Darmstoff Analogues. 3. Actions of Choline Esters of Acetal Phosphatidic Acids on Visceral Smooth Muscle†

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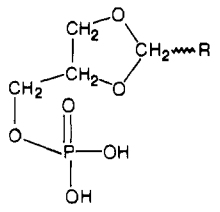
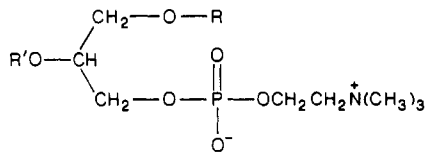
A number of naturally occurring phospholipids, e.g. the acetal phosphatidic acid derivatives that comprise Darmstoff (1) and the phosphatidylcholine derivative platelet activating factor (PAF), cause contraction of certain visceral smooth muscles and cause platelet activation. Because the Darmstoff phosphatidic acids and PAF are structurally similar, it was of interest to compare the biological actions of choline esters of Darmstoff with those of PAF and of the parent Darmstoff phosphatidic acids. To this end, [(2-pentadecyl-1,3-dioxolan-4-yl)methyl]phosphocholine (**3a**), [[2-(*cis*-8-heptadecenyl)-1,3-dioxolan-4-yl]methyl]phosphocholine (**3b**), and [[2-(*cis*-8-pentadecenyl)-1,3-dioxolan-4-yl]methyl]phosphocholine (**3c**) were synthesized. Compounds **3a**, **3b**, **3c**, and PAF caused dose-dependent relaxation of taenia coli strips. In contrast, the unesterified materials **1a** and **1b**, as well as lyso-PAF, caused contraction in taenia coli strips. Thus, the contractile effect of Darmstoff is reversed on esterification with choline. In preparations of whole trachea, both **1a** and **3a** had contractile effects similar to those of PAF.

Darmstoff, an acidic phospholipid mixture, was originally isolated by Vogt from horse intestine and shown to contract in vitro preparations of visceral smooth muscle.[1] Subsequently, a phospholipid material extracted from rabbit kidney also contracted visceral smooth muscle[2] and the major components of both the horse intestine and kidney extracts were shown to be phosphorylated glyceryl acetals of palmitaldehyde (**1a**), olealdehyde (**1b**), and linolealdehyde (**1c**).[3] Structures of the Darmstoff com-



1a: R = $(CH_2)_{14}CH_3$
b: R = $(CH_2)_7CH{=}CH(CH_2)_7CH_3$
c: R = $(CH_2)_7CH{=}CHCH_2CH{=}CH(CH_2)_4CH_3$

pounds were confirmed by synthesis and the synthetic olealdehyde derivative was shown to elicit contractions of the guinea pig ileum identical with those of the natural phospholipid mixture; in contrast the palmitaldehyde acetal had no ileal muscle contracting activity.[3] Palmitaldehyde–phosphatidic acid acetal (**1a**) had negative chronotropic and hypotensive actions in unanesthetized rats[4] and the three acetal phosphatidic acids **1a**, **1b**, and **1c** were shown to be potent inducers of platelet aggregation and release.[5,6] These properties suggest that Darmstoff phospholipids could have physiological or pathological significance, but as yet no biological role for these lipids has been demonstrated. A role in regulation of the rhythmicity of smooth muscle cells or of peristaltic function is conceivable for the visceral smooth muscle contracting acetal phosphatidic acid congeners. The structurally similar phospholipid 1-*O*-alkyl-2-*O*-acetyl-*sn*-glycero-3-phosphocholine (platelet activating factor, PAF, **2**) is produced by various cell types and organs and is a potent mediator of inflammation.[7] PAF also has smooth muscle activity, inducing acute bronchospasm[8] and con-



