International Journal of Pharmaceutics, 26 (1985) 227–241
Elsevier

IJP 00885

A comparison of the chemical stability and the enzymatic hydrolysis of a series of aliphatic and aromatic ester derivatives of metronidazole

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(Received March 25th, 1985)
(Accepted May 27th, 1985)

Key words: aliphatic and aromatic esters of metronidazole – hydrolytic degradation – chemical stability of metronidazole esters – enzymatic hydrolysis

Summary

The hydrolytic degradation rates of various aliphatic and aromatic esters of metronidazole in aqueous buffer solution and in human plasma were investigated at 37°C. Complete reversion to metronidazole was observed as determined by HPLC and in all cases the hydrolysis followed strict first-order kinetics. The susceptibility of the various ester derivatives to undergo enzyme-catalyzed hydrolysis was strongly influenced by the structure of the acyl moiety, but no unambiguous relationship between the degradation rate in aqueous buffer solution and in human plasma has been found. In aqueous solution the degradation of the aromatic esters was facilitated by low electron density at the reaction site as described by the Hammett equation. Concerning the decomposition of the aromatic esters in 2.5% human plasma a negative deviation from the Hammett plot was observed for the nitrobenzoic acid ester derivatives. A change in rate-determining step together with the polar nature of the nitro group is suggested to contribute to this deviation. It has been found that the length of the linear carbon chain in the aliphatic esters influences the enzyme catalyzed degradation rate. The ratio between the degradation rates in 2.5% human plasma and in aqueous buffer solution, pH 7.4, was greatest for the valerate ester, the value being 3800.

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Introduction

Considerable attention has been focused on the use of ester prodrugs in order to optimize delivery characteristics of various drugs. The objectives therefore are mainly: (i) to increase the water solubility of the active drug in order to prepare parenteral dosage forms (Johansen and Bundgaard, 1981; Cho et al., 1982; Bundgaard et al., 1984a and b); (ii) to provide sustained release preparations of various drug substances (for review see Sinkula, 1978); and (iii) to improve transport across different biological membranes through altering the lipophilic character of a drug compound (for review see Stella et al., 1980). Thus, profound general information on the stability of a broad spectrum of esters of the same parent compound both in aqueous solutions and in vivo is of great importance in the design of suitable prodrug candidates for drugs with a hydroxy functional group. Although studies on the stability within series of ester derivatives have been performed (Huang, 1979; Niphadkar, 1983), no attempts have been made to investigate systematically the stability characteristics of various ester types using the same drug compound possessing a hydroxy functional group. Together with our studies on amino acid esters (Bundgaard et al., 1984a and b) and the monosuccinate ester of metronidazole (Johansen and Larsen, 1984) and the reports on metronidazole phosphate (Cho et al., 1982) our aim at present has been to expand this general knowledge by investigation of the influence of structure on the rate of release of the active drug from the prodrug esters.

Materials and Methods

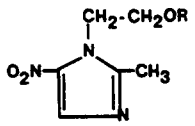
Apparatus

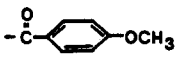
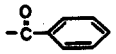
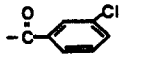
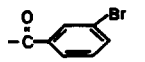
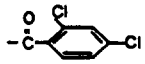
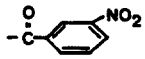
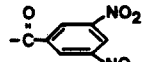
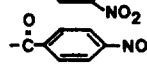
HPLC analysis was done with a Waters Associates Model 6000A constant-flow pump equipped with a Pye Unicam PU 4020 variable wave length detector and a Rheodyne Model 7125 injection valve with a 20 μl loop. Readings of pH were carried out on a Radiometer Type pH M 26 meter at the temperature of study. Infrared spectra were run on a Unicam SP 200 spectrophotometer using the potassium chloride disc technique. Melting points were taken in capillary tubes and are not corrected.

Synthesis of metronidazole esters

(a) Aromatic esters. All the substituted benzoic acid esters of metronidazole were prepared by the procedure described for metronidazole 4-methoxybenzoate (I): To a stirred solution of metronidazole (1.71 g, 10 mmol) in 15 ml of pyridine was added dropwise a solution of 4-methoxybenzoyl chloride (1.50 ml, 11 mmol) in 15 ml of pyridine. The mixture was stirred at room temperature overnight. The precipitate formed was filtered off and washed with water. The compound was recrystallized from ethanol. The esters were characterized by elemental and IR analysis and the melting points of the ester derivatives (I–X) are shown in Table 1.

