methylene chloride (3 × 100 mL), and the combined organic layers were dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure and the residue chromatographed (radial chromatography), eluting with 5% methanol in methylene chloride. The fractions containing the product were combined and evaporated under reduced pressure. The residue was triturated with ether and the insoluble product collected by filtration to give 1.62 g (85%) of **5b**: mp 133–134 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.04–1.16 (m, 6 H), 1.18 (s, 9 H), 1.94–2.06 (m, 4 H), 2.38 (t, 2 H, J = 7.37 Hz), 2.90–3.00 (m, 1 H), 3.00–3.10 (m, 1 H), 6.41 (m, 1 H), 6.47 (s, 1 H), 6.57–6.59 (AA′BB′, 2 H), 7.62–7.65 (AA′BB′, 2 H), 8.21 (d, 1 H, J = 7.40 Hz); mass spectrum, m/z (relative intensity) 381 (14), 260 (17), 249 (29), 248 (100), 247 (23), 164 (17), 131 (19), 119 (19). Anal. ($C_{29}H_{40}N_6O_7$) C, H, N.

Diethyl *N*-[*N*-[[2-(Pivaloylamino)-4-hydroxy-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl]methyl]-*N*^{10}-methyl-4-aminobenzoyl]glutamate (5e). To a stirred solution of **5b** (2.52 g, 4.31 mmol) and 37% formaldehyde (2 mL) in acetonitrile (20 mL) at 25 °C was added sodium cyanoborohydride (0.81 g, 12.93 mmol). Glacial acetic acid (0.5 mL) was added to the reaction mixture dropwise over a period of 10 min. After stirring for 2 h, additional glacial acetic acid (0.5 mL) was added, and the mixture was stirred for 18 h, poured into water (300 mL), and extracted with methylene chloride (3 × 100 mL). The combined organic layers were dried over anhydrous magnesium sulfate and the solution evaporated under reduced pressure. The residue was chromatographed (radial chromatography), eluting with 2% methanol in methylene chloride, to give 1.3 g of **5e** (50%). Recrystallization from ethyl acetate afforded pure **5e**: mp 181–183 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.11–1.18 (m, 6 H), 1.17 (s, 9 H), 1.92–2.02 (m, 4 H), 2.39 (t, 2 H, J = 7.43 Hz), 2.97 (s, 3 H), 2.93–3.02 (m, 1 H), 3.15–3.23 (m, 1 H), 3.65 (d, 2 H, J = 6.71 Hz), 3.98–4.10 (m, 4 H), 4.32–4.42 (m, 1 H), 6.41 (m, 1 H), 6.45 (s, 1 H), 6.70–6.73 (AA′BB′, 2 H), 7.69–7.72 (AA′BB′, 2 H), 8.28 (d, 1 H, J = 7.40 Hz); mass spectrum, m/z (relative intensity) 598 (4, M^+), 396 (33), 349 (41), 262 (42), 261 (40), 249 (100), 248 (45), 247 (26), 148 (20). Anal. ($C_{30}H_{42}N_6O_7$) C, H, N.

***N*-[*N*-[(2-Amino-4-hydroxy-5,6,7,8-tetrahydropyrido[2,3-*d*]pyrimidin-6-yl)methyl]-*N*^{10}-methyl-4-aminobenzoyl]glutamic Acid (7d).** A solution of **5e** (0.212 g, 0.35 mmol) in 1 N NaOH was stirred at 25 °C for 72 h and the reaction mixture acidified to pH 6 with 0.5 N HCl. The white solid was collected by filtration and washed with water. The product was allowed to air-dry on the filter and was then triturated with ether to remove residual pivalic acid to give 0.160 g (99%) of **7d** after drying overnight in a vacuum desiccator over P_2O_5: mp, gradually decomposes >180 °C; HPLC (retention time) diastereomer A, 6.46 min, and diastereomer B, 8.73 min; ^{1}H NMR (Me_2SO-d_6) δ 1.80–2.15 (m, 6 H), 2.31 (t, 2 H, J = 7.4 Hz), 2.37–2.39 (m, 1 H), 2.84–2.91 (m, 2 H), 2.96 (s, 1 H), 3.09–3.13 (m, 1 H), 3.3 (d, 2 H, J = 6 Hz), 4.30–4.37 (m, 1 H), 6.07 (s, 2 H), 6.35 (s, 1 H), 6.67–6.70 (AA′BB′, 2 H), 7.69–7.72 (AA′BB′, 2 H), 8.17 (d, 1 H, 7.4 Hz), 9.97 (br s, 1 H). Anal. ($C_{21}H_{26}N_6O_6$·0.75H_2O) C, H, N.

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Registry No. (6*R*)-**1**, 106400-81-1; (6*S*)-**1**, 106400-18-4; **2a**, 87373-56-6; **2b**, 120040-44-0; **2c**, 120040-43-9; **3a**, 115499-15-5; **3b**, 120145-37-1; **3c**, 115499-18-8; **3d**, 115499-19-9; **4**, 120145-38-2; (6*R*)-**5a**, 120203-03-4; (6*S*)-**5a**, 120203-04-5; (6*R*)-**5b**, 120145-39-3; (6*S*)-**5b**, 120145-40-6; (6*R*)-**5c**, 120203-05-6; (6*S*)-**5c**, 120203-06-7; (6*R*)-**5d**, 120203-07-8; (6*S*)-**5d**, 120203-08-9; (6*R*)-**5e**, 120145-41-7; (6*S*)-**5e**, 120145-42-8; **6**, 13726-52-8; (6*R*)-**7a**, 115587-73-0; (6*S*)-**7a**, 115587-72-9; (6*R*)-**7b**, 120203-09-0; (6*S*)-**7b**, 120203-10-3; (6*R*)-**7c**, 120145-43-9; (6*S*)-**7c**, 120145-44-0; (6*R*)-**7d**, 120145-45-1; (6*S*)-**7d**, 120145-46-2.

