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Hydrolysis of pivampicillin in buffer and plasma solutions. Formation of a 4-imidazolidinone from ampicillin and formaldehyde

Hans Bundgaard and Ulla Klixbüll

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

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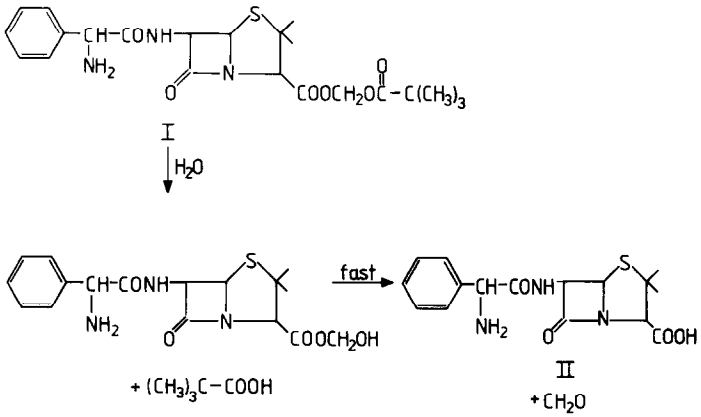
Summary

The degradation of pivampicillin, an ampicillin prodrug, was studied in phosphate buffer solutions of pH 7.40 and in human plasma solutions. The degradation course of pivampicillin was found to involve a quantitative hydrolysis of the pivaloyl ester moiety followed by a reversible reaction between the initial hydrolysis products, ampicillin and formaldehyde, to yield a 4-imidazolidinone derivative of ampicillin. This secondary reaction was shown to account for previously reported findings of decreased yields of both ampicillin and formaldehyde upon the enzymatic and non-enzymatic degradation of pivampicillin.

Introduction

Pivampicillin (I) is a widely used prodrug of ampicillin (II) showing improved oral absorption characteristics as compared to the parent drug (Daehne et al., 1970, 1971). The conversion of pivampicillin to ampicillin during and/or following the absorption from the intestine is generally thought to proceed by an enzymatic hydrolysis of the pivaloyl ester moiety to produce pivalic acid and ampicillin hydroxymethyl ester, which is highly unstable and spontaneously decomposes into

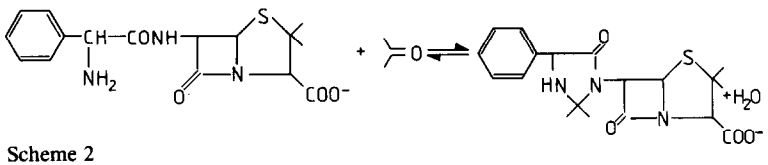
Correspondence: H. Bundgaard, Royal Danish School of Pharmacy, Department of Pharmaceutical Chemistry AD, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.



Scheme 1

ampicillin and formaldehyde (Daehne et al., 1971; Bundgaard, 1979; Ferres, 1983) (Scheme 1). Previous studies in our laboratory have, however, indicated that this degradation course may be complicated by some secondary reactions occurring between the reaction products. Thus, when the hydrolysis of pivampicillin in 20% human plasma solutions was followed by quantitating the formaldehyde released, the yield of aldehyde amounted to only about 50% (Johansen et al., 1983). Moreover, hydrolysis of pivampicillin in a phosphate buffer solution of pH 7.40 at 37°C was found to yield ampicillin in an amount of only about 60% (Bundgaard, 1979). This decreased yield of ampicillin was suggested to be due to cleavage of the β-lactam bond in pivampicillin simultaneously with hydrolysis of the pivaloyl ester moiety (Bundgaard, 1979).

