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Pro-drugs as drug delivery systems XXI. Preparation, physicochemical properties and bioavailability of a novel water-soluble pro-drug type for carbamazepine ***

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Summary

Various N-Mannich bases of carbamazepine with piperidine, diethylamine or dipropylamine as the amine component were prepared and evaluated as water-soluble pro-drugs. The hydrolysis, yielding carbamazepine, amine and formaldehyde in stoichiometric amounts, showed sigmoidal pH–rate profiles with maximum hydrolysis rates at pH > 9. At pH 7.40 and 37°C the half-lives of decomposition were 7, 19 and 165 min for the dipropylamine, diethylamine and piperidine derivative, respectively. The solubility of the hydrochloride salt of the dipropylamine Mannich base was found to exceed 50% w/v, i.e. more than 10^4-fold greater solubility than the parent drug. Following intramuscular administration in rats, higher and more rapidly appearing carbamazepine plasma levels were observed from aqueous solutions of the dipropylamino N-Mannich base pro-drug than from administering a suspension of the parent drug. In a similar comparative oral study, however, diminished carbamazepine plasma levels were observed from the pro-drug.

*** Part XX of this series: Bundgaard, H. and Johansen, M., Oxazolidines as potential pro-drug types for β-aminoalcohols, aldehydes or ketones. Int. J. Pharm., in press.

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Introduction

Previous studies explored the concept of N-aminomethylation of various carboxamides, thioamides, sulfonamides, imides and various other NH-acidic compounds as a potentially useful means of obtaining pro-drug forms of such not easily derivatizable chemical entities (Bundgaard and Johansen, 1980a and b; 1981b and c; Johansen and Bungaard, 1980a and b). Such N-Mannich bases were shown to decompose quantitatively to the parent compounds (for an amide Mannich base, see Scheme 1) in aqueous solution at rates determined by pH of the medium and by various structural factors.

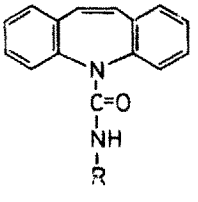
Scheme 1

$$R\text{-}CONHCH_2NR_1R_2 + H_2O \rightarrow R - CONH_2 + CH_2O + R_1R_2NH$$

Transformation of an amide-type compound into an N-Mannich base introduces an ionizable amine moiety which may allow the preparation of derivatives with improved aqueous solubility characteristics. Recently, it was shown that N-Mannich bases of various NH-acidic drug substances (phenytoin, chlorzoxazone, barbituric acids, acetazolamide, chlorothiazide, hydrochlorothiazide and allopurinol) possess markedly greater solubilities and dissolution rates in acidic aqueous solutions in comparison with the parent drugs and it was suggested that the derivatives might be of potential usefulness as pro-drugs for improving the oral bioavailability of these drugs of poor aqueous solubility (Bundgaard and Johansen, 1980c, 1981a).

The purpose of this investigation was to determine the feasibility of using this pro-drug approach for improving the water-solubility within the physiological pH range and hence the delivery characteristics of carbamazepine (I). This widely used anti-epileptic drug is marginally soluble in water or diluted hydrochloric acid (0.11 $mg \cdot ml^{-1}$) and, as expected, its absorption upon oral administration has been shown to be dissolution-rate limited (Levy et al., 1975b; Bertilsson, 1978; Frey and Löscher, 1980). Parenteral administration of the drug is seriously hampered by its low aqueous solubility and preparations used for such administration have contained large concentrations of co-solvents such as propylene glycol and ethanol (Levy et al., 1975a).

In this paper the synthesis and physicochemical properties including kinetics of decomposition of highly water-soluble N-Mannich bases (II–IV) of carbamazepine are described along with the results of a comparative study of the disposition of carbamazepine from the pro-drug II and the parent drug in the rat following oral and intramuscular administration.