Binary Antidotes for Organophosphate Poisoning: Aprophen Analogues That Are both Antimuscarinics and Carbamates

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Prophylaxis against organophosphate poisoning can be achieved by pretreatment with physostigmine or pyridostigmine, which are carbamates, and aprophen, which is an anticholinergic agent. Thus, a series of aprophen analogues was synthesized with carbamyl substitutions on the phenyl rings (carbaphens). The rationale behind this design is that such compounds might exhibit most of the therapeutic characteristics of aprophen, as well as the ability to protect prophylactically by chemically masking cholinesterase enzymes. Compounds **4** (dimethylhydroxycarbaphen), **15** (dimethylcarbaphen), and **16** (monomethylcarbaphen) were found to inactivate human butyrylcholinesterase in a time-dependent manner with potencies similar to those of physostigmine or pyridostigmine, and the latter two exhibited almost the same antimuscarinic profile as aprophen. In contrast to the potent inactivation of butyrylcholinesterase by these compounds, marginal inactivation of acetylcholinesterase activity was observed, and only at much higher drug concentrations. The noncarbamylated analogues had no effect on the activity of either cholinesterase. The carbaphen compounds are hence prototype drugs that can interact with either muscarinic receptors or butyrylcholinesterase. Furthermore, these compounds are prodrugs, since after carbamylation of the cholinesterase, the leaving group **14** (hydroxyaprophen) is a potent antimuscarinic itself.

Aprophen [(*N*,*N*-diethylamino)ethyl 2,2-diphenylpropionate] is a potent anticholinergic and antispasmodic agent possessing a wide number of distinct pharmacological actions, including both antimuscarinic and noncompetitive nicotinic antagonist activities.[1-9] The potent antimuscarinic and antinicotinic effects of aprophen make it a potential drug of choice in the therapy of poisoning by organophosphate/anticholinesterase agents.[3,10,11]

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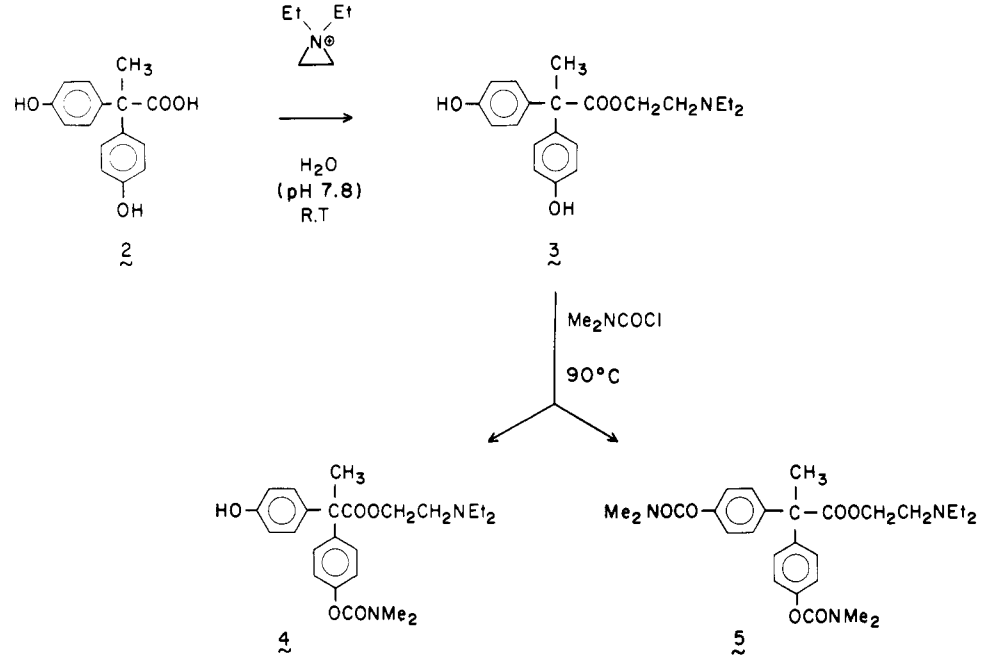
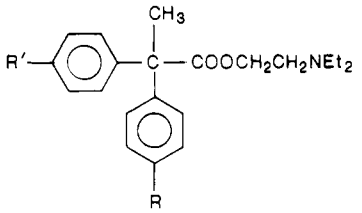
Scheme I. Synthetic Pathway of the Disubstituted Aprophen Derivatives



Organophosphates irreversibly inactivate cholinesterases. However, pretreatment with carbamates offers prophylactic protection against organophosphate poisoning because these drugs reversibly carbamylate the active site of cholinesterases. Therefore, the enzymes are chemically sequestered from the action of organophosphates, permitting recovery through decarbamylation.[12-16] The combination of aprophen and a carbamate, such as pyridostigmine or physostigmine, significantly improved the protection afforded by either drug against the physiological and behavioral symptoms of organophosphate poisoning, even if postpoisoning treatment were omitted.[7,8]

In view of the remarkable antidotal efficacy obtained by the combination of physostigmine and aprophen, we have synthesized a series of aprophen analogues with carbamyl substitutions on one or both of the phenyl rings. The rationale behind the design of such compounds, which we have termed carbaphens, is that they might exhibit most of the therapeutic characteristics of aprophen, along with the ability to protect prophylactically by chemically sequestering cholinesterase enzymes. In addition, the presence of two cholinergic recognition sites per molecule may cause higher local concentrations of the drug to be available at the postsynaptic sites.



aprophen: R = R' = H
3: R = R' = OH
4: R = OCONMe$_2$, R' = OH
5: R = R' = OCONMe$_2$
14: R = OH, R' = H
15: R = OCONMe$_2$, R' = H
16: R = OCONHMe, R' = H

Chemistry

Two series of carbamyl–aprophen compounds have been synthesized and tested for antimuscarinic and anticholinesterase potency. In the first series, both phenyl rings of the aprophen molecule were para-substituted, while in the second, only one phenyl ring was substituted at the para position by *N*-monomethylcarbamyl or *N,N*-dimethylcarbamyl groups.

