physical techniques. Three are esters of tiglic acid with the lactone ring closed to C-6. Two, euperfolin and euperfolitin, are 8-tigloyl-4,5-epoxy-1(10)-germacranolides with hydroxyl groups at the 3 and the 2 and 3 positions of the ring, respectively. The third, eufoliatin, is a 3-tigloyl-4,5,9,10-diepoxyguianolide with the lactone ring closed to C-6. The fourth, eufoliatorin, is a novel dilactone of the guaiane series whose stereochemistry was established by x-ray crystallography.

In the present paper we continue our reports on the constituents of *Eupatorium* species sensu stricto,[5] which have produced a number of cytotoxic and antitumor sesquiterpene lactones,[6] and describe the isolation of four polar new