J. Pharm. Pharmacol. 1997, 49: 843–851
Received March 14, 1997
Accepted June 2, 1997

Degradation Pathways of Ampicillin in Alkaline Solutions

V. A. ROBINSON-FUENTES*, T. M. JEFFERIES AND S. K. BRANCH

School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK

Abstract

Ampicillin trihydrate, sodium salt, in aqueous solution has a pH of about 8. No complete degradation pathway has been proposed to explain the degradation of ampicillin under alkaline conditions and the information available explains the formation of only certain products. The present work was carried out with the aim of providing this information.

The formation of degradation products of ampicillin trihydrate, sodium salt, produced in aqueous solutions (pH 12 and 37°C) have been studied as an accelerated form of the possible degradation that may occur in aqueous solutions at pH 8. Some of the degradation products formed under these conditions were then obtained either by synthesis or by degradation of ampicillin sodium followed by isolation using semi-preparative HPLC. These compounds were characterized by ^{1}H NMR spectroscopy.

The information obtained from the experiments by HPLC and NMR spectroscopy made it possible to propose a degradation pathway for ampicillin under the conditions described above. 5*R*-penicilloic acid is the first degradation product of ampicillin and subsequently undergoes epimerization at C-5 to form the 5*S* isomer via the imine tautomer. Mechanisms for the formation of compounds previously believed to form only under acidic conditions are proposed, i.e. ampicillin penilloic acid and 2-hydroxy-3-phenylpyrazine. The formation of ampicillin polymers was detected in dilute solutions (1% w/v) within a few hours of dissolution. The presence of ampicillin penicillenic acid and ampicillin penamaldic acid was detected by ^{1}H NMR and their main spectroscopic features determined.

Since the discovery of benzylpenicillin in 1944, a number of studies have been conducted on the stability of penicillins, including those by Hou & Poole (1971), Blaha et al (1976), Degelaen et al (1979), Kessler et al (1983) and Lipczynski (1988). Mechanisms of degradation under various conditions of temperature and pH have been proposed for this group of antibiotics based on the behaviour of benzylpenicillin as a model compound. This has led to some incorrect conclusions about the way other antibiotics of this group degrade, for example ampicillin, which is an aminopenicillin. Ampicillin is widely used in chemotherapy because of its stability in acid, low toxicity, efficient absorption and low minimum inhibitory concentration against bacteria (Masada et al 1980). This stability has been attributed to the incorporation of an electron-withdrawing substituent (NH_2) on its side chain causing a decrease in the nucleophilicity of the side-chain carbonyl (Kessler et al 1983). Since the protonated α-amino group in ampicillin plays a powerful electron-attacking role, these aminopenicillins are markedly stable to acids (Tsuji et al 1978). However, although the amino group on the side chain of ampicillin makes the molecule more stable against acids, it enhances its instability in neutral and alkaline conditions (Van Krimpen et al 1987).

The degradation of ampicillin in acidic conditions is very slow and so the study of its stability at this pH has less significance than its stability under alkaline conditions, since ampicillin sodium in aqueous solution has a pH of about 8. No degradation pathway has yet been proposed to explain the degradation of ampicillin under alkaline conditions and the information available only explains the formation of certain degradation products. There is no mechanism that suggests the interactions between all the degradation products, including those mentioned in the following paragraph.

Ampicillin can polymerise, via nucleophilic attack by the side-chain amino group in one molecule upon the β-lactam carbonyl of a second molecule (Tomlinson et al 1980). Polymers such as dimers, trimers and tetramers have been found in ampicillin preparations (Roets et al 1984) and these are known to possess strong antigenic properties (Bundgaard & Larsen 1977). Ampicillin, like any other β-lactam antibiotic with an amino group in the side chain, can, under certain circumstances, form a diketopiperazine (DKP) due to intramolecular aminolysis (Fogg & Fayad 1980). This reaction is favoured by the presence of carbohydrates or polyhydric alcohols (Bundgaard & Larsen 1979). Hydrolysis of ampicillin in-vivo yields ampicillin penicilloic acid (APC) and this type of derivative is common to all penicillins (Masada et al 1980). This compound is also the first degradation product in-vitro of ampicillin in either acid or alkaline conditions. Compounds such as ampicillin penillic acid (API), ampicillin penicillenic acid (APE) and ampicillin penamaldic acid (APENAM) have been described as playing an important role in the degradation of ampicillin. However, no experimental evidence has been reported.

The purpose of this work is to propose a pathway that explains in greater detail the degradation behaviour of ampicillin in basic conditions. Aged solutions of ampicillin were monitored by HPLC with UV detection, fractions collected, purified and their identities characterized by ^{1}H NMR spectroscopy to establish the order of appearance of the degradation compounds.

