International Journal of Pharmaceutics, 21 (1984) 201–209
Elsevier

IJP 00720

Stability and kinetics of hydrolysis of metronidazole monosuccinate in aqueous solution and in plasma

Marianne Johansen and Claus Larsen

Royal Danish School of Pharmacy, Department of Pharmaceutics, DK-2100 Copenhagen (Denmark)

(Received February 24th, 1984)
(Accepted May 7th, 1984)

Summary

The kinetics of hydrolysis of metronidazole monosuccinate in aqueous solution at pH 1.5–10 and 60 °C has been investigated. The decomposition was monitored by a high-performance liquid chromatographic method capable of determining the monosuccinate ester and the parent metronidazole simultaneously. At any given pH the reactions displayed strict first-order kinetics and were subject to specific acid–base catalysis; no general acid–base catalytic effect by various buffer substances was observed. Maximal stability was observed at pH 3–6, where the degradation rate was independent of pH indicating that intramolecular catalysis by the terminal carboxylate ion on the ester group is not involved in the hydrolysis reactions in this pH range. The lack of intramolecular catalysis is suggested to be due to the poor leaving ability of the strongly basic metronidazole alkoxide ion.

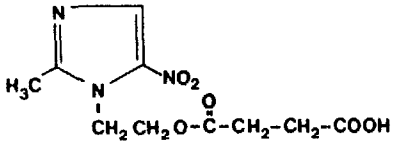
The rates of conversion of the ester to metronidazole at 37 °C in 80% human plasma and in 0.05 M phosphate buffer pH 7.40, respectively, were found to be nearly identical, $t_{1/2} \sim 580$ h, revealing that the degradation in plasma proceeds in the absence of enzymatic catalysis.

Introduction

Considerable attention has been focused on the potential use of macromolecular compounds as carriers or delivery systems for drugs and enzymes. Recently the objectives which may be achieved using this approach have been extensively re-

Correspondence: M. Johansen, Royal Danish School of Pharmacy, Department of Pharmaceutics, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

viewed by Poznansky and Cleland (1980). A means to improve as well as to control the rate of release of the active substance from the conjugate in vivo may be the incorporation of an appropriate spacer arm between the drug and the carrier. In our preliminary work on dextrans as carriers for various drugs in a prodrug manner we have examined the potential of using aliphatic dicarboxylic acids as spacer arms (to be published). The experiments have included an in vitro stability study of metronidazole monosuccinate (I) in aqueous solution and in plasma. pH–rate profiles for hydrolysis of various monosuccinate esters have been reported including hydrocortisone (Garrett, 1962), methylprednisolone (Anderson and Taphouse, 1981), acetaminophen (Rattie et al., 1970), substituted phenols (Gaetjens and Morawetz, 1960) and chlorzoxanzone (Johansen and Bundgaard, 1981). To our knowledge, however, this paper is the first describing the degradation kinetics in the pH range 2–10 of a succinic acid monoester derived from a simple aliphatic alcohol with basicity close to that of ethanol. In addition, the potential utility of monosuccinate esters as water-soluble prodrug candidates for drugs containing an alcoholic functional group is discussed.



I

Materials and Methods

Metronidazole was obtained from Dumex, Copenhagen. The solvents used in the mobile phase were of chromatographic grade. All other chemicals and buffer substances were of reagent grade.

Apparatus

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

Synthesis of metronidazole monosuccinate

A suspension of metronidazole (1.7 g, 10 mmoles) and succinic anhydride (2.5 g, 25 mmoles) in 20 ml of concentrated acetic acid was refluxed for 8 h. Upon cooling a white precipitate of succinic acid was formed and filtered off. The filtrate was evaporated to dryness in vacuo, and after addition of 20 ml of water the monosuccinate ester crystallized out. Recrystallization from ethanol–water gave crystals, m.p. 106–107 °C (reported m.p. 109 °C (Cosar et al., 1966)).

HPLC analysis

The monosuccinate ester and the parent metronidazole was determined by using a reversed-phase high-performance liquid chromatographic procedure. A column, 250 × 4 mm, packed with LiChrosorb RP-8 (7 μm particles) was eluted with a mobile phase consisting of methanol–0.02 M phosphate buffer pH 7.0 (3 : 7 v/v). The flow rate was 1.5 ml · min^{-1} and column effluent was monitored at 320 nm. Under these conditions the capacity factors of the ester and metronidazole were 0.8 and 1.3, respectively. Quantitation of the compounds was done from measurements of the peak heights in relation to those of standards chromatographed under the same conditions.

