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Cyclization-activated phenyl carbamate prodrug forms for protecting phenols against first-pass metabolism

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Summary

A series of phenyl carbamate esters derived from *N*-substituted 2-aminobenzamides was prepared and evaluated as prodrug forms with the aim of protecting phenolic drugs against first-pass metabolism following peroral administration. The stability of the derivatives was studied in aqueous buffer solutions and in various biological media. The carbamates showed a relatively high stability at pH 1–6 but underwent an apparent specific base-catalyzed cyclization in neutral and alkaline solution to a 2,4(1*H*,3*H*)-quinazolinedione with concomitant release of the parent phenol. The rate of this cyclization was not affected by liver, intestinal wall or plasma enzymes but depended on the pK_a of the phenol and the steric and polar properties of the *N*-substituent within the benzamide moiety. By appropriate selection of this substituent it is readily feasible to obtain prodrug derivatives having practically useful rates of cyclization and hence release of the parent phenolic drug at pH 7.4 and 37°C, corresponding to half-lives of 10–60 min. The results suggest that this prodrug principle involving a non-enzymatic but pH-dependent conversion may be a potentially useful approach to reduce the extent of first-pass metabolism of the vulnerable phenol group.

Introduction

Several drugs are efficiently absorbed from the gastrointestinal tract, but show limited systemic bioavailability due to presystemic (first-pass) metabolism or inactivation before reaching the systemic circulation. This metabolism can occur in the intestinal lumen, at the brush border of the intestinal cells, in the mucosal cells lining the gastrointestinal tract or in the liver. In addition to decreasing the percentage of dose reaching its intended site of action, extensive first-pass metabolism often results in significant variability in bioavailability.

A major class of drugs undergoing extensive first-pass metabolism are those containing phenolic hydroxyl groups. The inactivation of these drugs in the gut and/or liver is mainly due to sulphation, glucuronidation or methylation of the phenolic moieties (George, 1981; Pond and Tozer, 1984).

The traditional prodrug approach involving esterification of the metabolically vulnerable phenol group to prevent presystemic metabolism has been met with only limited success (Stella et al., 1985; Svensson and Tunek, 1988; Lokind et al., 1991; Bundgaard, 1992). The reason for this is the

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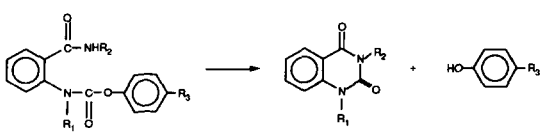
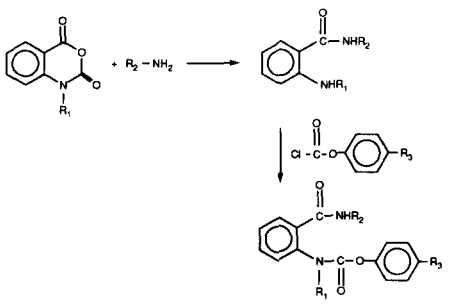
fact that the enzymatic hydrolysis of the phenol ester most often occurs already in the intestinal mucosa or liver during first-pass. The active parent phenol thereby released will then be available for metabolism within the same tissue.

A more promising approach to prevent or depress the first-pass metabolism of phenolic drugs may be development of prodrug derivatives where the regeneration of the parent phenol occurs by non-enzymatic means, e.g., by chemical hydrolysis or an intramolecular reaction occurring with an appropriate rate at the pH of the blood (7.4) and 37°C. To ensure passage of the prodrug in largely intact form through the stomach and upper intestine suitable prodrug forms should preferably be more stable at lower pH values.

Studies to exploit this new prodrug approach have been initiated in our laboratory (Hansen et al., 1992b; Bundgaard, 1992) as well as by others (Patel et al., 1991). In a previous paper (Hansen et al., 1992b), we have reported that various phenyl carbamate esters derived from amino acids or dipeptides possess properties close to the ideal ones mentioned above. The purpose of the present work was to examine a prodrug principle involving release of the phenol from the prodrug derivatives via a spontaneous ring closure reaction. The derivatives studied are phenyl carbamates of various *N*-substituted 2-aminobenzamides (anthranilamides). Several years ago Hegarty et al. (1974) reported that the phenyl carbamates of 2-aminobenzamide and 2-(methylamino)-benzamide underwent a cyclization to 2,4(1*H*,3*H*)-quinazolinediones with release of phenol (Scheme 1) in alkaline aqueous solutions at rates which may be appropriate for a suitable prodrug principle. We have prepared a number of such phenyl carbamates containing various R_1 and R_2 substituents at the two nitrogens (Scheme 1) and studied their stability in aqueous solutions as a function of pH and in plasma and other



Scheme 1.



Scheme 2.

biological media. Structure-reactivity relationships were established, making it possible to select prodrug derivatives with appropriate rates of conversion at physiological pH and temperature.

