International Journal of Pharmaceutics, 70 (1991) 137–146

ADONIS 037851739100142O

IJP 02361

Aminomethylbenzoate esters of chloramphenicol as a novel prodrug type for parenteral administration

Ejvind Jensen and Hans Bundgaard

The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, DK-2100 Copenhagen (Denmark)

(Received 21 October 1990)
(Accepted 26 November 1990)

Key words: Chloramphenicol; Prodrug; Enzymatic hydrolysis; Stability; Solubility

Summary

Various *N*-substituted 3-aminomethylbenzoate esters of chloramphenicol were synthesized and evaluated as water-soluble prodrugs with the aim of developing preparations more suitable for parenteral administration than the presently used monosuccinate ester. This ester shows a variable and incomplete bioavailability due to slow hydrolysis to the parent drug in vivo. Half-lives of hydrolysis of the aminomethylbenzoate esters in 80% human plasma ranged from 0.9 to 50 min. The solubility of some esters in weakly acidic solution was greater than 10%. The stability of 3-(diethylaminomethyl)benzoate ester was studied in detail as a function of pH at 80°C. It showed maximal stability at pH around 4. At this pH the stability was very high and even higher than that of chloramphenicol itself. The combination of high water solubility and stability in weakly acidic aqueous solution with a rapid rate of plasma-catalyzed hydrolysis makes the *N*-substituted 3-aminomethylbenzoate esters a promising new prodrug type for chloramphenicol suitable for parenteral administration.

Introduction

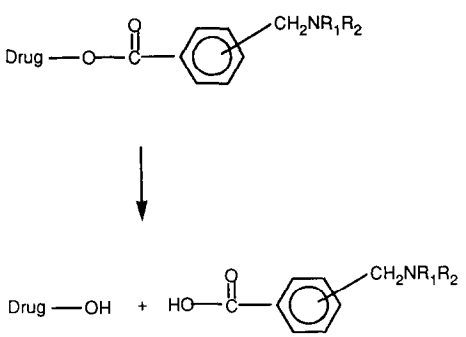
The broad spectrum antibiotic chloramphenicol (**I**) has in recent years gained increasing importance, predominantly because of its valuable effect in the treatment of meningitis and pneumonia caused by ampicillin-resistant strains of *Haemophilus influenzae* (Ambrose, 1984). Due to the limited water solubility (about 4 mg ml^{-1}) of chloramphenicol the highly water-soluble sodium salt of the 3-monosuccinate ester is used clinically as a prodrug for parenteral administration (Glazko et al., 1958). This ester is, however, not an optimal prodrug since it is incompletely converted to the parent active drug in vivo. Thus, Kauffman et al. (1981) have found that from 6 to 80% of a parenteral dose of chloramphenicol monosuccinate given to paediatric patients was recovered unchanged in the urine. Similar findings have been reported in studies including adults (Glazko et al., 1977; Slaughter et al., 1980; Nahata and Powell, 1981; Burke et al., 1982; Kramer et al., 1984). As summarized by Ambrose (1984) the bioavailability of chloramphenicol after intravenous administration of the succinate ester averages approx. 70%, but the range is quite variable. This incomplete and variable bioavailability is due to the resistance of the succinate ester to undergo rapid enzymatic

Correspondence: H. Bundgaard, The Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

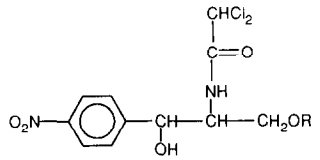
hydrolysis in the organism. In vitro studies have shown that the ester is stable in blood but can be hydrolyzed by esterases present in the liver, lung and kidney (Schmidt and Vömel, 1965).

More suitable water-soluble prodrugs of chloramphenicol may be amino acid esters such as the glycinate and morpholinoacetate esters (Concilio et al., 1958; Lauria and Defranceschi, 1958) since such esters are more readily hydrolyzed enzymatically to the parent drug (Trivellato et al., 1958; Azzollini et al., 1972). However, the α-amino acid esters exhibit a poor stability in aqueous solution (Concilio et al., 1958; Lanza, 1960), making it impossible to prepare ready-to-use solutions. The major reason for the high instability of such amino acid esters at pH 3–7 is the intramolecular catalytic effect exhibited by the protonated amino group on the ester bond cleavage (Bruice and Benkovic, 1976; Bundgaard et al., 1984). We have recently provided a solution of this problem which involves the incorporation of a phenyl group between the ester and amino group (Bundgaard et al., 1989). Such 3- or 4-aminomethylbenzoate esters (Scheme 1) of metronidazole, steroids and other hydroxyl-containing agents combine a high stability in weakly acidic aqueous solution, where the solubility of the esters is high, with a rapid rate of hydrolysis in plasma (Bundgaard et al., 1989; Jensen et al., 1990, 1991).