*Present address: V. A. Robinson-Fuentes, Departmento de Quimica y Biologia, Iniversidad de las Americas-Puebla, Santa Catarina Martir, Puebla 72820, Mexico.

Correspondence: T. M. Jefferies, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY, UK.

Materials and Methods

Preparation of degradation products

APC and DKP were synthesized following the methods of Munro et al (1978) and Bundgaard & Larsen (1979), respectively. The yields for APC and DKP were 38 and 16%, respectively. NMR and HPLC analysis showed only the presence of peaks corresponding to APC and DKP, indicating the good purity of the compounds. The method of Lebelle et al (1979) for 2-hydroxy-3-phenylpyrazine (HPP) was also followed, but produced 2-hydroxy-3-phenyl-6-methylpyrazine in 18% yield and poor purity as observed by NMR.

Ampicillin penilloic acid (APO), HPP and ampicillin dimer were obtained by semi-preparative HPLC. Ampicillin sodium (2·5 g) was dissolved in 250 mL buffer pH 12 (2·5 mM NaOH/50 mM KCl) and kept in a water bath at 37°C, and 10-mL samples were withdrawn from the degradation flask every 24 h for ten days. The pH of the buffer did not fall below 10 throughout this period. Each 10-mL sample was adjusted to pH 3 with diluted H_3PO_4 and injected in 2-mL portions, on the same day it was collected, into a reversed-phase column (Nucleosil ODS 5 μm, 250 × 10 mm i.d.) at 35°C fitted to an HPLC instrument (Milton Roy Constametric 3000 dual piston pump with a Dupont Instruments variable wavelength UV spectrophotometer, and a Rheodyne 7125 manual sample injector fitted with a 2-mL loop). The compounds were eluted with a mobile phase at 4 mL min^{-1} consisting of 2 mM sodium hexane sulphonate in water (pH 3) obtained by addition of diluted H_3PO_4 and acetonitrile in a step gradient mode (15% of acetonitrile from 0 to 6 min; 30% 6–12 min and 50% 12–40 min, Fig. 1). Detection was at 254 nm and 1–2 AUFS. Fractions of APO, HPP and ampicillin dimer were collected in an ice-bath and freeze-dried immediately after collection. These fractions were further purified by redissolving the samples in the minimum amount of mobile phase and injecting them again into the column using isocratic conditions. This time, the use of the ion-pairing reagent sodium hexane sulphonate was omitted from the mobile phase in order to avoid it contaminating the degradation products. For all the degradation products, retention times were much reduced, as expected.

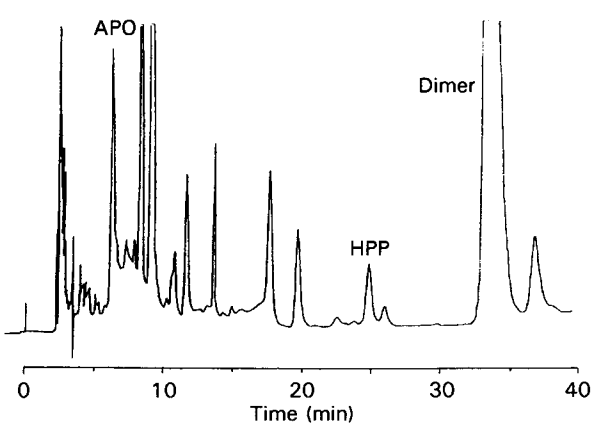


FIG. 1. Isolation of ampicillin penilloic acid (APO), 2-hydroxy-3-phenylpyrazine (HPP) and ampicillin dimer (Dimer) from ampicillin sodium degraded at pH 12, using semi-preparative HPLC with a step-gradient mobile phase containing sodium hexane sulphonate as ion-pairing reagent.

Under these conditions the APO fraction gave a peak at 3·5 min using 15% acetonitrile, (Fig. 2), HPP gave a peak at 6 min using 30% acetonitrile, (Fig. 3), and the ampicillin dimer gave a peak at 7 min using 50% acetonitrile, (Fig. 4). The main peak in each of these chromatograms contained the target compounds and was collected and freeze-dried for subsequent NMR characterization and confirmation of purity by HPLC analysis.

^1H NMR measurements

270-MHz ^1H NMR spectra were recorded on a Jeol JNM GX-270 FT instrument at ambient temperature. Scans (64–256 depending on the sample size available) with a frequency range of 3001·2 Hz were collected into 32 K data points giving a digital resolution of 0·18 Hz per point. Samples were dissolved in D_2O and d_6-DMSO and were referenced internally to HOD (4·8 ppm) and DMSO (2·5 ppm).

The overall degradation behaviour of ampicillin was monitored by dissolving a sample of the sodium salt in D_2O and adding a drop of NaOD (30% w/v). NMR spectra were acquired immediately after preparation and after 10, 20, 30 min and then every 24 h for a week.