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was performed with a Shimadzu system consisting of an LC-6A pump, an SPD-6A variable-wavelength UV detector and a Rheodyne 7125 injection valve with a 20 μl loop. A deactivated Supelcosil LC-8-DB reversed-phase column (33 × 4.6 mm i.d.) (3 μm particles) from Supelco Inc., U.S.A., was used in conjunction with a Supelguard precolumn. In some cases a Kontron system consisting of an LC pump T-414, a Uvikon 740 UV detector operated at a fixed wavelength (215 nm) and a 20 μl injection valve was used. A Chrompack column (100 × 3 mm) packed with CP Spher C-8 (5 μm particles) was used with this apparatus. Readings of pH were carried out on a Radiometer PHM Autocal instrument at the temperature of the study. Elemental analysis was performed at Leo Pharmaceuticals, Ballerup, Denmark.

Preparation of phenyl N-(2-carbamoylphenyl)carbamates

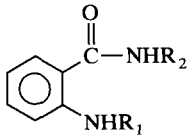
Various phenyl *N*-(2-carbamoylphenyl)carbamates were prepared by reacting the appropriate phenyl chloroformate with an anthranilamide, the latter being obtained by treating isatoic anhydride or an *N*-substituted isatoic anhydride with

a primary amine (Scheme 2). Isatoic anhydride and *N*-methylisatoic anhydride (recrystallized from methylene chloride) were purchased from Aldrich Chemie, Germany. *N*-Ethylisatoic anhydride was prepared by reacting isatoic anhydride with sodium hydride and ethyl iodide as described by Hardtmann et al. (1975).

Anthranilamides I–XII (Table 1) The compounds **I–IV** and **XI** were prepared according to a general method described by Lee (1964). A mixture of 10 mmol of the *N*-substituted isatoic anhydride and 30 mmol of the appropriate amine in water (15 ml) was stirred at 90°C for 1 h. The solid precipitates formed in the case of **I** and **III** were filtered off and dried. The oily residues obtained in the case of **II**, **IV** and **XI** were taken up in ethyl acetate. The solution was washed with water, dried over anhydrous sodium sulphate and evaporated in vacuo. The compounds were purified by recrystallization from methylene chloride-petroleum ether. The compounds **VIII**, **IX** and **XII** were prepared by adding 10 mmol of the *N*-substituted isatoic anhydride to a solution of 30 mmol of the hydrochlorides of glycinamide or α-alaninamide in 30 ml of water, the pH of the solution being adjusted to 8.5 with 10 M sodium hydroxide. The mixtures were stirred at 60°C for 2 h and then at room temperature for 3 days. The precipitates formed were filtered off, dried and recrystallized from ethanol-water. The compounds **V** and **VI** were prepared by adding 11 mmol of the appropriate amine to a solution of *N*-methylisatoic anhydride (10 mmol) and 4-dimethylaminopyridine (1 mmol) in 10 ml of *N,N*-dimethylformamide according to the method described by Venuti (1982). The mixtures were stirred at room temperature for 1 h (**VI**) or 3.5 h (**V**). Water was added and the precipitate formed was filtered off, dried and recrystallized from ethyl acetate-petroleum ether. The compounds **VII** and **X** were obtained by reacting *N*-methylisatoic anhydride (14.7 mmol) with the hydrochlorides of glycine ethyl ester or β-alanine ethyl ester, respectively (15.8 mmol), in 10 ml of pyridine according to the method described by Kim (1975). Melting points of the compounds along with some literature values are given in Table 1.

TABLE 1

Melting points of various N-substituted anthranilamides



Compound	R_1	R_2	m.p. (°C)
I	CH_3	H	161–162 (160–161) [a]
II	CH_3	CH_3	88– 89 (88) [b]
III	CH_3	CH_2CH_3	86– 87 (88–89) [b]
IV	CH_3	$CH_2CH_2CH_3$	58– 59
V	CH_3	$CH(CH_3)_2$	114–116 (118–119) [b]
VI	CH_3	$CH_2CH_2CH_2CH_3$	54– 55
VII	CH_3	$CH_2COOC_2H_5$	72– 73 (72–74.5) [c]
VIII	CH_3	CH_2CONH_2	182–184
IX	CH_3	$CH(CH_3)CONH_2$	232–234
X	CH_3	$CH_2CH_2COOC_2H_5$	oil
XI	C_2H_5	CH_3	60– 62 (60–62) [d]
XII	C_2H_5	CH_2CONH_2	177–178

[a] Horiuchi et al. (1981).
[b] Wagner and Rothe (1969).
[c] Kim (1975).
[d] Coppola and Mansukhani (1978).