I R = H

II R = $-CH_2-N(CH_2CH_2CH_3)(CH_2CH_2CH_3)$

III R = $-CH_2-N(CH_2CH_3)(CH_2CH_3)$

IV R = $-CH_2-N$ 

Materials and methods

Apparatus

Ultraviolet and visible spectral measurements were performed with a Zeiss PMQ II spectrophotometer and a Perkin-Elmer 124 recording spectrophotometer, using 1-cm cuvettes. PMR-spectra were run on a JEOL C-60-HL instrument using tetramethylsilane as an internal standard. Readings of pH were carried out on a Radiometer type PMH26 meter at the temperature of study. Microanalysis was carried out at the Microanalytical Department of Chemical Laboratory II, University of Copenhagen. The results were within ±0.4% of the theoretical values. Melting points were taken on a capillary melting-point apparatus and are uncorrected. Thin-layer chromatography (TLC) was done on precoated 0.25 mm silica gel 60 F_{254} glass plates (E. Merck, F.R.G.). High-performance liquid chromatography (HPLC) was done with an Altex liquid chromatograph equipped with a 280 nm detector, a Rheodyne 20 μl loop injector and a Chrompack column packed with a stationary phase of LiChrosorb 10RP18 (250 mm × 4.6 mm i.d.; particle size 10 μm). A small pre-column packed with RP18 column material was placed in front of the main column.

Preparation of N-Mannich bases of carbamazepine

The N-Mannich bases II–IV of carbamazepine were prepared by reacting the parent compound with paraformaldehyde and amine in ethyl acetate. The appropriate amine (piperidine, dipropylamine and diethylamine) (0.04 mol) and paraformaldehyde (0.04 mol) were added to a solution of carbamazepine (0.02 mol) in 100 ml of ethyl acetate and the solution was refluxed for 5–6 days. At this time almost all carbamazepine had reacted as determined by TLC (silica gel; tetrachloromethane–methanol 3:2). The solution was evaporated in vacuo and the residue was taken up in 30 ml of 2 M hydrochloric acid. Some undissolved residue (unreacted carbamazepine) was filtered off and the filtrate was shaken with 50 ml of ethyl acetate and 32 ml of 2 M sodium hydroxide. After separation of the phases the ethyl acetate layer was removed and the aqueous layer further extracted with 2 × 30 ml of ethyl acetate. The combined ethyl acetate extracts were dried with anhydrous sodium sulphate and evaporated under reduced pressure to give an oil which crystallized by standing overnight at 4°C. The yields obtained of the N-Mannich bases after recrystallization from ether–petroleum ether were within 60–80%. To prepare the hydrochloride salts, the Mannich bases (6 mmol) were dissolved in 15 ml of toluene, and 4 ml of 2.6 M HCl in methanol were added. The solutions were evaporated in vacuo and the solid residues were recrystallized from ethyl acetate.

The structures of the compounds were confirmed by PMR (in DMSO-d_6), UV and elemental analysis (C, H and N), as well as by molecular weight determination. The latter was performed by measuring the amount of formaldehyde released upon hydrolysis in alkaline solution as previously described (Johansen and Bundgaard, 1979). For all derivatives the molecular weights determined agreed within ±3% with the calculated values—m.p.: II, free base, 108–110°C; II, hydrochloride salt, 156–158°C dec.; III, hydrochloride salt, 183–185°C dec.; IV, free base, 114–116°C.

Kinetic measurements

All rate studies were performed in aqueous buffer solutions at 20 and 37 ± 0.1°C. Acetate, phosphate, borate and carbonate were used as buffers; the total buffer concentration was generally 0.1 M and a constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride.

The reactions were monitored by measuring the amount of formaldehyde released during decomposition of the N-Mannich bases using a previously described modification (Johansen and Bundgaard, 1979) of the colorimetric method of Sawicki et al. (1961). The compounds were dissolved in the buffer solutions to give a concentration of about 8×10^{-4} M. The solutions were kept in a water bath of constant temperature and at appropriate intervals 1000-μl samples were withdrawn and diluted to 10.00 ml with water. A 500-μl sample of the dilution was then analyzed for formaldehyde as described previously (Johansen and Bundgaard, 1979). Pseudo-first-order rate constants were calculated from the slopes of linear plots of log $(A_\infty - A_t)$ against time, where A_∞ and A_t are the absorbance readings (at 625 nm) at infinity and at time t, respectively.

Reactions in 0.05 M phosphate buffer, pH 7.40, containing 25% human plasma were performed and monitored in the same manner as reactions in pure aqueous solutions.

Solubility determinations

The solubility of the hydrochloride salt of the N-Mannich base II in water at 20°C was estimated by placing 50-mg fractions of the salt in 1 ml of water until a saturated solution was obtained.

Measurements of dissolution rates

Intrinsic dissolution rates were measured using the rotating disc method of Nogami et al. (1966). Discs of 100 mg and 11.3 mm diameter were used, the dissolution medium was 500 ml of 0.1 M hydrochloric acid, the temperature 24°C and the rotation velocity of the disc holder 200 rpm. Analysis of amount dissolved was carried out UV-spectrophotometrically at 285 nm.