(b) Aliphatic esters. The aliphatic esters of metronidazole were in general pre-



	R	compound
I	-H	Metronidazole
II		Metronidazole 4-methoxybenzoate
III		Metronidazole benzoate
IV		Metronidazole 4-chlorobenzoate
V		Metronidazole 3-chlorobenzoate
VI		Metronidazole 3-bromobenzoate
VII		Metronidazole 2,4dichlorobenzoate
VIII		Metronidazole 3-nitrobenzoate
IX		Metronidazole 3,5 dinitrobenzoate
X		Metronidazole 4-nitrobenzoate
XI	-C-CH3	Metronidazole acetate
XII	-C-CH2CH3	Metronidazole propionate
XIII	-C-CH2CH2CH3	Metronidazole butyrate
XIV	-C-CH2CH2CH2CH3	Metronidazole valerate
XV	-C-CH2CH2CH2CH2CH3	Metronidazole caproate

Formula 1

pared by adding to a solution of metronidazole (1.71 g, 10 mmol) in 15 ml of dimethylformamide a solution of the appropriate acid chloride (11 mmol) dissolved in 15 ml of dimethylformamide. The solution was left for 24 h under stirring and poured into a mixture of 40 g of ice, 0.93 g of sodium hydrogencarbonate and 75 ml of ethyl acetate. After separation of the ethyl acetate layer the water phase was extracted with a 75 ml portion of ethyl acetate. The combined extracts were washed with 50 ml of water, 50 ml of a 2% sodium bicarbonate solution and 50 ml of water. After drying with magnesium sulphate the ethyl acetate was evaporated under reduced pressure. In case of metronidazole acetate the residue was crystallized from ethanol (m.p. 73–74°C). The crude products of the other aliphatic esters appeared as an oily mass and was purified by column chromatography using ethyl acetate as eluent and silica gel (Silica Woelm, 63–100 μm) as the stationary phase. The

TABLE 1

MELTING POINTS OF METRONIDAZOLE AROMATIC ESTERS

Ester		m.p. (°C) [a]	m.p. (°C)
4-Methoxybenzoate	(I)	96	102 [b]
4-Methylbenzoate	(II)	103.5	
Benzoate	(III)	100.5	100 [b]
4-Chlorobenzoate	(IV)	159–160	
3-Chlorobenzoate	(V)	105	
3-Bromobenzoate	(VI)	100–101	
2,4-Dichlorobenzoate	(VII)	119	
3-Nitrobenzoate	(VIII)	140–141	
3,5-Dinitrobenzoate	(IX)	145.5	
4-Nitrobenzoate	(X)	162–164	166 [b]

[a] Experimental values, uncorrected.
[b] From Jacob et al. (1960).

structure of the compounds was confirmed by IR spectrophotometry.

Analysis of metronidazole esters by HPLC

The metronidazole esters were determined by using a reversed-phase high-performance liquid chromatographic procedure. A column 250 × 40 mm, packed with LiChrosorb RP-8 (7 μm particles) and equipped with a small pre-column was eluted with two different solvent systems depending on the compound in study:
(1) Acetonitrile—0.05 M phosphate pH 7.0 (55 : 45 v/v) (aliphatic esters of metronidazole);
(2) Acetonitrile—0.05 M phosphate pH 7.0 (6 : 4 v/v) (aromatic esters of metronidazole).

The flow rate was 1.0 $ml \cdot min^{-1}$ and the column effluent was monitored at 320 nm. Under these conditions the esters were well separated from metronidazole, the product of hydrolysis. Quantitation of metronidazole esters was done from peak height measurements in relation to those of the appropriate standards chromatographed under the same conditions.

Kinetic measurements

The study of the chemical stability of the esters was performed in aqueous buffer solution at 37°C. Phosphate, borate and carbonate were used as buffers, 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 a methanolic or an acetonitrilic solution of the esters to the pre-equilibrated buffer solutions to give an initial concentration of about 0.01–0.05 $mg \cdot ml^{-1}$. The concentration of organic solvent in the reaction solutions did not exceed 1%. At appropriate intervals samples were withdrawn and analyzed for metronidazole ester by HPLC.

