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Research Article

Water-Soluble, Solution-Stable, and Biolabile *N*-Substituted (Aminomethyl)benzoate Ester Prodrugs of Acyclovir

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Various *N*-substituted 3- or 4-(aminomethyl)benzoate esters of acyclovir were synthesized and evaluated as water-soluble prodrug forms with the aim of improving the delivery characteristics of acyclovir, in particular its parenteral administration. The esters showed a high solubility in weakly acidic solutions and, as demonstrated with the 3-(*N,N*-dipropylaminomethyl)benzoate ester, a high stability in such solutions, allowing storage for several years. The esters combine these properties with a high susceptibility to undergo enzymatic hydrolysis in plasma. The half-lives of hydrolysis in 80% human plasma ranged from 0.8 to 57 min, the rate being highly dependent on the position (3 or 4) of the aminomethyl group relative to the ester moiety. All esters were more lipophilic than acyclovir in terms of octanol–pH 7.4 buffer partition coefficients. These properties make *N*-substituted (aminomethyl)benzoate esters a promising new prodrug type for acyclovir to enhance its delivery characteristics.

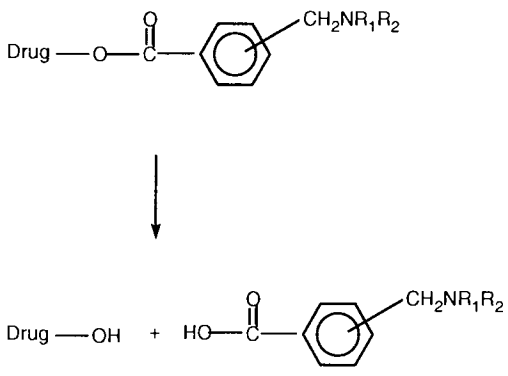
KEY WORDS: acyclovir; prodrugs; enzymatic hydrolysis; lipophilicity; solubility; stability.

INTRODUCTION

Acyclovir is a widely used agent to treat herpes virus infections, and intravenous, oral, and topical formulations are available. The delivery characteristics of acyclovir following administration by these routes is, however, far from being optimal, which most likely can be attributed to the poor aqueous solubility and/or low lipophilicity of the drug. Thus, the oral absorption of acyclovir can be highly variable and is only 15–20%, the percutaneous penetration is poor, and because of its limited solubility in water (1.4 mg ml^{-1}), the drug cannot be given as eyedrops or by intramuscular injection (for a recent review, see Ref. 1). Parenterally, acyclovir is presently given by infusion or by bolus intravenous injection in the form of strongly alkaline (pH 10–11) solutions of the sodium salt. Consequently, this administration may cause thrombophlebitis or perivascular inflammation (2).

A promising solution to these delivery problems may be the development of prodrugs of acyclovir with more desirable physicochemical properties. Thus, 6-deoxyacyclovir (desciclovir) was developed by Krenitsky *et al.* (3) and shown to be readily absorbed (about 75%) after oral administration (3,4). This prodrug is 18 times more water-soluble than acyclovir and is converted to the latter *in vivo* by xanthine oxidase (3). Colla *et al.* (5) have prepared various α-amino acid esters which are highly water-soluble and potentially useful to obtain, e.g., eyedrop formulations (6). However, a serious drawback of such esters is their poor stability in aqueous solution, making it impossible to prepare ready-to-use solutions (7). The major reason for the high instability of α-amino acid esters at pH 3–6 is the occurrence of intramolecular catalysis of ester hydrolysis by the neighboring amino group (8). We have recently shown that an effective and simple means to block totally this hydrolysis facilitating effect of the amino group is to incorporate a benzyl group between the ester moiety and the solubilizing amino group (9). Such *N*-substituted 3- or 4-(aminomethyl)benzoate esters have been prepared with various drugs containing a hydroxyl group including metronidazole (10), chloramphenicol (11), and paracetamol (12) and shown to combine a high solubility and stability in weakly acidic solutions with a high susceptibility to undergo enzymatic hydrolysis in the presence of plasma (Scheme I).

