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Syntheses and Antimicrobial Activities of Alkyl Lysophospholipids

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Twenty-seven alkyl analogs of lysophospholipid were synthesized and their structure-antimicrobial activity relationships were examined. These analogs differed in the structures of the long-chain alkyl moiety at position 1 and the β-*N*-substituted aminoethylphosphoryl moiety at position 3, and in the presence or absence of the 2-methoxy group of the glycerol moiety.

Many of the alkyl lysophospholipids were found to possess antimicrobial activities much more potent than those of naturally occuring lysolecithin and lecithin against *Tetrahymena pyriformis* W and a variety of fungi, including human pathogens. The maximal activity was observed with 2-methyl-1-tetradecylglycero-3-phosphocholines. 1-Alkyl-2-methylglycero-3-phosphocholines with longer as well as shorter alkyl chains tended to have lower antimicrobial activity.

Alkyl lysophospholipids with pyridinioethyl instead of the choline group showed potent antifungal activity comparable to alkyl glycerophosphocholines with the corresponding alkyl group but lower antiprotozoal activity. The tetradecyl congeners in these two classes of compounds showed potent inhibitory activity against *Trichophyton* species, comparable to that of clotrimazole. In contrast, alkyl lysophospholipids with an ethanolamine moiety in the polar head group showed decreased activity.

Changing the molecular backbone from glycerol to 1,3-propanediol had little effect upon the activity, and the resulting 1-alkyl-2-deoxyglycero-3-phosphocholines displayed antimicrobial properties similar to those of 1-alkyl-2-methylglycero-3-phosphocholines.

Keywords——alkyl lysophospholipids; 2-methyl-1-tetradecylglycero-3-phosphocholines; 2-methyl-1-tetradecylglyceryl-2-pyridinioethyl phosphate; 3-tridecyloxypropyl 2-aminoethyl phosphate; antitumor activity; antifungal activity; antiprotozoal activity; *Tetrahymena pyriformis* W; *Trichophyton* species; structure–activity relationship

Introduction

Alkyl lysophospholipids (ALPLs) are synthetic alkyl ether analogs of naturally occurring phospholipids with the general structures **1** and **2**. These alkyl ethers are of particular interest as potent antimetabolites of native phospholipids because they have approximately the same molecular geometry but should be metabolized in different ways. It thus seems important to know whether ALPL with a particular structure is correlated with one or more biological properties.

The striking antitumor property of 2-methyl-1-octadecylglycero-3-phosphocholine (the Max Planck group ALPL: ET 18-OMe, designated here as 1-C_{18}–A) has been extensively studied by Munder *et al.*[1–6] This compound has been shown to destroy tumor cells[5] selectively and to have prophylactic and therapeutic effects on several experimental tumors.[1–4] Tumor destruction by this compound is considered to be due to disturbance of the phospholipid metabolism of the tumor cells[6] and activation of macrophages in the host.[4]

However, these studies focused on only a limited number of ALPLs, including 1-C_{18}–A. To the best of our knowledge, ALPLs **1** with C_{13}–C_{15} alkyl or an alkyl chain longer than C_{18}

and ALPLs **1** and **2** with modified polar head groups have not been reported. Recently, Honma *et al.*[7] in our laboratories reported a potent inducing effect of 1-tetradecyl lysophospholipid (**1**-C_{14}–A) on differentiation[8] of myeloid leukemia cells into mature granulocytes and macrophages.

Since *Tetrahymena* and fungi are eukaryotic cells and metabolic differences from mammalian cells may be slight,[9] and inhibition test on the growth of these organisms should be useful as preliminary screening for the effects of given compounds on tumor cells.[10]

A diverse range of antifungal agents has been described in the literature,[11] but no report has yet appeared on the antifungal activity of alkyl lysophospholipids. This report deals with the synthesis and antimicrobial activity of alkyl lysophospholipids.

Chemistry

A number of publcations have described the biological properties of the following alkyl lysophospholipids: 1-dodecyl- and 1-octadecyl-2-methylglycero-3-phosphocholines[1–6,12] (**1**-C_{12}–A, **1**-C_{18}–A) and 3-alkoxypropyl 2-trimethylammonioethyl phosphate[12,13] (**2**-C_n–A, n=10, 12, 14, 16, 18, 22). However close examination of the reference[14] quoted in the reports[1–6,12,13] for the synthesis of these compounds showed that no actual information is given concerning their synthesis and physical data. Exceptionally, brief descriptions regarding 3-decyloxypropyl and 3-hexadecyloxypropyl 2-trimethylammonioethyl phosphates, **2**-C_{10}–A and **2**-C_{16}–A are given in another reference.[15]

Various ALPLs **1** and **2** which differ in the chain length of the alkyl group, the structure of the β-*N*-substituted aminoethylphosphoryl group and the structure of the molecular backbone which connects the polar and apolar regions of the molecules, *i.e.*, glycerol and 1,3-propanediol, were synthesized in our laboratories according to the method outlined in Chart 1.

