Carbamate Ester Prodrugs of Dopaminergic Compounds: Synthesis, Stability, and Bioconversion

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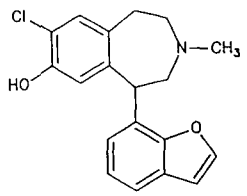
Abstract □ Various carbamic acid esters (CAE) of a new class of dopaminergic drugs, 5-substituted 8-chloro-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines, were synthesized and evaluated as prodrug forms with the aim of protecting the parent phenols against first-pass metabolism following oral administration. Monosubstituted CAE were found to be highly unstable at pH 7.4 and 37 °C, the half-lives of hydrolysis being between 4 and 40 min. Plasma from various species catalyzed the hydrolysis of the carbamates. *N,N*-Disubstituted carbamates, on the other hand, were stable both in buffer and plasma solutions. They showed a very potent inhibition of butyrylcholinesterase (EC 3.1.1.8), but were less potent inhibitors of the specific erythrocyte acetylcholinesterase (EC 3.1.1.17). In vitro incubations of an *N,N*-dimethylsubstituted carbamate ester (**10**) with liver microsomes from mouse and rat showed an appreciable formation of the parent phenolic compound. This bioconversion is suggested to occur via an initial cytochrome P-450-catalyzed hydroxylation to give an *N*-hydroxymethyl derivative which spontaneously decomposes to the *N*-monomethylcarbamate. It is concluded that *N,N*-disubstituted carbamate esters may be potentially useful prodrugs for the 7-hydroxy-3-benzazepines, whereas *N*-monosubstituted carbamates appear to be too chemically and enzymatically labile.

Several drugs containing a phenolic group show a limited and variable bioavailability following oral administration due to extensive first-pass metabolism in the gut and/or liver by conjugation reactions.[1,2] Bioreversible derivatization of the phenolic group to produce prodrugs has in some cases proved to be a valuable approach to reduce the extent of first-pass metabolism, as exemplified with salicylate and anthranilate esters of nalbuphine,[3] naltrexone,[4,5] and β-estradiol,[6] various carbamate esters of the dopamine agonist (−)-3-(3-hydroxyphenyl)-*N*-propylpiperidine,[7] and the bis-*N,N*-dimethylcarbamate ester of terbutaline (bambuterol).[8–12]

In our studies of a new class of dopaminergic drugs with potential antipsychotic effect, 5-substituted 8-chloro-7-hydroxy-3-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepines,[13,14] a low oral bioavailability in all species investigated was found, which seems to be a general feature of 7-hydroxy-3-benzazepines.[15] Thus, studies with **1** and **2** in rats showed a complete absorption but extensive first-pass metabolism, predominantly due to glucuronidation of the 7-phenolic group. Derivatization of this group to produce various alkyl and aryl ester prodrugs, including salicylate and anthranilate esters, was performed, but this approach met with only marginal success. The attention was then directed to the synthesis of carbamate esters in view of the previously reported[7,12] successful application of such derivatives to protect some phenolic drugs against first-pass metabolism.

In this paper, we describe the synthesis of various carbamate esters (**3–5**) of **1** and **2** and the evaluation of their stability and bioconversion characteristics. Some of the carbamate esters described were derived from amino acid esters and this type of carbamate has not previously been reported in the prodrug context.



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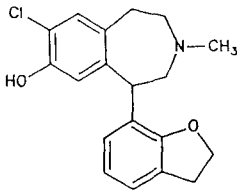

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Experimental Section

All chemicals used were of analytical or HPLC grade. The ^{1}H NMR spectra were run on a Bruker WM 400 (400 MHz) instrument, using tetramethylsilane as the internal standard. Low-pressure chromatography (flash chromatography) was performed as previously described,[16] using Merck silica 60 (40–63-μm particle size) as the stationary phase. Eluants were flushed through the column with a slight nitrogen pressure (~0.25 atm). Isocyanates and carbamoyl chlorides were obtained from Aldrich Chemie, F.R.G., or synthesized as described below. All prodrugs were obtained as amorphous compounds and their purity was checked by HPLC as described below.

High-Performance Liquid Chromatography Procedures—The purity of all prodrugs was checked with a HPLC system consisting of two Waters 6000 pumps, a Waters WISP 710 autosampler, and a Waters UV detector (UV 440) operated at 214 nm. The system was controlled by a Waters 721 datamodule. The samples were run on a C-18 Nucleosil column (5-μm particles, 4 × 200 mm; Machery-Nagel, F.R.G.), with a gradient procedure at a total flow rate of 1.0 mL/min. The gradient procedure was as follows: initial conditions of 0.1 M ammonium sulphate buffer (pH 3.3):acetonitrile (3:1 v/v) followed by a linear gradient of acetonitrile for a 25-min period to a final 75% (v/v) acetonitrile, and finally a return to the initial conditions after an additional 5 min.

Synthesis of *N*-Carbonyl Amino Acid Esters—Amino acid esters (as HCl salts, 25–50 mmol) were reacted with 2 equiv of phosgene reagent trichloromethylchloroformate (TCF) at 55–60 °C in dry dioxane (20–40 mL) with stirring. After dissolution of the HCl salt, the reaction was continued for 4 h. Excess reagent and dioxane was removed under reduced pressure. The product was purified by fractional distillation under reduced pressure and the identity of the product was confirmed by IR and ^{1}H NMR spectroscopy: *N*-carbonyl-D,L-Ala-OMe (yield, 67%; IR: 2245 cm^{-1}; ^{1}H NMR: 1.5 d, 2H; 3.8 s, 3H; 4.1 m, 1H); *N*-carbonyl-L-Phe-OMe (yield, 50%; IR: 2240 cm^{-1}).