In the present work, a series of such *N*-substituted 3- or 4-(aminomethyl)benzoate esters of acyclovir (Scheme II) has



Scheme I

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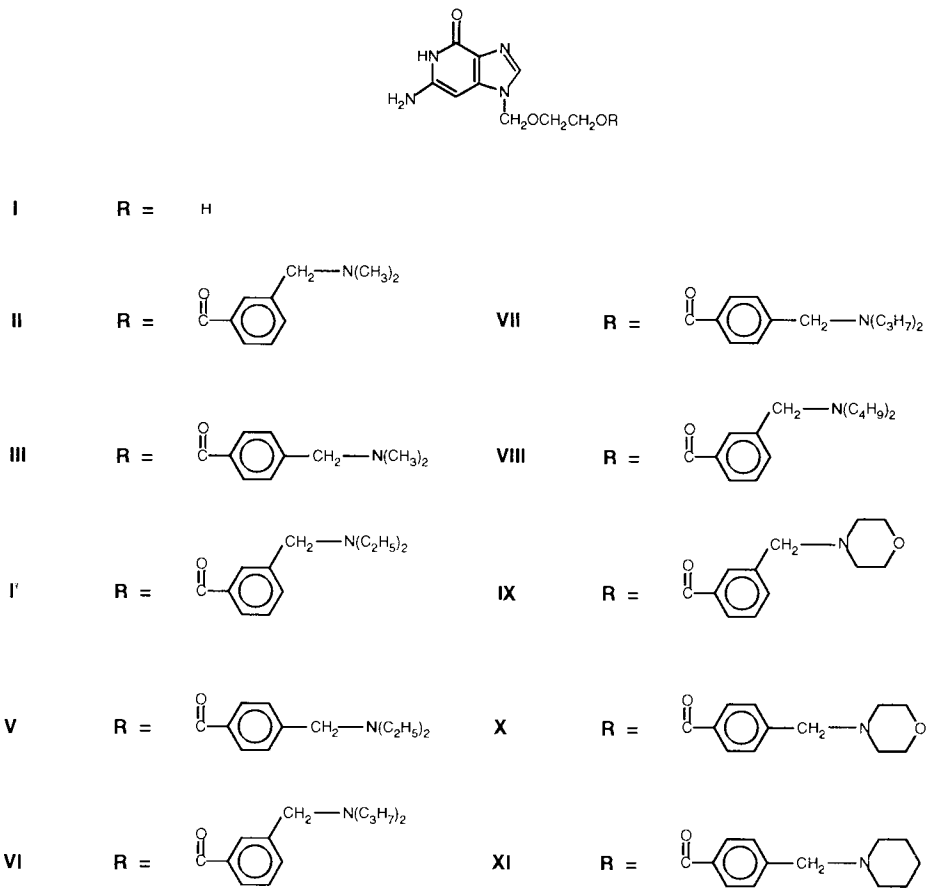

Scheme II

been prepared and evaluated as solution-stable, biolabile prodrug forms. To this end, the chemical stability and plasma-catalyzed hydrolysis of the new derivatives were investigated and their aqueous solubility and lipophilicity determined.

MATERIALS AND METHODS

Apparatus

High-performance liquid chromatography (HPLC) was performed with a Shimadzu apparatus consisting of an LC-6A pump, an SPD-6A variable-wavelength detector, and a 20-μl loop injection valve (Rheodyne). Separations were done on a deactivated reversed-phase Supelcosil LC-8-DB column (33 × 4.6 mm, 3-μm particles) equipped with a Supelguard column (from Supelco Inc., U.S.A.) or an Ultropac TSK ODU-120T column (250 × 4.6 mm, 5-μm particles) (obtained from LKB Products AB, Bromma, Sweden) fitted with a guard column (33 × 4.6 mm) filled with Pellicular Reversed Phase (purchased from Chrompack, The Netherlands). Readings of pH were carried out on a Radiometer PHM83 Autocal instrument at the temperature of study. ^{1}H-NMR spectra were obtained with a Varian 360L instrument. Melting points were taken in capillary tubes and are not corrected. Elemental analyses were performed at the Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Synthesis of Acyclovir Esters II–XI