Animal experiments

Five male Sprague–Dawley rats fasted overnight were used in a cross-over study to compare the plasma levels obtained after oral administration via an intubation tube of 100 mg/kg carbamazepine equivalent, of either carbamazepine (suspended) or II (dissolved) in 2% methylcellulose. After drug administration, ~200 μl blood samples from a tail clip were collected at 1/4, 1/2, 1, 2, 4, 6 and 8 h post-dosing and the plasma fraction assayed for carbamazepine. A one-week period was allowed for the cross-over.

In the intramuscular dosing study, 5 rats received either 100 mg/kg carbamazepine suspended in 2% methylcellulose (~100 μl) or a molar equivalent of II dissolved in water in the right hind leg. Blood samples were removed at the same times as for the oral study, and the plasma fraction assayed for carbamazepine.

Plasma assay

The plasma assay was similar to the method of Astier et al. (1979). Whole blood samples collected in vacutainers pretreated with EDTA were centrifuged and 50 μl of plasma deproteinized with 50 μl of acetonitrile containing 8 μg/ml of clonazepam (Hoffman-LaRoche, Nutley, NJ). After centrifugation, 20 μl of the clear supernatant was injected on HPLC which used a mobile phase of 40:60 v/v acetonitrile/water. A standard, clonazepam, was prepared daily from fresh rat plasma spiked with known quantities of carbamazepine and treated as above.

Results and Discussion

Kinetics and mechanism of decomposition

The kinetics of breakdown of the N-Mannich bases II–IV of carbamazepine was studied in aqueous solution at 37°C over the pH range 3–11. Under the experimental conditions used all reactions proceeded to completion as evidenced by the formation of formaldehyde in stoichiometric amounts. At constant pH and temperature the reactions displayed strict first-order kinetics over more than 4 half-lives.

The rates of decomposition were found to be independent of buffer concentration from 0.02 to 0.15 M. Such lack of significant general acid-base catalysis was also noticed for the decomposition of N-Mannich bases of various amides and imides (Bundgaard and Johansen, 1980a and b, 1981b; Johansen and Bundgaard, 1980b).

The influence of pH on the degradation rate for the N-Mannich bases is shown in Fig. 1, where the logarithms of the observed apparent first-order rate constants (k_{obs}) are plotted against pH. The sigmoidal shape of the pH–rate profiles is similar to that previously observed for the decomposition of N-Mannich bases of various carboxamides, thioamides, sulphonamides, imides and ureides with primary or secondary amines (Bundgaard and Johansen, 1980a and b, 1981b), and the dependence of k_{obs} on pH can be accounted for by assuming spontaneous decomposition of the free Mannich bases (B) and their conjugate acids (BH^+):

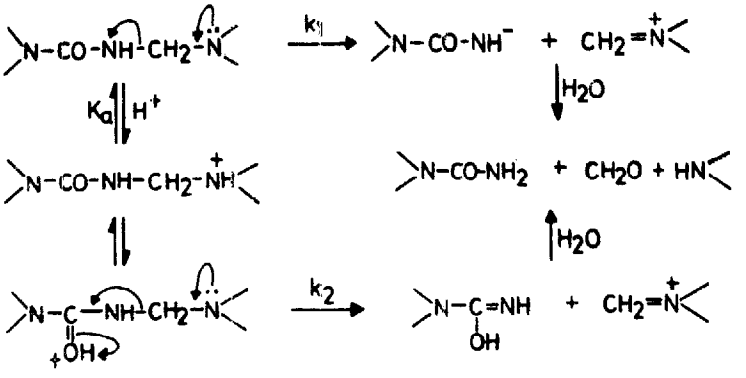
$$k_{obs} = \frac{k_1 K_a}{a_H + K_a} + \frac{k_2 a_H}{a_H + K_a} \quad (1)$$

where K_a is the apparent ionization constant of the protonated N-Mannich base, a_H is the hydrogen ion activity, and k_1 and k_2 are the apparent first-order rate constants for the spontaneous degradation of B and BH^+, respectively.

The rate constants and ionization constants were derived from the pH–rate profiles as previously described (Bundgaard and Johansen, 1980b) and the values are listed in Table I. The solid curves in Fig. 1 were constructed from Eqn. 1 and these values and the good fit observed demonstrates that Eqn. 1 adequately describes the degradation kinetics.