Pseudo-first-order rate constants were calculated from the slopes of linear plots of $\ln c_t$ against time, where c_t is the ester concentration at time t.

The conversion of the esters to metronidazole was also studied in 0.05 M phosphate buffer pH 7.4 containing varying amounts of human plasma at 37°C. The experimental conditions were as described above, but 500 μl samples were withdrawn and added to 1500 μl of methanol in order to deproteinize the plasma. The mixture was vortexed and after centrifugation for 2 min at 10,000 × *g*, 20 μl of the clear supernatant was injected on the column.

Results and Discussion

Hydrolysis of metronidazole esters in aqueous solution

The kinetics of hydrolysis of metronidazole 4-methylbenzoate (II) was studied in aqueous buffer solutions in the pH range 6.5–10 at 60°C, except in the studies on the influence of the temperature on the reaction rates. At constant pH and temperature the decomposition rates followed strict first-order kinetics. The variation (0.02–0.1 M) in the concentration of the buffers used was without significant influence on the reaction rates.

Fig. 1 shows the pH–rate profile for degradation of metronidazole 4-methylbenzoate. The pH–rate profile shows a straight-line portion with a slope of approximately +1, indicating that the degradation undergoes a specific base-catalyzed reaction in accordance with Eqn. 1:

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

where k_{obs} is the observed pseudo-first-order rate constant, k_{OH} is the second-order

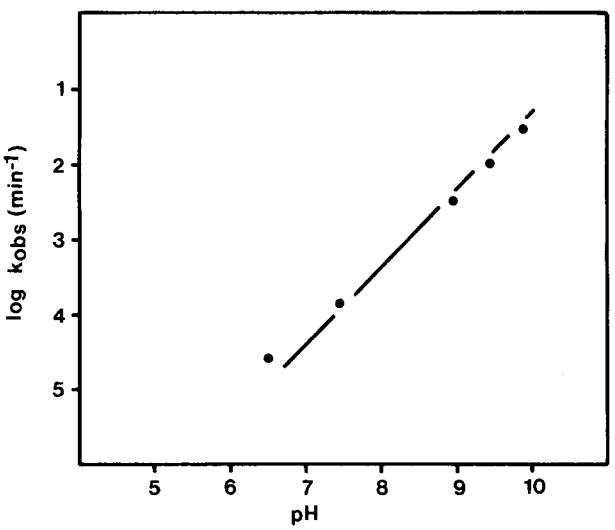


Fig. 1. pH–rate profile for hydrolysis of metronidazole 4-methylbenzoate at 60°C and $\mu = 0.5$. ●, experimental values and the curve is calculated from Eqn. 1

TABLE 2

DEGRADATION OF METRONIDAZOLE ESTERS AT 37°C

Ester	k_{obs} (min^{-1}) [a]	k_{OH} ($M^{-1} \cdot min^{-1}$) [b]	k_{obs} (min^{-1}) [c] pH 7.40	$\frac{k_{obs}}{k_{obs}(acetate)}$
4-Methoxybenzoate	5.43×10^{-3}	6.99	4.21×10^{-6}	
4-Methylbenzoate	1.02×10^{-2}	13.2	7.95×10^{-6}	
Benzoate	1.88×10^{-2}	24.2	1.46×10^{-5}	
4-Chlorobenzoate	4.74×10^{-2}	57.6	3.47×10^{-5}	
3-Chlorobenzoate	7.57×10^{-2}	97.5	5.87×10^{-5}	
3-Bromobenzoate	8.22×10^{-2}	106	6.38×10^{-5}	
2,4-Dichlorobenzoate	4.98×10^{-2}	64.2	3.87×10^{-5}	
3-Nitrobenzoate	1.49×10^{-2} [d]	241	1.45×10^{-4}	
3,5-Dinitrobenzoate	1.64×10^{-1} [d]	2660	1.60×10^{-3}	
4-Nitrobenzoate	2.27×10^{-2} [d]	368	2.22×10^{-4}	
Acetate	3.72×10^{-2}	47.9	2.89×10^{-5}	1
Propionate	2.94×10^{-2}	37.9	2.28×10^{-5}	0.79
Butyrate	1.69×10^{-2}	21.7	1.30×10^{-5}	0.45
Valerate	1.85×10^{-2}	23.9	1.44×10^{-5}	0.50
Caproate	1.73×10^{-2}	22.3	1.34×10^{-5}	0.46