The *N*-substituted 3- or 4-(aminomethyl)benzoate esters II–XI were prepared by reacting acyclovir (purchased from Sigma Chemicals Co., St. Louis, Missouri) with the appropriate *N*-substituted 3- or 4-(aminomethyl)benzoyl chloride (hydrochloride salt) in pyridine as exemplified for compound X.

4-(Morpholinomethyl)benzoyl chloride hydrochloride (12 mmol, 3.3 g) was added in four portions over 10 min to a mixture of acyclovir (8 mmol, 1.92 g) in 65 ml of pyridine. The mixture was stirred at room temperature for 20 hr and then evaporated under reduced pressure. The residue obtained was slurried in 25 ml of water and aqueous sodium hydroxide (2 *M*) was added to give a pH of 9 ± 0.2. Upon standing at 4°C for 5 hr, the precipitate formed was filtered off, washed with water, dried, and recrystallized from ethanol–water to give 2.2 g (62%) of acyclovir 4-(morpholinomethyl)benzoate ester (X) as the monohydrate.

The *N,N*-disubstituted 3- or 4-(aminomethyl)benzoyl chlorides were obtained by refluxing the corresponding aminomethylbenzoic acid hydrochloride with thionyl chloride (1 ml per 1 mmol of the acid) for 2 hr. The resulting solution was evaporated *in vacuo* to remove excess thionyl chloride

and reevaporated with benzene. The crude solid acid chloride (HCl salt) obtained was used in the esterification step without any further purification.

The *N,N*-disubstituted 4-(aminomethyl)benzoic acids (HCl salts) were prepared by reacting 4-(chloromethyl)-benzoic acid (from Aldrich-Chemie, F.R.G.) with the appropriate amine in ethanol as previously reported (13).

The *N,N*-disubstituted 3-(aminomethyl)benzoic acid hydrochlorides which have not been reported before were prepared via ethyl 3-(chloromethyl)benzoate obtained by reacting 3-(chloromethyl)benzoyl chloride (from Fluka AG, Switzerland) with ethanol. Reaction of ethyl 3-(chloromethyl)benzoate with an excess of the appropriate amine followed by hydrolysis yielded the 3-(aminomethyl)benzoic acids as exemplified by the synthesis of 3-(*N,N*-diethylaminomethyl)benzoic acid hydrochloride:

3-(Chloromethyl)benzoyl chloride (75 mmol, 10.7 ml) was dropwise added under stirring to ethanol (150 ml) cooled in an ice bath. After stirring for 10 min at room temperature the reaction mixture was evaporated *in vacuo*. The residue was dissolved in ethanol (200 ml), diethylamine (300 mmol, 31.2 ml) was added, and the mixture was stirred at 60°C for 20 hr. The mixture was evaporated *in vacuo* and hydrochloric acid (8 *M*, 150 ml) was added to the residue. The mixture was refluxed for 2 hr and evaporated *in vacuo*. The residue was dissolved in sodium hydroxide (2 *M*, 120 ml) and the solution was washed with three 100-ml portions of ether. The aqueous solution was acidified with concentrated hydrochloric acid and evaporated *in vacuo*. The residue was extracted with two 100-ml portions of boiling ethanol and the combined extracts were evaporated *in vacuo*. Crystallization of the residue from ethanol–acetonitrile–ether yielded 10.8 g (59%) of 3-(*N,N*-diethylaminomethyl)benzoic acid hydrochloride, m.p. 144–146°C.