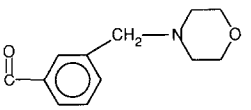
In this paper we report that such *N*-substituted 3-aminomethylbenzoate esters of chloramphenicol (**II–V**) may be promising prodrugs for parenteral administration. The enzymatic hydrolysis and chemical stability of the esters are described. Since the pH-rate profile for the degradation of chloramphenicol in aqueous solution has never been described this has also been studied and is included in the present paper.

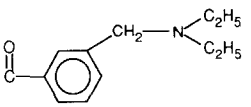


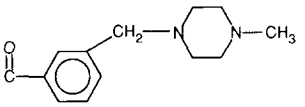
Scheme 1.

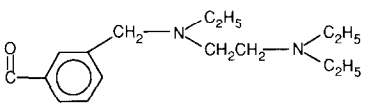


I R = H









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). 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 spectra were run on a Varian 360L instrument. Melting points were taken in capillary tubes and are not corrected. Elemental analysis was performed by G. Cornali,

Microanalytical Laboratory, Leo Pharmaceutical Products, Ballerup, Denmark.

Synthesis of chloramphenicol esters

The *N*-substituted 3-aminomethylbenzoate esters **II–V** were prepared by esterifying chloramphenicol with 3-chloromethylbenzoyl chloride (from Fluka AG, Switzerland) and subsequent reaction of the chloromethylbenzoate ester obtained with the appropriate amine in the presence of catalytic amounts of sodium iodide.

Chloramphenicol 3-(3-chloromethyl)benzoate

To a suspension of chloramphenicol (6.46 g, 20 mmol) in dichloromethane (100 ml) was added triethylamine (3.2 ml, 22 mmol) followed by 3-chloromethylbenzoyl chloride (3.14 ml, 22 mmol). The mixture was stirred overnight at room temperature, washed with a 2% aqueous solution of sodium bicarbonate, dried over anhydrous sodium sulphate and evaporated in vacuo. The solid residue obtained was recrystallized from ethanol-water to give 6.5 g of the title compound. M.p. 137–138°C.

Anal.: Calc. for $C_{19}H_{17}Cl_3N_2O_6$: C, 47.97; H, 3.60; Cl, 22.35; N, 5.89. Found: C, 47.95; H, 3.75; Cl, 22.23; N, 5.80.

Chloramphenicol 3-(3-N,N-diethylaminomethyl)benzoate, hydrochloride (III)

A mixture of chloramphenicol 3-(3-chloromethyl)benzoate (0.48 g, 1 mmol), sodium iodide (0.15 g, 1 mmol) and diethylamine (0.83 ml, 8 mmol) in 10 ml tetrahydrofuran was stirred at 50°C for 16 h. The mixture was filtered and evaporated in vacuo. The residue was taken up in ethyl acetate (50 ml) and water (40 ml) and the organic phase was separated, washed with water, dried and evaporated in vacuo. The residue was dissolved in ether and ethanol and 0.5 ml of a 2.5 M methanolic HCl solution added. Upon standing at 4°C for 20 h the precipitate formed was filtered off and recrystallized from ethanol to yield 0.38 g of the title compound.

The other esters were prepared in a similar way. Physical and analytical data for the esters **II–V** are given in Table 1. The NMR spectra of the compounds were consistent with their structures.

Kinetic measurements

Hydrolysis in aqueous solutions The hydrolysis of ester **III** and chloramphenicol (**I**) was studied in aqueous buffer solutions at constant temperature (±0.02°C). The buffers used were hydrochloric acid, acetate, phosphate, and borate buffers; the

TABLE 1

Physical and analytical data of various esters of chloramphenicol

Compound	Form	M.p. (°C)	Formula	Analysis (%)		
				Calculated		Found
II	HCl salt	184–185	$C_{21}H_{22}Cl_3N_3O_7$	C	47.16	47.01
				H	4.15	4.23
				N	7.86	7.79
III	HCl salt	210–211	$C_{23}H_{28}Cl_3N_3O_6$	C	50.33	50.31
				H	5.14	5.27
				Cl	19.38	19.29
				N	7.66	7.44
IV	HCl salt (2 equiv.)	90– 92	$C_{24}H_{30}Cl_4N_4O_6$, 2.5 H_2O	C	43.85	43.83
				H	5.36	5.55
				N	8.52	8.41
V	HCl salt (2 equiv.)	123–125	$C_{27}H_{38}Cl_4N_4O_6$, H_2O	C	48.08	48.12
				H	5.98	6.07
				N	8.31	8.23