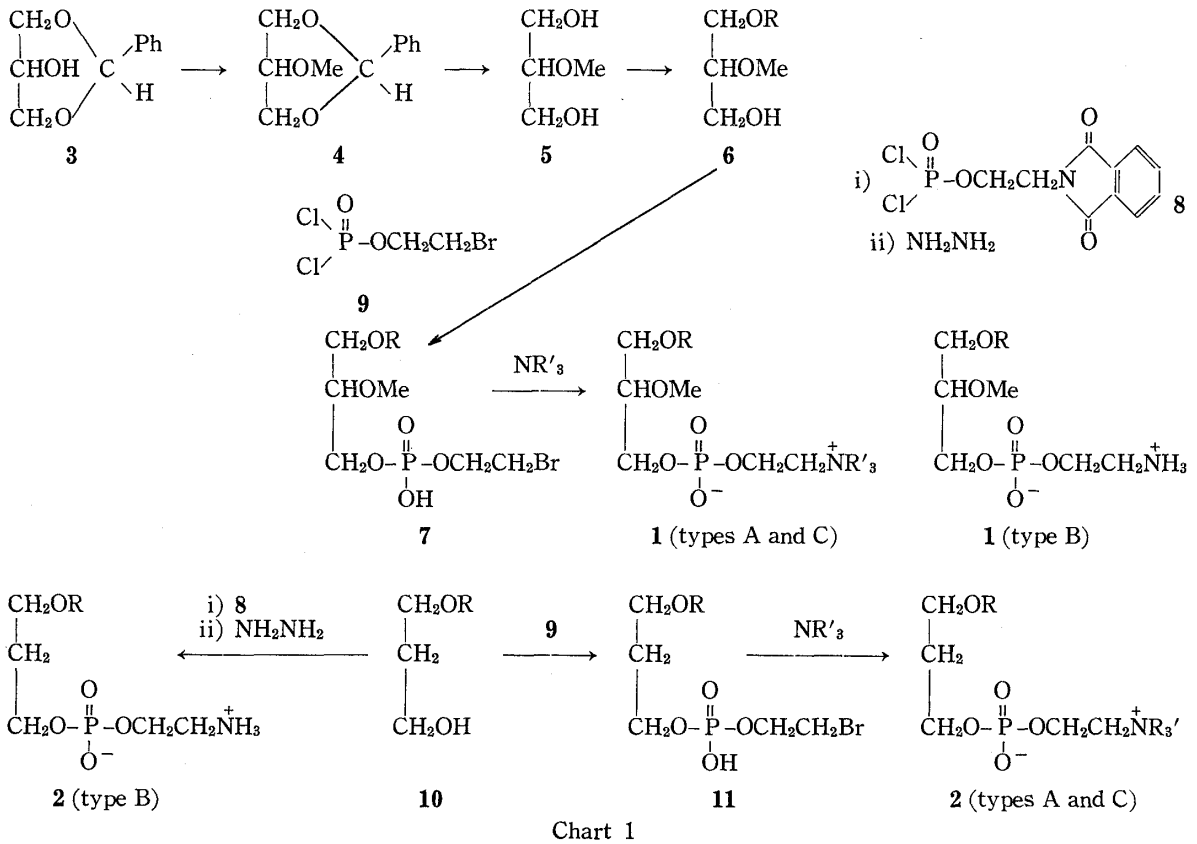

Chart 1

β-Methylglycerol, **5**, was prepared from 1,3-benzylideneglycerol,[16] **3**, *via* 1,3-benzyliden-2-methylglycerol[17] **4**. Alkylation of **5** with n-alkyl bromides of various carbon numbers in the presence of alkali gave the corresponding 1-alkyl-2-methylglycerols, **6**. Phosphorylation

of **6** with 2-bromoethyl phosphorodichloridate, followed by treatment with water and subsequent reaction of the intermediary bromoethyl glyceryl phosphate, **7**, with *N*-substituted amines gave the desired ALPL (**1**, types A and C). Those having a pyridinio moiety as a polar head group are new ALPL types.

A number of 3-alkoxypropyl 2-*N*-substituted ammonioethyl phosphates, another type of ALPL (**2**, types A and C), were synthesized similarly by starting from 1,3-propanediol instead of **5**.

Reaction of alkoxyalcohols, **6** and **10**, with 2-phthalimidoethyl phosphorodichloridate, **8**,[18] followed by treatment with water and subsequent hydrazinolysis of the intermediary *N*-protected ALPLs gave the corresponding alkyl lysophospholipids with the 2-aminoethylphosphate group; these are new ALPL types (**1** and **2**, type B).

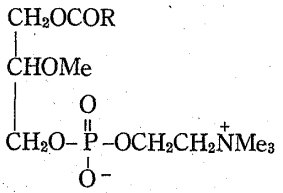

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Fig. 1

2-Methyl-1-tetradecanoylglycero-3-phosphocholine (**14**-C_{14}–A) and its congener (**14**-C_{18}–A) [17] were synthesized from **5** by esterification at position 1 with the corresponding long-chain acyl chloride and subsequent phosphorylation with 2-bromoethyl phosphorodichloridate followed by treatment with trimethylamine.

Compounds **1** and **2** were purified by column chromatography followed by crystallization from a suitable solvent system. The purity and structure of these ALPLs were confirmed by thin-layer chromatography (TLC), nuclear magnetic resonance (NMR) and elemental analysis. The physical data are listed in Table IV. TLC showed that the *Rf* values depend on the chemical structure. With silica gel as the stationary phase and $CHCl_3$–MeOH–H_2O (65 : 25 : 4) as the mobile phase, the *Rf* value increases as the alkyl ether chain length of ALPL increases, and in general, the *Rf* values of ethanolamine-type ALPL were higher than those of choline- and pyridinio-type ALPLs. ALPLs are zwitterionic and were found to be difficult to fuse, with no definite melting point. Those having a quaternary aminoethyl moiety were generally hygroscopic, especially the choline derivatives with shorter alkyl chains, and were commonly isolated as hydrates. They were amphiphilic and showed good solubility in a variety of solvents, which was advantageous for biological studies.

Compounds with the aminoethyl group (**1** and **2**, type B) crystallized very readily, yielding colorless nonhygroscopic needles or prisms. They were relatively less soluble in water, MeOH and EtOH than other ALPLs and the water solubility tended to decrease as the alkyl chain length increased.

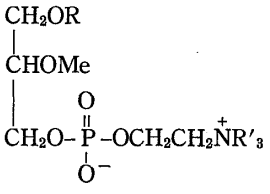
ALPL **1** has a chiral carbon in the glycerol moiety at position 2 and this chirality is presumed to have a profound influence upon the biological activity. Although compounds **1** listed in Table I are all racemic, syntheses of enantiomer pairs of several members are in progress.

Structure-Activity Releationship (SAR)

Preparation of chemically defined alkyl lysophospholipids (ALPLs) permitted investigation of the roles of the polar head group, the molecular backbone and the alkyl group of various chain lengths in the antimicrobial activity against a variety of eukaryotic microorganisms (fungi and protozoa). We found that many ALPLs have activities *in vitro* against *Tetrahymena pyriformis* W, a variety of human pathogenic and phytopathogenic fungi, but no appreciable activity against bacteria. As summarized in Tables I—III, we report here our initial attempts to correlate the structure of 31 phospholipids, including 27 new ALPLs, with specific biological effects.