By using essentially the same method the following 3-(aminomethyl)benzoic acids were obtained: 3-(*N,N*-dimethylaminomethyl)benzoic acid hydrochloride, m.p 178–180°C (from ethanol–ether); 3-(*N,N*-dipropylaminomethyl)benzoic acid hydrochloride, m.p. 203–205°C (from acetonitrile); 3-(*N,N*-dibutylaminomethyl)benzoic acid hydrochloride, m.p. 110–113°C (from ethanol–acetonitrile–ether); and 3-(morpholinomethyl)benzoic acid hydrochloride, m.p. 246–248°C (from ethanol).

Physical and analytical data for the acyclovir esters **II**–**XI** are given in Table I. The ^{1}H-NMR and UV spectra of the compounds were consistent with their structures. HPLC analysis as described below revealed a content of acyclovir less than 2%.

In the case of the ester **VII**, the hydrochloride salt was prepared as follows. Compound **VII** (1 mmol) was dissolved

Table I. Physical and Analytical Data of Various *N*-Substituted (Aminomethyl)benzoate Esters of Acyclovir

Compound	m.p. (°C)	Formula		Analysis (%) Calculated	Found
II	182–184	$C_{18}H_{22}N_6O_4$, 1.5 H_2O	C	52.30	52.40
			H	6.08	6.18
			N	20.33	20.16
III	185–186	$C_{18}H_{22}N_6O_4$, 1.5 H_2O	C	52.30	52.08
			H	6.08	5.89
			N	20.33	20.33
IV	190–192	$C_{20}H_{26}N_6O_4$, H_2O	C	55.46	55.36
			H	6.52	6.39
			N	19.43	19.53
V	195–197	$C_{20}H_{26}N_6O_4$, 1.75 H_2O	C	53.86	53.92
			H	6.66	6.64
			N	18.44	18.69
VI	194–195	$C_{22}H_{30}N_6O_4$, H_2O	C	57.38	57.58
			H	7.00	7.02
			N	18.24	18.31
VII	193–194	$C_{22}H_{30}N_6O_4$, 1.25 H_2O	C	56.82	56.65
			H	7.04	6.88
			N	18.07	18.14
VIII	200–201	$C_{24}H_{34}N_6O_4$, H_2O	C	61.00	60.88
			H	7.68	7.75
			N	17.78	17.75
IX	213–215	$C_{20}H_{24}N_6O_5$, H_2O	C	53.81	53.95
			H	5.87	5.99
			N	18.82	18.75
X	218–220	$C_{20}H_{24}N_6O_5$, H_2O	C	53.81	54.01
			H	5.87	5.93
			N	18.82	18.92
XI	187–189	$C_{21}H_{26}N_6O_4$, 1.5 H_2O	C	55.62	55.35
			H	6.44	6.38
			N	18.53	18.60

in 5 ml of 0.2 *M* hydrochloric acid. Upon the addition of 10 ml of acetone followed by about 10 ml of ether, the hydrochloride salt precipitated. It was filtered off and recrystallized from acetonitrile. Microanalysis showed the presence of 1.25 mol of water. A nitrate salt (isolated as a monohydrate) could be prepared in a similar way.

HPLC Analysis

A reversed-phase HPLC procedure was used for the quantitative determination of the (aminomethyl)benzoate esters of acyclovir. For the analysis of most esters, a Supelco column and a mobile phase consisting of a mixture of acetonitrile and 0.1% (v/v) phosphoric acid, with triethylamine added at a concentration of 10^{-3} *M* to improve peak shape, was used. For the esters IV, V, and XI the ratio (v/v) used was 10:90, for VI and VII it was 15:85, for VIII the ratio was 25:75, and for IX the ratio was 5:95. For the esters II, III, and X a mobile phase consisting of methanol–0.02 *M* phosphate buffer, pH 7.6 (40:60, v/v), was used. The flow rate was 1.0 ml min^{-1} and the column effluent was monitored at 254 nm. Under these conditions the esters showed retention times from 2 to 10 min and they were adequately separated from the products of hydrolysis, acyclovir and the corresponding (aminomethyl)benzoic acid. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

In the stability study of ester VI, the hydrolysis was monitored by following the formation of 3-(*N,N*-dipropylaminomethyl)benzoic acid. This acid was determined using a mobile phase system of acetonitrile–0.1% (v/v) phosphoric acid (5:95, v/v) containing triethylamine (10^{-3} *M*) and a flow rate of 2.0 ml min^{-1}, the column effluent being monitored at 215 nm. Under these conditions the acid showed a retention time of 4.5 min.