2 (PAF): R = $C_{16}H_{33}$, $C_{18}H_{37}$; R′ = CH_3CO
lyso-PAF: R = $C_{16}H_{33}$, $C_{18}H_{37}$, R′ = H

tracting lung tissue preparations from various animal species.[9,10] In view of the structural similarity between unesterified PAF and the Darmstoff phospholipids, it was of interest to study the visceral smooth muscle effects of the choline esters of Darmstoff compounds, particularly as the latter may be the biological precursors of Darmstoff in a pathway analogous to that described for formation of PAF;[11] moreover the choline esters may have important physiological actions per se. To this end esters of palmityl, oleyl, and palmitoleyl acetal phosphatidic acids (**3a**, **3b**, **3c**; Scheme II) were synthesized. The palmitoleyl congener (**3c**) was additionally selected for study in order to gain insight into the effect of alkyl side chain unsaturation on smooth muscle activity. Actions of the three glycerophosphocholines (**3a–c**) were evaluated with guinea pig taenia coli strips,[12] comparing responses with those of the parent Darmstoff acids **1a** and **1b** and with PAF. Some studies were also performed with guinea pig whole trachea preparations.[13]

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(1) Vogt, W. *Biochem. Pharmacol.* **1963**, *12*, 415.
(2) Dyer, D.; Walaszek, E. J. *J. Pharmacol. Exp. Ther.* **1968**, *160*, 360.
(3) Wiley, R. A.; Summer, D. D.; Walaszek, E. J. *Lipids* **1970**, *5*, 803.
(4) Wiley, R. A.; Milbert, A. N. *J. Med. Chem.* **1978**, *21*, 245.
(5) Bramner, J. P.; Maguire, M. H.; Walaszek, E. J.; Wiley, R. A. *Br. J. Pharmacol.* **1983**, *79*, 157.
(6) Bramner, J. P.; Maguire, M. M. *Br. J. Pharmacol.* **1984**, *82*, 61.
(7) Demopoulos, C. A.; Pinkard, R. N.; Hanahan, D. J. *J. Biol. Chem.* **1979**, *254*, 9355.
(8) Dejean, A.; Arnoux, B.; Masse, R.; Lockhart, A.; Benveniste, J. *J. Appl. Physiol.* **1983**, *55*, 799.
(9) Stimler, N. P.; Gerard, C.; O'Flaherty, T. J. In *Platelet Activating Factor*; Benveniste, J., Arnoux, B., Eds.; Elsevier: Amsterdam, 1983; p 195.
(10) Cerrina, J.; Raffestin, B.; Labat, C.; Boullet, C.; Bayor, C.; Gateau, O.; Brink, C. In *Platelet Activating Factor*; Benveniste, J., Arnoux, B., Eds.; Elsevier: Amsterdam, 1983; p 205.
(11) Snyder, F.; Blank, M. L.; Johnson, D.; Lee, T.; Malone, B.; Robinson, M.; Woodard, D. S. *Pharmacol. Res. Commun.* **1986**, *18-Suppl.*, 33.
(12) Maguire, M. H.; Satchell, D. G. *J. Pharm. Exp. Ther.* **1979**, *211*, 626.

