Synthesis of Lysophosphatidylethanolamine Analogs that Inhibit Renin Activity

Joseph G. Turcotte,* Cheng-Sein Yu, Hwei-Ling Lee, Sripada K. Pavanaram,

Department of Medicinal Chemistry, College of Pharmacy, University of Rhode Island, Kingston, Rhode Island 02881

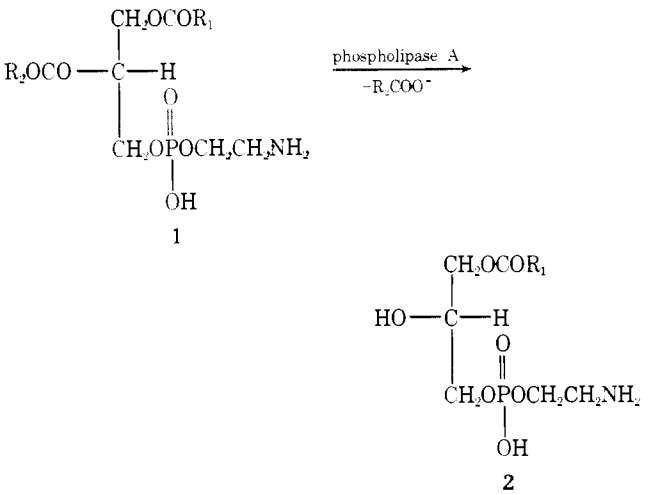
Subha Sen, and Robert R. Smeby

Research Division, Cleveland Clinic, Cleveland, Ohio 44106. *Received March 10, 1975*

A series of lysophosphatidylethanolamine analogs containing saturated and methylene-interrupted cis-olefinic fatty chains was synthesized by phosphorylation and phosphonylation of respective fatty alcohols. Arachidonyl- and linolenylphosphorylethanolamines (12, 13), arachidonyl (2-phthalimidoethyl)phosphonate (17), and arachidonyl (2-aminoethyl)phosphonate (18) were found to be effective inhibitors of the renin–renin substrate reaction in vitro; lysophosphatidylethanolamine analogs 14–16 of lesser unsaturation were either weakly active or inactive. In a preliminary study, intramuscular administration of 25 mg/kg/day of arachidonyl (2-aminoethyl)phosphonate (18) to the hypertensive rat caused pronounced reduction (50 mm) in blood pressure within 3 days; upon continued dosage (15 mg/kg/day) of 18 for an additional 4 days, plasma renin activity was found to be 16 ng/0.1 ml/15 hr as compared with 69 ng/0.1 ml/15 hr before initial drug administration. Arachidonic acid (3), arachidonyl alcohol (8), and several corresponding tetraenoid ester, amide, mesylate, and glyceryl ether derivatives (4–7, 10, 11), that are not phosphate or phosphonate esters, were found to exhibit negligible or modest inhibition of renin activity in vitro.

Renin, the enzyme component of the renin–angiotensin system, is inhibited in vitro[1–4] by kidney lysophosphoglycerides obtained by the action of phospholipase A on parent phosphoglycerides. Parenteral administration (im) of renal (dog, hog) phosphoglycerides and lysophosphoglycerides has been reported to cause reduction in blood pressure[1,5–9] and decrease in plasma renin activity[9,10] in the acute and chronic renal hypertensive rat; renal phosphoglyceride infusion (iv) into the anesthetized rat has prevented the pressor effect of exogenous renin.[6] Lysophosphoglyceride from plasma of normotensive patients has been found to inhibit (in vitro) the activity of human kidney renin.[11] This communication reports the chemical synthesis and structure–activity relationships of a number of lysophospholipid analogs, and lipids not containing phosphorus, that have been evaluated for inhibition of the renin–renin substrate reaction in vitro; a discussion of the results of a preliminary study on the activity of lysophosphatidylethanolamine analog 18 in the acute, renal hypertensive rat is presented.

The renal lysophosphoglycerides (renin inhibitor)† from several animal species (dog, hog, rat, shark) that have been utilized for in vitro and in vivo testing of renin inhibition have been reported by most investigators[2–5,12–15] to be *O*-(1-acyl-*sn*-glycero-3-phosphoryl)ethanolamines (**2**). The *sn*-1 fatty acyl chains (R_1CO) of **2** were found to be mainly saturated, although some monounsaturated species exist. The *sn*-2 fatty acyl chains (R_2CO) of the parent ethanolamine phosphoglycerides (**1**, renin preinhibitor)† have been shown to contain from one to six methylene-interrupted olefinic (cis) bonds. Arachidonic acid is reported to comprise the highest percentage of esterified fatty acid of renin preinhibitor phosphoglyceride isolated from hog (25–33%),[3,5,14] dog (38%),[16] and shark (26%)[4] kidney. Fresh kidney phospholipid from the dog and hog, each consisting of over 30 molecular species of ethanolamine phosphoglycerides, recently was determined by Yeung and Kuksis[19] to contain greater than 70% of *sn*-2 esterified arachidonic acid.‡ Furthermore, 40–50% of the ethanolamine phosphoglycerides were found to be *sn*-1 substituted vinyl ethers (plasmalogens); the renal plasmalogens were particularly rich (82–85%) in arachidonic acid acylated at the *sn*-2 position. The predominance of arachidonate in renal phosphoglycerides (**1**) prompted in part emphasis on utilization of this tetraenoid system for the synthetic studies reported herein, even though the corresponding lysophosphoglycerides (**2**), based on the majority of literature reports, would be expected to be arachidonate-poor. The occurrence and identification of individual molecular species of **2** in plasma have not been determined, and whether or not renal phosphoglycerides and lysophosphoglycerides have a physiologic role as regulators of the renin–angiotensin system in the body has yet to be demonstrated.



Studies on *synthetic* lysophosphoglycerides, phospho-

† In the literature parent renal phosphoglycerides demonstrated to inhibit renin in vivo and/or in vitro have been termed renin preinhibitor; corresponding lysophosphoglycerides obtained by phospholipase A cleavage of renin preinhibitor have been termed renin inhibitor. Renin preinhibitor and inhibitor from kidney have been identified by most investigators working in the area as phosphatidylethanolamines and lysophosphatidylethanolamines, respectively. On the basis of these experimental findings, these lipids may have erroneously acquired the status of being considered the only species capable of inhibiting renin activity. Sen et al.[16] presented evidence that dog kidney renin preinhibitor is a phosphoglyceride having a β-hydroxy-α-amino acid base different from that of serine or threonine; on the other hand, the data of Tinker et al.[15] argue convincingly that dog kidney phosphoglyceride that inhibits renin (in vitro) is phosphatidylethanolamine. The same authors[15] also found ox brain phosphatidylserine to be a highly potent inhibitor of renin in vitro. Renin preinhibitor and inhibitor should be understood to be *multispecies* phosphatidylethanolamine and lysophosphatidylethanolamine, respectively, which may differ in the composition of their fatty chains depending upon animal or organ source, but which usually have similar chromatographic (TLC, CC, etc.) properties and similar antirenin activities.