Synthesis of *N*-Methyl-*N*-Alkylcarbamoyl Chlorides—The amine (25–50 mmol), in its free base form, was dissolved in dry dioxane (50 mL) with 2 equiv of TCF (corresponding to 4 equiv of phosgene) at 0 °C. The solution was slowly heated to 55–60 °C with stirring, and then refluxed for 4 h. After removal of excess reagent and solvent under reduced pressure, the crude product was checked by IR for its characteristic absorption band at 1720–1740 cm^{-1}. This crude mixture was then reacted with the 7-hydroxybenzazepine as described below.

Synthesis of Carbamates 3–15—Compounds **3–8** were synthe-

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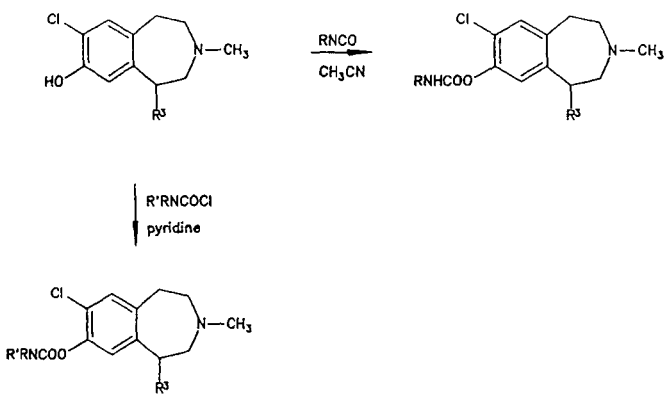
sized according to Scheme I. 7-Hydroxybenzazepine was reacted with 2–4 equiv of isocyanate in refluxing acetonitrile for 4–6 h. The crude product obtained by evaporating the reaction solution under reduced pressure was purified by flash chromatography using mixtures of dichloromethane and methanol with 1% aqueous ammonia. Fractions containing the product were pooled and evaporated under reduced pressure, leaving the product as an amorphous substance. A typical procedure referring to the synthesis of 6 is as follows. To a stirred solution of 1 (0.5 g, 1.5 mmol) in acetonitrile (20 mL) was added 0.3 mL (3.0 mmol) of isopropyl isocyanate dissolved in 5 mL acetonitrile in a dropwise manner under a nitrogen atmosphere at 80 °C. After the addition was complete, the reaction mixture was refluxed for 6 h and then evaporated under reduced pressure. The crude product obtained, a yellow oil, was purified by flash chromatography using a mixture of CH_2Cl_2:MeOH:conc. NH_3 (9:1:0.1). Fractions containing the product were collected and the eluant was removed under reduced pressure, leaving the product as a white amorphous substance. The obtained product was dried under reduced pressure for 24 h, and then subjected to ^1H NMR spectroscopy and HPLC analysis (Table I). *N*-Methyl-*N*-chloroformyl amino acid esters or *N*-methyl-*N*-alkyl carbamoyl chlorides were reacted with the 7-hydroxybenzazepine in dry pyridine under reflux for 10–24 h (Scheme I). In general, 2 equiv of reagent were used, and excess reagent was evaporated under reduced pressure after completion of the reaction. The crude product was purified on silica as described for the monosubstituted CAE. In some cases, the purified product was converted to the HCl salt with 1.0 M HCl in ether. As an example, 9 was synthesized by reacting 1 (0.5 g, 1.5 mmol) with dimethylcarbamyl chloride (0.28 mL, 3.0 mmol) in 20 mL of dry pyridine under reflux for 16 h. The mixture was evaporated under reduced pressure, leaving the crude product as an oil; this was then purified by flash chromatography using a CH_2Cl_2:MeOH:conc. NH_3 (49:1:0.1) mixture. Fractions containing the product were collected and, after removal of the solvent under reduced pressure, the product was obtained as white amorphous crystals. The identity of the product 9 was confirmed by ^1H NMR spectroscopy and HPLC analysis (Table I). The purity of the prodrugs (Table I) were checked by HPLC as described above and, in all cases, the purity was >97% based on chromatographic UV tracing at 214 nm.

Hydrolysis in Buffers—The prodrugs were incubated at 37 ± 0.2 °C in 0.01 M HCl (pH 2.0) and 0.02 M phosphate buffer (pH 7.4). The reactions were initiated by adding 100 μL of a stock solution of the compounds to 10 mL of preheated buffer, giving a final concentration of ~2.5×10^{-5} M. Aliquots (10 μL) were taken at various time intervals and injected into a HPLC system consisting of a Waters U6K injector valve with a 20-μL loop, a Waters 510 pump, a Shimadzu SPD-6A UV detector and a recorder (Instrument Innovators). The sample was chromatographed on a Knauer (Mikrolab, Aarhus, Denmark) cartridge (4 × 100 mm) packed with Spherisorb (3 μm silica, Phase Separations); a stainless steel cartridge (4 × 10 mm) packed with Polycosil silica (25–40 μm, Machery-Nagel) was used as a guard column. The column was eluted with MeOH:acetonitrile:5 mM ammonium hydrogen phosphate buffer (6:54:40), pH 7.8, at a rate of 1.0 mL/min. The column effluent was monitored at 244 nm. Quantitation was done by measuring the peak heights of the remaining intact prodrug. The hydrolysis was shown to follow pseudo-first-order kinetics according to eq 1:



Scheme I

$$\frac{dC}{dt} = k_{obs}C \quad (1)$$

where C is the concentration of the prodrug at time t and k_{obs} is the observed pseudo-first-order rate constant. Plotting the logarithm of the peak height of the remaining prodrug as a function of incubation time using least-square linear regression gave straight lines with correlation coefficients >0.99.