A more likely explanation of the decreased yield of both formaldehyde and ampicillin upon enzymatic or chemical hydrolysis of pivampicillin is, however, that the initial products of hydrolysis, ampicillin and formaldehyde, react with each other with the formation of a 4-imidazolidinone derivative. The α-aminoamide side-chain in ampicillin is known to react with various ketones or aldehydes to give the corresponding 4-imidazolidinones (Johnson and Panetta, 1965; Hardcastle et al., 1966). In a recent study (Klixbüll and Bundgaard, 1985) the kinetics of such reactions has been delineated and it was shown that the reaction of ampicillin with various aldehydes and ketones readily takes place in aqueous solutions at 37°C and is reversible (Scheme 2). The 4-imidazolidinone derivatives produced are very unstable except for the imidazolidinone derived from formaldehyde which possesses



Scheme 2

a half-life of hydrolysis of 29 h at pH 7.45 and 37°C (Klixbüll and Bundgaard, 1985).

The present study was undertaken to establish the enzymatic as well as the non-enzymatic degradation course of pivampicillin. To this end, the hydrolysis of pivampicillin was examined in aqueous buffer solutions of pH 7.40 and in plasma solutions, making use of analytical methods which enables the quantitation of the various reaction products including the ampicillin 4-imidazolidinone derived from formaldehyde.

Materials and Methods

Chemicals

Ampicillin sodium and pivampicillin hydrochloride were kindly provided by Leo Pharmaceuticals, Ballerup, Denmark. Formaldehyde was used in the form of a standardized 37% aqueous solution. Buffer substances and all other chemicals or solvents used were of reagent grade.

Apparatus

High-performance liquid chromatography (HPLC) was done with a Spectraphysics Model 3500 B instrument equipped with a variable-wavelength UV detector (8-μl 1 cm flow cells) and a 10-μl loop injection valve. The column used, 250 × 4 mm, was packed with LiChrosorb RP-8 (7 μm particles) (E. Merck, F.R.G.). Visible spectral measurements were performed with a Shimadzu UV-190 spectrophotometer, using 1 cm cuvettes. Readings of pH were carried out on a Radiometer Type PHM 26 meter at the temperature of study.

Kinetic measurements

The degradation of pivampicillin was studied in 0.02 M phosphate buffer solution of pH 7.40 at 37°C and 60°C and in 80% human plasma solutions (pH 7.40) at 37°C. The ionic strength of the buffer solutions was 0.5 (with potassium chloride). The degradation course for pivampicillin was followed by using a reversed-phase HPLC procedure which enabled separation and simultaneous quantitation of pivampicillin, ampicillin and their corresponding 4-imidazolidinones. The mobile phase used for the analysis of pivampicillin was 0.01 M phosphate pH 7.0–methanol (3 : 7 v/v) and that for ampicillin 0.01 M phosphate pH 7.0–acetonitrile (9 : 1 v/v). The elution was done at ambient temperature at a rate of 1.2 ml · min^{-1} and the column effluent was monitored at 220 nm. Quantitation was done from measurement of peak heights in relation to those of standards chromatographed under similar conditions. The 4-imidazolidinones derived from formaldehyde and ampicillin or pivampicillin were prepared in situ by reacting the penicillins with a large excess of formaldehyde according to a previous study (Klixbüll and Bundgaard, 1985). The reactions were initiated by adding 100 μl of an aqueous stock solution of pivampicillin hydrochloride to 10 ml of pre-heated buffer or plasma solution to produce an initial concentration of pivampicillin of 8×10^{-4} M. The reaction

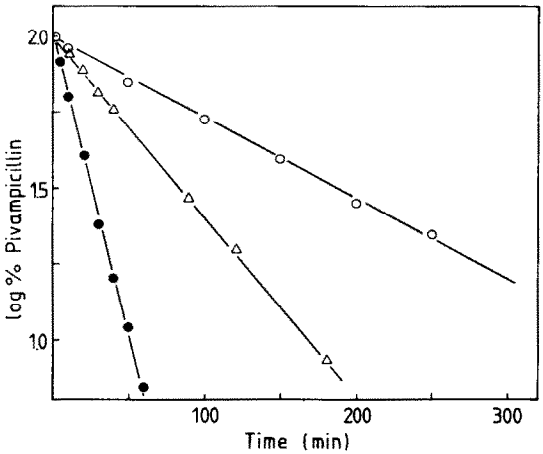


Fig. 1. First-order plots for the degradation of pivampicillin in 0.02 M phosphate buffer solution of pH 7.40 at 37°C (○) and 60°C (●) or in 80% human plasma solution at 37°C (△).

solutions were kept at 37 or 60°C and aliquots were removed at suitable intervals and chromatographed. In the case of the plasma solutions, aliquots of 200 μl were added to 1000 μl of ethanol. After mixing and centrifugation 10 μl of the clear supernatant obtained were chromatographed.