Phenyl N-(2-carbamoylphenyl)carbamates 1–13 (Table 2) These compounds were prepared by reacting the anthranilamides with the appropriate phenyl chloroformate in tetrahydrofuran. The preparation of compound **6** represents a typical procedure: phenyl chloroformate (0.44 ml, 3.5 mmol) was added over 5 min to a solution of compound **VI** (0.72 g, 3.5 mmol) and triethylamine (0.47 ml, 3.5 mmol) in 25 ml of tetrahydrofuran. The reaction mixture was stirred at 80°C for 30 min, cooled to room temperature and filtered. The filtrate was evaporated in vacuo and the residue obtained recrystallized from methylene chloride-petroleum ether. In the case of the compounds **8** and **12** the precipitate formed during the reaction consisted of both triethylamine hydrochloride and the carbamate products. Washing the precipitate with water removed the triethylamine hydrochloride. Physical and analytical data for compounds **1–13** are given in Table 2.

Ethyl N-methyl-N-(2-N-methylcarbamoylphenyl)carbamate Ethyl chloroformate (0.96 g, 10 mmol) was added to a solution of 2-(methylamino)-*N*-methylbenzamide (**II**) (1.64 g, 10 mmol)

and triethylamine (1.34 ml, 10 mmol) in tetrahydrofuran (10 ml). The mixture was stirred at 80°C for 1 h, cooled to room temperature and filtered. The filtrate was evaporated in vacuo and the residue obtained crystallized from methylene chloride, m.p. 101–103°C. Anal.: Calc. for $C_{12}H_{16}N_2O_3$: C, 61.00; H, 6.83; N, 11.86. Found: C, 61.15; H, 6.88; N, 11.78.

1,3-Dimethyl-2,4(1H,3H)-quinazolinedione This compound was prepared by a method described by Gadekar et al. (1964). Ethyl *N*-methyl-*N*-(2-*N*-methylcarbamoylphenyl)carbamate (0.47 g, 2 mmol) was added to a solution of potassium hydroxide (0.11 g, 2 mmol) in 10 ml ethanol and the mixture was stirred at 70°C for 2 h. The precipitate formed was filtered off, washed with ethanol and dried, m.p. 163–164°C (reported m.p. 164–165°C (Lee, 1964)).

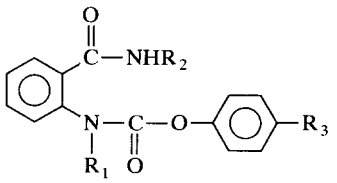
Kinetic measurements

Degradation in aqueous solutions The degradation of the compounds **1–13** was studied in aqueous buffer solutions at constant temperature (±0.2°C). The buffers used were hydrochloric acid, acetate, phosphate and borate solutions. The buffer concentration generally used was 0.02 M. A constant ionic strength (μ) of 0.5 was maintained for each buffer solution by adding a calculated amount of potassium chloride.

The rates of degradation of the compounds were determined by using reversed-phase HPLC procedures capable of separating the compounds from their products of degradation. Mobile phase systems of 0.1% phosphoric acid containing acetonitrile (15–35 v/v) or, in some cases, methanol (20 v/v) were used. The concentration of acetonitrile was adjusted for each compound to give a

TABLE 2

Physical and analytical data of various phenyl N-(2-carbamoylphenyl)carbamates



Compound	R_1	R_2	R_3	Formula [a]	m.p. (°C)
1a	CH_3	H	H	$C_{15}H_{14}N_2O_3$	146–147 [b]
1b	CH_3	H	Cl	$C_{15}H_{13}ClN_2O_3$	143–144
1c	CH_3	H	OCH_3	$C_{16}H_{16}N_2O_4$	148–150
2a	CH_3	CH_3	H	$C_{16}H_{16}N_2O_3$	106–107
2b	CH_3	CH_3	Cl	$C_{16}H_{15}ClN_2O_3$	120–121
2c	CH_3	CH_3	OCH_3	$C_{17}H_{18}N_2O_4$	129–131
3	CH_3	CH_2CH_3	H	$C_{17}H_{18}N_2O_3$	171–173
4	CH_3	$CH_2CH_2CH_3$	H	$C_{18}H_{20}N_2O_3$	98– 99
5	CH_3	$CH(CH_3)_2$	H	$C_{18}H_{20}N_2O_3$	92– 94
6	CH_3	$CH_2CH_2CH_2CH_3$	H	$C_{19}H_{22}N_2O_3$	107–108
7	CH_3	$CH_2COOC_2H_5$	H	$C_{19}H_{20}N_2O_5$	133–134
8	CH_3	CH_2CONH_2	H	$C_{17}H_{17}N_3O_4$	> 300
9	CH_3	$CH(CH_3)CONH_2$	H	$C_{18}H_{19}N_3O_4$	165–167
10	CH_3	$CH_2CH_2COOC_2H_5$	H	$C_{20}H_{22}N_2O_5$	87– 88
11	C_2H_5	CH_3	H	$C_{17}H_{18}N_2O_3$	112–113
12	C_2H_5	CH_2CONH_2	H	$C_{18}H_{19}N_3O_4$	189–190
13	H	H	H	$C_{14}H_{12}N_2O_3$	159–160 [c]

[a] Elemental analyses (C, H and N) were within ±0.4% of the calculated values.
[b] Reported m.p. 146–148°C (Hegarty et al., 1974).
[c] Reported m.p. 159–160°C (Hegarty et al., 1974).

retention time of 2–10 min. The column effluent was monitored at 215 nm.