Analysis of acyclovir was done by ion-paring with sodium 1-heptanesulfonate (SHS) on a Ultropac column. The mobile phase consisted of methanol and an aqueous solution of 0.02 *M* sodium acetate and 0.007 *M* SHS at the ratio 10:90 (v/v). The pH of the mixture was adjusted to 3.2. The column effluent was monitored at 250 nm and the flow rate was 1.2 ml min^{-1}. Under these conditions acyclovir showed a retention time of 5.0 min.

Stability Studies

The hydrolysis of ester VI was studied in aqueous buffer solutions at a 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 ester in ethanol–water to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the ester 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 ester were determined from the slopes of linear plots of the logarithm of residual ester against time.

For slowly proceeding reactions (at pH 1–7.4) the rate constants were obtained by measuring the initial rate of formation of 3-(*N,N*-dipropylaminomethyl)benzoic acid. In these cases, the initial ester concentration was 1×10^{-3} *M*. The formation of 3-(*N,N*-dipropylaminomethyl)benzoic acid 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 3-(*N,N*-dipropylaminomethyl)benzoic acid formed versus time with the initial ester concentration.

Hydrolysis Studies in Human Plasma

The esters II–XI 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 mixture of 0.1 *M* aqueous zinc sulfate solution–70% perchloric acid (1:1, v/v) or, in the cases of the esters II, III, and X, 500 μl of 0.1 *M* zinc sulfate–methanol (1:1, v/v) in order to deproteinize the plasma. After centrifugation for 3 min at 13,000 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.

Determination of Solubility and Partition Coefficients

The solubility of the esters in water was determined by the phase-solubility technique at 21°C. An excess of the compounds was added to 0.1 *M* buffer solutions of varying pH's and the suspensions were placed in an ultrasonic bath for 10 min and then rotated on a mechanical spindle for 24–48 hr 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 ester by HPLC.

The apparent partition coefficients of the esters were determined in an octanol–buffer system at 21°C. The aqueous phase was a 0.05 *M* phosphate buffer solution of pH 7.4. The buffer solution and octanol were mutually saturated at 21°C before use. The compounds were dissolved in the aqueous buffer phase at a concentration of 10^{-4} *M* and the octanol–water mixtures were shaken for about 2 hr to reach a distribution equilibrium. The volumes of each phase were chosen so that the solute concentration in the aqueous phase, before and after distribution, could readily be measured using the above-mentioned HPLC procedure. The partition coefficients (*P*) were calculated from Eq. (1):

$$P = \left(\frac{C_i - C_w}{C_w}\right)\left(\frac{V_w}{V_o}\right) \quad (1)$$

where C_i and C_w represent the solute concentration in the aqueous buffer phase before and after distribution, respectively, and V_w represents the volume of the aqueous and V_o the volume of the octanol phase.

RESULTS AND DISCUSSION

Enzymatic Hydrolysis of Acyclovir Esters

The rates of hydrolysis of the acyclovir esters II–XI were determined in 80% human plasma (pH 7.4) at 37°C. All esters underwent complete hydrolysis as indicated by the quantitative formation of acyclovir, and in all cases the hydrolysis exhibited strict first-order kinetics over several half-lives. Typical first-order plots are shown in Fig. 1. The half-lives obtained are listed in Table II. 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 the absence of plasma, i.e., in a pH 7.4 phosphate buffer at 37°C, exceeded 500 hr.