The synthetic pathway of the dicarbamyl–aprophen compounds is outlined in Scheme I. The key intermediate, 2,2-bis(4-hydroxyphenyl)propionic acid (**2**), was obtained by condensing phenol with pyruvic acid in HCl-saturated acetic acid.[17] The conversion of this acid to its (diethylamino)ethyl ester **3** did not proceed satisfactorily according to known conventional methods (acyl chloride, transesterification, etc.). In order to overcome this synthetic barrier, we have utilized our recent observation[18] that, when freshly distilled *N,N*-dialkylaminoethyl chlorides are treated for several hours at room temperature with water, a clear solution of the appropriate *N,N*-dialkylaziridinium chloride is formed quantitatively. Since

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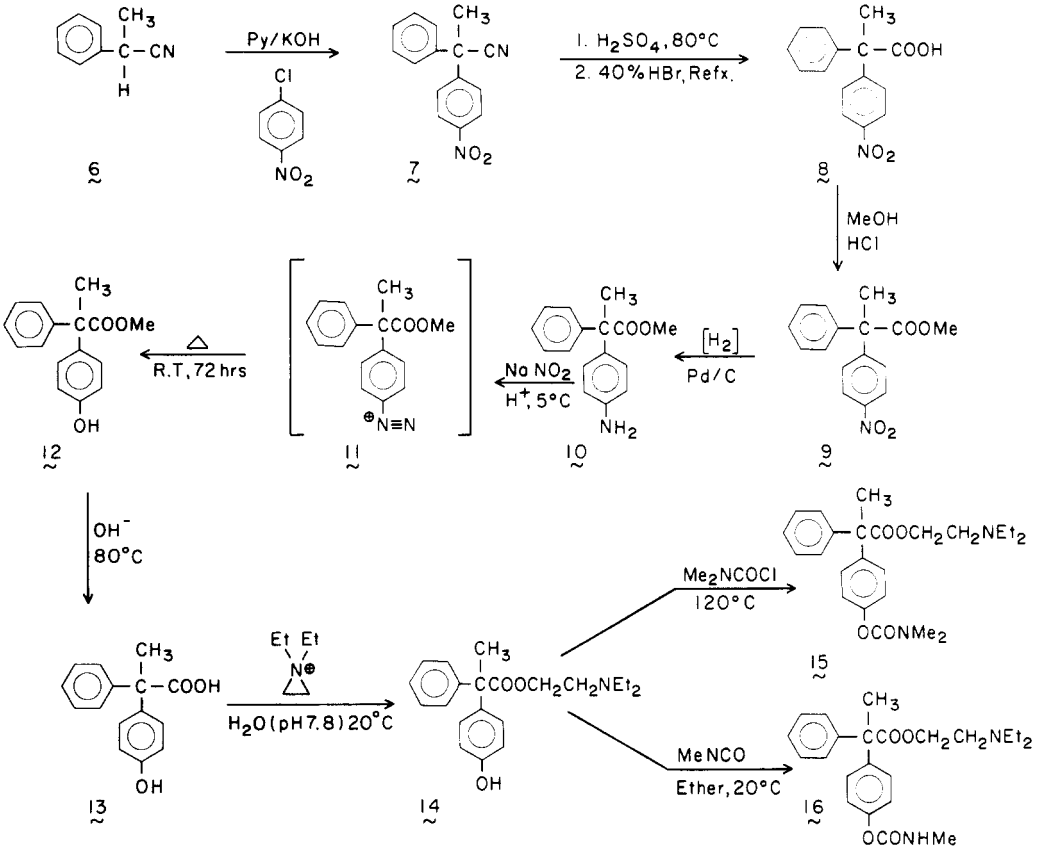
Scheme II. Synthetic Pathway of the Monosubstituted Aprophen Derivatives



even relatively highly concentrated solutions of the aziridinium salt (~20%) were found to be stable at room temperature for weeks (based on ^1H NMR and FAB mass spectrometry),[18] we used this source of aziridinium ions for the esterification reaction. Indeed, when a $NaHCO_3$ solution of **2** was mixed and stirred at room temperature with an aqueous solution of *N,N*-diethylaziridinium chloride, in the presence of ethyl acetate, the ester **3** was obtained in 86% yield. Treatment of this compound with an excess of dimethylcarbamyl chloride at 90 °C gave a mixture of the monocarbamate **4** and the dicarbamate **5**, which were separated by column chromatography.

The synthetic pathway for the monocarbamyl aprophen derivatives is outlined in Scheme II. 4-Chloronitrobenzene was reacted with α-methylbenzyl cyanide (**6**) and the *p*-nitrophenyl-substituted nitrile **7** was hydrolyzed via the amide to the appropriate propionic acid **8**. The methyl ester **9** was hydrogenated to the amine **10**, which was diazotized to **11** and decomposed at room temperature to give the hydroxy methyl ester derivative **12**.

Two important points should be noted with regard to the decomposition of the diazonium salt (**11** to **12**). First, an attempt to facilitate the generation of the *p*-hydroxy compound **12** by heating the acidic diazotization reaction mixture to temperatures above 40 °C failed, resulting in polymeric and other decomposition products. The optimal conditions for this reaction were to keep the reaction mixture at room temperature for at least 72 h. Second, a considerable amount (~25%) of an unidentified byproduct was isolated by chromatography from the above reaction. According to the ^1H NMR and CI-MS data (see Experimental Section), this byproduct is presumably a coupling reaction product resulting from the interaction between the unreacted diazonium salt **11** and the product **12** which took place during the relatively long period of time at room temperature.

Alkaline hydrolysis of the methyl ester **12** afforded the hydroxy acid **13**, which was esterified to the hydroxyaprophen derivative **14** by interacting with *N,N*-diethylaziridinium chloride in aqueous–bicarbonate solution. [[(Dimethylamino)carbonyl]oxy]aprophen (**15**) was obtained by reacting **14** with excess dimethylcarbamyl chloride at 120 °C for 2 h. [[(Methylamino)carbonyl]oxy]aprophen (**16**) was obtained by a Na-catalyzed addition reaction of methyl isocyanate to **14** in ether solution. All the new compounds were purified by column chromatography, and their structural elucidation and purity were confirmed by ^1H NMR, CI-MS, and TLC.