‡ Even though the occurrence of esterified saturated fatty acid in the *sn*-1 position and of unsaturated fatty acid in the *sn*-2 position is the distribution pattern common to most mammalian tissue phosphoglycerides, polyunsaturated fatty acid esterified at the *sn*-1 position of phosphatidylcholine has been reported[17] to be present in rabbit kidney cortex, but not in the medulla; renin inhibitory phosphoglyceride containing polyunsaturated fatty acid esterified at the *sn*-1 position also has been reported.[18]

glycerides, and related analogs have not settled with certainty the question of the structure(s) of renin-inhibitory lysophosphatides that may be possible bioactive natural product species, although considerable insight has been gained. Rakhit et al.[20] synthesized a number of long-chain (distearoyl, dioleoyl, dilinoleoyl, dilinolenoyl) racemic phosphatidylethanolamines and found that only the phospholipase A treated dilinolenoylphosphatidylethanolamine inhibited renin activity in vitro; the lesser unsaturated phosphatides had negligible or no activity. The inhibition, which was reported[20] to approach that of natural hog kidney renin inhibitor, could be interpreted as being due to *O*-(1-linolenoyl-*sn*-glycero-3-phosphoryl)ethanolamine; however, at least 50% of the racemic phosphoglyceride utilized in the test procedure should have remained as intact D-dilinolenoylphosphatidylethanolamine, since phospholipase A would be expected to cleave only the L-phosphatide enantiomeric molecules. In contrast, natural product renal phosphatidylethanolamine treated with phospholipase A should give solely multispecies *O*-(1-acyl-*sn*-glycero-3-phosphoryl)ethanolamines under proper incubation conditions.

Innovative studies by Pfeiffer and coworkers[3,21] on the synthesis and renin inhibition of a number of lysophosphoglyceride and phosphoglyceride analogs revealed that adamantane derivatives such as *O*-[1,2-di(1-adamantoyl)-*sn*-glycero-3-phosphoryl]ethanolamine, *O*-[1,2-di(1-adamantyl)-*sn*-glycero-3-phosphoryl]ethanolamine, and [2-(1-adamantyl)ethyl]2-aminoethyl phosphate showed good in vitro inhibition of renin. The biological results and those on other synthetic phosphatide analogs led the authors[3] to contend that good renin inhibitory activity in natural renal lysophosphoglycerides may not be dependent upon the degree of unsaturation of the fatty acyl chains or upon the presence of the *sn*-2 secondary alcohol group.

Turcotte, Yu, and coworkers[22] in preliminary communications reported the synthesis of a number of fatty alkylphosphorylethanolamine analogs (**12–16**) of lysophosphatidylethanolamine and their inhibitory effect on the renin–renin substrate reaction in vitro. Polyenoid species **12** and **13** were found to be comparable in activity to hog kidney lysophosphatidylethanolamine in an in vitro bioassay for renin inhibition; analogs **14–16** of lesser unsaturation were inactive. Under the bioassay conditions employed, the secondary hydroxyl and ester functions of species of naturally occurring **2** were concluded not to be essential for renin inhibition, but fatty chain polyunsaturation appeared to be intimately associated with activity in this class of lysophosphatidylethanolamine analogs. The details of this previous work[22] comprise in part the subject of this communication.

Two series of lipids were synthesized for evaluation of their effect on renin activity: **12–16** and **18** can be considered as simplified structural analogs of naturally occurring *sn*-1- or *sn*-2-lysophosphatidylethanolamines; derivatives **4**, **6**, and **9–11** are selected derivatives of arachidonic acid

$$CH_3(CH_2)_{14}COOCH_2CH_2\overset{+}{N}(CH_3)_3Cl^-$$

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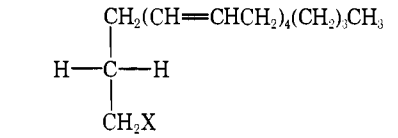
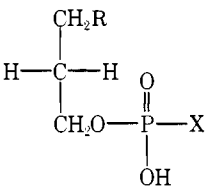
(**3**) and arachidonyl alcohol (**8**), respectively. Arachidonic acid (**3**), methyl ester **7**, and other fatty acid methyl esters employed in this study were obtained from commercial sources; lipid **5** is the saturated palmityl homolog of ester **4**.

Methods. The fatty alkylphosphorylethanolamines (**12–16**) were synthesized by pyridine-catalyzed phosphorylations of respective fatty alcohols with dichloro(*N*-2,2,2-trichloroethoxycarbonyl-2-aminoethyl) phosphate[23] followed by removal of the protective group and hydrolysis of the intermediate monochlorophosphates (Zn–95% HOAc). The fatty alcohols were obtained in near quantitative yields by hydride reduction of commercially available fatty acid methyl esters. Since the phosphate intermediates were not isolated, the desired lysophosphatide analogs **12–16** were elaborated in essentially two-step procedures in 30–50% average yields after one or more chromatographic (column) purification(s). Phosphonylation of arachidonyl alcohol (**8**) with (2-phthalimido)ethylphosphonic acid monochloride[24] in the presence of triethylamine gave the intermediate phthalimide **17**, which was converted via hydrazinolysis to **18**.



derivative	X
3	COOH
4	$COOCH_2CH_2\overset{+}{N}(CH_3)_3Cl^- \cdot HCl$
6	$CON(C_2H_5)_2$
7	$COOCH_3$
8	CH_2OH
9	$CH_2OSO_2CH_3$
10	$CH_2OCH_2\underset{\displaystyle O}{\underset{\diagup\quad\diagdown}{CH}}CH_2OC(CH_3)_2$
11	$CH_2OCH_2CHOHCH_2OH$



derivative	R	X
12	$CH_3(CH_2)_3(CH_2CH{=}CH)_4CH_2$	$OCH_2CH_2NH_2$
13	$CH_3(CH_2CH{=}CH)_3CH_2(CH_2)_4$	$OCH_2CH_2NH_2$
14	$CH_3(CH_2)_4(CH{=}CHCH_2)_2(CH_2)_4$	$OCH_2CH_2NH_2$
15	$CH_3(CH_2)_7CH{=}CH(CH_2)_5$	$OCH_2CH_2NH_2$
16	$CH_3(CH_2)_{12}$	$OCH_2CH_2NH_2$
17	$CH_3(CH_2)_3(CH_2CH{=}CH)_4CH_2$	$CH_2CH_2N(CO)_2C_6H_4$
18	$CH_3(CH_2)_3(CH_2CH{=}CH)_4CH_2$	$CH_2CH_2NH_2$

Heating (120°) arachidonoyl chloride and choline chloride neat according to a modification of the method of Phillips et al.[25] gave choline ester **4** containing an approximate mole equivalent of hydrogen chloride; spectroscopic data (^{1}H NMR, ir), however, indicated that the integrity of the fatty chain, i.e., a cis-polyenoid methylene-interrupted system, was largely maintained under the reaction conditions. No difficulty was encountered with the synthesis of palmitoylcholine chloride (**5**) under similar reaction conditions. Several attempts to prepare **4** according to the methods of Bremer[26,27] and Ziegler et al.,[28] who have synthesized long-chain fatty acylcarnitines, resulted in the recovery of large amounts of starting materials.

