A solution of the crude amine (8.41 g, 0.0352 mol) in EtOH (53 mL) was treated with a solution of KSCN (3.52 g, 0.0352 mol) in H_2O (88 mL) and 12 N HCl (8.81 mL), and the mixture was heated at reflux for 1 h. The solution was cooled, diluted with H_2O (100 mL), and extracted with Et_2O. The organic extracts were dried and concentrated under reduced pressure, and the residue was recrystallized from EtOAc–hexane to yield 1.48 g (18%) of **1p** as colorless crystals.

Method E. General Method for Cleavage of Aryl Methyl Ethers. 1-[3-(4-Hydroxyphenyl)propyl]imidazole-2-thione (1g). 1-[3-(4-Methoxyphenyl)propyl]imidazole-2-thione (**5**) (1.75 g, 0.007 mol) in CH_2Cl_2 (60 mL) was treated with a solution of BBr_3 (7.0 g, 0.028 mol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred for 1.5 h at ambient temperature, cooled to 0 °C, and *cautiously* treated with MeOH (50 mL). The solvents were evaporated, and the residue was recrystallized from EtOH to yield 1.02 g (67%) of **1g**.

Biology. In Vitro IC_{50} Determination. Each of the compounds in Table I was initially tested for DBH inhibitory activity at 1×10^{-4} M in duplicate assays. Where indicated, the IC_{50} value for a compound was determined in at least two separate experiments, each testing 4–6 concentrations of compound in duplicate. Each incubation vessel contained DBH (0.0145 unit/mL; Sigma D1893); test compound at the appropriate concentration; 0.2 M sodium acetate buffer, pH 5.0; 10 mM freshly prepared ascorbic acid; 10 mM freshly prepared sodium fumarate; crystalline catalase (65 000 units/mL); 1 mM pargyline; 30 mM freshly prepared *N*-ethylmaleimide; and 10 mM tyramine in a 1.0-mL volume. Samples were preincubated with compound for 2 h without tyramine in a shaking H_2O bath at 37 °C. Tyramine was added, and incubation was continued for 1 h. Tubes were removed and placed in ice, and then 0.2 mL of 3.0 M trichloroacetic acid was added and samples were centrifuged at 2000 rpm in a tabletop centrifuge for 10 min. The resulting supernatant was passed through Dowex 50 (H^+ form, 200–400 mesh, 0.3-mL packed volume) in a disposable column (1 × 11 cm). The sample tube and pellet were washed with 1.0 mL of H_2O, which was also poured onto the column. The column was washed two times with 2.0 mL of H_2O, and the washes were repassed through the column. The column was eluted with 1.5 mL of 4.0 M NH_4OH. The octopamine in the eluate was converted to 4-hydroxybenzaldehyde by the addition of 0.1 mL of 2% aqueous $NaIO_4$. After 5 min, 0.1 mL of 10% aqueous $Na_2S_2O_5$ was added to reduce excess $NaIO_4$. UV absorption was monitored against a reagent blank at 330 nm. An octopamine standard curve (50, 100, and 150 μM octopamine) and an H_2O blank were processed in each assay. Additionally, fusaric acid, a potent inhibitor of DBH, was processed with each assay as a control. Compounds whose H_2O solubilities were insufficient were dissolved in Me_2SO prior to dilution into the assay. Suitably treated controls were also assayed. A computer analysis of the data was used to establish the IC_{50} values and the 95% confidence limits. The IC_{50} value is defined as the concentration of the compound that produces a 50% inhibition of product formation when compared to the tyramine control.

Kinetic Assays with Purified DBH. The homogeneous bovine DBH (sp act. 25–42 μmol min^{-1} mg^{-1} at pH 5.0 in the tyramine assay) used for kinetic assays was isolated by our modified isolation procedure.[46] All experiments were carried out at 37 °C with incubation mixtures that contained 50 mM buffer (either NaOAc, pH 4.5, or NaH_2PO_4, pH 6.6), 200 μg/mL crystalline catalase, 10 μM (pH 4.5) or 5 μM (pH 6.6) $CuCl_2$, and enough NaCl to maintain a constant ionic strength of 0.2. The concentration of ascorbate was maintained at 10 mM when either tyramine or oxygen was the varied substrate. Tyramine was fixed at 1.0 mM when ascorbate or oxygen was varied. When tyramine or ascorbate was varied, oxygen was maintained at 0.24 mM by an equilibration in a reciprocating shaker-bath. When either tyramine or ascorbate substrates were varied, the production of octopamine was determined by using a procedure similar to the one described above. When oxygen was the varied substrate, a Yellow Springs Instrument Model 53 biological oxygen electrode was employed with 4-mL incubation mixtures. In a typical assay, buffer (with or without inhibitor) was stirred and equilibrated at 37 °C for 4 min while being saturated with oxygen or oxygen–nitrogen gas mixtures, the Lucite plunger holding the oxygen electrode was lowered into contact with the buffer, and crystalline catalase suspension (40 μL of 20 mg/mL) and 0.2 M aqueous ascorbic acid stock solution (20 μL) were added sequentially. After the rate of oxygen uptake due to nonenzymatic ascorbate autoxidation was monitored, DBH solution was added and oxygen uptake was followed for 1–4 min.