As can be seen from the data the (aminomethyl)-benzoate esters are readily converted to acyclovir under conditions similar to those prevailing *in vivo*. Although all the esters II–XI 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 plasma-catalyzed hydrolysis. Thus, the 3-substituted esters II, IV, and VI are markedly more reactive than their 4-substituted analogues, whereas the 3- and 4-(morpholinomethyl)benzoate esters show almost the same reactivity.

Table II. Rate Data for the Plasma-Catalyzed Hydrolysis, Partition Coefficients (P), Aqueous Solubilities, and pK_a Values for Various N-Substituted (Aminomethyl)benzoate Esters of Acyclovir

Compound	$t_{1/2}$ in 80% human plasma (min)[a]	log P[b]	B_s (mg ml^{-1})[c]	pK_a[d]
I (acyclovir)		−1.47	1.4[e]	
II	7.0	−0.94		
III	33	−0.92	1.3	8.2
IV	7.5	−0.37		
V	25	−0.35		
VI	0.8	0.60	0.13	8.3
VII	57	0.60		
VIII	2.3	1.50	0.006	8.4
IX	4.6	−0.04		
X	3.7	−0.05	0.77	6.1
XI	8.5	−0.11		

[a] At 37°C.
[b] Between octanol and 0.02 M phosphate buffer, pH 7.40 (21°C).
[c] Solubility of the free-base form of the acyclovir esters (21°C).
[d] Ionization constants for the amino group in the esters at 21°C.
[e] Solubility of acyclovir in water at 21°C.

Stability in Aqueous Solution

Due to its high rate of plasma-catalyzed hydrolysis, the 3-(*N,N*-dipropylaminomethyl)benzoate ester VI appears to be the most promising prodrug of acyclovir for parenteral delivery. The stability of this ester derivative in aqueous solution was therefore examined as a function of pH and temperature with the aim of predicting the shelf life of aqueous solutions of the prodrug.

The kinetics of hydrolysis of ester VI was studied in aqueous buffer solutions at 60°C over the pH range 1.1–10.8. At the buffer concentration used (0.02 M) no significant buffer catalysis was observed. Under the experimental conditions used, the degradation of the compound followed strict first-order kinetics. In alkaline and neutral solutions the disappearance of the ester was accompanied by the formation of acyclovir in stoichiometric amounts. At pH <7 and 60°C the stability of the ester was so high that it was not practical to monitor the degradation by measuring the intact ester. Instead, the initial rate method was used. In this method formation of the ester hydrolysis product 3-(*N,N*-dipropylaminomethyl)benzoic acid was monitored as a function of time up to an extent of degradation of 1–3%. The validity of this procedure was confirmed by following the rate of degradation of ester VI in 0.5 M hydrochloric acid both by the initial rate method and by measuring the amount of remaining ester. The rate constants obtained therefrom were of the same order of magnitude.

The influence of pH on the rates of hydrolysis at 60°C is shown in Fig. 2, in which the logarithm of the observed pseudo-first-order rate constants (k_{obs}) is plotted against pH. Maximal stability is seen to occur at pH 3–4. In the pH range investigated the acyclovir ester can exist in several ionic forms. The weakly basic imidazole moiety in acyclovir has a pK_a of 2.4, whereas the 2-NH moiety is weakly acidic, with a pK_a value of 9.1 at 20–25°C (14). These values are not expected to change significantly upon esterification of the

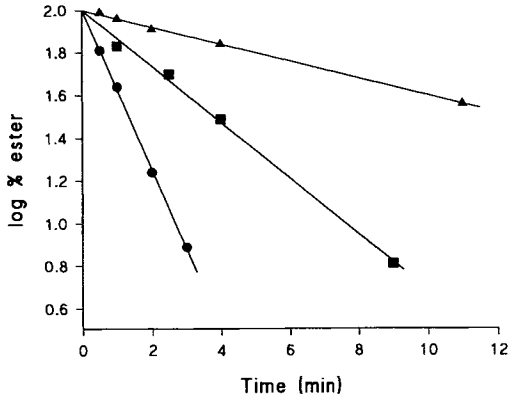

Fig. 1. First-order plots for the hydrolysis of the acyclovir esters VI (●), IV (▲), and VIII (■) in 80% human plasma at 37°C.

