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Spectrophotometric determination of the rates of hydrolysis of aldehyde-releasing pro-drugs in aqueous solution and plasma

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Summary

A previously described spectrophotometric method for the determination of formaldehyde or other aliphatic aldehydes has been modified and demonstrated to be a highly useful and convenient means for assessing the rate of hydrolysis of various pro-drugs which upon reconversion to their parent drugs release an aldehyde. The pro-drugs tested include a number of acyloxyalkyl esters of carboxylic acids (pivampicillin, bacampicillin, pivmecillinam and esters of isoguvacine), N-acyloxymethyl esters of 5-fluorouracil and theophylline, N-Mannich bases and N-hydroxymethyl derivatives. Rate data are given for the hydrolysis of these pro-drugs in buffer solutions as well as in solutions containing human plasma and are compared with data obtained by various other methods.

Introduction

A promising and increasingly applied approach to improve the delivery characteristics and therapeutic value of drugs is chemical transformation of the drug substances into per se inactive derivatives (pro-drugs) which convert to the parent active compounds by virtue of enzymic or chemical lability, or both, within the body system before or after reaching the site(s) of action (Stella et al., 1980; Bundgaard

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TABLE 1

SOME PRO-DRUG TYPES RELEASING AN ALDEHYDE DURING THEIR CONVERSION TO THE PARENT DRUGS

Parent drug	Pro-drug type	Reference
Penicillins	Acyloxyalkyl ester	Daehne et al. (1970, 1971)
Cephalosporins	Acyloxyalkyl ester	Bodin et al. (1975); Binderup et al. (1971); Wheeler et al. (1979); Wright et al. (1979)
Mecillinam	Acyloxyalkyl ester	Roholt (1977)
Penicillanic acid sulfone	Acyloxyalkyl ester	Foulds et al. (1980); Baltzer et al. (1980)
Isoguvacine	Acyloxyalkyl ester	Falch et al. (1981)
Cromolyn	Acyloxyalkyl ester	Bodor et al. (1980)
Methyldopa	Acyloxyalkyl ester	Saari et al. (1978)
	Imidoethyl ester	Vickers et al. (1978)
Amoxicillin	Methoxymethyl ester	Smyth et al. (1981)
Phenylbutazone	O-Acyloxymethyl derivatives	Bodor (1982)
	C-hydroxymethyl derivative	Bundgaard and Johansen (1980b)
	C-succinyloxymethyl derivative	Marunaka et al. (1980)
Theophylline	N-Acyloxyalkyl derivatives	Bodor and Sloan (1977)
5-Fluorouracil	N-Acyloxyalkyl derivatives	Ozaki et al. (1978)
Chlorzoxazone	N-acyloxymethyl derivatives	Johansen and Bundgaard (1981b)
Phenytoin	N-acyloxymethyl derivatives	Stella and Sloan (1979)
Tertiary or N-heterocyclic amines, e.g. pilocarpine	N-acyloxyalkyl derivatives ('soft quaternary salts')	Bodor (1977,1979); Bodor et al. (1980)
Various NH-acidic drug substances (e.g. hydantoins, amides, carbamazepine, allopurinol)	N-Mannich bases	Bundgaard and Johansen (1981); Bundgaard et al. (1982); Bundgaard (1982, and references cited therein)
	N-Hydroxymethyl derivatives	Johansen and Bundgaard (1979); Bundgaard and Johansen (1980c); Pitman (1981)

and Hansen, 1981). A basic requirement for a pro-drug is the capability to revert quantitatively and rapidly or with a controlled rate to the parent drug in vivo. Therefore, testing and evaluation of the pro-drug → drug transformation both as regards extent and rate constitutes an important step in the design and development of pro-drugs.