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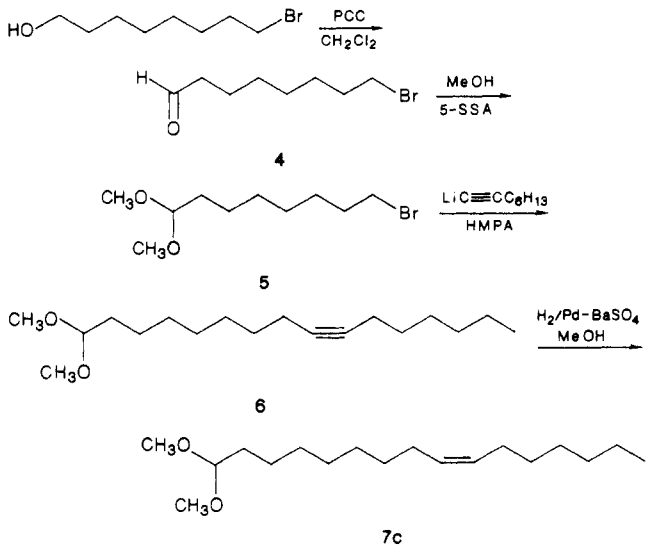
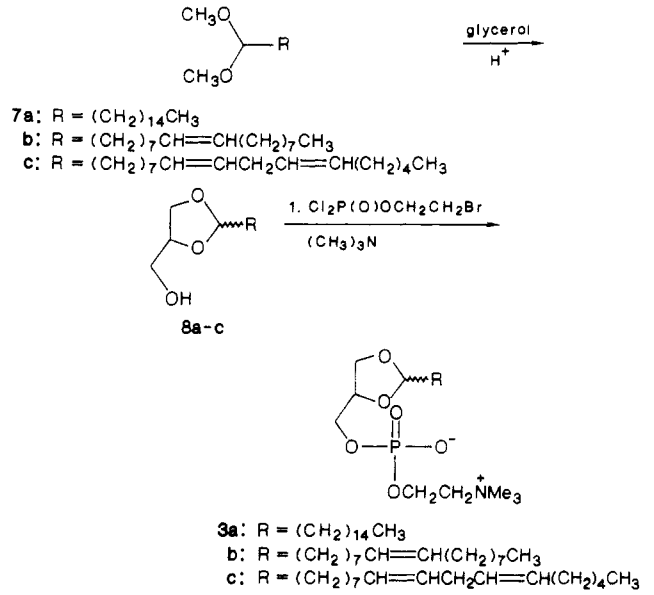
Scheme I



Scheme II



Results and Discussion

Chemistry. The alkyl side chains for **3a** and **3b** were prepared from the commercially available alcohols. The palmitoleyl side chain for **3c** was obtained in four steps from 8-bromooctanol[14] to give the acetal **7c** as shown in Scheme I. Known dioxolanes **8a** and **8c**[15] and the novel dioxolane **8b** were prepared by the transacetalation method of Piantadosi et al.[15,16] Finally, the phosphocholine group was introduced by using 2-bromoethyl phosphodichloridate by a modification of the method of Hirt[17] as shown in Scheme II. Thus, the reaction of **8a–c** with an excess of the phosphorylating reagent, followed by aqueous hydrolysis in diethyl ether gave the bromoethyl phosphodiesters which were converted to the phosphocholines **3a–c** by treatment with an excess of trimethylamine in a mixture of $CHCl_3$/DMF/*i*-PrOH. Other methods for introducing the phosphocholine group, such as esterification of an intermediate phosphodichloridate with a choline salt, were unsuccessful; the presence of aldehydes in the product mixture suggested that the acetal moiety may not withstand the acidic workup conditions of these alternate approaches.

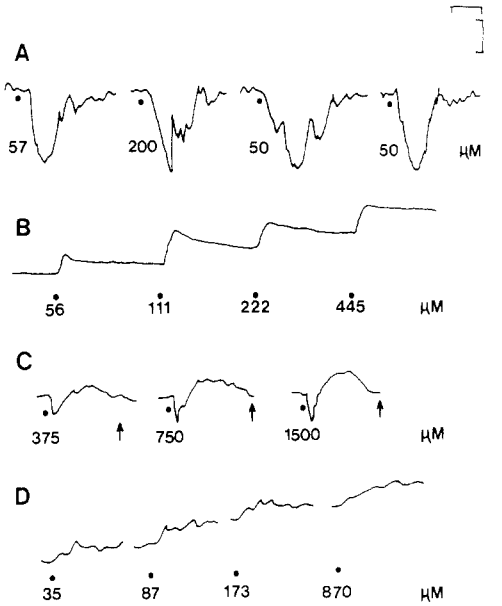


Figure 1. Typical responses of guinea pig taenia coli strip preparation to the acetal plasmalogens, **3a**, **3b** and **3c**, to PAF, to the acetal phosphatidic acids 1 and **1b**, and to lyso-PAF. Phospholipids were administered at the dot followed either by washout or by the next higher dose where cumulative dosing was used. Concentrations refer to final bath concentrations. Bars = abscissa 30 s, ordinate 300 mg. A. Comparison of relaxations to ED_{60} doses of **3a** (57 μM), **3b** (200 μM), **3c** (50 μM), and PAF (50 mM); washout was performed after 30 s. B. Dose-dependent contractions to cumulative doses of **1a**. C. Dose-dependent biphasic responses to **1b**; contraction recovered to base-line tone prior to washout indicated by the arrow. D. Dose-dependent contractions to cumulative doses of lyso-PAF.

