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Design of a water-soluble, solution-stable and biolabile prodrug of metronidazole for parenteral administration: *N*-substituted aminomethylbenzoate esters

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Summary

Various *N*-substituted aminomethylbenzoate esters of metronidazole were synthesized and evaluated as water-soluble prodrugs with the aim of developing preparations suitable for intravenous injection. The esters showed all a high solubility in weakly acidic solutions and were further characterized by possessing a high stability in such solutions allowing long-term storage combined with a high susceptibility to undergo enzymatic hydrolysis in plasma. Half-lives of hydrolysis in 80% human plasma were found to be less than 1 min for some esters. These compounds (the 4-(morpholinomethyl)benzoate and 3-[(4-methylpiperazin-1-yl)methyl]benzoate esters) were predicted to possess shelf-lives of more than 10 years in aqueous solution of pH 4.0 and at 25°C. These properties regarding solubility, chemical stability and enzymatic lability make *N*-substituted aminomethylbenzoate esters a promising new prodrug type for metronidazole as well as for other slightly soluble drugs containing an esterifiable hydroxyl group.

Introduction

Metronidazole (2-methyl-5-nitroimidazole-1-ethanol) (I) is a widely used drug for the prevention and treatment of infections caused by anaerobic bacteria. Although the drug is usually administered orally, intravenous infusion providing rapid onset of action is often required. Parenteral dosage forms for a simple injection are not available, presumably because of the relatively low solubility of metronidazole in water (~ 1% w/v at 25 °C). The intravenous administration of the drug is presently performed in the form of infusion, using 0.5% w/v aqueous solutions. To meet the required doses it is usually necessary to give 100–200 ml of such solutions every 8 h.

In recent years, the prodrug approach has been explored to overcome the solubility problem in formulating a parenteral solution of metronidazole to be administered by a single injection. An ideal prodrug should possess the following properties: it should be readily soluble (> 5%) in water at physiologically acceptable pH values, be sufficiently stable in aqueous solution to allow long-term storage (> 2 years) of ready-to-use solutions and yet it should be converted quantitatively and rapidly in vivo to the active parent drug. The alcoholic functional group in metronidazole is readily esterifiable and several types of water-soluble ester pro-

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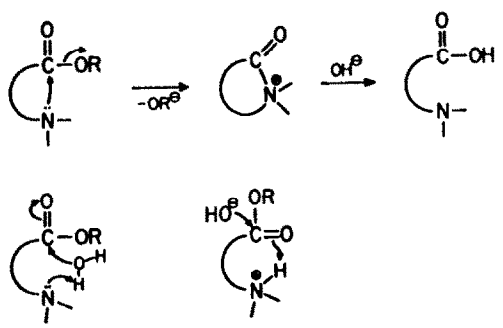
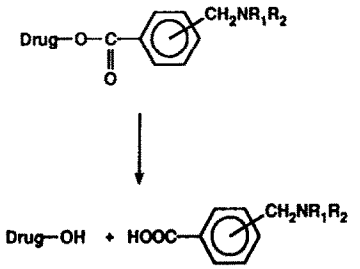
drugs of metronidazole have been developed (Bundgaard, 1985). However, none of these derivatives appear to fully satisfy the requirements listed above. Thus, dicarboxylic acid hemiesters such as hemisuccinates and hemiglutarates have limited stability in aqueous solution and are only slowly and incompletely converted in vivo to metronidazole (Bundgaard et al., 1984a; Larsen et al., 1988). A phosphate ester of metronidazole is more chemically stable but appears not to be rapidly and quantitatively converted to the parent drug in vivo (Cho et al., 1982). A third type of water-soluble metronidazole esters which have been described is α-amino acid esters or related short-chained aliphatic amino acid esters (Bundgaard et al., 1984a, b; Cho and Haynes, 1985). These esters are in general readily hydrolyzed by plasma enzymes but they exhibit a very poor stability in aqueous solution, making it impossible to prepare ready-to-use solutions.

The major reason for the high instability of such amino acid esters in aqueous solution at pH values affording their favourable water-solubility (i.e., pH 3–5) is partly due to the strongly electron-withdrawing effect of the protonated amino group which activates the ester linkage towards hydroxide ion attack and partly (and predominantly) to intramolecular catalysis or assistance by the neighbouring amino group of ester hydrolysis (Bruice and Benkovic, 1966; Kirby and Lloyd, 1976; Bundgaard et al., 1984b). The mechanisms involved include intramolecular nucleophilic catalysis, intramolecular general-base catalysis, or general-acid specific base catalysis (Bruice and Benkovic, 1966) as depicted in Scheme 1.



Scheme 1.



Scheme 2.