During the last few years an increasing number of pro-drug types have appeared

Scheme 1

$$R - COO - CHR_1 - OOC - R_2 \longrightarrow R - COOH + R_1CHO + R_2COOH$$

$$>N - CHR_1 - OOC - R_2 \longrightarrow >NH + R_1CHO + R_2COOH$$

$$R - CONH - CH_2 - NR_1R_2 \longrightarrow R - CONH_2 + CH_2O + R_1R_2NH$$

$$R - CONH - CH_2OH \longrightarrow R - CONH_2 + CH_2O$$

from which an aldehyde (mostly formaldehyde) is released during the reconversion to the parent drug substances. In Table 1 are listed several examples of such pro-drug types. Thus, as shown in Scheme 1, the conversion of acyloxyalkyl esters, N- or O-acyloxyalkyl derivatives and N-Mannich bases to the parent active drugs are accompanied by the release of formaldehyde or other simple aldehydes in stoichiometric amounts.

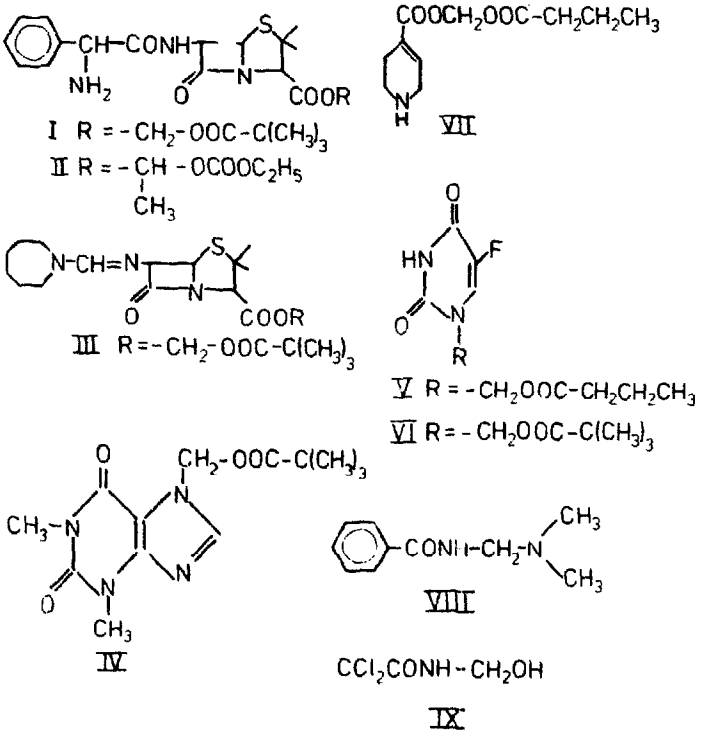

Fig. 1. Aldehyde-releasing pro-drugs investigated in this study: pivampicillin (I), bacampicillin (II), pivmecillinam (III), 7-pivaloyloxymethyl-theophylline (IV), 1-butyryloxymethyl-5-fluorouracil (V), 1-pivaloyloxymethyl-5-fluorouracil (VI), isoguvacine butyryloxymethyl ester (VII), N-(dimethylaminomethyl)benzamide (VIII) and N-(hydroxymethyl)dichloroacetamide (IX).

To study the conversion of aldehyde-releasing pro-drugs the availability of a sensitive, convenient and generally applicable analytical method may be highly useful. This paper reports on such a method based on the colorimetric assay of Sawicki et al. (1961). Its utility to determine the kinetics of hydrolysis of various pro-drugs (Fig. 1) in aqueous solutions containing human plasma is demonstrated and some new rate data are given.

Materials and methods

Apparatus

Spectral measurements were performed with a Perkin-Elmer 124 recording spectrophotometer, using 1-cm cuvettes. Readings of pH were carried out on a Radiometer Type PHM26 meter at the temperature of study. Melting points were taken on a capillary melting-point apparatus and are uncorrected. Microanalysis was carried out at the Microanalytical Department of Chemical Laboratory II, University of Copenhagen.

Chemicals

Bacampicillin hydrochloride and pivampicillin hydrochloride were from a previous study (Bundgaard, 1979) as were the butyryloxymethyl ester of isoguvacine (Falch et al., 1981), N-(dimethylaminomethyl)benzamide (Bundgaard and Johansen, 1980a) and N-(hydroxymethyl)dichloroacetamide (Johansen and Bundgaard, 1979). Pivmecillinam hydrochloride was provided by Leo Pharmaceuticals, Ballerup, Denmark. 7-Pivaloyloxymethyl-theophylline was prepared by reacting the potassium salt of theophylline with pivaloyloxymethyl chloride in acetone as previously described by Bodor and Sloan (1977), m.p. 108–109°C; reported m.p. 108–109.5°C.