Hydrolysis in Plasma—Plasma from rats, dogs, and humans was obtained by centrifugation of blood samples containing 0.3% citric acid at 3000 × *g* for 15–20 min. Plasma fractions (4 mL) were diluted with 0.02 M phosphate buffer (pH 7.4) to give a final volume of 5 mL (80% plasma). Incubations were performed at 37 ± 0.2 °C using a shaking water bath. The reactions were initiated by adding 100 μL of a stock solution of drug (1.0 mg/mL in acetonitrile) to 5 mL of preheated plasma. Aliquots (100 μL) were taken at various times and deproteinized by mixing with 200 μL of 0.01 M HCl in methanol. After centrifugation for 5 min at 5000 × *g*, 5 or 10 μL of the clear supernatant were chromatographed as described above. The amounts of remaining intact prodrug were plotted as a function of incubation time as described above under buffer hydrolysis.

Inhibition of Human Acetylcholinesterase (AcChE) and Butyrylcholinesterase (ChE)—Acetylcholinesterase (AcChE) and butyrylcholinesterase (ChE) activity was measured spectrophotometrically as previously described.[17] To 3.0 mL of 0.1 M phosphate buffer (pH 8.0) was added 20 μL of substrate solution (75 mM acetylthiocholine or 75 mM butyrylthiocholine, Sigma Chemical Company, St. Louis, MO), 100 μL of 0.01 M dithiobisnitrobenzoic acid reagent (DTNB, in 0.1 M phosphate buffer, pH 7.0, with 1.5 mg/mL of sodium hydrogencarbonate). The reactions were initiated by adding 50 μL of enzyme solution (2.5–5.0 units/mL in 1% aqueous gelatine, Sigma) to the sample cuvette. The absorbance was monitored at 412 nm using a double beam spectrophotometer (Lambda 5, Perkin Elmer). Correction for nonenzymatic hydrolysis was performed by adding 50 μL of water to the reference cuvette containing the same mixture as the sample cuvette. The initial velocity was determined from the slope of the absorbance versus time curves using a molar extinction coefficient of 13.6×10^3. The Michaelis–Menten constants (K_m and V_{max}) were determined using 10 different substrate concentrations and plotting the initial velocity as a function of substrate concentration. The data points were fitted to the Michaelis–Menten equation (eq 2) using nonlinear regression analysis on a IBM personal computer:[18]

$$V = \frac{V_{max}[S]}{K_m + [S]} \quad (2)$$

where V is the initial velocity, V_{max} is the maximum rate of substrate consumption, K_m is the Michaelis constant, and $[S]$ is the substrate concentration.

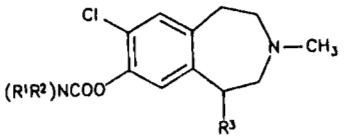
The inhibition of the enzymatic hydrolysis by the prodrugs was examined by adding 10–100 μL of aqueous solutions of the compounds (~1.0 mM) to the sample cuvette (an equivalent amount of water was added to the reference cuvette) and monitoring the absorbance change as described above. Inhibition constants (K_i) were determined by plotting the ratio between initial velocity (V) in the absence of inhibitor and initial velocity (V_i) in the presence of inhibitor according to eq 3:

$$\frac{V}{V_i} = 1 + \frac{IK_m}{K_i([S] + K_m)} \quad (3)$$

where I is the inhibitor concentration, K_i is the inhibition constant, and other constants are as defined above in eq 2. Plotting V/V_i against I gave a straight line, indicating that the inhibition is competitive and reversible. The parameter K_i was determined from the slope of the plot $(K_m/K_i(S)] + K_m)$ and known values of S and K_m.[19] All K_i values were determined using least-square linear regression plots obtained by eq 3.

Preparation of Liver Microsomes—Male NMRI mice (25 ± 5 g) and male Sprague-Dawley (180 ± 10 g) rats were dosed ip with saline or phenobarbital (80 mg/kg/day) for 4 days. Then the animals were fasted overnight with free access to water. On day 5, the animals were