Biological Activities

Antimuscarinic Activities. Table I shows that the most potent antimuscarinic analogue was obtained by substituting a monomethylcarbamate moiety at the para position of one phenyl ring of aprophen, monomethylcarbaphen (**16**). The pA_2 for monomethylcarbaphen was 7.3 ± 0.1 ($K_B = 5.0 \times 10^{-8}$ M) when tested on the acetylcholine-induced contraction of guinea pig ileum, which has predominantly the M_2 receptor subtype.[3,4,19-22] It inhibited the carbachol-induced release of α-amylase from pancreatic acinar cells, which have an M_2 receptor subtype that may be different from that of ileum,[23,24] with a K_i of

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Table I. Antimuscarinic Activity of Carbaphen Analogues and Standard Compounds[a]

compd	inhibition of guinea pig ileum contraction pA_2	inhibition of guinea pig ileum contraction K_B, M	inhibition of pancreatic acini α-amylase release K_i, M	inhibition of cerebral cortex [^{3}H]NMS binding K_i, M
monomethylcarbaphen (16)	7.3 ± 0.1	5.0×10^{-8}	$(2.0 \pm 0.9) \times 10^{-9}$	$(2.6 \pm 1.5) \times 10^{-8}$
dimethylcarbaphen (15)	7.1 ± 0.1	8.3×10^{-8}	$(4.2 \pm 0.9) \times 10^{-8}$	$(1.5 \pm 0.5) \times 10^{-7}$
hydroxyaprophen (14)	7.2 ± 0.3	7.0×10^{-8}	$(5.6 \pm 1.2) \times 10^{-9}$	$(1.0 \pm 0.5) \times 10^{-7}$
dihydroxyaprophen (3)	5.7 ± 0.1	2.0×10^{-6}	$(4.9 \pm 0.8) \times 10^{-6}$	$(2.1 \pm 0.2) \times 10^{-6}$
dimethylhydroxycarbaphen (4)	5.5 ± 0.2	3.2×10^{-6}	$(2.4 \pm 0.8) \times 10^{-6}$	$(3.4 \pm 0.3) \times 10^{-6}$
bis(dimethyl)carbaphen (5)	not active	not active	not active	marginal activity
aprophen	8.5 ± 0.1	3.1×10^{-9}	$(1.7 \pm 0.7) \times 10^{-9}$	$(5.1 \pm 1.0) \times 10^{-8}$
atropine	8.7 ± 0.1	2.0×10^{-9}	$(1.6 \pm 1.1) \times 10^{-9}$	$(2.4 \pm 0.7) \times 10^{-9}$
pirenzepine	4.3 ± 0.4	5.0×10^{-5}	$(1.2 \pm 0.2) \times 10^{-7}$	$(1.7 \pm 0.7) \times 10^{-7}$

[a] Each inhibition constant represents the mean of four to five independent experiments ± standard errors of the mean.

$(2.0 \pm 0.9) \times 10^{-9}$ M. With respect to the inhibition of [^{3}H]NMS binding to cerebral cortex membranes, which contain predominantly the M_1 receptor subtype,[19-21,25-27] the K_i was $(2.6 \pm 1.5) \times 10^{-8}$ M. Overall, the antimuscarinic profile of monomethylcarbaphen was very similar to that of aprophen (Table I), with the former showing more specificity for the pancreatic M_2 receptor subtype over the M_1 subtype of the cortex.

While the pA_2 value for the inhibition of ileum contraction by dimethylcarbaphen (**15**) was about the same as that of monomethylcarbaphen (**16**), the inhibition of α-amylase release and [^{3}H]NMS binding were each 1 order of magnitude lower (Table I). Substitution with a *p*-(dimethylcarbamate) on one phenyl ring of aprophen and a *p*-hydroxy group on the other [dimethylhydroxycarbaphen (**4**)] was not well tolerated. The antimuscarinic activities of **4** were 1–2 orders of magnitude lower than those of dimethylcarbaphen. Bis(dimethyl)carbaphen (**5**), a compound with *p*-(dimethylcarbamate) substitution on both phenyl rings of aprophen, was inactive. In comparing the inhibition constants of **15**, **4**, and **5**, it was evident that these carbaphen analogues did not exhibit any muscarinic subtype specificity.

Interestingly, the mono-*p*-hydroxy-substituted aprophen, hydroxyaprophen (**14**), which is both the synthetic precursor of mono- and dimethylcarbaphen and the decarbamylated end product, was almost as potent an antimuscarinic as monomethylcarbaphen and showed the most preference for the M_2 subtype of the pancreas over the M_1 subtype of the cerebral cortex (about 18-fold). However, the addition of a second *p*-hydroxy group to aprophen [dihydroxyaprophen (**3**)] led to a drastic decrease in the antimuscarinic activities with inhibition constants in the region of 10^{-6} M.

These structure–activity relationship data suggest that one of the phenyl rings of aprophen must remain unsubstituted in order to preserve antimuscarinic activity, while the substitution of a carbamate or hydroxy function in the para position of the other phenyl ring is well tolerated. This is evident from the poor antimuscarinic potency of dihydroxyaprophen (**3**) and dimethylhydroxycarbaphen (**4**), and from the lack of activity of bis(dimethyl)carbaphen (**5**), and contrasts with the potent antimuscarinic activity of **14**, **15**, and **16**, which have one unsubstituted phenyl ring.

Anticholinesterase Activity. When tested for their ability to inactivate human serum butyrylcholinesterase, i.e., carbamylating ability, the four carbaphen analogues, monomethylcarbaphen (**16**), dimethylcarbaphen (**15**), dimethylhydroxycarbaphen (**4**), and bis(dimethyl)carbaphen (**5**), showed a potent ability to carbamylate butyrylcholinesterase (Table II). It should be noted that aprophen, itself, was not subject to hydrolysis by human serum butyrylcholinesterase.[28] The K_I values were on the order of 10^{-6} M, similar to those of pyridostigmine and physostigmine, which are clinically useful carbamates.

Table II. Antibutyrylcholinesterase (Human) Activity of Carbaphen Analogues[a] and Standard Compounds

carbamates	K_I, M	bimolecular rate constant k_3', L/(mol·min)
monomethylcarbaphen (16)	$(1.4 \pm 0.6) \times 10^{-6}$	$(8.5 \pm 4.2) \times 10^5$
dimethylcarbaphen (15)	$(1.3 \pm 1.0) \times 10^{-6}$	$(6.6 \pm 3.5) \times 10^5$
dimethylhydroxycarbaphen (4)	$(0.8 \pm 0.5) \times 10^{-6}$	$(3.0 \pm 2.3) \times 10^5$
bis(dimethyl)carbaphen (5)	$(4.6 \pm 0.3) \times 10^{-6}$	$(1.4 \pm 0.8) \times 10^4$
pyridostigmine	$(3.5 \pm 1.3) \times 10^{-6}$	$(3.4 \pm 1.1) \times 10^4$
physostigmine	$(5.0 \pm 2.4) \times 10^{-6}$	$(7.0 \pm 0.9) \times 10^4$

[a] Each inhibition constant represents the mean of four to five independent experiments ± standard errors of the mean.