Ir, uv, and ^{1}H NMR data on the starting materials arachidonic acid and its methyl ester and synthetics **8–12**, **17**, and **18** indicated that the various reaction conditions employed caused no or negligible isomerization of the cis-tetraenoic system common to the latter derivatives. Examination of ir diagnostic bands at 3000–3100 (C=CH, carbon–hydrogen stretching vibrations) and 1580–1650 cm^{-1} (C=C stretching vibrations) revealed essentially identical absorptions in these regions for **3**, **7–12**, **17**, and **18**. Absorptions (965–975 cm^{-1}) characteristic of isolated trans-substituted olefins[29,30] were not apparent in the ir spectra of **3**, **6–9**, **12**, **17**, and **18**; some samples of naturally occurring lin-

Table I. Effect of Lysophosphatidylethanolamine Analogs and Nonphosphorus Lipids on the Renin-Renin Substrate Reaction in Vitro

Derivative	Amt, mg[a]	Inhibn,[a] %	Classification of act.[a]
3	21.5	0	Inactive
4	31.0	43	Active
5	15.0	0	Inactive
6	25.0	10	Weakly active
7	25.0	0	Inactive
8	25.0	0	Inactive
9	25.0	0	Inactive
10	8.8	26	Weakly active
11	16.0	24	Weakly active
12	15.0	48	Active–highly active
13	12.0	38	Active
14	11.9	0	Inactive
15	17.0	16	Weakly active
16	20.0	0	Inactive
17	24.3	77	Highly active
18[b]	5.0	62	Highly active

[a]Derivatives 3–18 (mg/3 ml of incubation mixture) were incubated with dog renin and dog renin substrate;[16] the amounts of angiotensin formed were assayed in the pentolinium-treated, vagotomized rat.[32] The amounts (control amounts) of angiotensin II released in the absence of lipid minus the amounts produced in the presence of respective lipids were divided by the control amounts and the results expressed as percent inhibition: weakly active = <30% inhibition; active = 30–50% inhibition; highly active = >50% inhibition. [b]Compound 18 from the same sample batch was utilized for preliminary studies in the hypertensive rat (see text).

oleic and arachidonic acids have been reported to exhibit weak absorptions at 970 cm^{-1}.[29] Spectra of **10** and **11** did contain low intensity bands at 970 cm^{-1} indicating that some isomerization might have occurred in the preparation of **10**. High-resolution ir spectra of **3** and **7–11** showed that several derivatives (**3**, **7**, **8**) exhibited very weak bands or shoulders at 985–995 cm^{-1} which possibly could be correlated with small amounts of trans- and/or cis-conjugated dienes and/or trienes.[30] The uv absorption spectra of **8–12**, **18**, commercial arachidonic acid (**3**), and its methyl ester (**7**) all included maxima at 233 nm indicative of small amounts of diene conjugation; no or negligible triene or tetraene conjugation was observed in the uv spectra. High-resolution (100 MHz) ^1H NMR spectra of **3** and **7–11** showed that the chemical shifts of protons common to the fatty chains of each derivative were almost identical. Allylic (CH_2CH=CH), intervening methylene (CH=CH-CH_2CH=CH), and olefinic absorptions integrated for 4, 6, and 8 protons, respectively, in each of the tetraenoids **3**, **7**, **8**, **10**, and **11**, and integration of these protons in nonpurified **9** was close to theoretical; lower resolution (60 MHz) ^1H NMR spectra of **12**, **17**, and **18** also were in accord with the structures of the molecules.

Compound **10** was obtained in 74% yield (after two-column chromatographic purifications) upon prolonged heating (50°, 43 hr) of a benzene solution of methanesulfonate **9** and 1,2-isopropylideneglycerol potassium alcoholate in excess isopropylideneglycerol initially added dropwise. When reverse addition using only benzene (gentle reflux, 20 hr) as solvent was carried out according to an established procedure[31] for the synthesis of saturated fatty alkyl glyceryl ethers, evidence of increased quantity of trans fatty chain isomer was indicated by the relative intensity of a weak ir band at 970 cm^{-1}. Vigorous reflux (benzene, 20 hr) caused isomerization. The ir spectrum of the isomerized product showed a strong band at 988 cm^{-1} characteristic of trans-trans conjugated double bonds. The proton integration of the ^1H NMR spectrum (60 MHz) of this material showed an increase of 2 protons at δ 1.45, 4 protons at δ 2.08 (CH_2CH=CH), and complete disappearance of the six protons at δ 2.83 (CH=CHCH_2CH=CH), as compared with the spectrum of **10**.

Derivatives **3–18** were assayed for inhibition of renin by incubation with dog renin in the presence of dog renin substrate;[16] the amounts of angiotensin formed (Table I) were assayed in the pentolinium-treated, vagotomized rat.[32] A preliminary evaluation of the antihypertensive effect of **18** in the acute hypertensive rat was carried out according to the method of Sen et al.[6]

Results and Discussion

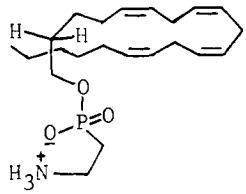
Tetraenoid derivatives **3–11**, which do not contain a phosphate or phosphonate function, exhibited modest or negligible inhibition of the renin–renin substrate reaction in vitro (Table I). Acid **3**, alcohol **8**, and diol **11** possess respective hydroxylic functions at the polar end of the polyunsaturated chain; polarity appeared to be a more important factor than ionizability among these three derivatives in view of the modest in vitro activity of **11**, as compared with the lack of activity of **3** and **8** under the experimental conditions employed.

The activity of the choline ester **4** revealed that polyunsaturated amine species that inhibit renin in vitro need not necessarily be phosphate or phosphonate betaines, although an ionic charge may be required. The minimal concentration of carboxylic acid ester **4** needed to inhibit renin compared with the concentrations of the arachidonyl and linolenyl phosphates (**12**, **13**) and the arachidonyl phosphonates (**17**, **18**) was not determined; the saturated palmitoyl choline ester **5** and the saturated palmityl phosphate ester **16** both were found to be inactive in the in vitro bioassay. Quaternary ammonium salt **5** showed a slight indication of enzyme stimulatory activity which has been observed[33] to a greater extent, tenfold stimulation of renin activity, with commercial beef lysophosphatidylcholine. Apparent stimulation of renin activity also was seen with the palmityl lysophosphatide analog **16**, the only fully saturated member of the series **12–16**. These results suggested a possible structure–activity correlation between saturated and/or poorly unsaturated lysophosphatidylcholines and the ability of these lipids to cause stimulation of renin activity in vitro. On the other hand, inhibition of renin by lysophosphatidylcholines may require a polyunsaturated fatty chain, as indicated by the activity of the somewhat structurally related cholinecarboxylic acid ester **4**.

In contrast to the activity of **4**, the carboxylic acid ester **7**, the sulfonate ester **9**, and the amide **6** lacked activity. Ketal **10** and diol **11** exhibited weak activity; the ether diol (**11**) derived from **10** can be considered as a nonhydrolyzable analog of a polyunsaturated monoglyceride. In summary, although the in vitro data on several of the tetraenes (**4**, **6**, **10**, **11**) of the series **3–11** revealed only modest activity, a possible trend in activity may be observed; i.e., polarity and, perhaps preferentially, ionizability at one end of the tetraene fatty chain apparently enhances activity.