Comparison of the inhibitory activities of various ALPLs showed that the chain length of the alkyl group connected to glycerol or 1,3-propanediol has a profound effect on the activity

TABLE I. Structures of Alkyl Lysophospholipids and Growth-inhibitory Activities against *Tetrahymena pyriformis* W



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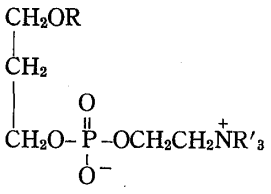

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Compound	R	$\overset{+}{N}R'_3$	MIC (μg/ml)	Compound	R	$\overset{+}{N}R'_3$	MIC (μg/ml)
1-C_8–A	C_8H_{17}	$\overset{+}{N}Me_3$	>4				
1-C_{12}–A	$C_{12}H_{25}$	$\overset{+}{N}Me_3$	0.4	**2**-C_{12}–A	$C_{12}H_{25}$	$\overset{+}{N}Me_3$	0.4
1-C_{12}–C	$C_{12}H_{25}$		4				
1-C_{13}–A	$C_{13}H_{27}$	$\overset{+}{N}Me_3$	0.4	**2**-C_{13}–A	$C_{13}H_{27}$	$\overset{+}{N}Me_3$	0.2—0.4
1-C_{13}–C	$C_{13}H_{27}$		4	**2**-C_{13}–B	$C_{13}H_{27}$	$\overset{+}{N}H_3$	0.4
1-C_{14}–A	$C_{14}H_{29}$	$\overset{+}{N}Me_3$	0.2	**2**-C_{13}–C	$C_{13}H_{27}$		4
1-C_{14}–B	$C_{14}H_{29}$	$\overset{+}{N}H_3$	2	**2**-C_{14}–A	$C_{14}H_{29}$	$\overset{+}{N}Me_3$	0.2
1-C_{14}–C	$C_{14}H_{29}$		4				
1-C_{15}–A	$C_{15}H_{31}$	$\overset{+}{N}Me_3$	0.2—0.4				
1-C_{15}–C	$C_{15}H_{31}$		2—4				
1-C_{16}–A	$C_{16}H_{33}$	$\overset{+}{N}Me_3$	0.4				
1-C_{16}–C	$C_{16}H_{33}$		2—4				
1-C_{17}–A	$C_{17}H_{35}$	$\overset{+}{N}Me_3$	0.4				
1-C_{17}–C	$C_{17}H_{35}$		4				
1-C_{18}–A	$C_{18}H_{37}$	$\overset{+}{N}Me_3$	1	**2**-C_{18}–A	$C_{18}H_{37}$	$\overset{+}{N}Me_3$	2—4
1-C_{18}–B	$C_{18}H_{37}$	$\overset{+}{N}H_3$	4	**2**-C_{18}–B	$C_{18}H_{37}$	$\overset{+}{N}H_3$	4
1-C_{18}–C	$C_{18}H_{37}$		4				
1-C_{19}–A	$C_{19}H_{39}$	$\overset{+}{N}Me_3$	4				
1-C_{22}–A	$C_{22}H_{45}$	$\overset{+}{N}Me_3$	>4				
1-C_{22}–C	$C_{22}H_{45}$		>4				
14-C_{14}–A	$C_{13}H_{27}$		>4				
14-C_{18}–A	$C_{17}H_{35}$		>4				
Egg lecithin (C_{16})			>4				
Egg lysolecithin (C_{16})			>4				

against *T. pyriformis*. Tetradecyl lysophospholipids (tetradecyl LPLs, **1**-C_{14}–A and **2**-C_{14}–A) showed extremely low minimum inhibitory concentration (MIC) values (0.2 μg/ml) against this microorganism and were not only more inhibitory than the octadecyl congener (**1**-C_{18}–A) but were also the most potent among the compounds listed in Table I. 2-Methyl-1-pentadecyl- and 2-methyl-1-tridecylglycero-3-phosphocholines (**1**-C_{15}–A, **1**-C_{13}–A) were almost as potent as **1**-C_{14}–A. Increasing as well as decreasing the alkyl carbon number from the optimal C_{14} tended to progressively lower the antiprotozoal activity. In fact, the octyl ether (**1**-C_8–A) and docosyl ether (**1**-C_{22}–A) showed essentially no inhibitory effect on *T. pyriformis*.

Replacement of alkyl ether with fatty acid ester having the same number of carbons (**14**-C_{14}–A, **14**-C_{18}–A) led to reduced activity against *T. pyriformis* (less than one-tenth). Similarly,

Table II. Antifungal Activity (MIC, μg/ml)

	Aspergillus niger IFO 6341	*Penicillium citrinum* IFO 6352	*Mucor spinescens* IFO 6350	*Rhodotorula rubra* IFO 0907	*Saccharomyces cerevisiae* IFO 0209	*Pyricularia oryzae* IFO 5279	*Helminthosporium oryzae* IFO 7503	*Botrytis cinerea* IFO 5365	*Helminthosporium sigmoideum* IFO 4867	*Colletotrichum lagenarium* IFO 6207
1-C_8–A	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1-C_{12}–A	25	25	50	50	25	25	>100	50	25	6.25
1-C_{12}–C	50	25	>100	50	50	25	50	25	50	50
2-C_{12}–A	25	25	25	25	12.5	12.5	6.25	25	25	3.12
1-C_{13}–A	25	25	50	25	12.5	12.5	>100	12.5	6.25	6.25
1-C_{13}–C	>100	12.5	50	25	25	6.25	25	6.25	12.5	12.5
2-C_{13}–A	12.5	12.5	12.5	12.5	6.25	6.25	6.25	6.25	12.5	1.56
2-C_{13}–B	25	25	100	50	25	25	100	100	>100	25
2-C_{13}–C	12.5	12.5	25	12.5	12.5	3.12	12.5	25	12.5	25
1-C_{14}–A	12.5	25	25	12.5	6.25	3.12	12.5	6.25	3.12	25
1-C_{14}–B	25	25	>100	25	25	25	>100	12.5	>100	50
1-C_{14}–C	25	12.5	100	12.5	12.5	6.25	25	6.25	6.25	25
2-C_{14}–A	12.5	12.5	12.5	12.5	6.25	6.25	12.5	6.25	6.25	6.25
1-C_{15}–A	12.5	12.5	6.25	>100	6.25	3.12	12.5	6.25	3.12	50
1-C_{15}–C	12.5	12.5	100	25	12.5	1.56	25	6.25	12.5	25
1-C_{16}–A	50	12.5	>100	>100	25	1.56	25	6.25	6.25	100
1-C_{16}–C	>100	50	>100	>100	100	1.56	>100	12.5	12.5	>100
1-C_{17}–A	100	25	>100	>100	50	1.56	50	6.25	6.25	>100
1-C_{17}–C	>100	50	>100	>100	>100	0.78	100	12.5	25	>100
1-C_{18}–A	>100	>100	>100	>100	>100	6.25	100	25	12.5	>100
1-C_{18}–B	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1-C_{18}–C	>100	>100	>100	>100	>100	6.25	50	6.25	25	>100
2-C_{18}–A	>100	100	>100	>100	>100	12.5	>100	25	50	>100
2-C_{18}–B	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1-C_{19}–A	>100	>100	>100	>100	>100	12.5	>100	100	25	100
1-C_{22}–A	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
1-C_{22}–C	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100
14-C_{14}–A	100	>100	100	100	50	50	>100	>100	100	>100
14-C_{18}–A	>100	>100	>100	>100	>100	>100	>100	>100	>100	>100