Fluorescence spectrum of HPP

The fluorescence spectrum of HPP was obtained from an aqueous solution of the compound isolated by HPLC. Scans of the solution were performed in a Shimadzu RF-540 spectrofluorophotometer in the regions of 200–400 nm and 350–

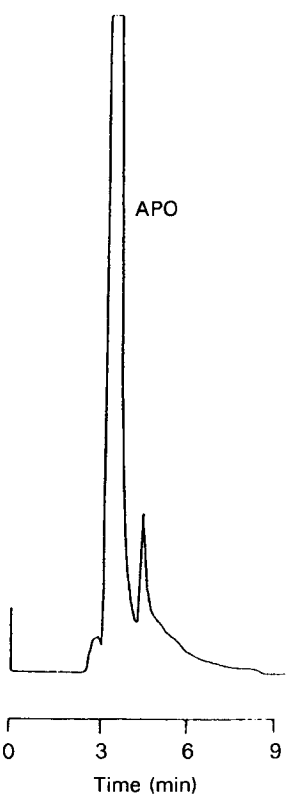


FIG. 2. HPLC purification of ampicillin penilloic acid (APO) fraction from Fig. 1, using acetonitrile:water adjusted to pH 3 with H_3PO_4 (15 : 85%). Major peak collected for NMR analysis.

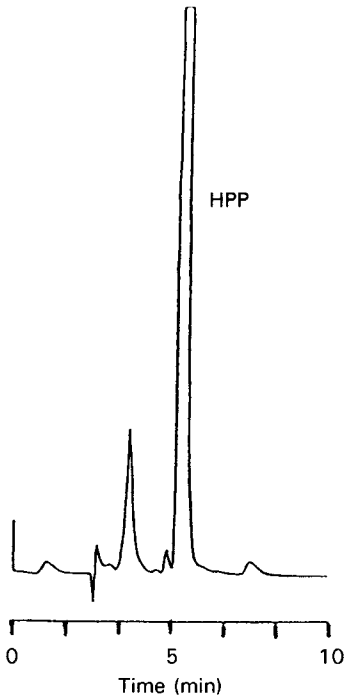


FIG. 3. HPLC purification of 2-hydroxy-3-phenylpyrazine (HPP) fraction from Fig. 1, using acetonitrile:water adjusted to pH 3 with H_3PO_4 (30%:70%). Major peak collected for NMR analysis.

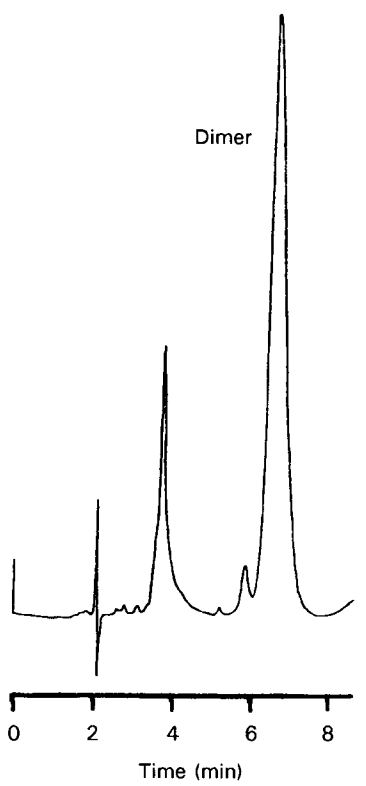


FIG. 4. HPLC purification of ampicillin dimer (Dimer) fraction from Fig. 1, using acetonitrile:water adjusted to pH 3 with H_3PO_4 (50%:50%). Major peak collected for NMR analysis.

600 nm in order to obtained the excitation and emission spectra of the compound.

Thin-layer chromatorgraphic identification of polymers

The identification of polymers was carried out using a solution of ampicillin aged for 7 days at pH 12. The silicagel plates (Merck) were used with a mixture of ethyl acetate:acetic acid:water (7:2:1, v/v/v), as described by Roets et al (1984).

Results and Discussion

Table 1 summarizes the NMR characteristics of the compounds obtained in these experiments whose structures can be seen in Scheme 1. The spectral features of APC and DKP in Table 1 correspond to the samples that were obtained by synthesis whereas APO, HPP and ampicillin dimer correspond to those samples obtained by semi-preparative HPLC isolation.

Ampicillin penicilloic acid (APC)

All penicillins degrade to the corresponding penicilloic acid, and it is very well documented as a degradation product of ampicillin in neutral and alkaline solutions (James & Riley 1985; Haginaka & Wakai 1986; Akanni & Ayim 1992) although it can also be found in acidic solutions (Masada et al 1980; Bird et al 1983; Haginaka & Wakai 1986). APC is microbiologically inactive and is probably associated with allergic reactions similar to those caused by benzylpenicilloic acid (Ghebre-Sellasie et al 1984).