The degradation reactions in buffer solutions were initiated by adding 100 μl of a stock solution of the compounds in acetonitrile to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration being 5×10^{-5}–10^{-4} M. The solutions were kept in a water-bath at constant temperature and at appropriate intervals samples were taken and chromatographed immediately. Pseudo-first-order rate constants for the degradation were determined from the slopes of linear plots of the logarithm of residual compound against time.

Degradation in biological media The degradation of the compounds was studied at 37°C in 40 or 80% human plasma. Compound **1a, 2a, 8** and **13** were also studied in 10 or 20% rabbit liver homogenate and compounds **2a** and **8** in a 20% rabbit gut homogenate. The initial concentration of the compounds was about 10^{-4} M. The reaction mixtures were kept in a water-bath at 37°C and at appropriate intervals samples of 250 μl were withdrawn and added to 500 μl of a 2% (w/v) solution of zinc sulphate in methanol-water (1:1, v/v) in order to stop the reactions and deproteinize the samples. After mixing and centrifugation for 3 min at 13000 rpm, 20 μl of the clear supernatant was analyzed by HPLC as described above.

Results and Discussion

Kinetics of degradation

At constant pH and temperature the degradation of all phenyl carbamates (**1–13**) studied displayed strict first-order kinetics for several half-lives. Typical first-order plots obtained are shown in Fig. 1. At the buffer concentration used (0.02 M) no significant catalysis by the buffer substances used to maintain constant pH was observed.

At pH 6.5–10 and 37°C the rate of degradation increased with increasing pH according to the following relationship:

$$k_{obs} = k_{OH} a_{OH} \qquad (1)$$

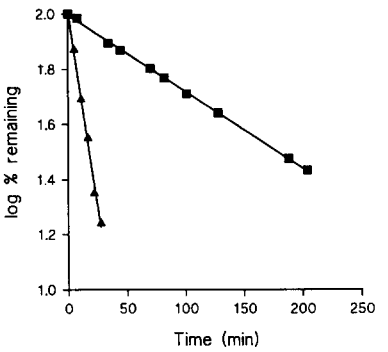


Fig. 1. First-order kinetic plot for the degradation of compound **2c** (■) and **7** (▲) in aqueous solutions (pH 7.4) at 37°C.

where k_{obs} is the observed pseudo first-order rate constant, a_{OH} is the hydroxide ion activity and k_{OH} is a second-order rate constant for the apparent specific base-catalyzed degradation. The pH-rate profiles obtained for some carbamates are shown in Fig. 2 whereas the k_{OH} values are listed in Table 3 along with the half-lives of degradation at pH 7.4.

In order to examine the influence of pH on the stability in acidic solutions the degradation of the carbamates **2a** and **8** was also studied at lower pH values at 60°C. The pH-rate profiles obtained are shown in Fig. 3. The shape of these profiles show that the compounds are subject to a spontaneous or water-catalyzed degradation and, in case of compound **8**, a specific acid-catalyzed degradation in addition to the apparent hydroxide ion-

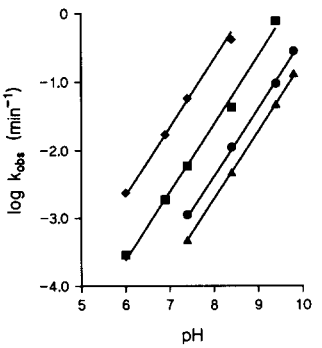


Fig. 2. The pH-rate profiles for the degradation of compound **2a** (■), **4** (▲), **9** (●) and **12** (◆) in aqueous solutions at 37°C.