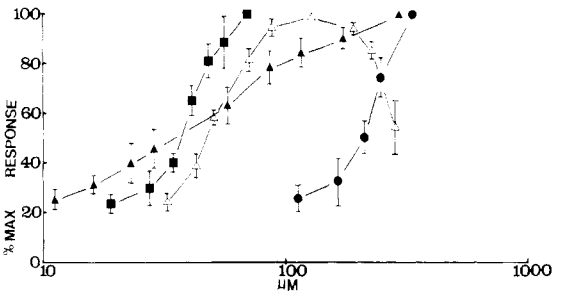


Figure 2. Concentration effect curves for relaxation of guinea pig taenia coli strips by acetal plasmalogens **3a** (solid triangles), **3b** (solid circles), and **3c** (solid squares) and PAF (open triangles). Values are means ± SEM of at least five responses from at least two different preparations. Drugs were administered to the bath in increasing order of concentration; each dose was followed by washout.

Pharmacology. Choline esters of acetal phosphatidic acids, i.e. **3a**, **3b**, and **3c** and PAF, relaxed the guinea pig taenia coli as indicated in the tracings shown in Figure 1. Relaxations were dose-dependent, but PAF differed from **3a**, **3b**, and **3c** in exhibiting tachyphylaxis at high concentrations (Figure 2). The unsaturated oleyl and palmitoleyl acetals **3b** and **3c** gave parallel log concentration–effect curves, but the oleyl acetal plasmalogen was 6 times less potent than the shorter chain congener; ED_{50} values were 200 and 37 μM, respectively. The concen-

(13) Smith, R. V.; Satchell, D. G. *Agents Actions* **1986**, *19*, 48.
(14) Rossi, R. *Synthesis* **1981**, 359.
(15) Piantadosi, C.; Anderson, C. E.; Brecht, E. A.; Yarbro, C. L. *J. Am. Chem. Soc.* **1958**, *80*, 6613.
(16) Piantadosi, C.; Anderson, C. E.; Yarbro, C. L.; Brecht, E. A. *J. Elisha Mitchell Sci. Soc.* **1965**, *81-Suppl.*, 1, 34.
(17) Hirt, R.; Berchtold, R. *Pharm. Acta Helv.* **1958**, *33*, 349.

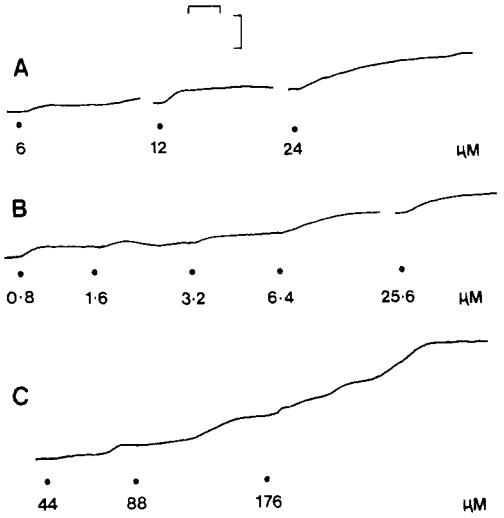


Figure 3. Typical responses of guinea pig whole trachea preparation to cumulative doses of PAF (uppermost tracing A), palmityl aldehyde acetal phosphatidic acid (**1a**) (middle tracing B), and **3a** (bottom tracing C). Intraluminal pressure was recorded. Phospholipids were added at the dot to give final bath concentrations shown. Bars = abscissa 2 min, ordinate 2 cm H_2O.