Registry No. **1a**, 17452-09-4; **1b**, 95333-67-8; **1c**, 23269-10-5; **1d**, 95333-64-5; **1e**, 95333-63-4; **1f**, 104489-48-7; **1g**, 95333-65-6; **1h**, 95333-55-4; **1i**, 104489-49-8; **1j**, 104489-50-1; **1k**, 104489-51-2; **1l**, 104489-52-3; **1m**, 104489-53-4; **1n**, 104489-54-5; **1o**, 104489-55-6; **1p**, 104489-56-7; **1q**, 104489-57-8; **1q**·HCl, 104489-61-4; **2**, 17452-14-1; **3**, 95460-09-6; **4**, 100134-69-8; **5**, 95333-89-4; **6**, 104489-45-4; **7**, 104489-46-5; **8**, 104489-47-6; 4-$MeOC_6H_4NCS$, 2284-20-0; 4-$MeOC_6H_4NHC{=}S(NHCH_2CH(OMe)_2)$, 95333-84-9; $H_2NCH_2CH(OMe)_2$, 22483-09-6; PhNCS, 103-72-0; 4-$MeOC_6H_4CHO$, 123-11-5; PhCHO, 100-52-7; 4-$MeOC_6H_4$-$(CH_2)_2CONHCH_2CH(OMe)_2$, 95333-86-1; 4-$MeOC_6H_4$-$(CH_2)_3NHCH_2CH(OMe)_2$, 95333-87-2; 4-$MeOC_6H_4(CH_2)_2COCl$, 15893-42-2; 4-$MeOC_6H_4(CH_2)_2CO_2H$, 1929-29-9; $CH_3CO(CH_2)_2Ph$, 2550-26-7; $(MeO)_2CHCH_2NHCH(CH_3)CH_2CH_2Ph$, 104489-58-9; $PhCOCONH_2$, 7505-92-2; $(EtO)_2CHCH_2N{=}C(Ph)CONH_2$, 104489-59-0; $PhCH(CONH_2)_2NHCH_2CH(OEt)_2$, 104489-60-3; $PhCH_2CH(OH)CH_2NHCH_2CH(OMe)_2$, 104489-62-5; $PhSO_2$-$(CH_2)_2NHCH_2CH(OMe)_2$, 104489-63-6; phenyl vinyl sulfone, 5535-48-8; 1-phenylpropane 2,3-epoxide, 4436-24-2; dopamine β-hydroxylase, 9013-38-1.

(46) DeWolf, W. E., Jr.; Kruse, L. I., unpublished results.

Studies of the Antitumor Activity of (2-Alkoxyalkyl)- and (2-Alkoxyalkenyl)phosphocholines

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Analogues of the synthetic antitumor phospholipid ALP (1-octadecyl-2-methyl-*sn*-glycero-3-phosphocholine; alkyl lysophospholipid) in which the 1-ether oxygen atom has been removed have been prepared and evaluated for in vitro and in vivo anticancer activity. Compounds are presented in which the saturated long chain varies in length from 8 to 25 carbon atoms. Sites of unsaturation are also incorporated into the framework in some examples. In particular, *rac*-(2-ethoxyeicosyl)phosphocholine (**10**) displays the best in vivo activity of the chemical series against a variety of transplanted tumors and activates murine peritoneal macrophages to express tumor cytotoxicity in vitro. However, **10** does not offer the wide spectrum of antitumor activity we feel necessary to warrant further study.

The recent discovery of platelet activating factor (1-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine, PAF) as a potent cell-derived mediator of allergy, inflammation, and cardiovascular function has provoked scientists of many

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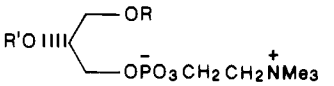
Table I. Antitumor Activity of Various Phosphocholines[a]

compd	CCRF-CEM (in vitro) IC_{50},[b] μg/mL	% inhibition of tumor growth of X5563 myeloma (in vivo) dose, mg/kg						
		70	50	35	25	17	10	5
24	2.9						(10/10)[c]	18 (4/10)
25	0.9		100 (9/10)	68 (3/10)	39 (2/10)	57 (0/10)		
26	≫20							
10	0.8	(10/10)	(10/10)	95 (4/10)	80 (1/10)	65 (0/10)		
11	1.5							
12	5.4			80 (4/10)	17 (2/10)	7 (3/10)		
13	55.0			45 (2/9)	39 (0/10)	41 (1/10)		
14	>200			17 (0/9)	16 (0/9)	17 (0/10)		
21	1.6	67 (9/10)	47 (1/10)	(1/10)				
22	6.0	31 (2/10)	(0/10)	(0/10)				

[a] The compounds were administered daily for 10 days as described in the Experimental Section. [b] Concentration to inhibit CCRF-CEM cell proliferation by 50% relative to untreated controls after 72 h of continuous drug exposure. [c] Number of toxic deaths over total number of mice per group (T/C).

disciplines to pay increasing attention to phospholipids and their roles in cellular function.[1] Munder and co-workers