TABLE 3

Half-lives of degradation of compound 1–13 and their k_{OH} values at 37°C

Compound	$t_{1/2}$ (min)		k_{OH} (M^{-1} min^{-1})
	pH 7.4 buffer	40% human plasma	
1a	485	125 (65) [b]	1.8×10^3
1b	260	20 [b]	3.0×10^3
1c	520	44 [b]	1.8×10^3
2a	120	70 (55) [b]	1.0×10^4
2b	60	25	2.0×10^4
2c	110	55	1.1×10^4
3	610	440	1.6×10^3
4	1.5×10^3	–	8.0×10^2
5	1.3×10^{5a}	–	1.6×10^1
6	1.8×10^3	580	5.5×10^2
7	11	13	1.0×10^5
8	10	5	1.5×10^5
9	625	360	1.7×10^3
10	165	85	6.0×10^3
11	145	85	7.6×10^3
12	12	5	8.9×10^4
13	9	5	1.2×10^5

[a] Calculated from the k_{OH} value determined in alkaline solutions.
[b] Data in 80% human plasma solutions.

catalyzed reaction according to the following rate expression:

$$k_{obs} = k_H a_H + k_0 + k_{OH} a_{OH} \quad (2)$$

where k_0 is a first-order rate constant for the spontaneous reaction and k_H is a second-order rate constant for specific acid-catalyzed degradation. The values obtained for these rate constants at 60°C are: compound **2a**: $k_0 = 2.5 \times 10^{-4}$ min^{-1}; $k_{OH} = 4.0 \times 10^4$ M^{-1} min^{-1}; compound **8**: $k_0 = 1.0 \times 10^{-5}$ min^{-1}; $k_H = 0.020$ M^{-1} min^{-1}; $k_{OH} = 6.0 \times 10^5$ M^{-1} min^{-1}.

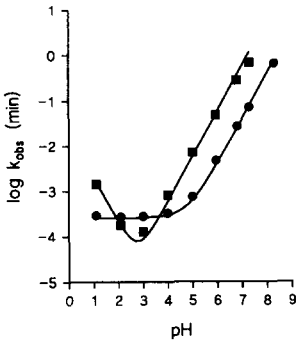


Fig. 3. The pH-rate profiles for the degradation of compound **2a** (●) and **8** (■) in aqueous solutions at 60°C.

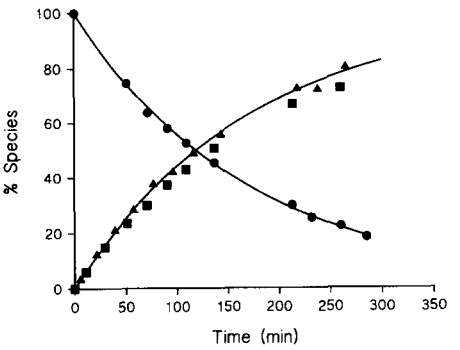


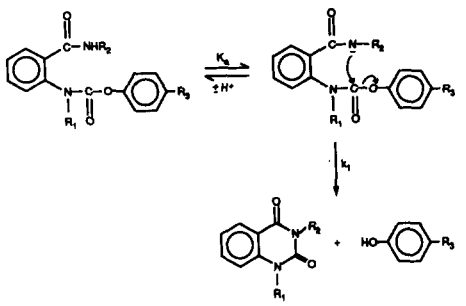
Fig. 4. Plots showing the time courses of degradation of compound **2a** (●) and formation of phenol (■) and 1,3-dimethyl-2,4(1*H*,3*H*)-quinazolinedione (▲) in aqueous solution (pH 7.4) at 37°C.

Mechanism of degradation

As noted in the Introduction, the high reactivity of the *N*,*N*-disubstituted phenyl carbamates studied in neutral and alkaline solution can be ascribed to a ring closure reaction resulting in the formation of phenol and a 2,4-quinazolinedione (Scheme 1). Hegarty et al. (1974) studied the kinetics of this reaction of compound **1a** at pH 10–12 and showed the products of the reaction to be phenol and 1-methyl-2,4(1*H*,3*H*)-quinazolinedione. With compound **2a** we confirmed this cyclization reaction and showed it to proceed in a quantitative fashion at pH 6.5–10 by means of HPLC analysis of the reaction solutions (Fig. 4). All the other carbamates studied were also shown to degrade quantitatively to the parent phenol at pH 6.5–10.

The reaction mechanism for the apparent hydroxide ion-catalyzed cyclization of the phenyl carbamates **1–12** most likely involves pre-equilibrium ionization of the *o*-amido group, the rate-determining step being attack of the amide anion on the carbamate carbonyl group (Hegarty et al., 1974) (Scheme 3). According to this mechanism, Eqn 1 should be written as:

$$k_{obs} = k_1 K_a / (a_H + K_a) \quad (3)$$



Scheme 3.

where K_a is the dissociation constant of the amide group and k_1 represents a first-order rate constant for the intramolecular attack of the amide anion on the carbamate carbonyl moiety. Since $K_a \leq 10^{-14}$ Eqn 3 can be reduced to