Concurrent with the HPLC measurements the formation of formaldehyde was monitored using a colorimetric method previously described (Johansen et al., 1983).

Results and Discussion

The overall degradation of pivampicillin in 0.02 M phosphate buffer solutions (pH 7.40) or in 80% human plasma solutions (pH 7.40) followed strict first-order kinetics for several half-lives when monitored by a specific HPLC procedure as shown in Fig. 1. The pseudo-first-order rate constants obtained from the slopes of the plots in Fig. 1 are listed in Table 1. For comparison the rate constants for the

TABLE 1

PSEUDO-FIRST-ORDER RATE CONSTANTS (IN MIN^{-1}) FOR THE OVERALL DEGRADATION OF PIVAMPICILLIN AND AMPICILLIN IN BUFFER AND HUMAN PLASMA SOLUTIONS

Compound	0.02 M phosphate pH 7.40		80% human plasma (at 37°C)
	At 60°C	At 37°C	
Pivampicillin	0.0461	0.0062	0.014
Ampicillin	0.0013	0.00010	-

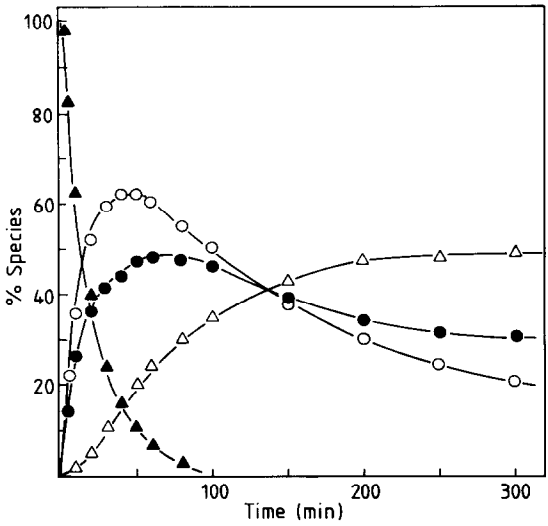


Fig. 2. Time-courses for pivampicillin (▲), ampicillin (○), formaldehyde (●) and the 4-imidazolidinone III (△) in the degradation of pivampicillin (8×10^{-4} M) in 0.02 M phosphate buffer solution of pH 7.40 at 60°C.

degradation of ampicillin in the same buffer at 37 and 60°C were also determined and are given in Table 1. It is noteworthy that the rate of degradation of pivampicillin in the plasma solution is only about two-fold greater than that for the degradation in the phosphate buffer solution. In whole blood the hydrolysis of pivampicillin proceeds, however, far more rapid than in plasma (Daehne et al., 1970). Phosphate ions are known to catalyze the degradation of penicillins including ampicillin in aqueous solution by a nucleophilic reaction mechanism (Bundgaard and Hansen, 1981) but the small difference observed in the rate of pivampicillin degradation in phosphate buffer and plasma solutions is not influenced by this since the 80% plasma solutions used also contained 0.02 M phosphate.

Chromatographic analysis of the reaction solutions revealed the formation of ampicillin and its 4-imidazolidinone derived from formaldehyde (III) whereas colorimetric analysis showed the formation of formaldehyde. The time-courses for these products in 0.02 M phosphate buffer solutions of pH 7.40 at 60 and 37°C are shown in Figs. 2 and 3. The data obtained, in particular those at 60°C, show that the overall reactions may be depicted as shown in Scheme 3. Pivampicillin is quantitatively (> 95%) hydrolyzed to yield ampicillin and formaldehyde. The latter compound then undergoes a reversible reaction with the ampicillin side-chain with formation of a 4-imidazolidinone derivative as described earlier (Klixbüll and Bundgaard, 1985). That the hydrolysis of pivampicillin to ampicillin is quantitative is evidenced by the fact that when up to 50% of the pivampicillin had disappeared the amount of ampicillin and compound III formed corresponded exactly (97–104%) to the loss in pivampicillin. Furthermore, kinetic analysis of the concentration–time data for pivampicillin and ampicillin according to a previously described procedure