PAF: R = $C_{16-18}H_{34-38}$. R' = $CH_3C(=O)$
ALP: R = $C_{18}H_{38}$. R' = CH_3

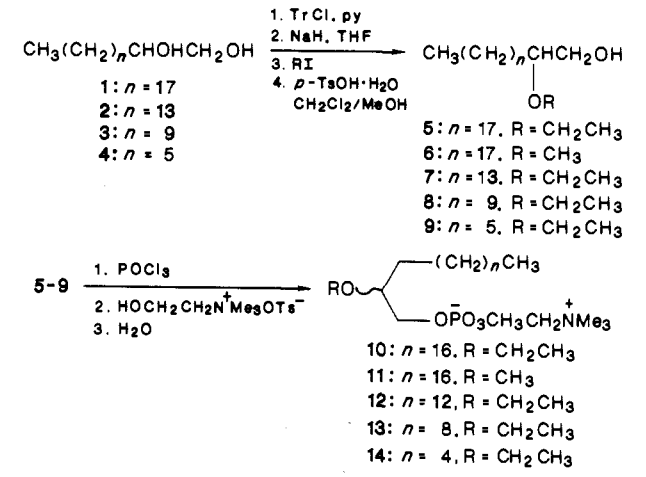
have also shown that synthetic 1-octadecyl-2-methyl-*sn*-glycero-3-phosphocholine (ALP, alkyl lysophospholipid) possesses a considerable degree of antitumor activity.[2] Recent publications have presented possible mechanisms of action of ALP including its inhibition of sialyltransferase,[3a] inhibition of a phospholipid-sensitive, Ca^{2+}-dependent protein phosphorylation system,[3b] and its membrane perturbation effect due to most tumor cells deficiency of 1-*O*-alkyl cleavage enzymes.[3c] In addition, ALP has been shown to activate murine and human macrophages in vitro to kill a variety of tumor cells.[4]

Our laboratory was interested in probing some of these areas of phospholipid pharmacology, and in this paper, we present our data regarding the in vitro and in vivo structure–antitumor activity relationship of some *rac*-(2-alkoxyalkyl)- and -(2-alkoxyalkenyl)phosphocholines. We also include, for comparison, our data on the recently published *rac*-1-carba-2-lysoglycerophosphocholine (lyso-1-carba PAF)[5], *rac*-1-carba-2-acetylglycerophosphocholine (1-carba PAF),[5,6] and the ALP analogue, *rac*-1-hexadecyl-2-ethylglycerophosphocholine.[7]

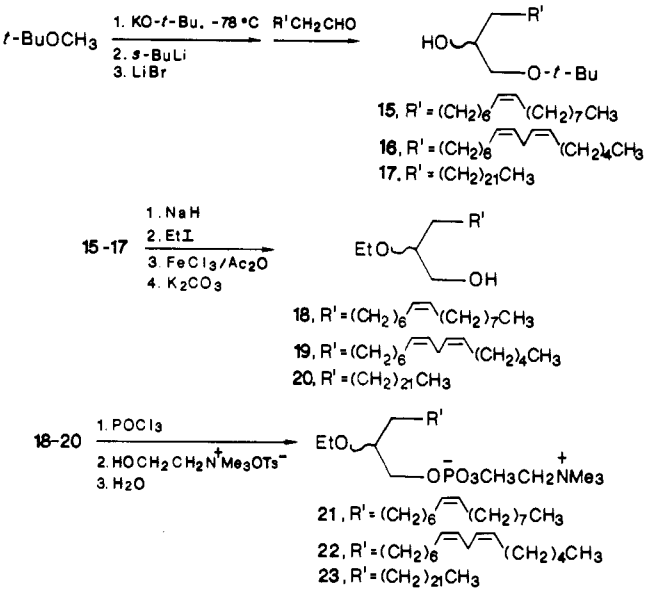
Chemistry

The preparation of the saturated (2-alkoxyalkyl)-phosphocholines **10–14** is depicted in Scheme I. 1,2-Eicosanediol was obtained in good yield from 1-eicosene by literature procedures,[8] and the remaining diols were commercially available. Selective protection of the primary alcohol using trityl chloride/pyridine was followed by alkylation of the secondary alcohol using standard tech-

Scheme I



Scheme II



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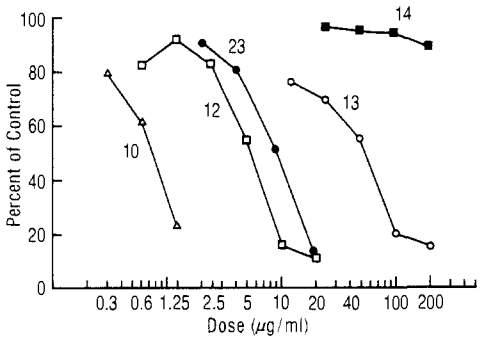


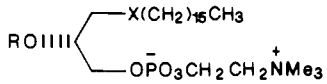
Figure 1. Growth inhibitory activity of *rac*-(2-ethoxyalkyl)-phosphocholines against CCRF-CEM human leukemia cells.

niques to generate the known 2-alkoxy 1-alcohols **5–9**.[9] These compounds were then reacted sequentially with $POCl_3$ and choline tosylate according to the method of Brockerhoff and Ayengar[10] to afford the desired phospholipids **10–14**.

On the other hand, the unsaturated diols were not known compounds. A method that provided a general entry into this compound type was recently reported by Corey and Eckrich[11] (Scheme II). Generation of (*tert*-butoxymethyl)lithium, followed by condensation with the appropriate unsaturated aldehyde,[12] reproducibly afforded the unsaturated *tert*-butyl ethers **15** and **16** in multigram quantities. Standard alkylation of the resulting secondary alcohols as above, followed by selective removal of the *tert*-butyl group,[13] led to the desired unsaturated alcohols **18** and **19**, which were moderately air labile. Generation of the unsaturated phospholipids proceeded without incident although compounds **21** and **22** were maintained under inert atmosphere. This synthetic route was also used to generate, for example, the saturated phospholipid **23** from tetracosanal, since in this case the analogous primary olefin was not readily available.