We have now found that a most effective and simple approach to totally block the hydrolysis facilitating effect of the amino group and yet retain a rapid rate of enzymatic ester hydrolysis is to incorporate a phenyl group between the ester moiety and the amino group. By doing so the intramolecular catalytic reactions of the amino group as outlined in Scheme 1 are no longer

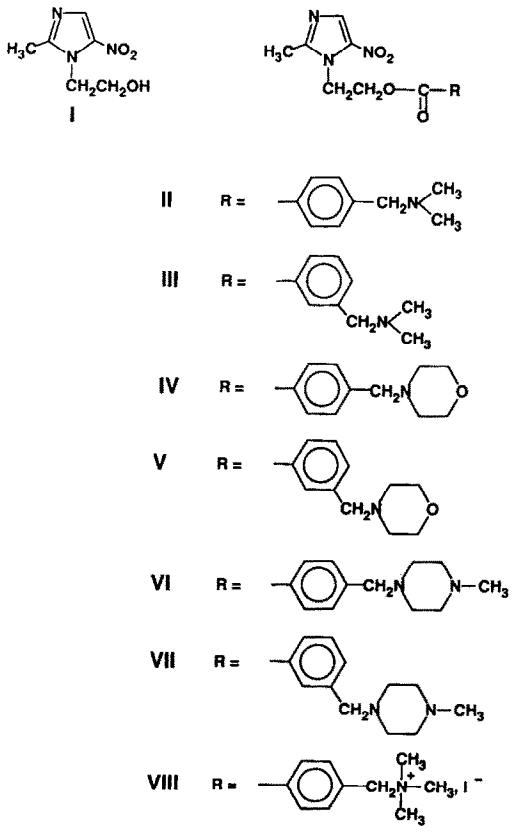


Fig. 1. Formula I–VIII.

possible for steric reasons and furthermore, the ester-labilizing effect of the protonated amino group due to its polar character is greatly diminished. Because of the requirement of a pK_a value greater than 5–6 for the amino group (for solubility reasons) the group is not directly attached to the phenyl nucleus (this will give an aromatic amine with a low pK_a) but separated from this by an alkylene group, in the most simple case a methylene group (Scheme 2).

In this paper we report that such *N*-substituted 3- or 4-aminomethylbenzoate esters of metronidazole (Fig. 1, **II–VIII**) may be promising prodrugs for parenteral administration due to their high solubility and chemical stability in aqueous solution combined with a high susceptibility to undergo enzymatic hydrolysis in plasma. Part of the present work has been described in a preliminary paper (Bundgaard et al., 1989).

Materials and Methods

Apparatus

High-performance liquid chromatography (HPLC) was done with a Kontron apparatus consisting of a LC Pump T-414, a Unikon 740 LC UV detector and a 20-μl loop injection valve. A deactivated reversed-phase Supelcosil LC-8-DB column (33 × 4.6 mm) (3-μm particles) equipped with a Supelguard column (purchased from Supelco Inc., U.S.A.) was used. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. ^{1}H NMR spec-

TABLE 1

Physical and analytical data of various esters of metronidazole

Compound	Form	M.p. (°C)	Formula	Analysis (%)	
				Calculated	Found
II	Free base	73– 74	$C_{16}H_{20}N_4O_4$	C 57.82	57.73
				H 6.07	6.10
				N 16.86	16.75
III	Fumarate (1.5 equiv.)	141–143	$C_{22}H_{26}N_4O_{10}$, 0.25 H_2O	C 51.71	51.64
				H 5.22	5.26
				N 10.96	10.94
IV	Free base	98– 99	$C_{18}H_{22}N_4O_5$	C 57.74	57.93
				H 5.92	5.96
				N 14.96	14.88
	Fumarate (1 equiv.)	144–146	$C_{22}H_{26}N_4O_9$	C 53.87	53.57
				H 5.34	5.51
				N 11.42	11.21
V	Fumarate (2 equiv.)	169–170	$C_{26}H_{30}N_4O_{13}$	C 51.49	51.52
				H 4.99	4.92
				N 9.24	9.24
VI	Fumarate (2 equiv.)	193–194	$C_{27}H_{33}N_5O_{12}$	C 52.34	52.10
				H 5.37	5.57
				N 11.30	11.12
VII	Fumarate (2 equiv.)	179–182	$C_{27}H_{33}N_5O_{12}$, 0.5 H_2O	C 51.59	51.56
				H 5.45	5.60
				N 11.14	11.15
VIII	Iodide	203–206	$C_{17}H_{23}IN_4O_4$	C 43.05	43.01
				H 4.89	4.98
				N 11.81	11.78

tra were run on a Varian 360L instrument. Melting points were taken in capillary tubes and are not corrected. Elemental analyses were performed by G. Cornali, Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Synthesis of metronidazole esters

The *N*-substituted 3- or 4-aminomethylbenzoate esters **II–VIII** were prepared by esterifying metronidazole with 3- or 4-chloromethylbenzoyl chloride and subsequent reaction of the chloromethylbenzoate esters obtained with the appropriate amine in the presence of catalytic amounts of sodium iodide.