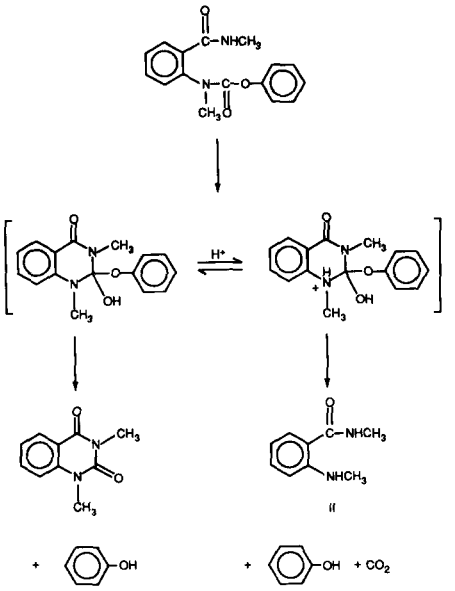
$$k_{obs} = k_1 K_a / a_H \quad (4)$$

or

$$k_{obs} = k_1 K_a a_{OH} / K_w \quad (5)$$

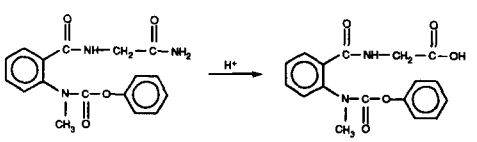
at the pH values studied (pH $\leq$ 10). Eqn 5 is seen to be of the same form as Eqn 1, showing that the mechanism proposed is in harmony with the observed reaction kinetics.

HPLC analysis of the reaction solution of compound **2a** at pH 1–6 showed the formation of the anthranilamide **II** in addition to 1,3-dimethyl-2,4(1*H*,3*H*)-quinazolinedione and phenol. Since the quinazolinedione was found to be highly stable in both acidic and alkaline solutions the formation of compound **II** must be due to a spontaneous cleavage of the carbamate bond in compound **2a**. The amount of **II** formed was 25% at pH 5 increasing to about 80% at pH 1. The increased formation of compound **II** with decreasing pH of solution can not be ascribed to simple acid-catalyzed cleavage of the carbamate moiety since the overall rate of degradation of compound **2a** is independent of pH in the range 1–4.5. The variation of the product distribution with pH may rather be ascribed to a degradation proceeding through rate-limiting formation of tetrahedral intermediates being in acid-base equilibrium and degrading to compounds **II** and 1,3-dimethyl-2,4(1*H*,3*H*)-quinazolinedione, respectively (Scheme 4). In the protonated intermediate expulsion of the aromatic amino group with the ultimative formation of 2-(methylamino)-*N*-methylbenzamide (**II**) and phenol may be the predominant reaction whereas at higher pH values the neutral intermediate preferentially expels phenolate anion with the formation of the quinazoline-2,4-dione.



Scheme 4.

In acidic solutions the degradation of the phenyl carbamate **8** showed no sign of formation of the corresponding anthranilamide (**VIII**). At pH 1–2, however, where the degradation showed specific acid catalysis (cf. Fig. 3), an additional peak was seen in the chromatograms of the reaction solutions. The structure of this major degradation product formed at pH 1–2 was not identified but is suggested to be the free acid form of compound **8** formed by acid-catalyzed hydrolysis of the terminal glycinamide bond (Scheme 5). When an aliquot of the reaction solution of pH 1



Scheme 5.

was added to a pH 7.4 buffer the product disappeared with a half-life of 15.5 h, presumably due to cyclization as for the other phenyl carbamates. Further evidence for the suggested structure of the product was provided by the fact that the retention time of the compound was identical to that of the product arising from hydrolysis of the ethyl ester analogue **7** in liver homogenates.

Structural effects on the rate of cyclization

A main objective of the present study was to examine the influence of the R_1, R_2 and R_3 substituents in the phenyl carbamates on the rate of cyclization at physiological pH. As seen from the data in Table 3 the reactivity of the derivatives varies widely, the half-lives of cyclization at pH 7.4 and 37°C ranging from 9 min to 2200 h.

Considering the substituents (R_3) in the phenolic group the reactivity increases with decreasing pK_a values of the phenol and hence with increasing leavability of the phenolate ion. This is illustrated by the Brønsted plots in Fig. 5 where log k_{OH} for the carbamates of 2-(methylamino)benzamide (**1a–1c**) and 2-(methylamino)-*N*-methylbenzamide (**2a–2c**) has been plotted against the pK_a of the parent phenols. The slopes of the straight lines are −0.30 and −0.37, respectively.

Both steric and polar properties of the R_2 substituent appear to have an influence on the rate of cyclization. In Fig. 6 log $t_{1/2}$ (at pH 7.4)

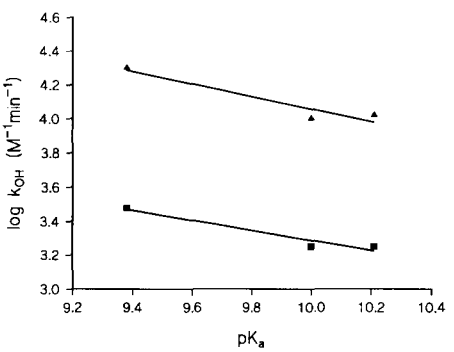


Fig. 5. Plots of log k_{OH} for the apparent specific base-catalyzed degradation of the carbamates of 2-(methylamino)-benzamide (**1a–1c**) (■) and 2-(methylamino)-*N*-methylbenzamide (**2a–2c**) (▲) against the pK_a value of the parent phenols.

