International Journal of Pharmaceutics, 6 (1980) 19–27

PRO-DRUGS AS DRUG DELIVERY SYSTEMS XI. PREPARATION AND CHARACTERIZATION OF A NOVEL WATER-SOLUBLE PRO-DRUG TYPE FOR BARBITURIC ACIDS [1]

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(Received March 11th, 1980)
(Accepted April 29th, 1980)

SUMMARY

The β-N,N-dimethylaminoethyl ester of diethylmalonuric acid was prepared and evaluated as a water-soluble pro-drug of barbital. The ester was found to undergo a rapid and quantitative cyclization in neutral and alkaline aqueous solution to barbital, the half-time of conversion being 2.8 min at pH 7.4 and 37°C. The kinetics of cyclization were studied in the pH range 4–10.2 and it was found that protonation of the dimethylamino moiety resulted in a 9-fold greater reactivity compared with the free base form. This rate-accelerating effect was attributed to electrostatic stabilization of a negatively charged reaction center. The solubility of the hydrochloride salt of the ester in water was found to be greater than 75% w/v. The results suggested that β-N,N-dimethylaminoethyl esters of malonuric acids may be potentially useful water-soluble pro-drugs of the respective barbituric acids with improved delivery properties for parenteral use.

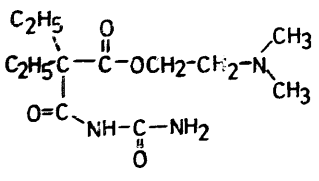
INTRODUCTION

A number of barbituric acids are widely used in medicine for their sedative, hypnotic, antiepileptic or anesthesic properties. However, the poor water-solubility (0.05–2 mg/100 ml (Breon and Paruta, 1970)) of the drugs combined with their weakly acidic nature has afforded various biopharmaceutical and formulation problems. Thus, the bioavailability of barbituric acids following peroral or rectal administration has been shown to be

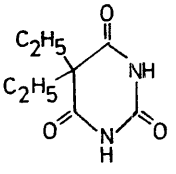
[1] Part 10 of this series: Bundgaard, H. and Johansen, M., N-Mannich bases as novel pro-drug candidates for amides, ureides, amines and other NH-acidic compounds. Kinetics and mechanism for decomposition and structure–reactivity relationships. Arch. Pharm. Chem., Sci. Edn., 8 (1980) 29–52.

dependent on various formulation variables (Sjögren et al., 1965; Singh et al., 1966; Finholt et al., 1972; Leucuta et al., 1977; Doluisio et al., 1978; Schlemmer et al., 1979). When given intramuscularly sodium phenobarbital, for example, has shown slow and incomplete absorption patterns (Pearce et al., 1977; Viswanathan et al., 1978). Parenteral barbituric acid preparations are alkaline solutions of the salt forms which, for solubility and stability reasons, often include the addition of large concentrations of co-solvents such as propylene glycol and ethanol. The suboptimal delivery pattern upon intramuscular injection of such preparations may be due to precipitation of the free acid form at the injection site as has been suggested to be the case in similar situations for a number of other poorly water-soluble drugs such as sodium phenytoin, digoxin, diazepam and chlordiazepoxide (for a review, see Tuttle, 1977).

The purpose of this investigation was to determine the feasibility of using the pro-drug approach for improving the water-solubility within the physiological pH range and hence the delivery characteristics of barbituric acids. In previous studies (Bundgaard et al., 1978, 1979a, b), various alkyl esters of malonuric acids including thiomalonuric acids were shown to undergo a rapid and quantitative intramolecular cyclization in neutral and alkaline aqueous solution to the corresponding barbituric acids and the derivatives were characterized as possible candidates as pro-drugs of their respective barbituric acid. By introducing an ionizable group in the alcohol portion of a malonuric acid ester, it should be possible to obtain a derivative with greatly increased aqueous solubility at physiological pH. In this paper the synthesis of a highly water-soluble β-N,N-dimethylaminoethyl ester of diethylmalonuric acid (I) is reported along with the kinetics and mechanism of its cyclization to barbital (II) in aqueous solution.



I



II

MATERIALS AND METHODS

Equipment

A recording Perkin-Elmer Model 124 spectrophotometer and a Zeiss PMQ II spectrophotometer with a thermostated cell compartment were used for all spectroscopic measurements. Infrared spectra were run on a Unicam SP 200 spectrophotometer using the potassium chloride disc technique. PMR spectra were run on a JEOL C-60-HL instrument using tetramethylsilane as an internal standard. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study. Melting points were taken on a capillary melting-point apparatus and are uncorrected. Thin-layer chromatography was done on precoated 0.25 mm silica gel 60 F_{254} glass plates (E. Merck, G.F.R.). Microanalysis was carried out at the Microanalytical Department of Chemical Laboratory II, University of Copenhagen.