Kinetics in aqueous solution

The buffers used were hydrochloric acid, formate, acetate, phosphate, borate and carbonate solutions. A constant ionic strength (μ) of 0.5 was maintained for each buffer by adding a calculated amount of potassium chloride. The reaction solutions were kept at a constant temperature of 60 ± 0.2 °C except in the studies of the influence of temperature on the reaction rates. The reactions were initiated by adding 50 μl of a stock solution of the monosuccinate ester in methanol to 10 ml of the appropriate buffer to give a final concentration of about 7×10^{-5} M. All buffer solutions were preheated to the temperature of study. At suitable intervals aliquots were withdrawn and analyzed immediately.

Pseudo first-order rate constants were calculated from the slopes of the logarithm of the concentration of intact monosuccinate ester versus time plots using linear regression. For the slow reactions in the pH range 3–6 the rate constants were derived using the initial rate method (Connors, 1973). At constant pH and temperature the formation of metronidazole was found to be a linear function of the initial concentration of the ester indicating first-order degradation kinetics. In some kinetic runs the observed first-order rate constant was determined by use of both methods and the obtained values agreed within ± 3%.

Kinetics in human plasma

An accurately weighed amount of metronidazole monosuccinate was dissolved in 80% human plasma pre-equilibrated at 37 °C to give a final concentration of about 250 μg · ml^{-1}. The solution was kept in a water bath of 37 °C and at appropriate intervals 500 μl samples were removed and deproteinized with 1500 μl of methanol. This mixture was vortexed and centrifuged for 2 min at 10,000 × *g*. The aqueous methanol layer was assayed for metronidazole by HPLC. The observed first-order rate constant was determined by use of the initial rate method.

Determination of the ionization constant

By potentiometric titration at 60 °C and $\mu = 0.5$ the ionization constant of the ester was determined to be $10^{-4.56}$, which compares well with the obtained values for other monosuccinate esters (Gaetjens and Morawetz, 1960; Anderson and Taphouse, 1981).

Results

Kinetics of hydrolysis of metronidazole monosuccinate

The kinetics of hydrolysis of metronidazole monosuccinate was studied in aqueous buffer solutions over the pH range 1.5–10.0. With the buffer concentration varying from 0.05 to 0.20 M it was observed that the rates of hydrolysis of I were not subject to catalysis by any of the buffer substances used in the kinetic studies.

The pH dependence of the buffer-independent first-order rate constant (k_{hyd}) for degradation of the monosuccinate ester at 60 °C and $\mu = 0.5$ is shown in Fig. 1. At pH < 2 and pH > 8 the pH–rate profile shows two straight-line portions with slopes of −0.98 and 1.0, respectively, while an almost pH-independent plateau is observed