egg lecithin and lysolecithin showed no significant inhibition of this microorganism, presumably due to the susceptibility of the ester group to the hydrolytic action of esterases present on the cell surface of the microorganism.[19]

Replacement of the glycerol moiety of 1-alkyl-2-methylglycero-3-phosphocholines with 1,3-propanediol affords another type of phospholipid, 3-alkoxypropyl 2-*N*-substituted aminoethyl phosphates (**2**). The antimicrobial activity was comparable to or slightly more potent than

Table III. Minimum Inhibitory Concentrations (μg/ml)

	Trichophyton mentagrophytes IFO 5809	*Trichophyton rubrum* IFO 5467
1-C_{12}–A	25	6.25
1-C_{12}–C	25	3.12
2-C_{12}–A	25	3.12
1-C_{13}–A	6.25	3.12
1-C_{13}–C	12.5	3.12
2-C_{13}–A	12.5	1.56
1-C_{14}–A	3.12	1.56
1-C_{14}–C	3.12	1.56
1–C_{15}–A	1.56	0.78
1-C_{15}–C	3.12	1.56
1-C_{18}–A	6.25	3.12
1-C_{18}–C	6.25	3.12
Clotrimazole	6.25	3.12

that of **1**, as exemplified by the comparison of **2**-C_{12-18}–A with the corresponding ALPLs of **1**. The SAR showed the same trends as those observed with ALPLs **1**.

Inhibition tests were conducted with 12 fungal species, including human pathogenic and phytopathogenic fungi. As shown in Tables II and III, the structure-antifungal activity relationships observed with these ALPLs were very similar to those for the antiprotozoal activity mentioned above. The maximal activity was generally observed with tetradecyl LPLs (**1**-C_{14}–A, **2**-C_{14}–A), though the activities of tridecyl and pentadecyl LPLs were comparable. The mechanism of the potent activity of tetradecyl LPLs against *T. pyriformis* as well as a variety of fungi is difficult to explain at present.

As shown in Table III, ALPLs which have a trimethylammonio (**1**-C_n–A, **2**-C_n–A: n=13 to 18) or pyridinio group (**1**-C_n–C) as the polar head group showed inhibitory activities against *Trichophyton mentagrophytes* and *T. rubrum* which were comparable to those of clotrimazole, one of the most useful antimycotic drugs currently in clinical use.

Modification of the polar head group of 1-alkyl-2-methylglycero-3-phosphocholines markedly influenced the antimicrobial activity. When the quaternary nitrogen in the choline moiety was replaced by a primary amino group, new ALPL types, 1-alkyl-2-methylglycero-3-phosphoethanolamines (**1**-C_{14}–B, **1**-C_{18}–B) and 3-alkoxypropyl 2-aminoethyl phosphates (**2**-C_{13}–B, **2**-C_{18}–B) were obtained as shown in Table I. This type of phospholipids showed significant antimicrobial activity against *T. pyriformis* and a variety of fungi, although the potency was lower than those of the corresponding trimethylammonio (**1**-C_{14}–A, **1**-C_{18}–A, **2**-C_{13}–A, **2**-C_{18}–A) and pyridinio compounds (**1**-C_{14}–C, **1**-C_{18}–C).

Replacement of the choline moiety of **1** with a pyridinioethyl group gave a new type of ALPL (**1**-C_{12-22}–C). The structure and antimicrobial activity are shown in Tables I—III. ALPLs of this type showed antifungal activities comparable to those of 1-alkyl-2-methylglycero-3-phosphocholines but less potent antiprotozoal activity. In general, the level of activity against *T. pyriformis* was approximately one-tenth of that observed with the corresponding choline-type phospholipids.

Morphological and metabolic resemblances noted between eukaryotic microorganisms

Table IV. Alkyl Lysophospholipids (1, 2)