The chemicals shifts obtained for this compound are in agreement with those previously reported by Bird et al (1983) for the sodium salt of APC. The chemical shifts indicate that the compound retained the 3*S*,5*R*,6*R*-configuration of the parent compound. HPLC analysis showed the presence of only one peak when the solution was analysed immediately after

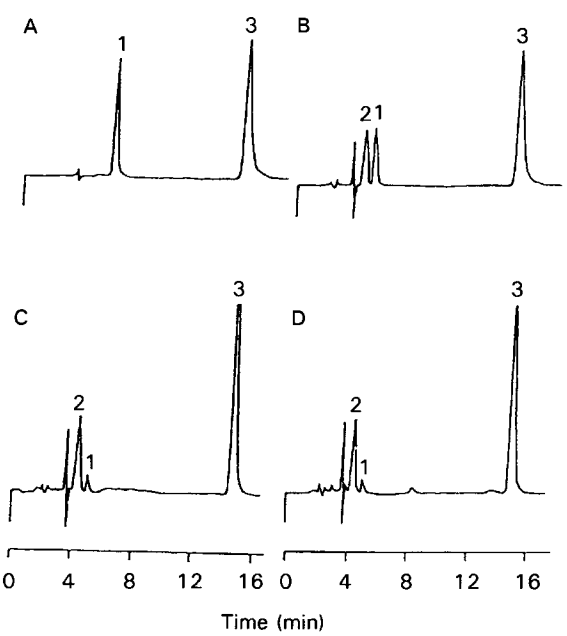


FIG. 5. Epimerization of 5*R*-ampicillin penicilloic acid to 5*S*-ampicillin penicilloic acid in 0·012 M NaOH/KCl buffer, pH = 12 at 37°C at various times. A. 0 h; B. 5 h; C. 24 h and D. 48 h. 1 = 5*R*-APC, 2 = 5*S*-APC, 3 = internal standard, sulphathiazole.

Table 1. ^{1}H NMR chemicals shift (δ in ppm) data for the degradation compounds of ampicillin obtained by either isolation or by synthesis.

Compound	2(α/β)CH_3	3H	5H,6H	Ar-CH	Ar	Solvent
5*R*-APC	1·10,1·16s	3·04s	4·25,5·05d	5·17s	7·5	D_2O
5*S*-APC	0·51,1·43s	3·26s	4·83,5d	5·27s	7·5	D_2O
5*R*-APO	0·9,1·43s	3·31s	4·81s*	4·56t	3·05-3·2dd	d_6-DMSO
5*S*-APO	1·07,1·45s	3·2s	4·8s*	4·49t	3·31-3·52dd	d_6-DMSO
Diketopiperazine	1·2,1·5s	3·58s	5·1d,3·8s	4·9s	7·4m	d_6-DMSO
2-Hydroxy-3-phenylpyrazine					7·4m, 8·27m	d_6-DMSO
Dimer						
Residue I	1·33,1·52s	3·92s	5·3d,5·47dd	5·8s		d_6-DMSO
Residue II	1·0,1·09s	3·3	4·8d,4·75dd	4·9d		

s = singlet, d = doublet, dd = doublet of doublets, m = multiplet, t = triplet. *CH_2–CH.

preparation by the method of Munro et al (1978), indicating the good purity of the sample. HPLC analysis also showed that the 5*S*-epimer was formed from the 5*R*-epimer under basic conditions at 37°C (Fig. 5). The 5*S*-epimer was then obtained in-situ for NMR analysis by leaving a solution of 5*R*-APC in D_2O containing one drop of NaOD to stand for 24 h. A second set of signals was then obtained which corresponded to those reported for the 5*S*-form (Bird et al 1983). Table 1 shows that the chemical shifts of both compounds are very similar, except for the 2(α/β)–CH_3 resonances where the signals of the 5*S*-form are more widely spaced. Branch et al (1987) reported that the separation of these two signals in the 5*R*- isomer of benzylpenicilloic acid is enhanced over that of the parent compound and even more so for the 5*S*-isomer. The NMR spectrum of the mixture of these two compounds shows that there was no deuterium incorporation at C-6 (Scheme 1). Therefore, the epimerization process occurs via the imine tautomer as was proposed previously by Davies et al (1991) for the epimerization of benzylpenicilloic acid.

Semi-preparative HPLC showed that APC was present in all samples, even after standing for 14 days. NMR revealed that the compound was present as a mixture in which the 5*S*-isomer was present in greater proportion, in an approximate ratio of 3:1 (5*S*:5*R*), when the equilibrium of these two compounds was established after 24 h.