[a] Determined in 0.05 M carbonate buffer solution pH = 10.51 and $\mu = 0.5$.
[b] Calculated from $k_{obs} = k_{OH} \cdot a_{OH}$ and $\log a_{OH} = pH - 13.62$.
[c] Calculated.
[d] Determined in 0.05 M borate buffer solution pH = 9.42 and $\mu = 0.5$.

rate constant for the specific base catalysis and a_{OH} is the hydroxide ion activity. The activity a_{OH} was calculated in accordance with Harned and Hamer (1933).

The effect of temperature on the rate of hydrolysis was determined in 0.05 M phosphate buffer solution (pH 7.40 and $\mu = 0.5$) in the range 60–80°C. From an Arrhenius type plot an apparent activation energy, E_a, of 112 $kJ \cdot mol^{-1}$ was calculated.

The pH–rate profiles for the degradation of the other compounds studied have similar courses as that of metronidazole 4-methylbenzoate. The reaction rates were studied at 37°C in the basic region of pH and the second-order rate constants for the specific base-catalyzed reactions were calculated in accordance with Eqn. 1. In Table 2 the catalytic constants are shown together with calculated degradation rate constants at physiological pH and temperature.

Structural effects on decomposition rate

From Table 2 it can be seen that for the aromatic esters the various substituents have a pronounced effect on the rates of the specific base-catalyzed hydrolysis. This effect is predominantly electric of nature and the rate data are well correlated by the Hammett equation (see Fig. 2):

$$\log k_{OH} = 1.6\sigma + 1.4 \quad (n = 8,\ r = 0.995)$$

Washkuhn et al. (1971) have described a correlation of relative rates of alkaline

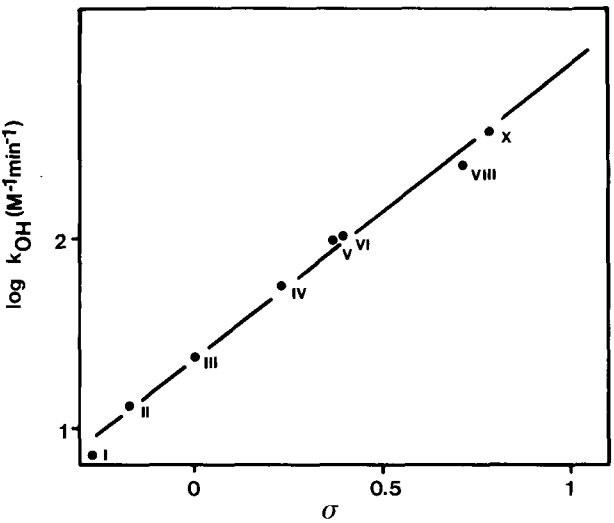


Fig. 2. Hammett plot of the specific base-catalyzed rate constants k_{OH} for decomposition of substituted benzoate esters of metronidazole at 37°C.

hydrolysis in 50% acetonitrile at 25°C with Hammett substituent constants (σ) for some benzoate esters with hydroxy component varying from simple aliphatic alcohols to substituted phenols. A reaction constant, ρ, corresponding to approximately 2.1 has been calculated for all esters. Taken into consideration that ρ is dependent on the reaction conditions, e.g. temperature, where ρ is inversely proportional with the absolute temperature (Connors, 1973), and medium, the calculated ρ of 1.6 from aqueous solution and 37°C therefore appears comparable to the results obtained by Washkuhn et al. (1971).

The length of the side-chain in the aliphatic esters also has an influence on the degradation rate as shown in Table 2. The acetate ester is degraded fastest and the rate decreases as the side-chain increases until a length corresponding to the butyrate ester. The degradation rate of the butyrate ester is approximately half the value of the acetate ester. These results are in favourable agreement with the findings of Tablot (1976), who found that the rate of the base catalyzed hydrolysis of ethyl acetate was twice that of ethyl butyrate. Higuchi et al. (1983) have determined the hydrolytic half-lives of acetaminophen propionate and caproate at pH 7.0 and 25°C. The former ester derivative underwent hydrolysis 1.8 times faster than the caproate ester. This is consistent with our findings, where the ratio of the corresponding rate constants has been determined to 1.7.