Physical and analytical data for the esters **II–VIII** are given in Table 1. The NMR spectra of the compounds were consistent with their structures.

Metronidazole 3-chloromethylbenzoate

A solution of 3-chloromethylbenzoyl chloride (from Fluka AG, Switzerland) (1.42 ml, 10 mmol) in methylene chloride (30 ml) was added dropwise to a mixture of metronidazole (1.71 g, 10 mmol), pyridine (1 ml) and methylene chloride (40 ml). The resulting clear solution was stirred at room temperature for 20 h and evaporated in vacuo. The residue was stirred with 1 M sodium carbonate solution (15 ml) for 15 min and the resulting solid was filtered off and washed with water to give 2.76 g (85%) of the title compound. After recrystallization from ethyl acetate-petroleum ether the compound melted at 78–80 °C.

Analysis: Calculated for $C_{14}H_{14}ClN_3O_4$: C, 51.94; H, 4.36; Cl, 10.95; N, 12.98; Found: C, 51.73; H, 4.38; Cl, 11.14; N, 13.06.

Metronidazole 4-chloromethylbenzoate

The compound was prepared by a method analogous to that described above, starting from 2.57 g (15 mmol) of metronidazole and 4-chloromethylbenzoyl chloride (15 mmol). The latter was prepared by refluxing 4-chloromethylbenzoic acid (from Fluka AG, Switzerland) with an excess of thionyl chloride for 1.5 h. The crude ester was purified by flash chromatography on silica gel (eluent: toluene containing ethyl acetate). Recrystallization from cyclohexane afforded 3.9 g (62%) of the title compound (m.p. 112–115 °C).

Analysis: Calculated for $C_{14}H_{14}ClN_3O_4$: C, 51.94; H, 4.36; Cl, 10.95; N, 12.98; Found: C, 51.77; H, 4.38; Cl, 11.25; N, 13.13.

Metronidazole 4-(dimethylaminomethyl)benzoate (II)

To a solution of metronidazole 4-chloromethylbenzoate (456 mg, 1.5 mmol) in acetone (20 ml) were added dimethylamine (0.82 ml of a 33% solution in ethanol, 6 mmol) and sodium iodide (20 mg). The mixture was stirred at room temperature for 20 h, filtered and evaporated in vacuo. Water (10 ml) was added to the residue and the mixture was extracted with methylene chloride (3×20 ml). The combined extracts were dried over anhydrous sodium sulphate and evaporated in vacuo. Flash chromatography of the residue on silica gel (eluent: methylene chloride containing acetone) gave 0.52 g of the title compound which was recrystallized from ether/petroleum ether.

Metronidazole 3-(dimethylaminomethyl)benzoate, fumarate (III)

This compound was prepared in a similar way as compound **II**. Treatment of the ester dissolved in ethyl acetate with a solution of fumaric acid in 2-propanol and subsequent addition of ether yielded a salt with 1.5 equivalent fumaric acid which crystallized from 2-propanol/ether with 0.25 mol of water.

Metronidazole 4-(morpholinomethyl)benzoate (IV)

A mixture of metronidazole 4-chloromethylbenzoate (648 mg, 2 mmol), morpholine (0.88 ml, 10 mmol), sodium iodide (20 mg) and acetone (20 ml) was refluxed for 20 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo. Water (20 ml) was added to the residue and the solid formed was collected and washed with water, yielding 625 mg (83%) of the title compound which was recrystallized from cyclohexane. A salt with 1 equivalent fumaric acid was prepared as described for ester **III**.

Metronidazole 3-(morpholinomethyl)benzoate, difumarate (V)

To a solution of metronidazole 3-chloromethylbenzoate (648 mg, 2 mmol) in acetone (25 ml)

were added morpholine (0.88 ml, 10 mmol) and sodium iodide (20 mg). The mixture was stirred at room temperature for 20 h, filtered and evaporated in vacuo. Water (10 ml) was added to the residue and the mixture was extracted with methylene chloride (3 × 20 ml). The combined extracts were dried and evaporated in vacuo. Flash chromatography of the residue on silica gel (eluent: methylene chloride containing acetone) gave 0.59 g (79%) of the title compound as an oil. The difumarate was prepared by adding a solution of fumaric acid in 2-propanol and precipitating the salt with ether. Recrystallization was done from 2-propanol/ether.