Derivatives **12–16** and **18** can be considered as analogs of lysophospho- and lysophosphonoglycerides, respectively. Of the series members, those (**12**, **13**, **17**, **18**) which had three or four methylene-interrupted cis-olefinic bonds all exhibited high inhibition of renin activity in vitro. Within the ethanolamine series (**12–16**), only the arachidonyl analog **12** and the linolenyl analog **13** showed inhibition. Irre-

spective of the uncertainty of the molecular structures of naturally occurring renal or other organ lysophosphatides that might be speculated to be regulators of the renin–angiotensin systems in vivo, these in vitro results convincingly demonstrate that, under the experimental conditions employed, side-chain polyunsaturation is required for inhibition of renin activity in this class of lysophosphatidylethanolamine analogs.



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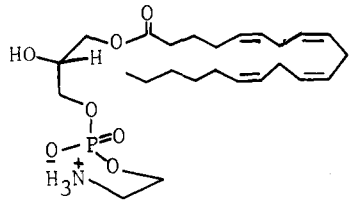

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The molecular structure of **18**, the simplest tetraenoid lysophosphatidylethanolamine analog, can be compared with that of *sn*-1-arachidonoyl-2 (**19**). Lysophosphatidylethanolamine **19** is the cis-20:4 fatty acyl homolog of linolenoyl-2 (*cis*-18:3-2), a synthetic molecule reported[20] to inhibit renin in vitro. Consideration of the structure–activity requirements (in vitro) of **18**, i.e., compared with **12–17** and hypothetical **19**, revealed the following.

(a) The number of cis-olefinic bonds in the fatty chain has a pronounced effect on activity; preliminary bioassay results[33] indicate a poly-yne system may also be effective; trans and/or conjugated systems have not yet been reported.

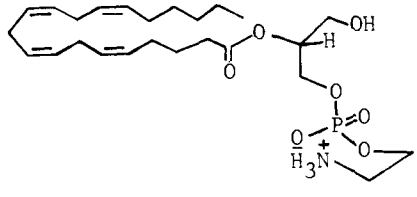
(b) The position of the methylene-interrupted cis double bond system from the polar end (i.e., P atom) of the molecule does not appear to be critical for good activity.

(c) A carboxylic acid ester function is not required for activity.

(d) A secondary hydroxyl function is not required for activity and, therefore, neither is optical activity at (*sn*-2) carbon.

(e) Interatomic distance between P and N in the polar end of lysophosphatide molecules and analogs apparently is not critical for activity.

Evidence for the existence of polyunsaturated species such as **19** that possibly could be derived from parent polyunsaturated phosphoglyceride in organs or plasma is limited. Phosphatidylcholine containing *sn*-1 esterified arachidonate has been demonstrated to occur in kidney (rabbit) cortex.[‡] Cleavage in vivo of the vinyl ether linkage of plasmalogens[34] such as those found in kidney[19] could give poly-



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unsaturated *sn*-2-lysophosphatidylethanolamines (e.g., **20**), i.e., positional isomers of species such as **19**. Under their experimental conditions employed, Tinker et al.[15] have presented evidence that quantities (0.16 mg/kg of kidney) of **20** may exist in dog kidney, but the lysophosphatidylethanolamine isolated (containing 66 mol % of esterified arachidonate) was found not to inhibit renin in vitro. These results appear to be in contradiction to those found in this investigation with synthetic **12**, **13**, **17**, and **18** and by Rakhit et al.[20] on phospholipase A treated racemic polyunsaturated phosphatidylethanolamines.

Further evidence that data from in vitro bioassays may be useful for prediction of structural requirements for in vivo activity was indicated by a preliminary assay of the activity of **18** in the experimentally hypertensive animal. Administration (im) of **18** (25 mg/kg/day) to the hypertensive rat (hypertension for 4-weeks duration due to clipping of the left renal artery, the contralateral kidney being untouched) resulted in a pronounced reduction (50 mm) in blood pressure within 3 days; following continued dosage (15 mg/kg/day) of **18** for four additional days, blood pressure reduction was maintained and plasma renin activity was found to be 16 ng/0.1 ml/15 hr as compared with 69 ng/0.1 ml/15 hr before drug administration (day 0). When the drug was withdrawn, blood pressure gradually rose to preadministration levels over a period of 1 week. The depressor effect of **18** on plasma renin activity and blood pressure was found to be comparable to that of naturally occurring kidney (dog, hog) phosphoglyceride and lysophosphoglyceride with an indication that onset of action was somewhat sooner than that of the naturally occurring kidney lipids. The minimal dosage of **18** needed to inhibit plasma renin activity and lower blood pressure has not yet been determined. The amount of **18** administered to the hypertensive rat, 25 mg/kg/day (~6 mg/rat/day), was taken as a convenient starting dose, but this would correspond to a relatively high dosage—about 1.7 g/day—in an adult weighing 150 pounds, for example. The phthalimido derivative **17**, although highly active in vitro (Table I), was inactive when administered (im, 25 mg/kg) to the hypertensive rat; however, inactivity possibly could be related to the bioavailability of the drug, a point that would be of interest for further investigation.

The effect of synthetic **18** on blood pressure and plasma renin activity appeared to closely parallel that reported for naturally occurring renal lysophosphoglyceride and phosphoglyceride.[5–8] Antonello et al.[8] administered (iv) 2 mg of hog kidney lysophospholipid renin inhibitor[†] prepared by the method of Smeby et al.[1] to rats (350–400 g) rendered hypertensive by unilateral renal ischemia and by continuous infusion of homologous renin; statistically significant reductions, 29.9 (11 rats) and 21.9% (5 rats), respectively, were observed. The hypotensive effect of acute administration of renin inhibitor, however, was found to be transient, with activity tending to disappear after 2 min; this led to the speculation[8] that rapid metabolism or removal from plasma of the lipid had occurred. Naturally occurring ethanolamine lysophosphoglycerides (e.g., **2**) may be expected to be metabolized by hydrolytic cleavage or by reacylation, etc. As compared with renal ethanolamine lysophosphoglyceride, which has three esteratic functions susceptible to metabolic hydrolytic attack, synthetic **18** has only one. The sustained hypotensive activity of **18** seen with single daily injections of about 6 mg/rat may be due to a resistance to catabolism or inability to be "reacylated" in the plasma relative to the natural product, as well as to its characteristic structural, chemical, and physical properties.