Biology

Figure 1 presents the dose–response curves of phospholipids **10**, **12–14**, and **23** with regards to their in vitro growth inhibitory activity against the human CCRF-CEM T-cell leukemia cell line. Table I displays the IC_{50} values (concentration required for 50% inhibition of growth) for all synthetic phospholipids prepared, including the known racemic compounds 1-carba PAF (**25**), lyso-1-carba PAF (**26**), and 1-hexadecyl-2-ethylglycero-3-phosphocholine, (**24**). In addition, Table I displays the results of our



24 (*rac*-ethyl AIP): R = C_2H_5, X = O
25 (*rac*-1-carba PAF): R = $CH_3C(=O)$, X = CH_2
26 (*rac*-lyso-1-carba PAF): R = H, X = CH_2

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Table II. Spectrum of Antitumor Activity of **10**[a]

tumor model	opt % inhibn: T/C (dose, mg/kg)	route of administrn	dosage schedule
X5563 plasma cell myeloma	56 (0/10, 50)[b]	po	daily × 10
	43 (1/10, 50)	iv	days 4, 8, 12
	77 (0/10, 25)	ip	daily × 10; 3-day delay
	31 (1/10, 50)	ip	days 4, 8, 12
C-6 colon	56 (1/10, 17)	ip	daily × 10; 5-day delay
6C3HED lymphosarcoma	85 (0/10, 35)	ip	daily × 8
AC755 breast carcinoma	42 (1/7, 25)	ip	daily × 10
C3H mammary carcinoma	37 (1/10, 25)	ip	daily × 10
M-5 ovarian carcinoma	22 (0/10, 17)	ip	daily × 10; 5-day delay
P388 leukemia	inactive		
B-16 melanoma	inactive		

[a] Compounds were administered via the indicated route and schedule in the various tumor models as described in the Experimental Section. [b] The number of toxic deaths over total number of mice per group at indicated dose in milligrams/kilogram per day.

Table III. Induction of Cytotoxic Macrophages by Various Phosphocholines

compd	concn, μg/mL	% macrophage cytotox[a,b]
10	1	29 (±6)**[c]
	0.1	37 (±9)**
	0.01	1 (±1)
13	1	32 (±5)**
	0.1	2 (±2)
	0.01	1 (±1)
14	1	0 (±0)
	0.1	0 (±0)
	0.01	0 (±0)
23	1	0 (±0)
	0.1	2 (±0)
	0.01	0 (±0)
24	1	31 (±9)**
	0.1	5 (±5)
	0.01	4 (±2)
25	1	23 (±3)**
	0.1	0 (±0)
	0.01	0 (±0)

[a] Mean and standard error derived from three individual experiments. [b] Lipopolysaccharide (LPS) control: 10 μg/mL gives 26 (±3)% activation. [c] ** denotes $p < 0.05$.

evaluation of selected entries for in vivo activity against the murine X5563 myeloma. In an attempt to better define the spectrum of tumors susceptible to our best compound, **10**, Table II presents the degree of inhibition of growth of a variety of murine tumor lines using the indicated dosage regimens and routes of administration. We also studied the ability of some of these synthetic phospholipids to activate murine macrophages to express tumor cytotoxicity, and those data are displayed in Table III.

Results and Discussion

To explore the biological consequences of deletion of the C-1 ether oxygen atom of *rac*-ALP, the ethyl and methyl ether containing phospholipids **10** and **11**, respectively, were prepared and studied initially since the literature suggested that these substituents afforded the best anticancer activity. As shown in Table I, the ethyl ether **10** showed consistently better in vitro activity against CCRF-CEM T-cell leukemia cells, leading to an evaluation of the biological consequences of changing the length of its carbon skeleton. Figure 1 displays the dose-response curves for the C-25 (**23**), C-20 (**10**), C-16 (**12**), C-12 (**13**),

and C-8 (14) alkyl phospholipids, and it is clear that, as in the case of ALP, substantial deviation from the 20-atom-length skeletal chain of the substrate leads to dramatic loss of activity. This trend is also borne out in vivo (Table I). In addition, introduction of unsaturation in the skeletal framework leads to a marked decrease in biological activity as shown with compounds **19** and **20**. This may well be due to their enhanced ability to undergo oxidative reactions as was evident chemically.

As the ethyl ether **10** seemed to be the best member of the series, further studies were undertaken to explore its breadth of antitumor activity (Table II). The compound is orally active, as well as effective when dosed intraperitoneally after a 3-day delay in the X5563 tumor model. Examination of **10** in other tumor systems revealed good activity against the C-6 colon and 6C3HED lymphosarcoma models and only slight activity against the AC755 and C3H breast carcinomas. The M-5 ovarian carcinoma, the P388 leukemia, and B-16 melanoma systems were not affected by **10**.

Antitumor activity of the reference compounds **24–26** in our test systems is also shown in Table I. Probably due to its chemical and known biological similarity to PAF, *rac*-ethyl-ALP (**24**) displayed markedly enhanced in vivo toxicity with little antitumor activity against the X5563 tumor model, although the in vitro cytotoxicity was substantial. *rac*-1-carba PAF (**25**), on the other hand, showed good in vitro and in vivo antitumor activity. *rac*-Lyso-1-carba PAF (**26**) was inactive in vitro and was not evaluated further.

Finally, we looked at the ability of selected members of our phospholipid series to activate murine macrophages toward target tumor cells.[14] In fact, as shown in Table III, the in vitro data tend to correlate well with the observed in vitro and in vivo antitumor results. 1-Carba PAF and ethyl ALP, **10** and **13**, significantly elicited a macrophage cytotoxic reaction toward allogeneic P815 mastocytoma target cells at a concentration of 1 μg/mL. Moreover, **10** still exhibited significant macrophage activating activity at a 10-fold lower concentration (0.1 μg/mL), whereas the rest were inactive. Thus, it seems that the ability of ether phospholipids to stimulate the formation of cytotoxic macrophages in vitro is also shared by these carbaphospholipids.