3-Methyl-benzothiazol-2-one hydrazone hydrochloride was obtained from Fluka AG, Switzerland and used as received. Other reagents and buffer substances used were of analytical grade. Plasma was obtained by centrifuging heparinized human blood.

Preparation of 1-butyryloxymethyl-5-fluorouracil

Potassium carbonate (690 mg, 5 mmol) was added to a solution of 5-fluorouracil (652 mg, 5 mmol) in 10 ml of dimethyl sulfoxide. Then a solution of chloromethyl butyrate (341 mg, 2.5 mmol) in 5 ml of dimethyl sulfoxide was added dropwise. The reaction mixture was stirred at 80°C for 3 h, cooled and poured onto 15 g of ice. The mixture was acidified with hydrochloric acid and extracted with three 20-ml portions of chloroform. The combined chloroform extracts were washed with 20 ml of water, dried over magnesium sulphate, and evaporated under reduced pressure. The residue was dissolved in toluene and transferred onto a column containing 50 g of silica gel (Silica Woelm 63-100 μm). The sample was eluted with toluene mixed with increasing amounts of ethyl acetate (10–50%). The eluate fractions containing pure title compound were pooled and evaporated in vacuo. Recrystallization of the residue from ether–petroleum ether gave 291 mg (51% yield): m.p. 92–93°C, reported m.p.

96–98°C (Ozaki et al., 1978). Anal.: calcd. for $C_9H_{11}FN_2O_4$: C, 46.96; H, 4.82; N, 12.17—found: C, 47.11; H, 4.92; N, 12.12.

Preparation of 1-pivaloyloxymethyl-5-fluorouracil

This compound was prepared from 5-fluorouracil and chloromethyl pivalate by the procedure described above. After recrystallization from ether–petroleum ether the yield was 24% and the m.p.: 158–160°C. Anal.: calcd. for $C_{10}H_{13}FN_2O_4$: C, 49.18; H, 5.36; N, 11.47—found: C, 49.32; H, 5.42; N, 11.35.

Determination of aldehyde

A 500-µl aliquot of the test solution (aldehyde concentration about 6×10^{-5} M) is mixed with 400 µl of a 0.1 M acetate buffer solution (pH 4.0) and 100 µl of a 0.5% aqueous solution of -methyl-benzothiazol-2-one hydrazone hydrochloride. After standing for 30 min at room temperature (20–25°C) 500 µl of a 0.25% aqueous solution of iron(III)chloride hexahydrate are added. After 10 min (formaldehyde) or 20 min (acetaldehyde) 1500 µl of water are added and the absorbance of the mixture is read at 625 nm (formaldehyde) or 620 nm (acetaldehyde) against a reagent blank. The concentration of aldehyde in the test solution is finally calculated by referring to a standard curve for the appropriate aldehyde.

Determination of rates of hydrolysis

The progress of hydrolysis of various pro-drugs was followed by measuring the production of aldehyde using the colorimetric procedure described above. The compounds were dissolved in water or ethanol and the reactions were initiated by adding 1000 µl of these solutions to 25.00 ml of buffer or plasma solutions pre-equilibrated in a water bath at 37°C. The initial concentrations of the pro-drugs in the reaction solutions were $5–8 \times 10^{-4}$ M. At appropriate times 1000 µl samples were withdrawn and diluted to 10.00 ml with water. A 500 µl sample of each dilution was then immediately analyzed as described. Pseudo-first-order rate constants were calculated from the slopes of linear plots of log $(A_\infty - A_t)$ against time, where A_∞ and A_t are the absorbance readings (at 625 or 620 nm) at infinity and at time t, respectively. To determine the extent of reaction the absorbance readings at infinity (periods corresponding to 8–10 half-lives) were transformed to aldehyde concentration using the appropriate standard curve. For reactions taking place in plasma solutions standard curves were constructed for solutions of aldehyde containing the same concentration of plasma.