Synthesis of β-N,N-dimethylaminoethyl diethylmalonurate hydrochloride (I)

The malonuric acid ester was prepared by alkylation of the triethylammonium salt of diethylmalonuric acid (m.p. 160–161°C, from aqueous ethanol) which was obtained by alkaline hydrolysis of barbital as described by Aspelund and Skoglund (1937). To a solution of 6.06 g (30 mmol) of diethylmalonuric acid in 150 ml of acetone were added 10.4 ml (75 mmol) of triethylamine and 5.41 g (37.5 mmol) of 2-dimethylaminoethylchloride hydrochloride. The mixture was refluxed for 7 h and filtered. The filtrate was evaporated in vacuo and the residue was taken up in a mixture of 100 ml of ethyl acetate and 40 ml of water. After separation of the phases the ethyl acetate layer was washed with 40 ml of water, 40 ml of a 5% sodium bicarbonate solution and finally with 50 ml of water. The ethyl acetate solution was dried with anhydrous sodium sulphate and evaporated under reduced pressure to give 3.05 g of crystalline ester I (37%). To prepare the hydrochloride salt, the ester was dissolved in 50 ml of ethyl acetate, and 50 ml of a saturated solution of HCl in ethyl acetate was added. After standing for 30 min at room temperature the precipitate was filtered off yielding 2.82 g of the title compound, m.p. 179–180°C dec. The compound was recrystallized by dissolution in 100 ml of ethanol at 40°C, filtration and subsequent addition of 50 ml of ethyl acetate and 250 ml of anhydrous ether. After standing overnight at 5°C 2.67 g were obtained, m.p. 180–182°C. IR (KBr): 3400, 3250, 2950, 2700, 1730, 1700 and 1585 cm^{-1}. PMR (D_2O)δ: 0.85 (t, 6H), – ($CH_2\underline{CH_3}$)$_2$; 1.95 (distorted q, 4H), – ($C\underline{H_2}CH_3$)$_2$; 3.00 (s, 6H), – ($N(C\underline{H_3})_2$); 3.65 (distorted t, 2H), – ($C\underline{H_2}N(CH_3)_2$); 4.70 (distorted t, 2H), – ($OC\underline{H_2}CH_2N(CH_3)_2$).

Analysis. Calculated for $C_{12}H_{24}ClN_3O_4$: C, 46.52; H, 7.81; Cl, 11.46; N, 13.56%. Found: C, 46.35; H, 7.92; Cl, 11.43; N, 13.38%.

Kinetic measurements

All kinetic measurements were either carried out directly in the thermostatted cell compartment of the spectrophotometer or in stoppered flasks placed in a constant temperature water bath of 23 and 37 ± 0.1°C. The reactions were performed in aqueous buffer solutions (acetate, phosphate, borate and carbonate) with a constant ionic strength (μ) of 0.5 adjusted with potassium chloride. The total concentration of the buffers was 0.1 M except in those runs in which buffer effects were specifically investigated. The initial concentration of ester I was about 10^{-4} M (pH > 8.2) and $4–10 \times 10^{-4}$ M (pH < 8.2). Reaction rates (at pH > 6.5) were followed by observing the increase of ultraviolet absorption at 238 nm due to the formation of barbital and the first-order rate constants determined as previously described (Bundgaard et al., 1979a). For runs performed at pH < 6.5, the reaction product barbital was quantitatively separated from unreacted ester by extracting aliquots of the reaction solutions with 3 parts of chloroform. The barbital was subsequently transferred to a 0.1 M borate buffer solution, pH 10.0, by extracting the chloroform solution with 3 parts of the buffer. The concentration of barbital in the buffer was finally determined by measuring the absorbance of the solution at 238 nm. First-order rate constants were determined from plots of $\log(A_\infty - A_t)$ against time, where A_∞ and A_t are the absorbance readings at infinity and at time, t, respectively.

Solubility determinations

The solubility of the ester I hydrochloride in water at 23°C was estimated by placing

50-mg fractions of the ester in 1 ml of water until a saturated solution was obtained. It was checked that no precipitation occurred by standing a solution containing 0.5 g/ml of the ester hydrochloride for 24 h at room temperature.