Compd. No.	R	$\overset{+}{N}R_3'$	TLC *Rf*	Physical form	Formula	Analysis (%) (Calcd) (Found) C	H	N	P
1-C_8–A	C_8H_{17}	$\overset{+}{N}Me_3$	0.17	a)	$C_{17}H_{38}NO_6P \cdot 1/2H_2O$	52.03 (52.05	10.02 9.80	3.57 3.60	7.89 7.75)
1-C_{12}–A	$C_{12}H_{25}$	$\overset{+}{N}Me_3$	0.19	a)	$C_{21}H_{46}NO_6P \cdot 1/3H_2O$	56.61 (56.71	10.56 10.61	3.14 3.25	6.95 7.01)
1-C_{12}–C	$C_{12}H_{25}$		0.24	a)	$C_{23}H_{42}NO_6P$	60.11 (60.23	9.21 9.45	3.05 3.23	6.74 6.56)
2-C_{12}–A	$C_{12}H_{25}$	$\overset{+}{N}Me_3$	0.18	a)	$C_{20}H_{44}NO_5P \cdot 1/3H_2O$	57.81 (57.81	10.83 11.15	3.37 3.56	7.45 7.65)
1-C_{13}–A	$C_{13}H_{27}$	$\overset{+}{N}Me_3$	0.21	a)	$C_{22}H_{48}NO_6P \cdot 1/3H_2O$	57.49 (57.46	10.68 10.71	3.05 3.11)	
1-C_{13}–C	$C_{13}H_{27}$		0.25	a)	$C_{24}H_{44}NO_6P \cdot H_2O$	58.64 (58.27	9.43 9.44	2.85 3.09	6.30 5.94)
2-C_{13}–A	$C_{13}H_{27}$	$\overset{+}{N}Me_3$	0.20	a)	$C_{21}H_{46}NO_5P \cdot H_2O$	57.12 (56.76	10.96 10.86	3.17 3.09	7.01 7.33)
2-C_{13}–B	$C_{13}H_{27}$	$\overset{+}{N}H_3$	0.40	b)	$C_{18}H_{40}NO_5P$	56.67 (57.11	10.57 10.55	3.67 4.02	8.12 8.31)
2-C_{13}–C	$C_{13}H_{27}$		0.24	a)	$C_{23}H_{42}NO_5P$	62.28 (62.19	9.54 9.51	3.16 3.25	6.98 6.74)
1-C_{14}–A	$C_{14}H_{29}$	$\overset{+}{N}Me_3$	0.22	a)	$C_{23}H_{50}NO_6P \cdot H_2O$	56.88 (56.66	10.79 10.86	2.88 3.08	6.38 6.42)
1-C_{14}–B	$C_{14}H_{29}$	$\overset{+}{N}H_3$	0.41	b)	$C_{20}H_{44}NO_6P$	56.46 (56.62	10.42 10.11	3.29 3.50	7.28 7.31)
1-C_{14}–C	$C_{14}H_{29}$		0.26	a)	$C_{25}H_{46}NO_6P \cdot H_2O$	59.39 (59.09	9.57 9.14	2.77 2.71	6.13 6.07)
2-C_{14}–A	$C_{14}H_{29}$	$\overset{+}{N}Me_3$	0.21	a)	$C_{22}H_{48}NO_5P \cdot H_2O$	58.00 (57.82	11.06 11.22	3.07 2.98	6.80 6.87)
1-C_{15}–A	$C_{15}H_{31}$	$\overset{+}{N}Me_3$	0.22	a)	$C_{24}H_{52}NO_6P \cdot 1/4H_2O$	59.29 (59.15	10.89 11.55	2.88 3.32)	
1-C_{15}–C	$C_{15}H_{31}$		0.26	a)	$C_{26}H_{48}NO_6P \cdot H_2O$	60.09 (60.03	9.70 9.40	2.70 2.87	5.96 5.75)
1-C_{16}–A	$C_{16}H_{33}$	$\overset{+}{N}Me_3$	0.22	a)	$C_{25}H_{54}NO_6P \cdot 1\,1/3H_2O$	57.78 (58.06	11.00 11.56	2.70 2.78)	
1-C_{16}–C	$C_{16}H_{33}$		0.28	a)	$C_{27}H_{50}NO_6P \cdot H_2O$	60.76 (60.92	9.82 9.72	2.63 2.58	5.80 5.74)
1-C_{17}–A	$C_{17}H_{35}$	$\overset{+}{N}Me_3$	0.23	a)	$C_{26}H_{56}NO_6P \cdot 2\,1/2H_2O$	56.29 (56.64	11.08 10.81	2.53 2.66	5.58 5.60)
1-C_{17}–C	$C_{17}H_{35}$		0.28	a)	$C_{28}H_{52}NO_6P \cdot H_2O$	61.40 (61.81	9.94 9.92	2.56 2.51	5.66 5.62)
1-C_{18}–A	$C_{18}H_{37}$	$\overset{+}{N}Me_3$	0.23	a)	$C_{27}H_{58}NO_6P \cdot 2\,1/2H_2O$	58.88 (59.02	11.16 11.19	2.54 2.78	5.62 5.36)
1-C_{18}–B	$C_{18}H_{37}$	$\overset{+}{N}H_3$	0.43	b)	$C_{24}H_{52}NO_6P$	59.85 (59.46	10.88 10.89	2.91 3.15	6.43 6.13)
1-C_{18}–C	$C_{18}H_{37}$		0.28	a)	$C_{29}H_{54}NO_6 \cdot 2\,1/2H_2O$	59.16 (59.19	10.10 10.04	2.38 2.39	5.26 5.81)
2-C_{18}–A	$C_{18}H_{37}$	$\overset{+}{N}Me_3$	0.23	a)	$C_{26}H_{56}NO_5P \cdot 2\,1/2H_2O$	59.97 (59.69	11.42 11.46	2.69 2.62	5.94 6.37)
2-C_{18}–B	$C_{18}H_{37}$	$\overset{+}{N}H_3$	0.43	b)	$C_{23}H_{50}NO_5P$	61.17 (60.91	11.16 10.95	3.10 3.33	6.86 6.84)
1-C_{19}–A	$C_{19}H_{39}$	$\overset{+}{N}Me_3$	0.24	a)	$C_{28}H_{60}NO_6P \cdot 2H_2O$	58.61 (58.31	11.24 11.08	2.44 2.64)	
1-C_{22}–A	$C_{22}H_{45}$	$\overset{+}{N}Me_3$	0.25	a)	$C_{31}H_{66}NO_6P \cdot 2H_2O$	60.46 (60.88	11.46 11.80	2.27 2.30	5.03 4.59)
1-C_{22}–C	$C_{22}H_{45}$		0.29	a)	$C_{33}H_{62}NO_6P \cdot 2H_2O$	62.33 (62.14	10.46 10.10	2.20 2.33	4.87 4.89)

a) Colorless powder, hygroscopic.
b) Colorless crystalline powder.