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 rates of hydrolysis were followed by monitoring the disappearance of the ester or chloramphenicol using a reversed-phased HPLC procedure. A deactivated Supelcosil column was eluted with a mobile phase consisting of 10% v/v acetonitrile in 0.05 M acetate buffer (pH 5.0) for the analysis of chloramphenicol whereas a solvent system of methanol-acetonitrile-0.1% phosphoric acid (5 : 30 : 65 v/v) containing triethylamine (10^{-3} M) to improve peak shape was used for the analysis of ester **III**. The flow rate was 1.0 ml min^{-1} and the column effluent was monitored at 280 nm. Under these conditions chloramphenicol showed a retention time of 1.6 min; the degradation products appeared in the solvent front. The ester **III** had a retention time of 3.2 min and was adequately separated from its hydrolysis products. Quantitation of the compounds was done by measuring the peak heights in relation to those of standards chromatographed under the same conditions.

The reactions were initiated by adding 100 μl of a stock solution of the compounds in ethanol or water to 10 ml of preheated buffer solution in screw-capped test tubes, the final concentration of the compounds being about 5×10^{-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 compounds were determined from the slopes of linear plots of the logarithm of the residual amount against time.

Hydrolysis in human plasma The derivatives **II**–**V** 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 solutions were withdrawn and added to 500 μl of a 1% w/v 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. Mixtures of methanol, acetonitrile and 0.1% phosphoric acid were used as eluents. For analysis of ester **II**, **III** and **V** the composition was 5 : 40 : 55 v/v and for ester **IV** it was 5 : 35 : 60 v/v. 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 were 2–3 min. It was ensured that in each case adequate separation of the ester from hydrolysis products, chloramphenicol and the corresponding aminomethylbenzoic acid, was achieved. Pseudo-first-order rate constants were calculated from the slopes of linear plots of the logarithm of residual ester against time.

*Rearrangement of ester **III** in aqueous solution* The kinetics of the rearrangement of ester **III** to the corresponding C-1 ester (**IIIa**) was studied in aqueous buffer solutions prepared as mentioned above. The procedure and analysis were done as described for the hydrolysis studies in aqueous solutions. It was established that the absorptivities of **III** and **IIIa** were equivalent under the HPLC conditions used, so a standard curve for **III** was used for the quantitation of both **III** and **IIIa**. Since the hydrolysis of ester **III** at 37°C was insignificant over the time of the study, the sum of the concentration of **III** and **IIIa** at all times was equal to the initial concentration of **III**. The sum of the forward (k_f) and reverse (k_r) rate constants describing the equilibrium of **III** and **IIIa** was calculated from the slopes of linear plots of the logarithm of (**IIIa**(eq) − **IIIa**(t)) against time. **IIIa**(eq) is the concentration of **IIIa** after equilibrium is attained and **IIIa**(t) is the concentration of **IIIa** at time t.

Determination of aqueous solubility The water solubility of the esters was determined at 21°C by adding excess amounts of the compounds to water in screw-capped test tubes. The mixtures were rotated on a mechanical spindle for 20–30 h to attain equilibrium. Upon filtration an aliquot of the filtrate was diluted with water and analyzed by HPLC.

Results and Discussion

Enzymatic hydrolysis of chloramphenicol esters

The rates of hydrolysis of the chloramphenicol esters **II**–**V** were determined in 80% human plasma

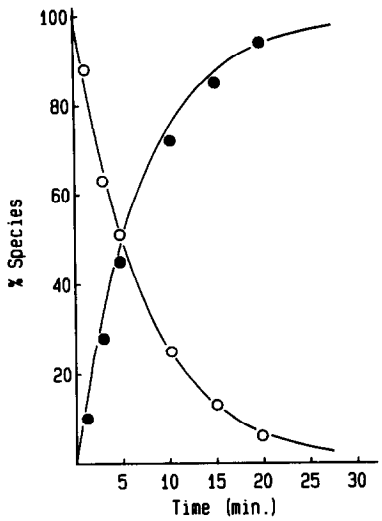


Fig. 1. Time courses for ester **III** (○) and chloramphenicol (●) during hydrolysis of the ester derivative in 80% human plasma at 37°C.

(pH 7.4) at 37°C. All esters underwent complete hydrolysis as indicated by the quantitative formation of chloramphenicol (Fig. 1), and in all cases the hydrolysis exhibited strict first-order kinetics over several half-lives. Typical first-order plots are shown in Fig. 2. The half-lives for the hydrolysis in 80% human plasma solutions are given in Table 2. A demonstration of the enzymatic conversion of

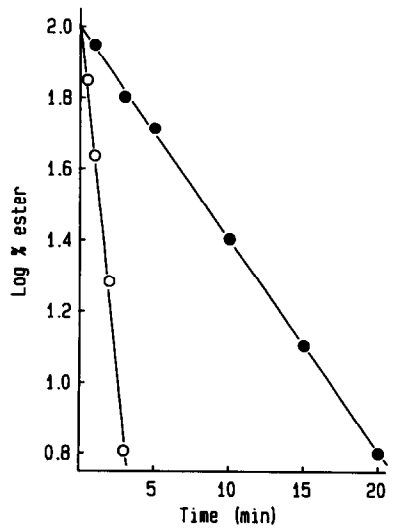


Fig. 2. First-order plots for the degradation of ester **IV** (●) and **V** (○) in 80% human plasma at 37°C.