RESULTS AND DISCUSSION

Kinetics and mechanism of cyclization of I to II

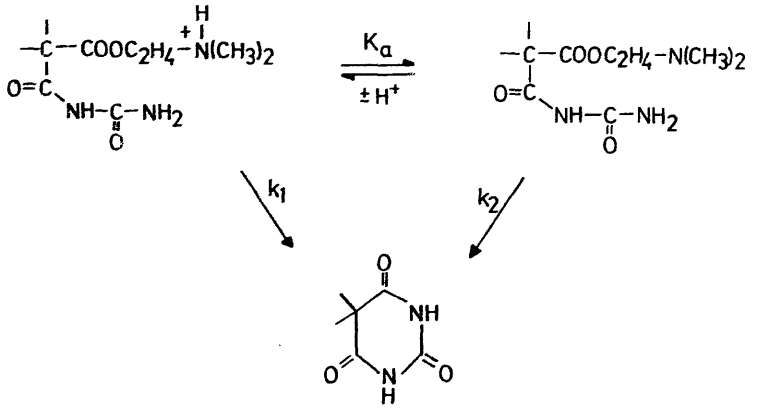
In the pH range studied, 4.2–10.2, and at temperatures of 23 and 37°C, the β-N,N-dimethylaminoethyl ester of 2,2-diethylmalonuric acid (I) was found to undergo a quantitative cyclization to the corresponding barbituric acid, barbital (II), as evidenced by ultraviolet spectral comparisons, TLC and product characterization in a similar way as previously described for other malonuric acid esters (Bundgaard et al., 1978, 1979a).

At constant pH and temperature the cyclization exhibited good first-order kinetics over more than 5 half-lives. As shown by some data listed in Table 1 the rate of cyclization was independent of buffer concentration from 0.02 to 0.2 M at constant ionic strength.

The effect of pH on the cyclization rate at 37°C is shown in Fig. 1 in which the logarithm of the observed first-order rate constants, k_{obs}, has been plotted against pH. In the ranges pH < 7.5 and pH > 8.5 the pH–rate profile shows two linear segments with slopes of unity while a partial plateauing is observed between pH 7.5 and 9.5. This pattern indicates that the free base and the protonated forms of the tertiary amino ester undergo cyclization with different rates and that the cyclization can be described in terms of specific base-catalyzed reactions of these species (Scheme 1):

$$k_{obs} = k_1 a_{OH} \frac{a_H}{a_H + K_a} + k_2 a_{OH} \frac{K_a}{a_H + K_a} \qquad (1)$$

where a_{OH} and a_H refer to the hydroxide ion and hydrogen ion activity, respectively, $a_H/(a_H + K_a)$ and $K_a/(a_H + K_a)$ are the fractions of total ester in the protonated and free base form, respectively, and K_a is the apparent ionization constant of the dimethylammonium group in the ester. The values of the second-order rate constants, k_1 and k_2,



Scheme 1

TABLE 1

Effect of varying buffer concentration on the observed first-order rate constants for cyclization of the β-N, N-dimethylaminoethyl ester (I) to barbital (μ = 0.5; 37°C)

Buffer	pH	k_{obs} (min^{-1})
Borate 0.05 M	9.05	2.31
Borate 0.10 M	9.05	2.28
Borate 0.20 M	9.05	2.28
Phosphate 0.02 M	7.40	0.251
Phosphate 0.05 M	7.40	0.238
Phosphate 0.10 M	7.40	0.260

for the apparently specific base-catalyzed cyclization of the two ester species were determined from the straight line portions of the pH–rate profile, and from Eqn. 1 and the values of k_{obs} in the pH range 7.5–9.5, K_a was determined. The hydroxide ion activity was calculated from the measured pH at 37 and 23°C as described previously (Bundgaard and Larsen, 1979). The following values were obtained at 37°C and μ = 0.5:

$k_1 = 4.6 \times 10^5\ M^{-1}\ min^{-1}$ ($3.3 \times 10^5\ M^{-1}$ at 23°C)

$k_2 = 5.2 \times 10^4\ M^{-1}\ min^{-1}$

$K_a = 10^{-8.15}$

The solid line drawn in Fig. 1 has been calculated from Eqn. 1 and these rate and equilib-