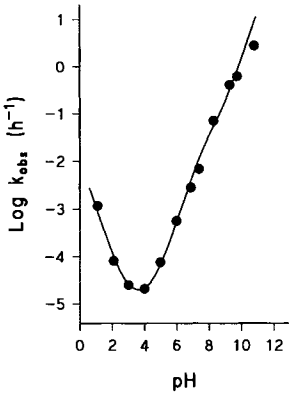

Fig. 2. The pH–rate profile for the degradation of acyclovir ester VI in aqueous solution (μ = 0.5) at 60°C.

hydroxyl group. As described below the pK_a of the *N,N*-dipropylamino group in the ester moiety is 8.3 at 21°C. The shape of the pH–rate profile can be accounted for in terms of a specific acid-catalyzed (k_H) and a water-catalyzed or spontaneous (k_0) reaction of the protonated form of the ester and a specific base-catalyzed hydrolysis of the protonated (k_{OH}) and unprotonated (k'_{OH}) forms of the ester, the ionization state of the acyclovir moiety being without significant influence on the reactivity in the pH range studied. Mathematically,

$$k_{obs} = k_H a_H \frac{a_H}{a_H + K_a} + k_0 \frac{a_H}{a_H + K_a} + k_{OH} a_{OH} \frac{a_H}{a_H + K_a} + k'_{OH} a_{OH} \frac{K_a}{a_H + K_a} \quad (2)$$

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activities, respectively, and K_a is the apparent ionization constant of the protonated amino group in the ester. The following values for the specific rate constants (at 60°C) were derived from the rate data:

$$k_H = 1.1 \times 10^{-2}\ M^{-1}\ hr^{-1}$$
$$k_0 = 1.5 \times 10^{-5}\ hr^{-1}$$
$$k_{OH} = 5.5 \times 10^{3}\ M^{-1}\ hr^{-1}$$
$$k'_{OH} = 1.5 \times 10^{3}\ M^{-1}\ hr^{-1}$$
$$K_a = 10^{-8.0}$$

The solid curve in Fig. 2 was constructed from these constants and Eq. (2). The slightly greater reactivity of the protonated form of the ester in hydroxide ion-catalyzed hydrolysis relative to the unprotonated form can be ascribed to the greater electron-withdrawing effect of the protonated amino group as discussed before in a study on the hydrolysis of similar esters of metronidazole (10).

In order to predict the stability of the ester in aqueous solution at normal storage temperatures, the rates of hydrolysis in a 0.02 *M* acetate buffer of pH 4.0 were also determined at 70 and 80°C. At this pH value the spontaneous or water-catalyzed hydrolysis [the k_0 term in Eq. (2)] is the predominant degradation reaction. In Fig. 3 the rate data obtained are plotted according to the Arrhenius equation:

$$\log k_{obs} = \log A - \frac{E_a}{2.303R} \frac{1}{T} \quad (3)$$

where *A* is the frequency factor, E_a is the apparent energy of activation, *R* is the gas constant, and *T* is the absolute temperature. From this plot values of the Arrhenius parameters *A* and E_a of 1.3×10^9 and 20.9 kcal mol^{-1}, respectively, were obtained. On the basis of these data it is possible to estimate the shelf life of aqueous solutions of the ester at pH 4.0 and various temperatures. Defining the shelf life as the time required to degrade 10% of the ester ($t_{10\%}$), the calculations show that a shelf life of 26 years is achieved at 25°C and 44 years at 20°C. Thus, the acyclovir ester can readily be formulated as highly stable, ready-to-use solutions at pH 3–4. This stability is of the same order of magnitude as that predicted for similar esters of metronidazole (10).