Enzymatic hydrolysis of metronidazole esters

The degradation of the metronidazole ester derivatives was studied at 37°C in 0.05 M phosphate buffer solution (pH 7.40) containing varying amounts of human plasma (2.5–80%). In all the kinetic runs the degradation rate followed strict first-order kinetics, indicating that the enzymes were present in excess. The hydro-

TABLE 3

HALF-LIVES FOR HYDROLYSIS OF METRONIDAZOLE ESTERS I–XV IN HUMAN PLASMA AND IN BUFFER SOLUTION AT 37°C and pH 7.40

Ester	$t_{1/2}$ (h)			$\frac{t_{1/2}(\text{buffer})}{t_{1/2}(2.5\%\ \text{plasma})}$
	buffer	2.5% plasma	80% plasma	
4-Methoxybenzoate	2740	11.3		242
4-Methylbenzoate	1450	1.3	0.13	1120
Benzoate	791	0.59	0.04	1340
4-Chlorobenzoate	333	0.38	0.07	876
3-Chlorobenzoate	197	0.50	0.09	394
3-Bromobenzoate	181	0.47	0.09	385
2,4-Dichlorobenzoate	299	0.40	0.08	748
3-Nitrobenzoate	79.7	2.3	0.23	34.6
4-Nitrobenzoate	52.0	0.86	0.07	60.5
Acetate	400	32	1.3	12.5
Propionate	507	3.2		158
Butyrate	890	0.45	0.002	1980
Valerate	802	0.21	0.002	3820
Caproate	862	0.24	0.003	3590

lytic rate data for degradation in human plasma and in pure buffer solution are presented in Table 3 revealing that the ester derivatives are subject to enzyme-catalyzed hydrolysis although the increment of the hydrolysis rate of the individual

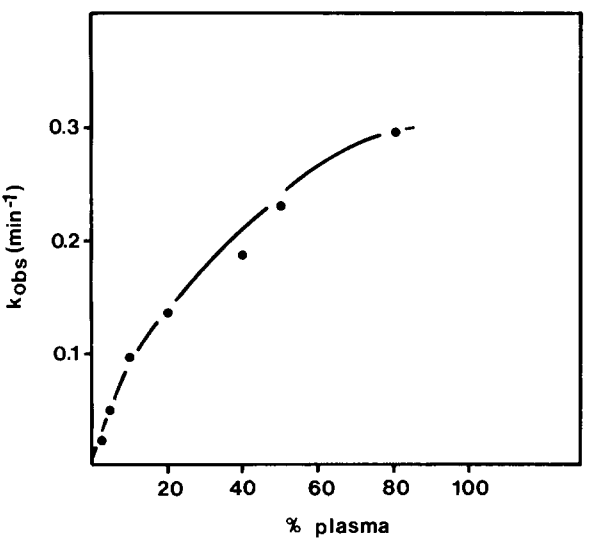


Fig. 3. Influence of the plasma concentration on the degradation of metronidazole benzoate, pH 7.40 and 37°C.

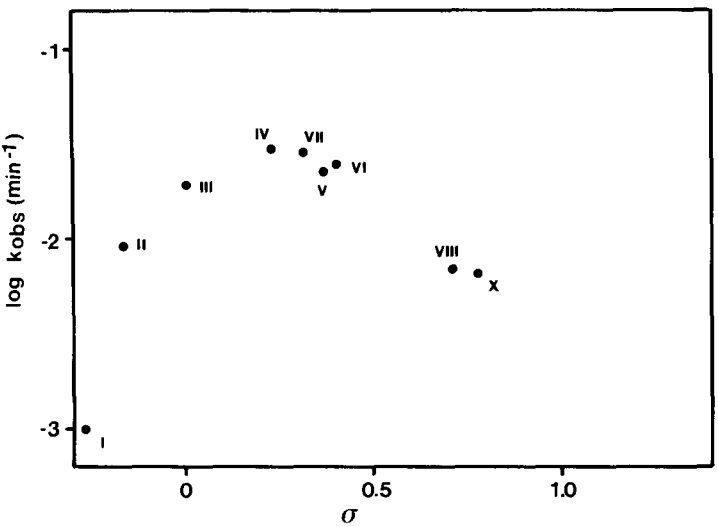


Fig. 4. Hammett plot of the rate constants k_{obs} for hydrolysis of substituted benzoate esters of metronidazole in 2.5% plasma, pH 7.40 and 37°C.

esters due to the presence of non-specific plasma carboxylases varies markedly. In Fig. 3 is shown the influence of the plasma enzyme concentration on the degradation rate of metronidazole benzoate. A 20,000-fold increase in rate of hydrolysis by changing the plasma concentration from 0 to 80% can be calculated. Therefore, in order to provide fully comparable kinetic data the reaction solutions were prepared containing identical amounts (on molar basis) of the individual metronidazole esters.