The reaction mechanism previously proposed for the decomposition of N-Mannich bases of various amides and imides (Bundgaard and Johansen, 1980a and b, 1981b) may also be valid for the N-Mannich bases of carbamazepine. As shown in Scheme 2

SCHEME 2



the mechanism involves as rate-determining step a unimolecular N–C bond cleavage with formation of carbamazepine and an immonium cation. The latter takes up a hydroxide ion from a solvent molecule, giving methylolamine, which rapidly dissociates to formaldehyde and amine. It should be noted that a mechanism involving the formation of N-hydroxymethylated carbamazepine as an intermediate can be ruled out because this compound (prepared by reacting carbamazepine with paraformaldehyde in absence of amine) degraded to parent drug and formaldehyde much slower than the N-Mannich bases.

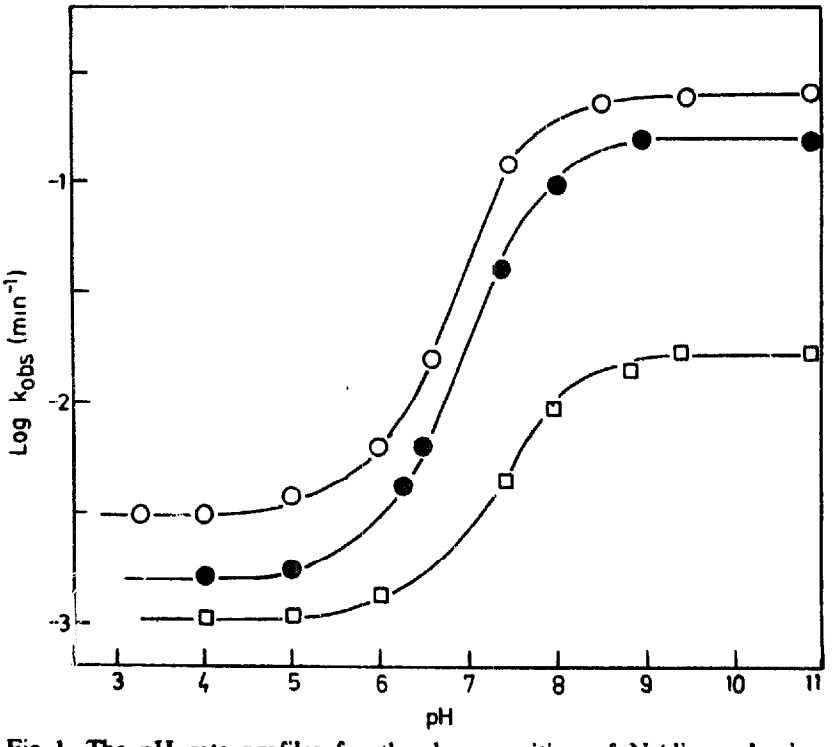


Fig. 1. The pH–rate profiles for the decomposition of N-(dipropylaminomethyl)carbamazepine (○), N-(diethylaminomethyl)carbamazepine (●) and N-(piperidinomethyl)carbamazepine (□) in aqueous solution at 37°C.

TABLE I

IONIZATION CONSTANTS AND RATE DATA FOR THE DECOMPOSITION OF VARIOUS N-MANNICH BASES OF CARBAMAZEPINE IN AQUEOUS SOLUTION (μ=0.5) AT 37°C

Compound	pK_a	k_1 (min^{-1})	k_2 (min^{-1})	$t_{1/2}$[a] (min)
N-(diethylaminomethyl)carbamazepine (I)	7.95	0.17	0.0017	19
N-(dipropylaminomethyl)carbamazepine (II)	7.75	0.27	0.0030	7
N-(piperidinomethyl)carbamazepine (III)	7.90	0.015	0.0011	165

[a] At pH 7.40

The pH–rate profiles for the decomposition of the N-piperidinomethyl derivatives of N-methylurea and N-phenylurea were previously found to be bell-shaped and markedly different from those of amide Mannich bases (Bundgaard and Johansen, 1980b). The similarity of the pH–rate profiles for the Mannich bases of carbamazepine (an N,N-substituted urea) to those of amide and imide Mannich bases may indicate that the behaviour of urea Mannich bases depends on the degree of substitution of H-atoms at the N-atom not aminomethylated. This question is currently being investigated with various substituted urea derivatives.