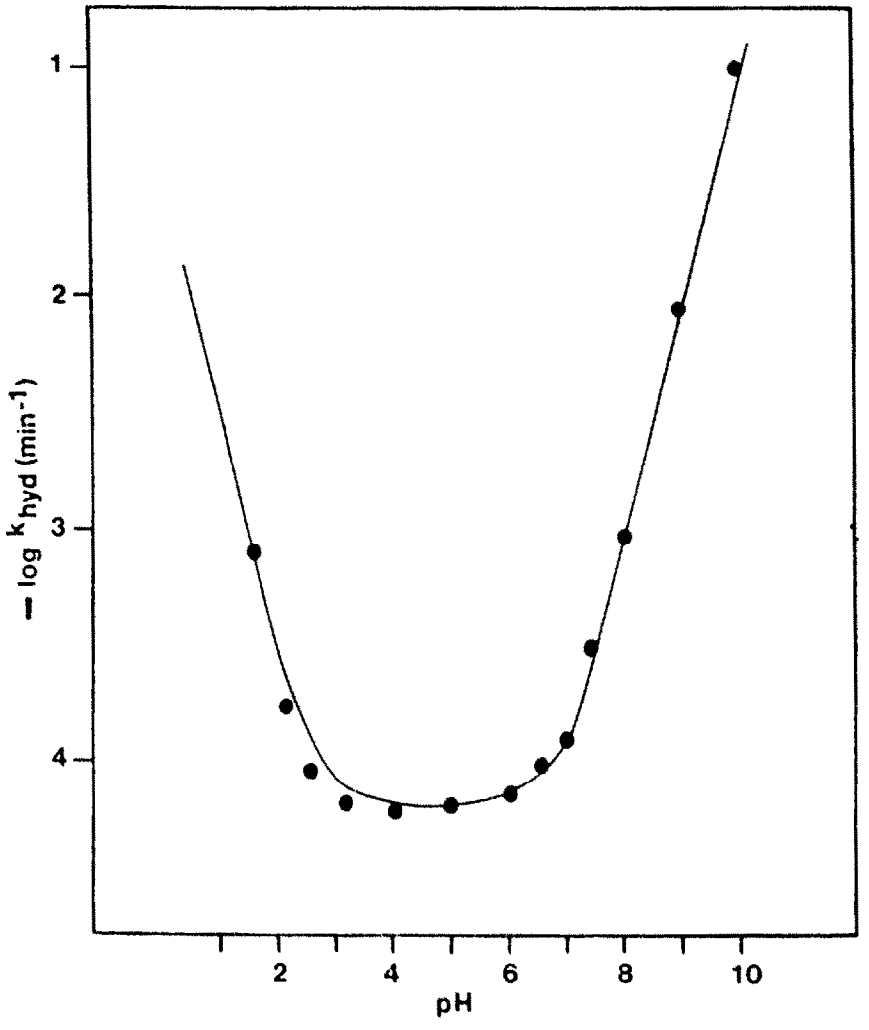


Fig. 1. pH–rate profile for the degradation of metronidazole monosuccinate at 60 °C and $\mu = 0.5$. The curve is calculated from Eqn. 1 and ● are the experimental values.

between pH 3 and 6. This profile shape is characteristic of degradation reactions susceptible to specific acid–base catalysis obeying the general rate law:

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

where a_H and a_{OH} refer to the hydrogen ion and hydroxide ion activity, respectively. k_H and k_{OH} are the second-order rate constants for specific acid and specific base catalysis, respectively, and k_0 is the first-order rate constant for spontaneous or water catalyzed degradation. The activity a_H was calculated in accordance with Harned and Hamer (1933). The smooth curve in Fig. 1 was calculated from Eqn. 1 and the following values for the rate constants (60°C and $\mu = 0.5$):

$$k_H = 1.63 \times 10^{-2}\ M^{-1} \cdot min^{-1}$$

$$k_0 = 3.96 \times 10^{-5}\ min^{-1}$$

$$k_{OH} = 11.6\ M^{-1} \cdot min^{-1}$$

The good agreement observed between the calculated and the experimental data demonstrates that the rate expression, Eqn. 1, adequately describes the degradation kinetics.

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 50–80°C. From an Arrhenius-type plot an apparent activation energy, E_a, of 87.3 $kJ \cdot mol^{-1}$ was calculated.

Degradation of I in human plasma

The rate constants for hydrolysis of I in 80% human plasma and in aqueous solution (pH 7.40) at 37°C are presented in Table 1, the latter rate constant being calculated from the Arrhenius equation. The results show that the degradation rate of I in plasma and in 0.05 M phosphate buffer, pH 7.40, is of the same order of magnitude indicating that the hydrolysis reaction in plasma proceeds without enzymatic catalysis.

TABLE 1

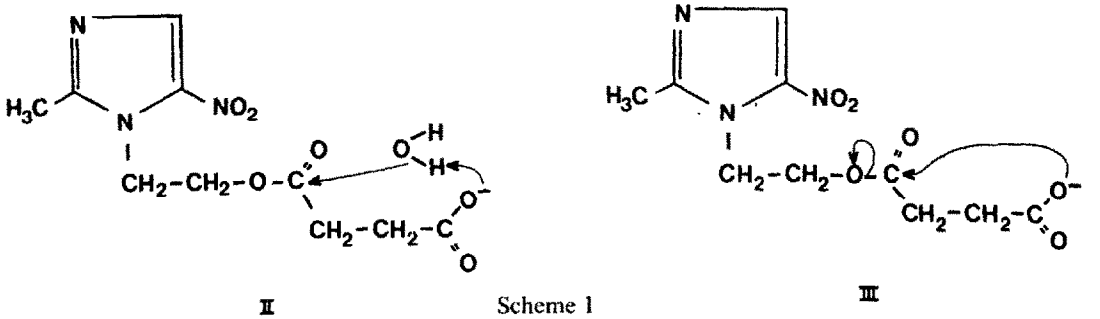
DEGRADATION OF METRONIDAZOLE ESTERS IN 0.05 M PHOSPHATE pH 7.40 AND PLASMA, 37°C