With regards to other pharmacological properties of the ether-containing phospholipids, preliminary data strongly suggest a dramatic decrease in the direct antihypertensive activity of **10** as compared to *rac*-ethyl ALP.[15]

In conclusion, we have demonstrated the in vitro and in vivo antitumor activity of some new synthetic phospholipids and have compared them to some previously published compounds. While **10** does not offer the desired in vivo spectrum of antitumor activity to warrant further evaluation, it does illustrate the potential of synthetic phospholipids as therapeutic agents.

Experimental Section

General Methods. 1-Eicosene, 1,2-hexadecanediol, 1,2-dodecanediol, and 1,2-octanediol were purchased from Aldrich Chemical Co. and converted by literature methods to desired starting materials for the syntheses of **5–9**. Tetracosanal was obtained by pyridinium chlorochromate oxidation of 1-tetracosanol (CTC Organics, Atlanta, GA) in benzene (24 h, 20 °C). Choline tosylate was prepared by the method of Brockerhoff and Ayengar.[10] LiBr was dried at 150 °C (5 mmHg) for 18 h. *tert*-Butyl methyl ether was freshly distilled from CaH_2. Et_3N and pyridine were stored over KOH. $CHCl_3$ was passed through activity I neutral alumina. All organic extracts were dried over anhydrous Na_2SO_4. TLC of the phospholipids was run on 5 × 10 cm EM Merck silica gel 60 F-254 plates in 10:5:1 $CHCl_3$/MeOH/NH_3 and visualized with H_2SO_4 or molybdenum blue. Preparative chromatographic purification was accomplished by flash column separation using EM Merck silica gel 60 or with the Waters Prep 500 HPLC system. Infrared (IR) spectra were recorded with a Perkin-Elmer 281 spectrophotometer using $CDCl_3$ as solvent. ^1H NMR spectra were recorded on Varian T-60 or JEOL FX-90Q instruments. All chemical shifts are expressed (ppm) downfield from Me_4Si. Mass spectral determinations were done with the CEC 21-110 (EI), Varian MAT 731 (FD), and VG analytical ZAB-3F (FAB and exact mass data) spectrometers. Elemental analyses were performed by analytical chemists at Lilly Research Laboratories facilities. Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical value.

Cell Culture Study. CCRF-CEM cells, a human leukemia cell line,[16] were grown as previously described.[17] Dose–response curves were generated for the various compounds as shown in Figure 1 to determine the concentration required for 50% inhibition of growth (IC_{50}). Cluster plates were prepared in duplicate with the compound at various concentrations. Test compounds were made initially in Me_2SO (or ethanol, in the case of **23**) at a concentration of 4 mg/mL and further diluted with solvent to the desired concentration. Cells in Roswell Park Memorial Institute 1640 media supplemented with 10% dialyzed fetal bovine serum, 16 mM HEPES, and 8 mM MOPS buffers[17] were added to the wells at a final concentration of 4.8×10^4 cells/well in a total volume of 2.0 mL. After 72 h of incubation (95% air, 5% CO_2), cell numbers were determined on a ZBI coulter counter. Cell number for indicated controls at the end of incubation is usually (4–6) × 10^5 cells/well.

In Vivo Antitumor Activity. For the various solid tumors, 1–2-mm^2 tumor fragments were implanted sc by trocar in the axillary region of syngeneic mice (C3H, C57BL6, BALB/C). Treatment was initiated 24 h after tumor implantation except where indicated. One day after the final dose, the inhibition of tumor growth was determined by comparing the tumor volume of the treated group to that of controls. Tumor volume (V) was calculated by measuring the tumor width (W) and length (L) and using the equation $V = LW^2/2$. For the P-388 leukemia, 1×10^6 cells were inoculated ip into DBA/2 mice and mean life span was determined for treated and control groups. For all studies, the compounds were suspended in 2.5% Emulphor in 0.9% saline and administered as indicated.

In Vitro Macrophage Activation. Animals. C3Heb/FeJ mice, 6–8 weeks old, were purchased from Jackson Laboratories (Bar Harbor, ME). They were fed autoclaved food and acid water ad libitum. All animals weighed at least 20 g before use as a source for peptone-elicited peritoneal macrophages.

Culture Media. All media used were endotoxin free as determined by the limulus lysate assay (M. A. Bioproducts, Walkersville, MD). Culture media (RPMI-FCS) was prepared by supplementing RPMI-1640 medium (M. A. BioProducts) with 20% heat-inactivated (56 °C, 30 min) fetal calf serum (M. A. Bioproducts), 50 μg/mL gentamicin (M. A. Bioproducts), and 25 mM HEPES buffer, pH 7.3 (Grand Island Biological Co., Grand Island, NY).

Collection of Peritoneal Macrophages. Elicited peritoneal macrophages were obtained 3 days after an ip injection of 1.5 mL of peptone broth. They were harvested by a peritoneal lavage with Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (HBSS: M. A. Bioproducts, Walkersville, MD). The cells were pooled from

(14) For additional experimental protocol, see: (a) Pidgeon, C.; Schreiber, R. D.; Schultz, R. *J. Immunol.* **1983**, *131*(1), 311. (b) Schultz, R.; Kleinschmidt, W. J. *Nature (London)* **1983**, *305*(5932), 239.

(15) Compound **10** shows mild but prolonged antihypertensive activity (tail cuff measurement) in spontaneously hypertensive rats at 30 mg/kg sc and no effect at 10 mg/kg sc, 5 mg/kg iv, or 50 mg/kg po. In contrast, **24** exhibited significant activity at 0.5 mg/kg sc and 5 mg/kg po.