and mammalian cells[9] have led to the use of *Tetrahymena* and fungi as a preliminary test system for screening cytotoxic antitumor agents.[10,20] Although the validity of the correlation between inhibitory effects on eukaryotic microorganisms and on tumor cell proliferation remains to be confirmed, we found that most ALPLs with antiprotozoal and antifungal activity inhibited tumor cell growth and *vice versa*.[7] In fact, no cytotoxic effect on tumor cells was observed with the compounds which showed no appreciable inhibition of any of these microorganisms (*e.g.* **1**-C_8–A, **1**-C_{22}–A). We did notice some discrepancy or complexity in the relationships between the structure dependency of ALPL antimicrobial activity and that of tumor cell inhibition. The maximal antimicrobial activity against *T. pyriformis* and a variety of fungi, observed with tetradecyl LPLs (**1**-C_{14}–A, **2**-C_{14}–A), appeared to coincide with the maximal effects on inductions of differentiation of human promyelocytic leukemia cells, HL-60, and mouse myeloid leukemia cells, Ml.[7,8] However, the inhibitory effects of ALPLs with a shorter alkyl chain (**1**-C_{14}–A, **2**-C_{14}–A) on tumor cell proliferation were found to be less potent than those of the compounds with a longer alkyl chain (*e.g.*, **1**-C_{18}–A).[7] This discrepancy and complexity can at least in part be explained by differences in the cell species employed in the test systems. Nevertheless, the assay system using *T. pyriformis* as well as fungi appears to be useful for monitoring the anti-tumor cell activity of compounds due to the simplicity of the procedure and the reproducibility of the results.

Elucidation of the mechanisms of the antimicrobial actions of ALPLs is a challenging theme for study. The cell membrane may be a possible site for the attack. However, the antimicrobial action of ALPLs cannot be explained by their surface activity[5] alone since the level of MIC ($0.1—1\times10^{-6}$ M) against *T. pyriformis* and a variety of fungi might be too low for ALPL to affect the surface tension of the cell membrane. The striking regularity that in each type, the most active ALPLs contained an alkyl group of C_{14} or similar chain length also suggests that the inhibitory effect on eukaryotic microorganisms is not due solely to the surfactant action of these ALPLs. Furthermore, we determined the hemolytic activity, a well-known property of naturally occurring lysophospholipids and ALPLs,[13] of a number of ALPLs with closely related structures and found that the compound with the maximal hemolytic activity (**1**-C_{16-18}–A)[21] differed from that with the maximal inhibitory activity against *T. pyriformis* (**1**-C_{14}–A). The antimicrobial activity of ALPLs seems to be at least partially due to the alteration of membrane functions through interaction (noncovalent bonding) between ALPL and some component of the cell membrane, such as membrane enzymes involved in phospholipid metabolism.[6] Further study is needed to more definitely elucidate the mode of antimicrobial action of ALPL.

ALPL **1** contains one asymmetric carbon in the glycerol moiety and this chirality seems to play an important role in mediating the biological activity. The biological tests mentioned here were performed with racemic samples of **1**. Synthesis and biological testing of enantiomers of **1** are in progress.

Experimental

Infrared (IR) spectra were recorded on a Hitachi EPI-G2 spectrometer. NMR spectra were measured with a Varian T-60 spectrometer. Chemical shifts are expressed as δ (ppm), using tetramethylsilane (TMS) as an internal standard. TLC was performed by using pre-coated silica gel plates (Kieselgel 60 F-254, Merck) in a solvent system of $CHCl_3$–MeOH–H_2O (65 : 25 : 4). A 5% solution of phosphomolybdic acid in EtOH was used for detection.

Materials——Lecithin (mainly dipalmitoyl phosphatidylcholine) and lysolecithin used were purchased from PL Biochemicals, Inc., Milwaukee, Wis. U.S.A.

Minimum Inhibitory Concentration——a) MICs of ALPLs against phytopathogenic fungi, saprophytic fungi, yeast and human pathogenic fungi (*Trichophyton* sp.) were determined by the agar dilution method after incubation at 28°C for 3 d. The assay medium used had the following composition: glucose (1.0%), meat extract (1.0%), polypeptone (1.0%), NaCl (0.25%) and agar (2.5%). In the case of *Trichophyton*, the medium containing polypeptone (1%), glucose (4%) and agar (2.5%) was used.

b) MIC against *Tetrahymena pyriformis* W was determined by a broth dilution method as described

by Tanida *et al.*[22]

1-Alkyl-2-methylglycerol (6, General Procedure)——The glyceryl ethers (**6**) were prepared by the treatment of 2-methylglycerol with an appropriate alkyl halide in the presence of KOH in an inert solvent. As a typical procedure, the preparation of 2-methyl-1-tetradecylglycerol is given in the following section.

2-Methyl-1-tetradecylglycerol——1-Bromotetradecane (8.75 g, 32 mmol) and 2-methylglycerol (10.0 g, 94 mmol) were dissolved in a mixture of dimethyl sulfoxide (DMSO) (25 ml) and tetrahydrofuran (THF) (25 ml). After addition of powdered KOH (7.0 g, 125 mmol), the whole was stirred at room temperature for 2 h, poured into water, neutralized with HCl and extracted with ethyl acetate. The extract was washed with water, dried and thoroughly concentrated. The residue was chromatographed on silica gel (100 g) with $CHCl_3$ as an eluent to give the desired product (4.2 g) as a colorless oil. NMR (DMSO-d_6) δ: 0.92 (3H), 1.13—1.73 (24H), 1.83 (2H), 3.33 (10H), 4.67 (1H). IR ν_{max}^{neat} cm^{-1}: 3425, 2925, 2850, 1460, 1115, 751. The following 1-alkyl-2-methylglycerols (**6**) were synthesized in a similar way. Their *Rf* values determined at 19°C were as follows (solvent system: *n*-hexane–ethyl acetate, 3: 2).

R	$C_{22}H_{45}$	$C_{18}H_{37}$	$C_{16}H_{33}$	$C_{14}H_{29}$	$C_{12}H_{25}$
Rf Values	0.46	0.44	0.43	0.41	0.37

3-Alkoxypropan-1-ol (10, General Procedure)——A series of these ether propanols was synthesized by a method similar to that described in the previous reports.[23] As a typical example, the preparation of 3-tridecyloxypropan-1-ol is described in the following section.

3-Tridecyloxypropan-1-ol——Powdered KOH (10.32 g, 184 mmol) was added to a solution of 1-bromotridecane (12.11 g, 46 mmol) and 1,3-propanediol (10.2 g, 138 mmol) in DMSO (40 ml) and THF (40 ml). After being stirred at room temperature for 2 h, the mixture was poured into water, neutralized with HCl and extracted with ethyl acetate. The extract was washed with water, dried and concentrated. The residue was chromatographed on silica gel to give the desired product as colorless crystals. Yield 10 g (84%). mp 29—30°C.