Ester	Plasma conc. (%)	k_{hyd} (min^{-1})
monosuccinate	80	1.83×10^{-5}
monosuccinate	0	2.6×10^{-5} (calc.)
butyrate	80	~ 0.5
butyrate	0	1.30×10^{-5} (calc.)
valerate	80	~ 0.5
valerate	0	1.44×10^{-5} (calc.)

Discussion

Mechanism of hydrolysis of I in aqueous solution

The previously reported pH–rate profiles for hydrolysis of monosuccinate esters do all exhibit a sigmoid curvature in the pH region near the pK_a value of the individual monosuccinate ester. Mechanistically this kinetic behaviour has been interpreted as being due to intramolecular attack of the terminal carboxylate ion on the ester group. Intramolecular catalysis by the neighbouring succinate anion



II Scheme 1 III

(Scheme 1) can proceed either in the form of general base catalysis of attack of water on the ester bond (II) or in the form of a nucleophilic mechanism leading to an intermediate formation of succinic anhydride (III). The latter mechanism has been shown to be involved in the hydrolysis of various phenyl monoesters of succinic acid (Bruice and Pandit, 1960; Gaetjens and Morawetz, 1960).

The U-shape of the pH–rate profile of metronidazole monosuccinate suggests that intramolecular catalysis by the terminal carboxylate ion is not involved in the hydrolysis reactions in the pH range 3–6. This is supported by the fact that the rate constants for hydrolysis of the butyrate, the valerate and the monosuccinate esters of metronidazole at neutral pH are almost identical as shown in Table 1. For the sake of comparison it should be noted that the degradation rates of I in acid and alkaline solutions are of the same order of magnitude as described for ethyl monosuccinate (Holba and Rievaj, 1973; Khalil and Hanna, 1978). Previously it has been shown that the efficiency of intramolecular catalysis depends strongly on the basicity of the leaving group. For substituted phenyl monosuccinate esters the rate of intramolecular catalyzed hydrolysis was increased significantly by introduction of electron withdrawing groups in the para-position (Gaetjens and Morawetz, 1960). Thus, the lack of intramolecular catalysis in the degradation of I is most likely explained by the fact that the metronidazole alkoxide ion is a strongly basic leaving group. The pK_a value of the hydroxy group might be expected to be about the same as that of ethanol, i.e. 15.9 (Ballinger and Long, 1960). In comparison to metronidazole monosuccinate the aliphatic monosuccinate esters of hydrocortisone (Garrett, 1962) and of methylprednisolone (Anderson and Taphouse, 1981) degrade much faster in the pH range 3–7 due to intramolecular catalysis. This difference in reactivity is presumed to be a reflection of the less basic character of the leaving groups of the two latter compounds. The apparent pK_a of the C-17 side-chain of methylpredniso-

lone and hydrocortisone is near 11 due to enolization (Hansen and Bundgaard, 1979). The present study together with the previous reports on stability of monosuccinate esters indicates that the susceptibility to undergo intramolecular catalyzed hydrolysis by the terminal carboxylate ion is strongly dependent on the basicity of the hydroxy group.