Table I—Structural Formulas for the Carbamate Esters 3–15



Compound	R^1	R^2	R^3	Yield, %	^{1}H NMR, ppm[a]
3	—H	$-CH_2CH=CH_2$	bf[b]	70	2.35 (s, 3H), 2.4–3.3 (m, 6H), 3.8 (t, 2H), 4.8 (t, 1H), 5.0–5.2 (m, 3H), 5.8 (m, 1H), 6.4 (s, 1H), 6.8 (s, 1H), 7.05 (d, 1H), 7.3 (m, 2H), 7.6 (m, 2H)[c]
4	—H	$-CH_2C_6H_5$	bf	90	2.3 (s, 3H), 2.4–3.4 (m, 6H, 4.85 (d, 1H), 5.1–5.3 (m, 3H), 6.5 (s, 1H), 6.8 (s, 1H), 7.0–7.6 (m, 10H)[c]
5	—H	$-C_4H_9$	bf	40	1.2 (m,7H), 2.3 (s, 3H), 2.4–3.3 (m, 6H), 4.7 (d, 1H), 5.0–5.2 (m, 3H), 6.4 (s, 1H), 6.8 (d, 1H), 7.05 (d, 1H), 7.25 (m, 2H), 7.6 (m, 2H)[c]
6	—H	$-CH(CH_3)_2$	bf	55	1.15–1.3 (m, 6H), 2.3 (s, 3H), 2.3–3.3 (m, 6H), 3.5 (m, 1H), 4.6 (d, 1H), 6.2 (s, 1H), 6.85 (m, 2H), 7.2 (m, 2H), 7.6 (m, 2H)[c]
7	—H	$-CH(COOCH_3)-CH_2C_6H_5$[d]	bf	25	2.25 (s, 3H), 2.4–3.2 (m, 6H), 3.8–4.1 (m, 4H), 4.55 (d, 1H), 5.1 (m, 2H), 6.3 (s, 1H) 6.75 (m, 2H), 7.15 (m, 2H), 7.55 (m, 2H)[c]
8	—H	$-CH(COOCH_3)-CH_3$[e]	bf	60	1.25 (d, 3H), 2.3 (s, 3H), 2.35–3.3 (m, 6H), 3.7–4.2 (m, 4H), 4.6 (d, 1H), 5.2 (m, 2H), 6.3 (s, 1H), 6.8 (m, 2H), 7.25 (m, 2H) 7.6 (m, 2H)[c]
9	$-CH_3$	$-CH_3$	bf	85	2.1 (t, 1H), 2.15 (s, 3H), 2.9 (s, 3H), 3.0 (s, 3H), 2.8–3.4 (m, 5H), 4.5 (d, 1H), 6.3 (s, 1H), 6.8 (m, 2H), 7.3 (m, 2H), 7.8 (m, 2H)[f]
10	$-CH_3$	$-CH_3$	2,3–bf[g]	87	2.2 (t, 1H), 2.3 (s, 3H), 2.85 (s, 3H), 3.0 (s, 3H), 2.6–3.3 (m, 7H), 4.35 (d, 1H), 4.4 (t, 2H), 6.3 (s, 1H), 6.95 (m, 2H), 7.2 (m, 2H), 7.4 (s, 1H)[f]
11	$-C_2H_5$	$-C_2H_5$	2,3-bf	75	1.15 (dt, 6H), 2.9 (s, 3H), 3.0–3.8 (m, 12H), 4.5 (t, 2H), 4.7 (d, 1H), 6.3 (s, 1H), 7.0 (m, 2H), 7.3 (d, 2H), 7.5 (s, 1H)[f]
12	$-CH_3$	$-C_2H_5$	2,3-bf	52	1.0–1.15 (dt, 3H), 2.15 (t, 1H), 2.25 (s, 3H), 2.7–3.4 (m, 12H), 4.4 (d, 1H), 4.45 (t, 2H), 6.35 (s, 1H), 6.9 (m, 2H), 7.2 (d, 2H), 7.5 (s, 1H)[f]
13	$-CH_3$	$-CH(CH_3)_2$	2,3-bf	70	1.0–1.2 (dd, 6H), 2.2 (t, 1H), 2.25 (s, 3H), 2.7–3.25 (m, 11H), 4.4 (d, 1H), 4.45 (t, 2H), 6.3 (s, 1H), 6.9 (m, 2H), 7.2 (d, 1H), 7.4 (s, 1H)[f]
14	$-C_2H_5$	$-C_2H_5$	bf	75	1.2 (m, 6H), 2.8 (s, 3H), 2.9–4.2 (m, 10H), 5.5 (s, 1H), 6.3 (s, 1H), 6.85 (s, 1H), 7.2–7.3 (m, 3H), 7.6 (m, 2H)[c]
15	$-CH_3$	$-CH(COOCH_3)-CH_3$[d]	2,3-bf	10	1.4 (dd,3H), 2.2 (t, 1H), 2.3 (s, 3H), 2.8–3.4 (m, 10H), 3.6 (t, 3H), 4.4 (d, 1H), 4.5 (t, 2H), 4.6 (m, 1H), 6.4 (d, 1H), 6.9 (m, 2H), 7.2 (d, 1H), 7.4 (d, 1H)[f]

[a] s: singlet, d: dublet, t: triplet, m: multiplet, dd: double dublet, dt: double triplet. [b] Benzofuran-7-yl. [c] $CDCl_3$. [d] *S*-Configuration. [e] *R,S*-Configuration. CD_3SOCD_3 (d_6-DMSO). [g] 2,3-Dihydrobenzofuran-7-yl.