In contrast to the potent inactivation of butyrylcholinesterase, the carbaphen analogues showed marginal time-dependent inactivation (about 20% inhibition at drug concentrations greater than 10^{-4} M) against acetylcholinesterase purified from fetal bovine serum[29] or obtained from electric eel (data not shown). These results are consistent with the inhibition, observed by using steady-state kinetics, produced by aprophen[30,31] and other aromatic/neurotoxic agents,[32-34] which preferentially inhibit butyrylcholinesterase over acetylcholinesterase. Similar results with additional compounds have led to the conclusion that butyrylcholinesterase, in comparison with acetylcholinesterase, contains a unique hydrophobic site which partially determines the substrate and inhibitor patterns of butyrylcholinesterase.[35,36]

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Conclusion

The magnitude of the inhibition constants of the carbaphen analogues against butyrylcholinesterase shows that, in contrast to the steric requirements of the muscarinic receptors, bulky phenyl ring substitutions of aprophen can be tolerated by butyrylcholinesterase. However, these analogues are only marginally active against acetylcholinesterase. It is important to note that both **15** and **16**, after carbamylating butyrylcholinesterase, produce as an end product hydroxyaprophen (**14**), itself a potent antimuscarinic (Table I). Thus, the carbaphen analogues monomethylcarbaphen (**16**) and dimethylcarbaphen (**15**) are binary prototype drugs that are potent both as antimuscarinic agents and as carbamates. Therefore, these compounds show promise as prophylactic/therapeutic antidotes for organophosphate poisoning, as well as being candidates for combination treatment modalities.

Experimental Section

Chemistry. Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were obtained on a Varian XL 300 (Me_4Si). Mass spectra (MS) were obtained on a Finnigan 1015 mass spectrometer (chemical ionization–NH_3). Elemental analyses were performed by Spang Micro Analytical Laboratory (Eagle Harbor, MI). For purity tests, TLC was performed on fluorescent silica gel plates (Polygram Sil G/UV254), and for each of the compounds, only one spot (visualized by UV light and I_2 vapor) was obtained. All new compounds gave satisfactory microanalyses for C, H, and N within ±0.4% and/or mass spectra consistent with the assigned structures.

β-(*N*,*N*-Diethylamino)ethyl 2,2-Bis(4-hydroxyphenyl)-propionate (3). β-(*N*,*N*-Diethylamino)ethyl chloride (obtained from the HCl salt, and freshly distilled, 2.7 g, 0.02 mol) was suspended in 20 mL of H_2O. After vigorous stirring for 4 h at room temperature, the resulting clear aqueous solution (which contained *N*,*N*-diethylaziridinium chloride[18]) was added in one portion to a solution of 2,2-bis(4-hydroxyphenyl)propionic acid[17] (2.6 g, 0.01 mol) in 10% $NaHCO_3$ (20 mL). Ethyl acetate (100 mL) was added, and the biphasic reaction mixture (pH 7.8) was stirred at room temperature for 48 h. The organic phase was separated, washed with brine (2 × 50 mL), dried ($MgSO_4$), and evaporated to give 3.1 g (80%) of a viscous oil which solidified on standing at room temperature. Purification by column chromatography (silica gel, ether saturated with NH_3) afforded 2.9 g of **3** as a white solid, mp 140–141 °C. No alkylation product of the phenolic OH was detected and/or isolated from the crude product of **3**, which was found to be almost pure by TLC and ^{1}H NMR.

^{1}H NMR [$(CD_3)_2CO$] δ 7.02 (d, 4 H, *J* = 8.7 Hz), 6.71 (d, 4 H, *J* = 8.7 Hz), 4.10 (t, 2 H, *J* = 5.9 Hz), 2.57 (t, 2 H, *J* = 5.9 Hz), 2.42 (q, 4 H, *J* = 7.0 Hz), 1.76 (s, 3 H), 0.88 (t, 6 H, *J* = 7.0 Hz). Anal. ($C_{21}H_{27}NO_4$) C, H, N.

β-(*N*,*N*-Diethylamino)ethyl 2-(4-Hydroxyphenyl)-2-[4-[[(dimethylamino)carbonyl]oxy]phenyl]propionate (4) and β-(*N*,*N*-Diethylamino)ethyl 2,2-Bis[4-[[(dimethylamino)-carbonyl]oxy]phenyl]propionate (5). Compound **3** (1.8 g, 0.005 mol) was dissolved in an excess of dimethylcarbamyl chloride (6.0 mL). The solution was heated to 90 °C for 6 h, cooled to room temperature, and poured on ice (100 mL). After the evolution of the CO_2 ceased, the reaction mixture was basified with solid $NaHCO_3$ to pH 8.8 and extracted with ethyl acetate (3 × 100 mL). The organic phase was washed with brine (2 × 50 mL) and dried ($MgSO_4$), and the solvent was evaporated to give 2.0 g of a pale brown viscous oil, which according to TLC and ^{1}H NMR analysis was found to be a mixture of **4** and **5** (40:60). Separation and purification of **4** and **5** were achieved by column chromatography (silica, 5% MeOH–$CHCl_3$).

4: 0.7 g, TLC (silica, 10% MeOH–$CHCl_3$) R_f 0.35; ^{1}H NMR ($CDCl_3$) δ 7.10 (d, 2 H, *J* = 8.7 Hz), 6.95 (d, 2 H, *J* = 8.7 Hz), 6.94 (d, 2 H, *J* = 8.7 Hz), 6.56 (d, 2 H, *J* = 8.7 Hz), 4.20 (t, 2 H, *J* = 5.8 Hz), 3.02 and 2.93 (2 s, 6 H, Me_2N–), 2.68 (t, 2 H, *J* = 5.8 Hz), 2.47 (q, 4 H, *J* = 7.1 Hz), 1.77 (s, 3 H), 0.90 (t, 6 H, *J* = 7.1 Hz). Anal. ($C_{24}H_{32}N_2O_5$) C, H, N.

5: 1.0 g, TLC (silica, 10% MeOH–$CHCl_3$) R_f 0.65; ^{1}H NMR ($CDCl_3$) δ 7.26 (d, 4 H, *J* = 8.7 Hz), 7.08 (d, 4 H, *J* = 8.7 Hz), 4.28 (t, 2 H, *J* = 6.0 Hz), 3.13 and 3.05 (2 s, 12 H, Me_2N–), 2.73 (t, 2 H, *J* = 6.0 Hz), 2.55 (q, 4 H, *J* = 7.1 Hz), 1.93 (s, 3 H), 1.02 (t, 6 H, *J* = 7.1). Anal. ($C_{27}H_{37}N_3O_6$) C, H, N. HCl salt: 175–176 °C.