tration–effect curve of the saturated acetal plasmalogen **3a** was shallower than those of **3b** and **3c**, and covered 2 orders of magnitude of concentration; the ED_{50} and 34 μM. These findings suggest that **3b** and **3c** act similarly to relax the taenia and suggest that the mode of action of **3a** may be different. Moreover, the C_{15} side chain is seen to be associated with greater smooth muscle relaxing activity than an unsaturated C_{17} side chain, and unsaturation of the C_{15} side chain is found to enhance smooth muscle relaxing activity. Because PAF exhibited pronounced tachyphylaxis at higher concentrations, the log concentration–effect curve was bell-shaped. However, the ascending component of the PAF curve was parallel to the curves of **3b** and **3c**, suggesting that at lower concentrations PAF, **3b**, and **3c** act similarly.

Relaxation of taenia coli strips to ED_{50} concentrations of **3a**, **3b**, or **3c** were not antagonized by pretreatment for 30 min with 7 μM phentolamine, 5 μM propanolol, or 1.3 μM hyoscine, suggesting that these compounds do not act via cholinergic, muscarinic, or adrenergic receptors, and that they do not act by releasing acetylcholine or noradrenaline.

In contrast to the relaxation elicited by **3a**, **3b**, and **3c**, the unesterified acetal phosphatidic acids **1a** and **1b** caused dose-dependent contractions of the taenia coli (Figure 1). Contractions due to the palmitaldehyde derivative **1a** quickly reached a steady state and allowed cumulative dosing. Contractions in response to the olealdehyde derivative (**1b**) were preceded by transient rapid relaxations. The contractions were not sustained so that cumulative dosing could not be used. Lyso-PAF, a derivative of PAF that lacks the 2-acetyl substituent and the potent biological actions of PAF, also caused dose-dependent contraction of taenia coli (Figure 1). In the guinea pig whole tracheal preparation PAF, **1a**, and **3a** all caused dose-dependent increases in intraluminal pressure (Figure 3).

These findings show that contractile effects of acetal phosphatidic acids **1a** and **1b** on intestinal smooth muscle are reversed on esterification with choline. Unlike PAF, the choline esters **3a**, **3b**, and **3c** do not show tachyphylaxis in their actions on the taenia coli. It is tempting to speculate that **1a** and **3a** and **1b** and **3b** may be involved in regulation of visceral smooth muscle contraction in a cyclical fashion, with **3a** and **3b** being inactivated by deesterification and re-formed by esterification. The rhythmicity seen in a homogeneous layer of intestinal smooth muscle such as taenia coli may be related to such a cycle. However isolation of **3a** and **3b** from intestinal smooth muscle will be necessary to substantiate these conjectures. In contrast to its actions on the taenia coli, **1a** was previously shown to be without activity on the guinea pig ileum preparation while **1b** was quite potent.[3] The significance of these tissue-specific differences is not clear at present, but it should be noted that studies on the ileum were performed in the absence of hyoscine, so that indirect effects due to acetylcholine release are not ruled out.

On the whole trachea preparation both **1a** and **3a** had contractile actions similar to those of PAF. If present in tracheal smooth muscle, these phospholipids could contribute to asthma or other bronchial conditions.

After this work was completed, a report appeared that described the effects of a series of "acetal plasmalogens", bearing saturated alkyl side chains and similar to **3** on rabbit aortic strips.[18] Criteria of identity were not provided, but in general these materials produced a relaxation in the aortic preparations.

Experimental Section

Chemistry. Proton nuclear magnetic resonance spectra were recorded in $CDCl_3$ on a Varian FT-80A spectrometer and chemical shifts are reported in parts per million relative to internal tetramethylsilane (0.00 ppm). Infrared spectra (IR) were recorded on an IBM FT-IR 32 or a Beckman IR-33 instrument as thin films (liquids) or KBr pellets (solids). Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Electron-impact mass spectra (EIMS) and chemical-ionization mass spectra (CIMS) were recorded on a Ribermag R 10-10 mass spectrometer. Microanalyses were performed on a Hewlett-Packard 185 CHN analyzer. All reactions requiring anhydrous conditions and/or an inert atmosphere were performed under a positive pressure of dry argon. Anhydrous solvents were prepared by distillation under dry nitrogen and stored over activated molecular sieves. Solvents for chromatography were distilled before use.