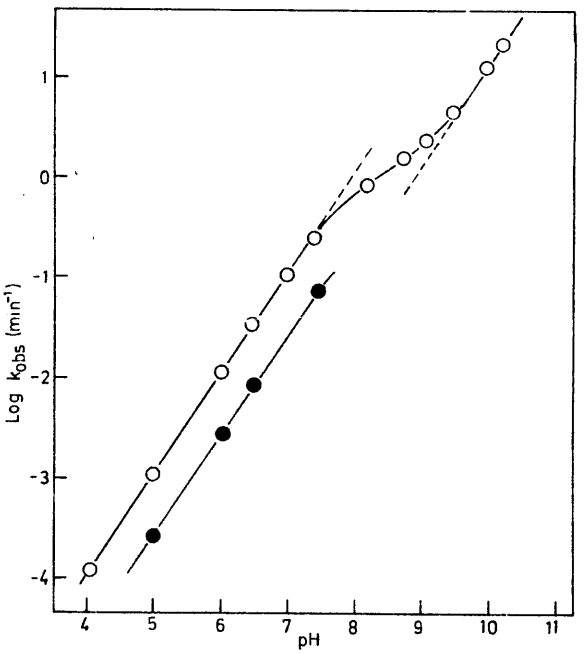
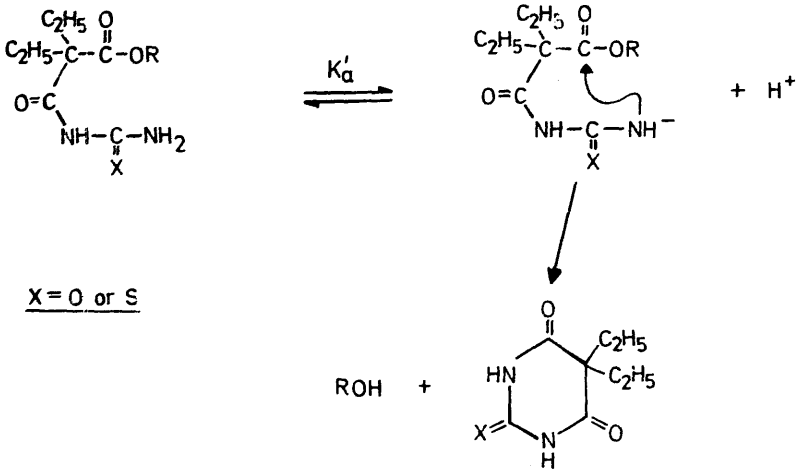


Fig. 1. pH–rate profiles for the cyclization of β-N,N-diethylaminoethyl diethylmalonurate to barbital in aqueous solutions (μ = 0.5) at 23 (●) and 37°C (○).

rium constants and the good agreement observed between the calculated and experimental data demonstrates that the rate expression adequately fits the rate data. Due to the instability of the ester at pH values near pK_a, the ionization constant could not be determined by potentiometric titration but the kinetically obtained value is in the expected range. Thus a pK_a of 8.47 at 25°C has been reported for the related 2-dimethylaminoethyl benzoate (Chu and Mautner, 1966).

Although the reactions have been expressed in terms of specific base catalytic cyclizations, a kinetically equivalent reaction mechanism involving intramolecular nucleophilic attack of the terminal ureido nitrogen anion upon the ester carbonyl moiety (Scheme 2)

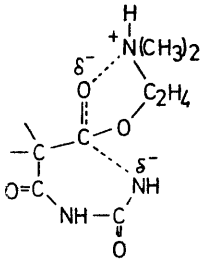


Scheme2

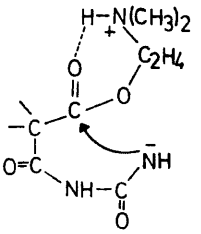
is preferred as argued in a previous study of the cyclization of various alkyl esters of malonuric acids (Bundgaard et al., 1979a, b). The pK_a value of the ureido group might be expected to be greater than 14 (Hegarty and Bruice, 1970), which agrees with the rate expression of Eqn. 1 being valid at pH values less than the pK_a value for the ureido group.