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10 mice, washed, and resuspended in RPMI-1640 medium prior to counting in a hemacytometer.

Macrophage Activation Assay. Macrophage activation was determined by a tumor growth inhibition assay as described.[14] Approximately 4×10^5 macrophages were seeded into 16-mm wells on tissue culture plates (Costar, Cambridge, MA) in 1.0 mL of RPMI-FCS. After (5% CO_2 in air, 37 °C) cultures were incubated for 90 min, adherent macrophage monolayers were washed three times with RPMI-FCS; it was estimated that more than 95% of the resultant adherent cells had morphologic and phagocytic properties of macrophages. Test compounds were made initially in Me_2SO at a concentration of 3 mg/mL, sonicated, and then diluted to the final test concentration in RPMI-FCS. One milliliter of RPMI-FCS containing 4×10^4 allogeneic P815 mastocytoma target cells and 1 mL of RPMI-FCS containing the appropriate concentration of test compound were added to replicate wells. Cultures were incubated at 37 °C in 5% CO_2 in air. P815 target cells have a doubling time of approximately 13 h in the presence of untreated, resting macrophages. After 48 h of incubation, nonadherent cells from duplicate wells were transferred to individual wells, centrifuged at 1000 rpm (10 min), resuspended in 2 mL of serum-free trypan blue, and counted with a hemacytometer. The ratio of macrophages to target cells was approximately 10:1 at the beginning of the experiment. Macrophage cytotoxicity to target cells was estimated by

$$\% \text{ cytotoxicity} = [(A - B)/A]100$$

in which A and B are the number of tumor cells surviving macrophages in the absence or presence of drug, respectively. The direct cytotoxicity of drug on target cells in the absence of macrophages was substracted from the above equation before calculating percent macrophage cytotoxicity.

***rac*-(2-Ethoxyeicos-1-yl)phosphocholine (10).** To a solution of $POCl_3$ (1.69 mL, 12.1 mmol) in THF (12 mL) was added dropwise a solution of 2-ethoxy-1-eicosanol (**5**; 4.14 g, 12.1 mmol) and Et_3N (3.37 mL, 24.2 mmol) in 60 mL of THF. After 35 min, the resulting suspension was quickly filtered, evaporated, and redissolved in a mixture of pyridine (7.54 mL, 93.2 mmol) in $CHCl_3$ (72.6 mL). Choline tosylate (7.33 g, 26.6 mmol) was then added, and after 4.5 h, 2.4 mL of H_2O was introduced. After an additional 30 min, the reaction mixture was diluted with a double volume of a 3:2:1 mixture of $CHCl_3/H_2O$/MeOH. The organic phase was washed with saturated $NaHCO_3$ solution, dried, concentrated, and then chromatographed on a silica column, eluting first with $CHCl_3$, and after a $CHCl_3$/MeOH gradient, the desired product, **10**, was obtained as a white solid (2.22 g, 36.1%) with 1:10 $CHCl_3$/MeOH: NMR ($CDCl_3$) δ 3.40 (s, 9, $N(CH_3)_3$), 3.4–3.85 (m, 7 H), 4.25 (m, 2 H). Anal. ($C_{27}H_{59}NO_5P$) C, H, N.

***rac*-(2-Methoxyeicos-1-yl)phosphocholine (11).** By the procedure described above, 2-methoxy-1-eicosanol (**6**; 0.657 g, 2 mmol) was converted to the desired product 11: 0.403 g (40.8%); NMR ($CDCl_3$) δ 3.39 and 3.40 (2 s, 12 H), 3.65–3.95 (m, 5 H), 4.25 (m, 2 H). Anal. ($C_{26}H_{57}NO_5P$) C, H, N.

***rac*-(2-Ethoxyhexadec-1-yl)phosphocholine (12).** Using the above procedure, 2-ethoxy-1-hexadecanol (**7**; 1.86 g, 6.2 mmol) was converted to the desired product **12**, 1.19 g (25.6%). Anal. ($C_{23}H_{50}NO_5P \cdot {}^1/_2H_2O$) C, H, N.

***rac*-(2-Ethoxydodec-1-yl)phosphocholine (13).** By the above procedure, 2-ethoxy-1-dodecanol (**8**; 2.13 g, 9 mmol) was converted to the desired product **13**, 1.23 g (35%). Anal. ($C_{19}H_{42}NO_5P \cdot H_2O$) C, H, N.

***rac*-(2-Ethoxyoct-1-yl)phosphocholine (14).** By the above procedure, 2-ethoxy-1-octanol (**9**; 8.88 g, 51 mmol) was converted to the desired product 14, 3.5 g (20%). Anal. ($C_{15}H_{34}NO_5P \cdot 2H_2O$) C, H, N.

(*Z*)-1-(1,1-Dimethylethoxy)-10-nonadecen-2-ol (15). To a suspension of powdered KO-*t*-Bu (28.3 g, 0.252 mol) in dry *tert*-butyl methyl ether (400 mL) at −78 °C under inert atmosphere was injected a 1.4 M solution of *s*-BuLi in cyclohexane (180 mL, 0.252 mol) over 10 min. The resulting orange mixture was stirred for 2 h, at which time 252 mL of a 2.0 M solution of dry LiBr in THF was added. The yellow reaction mixture was allowed to warm to −10 °C over a 30-min period. Recooling to −78 °C, followed by addition of freshly prepared oleyl aldehyde (15.99 g, 60 mmol), afforded a pale yellow suspension. After 15 min, 50 mL of 10% aqueous NH_4Cl was slowly introduced and the mixture was allowed to warm to room temperature. Following dilution with Et_2O and H_2O, the organic phase was washed with H_2O and then brine, dried, and then concentrated to afford 20.32 g of crude material. Purification was achieved by preparative HPLC (Waters Prep 500) over a silica column using a cyclohexane/Et_2O gradient. The desired product **15** was eluted with 5:1 cyclohexane/Et_2O: 11.58 g (54%); NMR ($CDCl_3$) δ 2.0 (m, 4, CH_2CH=CH), 2.5 (m, 1, OH), 3.0–3.8 (m, 3 H), 5.3 (m, 4, vinyl H). Anal. ($C_{22}H_{44}O$) C, H.