TABLE 2

Half-lives of hydrolysis of various N-substituted 3-aminomethylbenzoate esters of chloramphenicol in 80% human plasma at 37°C

Ester	Half-life (min)
II	50
III	8.0
IV	5.0
V	0.9

the esters in plasma is provided by the fact that the half-lives of hydrolysis in absence of plasma, i.e. in a pH 7.4 phosphate buffer at 37°C, exceeded 50 h.

Inspection of the rate data shows that the derivatives except the 3-morpholinomethylbenzoate ester (**II**) are rapidly converted to chloramphenicol at conditions similar to those prevailing in vivo. It is noticeable that even a minor change in the structure of the substituted amino group has a pronounced effect on the enzymatic lability of the ester bond. This has also been observed for similar aminomethylbenzoate esters of other hydroxyl-containing agents like metronidazole and paracetamol (Bundgaard et al., 1989; Jensen et al., 1990, 1991).

Stability in aqueous solution

With the aim of obtaining knowledge of the chemical stability of the chloramphenicol prodrugs and to compare this with the stability of chloramphenicol itself the kinetics of hydrolysis of ester **III** and of chloramphenicol was studied in detail in aqueous buffer solution over the pH range 1.1–9.8 at 80°C.

At constant pH and temperature the degradation of the compounds displayed strict first-order kinetics and no significant buffer catalysis occurred at the low buffer concentration (0.02 M) used.

The influence of pH on the rates of hydrolysis of chloramphenicol and ester **III** are shown in Figs 3 and 4 in which the observed pseudo-first-order rate constants (k_{obs}) are plotted against pH. The pH-rate profile for chloramphenicol (Fig. 3) can

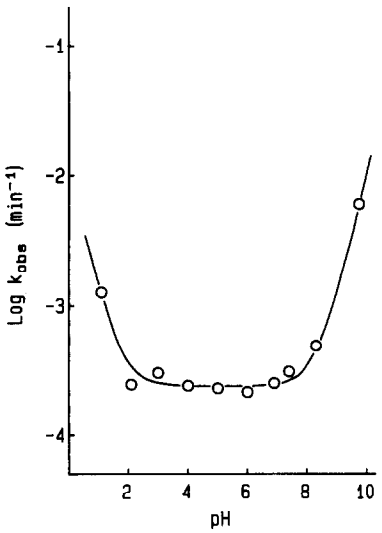


Fig. 3. The pH-rate profile for the degradation of chloramphenicol in aqueous solution ($\mu = 0.5$) at 80°C.

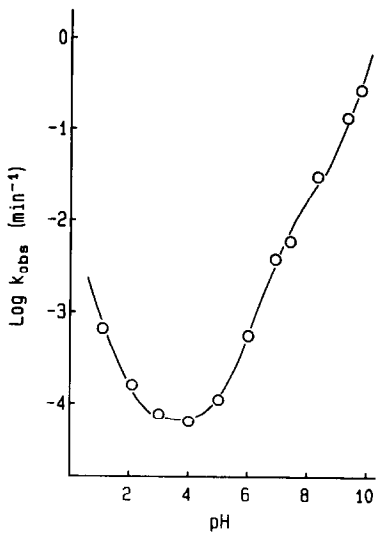


Fig. 4. The pH-rate profile for the degradation of the chloramphenicol ester **III** in aqueous solution ($\mu = 0.5$) at 80°C.

be accounted for by the following rate expression:

$$k_{obs} = k_H a_H + k_0 + k_{OH} a_{OH} \quad (1)$$

where a_H and a_{OH} refer to the hydrogen and hydroxide ion activities, respectively, k_H and k_{OH} are second-order rate constants for specific acid and base catalysis, respectively, and k_0 is the first-order rate constant for spontaneous or water-catalyzed degradation. The a_{OH} values were calculated from the measured pH at 80°C according to the following equation:

$$\log a_{OH} = \text{pH} - 12.60 \quad (2)$$

where 12.60 is the pK_w value at 80°C obtained by extrapolation of values at lower temperatures given by Conners et al. (1986). The various rate constants derived are listed in Table 3.