Structural effects

Aromatic esters

Attempts to determine the influence of the structure on the rate of enzyme-mediated cleavage of the esters were not conclusive as seen from the Hammett plot of log k_{obs} (2.5% plasma) against σ in Fig. 4. A reasonable agreement with the Hammett equation (log $k_{obs} = \rho\sigma$ + constant) might be obtained if the nitro compounds are excluded. The best fit of the equation is found for the *p*-substituted derivatives. Dividing σ into contributions from two different electric effects, e.g. a localized (inductive) effect σ_L and a delocalized (resonans) effect σ_D (Charton, 1977) the following equation is found:

$$\log k_{obs} = 1.9\sigma_L + 3.3\sigma_D - 1.6$$

A similar equation is found for the non-enzymatic catalyzed reaction:

$$\log k_{OH} = 1.4\sigma_L + 1.5\sigma_D + 1.7$$

By comparison of the two equations it is seen that the delocalized effect seems to

contribute more to the enzymatic degradation than to the reaction in pure buffer solution.

Omitting the data from the nitro-substituted compounds an overall regression line including *p*- and *m*-substituted compounds can be calculated:

$$\log k_{obs} = 1.6\sigma - 2.1 \quad (n = 7,\ r = 0.80)$$

giving a ρ value (1.6) identical with the one found for the non-enzymatic reaction.

The correlation is not convincing (regression coefficient $r = 0.80$), but suggests that the reaction is facilitated by low electron density at the reaction site. The negative deviation from the Hammett plot for the nitrobenzoic acid esters, however, indicates that in addition to the electronic effects other factors contribute to the susceptibility to undergo enzyme-catalyzed hydrolysis. The experiments do not allow an unambiguous explanation for the observed deviation, but several possibilities might be taken into consideration:

(a) the enzymatic reaction is subject to product inhibition;
(b) the lipophilicity of the nitro compounds are unfavourable for enzymatic attack;
(c) there is a change in the rate-determining step; or
(d) the nitro groups are partly charged and thus the nitro derivatives are poor substrates for non-specific plasma carboxylesterases.

(a) The difference in reactivity of the nitro esters compared with the other esters studied might be due to product inhibition by the released 4- or 3-nitrobenzoic acid. The possible inhibitory effect was examined by adding 4-nitrobenzoic acid to sample solutions in a molar concentration equivalent to the compound under study, and the degradation rate constant was determined. As seen from Table 4 the obtained mutual rate constants are in favourable agreement, thus excluding a mechanism involving product inhibition.

(b) It is likely that plasma protein binding is favoured by compounds with relative high lipophilicity and therefore might result in a decrease in the enzymatic catalyzed degradation rate (Hansch, 1968). Inspection of the hydrophobicity parameter, π_x, reveals, however, that the nitro compounds are less lipophilic than the benzoate ester ($\pi_{NO_2} = -0.28$ and $\pi_H = 0$). Calculating the multiple regression equation for the 4-methoxy, benzoate, 4-Cl, 3-Cl, 3-Br, 3-NO_2 and 4-NO_2 derivative

TABLE 4

INFLUENCE OF 4-NITROBENZOIC ACID ON THE DEGRADATION RATE OF BENZOATE ESTERS OF METRONIDAZOLE IN THE PRESENCE OF PLASMA (pH 7.40 and 37°C)

Ester	k_{obs} (min^{-1}) 80% plasma	k_{obs} (min^{-1}) 80% plasma containing 4-nitrobenzoic acid [a]
3-Nitrobenzoate	0.0508	0.0502
4-Methylbenzoate	0.0935	0.0940

[a] The 4-nitrobenzoic acid concentration is equivalent to the concentration of the ester compound studied.

gives the following result:

$$\log k_{obs}(2.5\% \text{ plasma}) = 0.68\sigma + 0.70\pi - 2.33 \quad (n = 7,\ r^2 = 0.56)$$

indicating that no correlation is present. Omitting the σ substituent parameter gives

$$\log k_{obs}(2.5\% \text{ plasma}) = 0.61\pi - 2.1 \quad (n = 7,\ r^2 = 0.33)$$

which is even worse, indicating that the lipophilicity is without significant importance in the degradation of metronidazole benzoate esters in plasma.