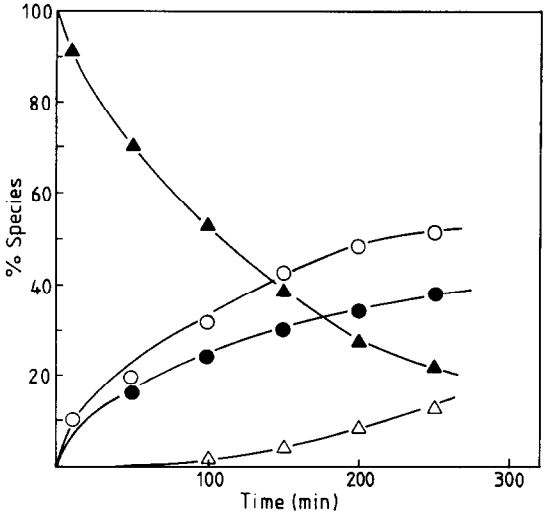
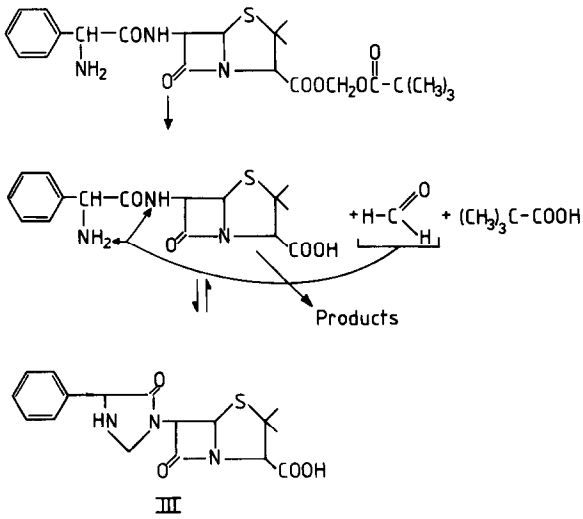


Fig. 3. Time-courses for pivampicillin (▲), ampicillin (○), formaldehyde (●) and the 4-imidazolidinone III (△) in the degradation of pivampicillin (8×10^{-4} M) in 0.02 M phosphate buffer solution of pH 7.40 at 37°C.

(Larsen and Bundgaard, 1978; Bundgaard and Larsen, 1978) afforded pseudo-first-order rate constants for the formation of ampicillin of 0.045 min^{-1} (at 60°C) and 0.0060 min^{-1} (at 37°C). These values are seen to be almost identical to the pseudo-first-order rate constants for the disappearance of pivampicillin (Table 1).

Fig. 2 shows a pronounced lag-time in the formation of compound III which



Scheme 3

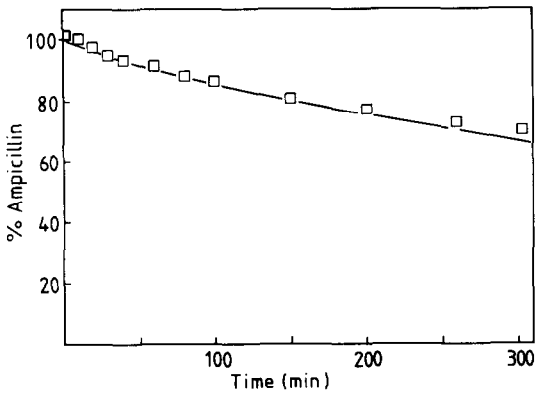


Fig. 4. Plot showing the rate of hydrolysis of ampicillin (———) in 0.02 M phosphate buffer solution of pH 7.40 at 60°C and the decrease of the sum (□) of the percentage concentrations of the compounds of Fig. 2.