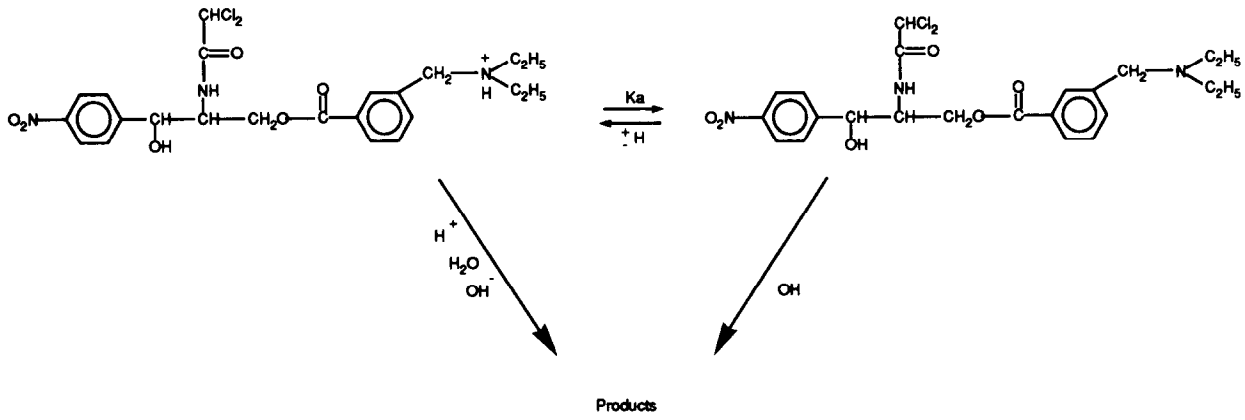
In the pH-range studied, ester **III** occurs in two forms with the amino function being unprotonated or protonated. The shape of the pH-rate profile (Fig. 4) indicates that the free base and the protonated forms of the ester undergo hydrolysis at different rates and that the hydrolysis can be described in terms of specific base-catalyzed reactions involving both species and a spontaneous and specific acid-catalyzed reaction of the protonated ester (Scheme 2). Accordingly, the following rate expression can be formulated:

$$\begin{aligned} k_{obs} = {} & k_H a_H (a_H/(a_H + K_a)) \\ & + k_0 (a_H/(a_H + K_a)) \\ & + k_{OH} a_{OH} (a_H/(a_H + K_a)) \\ & + k'_{OH} a_{OH} (K_a/(a_H + K_a)) \end{aligned} \quad (3)$$

TABLE 3

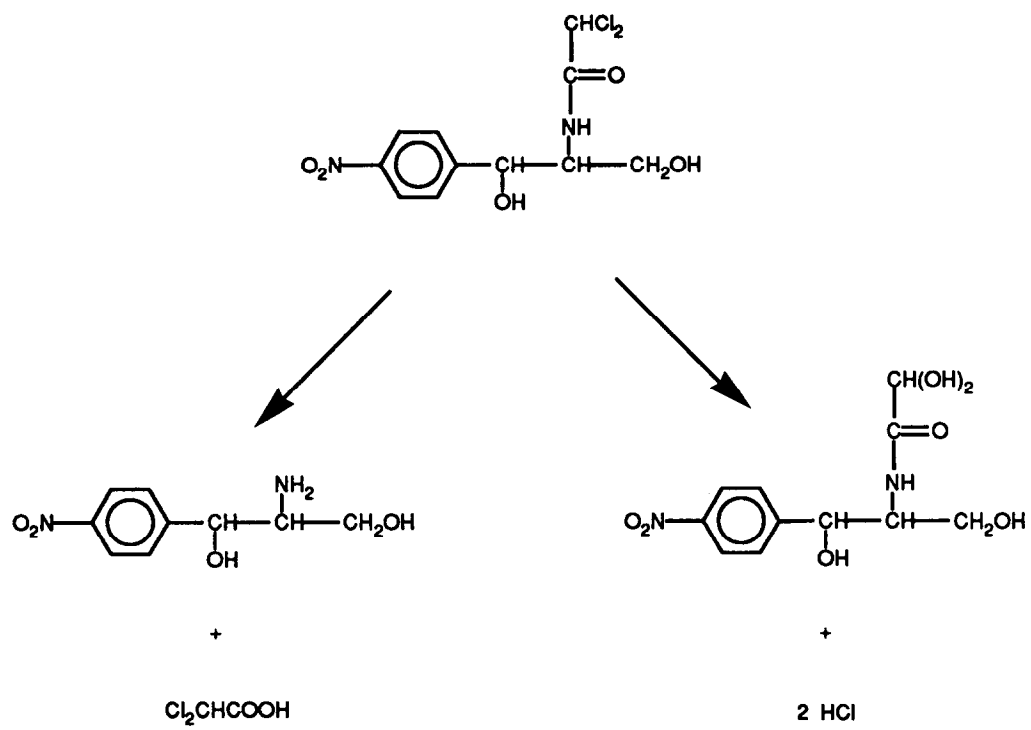
*Rate data for the degradation of chloramphenicol (**I**) and the chloramphenicol ester **III** in aqueous solution ($\mu = 0.5$) at 80°C*