Metronidazole 4-[(4-methylpiperazin-1-yl)methyl]-benzoate, difumarate (VI)

A mixture of metronidazole 4-chloromethylbenzoate (648 mg, 2 mmol), *N*-methylpiperazine (1.12 ml, 10 mmol), sodium iodide (20 mg) and methylene chloride (25 ml) was refluxed for 20 h. The reaction mixture was washed with water (2 × 25 ml), dried and evaporated in vacuo. To the residue was added a solution of 464 mg (4 mmol) of fumaric acid in 2-propanol (12 ml) followed by ether precipitating the difumarate of the title compound. Yield: 754 mg (60%).

Metronidazole 3-[(4-methylpiperazin-1-yl)methyl]-benzoate, difumarate (VII)

The compound was prepared by the same procedure as used for compound **VI**. The yield of the difumarate of the ester was 52%.

Metronidazole 4-(trimethylammoniummethyl)benzoate iodide (VIII)

A mixture of metronidazole 4-(dimethylaminomethyl)benzoate (**II**) (60 mg, 0.18 mmol), methyl iodide (0.2 ml, 2.8 mmol) and methanol (4 ml) was stirred at 50 °C for 4 h. After cooling, ether (10 ml) was added and the title compound was filtered off and recrystallized from ethanol to give 76 mg.

Analysis of metronidazole and the esters II–VIII by HPLC

A reversed-phase HPLC procedure was used for the quantitative determination of the aminomethylbenzoate esters and the parent metronidazole. A deactivated Supelcosil column was eluted with a mobile phase consisting of methanol/acetonitrile/0.1% phosphoric acid (5 : 20 : 75, v/v) in the analysis of the esters whereas a solvent system consisting of acetonitrile/0.1% phosphoric acid (5 : 95, v/v) was used for the analysis of metronidazole. In both cases triethylamine was added to the solvent systems at a concentration of 10^{-3} M in order to improve the peak shapes. The flow rate was 1.0 ml · min^{-1} and the column effluent was monitored at 215 nm. Under these conditions the retention times of the esters and metronidazole were about 2–3 min. It was assured that in each case adequate separation of the ester from the hydrolysis products metronidazole and the corresponding aminomethylbenzoic acid was achieved. Quantitation of the compounds was done from measurements of the peak heights in relation to those of standards chromatographed under the same conditions.

Kinetic measurements

Hydrolysis in aqueous solutions The hydrolysis of the esters **II–VIII** was studied in aqueous buffer solutions at constant temperature (±0.2 °C). The buffers used were hydrochloric acid, acetate, phosphate, borate and carbonate buffers; the total buffer concentration was generally 0.02 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 initiated by adding 100 μl of a stock solution of the esters in ethanol or water to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 3×10^{-5} 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 of the esters were determined from the slopes of linear plots of the logarithm of residual ester against time.

For slowly proceeding reactions (at pH 2–7.4) the rate constants were obtained by measuring the initial rates of metronidazole formation. In these cases, the initial concentration was 6×10^{-4} M. The formation of metronidazole was followed up to 1–3% of the initial ester concentration.

Pseudo-first-order rate constants for the hydrolysis were obtained by dividing the slopes of linear plots of metronidazole formed vs. time with the initial ester concentration.

Hydrolysis in human plasma The esters **II–VIII** were incubated at 37 °C in human plasma diluted to 80% with 0.05 M phosphate buffer of pH 7.40. The initial concentration of the esters was 6×10^{-5} M. At appropriate intervals, samples of 250 µl of the plasma reaction solutions were withdrawn and added to 500 µl of a 2% solution of zinc sulphate in acetonitrile/water (1 : 1, v/v) in order to deproteinize the plasma. After mixing and centrifugation for 3 min at 13000 rpm, 20 µl of the clear supernatant was analyzed by HPLC as described above. Pseudo-first-order rate constants were calculated from the slopes of linear plots of the logarithm of residual ester against time.

Solubility measurements The pH-solubility profile of ester **IV** was determined by the phase-solubility technique at 21 °C. An excess of the compound was added to 0.05 M buffer solutions of varying pH and the suspensions were rotated on a mechanical spindle for 24–30 h to attain equilibrium. The pH of the saturated solutions was measured and an aliquot of the filtrate was diluted with an appropriate amount of water and analyzed for the ester by HPLC.

Determination of ionization constants The pK_a values of the esters were determined by potentiometric titration of 50 ml of 3×10^{-3} M solutions of the compounds in water with 0.1 M sodium hydroxide. Esters present in free base forms were initially dissolved in an equivalent amount of 0.1 M hydrochloric acid.