3-Alkoxy-2-methoxypropyl 2-*N*-Trisubstituted Ammonioethyl Phosphate[24] (1-C_n-A and 1-C_n-C, General Procedure)——An appropriate 1-alkyl-2-methylglycerol (**6**) was treated with 2-bromoethyl phosphorodichloridate (**9**) in an anhydrous solvent containing pyridine at room temperature. The reaction mixture was concentrated, and the residue of the diesterified phosphorochloridate was warmed in water affording the intermediary 3-alkoxy-2-methoxypropyl 2-bromoethyl phosphate (**7**). This was treated with an excess of trimethylamine or pyridine to give the corresponding alkyl lysophospholipid (**1**, types A and C). Thus, 1-C_8-A, 1-C_{12}-A(C), 1-C_{13}-A(C), 1-C_{14}-A(C), 1-C_{15}-A(C), 1-C_{16}-A(C), 1-C_{17}-A(C), 1-C_{18}-A(C), 1-C_{19}-A and 1-C_{22}-A(C) were each synthesized and their physical data are shown in Table IV. As typical examples, the syntheses of 1-C_{14}-A and 1-C_{14}-C are described in the following section.

2-Methoxy-3-tetradecyloxypropyl 2-Trimethylammonioethyl Phosphate (1-C_{14}-A)——Pyridine (2.61 g, 33 mmol) was added dropwise with stirring to a solution of 2-methyl-1-tetradecylglycerol (5.0 g, 16.5 mmol) and 2-bromoethyl phosphorodichloridate (**9**) (5.19 g, 21.5 mmol) in benzene (50 ml) under cooling in ice-water. The mixture was stirred at room temperature for 2 h, then thoroughly concentrated *in vacuo*. After addition of water (40 ml), the mixture was refluxed for 1.5 h, then cooled and extracted with ether. The extract was washed with water, dried and evaporated to dryness. The residue was dissolved in a toluene solution (25 ml) of trimethylamine (4 g), and the mixture was allowed to stand at room temperature for 3 d, then evaporated to dryness. To this residue, Ag_2CO_3 (6.4 g) and MeOH (50 ml) were added. The mixture was refluxed for 1.5 h and filtered. After concentration of the filtrate, the resulting residue was chromatographed on a column of silica gel (70 g) using $CHCl_3$–MeOH–H_2O (65: 25: 4) as an eluent. After work-up, the desired product was obtained as a colorless powder (1.0 g). NMR ($CDCl_3$) δ: 0.86 (3H), 1.22 (24H), 3.32 (9H, s), 3.38 (3H, s), 3.2—4.7 (11H, m). IR ν_{max}^{film} cm^{-1}: 3350, 2920, 2850, 1650, 1460, 1080, 1050, 960, 760.

2-Methoxy-3-tetradecyloxypropyl 2-Pyridinioethyl Phosphate (1-C_{14}-C)——The intermediary 2-methoxy-3-tetradecyloxypropyl 2-bromoethyl phosphate (**7**) was prepared from 2-methyl-1-tetradecylglycerol (2.46 g, 8.1 mmol) and 2-bromoethyl phosphorodichloridate (**9**) (2.93 g, 12 mmol) as described above. The diesterified phosphate obtained was dissolved in pyridine (12 ml) and refluxed for 1.5 h. After concentration *in vacuo*, followed by addition of Ag_2CO_3 (2.9 g) and MeOH (35 ml), the mixture was refluxed for 1 h. An insoluble substance was removed by filtration and the filtrate was evaporated to dryness. The residue was purified by chromatography on a column of silica gel (60 g) with $CHCl_3$–MeOH–H_2O (65: 25: 4). After work-up, the desired product was obtained as a colorless powder (0.61 g). NMR ($CDCl_3$) δ: 0.86 (3H), 1.22 (24H), 3.44 (3H, s), 3.1—4.7 (9H, m), 4.95 (2H, br), 7.74—8.60 (3H, m), 9.22 (2H). IR ν_{max}^{KBr} cm^{-1}: 3420, 2920, 2850, 1630, 1490, 1463, 1230, 1070.

3-Alkoxypropyl 2-Trisubstituted Ammonioethyl Phosphate (2-C_n-A and 2-C_n-C, General Procedure)——The title phosphate was synthesized starting from an appropriate 3-alkoxypropan-1-ol (**10**) by reaction with 2-bromoethyl phosphorodichloridate (**9**) and the subsequent hydrolysis of the intermediary diesterified phosphorochloridate, followed by reaction of the corresponding phosphate (**11**) with trimethylamine or pyridine as described in the previous section. Thus, 2-C_{12}-A, 2-C_{13}-A(C), 2-C_{14}-A, 2-C_{18}-A were each synthesized and their physical data are given in Table IV. As a typical example, the preparation of 2-C_{13}-A is described in the following section.

3-Tridecyloxypropyl 2-Trimethylammonioethyl Phosphate (2-C_{13}-A)——Pyridine (1.83 g, 23.2 mmol) was added dropwise to a solution of 3-tridecyloxypropan-1-ol (4.0 g, 15.5 mmol) and 2-bromoethyl phosphorodichloridate (**9**) (5.62 g, 23.2 mmol) in benzene (50 ml). The mixture was stirred at room temperature for 2 h, then concentrated. After addition of water (50 ml), the mixture was refluxed for 1.5 h, then cooled and extracted with ether. The extract was washed with water, dried and evaporated to dryness. The residue was dissolved in a toluene solution (40 ml) of trimethylamine (8 g) and allowed to stand at room temperature for 3 d. After removal of the solvent, Ag_2CO_3 (1.8 g) and MeOH (40 ml) were added to the residue, and the mixture was refluxed for 1.5 h. The insoluble material was removed by filtration and the filtrate was evaporated to dryness. The residue was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (65: 25: 4) as an eluent. After work-up, the desired compound (2.5 g) was obtained as a colorless powder. NMR ($CDCl_3$) δ: 0.90 (3H), 1.33 (22H), 1.93 (2H), 3.40 (9H), 3.17—4.85 (10H). IR ν_{max}^{KBr} cm^{-1}: 3400, 2920, 2850, 1472, 1255, 1085.