anesthetized with ether and the livers were rapidly removed and placed in 10 mL of ice-cold 0.15 M KCl. After a few minutes wash, the livers were blotted dry, weighed, and homogenized in four volumes of ice-cold 0.25 M sucrose using a glass Teflon Potter-Elvehjem homogenizer (Polytron, Kinematica). The homogenate was then centrifuged for 15 min at 12 000 × *g*. The supernatant was transferred to nitrocellulose vials (Beckman) and centrifuged for 60 min at 100 000 × *g*. The pellets obtained were resuspended in 5 mL of ice-cold 0.1 M Tris buffer (pH 7.4) and centrifuged for 60 min at 100 000 × *g*. The final pellets were resuspended in ice-cold 0.1 M Tris buffer (pH 7.4) with 20% (v/v) glycerol to give a protein concentration of ~10 mg/mL. Aliquots were taken and analyzed for protein by the Bradford method after solubilizing with 0.5 M NaOH[20] (Coomassie blue reagent, Bio-Rad Lab.). Microsomes were stored at −80 °C until used. All operations described above were performed at 4 °C.

Incubation with Liver Microsomes—Compound **10** was incubated in capped polyethylene Eppendorf vials (Nunc, Denmark) at 37 °C in a shaking water bath with a microsomal suspension containing 7 mM $MgCl_2$, 24 mM nicotinamide, 1.9 mg/mL of isocitric acid (sodium salt), 0.2 units of isocitrate dehydrogenase, and 0.5 mM $NADP^+$ in 0.5 mL of 0.1 M Tris buffer (pH 7.4). The incubation mixture was mixed with microsomal protein suspension (final concentration, 1 mg/mL protein) and preincubated for 2 min. A stock solution of **10** in acetonitrile (20 μL) was added, giving a final concentration of either 0.01 or 0.1 mM. At various time intervals, an aliquot (0.5 mL) was deproteinized by the addition of 50 μL of 70% perchloric acid. After standing for 5 min at 0–4 °C, the sample was centrifuged 10 min at 5000 × *g* to precipitate the proteins, and 20 μL of the clear supernatant was injected into the following HPLC system. The system consisted of two Kontron 420 pumps with dynamic eluant mixing, a Kontron 460 autosampler, a Linear 206 PHD UV detector, and a Kontron MT450 system controller. Compounds **2** and **10** were detected at 280 nm after separation on a C-18 Nucleosil column (5 μm, 4 × 200 mm) and a Vydac guard column (30–40 μm, C-18; Machery-Nagel), using an eluant comprising 42% (v/v) acetonitrile and 58% (v/v) 20 mM perchloric acid adjusted to pH 2.5 with 1 M NaOH. The retention times for **2** and **10** were 5.1 and 7.9 min, respectively.

Results and Discussion

Hydrolysis in Buffer and Plasma—The hydrolytic stability of the carbamates **3–15** was examined in aqueous solution at both acidic (pH 2.0) and physiological (pH 7.4) pH, as well as in plasma solutions. All the monosubstituted carbamates (**3–8**) were rapidly hydrolyzed at pH 7.4, and, in plasma solutions, the hydrolysis following strict first-order kinetics over several half-lives (Figure 1). The half-lives observed for the hydrolysis are shown in Table II. In contrast, the disubstituted carbamates **9–15** proved highly stable at pH 7.4, as well as in the plasma solutions. Less than 5% degradation occurred following incubation of the derivatives for 96 h in both rat, dog, and human plasma and in aqueous buffer solution at physiological pH.

The reason for this difference in stability is that monosubstituted carbamates of phenols or alcohols with pK_a values less than ~12 decompose via an E1cB mechanism involving an intermediate formation of an unstable isocyanate, whereas disubstituted carbamates are structurally incapable of undergoing this degradation and instead are hydrolyzed via the common ester $B_{AC}2$ mechanism.[21–25] The phenolic group in **1** and **2** has a pK_a value of 8.3, which is favorable for the occurrence of an E1cB mechanism for **3–8**.

Except for **4**, the hydrolysis of the monosubstituted carbamates is catalyzed by plasma from various species (Table II). In rat and dog plasma, the degradation of **4** is inhibited, which may be due to binding of this lipophilic compound to plasma proteins. The failure of the *N,N*-disubstituted carbamates **9–15** to undergo significant plasma-catalyzed hydrolysis is in accordance with the behavior of other disubstituted carbamates.[26]

Although being rather stable at acidic pH—<10% degradation at pH 2 after incubation for 1 h—the monosubstituted carbamates **3–8** appear not to be suitable as prodrugs for oral administration. Their ease of decomposition at neutral pH indicates that they would be cleaved in the intestine, leaving the metabolically labile phenol group unprotected against first-pass metabolism.

In Vitro Inhibition of Human Esterases—Disubstituted CAE are known as reversible inhibitors of plasma enzymes like cholinesterases (ChE) and acetylcholinesterase (AcChE).[26–28] All disubstituted CAE described here (**9–15**) were found to be potent and reversible inhibitors of human plasma ChE, also known as unspecific plasma esterase or pseudocholinesterase. Figure 2 is a typical plot showing this inhibition, and the values of K_i obtained are given in Table III. The most potent inhibitors are the symmetrical *N,N*-dimethyl- or diethyl-substituted CAE, whereas the *N*-methylalanine methyl ester **15** is an ~100-fold less potent inhibitor of butyrylcholinesterase.