Results and Discussion

Enzymatic hydrolysis of metronidazole esters

The rates of hydrolysis of the metronidazole esters **II–VIII** were determined in 80% human plasma (pH 7.4) and 37 °C. All esters underwent complete hydrolysis as indicated by the quantitative formation of metronidazole (Fig. 2), and in all cases the hydrolysis exhibited strict first-order kinetics over several half-lives. Typical first-order plots are shown in Fig. 3. The half-lives for the

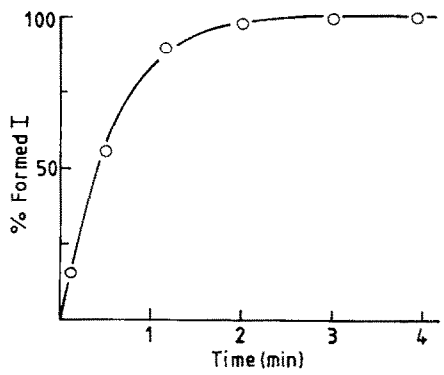


Fig. 2. Plot showing the formation of metronidazole (**I**) by hydrolysis of the ester **IV** in 80% human plasma at 37 °C.

hydrolysis in 80% human plasma solutions are given in Table 2. A demonstration of the enzymatic conversion of the esters in plasma is provided by the fact that the half-lives of hydrolysis of the esters in absence of plasma, i.e. in a pH 7.4 phosphate buffer at 37 °C, exceeded 800 h.

As can be seen from the data the aminomethylbenzoate esters are readily converted to metronidazole at conditions similar to those prevailing in vivo. Although all the esters **II–VII** are rapidly hydrolyzed by plasma enzymes the data show that both the structure of the amino group and the position of the aminomethyl group relative to the ester moiety have an influence on the rate of the plasma-catalyzed hydrolysis. Thus, for the morpholinomethylbenzoate esters the 4-substituted es-

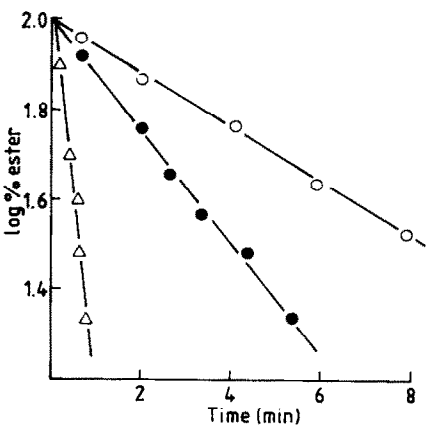


Fig. 3. First-order plots for the hydrolysis of the metronidazole esters **IV** (△), **V** (○) and **VI** (●) in 80% human plasma at 37 °C.

TABLE 2

Half-lives of hydrolysis of various N-substituted aminomethylbenzoate esters of metronidazole in 80% human plasma (pH 7.4) at 37 °C, values of the specific base-catalytic rate constants (k'_{OH}) at 37 °C and the pK_a values at 21 °C

Ester	$t_{1/2}$ in human plasma (min)	k'_{OH} [a] ($M^{-1} \cdot min^{-1}$)	pK_a
II	4.7	8.9	7.90
III	5.1	9.5	7.90
IV	0.4	12.0	6.15
V	5.0	11.6	6.15
VI	2.4	11.0	7.85 [b]
VII	0.6	12.0	7.85 [b]
VIII	51	29.1	–

[a] Determined in the pH range 10–11 at 37 °C.

[b] This value represents the second ionization constant of the *N*-methylpiperazino group. The value for the first ionization constant (estimated to be in the range 4–5) could not be determined due to interference from fumaric acid.

ter (**IV**) is more reactive than its 3-substituted analogue (**V**) whereas the opposite is the case for the *N*-methylpiperazinomethylbenzoate esters (**VI** and **VII**). For the dimethylaminomethylbenzoate esters (**II** and **III**) the position of substitution has only a minor influence on the enzymatic reactivity. The quaternary ammonium compound **VIII** is hydrolyzed significantly slower than the other esters and since its water-solubility also is much lower than that of esters containing a tertiary amino group, this derivative is less suitable as a prodrug form. For the sake of comparison it is of interest to note that the half-life for the hydrolysis of the plain benzoate ester of metronidazole in 80% human plasma (3.5 min) (Bundgaard et al., 1983) is in the same range as that for the aminomethylbenzoate esters. It is also of interest to note that the rates of hydrolysis observed for the esters **II**–**VII** are considerably higher than those for most aliphatic amino acid esters of metronidazole previously studied (Bundgaard et al., 1984a; Cho and Haynes, 1985).