2-(4-Nitrophenyl)-2-phenylpropionitrile (7). This compound was synthesized according to Makosza et al.[37] with some modifications. A solution of 4-chloronitrobenzene (31.5 g, 0.2 mol) in pyridine (100 mL) was added dropwise to a vigorously stirred suspension of KOH (fine powder, 70.0 g) and α-methylbenzyl cyanide (26.2 g, 0.2 mol) in pyridine (150 mL), with the reaction temperature being maintained at 10 °C. The reaction mixture was stirred for 24 h at 25 °C and poured onto an excess of HCl–ice mixture. The acidic aqueous mixture was extracted with benzene, the organic phase washed with brine and dried (Na_2SO_4), and the benzene evaporated under vacuum. A crude product, 42.5 g (84%), resulted and was recrystallized from 2-propanol–petroleum ether to give **7** as white crystals, mp 76–78 °C (lit. 76 °C). Anal. ($C_{15}H_{12}N_2O_2$) C, H, N.

2-Phenyl-2-(4-nitrophenyl)propionic Acid (8). The nitrile **7** (5.0 g, 0.02 mol) was dissolved in concentrated H_2SO_4 and the mixture heated to 80 °C for 2 h. The pale brown solution was then poured onto ice and extracted with ethyl acetate (2 × 100 mL). The organic solvent was evaporated under reduced pressure, and 48% HBr solution (100 mL) was added to the remaining viscous residue. After refluxing for 8 h, the viscous oil turned to a yellow crystalline material. The aqueous acidic phase was then removed by decantation, and the crystalline material was washed and recrystallized with H_2O to give 4.2 g (77%) of **8**, mp 191–192 °C (lit. 192 °C).[38]

^{1}H NMR ($CDCl_3$) δ 8.27 (d, 2 H, *J* = 9.0 Hz), 7.55–7.40 (m, 9 H), 2.1 (s, 3 H). Anal. ($C_{15}H_{13}NO_4$) C, H, N.

Methyl 2-Phenyl-2-(4-nitrophenyl)propionate (9). A solution of the acid **8** (5.4 g, 0.02 mol) in a saturated solution of HCl/MeOH (150 mL) was refluxed for 16 h. The product was isolated by conventional procedures to give 5.0 g (87%) of **9** as a viscous oil. TLC (silica, 5% MeOH–$CHCl_3$) showed one spot (R_f 0.65). This crude compound was used in the next step without any purification.

^{1}H NMR ($CDCl_3$) δ 8.06 (d, 2 H, *J* = 9.0 Hz), 7.29 (d, 2 H, *J* = 9.0 Hz), 7.25–7.15 (m, 5 H), 3.68 (s, 3 H), 1.89 (s, 3 H).

Methyl 2-Phenyl-2-(4-aminophenyl)propionate (10). A solution of **9** (2.85 g, 0.01 mol) in 95% ethanol (200 mL) was hydrogenated in the presence of 5% Pd/C under 35 psi of hydrogen. After 24 h, the catalyst was removed by filtration and the solution evaporated to yield 2.3 g (90%) of crude viscous oil which crystallized on standing, mp 79–80 °C (*n*-hexane).

^{1}H NMR ($CDCl_3$) δ 7.29–7.17 (m, 5 H), 7.03 (d, 2 H, *J* = 8.5 Hz), 6.62 (d, 2 H, *J* = 8.5 Hz), 3.71 (s, 3 H), 3.64 (br s, 2 H, NH_2), 1.87 (s, 3 H). Anal. ($C_{16}H_{17}NO_2$) C, H, N.

Methyl 2-Phenyl-2-(4-hydroxyphenyl)propionate (12). A solution of sodium nitrite (1.12 g, 0.013 mol) in 3 mL of water was added to a stirred solution of **10** (2.5 g, 0.01 mol) in 25% H_2SO_4 (15 mL) at 0 °C. The mixture was stirred for 30 min at 0 °C and then for 72 h at room temperature. The dark brown oil that separated from the aqueous reaction mixture was extracted with ethyl acetate (3 × 100 mL) and dried over $MgSO_4$. The filtrate was evaporated and the viscous residue (2.2 g) purified by column chromatography (silica, $CHCl_3$ and 5% MeOH–$CHCl_3$). After the first fractions, which contained a considerable amount (0.6 g) of an unidentified nonpolar byproduct [TLC (silica, $CHCl_3$) R_f 0.9; ^{1}H NMR ($CDCl_3$) δ 7.5–7.1 (m, 17 H), 3.75 (s, 6 H), 1.94 (s, 6 H); CI-MS *m/e* 523 (M^+)], compound **12** (1.4 g, 55%) was isolated as a pale brown viscous oil: TLC (silica, 5% MeOH–$CHCl_3$) R_f 0.65.

^{1}H NMR ($CDCl_3$) δ 7.30–7.19 (m, 5 H), 7.10 (d, 2 H, *J* = 8.7 Hz), 6.75 (d, 2 H, *J* = 8.7 Hz), 4.87 (s, 1 H, OH), 3.73 (s, 3 H), 1.89 (s, 3 H). Anal. ($C_{16}H_{16}O_3$) C, H.

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Additional quantities of **12** were afforded by reextracting the original aqueous diazotization reaction mixture after allowing it to stand at room temperature for 3–7 days, thus increasing the total yield of **12** to 1.8 g (70%). Attempts to facilitate the decomposition of the diazonium salt by increasing the temperature reduced the yield considerably.

2-Phenyl-2-(4-hydroxyphenyl)propionic Acid (13). A solution of **12** (1.8 g, 0.007 mol) in 70 mL of 10% KOH was warmed to 80 °C for 2 h. After cooling, the reaction mixture was acidified with 35% HCl, and the pale brown oil was extracted with ether (4 × 50 mL). After drying ($MgSO_4$), the ether was evaporated to give 1.6 g (94%) of viscous oil which solidified on standing. Recrystallization from H_2O afforded **13** as white crystals, mp 136–137 °C.

^1H NMR ($CDCl_3$) δ 7.30–7.24 (m, 5 H), 7.17 (d, 2 H, J = 8.8 Hz), 6.77 (d, 2 H, J = 8.8 Hz), 1.91 (s, 3 H). Anal. ($C_{15}H_{14}O_3$) C, H.