Hydrolysis of monosuccinate ester prodrugs in vivo and in plasma

Dicarboxylic acid monoesters of several drugs have been synthesized with the purpose of developing water-soluble prodrugs suitable for parenteral administration. Although salts of monosuccinate esters of various drugs, e.g. corticosteroids and chloramphenicol, have been marketed, only few experimental data with respect to enzyme-mediated cleavage of such esters in vivo and in plasma are available in the literature. It has been shown (Melby and St. Cyr, 1961) that parenteral administered 21-monosuccinate esters of prednisolone and hydrocortisone produced plasma levels of the parent steroids, but the bioavailability of the compounds from the corresponding ester prodrugs was not accessed. The results indicate, however, that the 21-monosuccinate esters to some extent might be excreted unchanged in the urine after parenteral administration. In case of chloramphenicol-3-monosuccinate pharmacokinetic studies have revealed an excretion of unhydrolyzed prodrug after intravenous injection corresponding to about 30% of the dose (Kauffman et al., 1981; Nahata and Powell, 1981; Burke et al., 1982). In vitro experiments have shown that the metabolic organs and particularly the liver were capable to release the parent chloramphenicol from the prodrug ester, while plasma enzyme-catalyzed hydrolysis was found to be negligible (Schmidt and Vömel, 1965). This lack of enzyme catalysis of hydrolysis of the monosuccinate ester is in accordance with the results of the present study and the findings of Johansen and Bundgaard (1981) in the stability study of chlorzoxazone monosuccinate. Apparently plasma enzymes have no significant influence on the degradation rate of the hitherto investigated monosuccinate ester prodrugs. Reports indicating that charged polar compounds in general are either not attacked by or are poor substrates for non-specific plasma carboxylesterases (Krisch, 1971) reflect that the observations mentioned above might be expanded to encompass other monosuccinate ester derivatives.

In the design of ester prodrugs with the express purpose of improving the water-solubility of the hydroxy compound several properties of the prodrug ester have to be considered. Fundamentally, the ideal prodrug candidate suitable for parenteral administration must possess a reasonable stability in aqueous solution in vitro together with an ability to regenerate the active drug rapidly and quantitatively after entering the body. Although the formation of succinic acid monoesters of hydroxy compounds does provide a desired improvement in water solubility, the available data for degradation of such ester prodrugs in vivo and in plasma suggest that alternative types of water-soluble ester derivatives also should be taken into consideration. In this connection phosphate esters are freely soluble and stable in vitro at physiological pH 7.4 and in the past, several phosphate ester prodrugs have been used in preparing parenteral dosage forms of various drugs containing an alcoholic hydroxy group (Stella, 1975; Sinkula and Yalkowsky, 1975; Cho et al.,

1982). A third and perhaps the most promising type of water-soluble ester derivatives is esters with an ionizable amino function in the acid portion (Nudelman et al., 1974; Kawamura et al., 1971; Kigasawa et al., 1979; Fogt et al., 1980; Johansen and Bundgaard, 1981; Kovach et al., 1981; Bundgaard et al., 1984a; Bundgaard et al., 1984b).

References

Anderson, B.D. and Taphouse, V., Initial rate studies of hydrolysis and acyl migration in methylprednisolone 21-hemisuccinate and 17-hemisuccinate. J. Pharm. Sci., 70 (1981) 181–186.

Ballinger, P. and Long, F.A., Acid ionization constants of alcohols II. Acidities of some substituted methanols and related compounds. J. Am. Chem. Soc., 82 (1960) 795–798.

Bruice, T.C. and Pandit, U.K., The effect of geminal substitution ring size and rotamer distribution on the intramolecular nucleophilic catalysis of the hydrolysis of monophenyl esters of dibasic acids and the solvolysis of the intermediate anhydrides. J. Am. Chem. Soc., 82 (1960) 5858–5865.

Bundgaard, H., Larsen, C. and Thorbek, P., Prodrugs as drug delivery systems. XXVI. Preparation and enzymatic hydrolysis of various water-soluble amino acid esters of metronidazole. Int. J. Pharm., 18 (1984a) 67–77.

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

Burke, J.T., Wargin, W.A., Sheretz, 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. Pharmacokin. Biopharm., 10 (1982) 601–614.

Cho, M.J., Kurtz, R.R., Lewis, C., Machkovech, S.M. and Houser, D.J., Metronidazole phosphate—a water-soluble prodrug for parenteral solutions of metronidazole. J. Pharm. Sci., 71 (1982) 410–414.

Connors, K.A., Reaction Mechanisms in Organic Analytical Chemistry, John Wiley, New York, 1973, pp. 41–110.

Cosar, C., Crisan, C., Horclois, R., Jacob, R.M., Robert, J. Tchelitcheff, S. and Vaupré, R., Nitro-imidazoles—préparation et activité chimiothérapeutique. Arzneim.-Forsch., 16 (1966) 23–29.