It appears from the values of k_1 and k_2 that the ester with a protonated dimethylamino moiety possesses a 9-fold greater susceptibility to undergo ring closure as compared with the free base form which in turns shows almost the same reactivity as the corresponding methyl ester (for which $k = 5.5 \times 10^4\ M^{-1}$ at 37°C (Bundgaard et al., 1979a)). This increased rate of reaction of protonated amino ester may be attributed to the positively charged nitrogen atom being situated in such a way that electrostatic interactions with the developing negative charge on the carbonyl oxygen atom may occur and cause stabilization of the transition state III. However, a mechanism involving intramolecular general acid catalysis by the protonated dimethylamino group of the nucleophilic attack of the ureido nitrogen anion (IV) cannot be fully discounted. A kinetic equivalent of either of these mechanisms is intramolecular general base catalysis by the unprotonated dimethylamino group of attack of the neutral ureido group (V), but this mechanism seems less likely, since reactions involving participation by the neutral ureido group are not significant for simple alkyl esters of malonuric acids (Bundgaard et al., 1979a) and

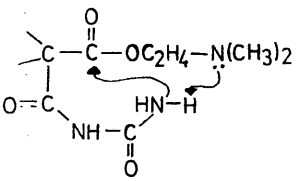
also because of the lack of significant general base catalysis by various buffers.



III



IV



V

Due to the intramolecular anchimeric effect of the protonated dimethylamino moiety, the conversion of the ester to barbital is very efficient at pHs below the pK_a of the amino moiety. Thus, at pH 7.40 and 37°C the half-life is 2.8 min which may be compared with the half-life of 21 min for the conversion of the methyl ester of the corresponding malonuric acid under the same conditions (Bundgaard et al., 1979a). It is to be expected that in vivo the amino ester would undergo ring closure as efficiently as observed in vitro and thus, it may act as a pro-drug of the barbituric acid. The generation of the barbituric acid is independent of the need of enzymatic mediation and furthermore, by examining the reaction of the ester in a phosphate buffer, pH 7.4, containing 75% human serum at 37°C as previously described (Bundgaard et al., 1979a), it was ascertained that a possible enzymatic hydrolysis of the ester grouping is insignificant and unable to compete with the fast intramolecular cyclization at conditions simulating those prevailing in vivo.

Intramolecular nucleophilic displacement reactions by ureido groups at the carbonyl moiety of esters have previously been demonstrated for hydantoic acid esters (Stella and Higuchi, 1973; Stella et al., 1975). These investigators also showed that β-N,N-diethylaminoethyl esters, in particular, were susceptible of undergoing ring closure reactions at neutral pH.

The potential usefulness of amino esters like I as water-soluble pro-drugs of barbituric acids

The hydrochloride salt of the amino ester I displayed a high aqueous solubility. The solubility in water was found to exceed 75% w/v or 2.4 M at 22°C, the pH of the concentrated solution being about 4. The solubility of barbital under similar conditions is 3.7×10^{-2} M or 0.68% w/v (Bundgaard et al., 1979a) which means that the increase in water-solubility of the pro-drug hydrochloride salt over barbital is a factor of more than 65 in terms of barbital equivalents. Simple alkyl esters of malonuric acids are more lipophilic

and less water-soluble than the corresponding barbituric acids (Bundgaard et al., 1979a) and it appears, therefore, that the dimethylamino moiety greatly helps to increase the aqueous solubility at pH values where it is protonated which include the physiological pH range.

The rapid rate of ring closure of the amino ester at pH 7.4 and 37°C and the high aqueous solubility of its hydrochloride salt make this a potentially useful water-soluble pro-drug of barbital. At slightly acidic pH values the stability is adequate for using the ester as a component in a reconstitutable injection. For example, at pH 4 and 5 and at 23°C the times for 10% conversion are 63 and 6.3 h, respectively. The high water-solubility of the pro-drug candidate makes it unnecessary to incorporate any co-solvent as propylene glycol in an injection preparation and further, it would expectedly ensure that no precipitation would occur in the blood stream or at the injection site. Thus, the pro-drug may result in an improved delivery of the barbituric acid after parenteral administration compared with that achieved with the presently used preparations.

Although only barbital has been concerned in this study it is to be expected that amino esters like I of other malonuric acids corresponding to various medically important barbituric acids would possess similar properties regarding facile ring closure and superior aqueous solubility to that of the parent drug. It has previoulsy been found (Bundgaard et al., 1979a) that the rate of ring closure of malonurate alkyl esters vary only slightly within a series comprising barbital, phenobarbital, ethallobarbital, allobarbital and hexobarbital and it may be predicted with some confidence from the rate data for these esters and those presented in this paper that the half-time of conversion of β-N,N-dimethylaminoethyl esters of the malonuric acids corresponding to these drugs would be in the range 1–9 min at pH 7.4 and 37°C. For thiobarbituric acids, esters of the corresponding thiomalonuric acids show about a 40-fold greater reactivity than the oxy analogues (Bundgaard et al., 1979b).