β-(*N*,*N*-Diethylamino)ethyl 2-Phenyl-2-(4-hydroxyphenyl)propionate (14). β-(*N*,*N*-Diethylamino)ethyl chloride (freshly distilled, 2.0 g, 0.014 mol) was suspended in 15 mL of H_2O. After vigorous stirring for 4 h, all the oil disappeared, and the clear aqueous solution obtained was added in one portion to a solution of **13** (1.7 g, 0.007 mol) in 30 mL of 10% $NaHCO_3$. Ethyl acetate (50 mL) was added, and the biphasic reaction mixture (pH 7.8) was stirred at room temperature for 36 h. The organic phase was separated, washed with brine (2 × 50 mL), dried ($MgSO_4$), and evaporated to leave 2.2 g (64%) of viscous oil, which was chromatographed (silica, ether saturated with NH_3) to afford 2.1 g of **14** as a pale yellow viscous oil.

^1H NMR ($CDCl_3$) δ 7.31–7.18 (m, 5 H), 7.10 (d, 2 H, J = 8.7 Hz), 4.23 (t, 2 H, J = 6.1 Hz), 2.68 (t, 2 H, J = 6.1 Hz), 2.49 (q, 4 H, J = 7.1 Hz), 1.89 (s, 3 H), 0.96 (t, 6 H, J = 7.1 Hz). Anal. ($C_{21}H_{27}NO_3$) C, H, N.

β-(*N*,*N*-Diethylamino)ethyl 2-Phenyl-2-[4-[[(dimethylamino)carbonyl]oxy]phenyl]propionate (15). Compound **14** (1.05 g, 0.003 mol) was dissolved in excess dimethylcarbamyl chloride (5 mL). The solution was warmed to 120 °C for 10 h, cooled to room temperature, and poured onto ice–H_2O (100 mL). After the evolution of the CO_2 ceased, the reaction mixture was basified with solid $NaHCO_3$ to pH 8.8 and extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with brine (2 × 50 mL) and dried ($MgSO_4$), and the solvent was evaporated. The residue was purified by column chromatography (silica, 5% MeOH–$CHCl_3$) affording 1.2 g of **15** (87%) as a viscous pale yellow oil.

^1H NMR ($CDCl_3$) δ 7.28–7.21 (m, 7 H), 7.04 (d, 2 H, J = 8.7 Hz), 4.20 (t, 2 H, J = 6.2 Hz), 3.09 and 3.01 (2 s, 6 H, Me_2N–), 2.64 (t, 2 H, J = 6.2 Hz), 2.47 (q, 4 H, J = 7.1 Hz), 1.90 (s, 3 H), 0.95 (t, 6 H, J = 7.1 Hz). Anal. ($C_{24}H_{32}N_2O_4$) C, H, N.

β-(*N*,*N*-Diethylamino)ethyl 2-Phenyl-2-[4-[[(methylamino)carbonyl]oxy]phenyl]propionate (16). A small piece of Na (~10 mg)[39] was added to a solution of **14** (1.5 g, 0.004 mol) and methyl isocyanate (7.5 mL) in dry ether (100 mL). The reaction mixture was kept at room temperature with occasional shaking for 3 days, after which it was filtered, the filtrate evaporated, and the residue purified by column chromatography (silica, 5% MeOH–$CHCl_3$) to afford 1.6 g (91%) of **16** as a pale yellow viscous oil.

^1H NMR ($CDCl_3$) δ 7.30–7.21 (m, 7 H), 7.05 (d, 2 H, J = 8.6 Hz), 5.0 (br q, 1 H, NH), 4.21 (t, 2 H, J = 6.1 Hz), 2.89 (d, 3 H, J = 4.9 Hz, MeNH), 2.64 (t, 2 H, J = 6.1 Hz), 2.47 (q, 4 H, J = 7.1 Hz), 1.91 (s, 3 H), 0.95 (t, 6 H, J = 7.1 Hz). Anal. ($C_{23}H_{30}N_2O_4$) C, H, N.

Pharmacological Assays. α-Amylase Secretion from Pancreatic Acinar Cells. Pancreatic acinar cells were prepared from male Sprague-Dawley rats by three successive incubations with collagenase (0.8 mg/mL).[40,41] The cells were suspended in 16 mL of Dulbecco's minimal essential medium containing 0.2% albumin, 0.01% trypsin inhibitor, and 0.09% theophylline, aerated with 100% O_2, and diluted 5-fold before use. Viability test by trypan blue exclusion was greater than 99%. The acinar cells were incubated with varied doses of each compound to be tested and 10^{-5} M carbachol in 0.5 mL. α-Amylase secreted from the acinar cells was determined with a Pharmacia Phadebas kit. I_{50} values, the concentration causing a 50% decrease in α-amylase secretion, were determined by using ALLFIT, a computer program for the analysis of inhibition curves, and converted to K_i values by using the K_d determined from the binding of [^{14}C]carbachol to pancreatic acinar cells ($K_d = 3.7 \times 10^{-7}$ M), by the method of Cheng and Prusoff.[42]

Acetylcholine-Induced Contraction of Guinea Pig Ileum. Distal ileum was obtained from male albino guinea pigs (350–500 g), and a segment approximately 2 cm in length was suspended in each 10-mL organ bath in oxygenated Krebs–Ringer solution maintained at 37 °C.[43] Isometric contractions were recorded by means of a free-displacement transducer (Harvard Apparatus, Natick, MA) set at 1-g tension. After a stabilization period of 45 min, acetylcholine (ACh) was added to the bath, allowed to act for 1 min, and then washed out. The tissue was allowed 5 min to recover prior to the next addition. The maximal contractile response was designated as 100%, and other responses were reported as a percentage of that response. After a recovery period of 15 min, test compounds, followed 30 s later by ACh, were added to each bath and the contractile responses recorded. The K_B or pA_2 values, measuring the affinity of an antagonist for the muscarinic receptor, were calculated by using computer programs for the Schild plot.[44]

Binding Assays. Freshly obtained bovine cerebral cortex was minced, mixed with an equal volume (w/v) of 50 mM potassium phosphate buffer, pH 7.2, containing a cocktail of protease inhibitors (0.1 mM ethylenediaminetetraacetate, 0.1 mM phenylmethanesulfonyl fluoride, and 0.02% sodium azide), and homogenized at 4 °C. The homogenate was centrifuged at 16000*g* for 1 h at 4 °C, and the pellet was resuspended in the same volume of buffer and centrifuged again. The pellet was resuspended in buffer and frozen in aliquots at −30 °C. [^{3}H]-*N*-Methylscopolamine ([^{3}H]NMS, 72 Ci/mmol, New England Nuclear, Boston, MA) binding to homogenized cortex was carried out in 96-well plates at 25 °C in a final volume of 0.2 mL.[45] The concentration of [^{3}H]NMS was 2 nM, and nonspecific binding was determined in the presence of 1 μM atropine. K_i values were determined as for the α-amylase assay by using the K_d calculated for [^{3}H]NMS binding to cortex by Scatchard analysis ($K_d = 3.2 \times 10^{-10}$ M).