The structural effects on the decomposition rate of N-Mannich bases derived from various amides and imides have previously been shown to involve steric effects and basicity of the amine component and acidity of the amide-type component (Bundgaard and Johansen, 1980a and b, 1981b and c). The rates of the reactions of unprotonated Mannich bases, and accordingly the decomposition rates in weakly acidic to basic solutions, were accelerated strongly by increasing acidity of the parent amide-type compound and by increasing steric effects and basicity of the amine component. The present results for carbamazepine Mannich bases are fully compatible with these relationships. The pK_as of piperidine, diethylamine and dipropylamine are of the same size and the different reactivity of the Mannich bases (cf. k_1 in Table I) can be accounted for in terms of different steric effects within the amines (cf. Bundgaard and Johansen, 1980b). For benzamide Mannich bases the ratio of k_1 for the diethylamino and the piperidino derivative was found to be 10 (Bundgaard and Johansen, 1980b) and the corresponding ratio for the carbamazepine derivatives (11) is seen to be quite similar.

The reactivity of piperidine N-Mannich bases of a number of various carboxamides, a thioamide and a sulfonamide has been shown to be correlated ($r = 0.995$, $n = 9$) with the pK_a values of the Mannich bases by the following expression (Bundgaard and Johansen, 1980b):

$$\log k_1 = -2.30\, pK_a + 16.4 \quad (k_1 \text{ in } min^{-1};\ 37°C) \qquad (2)$$

This highly significant correlation can now be seen to encompass a urea derivative such as carbamazepine. The pK_a of the carbamazepine Mannich base with piperidine is 7.90 and according to Eqn. 2, a k_1 value of 0.017 min^{-1} is predicted which is quite close to the value obtained experimentally (0.015 min^{-1}).

The correlation of the rate constants k_1 for piperidine Mannich bases of various amides with the acidity of the parent amides was expressed by the following regression equation ($r = 0.995$, $n = 5$) (Bundgaard and Johansen, 1980b):

$$\log k_1 = -1.42pK_a + 19.3 \quad (3)$$

where pK_a refers to the parent amides. The pK_a for carbamazepine is not known but in view of the excellent fit of the rate data for its piperidino Mannich base to those for amide derivatives it is tempting to use Eqn. 3 for the calculation of a pK_a value. Using the k_1 value found to be 0.015 min^{-1}, Eqn. 3 predicts a pK_a value for carbamazepine of 14.9 which appears to be reasonable.

Based on the rate data obtained the dipropylamino derivative (II) appeared to be the most promising candidate as a water-soluble pro-drug of carbamazepine. As seen from Table I the half-life of decomposition of II at pH 7.4 and 37°C is 7 min. In accord with the results of a previous study on the decomposition of amide Mannich bases in neutral aqueous solutions containing human plasma (Johansen and Bundgaard, 1981), the rate of breakdown of the derivative II in a phosphate buffer solution (pH 7.4) containing 25% plasma ($k_{obs} = 0.095$ min^{-1}) was found to be the same as that in pure buffer solution ($k_{obs} = 0.010$ min^{-1}).

Solubility and stability of compound II

The hydrochloride salt of the dipropylamino Mannich base II displayed a high aqueous solubility. The solubility in water was found to exceed 50% w/v or 1.3 M at 20°C, the pH of a 10% w/v solution being 4.8. The solubility of carbamazepine under similar conditions was determined to be 0.011% w/v or 4.7×10^{-4} M which means that the increase in water-solubility of the pro-drug salt over carbamazepine is a factor of at least 2.8×10^3 in terms of carbamazepine equivalents.

The rate of decomposition of II was followed at 20°C at pH 4 and 5 to stimulate storage conditions. For 8×10^{-4} M aqueous solutions the following rate data were obtained:

pH 4.00: $k_{obs} = 2.7 \times 10^{-4}$ min^{-1}, $t_{1/2} = 42.5$ h

pH 5.00: $k_{obs} = 2.9 \times 10^{-4}$ min^{-1}, $t_{1/2} = 40.0$ h

It should be stressed, however, that the determining factor for the stability of an aqueous solution of the pro-drug at concentrations of, for example, 10% w/v will not be the loss of the pro-drug per se but rather the gradual formation of carbamazepine and its subsequent precipitation as the saturation solubility of the drug is reached. The formation of a saturated solution of carbamazepine will be a function of the initial concentration of the pro-drug so the initial rate of formation of carbamazepine (C) would be:

$$\left(\frac{d[C]}{dt}\right)_i = k[II]_0 \quad (4)$$

TABLE 2

APPARENT ZERO-ORDER INITIAL RATES OF FORMATION OF CARBAMAZEPINE (C) FROM ITS PRO-DRUG II AND TIMES FOR C TO BEGIN PRECIPITATING (t_{pt}) FROM SOLUTIONS OF II AS CALCULATED FROM EQN. 4 (AT 20°C)