8-Bromooctanal Dimethyl Acetal (5). 8-Bromooctanal (**4**) was prepared from 8-bromooctanol[14] by oxidation with pyridinium chlorochromate in 72% yield (bp 70–72 °C/0.12 mm). A solution of **4** (7.10 g, 34.3 mmol) in 150 mL of anhydrous methanol containing 25 mg of 5-sulfosalicyclic acid was refluxed for 8 h. After cooling, 25 mL of 1 N NaOH was added and the mixture stirred for 1 h. This was extracted with 3 × 75 mL of hexane, dried (Na_2SO_4), and evaporated to dryness. The residue was distilled to give 6.30 g of **5** (73%, bp 84 °C/0.05 mm) as a colorless oil. ^1H NMR: δ 4.40 (t, 1 H, acetal H), 3.40 (t, 2 H, CH, B_2R), 3.30 (s, 6 H, OCH_3). IR: 2984, 1458, 1230, 1055 cm^{-1}. Anal. ($C_{14}H_{21}BrO_2$) C, H. EIMS: 109, 95, 75 (base).

9-Hexadecynal Dimethyl Acetal (6). A cooled (0 °C) solution of 1-octyne (0.441 g, 4.00 mmol) in 2 mL of anhydrous HMPA was treated with *n*-butyllithium (9.5 M, 0.42 mL, 4 mmol) and stirred for 35 min at 4 °C. A solution of **5** (1.000 g, 4.00 mmol) in 1 mL of HMPA was added and the mixture was stirred overnight. After any unreacted butyllithium was decomposed with 0.1 mL water, the mixture was diluted with 5 mL of water and extracted with 3 × 60 mL of hexane. The combined extracts were washed with 25 mL of water and 25 mL of saturated aqueous NaCl, dried (K_2O_3), and evaporated. Distillation gave 500 mg of **6** (44%) as a light yellow oil (bp 125–127 °C/0.2 mm). ^1H NMR: δ 4.34 (t, 1 H, acetal H), 3.30 (s, 6 H, OCH_3), 2.12 (t, 4 H, CH_2CCCH_2). IR: 2932, 1462, 1126, 1057 cm^{-1}. Anal. ($C_{18}H_{34}O_2$) C, H. EIMS: 250 (M − 32), 75 (base).

(18) Braquet, P.; Braquet, C.; Auget, M.; Baranes, J.; Etiemble, E.; Godfroid, J. J. *Prostaglandins, Leukotrienes, Med.* **1987**, *26*, 209.

(19) Farmer, J. B.; Coleman, R. A. *J. Pharm. Pharmacol.* **1970**, 22, 46.

Hexadecenal Dimethyl Acetal (7a). This compound was prepared from hexadecanal as described for acetal **5** to give 18.7 g of **7a** (84%, bp 126–130 °C/0.5 mm) as a colorless oil.[15]

9-*cis*-Octadecenal Dimethyl Acetal (7b). This compound was prepared from 9-*cis*-octadecenal as described for acetal **5** to give 19.16 g of **7c** (84%, bp 123–135 °C/0.3 mm) as a colorless oil.

9-*cis*-Hexadecenal Dimethyl Acetal (7c). A solution of **6** (1.129 g, 4 mmol) in 50 mL of anhydrous methanol was added to a suspension of prereduced 5% $Pd/BaSO_4$ (120 mg) and quinoline (120 mg) in 5 mL of anhydrous methanol. After 2 h at room temperature (H_2, 1 atm), the mixture was filtered and evaporated and the residue was distilled to give 0.93 g of **7c** (81%) as a colorless oil (bp 120–121 °C/0.05 mm). Analysis by GLC (DB-5 column) indicated a single product and proton NMR (5.34 t, 2 H, J = 4.5 Hz) confirmed the presence of a cis double bond. Anal. ($C_{16}H_{36}O_2$) C, H. EIMS: 253 (M − 31), 75 (base).