Ampicillin penilloic acid (APO)

Penilloic acids may, in general, be regarded as the decarboxylation products from the corresponding penicilloic acids under acidic conditions, according to Hou & Poole (1971), Schwartz (1969) and Blaha et al (1976). Hou & Poole (1971) reported the presence of this compound in strong basic conditions by TLC analysis, although no experimental evidence was provided. The loss of one molecule of carbon dioxide at C-6 removes one of the molecule's chiral centres. Nevertheless, the centre at C-5 remains intact and therefore two isomers can be found, 5*R*-APO and 5*S*-APO.

There are several methods in the literature describing the production of this compound but only one of them was reported to produce successful results (Suwarumpha & Freas 1989).

Semi-preparative HPLC was used to obtain APO because none of the reported experimental procedures, including that of Suwarumpha & Freas (1989), produced positive results. Ampicillin sodium was degraded at pH 12.

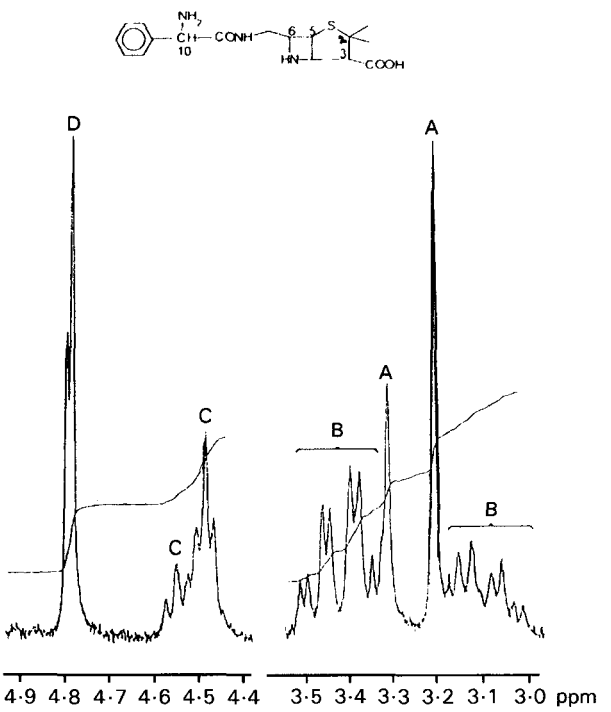
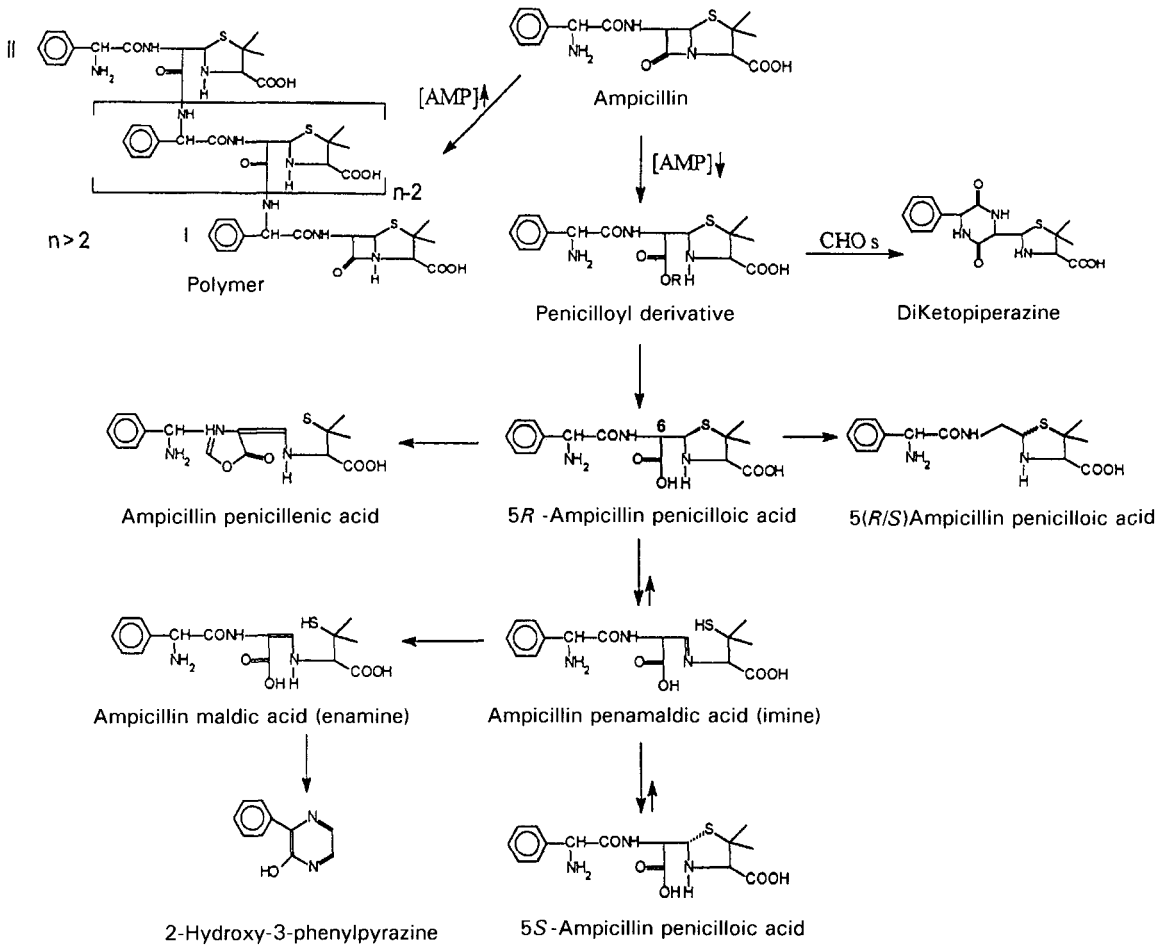