Compounds **10–15** were much less potent as inhibitors of the specific esterase acetylcholinesterase (Table III). The results show that *N,N*-dimethylsubstituted CAE (**9** and **10**) are the most potent inhibitors of both ChE and AcChE, and also that small changes in molecular structure alter the affinity of the compound for the enzymes (e.g., comparing **9**

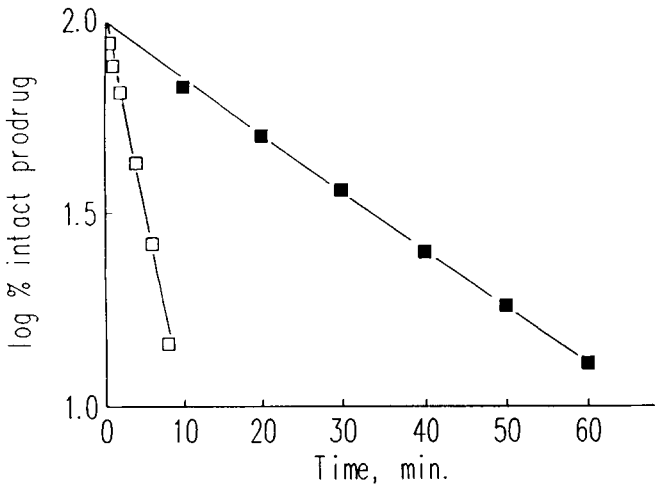


Figure 1—First-order plots for the degradation of monosubstituted CAE **3** (■) in 0.02 M phosphate buffer and of CAE **7** (□) in 80% dog plasma (pH 7.4 and 37 °C).

Table II—Half-Lives of Monosubstituted Carbamic Acid Esters 3–8 in Aqueous Buffer Solution and 80% Plasma from Rats, Dogs, and Humans[a]

Compound	$t_{1/2}$, min			
	Buffer Solution	Rat	Dog	Human
3	23.0	13.0	5.4	1.2
4	5.7	13.1	9.5	4.6
5	40.0	34.0	10.0	2.3
6	28.4	22.4	12.4	0.9
7	11.6	<0.5	3.7	1.3
8	4.0	0.5	0.9	0.7

[a] pH 7.4 and 37 °C.

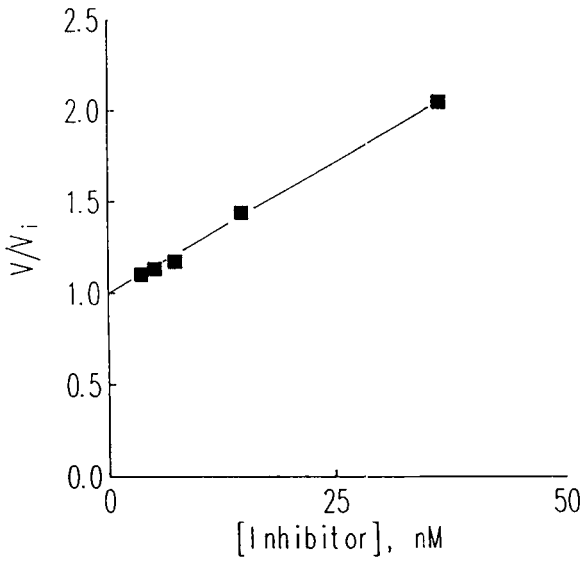


Figure 2—Plot showing the reversible and competitive inhibition of human cholinesterase in vitro with CAE **10**.

Compounds **9** and **10** are the most potent inhibitors of both ChE and AcChE, and also that small changes in molecular structure alter the affinity of the compound for the enzymes (e.g., comparing **9**

Table III—In Vitro Inhibition of Human Plasma Butyrylcholinesterase (ChE) and Human Erythrocyte Acetylcholinesterase (AcChE) by Disubstituted Carbamic Acid Esters 9–15

Compound	K_i, nM[a]		$K_{i,AcChE}/K_{i,ChE}$
	AcChE	ChE	
9	n.d.	8.8	n.d.
10	17 000	3.2	5400
11	>100 000	2.7	>37 000
13	83 000	68.0	1200
14	>100 000	28.2	>3500
15	>100 000	590.0	>170

[a] The K_i values were calculated using the following Michaelis–Menten constants (mean ± SD): $K_{m,AcChE}$, 7.12 ± 0.95 × 10^{-4} M; $V_{max,AcChE}$, 3.56 ± 0.17 × 10^{-5} M/min; $K_{m,ChE}$, 9.08 ± 1.7 × 10^{-5} M; $V_{max,ChE}$, 9.47 ± 0.2 × 10^{-6} M/min; n.d., not determined.

and **10** where the only difference is a reduction of a double bond in the benzofuranyl substituent at the 5 position). Also, the relatively small inhibitory effect of **15** on the unspecific esterase may reflect some structural requirements of the enzyme active site (e.g., that bulky substituents in the carbamic acid ester moiety may lower the affinity).