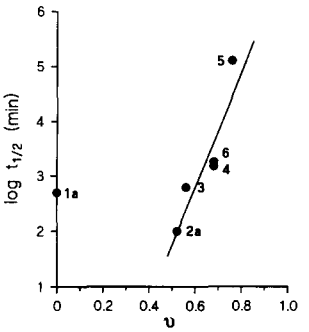


Fig. 6. Log $t_{1/2}$ (pH 7.4, 37°C) plotted against Charton's steric parameter (υ) for the R_2 substituents.

has been plotted against Charton's steric parameter (υ) (Charton, 1977) for compounds wherein $R_1 = CH_3$, $R_3 = H$ and R_2 is hydrogen or various alkyl groups. It can be seen that the difference in reactivity of the compounds, excluding compound **1a** where R_2 is hydrogen, can be correlated reasonably well with the difference in steric effects of the alkyl groups on the amide nitrogen. The regression equation between log $t_{1/2}$ and υ for the compounds is given by Eqn 6:

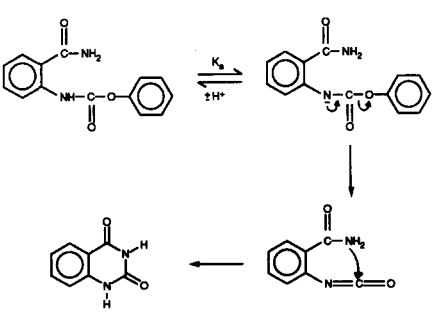
$$\log t_{1/2} = 10.6\upsilon - 3.54 \quad (n = 5;\ r = 0.909) \qquad (6)$$

Thus, the reactivity of these phenyl carbamates decreases very markedly with increasing steric effects within the R_2 substituent. This is readily understandable from the reaction mechanism in that the attack of the amide nitrogen on the carbamate moiety should be expected to be more difficult when bulky R_2 groups are attached to the nitrogen. It is, however, of interest to note that the unsubstituted amide (compound **1a**) falls greatly outside the plot in Fig. 6, showing a much lower reactivity than predicted. The large influence of the steric properties of the R_2 substituent on the rate of cyclization is also apparent by comparing the compounds **8** and **9**. The polar effects of these substituents are almost the same but the branched R_2 substituent in compound **9** makes this derivative 63-times less reactive than the unbranched compound **8**.

Polar effects within the R_2 substituent also influence the reaction rate. Thus, the introduction of a strongly electron-withdrawing amide or

ester group as in compounds **7** and **8** is seen to greatly accelerate the rate of cyclization. This is also apparent by comparing compounds **7** and **10**. The former is 15-times more reactive than compound **10** in which the polar ethyl ester group is placed more far away from the amide nitrogen than in compound **7**. According to the reaction mechanism depicted in Scheme 3 increased polar effects within the R_2 substituent should lower the pK_a value of the amide moiety and thus increase the concentration of the reacting amide anionic species at neutral pH. On the other hand, the increased acidity of the amide moiety should result in decreased nucleophilicity of the amide anion and hence a decreased k_1 value. Apparently, the former effect more than offsets the effect on k_1.

Replacement of a methyl group at the carbamate nitrogen (R_1) with an ethyl group has only a minor influence on the reaction rate. Thus, the compounds **11** and **12** containing an ethyl group are 1.2-times more stable than the analogous derivatives (**2a** and **8**) with R_1 = methyl. When R_1 is hydrogen, however, the cyclization is greatly accelerated. Thus, compound **13** is 54-fold more reactive than compound **1a** in which R_1 is methyl and R_2 and R_3 are hydrogen as in **13**. Compound **13** has previously been studied by Hegarty et al. (1974) and various pieces of evidence were provided for an elimination-addition (E1cB) mechanism involving a rate-determining formation of an isocyanate which subsequently is trapped by the *o*-amido group to yield a 2,4-quinazolinedione (Scheme 6). Such an isocyanate pathway is structurally impossible in the other phenyl carbamates where R_1 is methyl or ethyl.



Scheme 6.