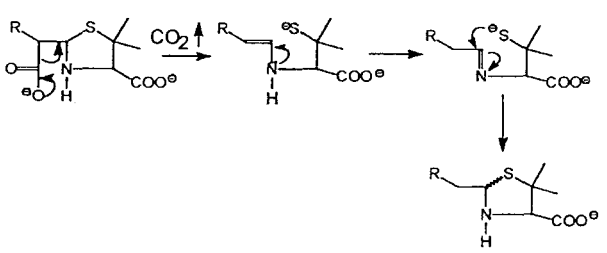
FIG. 6. Expansion of the 270-MHz ^{1}H NMR spectrum of a mixture of 5*R*- and 5*S*- ampicillin penilloic acids isolated by semi-preparative HPLC. A. 3-H; B. 6-H_2; C. 5-H; D. 10-H. The signals in greater proportion correspond to 5*S*-APO.

The APO sample isolated by semi-preparative HPLC (Fig. 2), showed a double set of signals when it was analysed by NMR, indicating the isomeric nature of the sample (Fig. 6). The two multiplets at 3·05–3·2 and 3·31–3·52 ppm confirmed the identity of the sample as APO, since both multiplets were composed of 8 lines as required by the ABX system (CH_2–CH). The height of the two sets of signals indicated that one of the isomers was in a greater proportion. There was no NMR information available for this compound and so the assignment was performed by comparison with chemical shifts reported for benzylpenicilloic acid (Branch et al 1987), benzylpenilloic acid (Kiener & Waley 1978) and those found in the experiments undertaken here for APC. The complete results are summarized in Table 1.



Scheme 1. Proposed sequence of the formation of degradation products of ampicillin sodium dissolved in aqueous solution at pH 12 and 37°C.

As indicated before, this compound was known to be a degradation product of ampicillin only under acidic conditions and these experiments show that it can also be formed in basic conditions. Scheme 2 is proposed to explain the decarboxylation of APC to form APO, under the conditions used in these experiments (pH 12).



Scheme 2. Proposed method of decarboxylation of ampicillin penicilloic acid (APC) to form ampicillin penilloic acid (APO) in aqueous solution at pH 12 and 37°C.

Diketopiperazine (DKP)

DKP is also known as piperazine-2,5-dione and is a cyclic derivative of ampicillin (and aminopenicillins in general) which is formed in solutions containing carbohydrates or polyalcohols. The formation of this compound is optimal between pH 9 and 9·5; above pH 9·5 it decreases (Bundgaard & Larsen 1979). Above this pH, hydroxide-catalysed hydrolysis becomes increasingly predominant in relation to the spontaneous cyclization to form DKP. This compound was not found in the degradation mixture prepared for the isolation of degradation products probably because the pH of the solution was 12, well above 9·5. However, in an independent experiment, in the presence of glucose, it was found that DKP was formed at pH 9.

2-Hydroxy-3-phenylpyrazine (HPP)

HPP was isolated by semi-preparative HPLC (Fig. 3), from ampicillin sodium degraded at pH 12 and 37°C in aqueous 2·5 mM NaOH/50 mM KCl. The fluorescence spectrum of the isolated compound exhibited excitation and emission maxima at 345 and 425 nm, respectively, which is in agreement with the maxima reported in the literature (Barbhaiya & Turner 1976; Lebelle et al 1979). The identification of this compound was supported by ^{1}H-NMR evidence.

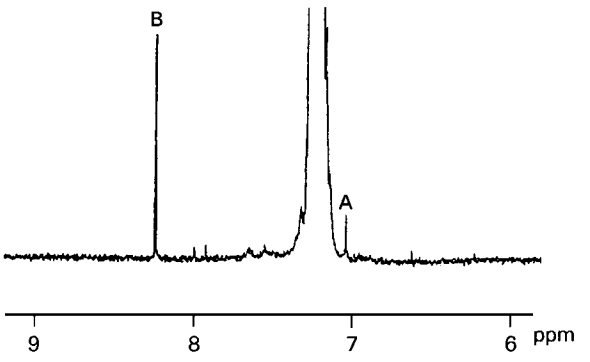


FIG. 7. Expansion of the 270-MHz ^{1}H NMR spectrum of a solution of ampicillin in D_2O with NaOD aged for six days. The singlets correspond to protons in position 5 of ampicillin penicillenic acid (A) and ampicillin penamaldic acid (B).