Bioconversion in Liver Microsomes—To examine whether bioconversion of the disubstituted CAE may take place in the liver, **10** was incubated with liver microsomes from male mice and rats. Drug metabolism enzymes in the liver were stimulated by pretreatment of the animals with a known inducer of drug metabolism, phenobarbital (PB), which induces the major family of the cytochrome P-450 superfamily, cytochrome P450IIB.[29] Two concentrations of **10** were selected, 0.01 and 0.1 mM. The lower concentration should reflect the in vivo situation following the absorption phase and the high concentration substrate saturation in the liver during absorption from the gut. Figures 3 and 4 show the time courses for the degradation of **10** and the formation of **2**. In some cases, the rate data for the formation of **2** could be fitted to a first-order rate equation. The rate constants and the corresponding half-lives obtained are listed in Table IV. In both species there was a reduced rate of formation of **2** at the highest concentration using noninduced liver microsomes, which may be a reflection of substrate inhibition. This

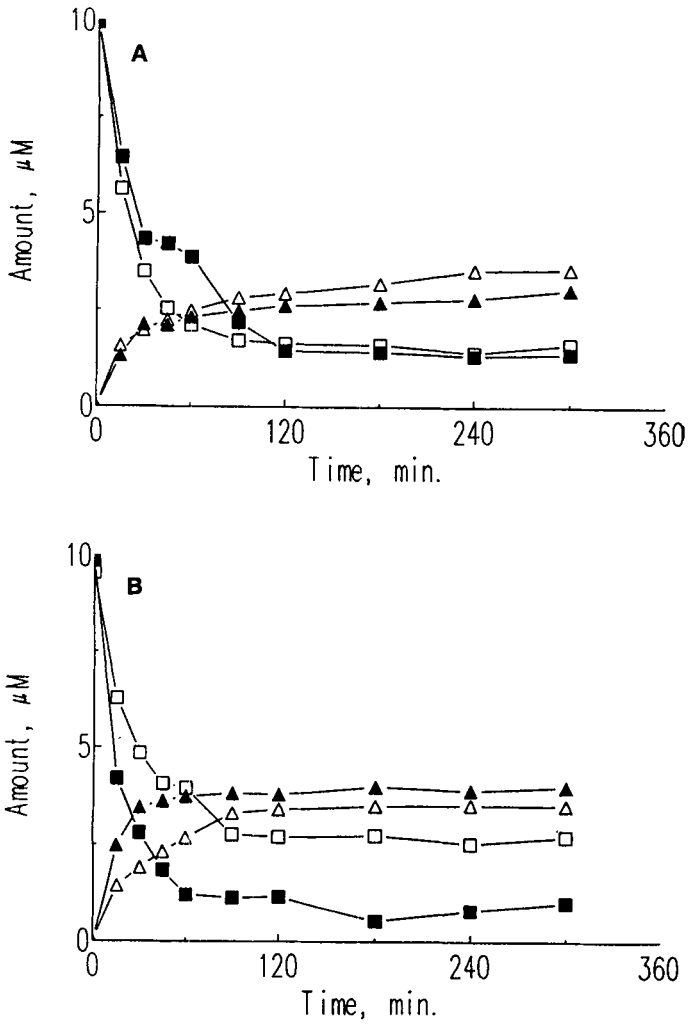


Figure 3—Plots showing the time courses for the degradation of **10** (■, □) and formation of **2** (▲, △) in mouse (■, ▲) and rat (□, △) liver microsomes: (**A**) 0.01 mM **10**, saline pretreatment; (**B**) 0.01 mM **10**, PB pretreatment. The data are the means of three experiments.

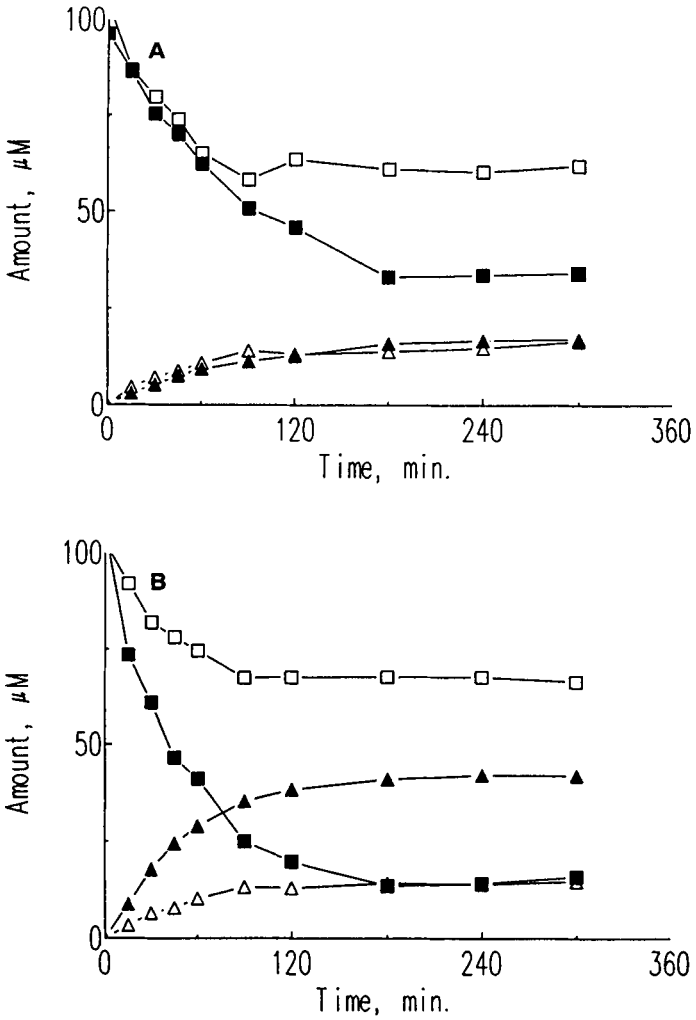


Figure 4—Plots showing the time courses for the degradation of **10** (■, □) and formation of **2** (▲, △) in mouse (■, ▲) and rat (□, △) liver microsomes: (**A**) 0.1 mM **10**, saline; (**B**) 0.1 mM **10**, PB pretreatment. The data are the means of three experiments.