Compound	k_H (M^{-1} min^{-1})	k_0 (min^{-1})	k_{OH} (M^{-1} min^{-1})	k'_{OH} (M^{-1} min^{-1})	pK_a
I	1.2×10^{-2}	2.3×10^{-4}	4.1		
III	8.0×10^{-3}	6.0×10^{-5}	2.0×10^{3}	2.0×10^{2}	7.5



Scheme 2.

where $a_H/(a_H + K_a)$ 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 ester. The rate constant k_0 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 the protonated ester, and k_{OH} and k'_{OH} are the second-order rate constants for the hydroxide ion-catalyzed hydroly-



Scheme 3.

sis of the protonated and unprotonated ester species, respectively. The various constants derived from the pH-rate profile are listed in Table 3. Using these constants, the solid curve in Fig. 4 was constructed.

The data obtained show that the protonated form of the ester **III** is 10-fold more reactive in hydroxide ion-catalyzed degradation than the unprotonated form. This difference in reactivity can most likely be ascribed to polar effects, the protonated amino group having a greater electron-withdrawing effect relative to the unprotonated form.

Two hydrolytic processes have been shown to be the major causes of chloramphenicol degradation in aqueous solution, hydrolysis of the amide bond and hydrolysis of the covalently bound chlorine in the dichloroacetamide moiety (Higuchi and Bias, 1953; Higuchi and Marcus, 1954; Higuchi et al., 1954) (Scheme 3). At pH < 7 the amide hydrolysis was found to be the predominant reaction whereas both reactions contribute to the overall degradation in alkaline solution.

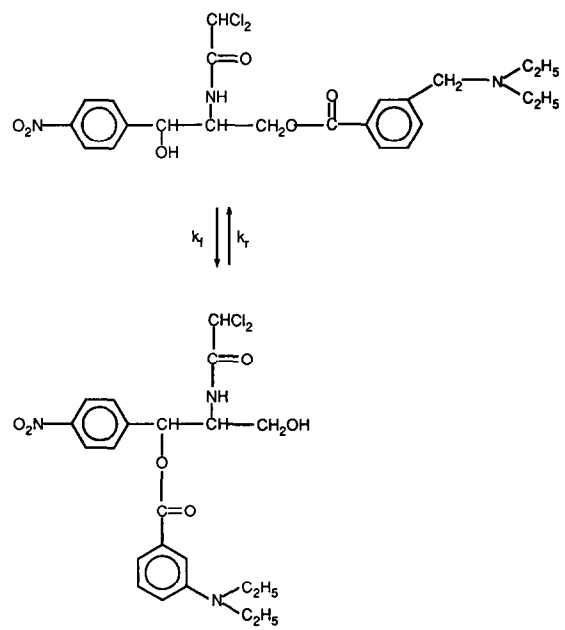
Comparing the stability of the ester **III** and chloramphenicol shows that the ester is less stable in neutral and basic solutions but, surprisingly, more stable in solutions at pH < 5. The predominant degradation pathway of the ester at basic pH is hydrolysis of the ester bond to yield chloramphenicol as revealed by product analysis studies using the HPLC assay for chloramphenicol. It was thus found that the loss of ester at pH 7–9 corresponded closely to the amount of chloramphenicol formed.

In solutions at pH < 5 the degradation of ester **III** may involve both ester hydrolysis and cleavage of its amide bond. As noted above the latter reaction is the only significant route of degradation of chloramphenicol at these conditions. The lower k_H and k_0 values observed for the ester relative to chloramphenicol (Table 3) therefore indicate that esterification of chloramphenicol stabilizes its amide function towards hydrolysis. This stabilizing effect is most likely due to the greater steric hindrance within the amide moiety obtained by esterification of the C-3 hydroxyl group. The greater polar effect of the ester group relative to the hydroxyl group should make the amide function more susceptible to hydrolysis but this effect is apparently counteracted by the steric effect.

From the Arrhenius equation and using an activation energy of 24.2 kcal mol^{-1} (Higuchi and Marcus, 1954) the shelf-life (i.e the time for 10% degradation) for chloramphenicol solutions at pH 4–6 and 25°C can be predicted to be about 6 months. In view of this limited stability the greater stability of the ester prodrug **III** at pH 4–5 may be of practical importance.