For concentrated prodrug solutions the shelf life may often be limited by precipitation of the parent drug formed upon hydrolysis rather than by loss of prodrug (15–17). This problem, however, is unlikely to occur because of the extremely high stability of the ester. Thus, it can be calculated that for a 20.2% (w/v) solution of ester VI at pH 4.0, which is equivalent to 10% acyclovir on a molar basis, the extent of ester degradation needed to form acyclovir at a concentration corresponding to its water solubility (1.4 mg ml^{-1}) at 21°C is 1.4%. The time required to achieve this extent of hydrolysis can be calculated to be 3.6 years at 25°C and 6.2 years at 20°C.

Solubility and Lipophilicity

Being weak bases with a pK_a value of about 8 (for the morpholine-containing esters the pK_a is 6.1), the (aminomethyl)benzoate esters are readily soluble at weakly acidic pH values. The aqueous solubility of the free-base forms of some esters at 21°C is listed in Table II. For the esters VI and VIII the pH–solubility profiles were obtained at 21°C (Fig. 4). In the pH range studied the total solubility (S_T) of the compounds can be expressed by the following equation:

$$S_T = B_S \frac{a_H + K_a}{K_a} \quad (4)$$

where B_S is the solubility of the free-base form and K_a is the ionization constant of protonated ester. The curves drawn in Fig. 4 were constructed from Eq. (4) and the values of B_S and pK_a listed in Table II. To obtain a 20% (w/v) solution of ester

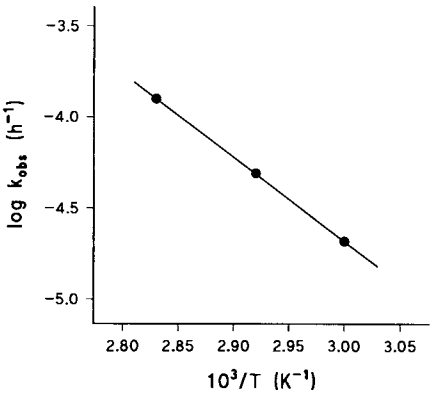

Fig. 3. Arrhenius plot of the rate of hydrolysis of the acyclovir ester VI in 0.02 *M* acetate buffer solution (μ = 0.5) of pH 4.0.

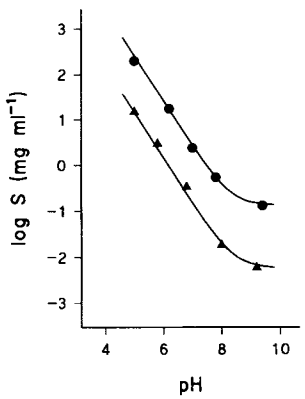

Fig. 4. Aqueous solubility–pH profiles for the acyclovir esters VI (●) and VIII (▲) at 21°C. The points are experimental, while the curves are calculated from Eq. (4).

VIII, the pH should be 3.9 or less. For the more soluble ester VI a 20% (w/v) solution can be made at a pH up to 5.1.

The lipophilicity of the ester prodrugs and of acyclovir was assessed by measuring the partition coefficients (P) between octanol and 0.02 M phosphate buffer of pH 7.40. At this pH the derivatives are partly protonated and the P values are therefore rather distribution coefficients. The log P values obtained are shown in Table II. The data show that the esters are much more lipophilic than the parent drug and that (aminomethyl)benzoate esters with varying lipophilicity can readily be obtained by selecting appropriate amino substituents. The log P value found for acyclovir is close to that (-1.62) reported by Kristl *et al.* (14).

CONCLUSIONS

This study shows that N-substituted 3- or 4-(aminomethyl)benzoate esters of acyclovir are promising prodrugs for improving the parenteral delivery of the parent drug. The derivatives combine a high stability and solubility in weakly acidic aqueous solution with a facile enzymatic conversion in plasma. In addition, the esters show greatly increased lipophilicity at physiological pH as compared with acyclovir itself. These properties make the novel esters promising prodrug forms for acyclovir to improve not only the parenteral delivery characteristics of the drug but also the ocular, dermal, or oral delivery. Studies are in progress to explore these possibilities.

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