When Antonello et al.[8] administered (iv) 2 mg (per animal) of hog kidney renin preinhibitor[†] to hypertensive rats under the same experimental conditions used with renin inhibitor, no effect on blood pressure was observed; the authors suggested either the absence of circulating specific phospholipase(s) or nonactivation of renin preinhibitor to explain the observed results. Several independent laboratories, however, have reported[5–7] that administration of renin preinhibitor phosphoglyceride at doses higher than 2 mg/kg caused sustained reduction in blood pressure. Sen et al.[6] found that blood pressure in acute renal hypertensive

rats (average systolic pressure 244 mmHg; range 220–260) fell 80–130 mmHg when injected (im) with daily doses of 8–15 mg/kg of hog kidney renin preinhibitor. Peterhouse[5] administered 4.5 mg/day of a hog kidney phosphatidylethanolamine fraction to a number (unspecified) of rats with renal hypertension and found an average decrease of 36 mmHg in two of the rats; in other rats the variation in blood pressure was ±5 mmHg; dosage with different hog kidney lipid fractions—neutral lipids, phosphatidylcholine, lysophosphatidylcholine plus lysophosphatidyl serine—resulted in no antihypertensive effect. Ripa et al.[7] treated (sc) rats made hypertensive by the Grollman technique (8-type ligature of one kidney and counterlateral nephrectomy 2 months later) with hog renal phospholipid mixture at a dosage of 30 mg/animal twice weekly for 120 days starting from the day of the first intervention until the 60th day after the second intervention. Reductions in blood pressure of 15 and 20% relative to control animals were observed after 60 and 180 days, respectively, from the second kidney intervention; pressure differences between control rats and rats treated with phospholipids were found to be greatest 180 days from the second intervention, i.e., 4 months after stoppage of phospholipid administration. Tinker et al.[15] infused hog and dog phosphatidylethanolamine and ox brain phosphatidylserine (10 mg/ml in Na deoxycholate) into rats under experimental conditions[§] claimed to be the same as those used by Sen and coworkers[9] and found that the lipids failed to inhibit renin activity in vivo. These results as well as other studies[35] on the activity and stereospecificity of rat plasma phospholipase on rat liver phosphatidylethanolamine led Osmond and coworkers[15,36] to question the existence of an arachidonate-rich lysophosphatidylethanolamine inhibitor in plasma and the relevance of phospholipids as physiological regulators of the renin–angiotensin system.

Differences in results and interpretation of experimental data among investigators still exist concerning the structures of individual species of renin inhibitor molecules, their in vivo effects on blood pressure and plasma renin activity, and their possible involvement as regulators of the renin–angiotensin system; however, more data exist now to support this type of activity by exogenously administered lipid than existed when phospholipid inhibition of renin was first reported.[1,16] Endogenously, the possible role of a phosphoglyceride–phospholipase–lysophosphoglyceride system operant in the regulation of the renin–angiotensin system has yet to be proven. If lysophosphoglycerides have a physiologic role as regulators of the renin angiotensin system, it can be suggested that such lipids are polyunsaturated lysophosphatidylethanolamines containing arachidonate as a principal bioactive moiety of molecular structure; speculation is made on the basis of preliminary results on the in vivo activity of **18**, in vitro studies with **3–18** and related analogs,[33] and an attempt to discern trends in structure–activity relationships of renal and synthetic phosphoglycerides, lysophosphoglycerides, and structurally related analogs reported in the literature that inhibit renin activity.[&]

Experimental Section

Arachidonic acid (**3**), arachidonic acid methyl ester (**7**) (97–99%), and other fatty esters were obtained from the Hormel Institute, Austin, Minn. 55912, and Nu-Check Prep., Inc., Elysian, Minn. 56028. Unless otherwise specified, silica gel (0.05–0.2 mm) for column chromatography and precoated 0.25-mm silica gel G plates used for TLC analysis were obtained from Brinkmann Instruments, Inc., Westbury, N.Y. 11590; silicic acid (100 mesh) for column chromatography was obtained from Mallinckrodt Chemical Works, St. Louis, Mo. 63160. Identification of thin-layer chromatographic spots was carried out with dichromate–H_2SO_4, ethanolic ninhydrin (2%),[37] and molybdenum[38] reagents, and with iodine vapor. Ir spectra (cm^{-1}) were determined with a Beckmann IR 8 spectrophotometer; high-resolution ir spectra of **3** and **7–11** were taken with a Perkin-Elmer 621 spectrophotometer. ^1H NMR spectra (δ, ppm) were recorded in $CDCl_3$ (internal Me_4Si) with Varian A-60 and Jeol JNMPS-100 NMR spectrometers. Description of 60-MHz ^1H NMR data only is included in the Experimental Section; unless otherwise specified, chemical shift (δ) values are reported as the actual or approximate centers of respective proton resonances. A Cary 15 recording spectrophotometer was used for determination of uv spectra; λ max (cyclohexane) of tetraenoids **3–11**, **12**, **17**, and **18** was 233 nm. Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Chemical reactions and column chromatography generally were carried out under N_2; under carefully controlled conditions, derivatives of high purity were obtained without employment of antioxidants. In some preparations N_2 was purified by bubbling it first through a solution of benzopinacolone in pyridine treated with $LiAlH_4$[39] and then through concentrated H_2SO_4. Elemental analyses were performed by Micro-Analysis, Inc., Marshallton, Wilmington, Del. 19808; microanalytically acceptable samples of polyunsaturated lipids did not necessarily indicate that such derivatives did not contain trace amounts of autooxidized products. Ir spectra of **4**, **5**, and **12–17** clearly showed the presence of H_2O; only **17** and **18** were dried (P_2O_5, 0.1 mm/24 hr) thoroughly prior to elemental analysis. Polyunsaturated derivatives for microanalysis and biologic testing were sealed and stored in ampoules (−40°) under N_2.

Dichloro(*N*-2,2,2-trichloroethoxycarbonyl-2-aminoethyl) phosphate was prepared by the method of Pfeiffer et al.;[23] (2-phthalimido)ethylphosphonic acid monochloride was prepared according to the method of Baer and Stanacev.[24] Derivatives **12–15** were synthesized under the same reaction conditions, except for stoichiometry, so that details of the preparation of **12** only are given; column chromatographic purifications of **13–16** were similar, but not identical, to that employed for **12**.

Arachidonoylcholine Chloride (4). A solution of 1 g (0.003 mol) of **3** and 0.8 g (0.006 mol) of freshly distilled oxalyl chloride in 30 ml of anhydrous C_6H_6 was refluxed for 4 hr under N_2. The reaction mixture was cooled and the solvent was removed under reduced pressure (2 mm) to obtain a quantitative yield of arachidonoyl chloride. A mixture of 0.5 g (0.0015 mol) of the acid chloride and 0.22 g (0.0015 mol) of choline chloride (P_2O_5 dried) was heated (oil bath) at 120° for 12 hr. The reaction mixture was cooled and taken in a small volume of $CHCl_3$ and the product was purified by chromatography on a column (24 × 19 mm) of silica gel (75-325 mesh, 30 g) using $CHCl_3$ (100 ml), $CHCl_3$–MeOH (4:1, 150 ml), and $CHCl_3$–MeOH (3:2) as eluents. After removal of the solvents under reduced pressure, there was obtained 0.42 g (62.9%) of a brown-colored waxy hygroscopic semisolid: R_f (TLC) 0.68 [silica gel, $CHCl_3$–MeOH–0.1 *M* NaOAc (4:4:1)]; ir (neat) 3020, 2925, 2850, 1750, 1640, 1450, 700; ^1H NMR 0.85 (t, 3, CH_3), 1.31 (s, 8, CH_2), 1.61–2.58 (overlapping multiplets, 10, CH_2CH_2CO, CH_2CHCl, $CH_2CH{=}CH$, CH_2CO), 2.78 (m, 4, $CH{=}CHCH_2CH{=}CH$), 3.52 [s, 9, $^+N(CH_3)_3$], 4.05 (broad, 2, CH_2N^+), 4.60 (broad, >2, $CHCl$, CH_2O), 5.34 (m, 6, $CH{=}CH$). Anal. ($C_{25}H_{44}ClNO_2{\cdot}2H_2O{\cdot}HCl$) C, H, N; Cl: calcd, 14.23; found, 14.70.