REFERENCES

Aspelund, H. und Skoglund, L., Die Beständigkeit der Natriumsalze einiger Schlafmittel der Barbitursäure-Reihe in Lösung. Acta Acad. Aboensis Math. Phys., 10 (1937) (No. 10) 1–22.

Breon, T.L. and Paruta, A.N., Solubility profiles for several barbiturates in hydroalcoholic mixtures. J. Pharm. Sci., 59 (1970) 1306–1313.

Bundgaard, H. and Larsen, C., Pro-drugs as drug delivery systems. II. Open-ring ester derivatives as novel pro-drug candidates for trimethadione. Arch. Pharm. Chem., Sci. Edn., 7 (1979) 41–50.

Bundgaard, H., Hansen, A.B. and Larsen, C., Pro-drugs as drug delivery systems. I. Esters of malonuric acids as novel pro-drug candidates of barbituric acids. Arch. Pharm. Chem., Sci. Edn., 6 (1978) 231–240.

Bundgaard, H., Hansen, A.B. and Larsen, C., Pro-drugs as drug delivery systems III. Esters of malonuric acids as novel pro-drug types for barbituric acids. Int. J. Pharm., 3 (1979a) 341–353.

Bundgaard, H., Hansen, A.B. and Larsen, C., Pro-drugs as drug delivery systems. VII. Rapid cyclization of methyl diethylthiomalonurate to thiobarbital in aqueous solution. Arch. Pharm. Chem., Sci. Edn., 7 (1979b) 193–198.

Chu, S.-H. and Mautner, H.G., Analogs of neuroeffectors. V. Neighboring-group effects in the reaction of esters, thiolesters, and selenolesters. The hydrolysis and aminolysis of benzoylcholine, benzoylthiolcholine, benzoylselenocholine, and of their dimethylamino analogs. J. Org. Chem., 31 (1966) 308–312.

Doluisio, J.T., Smith, R.B., Chun, A.H.C. and Dittert, L.W., Pentobarbital absorption from capsules and suppositories in humans. J. Pharm. Sci., 67 (1978) 1586–1588.

Finholt, P., Haabrekke, O., Holme, I., Jansholt, L., Paulssen, R.B. and Sveen, K., Absorption of phenobarbital from tablets. Rate studies and statistical interpretation. Medd. Norsk Farm. Selskap, 34 (1972) 101–116.

Hegarty, A.F. and Bruice, T.C., Acyl transfer reactions from and to the ureido functional group. III. The mechanism of intramolecular nucleophilic attack of the ureido functional group upon acyl groups. J. Am. Chem. Soc., 92 (1970) 6575–6588.

Leucuta, S.E., Popa, L., Ariesan, M., Popa, L., Pop, R.D. and Toader, S., Bioavailability of phenobarbital from different pharmaceutical dosage forms. Pharm. Acta Helv., 52 (1977) 261–266.

Pearce, J.L., Sharman, J.R. and Forster, R.M., Phenobarbital in the acute management of febrile convulsions. Pediatrics, 60 (1977) 569–572.

Schlemmer, W., Stanislaus, F. and Rehm, K.-D., Zur biologischen Verfügbarkeit von Hexobarbital – in vitro- und in vivo – Untersuchungen. Acta Pharm. Techn., 25 (1979) 81–91.

Singh, P., Guillory, J.K., Sokoloski, T.D., Benet, L.Z. and Bhatia, V.N., Effect of inert tablet ingredients on drug absorption. I. Effect of polyethylene glycol 4000 on the intestinal absorption of four barbiturates. J. Pharm. Sci., 55 (1966) 63–68.

Sjögren, J., Sölvell, L., and Karlsson, I., Studies on the absorption rate of barbiturates in man. Acta Med. Scand., 178 (1965) 553–559.

Stella, V. and Higuchi, T., Esters of hydantoic acids as pro-drugs of hydantoins. J. Pharm. Sci., 62 (1973) 962–967.

Stella, V., Higuchi, T., Hussain, A. and Truelove, J., The chemistry of a novel 5,5-diphenylhydantoin pro-drug. In Higuchi, T. and Stella, V. (Eds.), Pro-drugs as Novel Drug Delivery Systems, American Chemical Society, Washington, D.C., 1975, pp. 154–183.

Tuttle, C.B., Intramuscular injections and bioavailability. Am. J. Hosp. Pharm., 34 (1977) 965–968.

Viswanathan, C.T., Booker, H.E. and Welling, P.G., Bioavailability of oral and intramuscular phenobarbital. J. Clin. Pharmacol., 18 (1978) 100–105.