The conversion of ampicillin into fluorescent compounds, including HPP, has been the basis for a number of fluorometric methods for the determination of ampicillin. Uno et al (1981), published a thorough examination of the degradation products of ampicillin formed by using six different procedures that were typical of those published by Jusko (1971), Barbhaiya et al (1978), Lebelle et al (1979) and Miyazaki et al (1974). They found that the Jusko (1971) and Barbhaiya et al (1978) methods produced 2-hydroxy-3-phenyl-6-methylpyrazine. The methods of Lebelle et al (1979) and Miyazaki et al (1974) produced 2-hydroxy-3-phenylpyrazine (HPP), but only as a minor product. Uno et al (1981) succeeded in isolating a new, water-soluble compound that, unlike HPP, was not soluble in ethyl acetate, and identified it as 2-hydroxy-3-phenyl-6-penillomethylpyrazine—the major fluorescent product.

The formation of HPP by the methods of Lebelle et al (1979) and Miyazaki et al (1974) both required alkaline degradation of ampicillin to ampicillin penicilloic acid (5*R*-APC) as a first step, followed by either acidic conditions (pH 2 at 50°C for 2 h; Lebelle et al 1979) or 0·04% $HgCl_2$ in citrate buffer pH 2·5, then phosphate buffer pH 6 at 40°C for 20 min (Miyazaki et al 1974). The experiments in this report show that HPP can also form under solely basic conditions, and a proposed mechanism for this is illustrated in Scheme 3.

It is considered that APENAM is formed and subsequently converted to ampicillin penaldic acid which gives ampicillin penilloaldehyde after alkaline hydrolysis and finally, cyclization and oxidation yields HPP.

Polymers

Aminopenicillins can undergo intermolecular reaction resulting in the formation of polymers (Stanfield et al 1978; Van Krimpen at al 1987). The formation of these compounds is accelerated in concentrated solutions (Bundgaard 1976; Bundgaard & Larsen 1977; Ueno et al 1981; Aki et al 1991). The rate and degree of polymerization depends upon pH and the initial concentration of the ampicillin solution. Increases in pH and concentration lead to a higher degree of polymerization. Oligomers of up to nine units have been reported by different authors (Kuchinskas & Levy 1972; Bundgaard & Larsen 1977; Larsen & Johansen 1982). However, Roets et al (1984) used mass spectrometry to show that ampicillin formed dimers, trimers and tetramers in 20% w/v solutions at pH 8·5 rather than the dimers, tetramers and hexamers previously suggested (Bundgaard & Larsen 1977) on the basis of functional group analysis. These polymers have been found to be present in some clinically used ampicillin solutions (Bundgaard 1978) and are associated with allergic reactions (Larsen & Johansen 1982; Takagi et al 1983; Urleb et al 1985).