Rearrangement of chloramphenicol esters in aqueous solution

The 3-monosuccinate ester of chloramphenicol has previously been shown to exist in equilibrium with the corresponding 1-monosuccinate ester at pH 7.4 (Brent et al., 1980; Burke et al., 1980). At pH 7.4 and 37°C this acyl migration to form a mixture of 77% of the 3-ester and 23% of the 1-ester occurred with a half-time of 18 min (Burke et al., 1980). A similar rapid reversible rearrangement was seen with the esters **II**–**V** as revealed by HPLC (Scheme 4). The kinetics of the reaction was studied in detail with ester **III**. When this



Scheme 4.

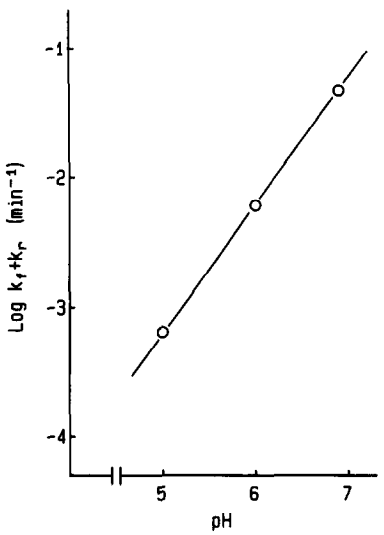


Fig. 5. The pH-rate profile for the reversible rearrangement of the chloramphenicol ester **III** to **IIIa** in aqueous solution at 37°C.

compound was incubated in buffer solutions a new peak (assigned to the 1-ester **IIIa**) in the HPLC chromatograms showing a retention time of 2.8 min rapidly appeared. Under the same chromatographic conditions ester **III** and chloramphenicol showed a retention time of 3.2 and 1.6 min, respectively.

The composition of the reaction solutions at pH 5–9 and 37°C after equilibrium between **III** and **IIIa** was attained was 85% of **III** and 15% of **IIIa** which is similar to the behaviour of the succinate ester. The influence of pH on the rate of reversible acyl migration is shown in Fig. 5 in which the logarithm of the pseudo-first-order rate constants $(k_f + k_r)$ is plotted against pH. The pH-rate profile obtained is a straight line with a slope of 0.98, indicating that the rearrangement is specific base-catalyzed:

$$(k_f + k_r) = a_{OH} k''_{OH} \quad (4)$$

where k''_{OH} is a second-order rate constant for the apparent hydroxide ion-catalyzed reaction. At 37°C k''_{OH} has a value of 2.4×10^5 M^{-1} min^{-1}. The half-times for the establishment of the equilibrium between **III** and **IIIa** can be calculated from the equation:

$$t_{1/2} = 0.693/a_{OH} k''_{OH} \quad (5)$$

The half-times calculated at pH 5, 6 and 7.4 at 37°C are 1200, 120 and 4.8 min, respectively.

Water solubility

The solubility of the chloramphenicol esters as hydrochloric acid salts in water was determined at 21°C. The ester **III** showed a solubility of 1.0 mg ml^{-1}, the pH of the saturated solution being 5.9. A much greater solubility was observed for the esters **IV** and **V**, the solubility of the dihydrochlorides being greater than 15% w/v.

Conclusions

The results described above show that *N*-substituted 3-aminomethylbenzoate esters of chloramphenicol may be suitable prodrug forms for parenteral use. In contrast to the presently used parenteral product, the 3-monosuccinate ester, these esters are rapidly hydrolyzed to the parent drug by plasma enzymes. Futhermore, the esters are characterized by possessing a high chemical stability at pH values (3–5) favouring their solubility in water.

Acknowledgements

This study was supported by the PharmaBiotec Research Centre and the Lundbeck Foundation.

References

Ambrose, P.J., Clinical pharmacokinetics of chloramphenicol and chloramphenicol succinate. *Clin. Pharmacokinet.*, 9 (1984) 222–238.

Azzollini, F., Gazzaniga, A., Lodola, E. and Natangelo, R., Elimination of chloramphenicol and thiamphenicol in subjects with cirrhosis of the liver. *Int. J. Clin. Pharmacol. Ther. Toxicol.*, 6 (1972) 130–134.

Brent, D.A., Chandrasurin, P., Ragouzeos, A., Hurlbert, B.S. and Burke, J.T., Rearrangement of chloramphenicol-3-monosuccinate. *J. Pharm. Sci.*, 69 (1980) 906–908.

Bruice, T.C. and Benkovic, S.J., *Bioorganic Mechanisms*, Vol. I, W.A. Benjamin, New York, 1966, pp. 134–145.

Bundgaard, H., Falch, E. and Jensen, E., A novel solution stable, water-soluble prodrug type for drugs containing a hydroxyl or an NH-acidic group. *J. Med. Chem.*, 32 (1989) 2503–2507.

Bundgaard, H., Larsen, C. and Arnold, E., Prodrugs as drug delivery systems. XXVII. Chemical stability and bioavailability of a water-soluble prodrug of metronidazole for parenteral administration. *Int. J. Pharm.*, 18 (1984) 79–87.