Concentration of II, hydrochloride salt ($mg \cdot ml^{-1}$)	$(d[C]/dt)_i$ ($mg \cdot ml^{-1} \cdot min^{-1}$)	t_{pt} (min)
25	0.0043	26
50	0.0086	13
100	0.0162	7

where k is the observed pseudo-first-order rate constant for the decomposition of II to carbamazepine under the designated experimental conditions. At pH 4–5, corresponding to the pH range for 1–20% aqueous solutions of the hydrochloride salt of II, and 20°C, k is equal to 2.8×10^{-4} min^{-1}. The time for carbamazepine to potentially begin nucleating from solutions of II hydrochloride salt (t_{pt}) can be calculated from this equation, knowing that the solubility of carbamazepine is 0.11 $mg \cdot ml^{-1}$ at 20°C. Table II gives the calculated zero-order rates of formation of carbamazepine as a function of the initial pro-drug concentration at 20°C. The results indicate that solutions of II have quite short utilization times. However, it was repeatedly found experimentally that the time required for carbamazepine precipitation to occur was much higher (more than 5–6 h) than the predicted figures. This discrepancy may possibly be due to the occurrence of supersaturation. A similar lack of correlation between theoretical and experimental precipitation times when water-soluble pro-drugs degrade to relatively insoluble parent drugs has been described by Stella et al. (1975) for phenytoin pro-drugs.

Dissolution rates

The intrinsic dissolution rate of the hydrochloride salt of II was determined in 0.1 M hydrochloric acid and compared to that of carbamazepine. The initial rates of dissolution followed apparent zero-order kinetics in accordance with the experimental conditions of constant dissolution surface area and apparent sink conditions. The rates were calculated from the slopes of amount dissolved versus time plots divided by the surface area of the compressed disc and the following values were obtained (at 24°C): 1.6×10^{-7} $mol \cdot cm^{-2} \cdot min^{-1}$ for carbamazepine and 4.6×10^{-5} $mol \cdot cm^{-2} \cdot min^{-1}$ for the hydrochloride salt of II. As expected on basis of the solubility data, the N-Mannich base in salt form possesses greatly increased dissolution rate as compared with the neutral parent drug. Similar dissolution rate enhancements in acidic solutions have been observed for various other N-Mannich bases, both in free base form and as salts, in comparison with the parent drugs (Bundgaard and Johansen, 1980c, 1981a).

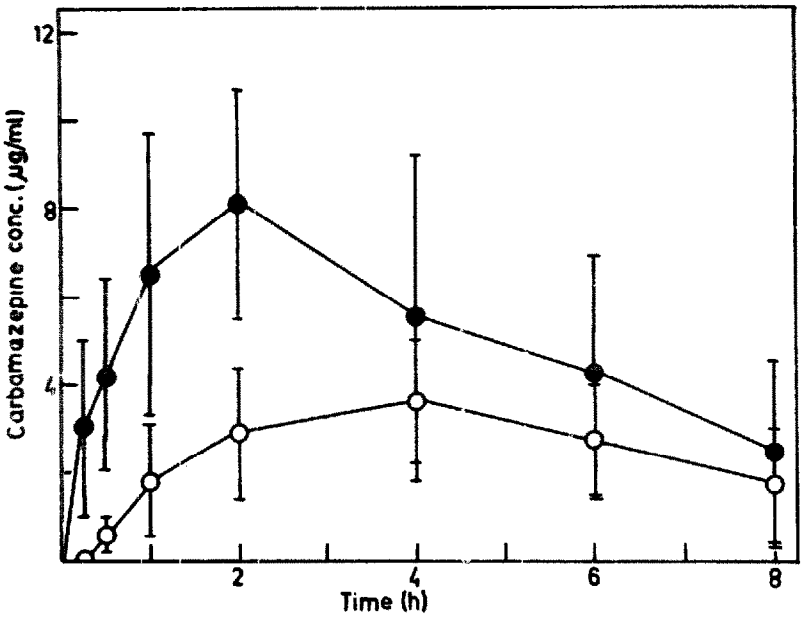


Fig. 2. Mean carbamazepine plasma levels (±S.D.) versus time plots obtained after oral administration of carbamazepine (●) and II (○) to rats at a dose of 100 mg/kg carbamazepine equivalents.