Stability in aqueous solution

Due to their exceptionally high rate of plasma-catalyzed hydrolysis the esters **IV** and **VII** appear to be the most promising prodrugs for parenteral delivery. The stability of these esters in aqueous solution was therefore examined in detail as a function of pH and temperature whereas the other esters were only examined in alkaline solutions (pH 10–11).

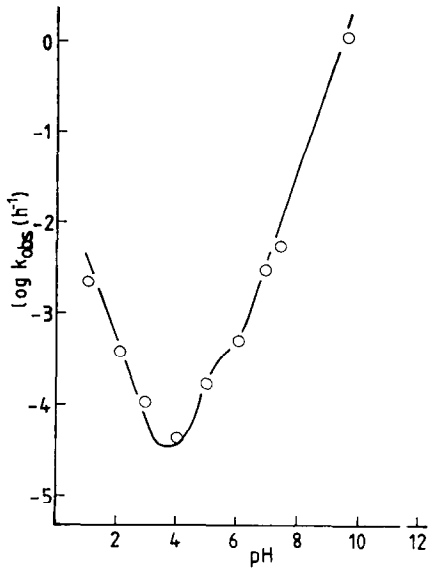


Fig. 4. The pH-rate profile for the degradation of the metronidazole ester **IV** in aqueous solution ($\mu = 0.5$) at 60 °C.

The kinetics of hydrolysis of the esters **IV** and **VII** were studied in aqueous buffer solutions at

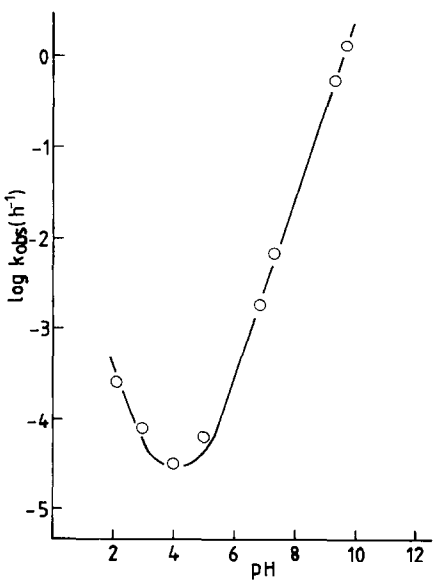
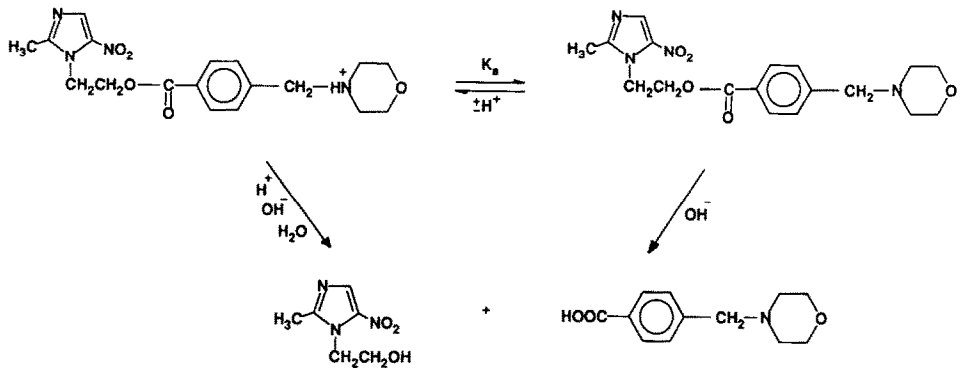


Fig. 5. The pH-rate profile for the degradation of the metronidazole ester **VII** in aqueous solution ($\mu = 0.5$) at 60 °C.



Scheme 3.

60 °C over the pH range 1.1–9.7. Under the experimental conditions used, the hydrolysis of the compounds followed strict first-order kinetics and proceeded with the quantitative formation of metronidazole. At the buffer concentration used (0.02 M) no significant buffer catalysis was observed.