agrees with the proposed reaction scheme in that ampicillin and formaldehyde have to be formed before the formation of III can take place. When all pivampicillin had degraded almost equal amounts of ampicillin and the 4-imidazolidinone derivative III are present (cf. Fig. 2). As seen from Fig. 2 the latter derivative continues to be formed, which is paralleled by a decrease in the amounts of ampicillin and formaldehyde. Compound III reaches a maximal concentration at 60°C corresponding to 49%, on a molar basis, of the initial pivampicillin concentration (8×10^{-4} M). The sum of all products present in the phosphate buffer solution during the degradation of pivampicillin is depicted as a function of time in Fig. 4. It was found that during the initial stage ($< 50\%$) of pivampicillin degradation this sum amounts to 100%. Thereafter, it decreases continuously and as seen from Fig. 4 the progress of this decrease corresponds closely to the rate of hydrolysis of ampicillin in the buffer solution without formaldehyde.

It may be envisaged that the formaldehyde released upon the hydrolysis of pivampicillin may also react with the side-chain in the remaining pivampicillin to produce a 4-imidazolidinone in addition to its reaction with ampicillin. The HPLC analysis revealed, however, no evidence for the occurrence of such a reaction (i.e. $< 2\%$) and furthermore, the strict first-order kinetics observed for the disappearance of pivampicillin (Fig. 1) is indicative of the insignificance of such a reaction.

It is of interest to note that the rate of liberation of ampicillin from pivampicillin proceeds according to first-order kinetics with no indication of a lag-time. This lends evidence to the suggestion (Daehne et al., 1970, 1971; Bundgaard, 1979; Ferres, 1983) that the intermediary ampicillin hydroxymethyl ester (cf. Scheme 1) is highly unstable and spontaneously decomposes into ampicillin and formaldehyde.

The degradation course of pivampicillin observed in phosphate buffer solutions at physiological pH was also found to apply for the degradation occurring in 80% human plasma solutions. At an initial pivampicillin concentration of 8×10^{-4} M the maximal amounts of ampicillin formed in plasma solutions corresponded to 45%

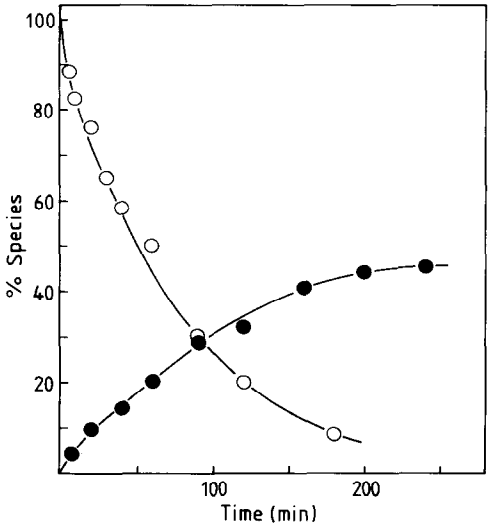


Fig. 5. Plots showing the rate of disappearance of pivampicillin (○) and the rate of formation of ampicillin (●) in the degradation of pivampicillin (8×10^{-4} M) in 0.02 M phosphate buffer solution (pH 7.40) containing 80% human plasma (at 37°C).

(Fig. 5) which is close to the value (53%) observed in buffer solutions at 37°C. The yield of formaldehyde formed corresponded to 50%. Both figures indicate the formation of compound III to an extent of about 50%. HPLC analysis of the plasma reaction solutions showed the formation of compound III but due to insufficient separation between the peaks in the chromatograms for the plasma solutions no quantitative data could be obtained.

The results obtained thus demonstrate unequivocally that the less than expected yield of both ampicillin and formaldehyde formed upon hydrolytic degradation of pivampicillin is due to a secondary reversible reaction between these products with formation of a 4-imidazolidinone derivative. The data obtained are for an initial pivampicillin concentration of 8×10^{-4} M. At higher initial concentrations the amounts of compound III formed would be increased since the formation of the compound is an overall second-order process, i.e. it shows a first-order dependence on both ampicillin and formaldehyde concentration (Klixbüll and Bundgaard, 1985).

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