2-(8-*cis*-Heptadecenyl)-4-(hydroxymethyl)-1,3-dioxolane (8b). This compound was prepared from **7b** as described for **8c** to give 19.5 g of **8b** (78%) after chromatography. Anal. ($C_{21}H_{40}O_3$) C, H.

2-(8-*cis*-Pentadecenyl)-4-(hydroxymethyl)-1,3-dioxolane (8c). A mixture of **7c** (0.468 g, 1.65 mmol), glycerol (0.303 g, 3.29 mmol), and 25 mg of 5-sulfosalicyclic acid was stirred vigorously at 140 °C for 2 h and then at 170 °C for 15 min to ensure the complete removal of methanol. After cooling, 50 mL of 1 N NaOH was added and the mixture was stirred for 1 h. This was diluted with 50 mL of water and extracted with 3 × 75 mL of hexane. The combined extracts were washed with 25 mL of H_2O and 25 mL of saturated NaCl, dried (Na_2SO_4), treated with activated carbon, filtered, and evaporated. Chromatography (silica gel, hexane/EtOAc 4:1) gave 0.381 g of **8c** (78%) as a colorless oil. ^1H NMR: δ 5.40 (t, 2 H, HC=CH), 4.89 (m, 1 H, acetal H), 4.00–3.50 (m, 6 H, glycerol H), 2.00 (s, 6 H, OCH_2-H_2 aliph, CH_2HC=$CHCH_2$). IR: 3426, 3003, 2924, 1458, 1142, 1043 cm^{-1}. Anal. ($C_{19}H_{36}O_3$) C, H. EIMS: 312 (M^+), 311, 103 (base), 57.

[(2-Pentadecyl-1,3-dioxolan-4-yl)methyl]phosphocholine (3a). To a cooled (0 °C) solution of 2-bromoethyl phosphorodichloridate[17] (0.288 g, 1.19 mmol) in 5 mL of anhydrous trichloroethylene (TCE) was added a solution of **8a**[15,16] (0.250 g, 0.795 mmol) and NEt_3 (0.483 g, 4.77 mmol) in 10 mL of TCE. After 4 h, 10 mL of toluene was added, and the mixture was filtered and evaporated. The residue was stirred in a mixture of 1 mL of H_2O and 0.1 mL of NEt_3 in 25 mL of Et_2O for 2 h at room temperature. This was diluted with 25 mL of water and extracted with 3 × 20 mL of Et_2O. The ether extracts were washed with 50 mL of 1 N Na_2CO_3, dried ($MgSO_4$), and evaported. The residue was dissolved in 2 mL of $CHCl_3$ and precipitated with 15 mL of anhydrous acetone. The crude intermediate was dissolved in 20 mL of a mixture of 25% trimethylamine (aqueous) in $CHCl_3$/DMF/isopropanol (7:3:5:5) and stirred for 8 h at 50 °C. The solvents were removed, and the residue was refluxed for 1 h in 25 mL of MeOH containing 100 mg of Ag_2O_3. The mixture was filtered and evaporated. The residue was purified by chromatography (silica gel, $CHCl_3$/MeOH/H_2O 65:30:4) to give 0.201 g of **3a** (27%) as a hygroscopic solid. ^1H NMR: δ 4.80 (m, 1 H, acetal H), 4.30 (m, 5 H, glycerol H), 3.81 (m, 4 H, $POCH_2CH_2N$), 3.35 (s, 9 H, $N(CH_3)_3$), 2.76 (s, 2 H, H_2O). IR: 3200 (H_2O), 2900, 2820, 1450, 1200, 1050, 940 cm^{-1}. Anal. ($C_{24}H_{50}NO_6P{\cdot}2H_2O$) C, H, N. CIMS: 537 ($MH^+$ + 57), 480 (MH^+), 421, 209, 181, 103, 72 (100), 60.