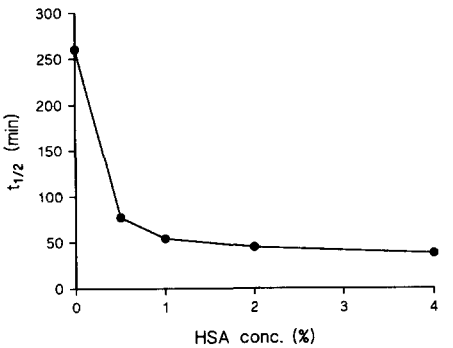


Fig. 7. Influence of human serum albumin (HSA) concentration on the rate of degradation of compound **1b** in aqueous solution (pH 7.4) at 37°C.

Stability in biological media

The stability of the phenyl carbamate esters was studied at 37°C in human plasma solutions as well as in rabbit liver or gut homogenates (all at pH 7.4) to examine the possible influence of these biological media on the degradation. In all cases the rates of degradation were found to follow good first-order kinetics and the parent phenol was released in stoichiometric amounts. For compound **2a** it was also found that the corresponding 2,4-quinazolinedione was formed in quantitative amounts. The observed half-lives of degradation (cyclization) in 40 or 80% plasma solutions are listed in Table 3. As can be seen from the rate data plasma showed a slight catalytic effect on the rate of cyclization of most compounds. For compounds **1a–1c**, however, the catalytic effect was more pronounced, amounting to a factor of 8–13 in 80% plasma solutions. These compounds differ from the other carbamates by having an unsubstituted benzamide group.

The rate-accelerating effect of human plasma does not appear to be due to enzymatic catalysis since heating the plasma to 80°C prior to the experiments had no significant influence on the rates of degradation. The effect may most likely be due to reversible binding to proteins, affording a facilitation of the cyclization reaction. It was thus found that human serum albumin (HSA) also accelerated the rate of reaction. When compound **1b** was incubated in a phosphate buffer containing 0.5–4% HSA (pH 7.4), the half-lives

TABLE 4

Half-lives of degradation of various phenyl carbamates in 0.02 M phosphate buffer solution (pH 7.4) and various biological media

Compound	$t_{1/2}$ (min)		
	pH 7.4 buffer	rabbit liver homogenate	rabbit gut homogenate (20%)
1a	485	450 [a]	
1b	260	40 [b]	
2a	120	170 [b]	140
8	10	15 [a]	11
13	9	9 [a]	

[a] 10% homogenate.
[b] 20% homogenate.

of degradation decreased with increasing concentration of HSA (Fig. 7).

Rabbit liver and gut homogenates showed no catalytic effect on the rate of degradation of compound **1a, 2a, 8** and **13** but some effect was seen for compound **1b** (Table 4). The carbamates **7** and **10** were rapidly degraded in the liver homogenates ($t_{1/2} < 1$ min) but this reaction was due to enzymatic hydrolysis of the ester groups in the R_2 substituent.

It is of interest to note that the degradation of the monosubstituted phenyl carbamate **13** was not catalyzed by liver homogenate and only to a minor extent by human plasma. In contrast to *N,N*-disubstituted phenyl carbamates monosubstituted phenyl carbamates are generally subject to pronounced enzymatic hydrolysis by liver or plasma enzymes (Hansen et al., 1991, 1992a). This lack of significant enzymatic hydrolysis may be due to the facility of the spontaneous intramolecular cyclization and/or to the steric hindrance exhibited by the *o*-amido group.

Conclusions

The results of this study show that derivatization of the phenolic group in the form of carbamates of *N*-substituted 2-aminobenzamides may be a promising prodrug approach to protect phenolic drugs against presystemic metabolism. The carbamates possess a sufficient stability in solutions of pH 1–6 to ensure minimal degradation during passage through the stomach and upper intestine but are degraded more facilely at physiological pH by an apparent hydroxide ion-catalyzed cyclization with release of the parent phenol. This process is not affected by plasma, liver or intestinal wall enzymes and, in most cases, to only a minor extent by protein binding phenomena. The latter effect may, however, be dependent on the structure of the phenolic drug being derivatized. The nature of the *N*-substituent in the benzamide moiety has a large influence on the rate of cyclization, the effect being largely due to steric and polar properties of the substituent. By appropriate selection of this substituent it is readily feasible to obtain prodrug derivatives having practically useful rates of conversion to the parent phenols at pH 7.4 and 37°C, i.e. the half-lives being in the order of 10–60 min.

A monosubstituted phenyl carbamate like compound **13** can also be designed to possess an useful rate of cyclization but such carbamates are considered to be less favourable prodrug candidates than the *N,N*-disubstituted carbamates. In contrast to the latter monosubstituted phenyl carbamates appear to undergo cyclization via an isocyanate intermediate and it may be envisaged that this reactive species may react covalently with nucleophilic groups in tissue components, e.g., proteins, at the expence of being trapped by the *o*-amido group in the molecule.

The pro-moieties released by the cyclization of the phenyl carbamates are 2,4-quinazolinediones and an evaluation of the possible biological effects of these compounds is evidently a necessary step in the further assessment of the potential utility of the prodrug concept described.

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