(*Z*,*Z*)-1-(1,1-Dimethylethoxy)-10,13-nonadecadien-2-ol (16). This compound was synthesized as described above from 15.9 g (0.06 mol) of freshly prepared linoleyl aldehyde. The alcohol **16** was obtained in a yield of 56% (11.8 g) after chromatographic purification: NMR ($CDCl_3$) δ 2.05 (m, 4, CH_2CH=CH), 2.45 (d, 1, OH), 2.8 (m, 2 H), 3.0–3.5 (m, 2 H), 3.7 (m, 1 H), 5.4 (m, 4, vinyl H); MS (FD) m/e 353 $(M + 1)^+$, 338 $(M - CH_3)^+$, 296 (M − O-*t*-Bu)$^+$.

1-(1,1-Dimethylethoxy)-2-pentacosanol (17). This compound was synthesized as described above using 1.5 g (4.2 mmol) of freshly prepared 1-tetracosanal.[17] Due to solubility problems, following addition of this aldehyde at −78 °C, the reaction mixture was then warmed to −10 °C for 30 min prior to NH_4Cl quench. Thus, alcohol **17** was obtained in a yield of 66% (0.91 g) after chromatographic purification: NMR ($CDCl_3$) δ 1.2 (s, 9 H), 2.45 (s, 1 H, OH), 3.15 (app t, 1 H), 3.39 (dd, 1 H), 3.65 (br m, 1 H); MS (FD) m/e 441 $(M + 1)^+$, 425 $(M - CH_3)^+$, 354 (M − O-*t*-Bu)$^+$. Anal. ($C_{29}H_{60}O_2$) C, H.

(*Z*)-2-Ethoxy-10-nonadecen-1-ol (18). To a solution of **15** (12.64 g, 0.035 mol) in 140 mL of dry THF was added a 60% dispersion of NaH in mineral oil (3.564 g, 2.5 equiv) under inert atmosphere. The mixture was heated to 65 °C for 1 h, after which time ethyl iodide (14.3 mL, 5 equiv) was introduced, and the reaction was further heated for 1.5 h. The mixture was then allowed to cool to room temperature and then quenched carefully with H_2O. The organic phase was washed with additional H_2O and then brine and dried. Concentration afforded 15 g of crude ether.

This material was then suspended in acetic anhydride (178 mL) and chilled to 0 °C. Anhydrous $FeCl_3$ (578 mg, 0.1 equiv) was then added, and after 15 min reaction was complete.[13] The mixture was extracted into hexane/H_2O, and the resulting organic phase was washed with H_2O and then saturated $NaHCO_3$ (3×), dried, and concentrated. The crude acetate was then dissolved in 90 mL of CH_2Cl_2 and then diluted with 180 mL of MeOH. K_2CO_3 (5.42 g, 1.1 equiv) was added, and solvolysis was allowed to proceed for 1.5 h. The mixture was diluted with CH_2Cl_2 and H_2O. The organic layer was washed sequentially with H_2O and then brine, dried, and concentrated to afford 14.05 g of crude alcohol. Preparative HPLC purification (cyclohexane → 4:1 cyclohexane/EtOAc) generated 9.4 g (80%) of **17**: NMR ($CDCl_3$) δ 2.0 (m, 5, CH_2CH=$CHCH_2$ and OH), 3.2–3.8 (m, 5 H); 5.4 (m, 2, vinyl H); MS (FD) m/e 326 (M^+), 295 $(M - CH_2OH)^+$. Compound **18** was used directly in the next step.

***rac*-(*Z*)-(2-Ethoxy-10-nonadecen-1-yl)phosphocholine (21).** Compound **18** (9.41 g, 28.8 mmol) was converted to the desired product by the procedure described previously. Phospholipid **21** was obtained with a yield of 25%: 3.54 g; NMR ($CDCl_3$) δ 2.0 (br d, 4 H, CH_2CH=$CHCH_2$), 3.35 (s, 9 H), 4.3 (m, 2 H), 5.3 (app t, 2 H, vinyl H); MS (FAB) m/e 492 $(M + 1)^+$, 184, 86. M_r for $C_{26}H_{55}NO_5P$: calcd, 492.3818; found, 492.3786. (Satisfactory EA was not obtained.) This material was relatively stable in an O_2-free and anhydrous environment.

(*Z*,*Z*)-(2-Ethoxy-10,13-nonadecadien-1-ol (19). Compound **16** (5.9 g, 16.7 mmol) was converted to the desired product, **19** (3.92 g, 72%), by the procedure described above. It was also kept under O_2-free atmosphere and used directly in the next step: NMR ($CDCl_3$) δ 2.0 (m, 5, 2 CH_2CH=CH- and OH), 2.75 (m, 2 H), 3.2–3.8 (m, 5 H), 5.3 (m, 4, vinyl H); MS (FD) m/e 324 (M^+), 293 $(M - CH_2OH)^+$. This material failed to provide acceptable EA.