Palmitoylcholine Chloride (5). A mixture of palmitoyl chloride, 1.3 g (0.005 mol), and P_2O_5-dried choline chloride, 0.7 g (0.005 mol), was heated (oil bath) at 140° for 2 hr. The reaction mixture was cooled and 20 ml of anhydrous Et_2O was added which caused separation of a white solid which then was collected and dissolved in CH_3OH. Recrystallization several times from CH_3OH–Et_2O gave a white amorphous solid: mp 200–203°; ir (KBr) 2960, 2845, 1750, 1450; ^1H NMR 0.90 (t, 3, CH_3), 1.27 (s, 26, CH_2), 2.35 (t, 2, CH_2CO), 3.55 [s, 9, $^+N(CH_3)_3$], 4.12 (broad, 2, CH_2N^+), 4.62 (broad, 2, CH_2O). Anal. ($C_{21}H_{44}ClNO_2{\cdot}H_2O$) C, H, N, Cl, O.

***N,N*-Diethyl-5,8,11,14-eicosatetraenamide (6).** Arachidonoyl chloride (1.05 g, 0.003 mol) was cooled in an ice bath and 0.438 g (0.006 mol) of freshly distilled diethylamine was added. The reaction mixture was stirred (magnetic bar) for 10 min and then 20 ml

[§] The techniques utilized by Tinker et al.[15] and by Sen et al.[9] were not exactly the same; the former group infused phospholipid and then administered renin, whereas the latter group simultaneously infused phospholipid and renin.

[&] The fact that lysophospholipids can inhibit renin activity does not necessarily mean that these species associate only with the enzyme (renin) itself. Angiotensinogen (renin substrate) is also a protein (α-globulin), so that the possibility exists that angiotensin I generation could be inhibited by lipid interaction with renin and/or its substrate.

of $CHCl_3$ and 5 ml of H_2O were added. After separation of the aqueous phase, the organic phase was extracted with three 5-ml portions of H_2O; the original aqueous phase and the other aqueous extracts were combined and extracted with three 20-ml portions of $CHCl_3$. The chloroformic extracts were combined and the solvent was removed under reduced pressure (25–30°). The crude product, 1.0 g, was purified by chromatography on a column (24 × 19 mm) of silicic acid (30 g, 100 mesh), starting with C_6H_6 (100 ml), C_6H_6–$CHCl_3$ (9:1, 120 ml), and C_6H_6–$CHCl_3$ (8:2, 200 ml). Most of the product then was eluted with C_6H_6–$CHCl_3$ (7:3) to obtain 0.8 g (70%) of a yellow oil: R_f (TLC) 0.5 [silica gel, hexane–acetone (8:2)]; ir (neat) 3020, 2925, 2850, 1640, 1450, 700; ^1H NMR 0.93 (m, 9, CH_3), 1.27 (m, 8, CH_2), 1.52–2.53 (m, 6, CH_2CH=CH, CH_2CO), 2.76 (m, 6, CH=CHCH_2CH=CH), 3.27 [m, 4, N(CH_2CH_3)$_2$], 5.27 (m, 8, CH=CH). Anal. ($C_{24}H_{41}NO\cdot\frac{1}{8}H_2O$) C, H, N.

Arachidonyl Alcohol (8). A solution of 0.31 g (0.0082 mol) of dry powdered $LiAlH_4$ in 30 ml of anhydrous Et_2O was refluxed for 2 hr under purified N_2. A solution of 3.42 g (0.011 mol) of **7** in 15 ml of anhydrous Et_2O then was added (dropwise) at a rate such as to produce gentle reflux. After the addition was completed, the solution was refluxed for another hour and was cooled (ice bath) with continued stirring. The unreacted hydride and reaction complex were decomposed by the dropwise addition of 30 ml of cold O_2-free 10% aqueous H_2SO_4. After filtration, the reaction product was extracted with six successive 10-ml portions of Et_2O, and the combined ether extracts were washed with 10 ml of H_2O and then dried over anhydrous Na_2SO_4 under N_2. Removal of the solvent under reduced pressure gave 3.16 g (quantitative, crude) of a light-yellow-colored oil: R_f (TLC) 0.2 [silica gel, hexane–Et_2O (7:3)]; ir (neat) 3200–3400, 3020, 2925, 2850, 1640, 1450, 1050, 985, 700; ^1H NMR 0.92 (t, 3, CH_3), 1.34 (m, 10, CH_2), 2.08 (m, 4, CH_2CH=CH), 2.84 (t, 6, CH=CHCH_2CH=CH), 3.64 (t, 2, CH_2OH), 5.38 (m, 8, CH=CH).

Arachidonyl Methanesulfonate (9). To a cooled solution (ice bath) of 2.56 g (0.009 mol) of 8 in 13 ml of anhydrous pyridine was added (dropwise) 2 ml of freshly distilled methanesulfonyl chloride over a period of 1 hr. The ice bath was removed and stirring was continued for another 5 hr at room temperature. The resultant brown slurry was treated with 20 ml of H_2O and 30 ml of Et_2O with stirring and cooling; the aqueous layer was separated and kept in an ice bath. The ether phase was extracted consecutively with 8 ml of H_2O, 2 ml of 2 *N* aqueous H_2SO_4, 8 ml of H_2O, 1 ml of 1% aqueous K_2CO_3, and 8 ml of H_2O and then was dried over anhydrous Na_2SO_4 under N_2. The original aqueous phase and the other basic aqueous layers were combined and extracted with 10 ml of Et_2O, and after washing the ether extract with 8 ml of H_2O, the ether extract was used to extract the original acidic layers. The ethereal phase was treated with 8 ml of H_2O, 1 ml of 1% aqueous K_2CO_3 solution, and 8 ml of H_2O and then was dried with the original ether extract over anhydrous Na_2SO_4 (N_2). After filtration and removal of the solvent under reduced pressure and then at vacuum (2 mm), there was obtained 2.94 g (91% crude) of a brown syrup: R_f (TLC) 0.17 [silica, hexane–Et_2O (7:3)]; ir (neat) 3020, 2925, 2850, 1640, 1450, 1170, 1350; ^1H NMR 0.92 (t, 3, CH_3), 1.34 (m, 10, CH_2), 2.08 (m, 4, CH_2CH=CH), 2.84 (t, 6, CH=CHCH_2CH=CH), 2.96 (s, 3, CH_3SO_3), 4.22 (t, 2, CH_2O), 5.37 (m, 8, CH=CH).