(c) Huang (1979) has studied the degradation rate of *p*-substituted ethyl benzoates in presence of non-specific esterases (EC 3.1.1.1). He found good correlation with the Hammett equation by plotting the maximal velocity for degradation, V_{max}, against the σ substituent parameter for *p*-NH_2, *p*-OH, *p*-F and *p*-CN substituents. However, he also observed a negative deviation for the *p*-NO_2 compound in the Hammett plot. Huang (1979) describes that this deviation probably is due to a change in the rate-determining step. Enzymes forming an acyl–enzyme intermediate catalyze reactions by a three-step mechanism (Scheme 1)

$$AcX + E \underset{K_s}{\rightleftharpoons} AcX \cdot E \overset{k_2}{\rightarrow} Ac\text{–}E + X \overset{k_3}{\rightarrow} AcOH + E$$

involving an acylation and a deacylation step characterized by the rate constants k_2 and k_3, respectively. Huang (1979) suggests that for low σ values $k_2 < k_3$ whereas as σ increases $k_2 \approx k_3$ or $k_2 > k_3$ resulting in the observed negative deviation in the Hammett plot. The suggestions are partly based upon the fact that the smaller the σ values are, the closer is the value of the slope of the Hammett plot for the enzyme-mediated reactions to that observed for the specific base-catalyzed reaction. The latter observation is in accordance with our results, where the reaction constants are identical for the specific base and the enzyme-catalyzed reactions omitting the rate data of the nitro compounds. It therefore seems most likely to suggest that a change in rate-determining step is responsible for the non-linear Hammett plot.

(d) Another reason for the low reactivity of the nitrobenzoate esters in plasma can be the polar nature of the nitro group. Nitrobenzene is polarized as shown in Fig. 5 with a partial negative charge on the oxygen atom (Urbanski, 1970). Recently it has been reported that charged polar compounds in general are either not attacked by or are poor substrates for non-specific plasma carboxylesterases (Krisch, 1971; Higuchi et al., 1983; Johansen and Larsen, 1984). The polar structure might also contribute to the observed deviation from the Hammett plot.

Aliphatic esters

In case of aliphatic esters the catalytic cleavage rate by carboxylesterases has been reported to be influenced by the chain length of both the acyl and the alcohol portion of the ester compounds (Krisch, 1971). Hofstee (1954) found, using a purified horse liver esterase, increasing rate of catalytic hydrolysis of fatty acid esters

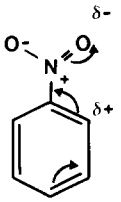


Fig. 5. Polarization of nitrobenzene.

of *m*-hydroxybenzoic acid when altering the acyl carbon chain length from C_2 to C_{12}. Similarly, Webb (1971) has studied a series of aliphatic esters with varying length of the acyl side-chain as substrates for a horse liver carboxylesterase preparation. The experiments showed that an elongation of the acyl side chain to C_4–C_6 resulted in an increase in both affinity and reactivity of the substrates. These observations are in very fine agreement with our results as shown in Table 3, where the half-lives for degradation in pure buffer solution and in plasma are presented. The most efficient catalysis of plasma carboxylesterases appears to occur for the valerate ester, where the hydrolysis proceeds 3800 times faster in 2.5% plasma than in aqueous buffer solution.