2-Ethoxy-1-pentacosanol (20). Compound **17** (0.91 g, 2 mmol) was converted to the desired product, **20** (0.70 g, 80%), by the procedure described above with the following modification: removal of the *tert*-butyl group was achieved with a 1:1 volume of acetic anhydride/cyclohexane, followed by the appropriate amount of $FeCl_3$. Reaction time was lengthened to 2.5 h: NMR ($CDCl_3$)

δ 1.98 (dd, 1 H, OH), 3.32–3.72 (4 m, 5 H); MS (FD) *m/e* 413 (M + 1)$^+$, 381 (M − CH_2OH)$^+$. Anal. ($C_{27}H_{56}O_2$) C, H.

***rac*-(*Z*,*Z*)-(2-Ethoxy-10,13-nonadecadien-1-yl)phosphocholine (22).** The above alcohol **19** (3.92 g, 12 mmol) was transformed to the desired phospholipid **22** (1.80 g, 30.5%) by the previously described procedure. As in the previous instance, this material was stable in an O_2-free/anhydrous environment: NMR ($CDCl_3$) δ 2.0 (m, 4 H), 2.75 (m, 2 H), 3.35 (s, 9 H), 4.3 (m, 2 H), 5.3 (m, 4, vinyl H); MS (FAB) *m/e* 490 (M + 1)$^+$, 184. M_r for $C_{26}H_{53}NO_5P$: calcd, 490.3662; found, 490.3660.

***rac*-(2-Ethoxypentacos-1-yl)phosphocholine (23).** By the above procedure, alcohol **20** (0.206 g, 0.5 mmol) was converted to the desired product **23**: 0.175 g (60%); MS (FAB) *m/e* 578 (M)$^+$. Anal. ($C_{32}H_{68}NO_5P$) C, H, N.

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Registry No. **5**, 104532-20-9; **6**, 104532-21-0; **7**, 104548-62-1; **8**, 104532-22-1; **9**, 104532-23-2; **10**, 104532-24-3; **11**, 104532-25-4; **12**, 104548-80-3; **13**, 104532-26-5; **14**, 104532-27-6; **15**, 104532-28-7; **15** (ethyl ether), 104532-34-5; **16**, 104532-29-8; **17**, 104532-30-1; **18**, 104532-31-2; **18** (acetate), 104532-35-6; **19**, 104532-32-3; **20**, 104532-33-4; **21**, 104548-81-4; **22**, 104548-82-5; choline tosylate, 55357-38-5; oleyl aldehyde, 2423-10-1; linoleyl aldehyde, 2541-61-9; 1-tetracosanal, 57866-08-7.

Chemically Stable Homocinnamyl Analogues of the Leukotrienes: Synthesis and Preliminary Biological Evaluation[1]

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The synthesis and biological characterization of a series of stable leukotriene analogues (**2**) are reported. They are derivatives of (5*S*,6*R*,7*Z*)-6-peptidyl-5-hydroxy-9-phenyl-7-nonenoic acid, in which the phenyl group is variously substituted with a heptanyl, 2-heptenyl, or hexanyloxy chain (R^1) and the peptide is either glutathionyl, cysteinylglycinyl, or cysteinyl. The most potent agonist is (5*S*,6*R*,7*Z*)-6-*S*-glutathionyl-5-hydroxy-9-(4-heptanylphenyl)-7-nonenoic acid. This analogue has an EC_{50} value of 74.5 nM, in the presence of 1-serine borate (45 mM), on guinea pig tracheal spirals. The agonist activity of the cysteinylglycinyl- and the cysteinyl-substituted analogues was inhibited by FPL-55712. Three of the analogues were weak leukotriene antagonists in vitro on guinea pig tracheal spirals. The most potent of these was (5*S*,6*R*,7*Z*)-6-*S*-cysteinyl-5-hydroxy-9-(2-heptanylphenyl)-7-nonenoic acid. At 10 μM, this analogue inhibited by 28% the contraction induced by 8 nM LTE_4.

Reported herein is a series of chemically stable, biologically active analogues of the peptidoleukotrienes **1**.[1,2] The leukotrienes are a class of potent spasmogenic agents,[3] which make up the active components of the "Slow Reacting Substance of Anaphylaxis" (SRS-A) first discovered by Kellaway over 40 years ago.[4] The early research on SRS-A was hampered because its structure was unknown. Key factors delaying its structural elucidation were the limited availability of crude SRS-A (leukotrienes) from biological sources and its intrinsic instability.[5] The elucidation of the leukotriene structures by Corey and Samuelsson prompted a resurgence of interest in these mediators,[6] which are believed to play a significant role in the mediation of asthma and other diseases. Recently, reports of clinical studies on the effects of pure leukotrienes in man have shown them to be bronchospastic and cause an asthmatic-like response in normal individuals.[7]

Since our team's primary goal is the development of clinically useful antiasthma drugs, we decided to explore the possible utility of leukotriene antagonists as such drugs. A classical approach to the synthesis of antagonists is via structural modification of the corresponding agonist.[8] In the case of the leukotrienes, we felt that their inherent chemical instability would preclude the development of drugs that retained those structural elements responsible for instability. As a first step, it was necessary to develop relatively stable analogues that interacted with the leukotriene receptors. Ideally these would be antagonists. If, however, they were potent leukotriene receptor agonists, we were confident that they would serve as a new starting

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(1) A preliminary account of this work was presented at the 1984 International Chemical Congress of Pacific Basin Societies, Honolulu, HI; Bernstein, P. R.; Adams, E. J.; Maduskuie, T. P.; Snyder, D. W.; Vacek, E. P.; Willard, A. K.; Abstract 10E0098.
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