The influence of pH on the rates of hydrolysis at 60 °C is shown in Figs. 4 and 5 in which the logarithm of the observed pseudo-first-order rate constants (k_{obs}) is plotted against pH. For both esters maximal stability occurs at pH values about 4. In the pH-range investigated the esters can occur in two forms with the amino function being unprotonated or protonated as shown in Scheme 3 for ester **IV**. The shapes of the pH-rate profiles indicate that the free base and the protonated forms of the esters undergo hydrolysis at different rates (for ester **IV**) and that the hydrolysis can be described in terms of specific reactions involving both species and a spontaneous and specific acid-catalyzed reaction of the protonated ester (Scheme 3). Mathematically,

$$k_{obs} = k_H a_H \frac{a_H}{a_H + K_a} + k_o \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (1)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activities, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total ester in the protonated and free base forms, respectively, and K_a is the apparent ionization constant of the protonated amino group in the esters. The rate constant k_o refers to the spontaneous or water-catalyzed hydrolysis of the protonated form of the ester, k_H is the specific acid-catalyzed rate constant for protonated ester, and k_{OH} and k'_{OH}

TABLE 3

*Ionization constants and rate data for the hydrolysis of the metronidazole esters **IV** and **VII** in aqueous solution ($\mu = 0.5$; 60 °C)*

Compound	k_H ($M^{-1} \cdot h^{-1}$)	k_o (h^{-1})	k_{OH} ($M^{-1} \cdot h^{-1}$)	k'_{OH} ($M^{-1} \cdot h^{-1}$)	pK_a
IV	5.6×10^{-2}	$< 10^{-6}$	1.6×10^{4}	3.1×10^{3}	5.8
VII	4.0×10^{-2}	2.1×10^{-5}	3.0×10^{3}	3.0×10^{3}	– [a]

[a] No kinetically derived values were obtained from the rate data.

are the second-order rate constants for the hydroxide ion-catalyzed hydrolysis of the protonated and unprotonated ester species, respectively. The a_{OH} values were calculated from the measured pH at 60 °C according to the following equation (Harned and Hamer, 1933):

$$\log a_{OH} = pH - 13.02 \quad (2)$$

The various rate and ionization constants derived from the pH-rate profiles are listed in Table 3. Using these constants, the solid curves in Figs. 4 and 5 were constructed. The good agreement between calculated and experimental data demonstrates that Eqn. 1 adequately describes the hydrolytic mechanism. Taking the temperature difference into account, the kinetically derived pK_a value for the ester **IV** agrees satisfactorily with that determined by titrimetry (Table 2).

The data obtained show that the protonated form of the ester **IV** is somewhat more reactive in hydroxide ion-catalyzed hydrolysis than the unprotonated form which can be ascribed to the greater electron-withdrawing effect of the protonated amino group relative to the unprotonated form. This effect is also apparent by comparing the specific base catalytic rate constants k'_{OH} for compound **II** and its quaternized derivative **VIII** (Table 2). The latter is about 3 times more reactive than ester **II** which is in accordance with the rate data previously reported by Smith and Menger (1969) on alkaline hydrolysis of a similar set of esters of methanol.

In the pH-rate profile for ester **VII** no significant curvature is apparent at a pH corresponding to its second pK_a value (7.85 at 21 °C). This value is most likely assigned to the methyl-substituted nitrogen and the lack of significant influence of protonation of this group on the hydrolysis of the ester bond is probably due to its long distance from the phenyl group and hence its reduced electron-withdrawing effect. Likewise, the rate data observed at pH values corresponding to the first ionization constant of the *N*-methylpiperazino group (about 4–5) do not allow one to distinguish clearly between the reactivities of the protonated and unprotonated species. Therefore, k_{OH} and

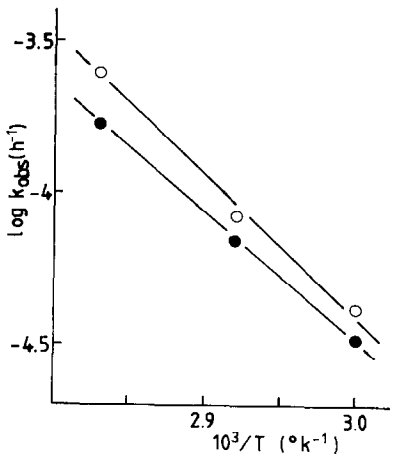


Fig. 6. Arrhenius plots of the rates of hydrolysis of the metronidazole esters **IV** (○) and **VII** (●) in 0.02 M acetate buffer solution ($\mu = 0.5$) of pH 4.0.

k'_{OH} given in Table 3 have been given the same values.

As appears from the k'_{OH} values in Table 2 the amino substituents as well as the position of substitution (3- or 4-position) have almost no influence on the chemical stability in alkaline solution. In fact, the k'_{OH} values were found to be quite similar to that (11.8 $M^{-1} \cdot min^{-1}$) for the plain unsubstituted benzoate ester of metronidazole.