The relative rates of enzymatic hydrolysis of *p*-nitrophenyl esters by human serum have been described by the following equation (Hansch, 1968):

$$\log A_x = -7.614\sigma^* + 0.389\pi + 3.808E_s + 1.552 \quad (n = 6,\ r = 0.991)$$

where A_x is the relative rate of hydrolysis, σ^* is a measure of the electronic effects of substituents in aliphatic systems, E_s refers to Taft's steric parameter and π is the lipophilic parameter. Using the values for E_s, π and σ^* reported by Hansch (1972) the results of the present study lead to the following equation as calculated by multiple regression analysis.

$$\log k_{obs}(2.5\%\ \text{plasma}) = -6.90\sigma^* + 0.1\pi - 2.7E_s - 3.48 \quad (n = 5,\ r^2 = 0.989)$$

Although the equation is based on relatively few data, it can be seen that the coefficients of the σ^* and π parameters are of the same order of magnitude as obtained by Hansch (1968). The difference in sign of the coefficient for E_s might be due to the fact that our study has encompassed only esters with unbranched aliphatic acyl side chains, which only to a minor extent contribute to the overall expression.

Ester prodrugs

In the design of ester prodrugs with the purpose of optimizing the physicochemical characteristics of a drug compound, the properties of the prodrugs in vitro as well as in vivo have to be considered. The present study reveals very large differences in the chemical stability of the ester derivatives in aqueous solution and in plasma (Table 3). Furthermore it is shown that the structure of the derivatives play an

TABLE 5

HALF-LIVES FOR THE HYDROLYSIS OF THE METRONIDAZOLE ESTERS IN HUMAN PLASMA (pH 7.4) AND 0.05 M PHOSPHATE BUFFER pH 7.40 AT 37°C

Ester	$t_{1/2}$ in 2.5% human plasma [a] (h)	$t_{1/2}$ in 80% human plasma [b] (h)	$t_{1/2}$ in buffer [b]	$\frac{t_{1/2}(\text{buffer})}{t_{1/2}(2.5\%\ \text{plasma})}$	$\frac{t_{1/2}(\text{buffer})}{t_{1/2}(80\%\ \text{plasma})}$
N,N-Dimethylglycinate	1.5	0.2	4.2	2.8	21
3-Dimethylamino-propionate	0.53	0.77	0.87	1.6	1.1
4-Methyl-1-piperazino-acetate	9.4	8.7	29	3.1	3.3
4-Morpholino-acetate	4.5	0.5	31.3	7.0	62.6
Monosuccinate		630 [c]	445 [c]		0.7

[a] Experimental determined values, using the method described by Bundgaard et al. (1984a).

[b] From Bundgaard et al. (1984a).

[c] From Johansen and Larsen (1984).

important role in determining the susceptibility of the various esters to undergo enzyme-catalyzed hydrolysis, although no unambiguous relationship between the degradation rates in aqueous buffer solution and in plasma seems to exist.

In Table 5 are presented the half-lives for degradation of various amino acid esters of metronidazole (from a previous study, Bundgaard et al., 1984a) and for the monosuccinate ester (Johansen and Larsen, 1984). Comparison of Table 3 with Table 5 shows that the increment in degradation rate [$t_{1/2}$(buffer)/$t_{1/2}$(plasma)] is most pronounced for the aliphatic esters, indicating that the esters with no charge and minor steric hindrance are the best substrates. This observation is in accordance with Krisch (1971). However, if the half-lives for the amino acid esters at 80% plasma is compared with the stability in buffer solution, the following ratios for $t_{1/2}$(buffer)/$t_{1/2}$(plasma) have been calculated: 1.1 (3-dimethylaminopropionate), 3.3 (4-methyl-1-piperazinoacetate), 20.8 (N,N-dimethylglycinate) and 62.7 (4-morpholinoacetate) (Bundgaard et al., 1984a). The two latter compounds are much better substrates than the other amino acid esters. This might be a reflection of the fact that the two latter amino acid esters only partly are protonated at pH 7.40. A pK_a value of 6.5 at 37°C has been determined for the N,N-dimethylglycinate ester (Bundgaard et al., 1984b). The pK_a of the 4-morpholinoacetate ester is 4.5 (37°C) as determined by titrimetry according to the method described by Albert and Serjeant (1971). Krisch (1971) reported that positively and negatively charged polar compounds are either poor substrates or are not substrates at all for carboxylesterases. This observation in combination with the above-described results indicate that amino acid esters, which mainly are unprotonated at physiological pH, are subject to enzyme catalysis to a larger extent compared with esters which are fully protonated at pH 7.40. It should be mentioned that the observed decrease in pK_a of the amino groups by esterification only is valid for N,N-dimethylglycinate and 4-morpholinoacetate esters, whereas the other two esters have pK_a values near the parent amino acid, e.g. 9.5–10.5.

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