Arachidonylacetone Glycerol 1-Ether (10). A solution of 0.403 g (0.01 mol) of potassium in 7 ml of 1,2-isopropylideneglycerol was added (dropwise) to a solution of 4.3 g (0.012 mol) of crude **9** in 15 ml of dry C_6H_6. The mixture was warmed in an oil bath (50°) under purified N_2 for 43 hr. The mixture was allowed to cool to room temperature and was extracted with 70 ml of Et_2O and 70 ml of H_2O. The water layer then was extracted with successive 50- and 30-ml portions of Et_2O. The combined ether layers were washed with H_2O (20 ml) and the solvent was removed under reduced pressure and then under vacuum (2 mm) at room temperature to give a light-brown-colored syrup. The crude product was purified by chromatography on a column (2.5 × 40 cm) of silica gel (100 g) using hexane–Et_2O (7:3) as eluent, followed by rechromatography on a column (24 in. × 19 mm) of silica gel (75 g) using hexane–Et_2O (9:1) and then hexane–Et_2O (7:3) as eluents. After removal of the solvents under reduced pressure and then under vacuum (2 mm) at room temperature, there was obtained 3.51 g (74.4%) of an almost colorless oil: ir (neat) 3010, 2980, 2925, 2850, 1640, 1385–1365, 1140–1085, 970, 700: ^1H NMR 0.93 (t, 3, CH_3), 1.21–1.72 (m, 10, CH_2), 1.35, 1.42 [2, s, 6, $(CH_3)_2C$], 2.08 (m, 4, CH_2CH=CH), 2.83 (t, 6, CH=CHCH_2CH=CH), 3.30–4.35 (m, 7, $CHOH$, CH_2O), 5.38 (m, 8, CH=CH). Anal. ($C_{26}H_{44}O_3$) C, H.

1-*O*-Arachidonylglycerol (11). To a solution of 0.20 g (0.49 mmol) of **10** in 10 ml of freshly distilled CH_3OH was added (dropwise) 1 ml of concentrated aqueous HCl with continuous stirring. The solution was kept under N_2 for 24 hr at room temperature. The product was treated with 10 ml of H_2O and the solution was extracted with three 20-ml portions of Et_2O. The combined ether extracts were washed consecutively with 4 ml of H_2O, 1 ml of 1% aqueous K_2CO_3, and 4 ml of H_2O and were dried over anhydrous Na_2SO_4 (N_2). After removing the solvent under vacuum (2 mm) at room temperature, there was obtained 0.18 g (quantitative, crude) of an almost colorless oil: R_f (TLC) 0.18 [silica gel; hexane–Et_2O–MeOH (9:1:2)]; ir (neat) 3400, 3020, 2925, 2850, 1640, 1450, 1045, 1115, 970; ^1H NMR 0.93 (t, 3, CH_3), 1.35 (m, 10, CH_2), 2.08 (m, 4, CH_2CH=CH), 2.83 (t, 6, CH=CHCH_2CH=CH), 3.35–4.00 (m, 7, $CHOH$, CH_2O), 5.38 (m, 8, CH=CH). An analytical sample was prepared by chromatography on a column (18 in. × 10 mm) of silica gel (20 g/500 mg of crude product) using $CHCl_3$–CH_3OH (9:1) as the eluent followed by rechromatography on the same column using $CHCl_3$–MeOH (95:5). Anal. ($C_{23}H_{40}O_3\cdot\frac{1}{8}H_2O$) C, H.

Arachidonylphosphorylethanolamine (12). A solution of 4.6 g (0.016 mol) of 8 in 40 ml of dry $CHCl_3$ and 2.53 g (0.032 mol) of anhydrous pyridine was added to an ice-cold solution of 11.3 g (0.032 mol) of dichloro(*N*-2,2,2-trichloroethoxycarbonyl-2-aminoethyl) phosphate in 15.6 ml of dry C_6H_6. The addition took about 2 hr and after being stirred 1 hr at 0°, the solution was stirred at room temperature under N_2 for 20 hr. Et_2O (700 ml) was added and the mixture was washed with 70 ml of cold H_2O, 70 ml of cold dilute aqueous HCl, 70 ml of cold saturated aqueous NaCl solution, 70 ml of cold 5% aqueous $NaHCO_3$, and 70 ml of cold saturated aqueous NaCl solution. The ether layer was evaporated without drying to give a suspension which was dissolved in 60 ml of 95% HOAc and 60 ml of Et_2O–25 g of activated Zn then was added and the suspension was stirred at room temperature under N_2 for 20 hr. The suspension was diluted with Et_2O (700 ml), the Zn and inorganics were filtered, and the filtrate was washed with four 200-ml portions of H_2O and then with 200 ml of saturated NaCl solution. Evaporation of the dried (Na_2SO_4) solvent gave 7.62 g of a brown syrup. The crude product (4 g) was purified by chromatography on a column (2.5 × 40 cm) of silica gel (100 g) using $CHCl_3$–MeOH–H_2O (1:5:0.7) as eluent. Removal of the solvent was followed by rechromatography on a column (2.5 × 40 cm) of silica gel (100 g) using the same eluent. After removal of the solvent under reduced pressure and then under vacuum (2 mm, 10 hr), 0.85 g of homogeneous (TLC) product and an additional 0.56 g containing trace impurities were obtained (41% yield from **8**) of a light-brown-colored wax: R_f (TLC) 0.37 [silica gel, $CHCl_3$–MeOH–H_2O (11:5:0.7)]; ir (neat) 3020, 2925, 2850, 2700–2500, 2140, 1640, 1450, 1240, 1080, 1050–980; ^1H NMR 0.92 (t, 3, CH_3), 1.34 (m, 10, CH_2), 2.07 (m, 4, CH_2CH=CH), 2.83 (m, 6, CH=CHCH_2CH=CH), 3.10 (m, 2, CH_2N), 3.92 (m, 4, CH_2O), 5.38 (m, 8, CH=CH). Anal. ($C_{22}H_{40}NO_4P\cdot 0.5\ H_2O$) C, H, N, P.

Linolenylphosphorylethanolamine (13). Crude brown-colored oil **13** (3.62 g) was purified by chromatography on a column (2.5 × 40 cm) of silica gel (100 g) using $CHCl_3$–MeOH (8:2) and then $CHCl_3$–MeOH–H_2O (11:5:0.7) as eluents. Removal of solvents was followed by rechromatography (silica gel, 100 g) with $CHCl_3$–MeOH–H_2O (11:5:0.7); a third chromatographic purification (silica gel, 100 g) with $CHCl_3$, $CHCl_3$–MeOH (7:3), and then $CHCl_3$–MeOH–H_2O (11:5:0.7) gave, after removal of solvents under reduced pressure and then under vacuum (2 mm, 10 hr), a homogeneous (TLC) light-yellow-colored oil in about 40% yield (estimated, due to mechanical loss). Anal. ($C_{20}H_{38}NO_4P\cdot H_2O$) C, N; H: calcd, 9.94; found, 9.30; Cl: calcd, 7.64; found, 7.21.

Linoleylphosphorylethanolamine (14). Crude yellow-colored oil **14** (16.9 g) was purified by chromatography on a column (4 × 75 cm) of silica gel G (300 g) using $CHCl_3$–MeOH–H_2O (11:5:0.7) as eluent. Removal of solvents under reduced pressure and then under vacuum (2 mm, 10 hr) gave 2.62 g (36%) of a white wax-like solid. Anal. ($C_{20}H_{40}NO_4P\cdot 0.5\ H_2O$) C, H, N, P.