In order to predict the stability of the aminomethylbenzoate esters in aqueous solution at normal storage temperatures, the rates of hydrolysis of the esters **IV** and **VII** in a 0.02 M acetate buffer of pH 4.0 were also determined at 70 and 80 °C. By plotting the rate constants obtained according to the Arrhenius equation (Fig. 6), energies of activation of 21.0 and 19.0 kcal · mol^{-1} were obtained for ester **IV** and **VII**, respectively. On the basis of these data it is possible to estimate the shelf-life of aqueous solutions of the esters at pH 4.0 at various temperatures. Defining the shelf-life as the time required to degrade 10% of the ester ($t_{10\%}$) the calculations show that shelf-lives of 12–14 years are achieved at 25 °C and 19–24 years at 20 °C. Thus, these esters can readily be formulated as highly stable, ready-to-use solutions at pH 3–4.5. It should be noted that for concentrated prodrug solutions the shelf-life may

often be limited by precipitation of parent drug formed upon hydrolysis rather than by loss in prodrug (Bundgaard et al., 1984b; Varia et al., 1984; Anderson et al., 1985). This will, however, not be a problem in this case because of the not so low solubility of metronidazole (10 mg · ml^{-1} at 25 °C) (Cho et al., 1982) and the high stability of the esters. Thus, it can be calculated that for a 21.8% w/v solution of ester **IV**, which is equivalent to 10% metronidazole on a molar basis, the time needed to form metronidazole at a concentration of 10 mg · ml^{-1} corresponds to $t_{10\%}$ in terms of prodrug loss, i.e. 14 years at 25 °C.

Water-solubility of the esters

Being weak bases (Table 2 lists the pK_a values) the aminomethylbenzoate ester derivatives readily form water-soluble salts with hydrochloric acid or other acids. The esters **II–VII** were all soluble to an extent higher than 10% w/v as assessed by dissolving the fumarate salts in 10 parts of water or, when the esters were isolated as free bases, in 10 parts of diluted hydrochloric acid. For ester **IV** (the free base form) the aqueous solubility was determined at 21 °C as a function of pH. The pH-solubility profile obtained is shown in Fig. 7. In the pH range studied the total solubility (S_T) of the compound can be expressed by the following equation:

$$S_T = [B]_s \frac{a_H + K_a}{K_a} \quad (3)$$

where $[B]_s$ is the concentration of the free base in the saturated solution, a_H is the hydrogen ion activity and K_a is the ionization constant of protonated **IV**. The intrinsic solubility, $[B]_s$, of the free base was determined to be 0.48 mg ml^{-1} from experiments performed at pH 9.0. Using this value and the solubility data at pH 5–8 along with Eqn 3 a pK_a value of 6.10 was obtained, in agreement with the value (6.15) obtained by titration. In Fig. 7 the curve drawn is constructed from these values and Eqn 3. To obtain a 20% w/v solution of ester **IV** the pH should be 3.5 or less. For the more basic ester **VII** a 20% w/v solution can be made at higher pH values.

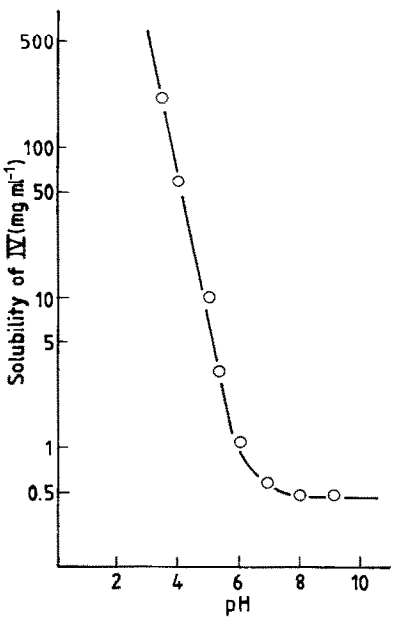


Fig. 7. Aqueous solubility-pH profile for the metronidazole ester **IV** at 21 °C. The points are experimental while the curve is calculated from Eqn. 3.

Conclusions

This study shows that it is possible to design water-soluble prodrugs of metronidazole satisfying the criteria of possessing a high stability in aqueous solution at pH values affording good solubility (i.e., weakly acidic pH values) and at the same time being rapidly hydrolyzed in the presence of plasma. The solution stability of the *N*-substituted aminomethylbenzoate esters studied is markedly higher than that of other amino-containing ester prodrugs such as esters formed with α-amino acids which only show shelf-lives of a few days (Bundgaard et al., 1984b). The phenyl moiety separating the ester moiety and the amino group in the former derivatives can be said to function as a spacer group hindering any instability-causing interactions between the ester moiety and the solubilizing amino function. Studies are in progress to examine toxicological and pharmacokinetic aspects of the metronidazole prodrugs and to explore the potential applicability of the new ester prodrug type to improve the solubility and

hence bioavailability characteristics of several other slightly soluble drugs containing an hydroxyl group.

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