Results and discussion

The colorimetric method used for the quantitative determination of formaldehyde or other aliphatic aldehydes is a modification of the procedure described by Sawicki et al. (1961). It is based on the condensation of aldehyde with 3-methyl-benzothiazol-2-one hydrazone to form an azine which subsequently is oxidized by iron(III) ions to give a blue cationic dye. In the present modification of the method the

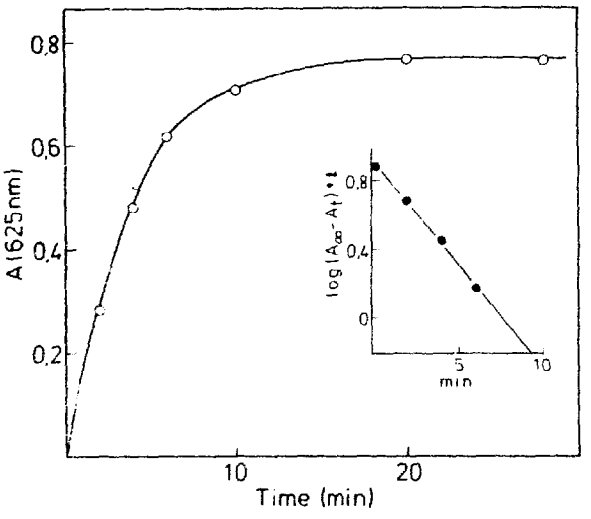

Fig. 2. Time-course for the formation of formaldehyde upon decomposition of 7-pivaloyloxymethyl-theophylline in 0.05 M phosphate buffer solution (pH 7.40) containing 10% human plasma at 37°C. The inset is a first-order plot of the data of the figure.

condensation step was found to be complete after a reaction time of 25–30 min at room temperature. The pH of the acetate buffer may vary between 3.5 and 4.5 without affecting the reaction time. The color produced in the oxidation step showed a high stability, the absorbance remaining constant for at least 1 h.

TABLE 2

PSEUDO-FIRST-ORDER RATE CONSTANTS (k) FOR THE HYDROLYSIS OF VARIOUS PRODRUGS AT 37°C IN 0.05 M PHOSPHATE BUFFER SOLUTION (pH 7.40) WITH AND WITHOUT ADDITION OF HUMAN PLASMA AS DETERMINED ON BASIS OF ALDEHYDE [a] RELEASE

Compound [b]	k(min^{-1})	
	In buffer	In buffer with 10% plasma
Pivampicillin		0.016 [c]
Bacampicillin	0.0034	0.027
Pivmecillinam		0.010 [c]
7-Pivaloyloxymethyl-theophylline	0.0005	0.24
1-Butyryloxymethyl-5-fluorouracil	0.00015	0.0018
1-Pivaloyloxymethyl-5-fluorouracil		0.00030
Isoguvacine butyryloxymethyl ester	0.0011	0.27
N-(Dimethylaminomethyl)-benzamide	0.027	0.025 [d]
N-(Hydroxymethyl)dichloroacetamide	0.0087	0.0083 [d]

[a] Acetaldehyde for bacampicillin and formaldehyde in all other cases.

[b] The initial concentrations of the compounds in the reaction solutions were $3–8 \times 10^{-4}$ M.

[c] In the presence of 20% plasma.

[d] The same values were obtained in buffer solutions containing 25% plasma (Johansen and Bundgaard, 1981a).

The method showed a high reproducibility. Thus, 10 determinations made on the same formaldehyde or acetaldehyde solutions resulted in relative standard deviations within the range 1–2%. The method has also a high sensitivity: the molar absorptivities for formaldehyde and acetaldehyde were determined to be 6.0×10^4 and 6.5×10^4, respectively.