Oleylphosphorylethanolamine (15). Crude colorless oil **15** (9.0 g) was purified by chromatography on a column (4 × 95 cm) of silica gel G using $CHCl_3$–MeOH–H_2O (11:5:0.7) as eluent; removal of solvent and drying in vacuo (2 mm, 10 hr) gave 3.66 g (50%) of a white wax-like solid. Anal. ($C_{20}H_{42}NO_4P\cdot\frac{1}{3}H_2O$) C, H, N; P: calcd, 7.79; found, 7.24.

Palmitylphosphorylethanolamine (16). A solution of 1.0 g (0.0041 mol) of palmityl alcohol in 8 ml of dry $CHCl_3$ and 0.64 ml of dry pyridine was added (90 min) to an ice-cold solution of 2.92 g (0.00825 mol) of dichloro(*N*-2,2,2-trichloroethoxycarbonyl-2-aminoethyl) phosphate in 5 ml of dry C_6H_6; after being stirred 1 hr at 0°, the solution then was stirred at room temperature under N_2 for 34 hr. Absolute Et_2O (200 ml) was added, and the mixture was

washed with 20 ml of H_2O, 20 ml of dilute aqueous HCl, 20 ml of saturated aqueous NaCl solution, 20 ml of 5% aqueous $NaHCO_3$, and 20 ml of saturated aqueous NaCl solution. The ether layer was evaporated without drying to give a suspension, which was dissolved in 12.5 ml of 95% HOAc and 10 ml of absolute Et_2O; 5.0 g of activated Zn then was added. The suspension then was stirred at room temperature for 21 hr and after dilution with absolute Et_2O (150 ml), the zinc and inorganics were filtered, and the filtrate was washed with four 50-ml portions of H_2O. The ether layer was exposed to a stream of HCl gas[20] to give a white precipitate. After centrifugation, the supernatant was separated and concentrated to obtain another precipitate; the procedure was repeated several times. The combined precipitates were purified by chromatography on a column (2.5 × 40 cm) of silica gel (100 g) using $CHCl_3$–MeOH–H_2O (11:5:0.7) as eluent. After removal of the solvent, 400 mg (27%) of an almost pure product was obtained. The product was crystallized from $CHCl_3$–MeOH–H_2O (11:5:0.7) and then from $CHCl_3$–MeOH (1:1). The twice crystallized product was heated with $CHCl_3$ on a steam bath, allowed to cool to room temperature, and filtered to obtain a white solid: mp 236.5–238°; R_f (TLC) 0.47 [silica gel, $CHCl_3$–MeOH–H_2O (11:5:0.7)]; ir (KBr) 3300–3030, 2900, 2850, 2700–2500, 2130, 1450, 1220, 1080, 1050–980. Anal. ($C_{18}H_{40}NO_4P·0.25H_2O$) C, H, N, P.

Arachidonyl (2-Phthalimidoethyl)phosphonate (17). 2-Phthalimidoethylphosphonic acid monochloride freshly prepared from 2.04 g (0.008 mol) of 2-phthalimidoethylphosphonic acid and 1.68 g (0.008) mol) of PCl_5 was dissolved in 100 ml of anhydrous, EtOH-free $CHCl_3$. The solution was added under anhydrous conditions during 1 hr to an ice-cold solution of 1.1 g (0.004 mol) of 8 and 4 ml (0.028 mol) of dry Et_3N in 100 ml of anhydrous EtOH-free $CHCl_3$. The reaction mixture was stirred at room temperature (25°) under purified N_2 for 5 days; 2 ml of Et_3N and 4 ml of water then were added and the mixture was stirred vigorously for 2 hr. The solvents were evaporated under reduced pressure (30–35°) and 100 ml of C_6H_6 was added to the residue. The C_6H_6 was distilled under reduced pressure and the procedure was repeated twice more with additional 100-ml portions of C_6H_6. The residue was extracted with three 100-ml portions of anhydrous Et_2O, and the combined ether extracts were distilled under reduced pressure (20°). The semisolid was dissolved in 200 ml of $CHCl_3$–MeOH–H_2O (5:4:1); 40 g of Amberlite IR-120 (H^+ form) was added to the solution and the mixture was stirred vigorously for 15 min. The Amberlite was filtered and washed with two 75-ml portions of $CHCl_3$ and the combined filtrates were evaporated to dryness under reduced pressure (25–30°). The crude product weighed 1.5 g and was purified by chromatography on a column (24 in. × 19 mm) of silicic acid (50 g, 100 mesh) using consecutive 200-ml portions of $CHCl_3$–C_6H_6 (1:1), $CHCl_3$–C_6H_6 (3:1), $CHCl_3$, and $CHCl_3$–MeOH (19:1) as eluents. After removal of the solvents under reduced pressure, there was obtained 0.7 g (50% yield) of a brown-colored waxy semisolid: R_f (TLC) 0.6 [silica gel, $CHCl_3$–MeOH–H_2O (6.5:3:0.5)]; ir (neat) 3020, 2925, 2850, 2700–2500, 2130, 1780, 1730, 1640, 1450, 1230, 1050, 1050; ^1H NMR 0.92 (t, 3, CH_3), 1.31 (m, 10, CH_2), 1.65 (m, 2, PCH_2), 2.17 (m, 4, CH_2CH=CH), 2.80 (m, 6, CH=CHCH_2CH=CH), 3.85–4.50 (m, 4, CH_2O, CH_2N), 5.36 (m, 8, CH=CH), 7.73 (m, 4, aromatic). Anal. ($C_{30}H_{42}NO_5P·H_2O$) C, H, N, P.

Arachidonyl (2-Aminoethyl)phosphonate (18). Phthalimide 17, 0.9 g (0.0017 mol), was stirred with 95% EtOH (60 ml) and 50% aqueous hydrazine (0.6 ml) at 25° for 30 hr; a precipitate was observed after about 6 hr, which was voluminous at the end of the reaction period. The solvents were evaporated, the residue was stirred with 40 ml of $CHCl_3$, and the suspension was filtered. The filtrate was concentrated, the yellow-colored semisolid was taken up in $CHCl_3$, and the solution was applied to a column of 20 g of silicic acid (100 mesh) and chromatographed using approximately 100-ml volumes of C_6H_6–$CHCl_3$ (1:1), $CHCl_3$, $CHCl_3$–MeOH (95:5), and $CHCl_3$–MeOH (9:1) as eluents to give 0.25 g of a yellow-colored waxy semisolid: R_f (TLC) 0.45 [silica gel, $CHCl_3$–MeOH–H_2O (6.5:3:0.5)]; ir (neat) 3020, 2925, 2850, 2700–2500, 2130, 1640, 1580, 1450, 1230, 1050, 1050; ^1H NMR 0.92 (t, 3, CH_3), 1.31 (m, 10, CH_2), 1.65 (m, 2, PCH_2), 2.17 (m, 6, CH_2CH=CH, CH_2N), 2.80 (m, 6, CH=CHCH_2CH=CH), 3.85 (m, 2, CH_2O), 5.36 (m, 8, CH=CH), 8.67 (broad, 2, NH_2). Anal. ($C_{22}H_{40}NO_3P$) C, H, N, P.

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