Animal studies

To test the hypothesis that II should be a suitable water-soluble pro-drug of carbamazepine for oral and parenteral (intramuscular) administration, carbamazepine and II were administered to rats. Fig. 2 shows the mean carbamazepine plasma levels obtained in 5 rats from the oral cross-over study. It can be seen that superior carbamazepine levels were obtained from carbamazepine rather than from the more water-soluble pro-drug, II. Fig. 3 shows the mean carbamazepine plasma levels

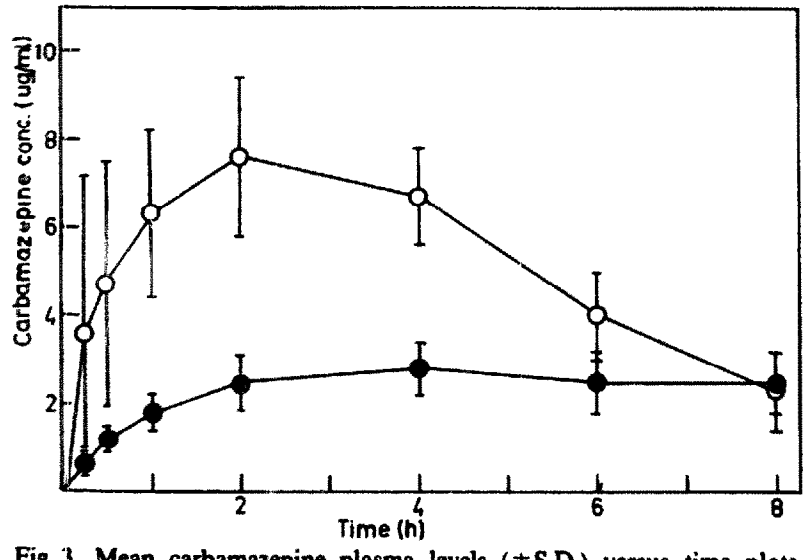


Fig. 3. Mean carbamazepine plasma levels (±S.D.) versus time plots obtained after intramuscular administration of carbamazepine (●) and II (○) to rats at a dose of 100 mg/kg carbamazepine equivalents.

obtained for the intramuscular study. The more rapid appearance of plasma carbamazepine from II compared to the carbamazepine suspension can be readily seen.

In the oral study, the peaking of carbamazepine levels at 2 h from the carbamazepine suspension showed that the carbamazepine absorption was reasonably slow. This suggested that the dissolution step was contributing to the rate of appearance of the drug in systemic circulation. The diminished carbamazepine levels from II are difficult to explain. Two possible explanations are: (1) the polarity of II (pK_a 7.75) may limit its ability to be absorbed because it will be largely ionized in the upper gastrointestinal tract; or (2) II undergoes pre-systemic clearance to a metabolite other than carbamazepine. For example, N-depropylation of II by oxidative enzymes would give a Mannich base which would then degrade to carbamazepine more slowly than II (Bundgaard and Johansen, 1980b). This would allow time for other metabolic processes such as epoxidation of the carbamazepine nucleus to occur preventing the appearance of carbamazepine in the plasma. Alternatively, II may be a better substrate for epoxidation than carbamazepine so preventing carbamazepine being formed from II, i.e. breakdown of II results in the formation of the epoxide or other metabolites rather than carbamazepine.

The delayed and prolonged appearance of carbamazepine from the intramuscular suspension of carbamazepine was consistent with dissolution of carbamazepine from the injection site being slow. The more rapid appearance of carbamazepine from II resulted from its more rapid clearance from the injection site and subsequent conversion to carbamazepine.

In conclusion, it appears from the physicochemical studies and animal experiments that Mannich bases such as II may be suitable as parenteral pro-drug forms of amide-type compounds such as carbamazepine. More in vivo studies on oral dosing of N-Mannich bases are needed to decipher the cause of the diminished availability of the parent drug, carbamazepine, from the pro-drug Mannich base, II, seen in rats in the present study. Of particular importance is whether the diminished availability seen here is unique to carbamazepine.

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