difference was diminished in mice after induction of liver enzymes with PB, indicating that the enzymes responsible for the formation of **2** in mice belong to the cytochrome P450IIB family. In general, the mouse shows the same hydrolytic activity as the rat, but the formation of **2** is enhanced in the mouse after PB pretreatment (Figures 3 and 4). In the rat, no effect of PB treatment was observed, as seen in Figures 3 and 4. In mice, the formation of **2** amounted to 22% (0.01 mM) and 13% (0.1 mM) without PB pretreatment and to 40% (0.01 mM) and 42% (0.1 mM) after PB pretreatment. In rats, these figures were 36% (0.01 mM, saline), 15% (0.1 mM, saline), 35% (0.01 mM, PB), and 15% (0.1 mM, PB). The total recovery (**2** + **10**) ranged from 36 to 81%, the recovery being slightly higher in rats (52–81%) as compared with mice (36–58%). The existence of other metabolic pathways (e.g., glucuronidation of the liberated phenol group) could explain the lack of total recovery. However, using the *N*-demethyl derivative of **2** (no methyl group in the 3-position), it was shown that *N*-demethylation of the liberated parent compound **2** was absent after incubation of **10** with liver microsomes, and the occurrence of this metabolic pathway was therefore excluded as the reason for the incomplete recovery.

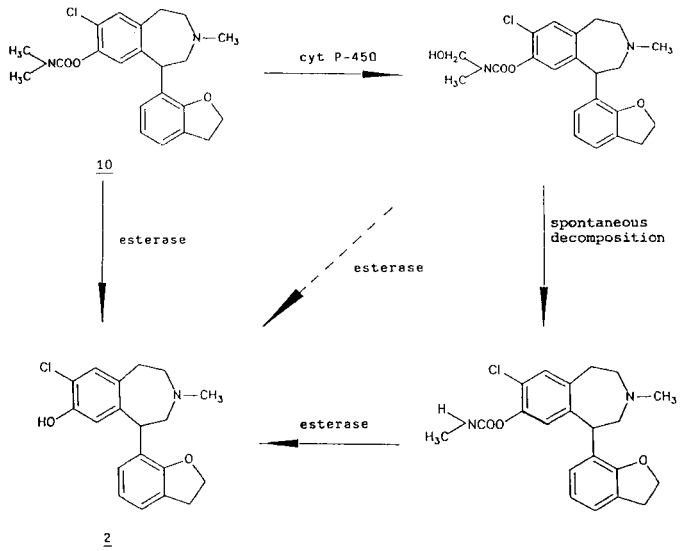
The mechanism for the bioconversion of **10** to **2** could be a cytochrome P-450-catalyzed hydroxylation on one or both of the methyl groups in the carbamic acid ester moiety. Such a

Table IV—Kinetic Data for the In Vitro Formation of 2 in Mouse and Rat Liver Microsomes

Animal	Concentration of 10, mM	k_{obs}, min^{-1}	$t_{1/2}$, min
Mouse, PB	0.01	6.8×10^{-2}	10.2
Mouse, PB	0.10	1.9×10^{-2}	36.1
Rat, saline	0.01	2.7×10^{-2}	26.0
Rat, PB	0.01	2.8×10^{-2}	25.0

mechanism has been suggested for other *N,N*-dimethylsubstituted carbamates and in some cases, the intermediate, an *N*-hydroxymethyl derivative, has been isolated.[11,30] This intermediate is unstable and spontaneously releases formaldehyde with the formation of a monosubstituted CAE, which then readily undergoes enzymatic hydrolysis to release the parent compound.[11] Accordingly, the bioconversion of **10** to **2** in the liver could occur as outlined in Scheme II. The direct hydrolysis of **10** is probably only a minor pathway, since **10** is a very potent inhibitor of esterases (Table III).

In liver microsomes showing low hydrolytic activity against disubstituted CAE, addition of plasma to in vitro incubations has resulted in almost quantitative recovery of the parent compound, suggesting that after the initial cytochrome P-450 hydroxylation on one of the methyl groups, the *N*-hydroxymethyl intermediate becomes a better substrate for plasma esterases than the carbamic acid ester prodrug.[12] However, incubations of **10** in liver microsomes with 50% plasma only marginally changed the rate of formation of **2** in the mouse or rat. Actually, there was a slight tendency towards a decreased formation rate, which could be explained by plasma protein binding of **10**, resulting in a reduced free fraction available for the initial hydroxylation by liver cytochrome P-450s.



Scheme II

In conclusion, the *N,N*-dimethylcarbamate **10** may be a potentially useful prodrug for the protection of the parent compound against first-pass metabolism. Preliminary studies in dogs have confirmed that oral administration of the prodrug **10** results in significantly increased bioavailability of the parent compound **2**, which when administered per se showed a bioavailability of only ~5%. Pharmacokinetic studies with some of these carbamate ester prodrugs have been described.[31]

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