Burke, J.T., Wargin, W.A. and Blum, M.R., High-pressure liquid chromatographic assay for chloramphenicol, chloramphenicol-3-monosuccinate, and chloramphenicol-1-monosuccinate. *J. Pharm. Sci.*, 69 (1980) 909–912.

Burke, J.T., Wargin, W.A., Sherertz, R.J., Sanders, K.L., Blum, M.R. and Sarubbi, F.A., Pharmacokinetics of intravenous chloramphenicol sodium succinate in adult patients with normal renal and hepatic function. *J. Pharmacokinet. Biopharm.*, 10 (1982) 601–614.

Concilio, C., Tezza, A. and Perlotto, T., Su alcuni nuovi esteri del cloramfenicolo. Nota III - il glicinato come derivato idrosolubile. *Il Farm. Ed. Sci.*, 13 (1958) 393–398.

Conners, K.A., Amidon, G.L., and Stella, V.L., *Chemical Stability of Pharmaceuticals*, 2nd Ed, Wiley, New York, 1986, p. 818.

Glazko, A.J., Carnes, H.E., Kazenko, A., Wolf, L.M. and Reutner, T.F., Succinic acid esters of chloramphenicol. *Antibiot. Ann.*, (1958) 792–802.

Glazko, A.J., Dill, W.A., Kinbel, A.W., Goulet, J.R., Halloway, M.D. and Buchanan, R.A., Absorption and excretion of parenteral doses of chloramphenicol sodium succinate (CMS) in comparison with peroral doses of chloramphenicol (CM). *Clin. Pharmacol. Ther.*, 21 (1977) 104–104.

Higuchi, T. and Bias, C.D., The kinetics of degradation of chloramphenicol in solution. I. A study of the rate of formation of chloride ion in aqueous media. *J. Am. Pharm. Assoc.*, 42 (1953) 707–714.

Higuchi, T. and Marcus, A.D., The kinetics of degradation of chloramphenicol in solution. III. The nature, specific hydrogen ion catalysis, and temperature dependencies of the degradative reactions. *J. Am. Pharm. Assoc.*, 43 (1954) 530–535.

Higuchi, T., Marcus, A.D. and Bias, C.D., The kinetics of degradation of chloramphenicol in solution. II. Over-all disappearance rate from buffered solutions. *J. Am. Pharm. Assoc.*, 43 (1954) 129–134.

Jensen, E., Bundgaard, H. and Falch, E., Design of a water-soluble, solution-stable and biolabile prodrug of metronidazole for parenteral administration: N-substituted aminomethylbenzoate esters. *Int. J. Pharm.*, 58 (1990) 143–153.

Jensen, E., Falch, E. and Bundgaard, H., Water-soluble aminomethylbenzoate esters of phenols as prodrugs: Synthesis, enzymatic hydrolysis and chemical stability of paracetamol esters. *Arch. Pharm. Nord.*, (1991) in press.

Kauffman, R.E., Miceli, J.N., Strebel, L., Buckley, J.A., Done, A.K. and Dajani, A.S., Pharmacokinetics of chloramphenicol and chloramphenicol succinate in infants and children. *J. Pediatr.*, 98 (1981) 315–320.

Kramer, W.G., Rensimer, E.R., Ericsson, C.D. and Pickering, L.K., Comparative bioavailability of intravenous and oral chloramphenicol in adults. *J. Clin. Pharmacol.*, 24 (1984) 181–186.

Lanza, P., Su alcuni nuovi esteri del cloramfenicolo. Nota VI: Idrolisi alcalina di esteri basici del cloramfenicolo. *Il Farm. Ed. Sci.*, 15 (1960) 235–245.

Lauria, F. and Defranceschi, A., Basic chloramphenicol esters. *Chem. Ind.*, (1958) 1002.

Nahata, M.C. and Powell, D.A., Bioavailability and clearance of chloramphenicol after intravenous chloramphenicol succinate. *Clin. Pharmacol. Ther.*, 30 (1981) 368–372.

Schmidt, F.H. and Vömel, W., Über die Spaltung von Chloramphenicol-succinat durch tierische und menschliche Gewebe. *Klin. Wochenschr.*, 43 (1965) 535–539.

Slaughter, R.L., Pieper, J.A., Cerra, F.B., Brodsky, B. and Koup, J.R., Chloramphenicol sodium succinate kinetics in critically ill patients. *Clin. Pharmacol. Ther.*, 28 (1980) 69–77.

Trivellato, E., Malesani, L. and Concilio, C., Su alcuni nuovi esteri del cloramfenicolo: Nota IV - Ricerche farmacologiche sul glicinato di cloramfenicolo. *Il Farm. Ed. Sci.*, 13 (1958) 399–405.