[[2-(*cis*-8-Heptadecenyl)-1,3-dioxolan-4-yl]methyl]phosphocholine (3b). This compound was prepared as described for **3a** from **8b** (0.250 g, 0.734 mmol), NEt_3 (0.446 g, 4.40 mmol), and the phosphorus reagent (0.226 g, 1.01 mmol) to give, after chromatography, 0.113 g of **3b** (31%) as a hygroscopic solid. Anal. ($C_{26}H_{52}NO_6P{\cdot}2H_2O$) C, H, N. CIMS: 563 ($MH^+$ + 57), 506 (MH^+), 474, 209, 181, 103, 72, 60 (base).

[[2-(*cis*-8-Pentadecenyl)-1,3-dioxolan-4-yl]methyl]phosphocholine (3c). This compound was prepared as described for **3a** from **8c** (0.381 g, 1.22 mmol), NEt_3 (0.740 g, 7.32 mmol), and the phosphorus reagent (0.442 g, 1.83 mmol) to give, after chromatography, 0.168 g of **3c** (29%) as a hygroscopic solid. Anal. ($C_{24}H_{48}NO_6P{\cdot}2H_2O$) C, H, N. CIMS: 535 ($MH^+$ + 57), 478 (MH^+), 209, 181, 103, 90, 72, 60 (base).

Pharmacology. Taenia coli smooth muscle strips were obtained from guinea pigs of either sex weighing 500–700 g. Strips 2–3 cm in length were mounted in 3-mL organ baths in a modified Krebs solution (mM: NaCl 133, KCl 4.5, $CaCl_2$ 2.6, $MgSO_4$ 1.2, $NaHCO_3$, 16.3, NaH_2PO_4 1.5, and dextrose 7.8) containing 1.5 μM hyoscine. Three hundred milligrams of tension was applied to each preparation and muscle activity was measured isometrically by means of Grass FT:03 force transducers and recorded on a Grass polygraph. Dosing of PAF was performed with increasing concentrations each followed by washout. Dosing of other phospholipids was either random with each dose followed by washout or was cumulative. Trachea were dissected out and mounted in organ baths in Krebs' solution according to the method of Farmer and Coleman.[19] Intraluminal pressure was recorded on a Grass polygraph via Gould Statham P23ID pressure transducers. Preparations were gassed with 95% O_2 and 5% CO_2 and maintained at 37 °C.

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Cyclic Carbamate Analogues of Pilocarpine

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A number of pilocarpine analogues containing the (*S*)-3-ethyl-4-[(4′-imidazolyl)methyl]-2-oxazolidinone (**9**) structural feature were synthesized from L-histidine. With 1-benzyl-L-histidine as the key intermediate, a regiospecific synthetic route was developed to the N^{π}-methyl derivative **8**. The regiochemistry of the alkylation of the imidazole nucleus was determined by measuring proton cross-ring coupling constants in the high-field ^1H NMR. The effects on muscarinic receptors of these variously alkylated derivatives **6–10** were studied on isolated guinea pig ileum. The derivatives in which the imidazole nitrogen was unsubstituted (**9**), N^{τ}-methylated (**10**), and N^{π}-methylated (**8**) were cholinergic muscarinic agonists with an increasing order of potency; compounds **6** and **7** were inactive. Analogue **8** with the same substitution pattern as pilocarpine was equipotent with pilocarpine, making these hydrolytically stable carbamate derivatives potentially useful drugs.

Pilocarpine, the only cholinergic muscarinic agonist in clinical use, is widely employed as a topical miotic for controlling the elevated intraocular pressure associated with glaucoma. In spite of its disadvantage of a short duration of action, pilocarpine has enjoyed widespread use. The duration of lowering of the intraocular pressure caused by pilocarpine lasts only for about 3 h, and consequently the frequency of administration is 3–6 times a day.[1] This

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