The applicability of the colorimetric method for assessing the stability of aldehyde-releasing compounds was investigated for a number of pro-drugs (Fig. 1) with these characteristics, 0.05 M phosphate buffer solutions (pH 7.40) with or without addition of human plasma being used as test solutions. As described in the experimental section the hydrolysis of the compounds was monitored by measuring the amount of aldehyde released during the reaction. As a typical example, the time-course for formaldehyde formation upon degradation of 7-pivaloyloxymethyltheophylline in a buffer solution containing 10% plasma is shown in Fig. 2. The inset in the figure shows that the hydrolysis follows strict first-order kinetics when monitored by the method.

For all pro-drugs studied the release of formaldehyde or, in case of bacampicillin, acetaldehyde showed strict first-order kinetic behaviour with the rate constants listed in Table 2. Under the experimental conditions the reactions proceeded to completion as revealed by the formation of aldehyde in stoichiometric amounts except those of the ampicillin esters where the yield of aldehyde only amounted to 50–70%. A most likely explanation for this decreased yield is that the aldehyde released reacts with the adjacent amino and amido groups in the side-chain of the ampicillin pro-drugs and of ampicillin itself with formation of a 4-imidazolidinone derivative. Thus, such a compound (hetacillin) is known to be easily formed by reaction of ampicillin with acetone in aqueous solution (Hardcastle et al., 1966; Durbin and Rydon, 1970). In separate experiments we found that when a phosphate buffer solution of pH 7.4 containing equimolar concentrations (8×10^{-4} M) of ampicillin and formaldehyde or acetaldehyde was kept at 37°C, the aldehyde concentration decreased until an equilibrium was reached. The extent of aldehyde loss was 50% for formaldehyde and 23% for acetaldehyde and the half-times for the reactions were 5.6 h and 6 min, respectively.

The usefulness of the spectrophotometric method as a convenient means of assessing the stability of aldehyde-releasing pro-drugs in aqueous solutions may further be substantiated by comparing some of the rate data obtained using this method with those obtained with the use of other methods. For 1-butyryloxymethyl-5-fluorouracil in the pH 7.4 buffer solution containing 10% plasma, a pseudo-first-order rate constant of 0.0020 min^{-1} was obtained by following the formation of 5-fluorouracil using an HPLC-procedure (Møllgaard et al., 1982) which is in good agreement with the value (0.0018 min^{-1}) given in Table 2. Also, the rate data for the isoguvacine butyryloxymethyl ester agree favourably with those previously obtained under the same reaction conditions and using a titrimetric procedure (Falch et al., 1981). Finally, a half-life of 28 min has been reported for the hydrolysis of bacampicillin to ampicillin in a pH 7.4 phosphate buffer containing 10% human serum at 37°C as determined by a combined extraction-microbiological assay procedure (Bodin et al., 1975); this value is similar to that (26 min) obtained on the

basis of the present spectrophotometric method. It should also be mentioned that the method has been widely applied in our recent studies on the stability of various N-Mannich bases and N-hydroxymethyl derivatives (Bundgaard and Johansen, 1981; Bundgaard, 1982, and references cited therein).

Rate data for hydrolysis of the N-acyloxymethyl derivatives of theophylline and 5-fluorouracil have not been reported before. The results given in Table 2 reveal a large difference in reactivity of these derivatives. Whereas the theophylline derivative shows a reactivity of the same order as for acyloxymethyl esters of carboxylic acids, the 5-fluorouracil derivatives are surprisingly resistant to undergoing hydrolysis both in neutral buffer solution and in the presence of plasma. No reasons can presently be offered to explain this difference in reactivity.

It should finally be pointed out that the mild reaction conditions of the present method (i.e. condensation of aldehyde at pH 3.5–4.5 for 30 min at room temperature) may also be advantageous for determining the spontaneous decomposition of some N-hydroxymethylated amines to amine and formaldehyde. Such compounds are intermediates in the metabolic dealkylation of various N-methyl substituted amino compounds as, e.g. hexamethylmelamine and procarbazine (Hickman, 1978; Gescher et al., 1979, 1980), and the method may be mild enough to allow a differentiation between some N-hydroxymethylated amines and formaldehyde.

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