Fogt, S.W., Scozzie, J.A., Heilman, R.D. and Powers, L.J., Lipophilic and hydrophilic esters of 4-acetyl-2-(2-hydroxyethyl)5,6-bis(4-chlorophenyl)-2H-pyridazin-3-one as antihypertensive agents. J. Med. Chem., 23 (1980) 1445–1448.

Gaetjens, E. and Morawetz, H., Intramolecular carboxylate attack on ester groups. The hydrolysis of substituted phenyl acid succinates and phenyl acid glutarates. J. Am. Chem. Soc., 82 (1960) 5328–5335.

Garrett, E.R., The solvolysis of 21-hydrocortisone esters and hemiesters. J. Med. Pharm. Chem., 5 (1962) 112–133.

Hansen, J. and Bundgaard, H., Studies on the stability of corticosteroids I. Kinetics of degradation of hydrocortisone in aqueous solution. Arch. Pharm. Chemi, Sci. Edn., 7 (1979) 135–146.

Harned, H.S. and Hamer, W.J., The ionization constant of water and the dissociation of water in potassium chloride solutions from electromotive forces of cells without liquid junction. J. Am. Chem. Soc., 55 (1933) 2194–2206.

Holba, V. and Rievaj, M., The effect of ionic strength on the alkaline hydrolysis of sodium ethyl succinate and sodium ethyl glutarate. Collect. Czech. Chem. Commun., 38 (1973) 3283–3289.

Johansen, M. and Bundgaard, H., Prodrugs as drug delivery systems. XVI. Novel water-soluble pro-drug types for chlorzoxazone by esterification of the N-hydroxymethyl derivatives. Arch. Pharm. Chemi, Sci. Edn., 9 (1981) 43–54.

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

Kawamura, M., Yamamoto, R. and Fujisawa, S., Pharmaceutical studies on water-soluble corticosteroid derivatives. II. Stability of hydrocortisone 21-aminoalkylcarboxylates in solution. Yakugaku Zasshi, 91 (1971) 863–870.

Khalil, F.Y. and Hanna,M.T., Kinetic study of the acid hydrolysis of ethyl hydrogen succinate in binary solvent mixtures. Z. Naturforsch. Teil. B, 33 (1978) 1479–1483.

Kigasawa, K., Shimizu, H., Ohtani, H., Hayashida, S. and Ishiodori, T., Decomposition and stabilization of drugs. XVIII. Studies on the stability of carboxylic acid esters of phenols and their effectiveness as prodrugs. Yakugaku Zasshi, 99 (1979) 402–412.

Kovach, I.M., Pitman, I.H. and Higuchi, T., Amino acid esters of phenols as prodrugs: synthesis and stability of glycine, β-aspartic acid and α-aspartic acid esters of *p*-acetamidophenol. J. Pharm. Sci., 70 (1981) 881–885.

Krisch, K., Carboxylic ester hydrolases. In Boyer, P.D. (Ed.), The Enzymes. Vol. 5, 3rd edn., Academic Press, New York, 1971, pp. 43–69.

Melby, I.C. and St. Cyr, M., Comparative studies on absorption and metabolic disposal of water-soluble corticosteroid esters. Metab. Clin. Exp., 10 (1961) 75–82.

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

Nudelman, A., McCaully, R.J. and Bell, S.C., Water-soluble derivatives of 3-oxy-substituted 1,4-benzodiazepines. J. Pharm. Sci., 63 (1974) 1880–1885.

Poznansky, M.J. and Cleland, L.G., Biological macromolecules as carriers of drugs and enzymes. In Juliano, R.L. (Ed.), Drug Delivery Systems, Oxford University, New York and Oxford, 1980, pp. 253–315.

Rattie, E.S., Shami, E.G., Dittert, L.W. and Swintosky, J.V., Acetaminophen prodrugs III: Hydrolysis of carbonate and carboxylic acid esters in aqueous buffers. J. Pharm. Sci., 59 (1970) 1738–1741.

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

Sinkula, A.A. and Yalkowsky, S.H., Rationale for design of biologically reversible drug derivatives: prodrugs. J. Pharm. Sci., 64 (1975) 181–210.

Stella, V., Prodrugs: an overview and definition. In Higuchi, T. and Stella, V. (Eds.), Pro-drugs as Novel Drug Delivery Systems, Am. Chem. Soc., Washington, DC, 1975 pp. 1–115.