Cholinesterase Assay and Inhibition Kinetics. The activities of human serum butyrylcholinesterase, electric eel acetylcholinesterase (EC 3.1.1.8 and EC 3.1.1.7, respectively, Sigma Chemical Co., St. Louis, MO), and fetal bovine serum acetylcholinesterase[29] were determined colorimetrically by the method of Ellman,[46] as described by Main et al.[47] Reactions were carried out at 30 °C in 0.1 M sodium phosphate buffer at pH 8.0 in the presence of 10^{-3} M butyrylthiocholine or acetylthiocholine and 3.3×10^{-4} M 3-carboxy-4-nitrophenyl disulfide. Aliquots of incubating mixtures containing enzyme alone, or enzyme in the presence of each carbamate, were withdrawn at selected time intervals and assayed for enzyme activity in order to obtain kinetic data. From the kinetic data, inhibition and bimolecular rate constants were calculated by the equation:[47–50]

$$\frac{1}{K_{app}} = \frac{1}{k_3} + \frac{K_I}{k_3}\frac{1}{[I]}$$

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in which k_{app} is the pseudo-first-order rate constant. The bimolecular rate constant (k_3')[48] is equal to k_3/K_I.

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Registry No. 2, 92549-67-2; 3, 120665-63-6; 4, 120637-92-5; 5, 120637-93-6; 6, 1823-91-2; 7, 22156-51-0; 8, 22156-69-0; 9, 120637-94-7; 10, 120637-95-8; 12, 120637-96-9; 13, 120637-97-0; 14, 120637-98-1; 15, 120637-99-2; 16, 120665-64-7; β-(*N*,*N*-diethylamino)ethyl chloride, 100-35-6; 4-chloronitrobenzene, 100-00-5; cholinesterase, 9001-08-5.

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Organic Phosphorus Compounds. 2. Synthesis and Coronary Vasodilator Activity of (Benzothiazolylbenzyl)phosphonate Derivatives[1]

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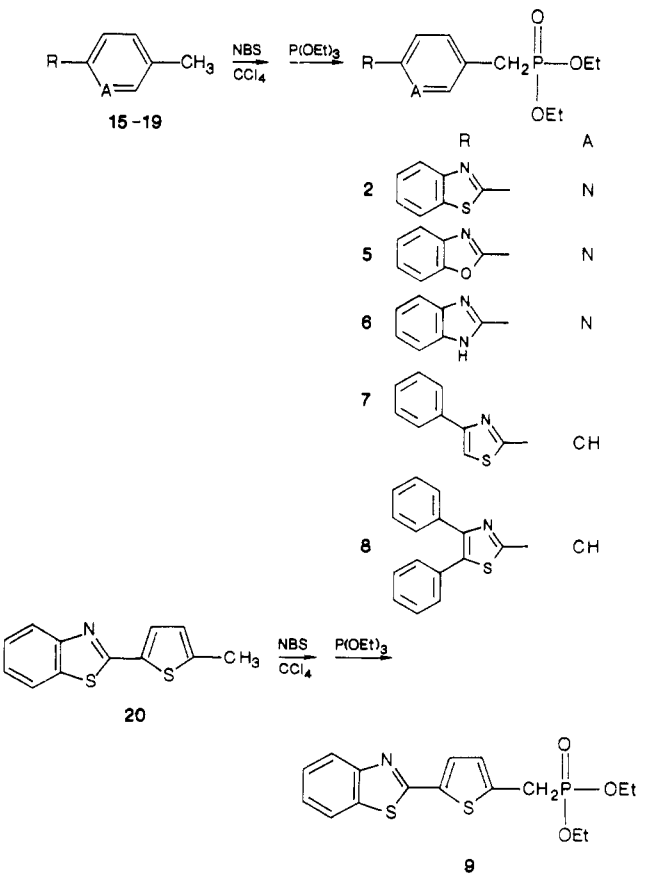
Structural modification and the coronary vasodilator activity of the calcium antagonist fostedil (KB-944) are described. Elimination of the benzothiazole ring or replacement of the benzothiazole ring of fostedil with other hetero rings leads to a decrease in vasodilator activity. Change of the distance from the aromatic ring to the phosphorus of fostedil causes a decrease in the activity. The present study indicates that the presence of an aromatic ring substituted thiazole ring and the presence of phosphonate at an appropriate distance from the thiazole ring are important for the coronary vasodilator action of fostedil.

Previously, we reported a new calcium antagonist fostedil[1,2] whose structure is totally different from that of conventionally known calcium antagonists. At present, three types of calcium antagonists (nifedipine and its derivatives,[3] diltiazem,[4] and verapamil[5]) are widely used. These agents exert their action by binding to the protein of the voltage-operated calcium channel present on the membrane of smooth muscles. The three calcium antagonists bind to different sites; they have allosteric effects on each other.[6] Fostedil seems to bind to the diltiazem binding site from the results of the binding studies with ^{3}H-labeled calcium antagonists.[7-9] Fostedil inhibited [^{3}H]diltiazem binding to the calcium channel in rat cerebral cortex.[7] Like diltiazem, fostedil promoted the binding of nifedipine through an allosteric effect.[8] It is very interesting that fostedil, whose structure is quite different from that of diltiazem, binds to the diltiazem binding site and has an effect similar to that of diltiazem. For this reason, we have studied which sites are essential for the action of fostedil.

Scheme I



In our previous study,[1] the presence of the phosphonate moiety proved to be important to the action of fostedil. In this paper, three types of structural modification of fostedil and the coronary vasodilator activity are reported (see Figure 1): (1) the benzene ring of fostedil was replaced

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