3-Tridecyloxypropyl 2-Pyridinioethyl Phosphate (2-C_{13}-C)——Pyridine (0.48 g, 6 mmol) was added dropwise to a solution of 3-tridecyloxypropan-1-ol (1.03 g, 4 mmol) and 2-bromoethyl phosphorodichloridate (**9**) (1.45 g, 6 mmol) in benzene (8 ml), and the mixture was stirred at room temperature for 3 h, then concentrated thoroughly *in vacuo*. After addition of water (30 ml), the mixture was refluxed for 1 h, then cooled and extracted with ether. The extract was washed with water, dried and concentrated. The residue was dissolved in pyridine (30 ml) and warmed at 73°C for 3 d. After addition of Ag_2CO_3 (1.03 g) and MeOH (30 ml), the mixture was stirred at room temperature for 2 h. An insoluble substance was removed by filtration and the filtrate was concentrated. The residue was chromatographed on a column of silica gel (20 g) using MeOH as an eluent. After work-up, the desired product was obtained as a colorless powder (1.17 g). NMR ($CDCl_3$) δ: 0.90 (3H), 1.27 (22H), 1.87 (2H), 3.37 (2H), 3.77 (2H), 4.10 (2H), 4.30 (2H), 5.07 (2H), 8.08 (2H), 8.48 (1H), 9.37 (2H). IR ν_{max}^{KBr} cm^{-1}: 3320, 2930, 2855, 1490, 1465, 1260, 1075, 1055.

3-Alkoxy-2-methoxypropyl 2-Aminoethyl Phosphate and 3-Alkoxypropyl 2-Aminoethyl Phosphate (1-C_n-B and 2-C_n-B, General Procedure)——The title compound was synthesized from an appropriate alkoxyalcohol (**6** or **10**) by reaction with 2-phthalimidoethyl phosphorodichloridate (**8**) and subsequent hydrolysis of the diesterified phosphorochloridate, followed by hydrazinolysis of the intermediary diesterified phosphate. 1-C_{14}-B, 1-C_{18}-B, 2-C_{13}-B and 2-C_{18}-B were each synthesized by this method. The physical data are listed in Table IV. As typical examples, the syntheses of 1-C_{14}-B and 2-C_{13}-B are described in the following sections.

2-Methoxy-3-tetradecyloxypropyl 2-Aminoethyl Phosphate (1-C_{14}-B)——Pyridine (1.2 g, 14.9 mmol) was added dropwise to a solution of 2-methyl-1-tetradecylglycerol (3.0 g, 9.9 mmol) and 2-phthalimidoethyl phosphorodichloridate (**8**) (3.97 g, 12.9 mmol) in benzene (23 ml), and the mixture was stirred at room temperature for 3 h, then concentrated. Water (4.5 ml) and pyridine (10.5 ml) were added to the residue and the mixture was warmed at 70°C for 15 min. The reaction mixture was neutralized and extracted with ether. The extract was dried and concentrated, then the resulting residue was dissolved in a MeOH solution (75 ml) of hydrazine hydrate (1.5 g). The mixed solution was refluxed for 1 h and concentrated. $CHCl_3$ was added to the residue and the precipitated phthalhydrazide was filtered off. After concentration of the filtrate, the resulting residue was chromatographed on silica gel using $CHCl_3$–MeOH–H_2O (65: 25: 4) as an eluent. After recrystallization from MeOH, the desired product was obtained as a colorless crystalline powder (2.31 g). mp 195—200°C. NMR ($CDCl_3$) δ: 0.90 (3H), 1.24 (24H), 3.46 (3H, s), 2.8—4.4 (11H, m), 8.60 (2H, br). IR ν_{max}^{KBr} cm^{-1}: 2920, 2845, 1560, 1460, 1218, 1070, 910, 800.

3-Tridecyloxypropyl 2-Aminoethyl Phosphate (2-C_{13}-B)——A mixture of 3-tridecyloxypropan-1-ol (2.0 g, 7.7 mmol), 2-phthalimidoethyl phosphorodichloridate (**8**) (3.1 g, 10 mmol) and pyridine (0.79 g, 10 mmol) in benzene (12 ml) was stirred at room temperature for 2 h, then concentrated. Water (3 ml) and pyridine (7 ml) were added to the residue and the mixture was warmed at 70°C for 15 min. The reaction mixture was neutralized, extracted with ether and concentrated. The residue was dissolved in a MeOH solution (50 ml) of hydrazine hydrate (1 g) and the mixture was refluxed for 1 h. Concentration of the reaction mixture gave a residue, to which $CHCl_3$ was added. The precipitate was filtered off and the filtrate was concentrated to give a residue. Chromatography on a column of silica gel using $CHCl_3$–MeOH–H_2O (65: 25: 4), and work-up of the eluate gave the desired product as a colorless crystalline powder (1.8 g). This product was slighly soluble in $CHCl_3$, MeOH and DMSO at room temperature. mp >200°C (from MeOH). IR ν_{max}^{KBr} cm^{-1}: 2910, 2850, 1640, 1560, 1470, 1250, 1230, 1120, 1080, 1005.

2-Methoxy-3-myristoyloxypropyl 2-Trimethylammonioethyl Phosphate (14-C_{14}-A)——This new compound (1.6 g) was prepared as a colorless powder from 2-methyl-1-myristoylglycerol (3.16 g) according to the method described in previous report.[17] Rf=0.20 on TLC (solvent system, $CHCl_3$–MeOH–H_2O, 65: 25: 4). NMR ($CDCl_3$) δ: 0.87 (3H, t), 1.27 (25H, m), 2.30 (2H, t), 3.35 (9H, s), 3.42 (3H, s), 3.2—4.5 (9H, m). IR ν_{max}^{film} cm^{-1}: 3350, 2925, 2850, 1730, 1460, 1240, 1220, 1060, 960. *Anal.* Calcd for $C_{23}H_{48}NO_7P$ 0.74H_2O: C, 55.84; H, 10.08; N, 2.83; P, 6.26. Found: C, 55.74; H, 10.04; N, 2.69; P, 5.81.

2-Methoxy-3-stearoyloxypropyl 2-Trimethylammonioethyl Phosphate (14-C_{18}-A)——This compound was prepared according to the method described in the literature.[17]

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