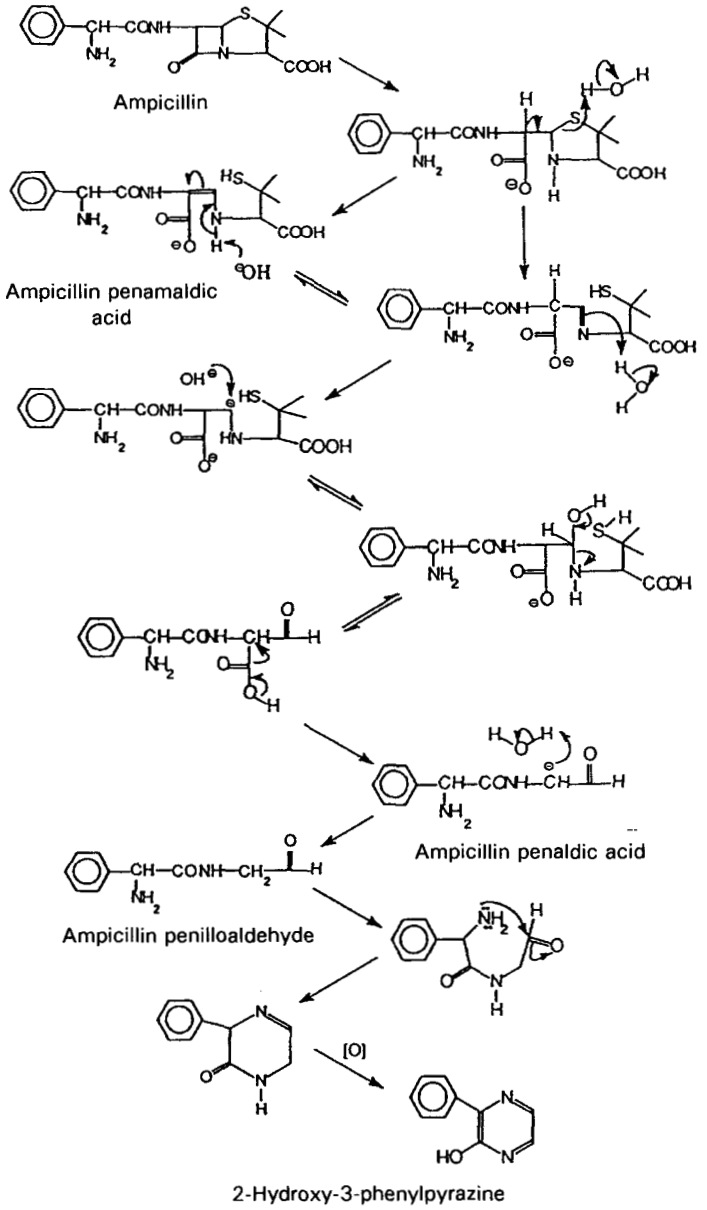
Existing methods to isolate ampicillin polymers use cation-exchange or size-exclusion chromatography. In the experiments described here, isolation was carried out using a reversed-phase Nucleosil column (Fig. 4) and gave the salt form. The compound isolated by these means, and suspected to be a polymer, produced a fairly complicated NMR spectrum. The two sets of signals for the methyls at C-2 produced similar intensities and indicated a dimer. Roets et al (1984) suggested that the degree of polymerization could easily be observed from these signals since each residue shows its own set. Assignments were made with the aid of homo-nuclear decoupling experiments and the results are shown in Table 1. The chemical shifts in residue II are all shifted upfield indicating cleavage of the β-lactam ring (Aki et al 1991) to give the dimeroate.

The formation of the dimer could be detected by HPLC even in a 1% solution (pH 12, 37°C) after 4 h. Aki et al (1991) reported the formation of the dimer within 1 h in a 50% solution, followed by polymers of higher molecular weight. The sample used here for isolation of the dimer was collected from an ampicillin solution aged for four days but its presence could be detected even after 6 days. The ampicillin solution used to identify the presence of higher polymers by TLC was aged for longer (2 weeks) and the trimer was suspected from comparison with reported R_f values using the same TLC conditions (Roets et al 1984).

Degradation pathways

An NMR experiment was carried out to give a better understanding of the degradation behaviour of ampicillin. Spectra were recorded at intervals using D_2O as the solvent with the addition of NaOD, and in this way it was possible to monitor the appearance and disappearance of breakdown products through the chemical shifts already known from pure standards. The first sample was recorded approximately 5 min after the reaction started and the only signals present corresponded to 5*R*-APC. This rapid hydrolysis of ampicillin indicated that the pH was 12 or greater. Epimerisation of 5*R*-APC followed, equilibrium being established after 24 h, when 5*S*-APC was predominant. The presence of several sets of 2-methyl signals was indicative of the formation of other degradation compounds. This region can be considered as the fingerprint region for ampicillin derivatives (Connor et al 1994) if the chemical shifts of pure compounds are known. Since there were only a few pure compounds available, some deductions were made from the NMR data, especially after comparing the results in this report with those obtained from similar studies of benzylpenicillin degradation (Degelaen et al 1979; Lipczynski 1988).

Two singlets near the aromatic region (Fig. 7) seemed indicative of the presence of degradation products not recorded before by NMR. One singlet at 7·04 ppm appeared after 20 min of reaction and its intensity increased six times from the original intensity in 24 h, after which time the peak



Scheme 3. Proposed method for the formation of 2-hydroxy-3-phenylpyrazine (HPP) from ampicillin sodium dissolved in aqueous solution at pH 12 and 37°C.

decreased in height. However the signals were always very small. The other singlet appeared at 8·25 ppm and was evident after 24 h. Its intensity also increased with time (14 times from the original) and its maximum occurred after 6 days. Comparing the information available for benzylpenicillin, it was found that the protons at position five in penicillenic and penamaldic acids are shifted downfield from those of the corresponding parent compound or other degradation products. The aforementioned acid derivatives from benzylpenicillin produce singlets at 7·34 and 7·78 ppm, respectively (Degelaen et al 1979; Lipczynski 1988). By analogy, the singlet at 7·04 ppm in our experiments must correspond to the penicillenic acid of ampicillin since it appears from the very beginning of the reaction, with very low intensity, coinciding with results obtained in an independent experiment by UV-spectrophotometry. This independent experiment consisted of degrading ampicillin trihydrate in buffer pH 12 and recording UV scans of the solution at different times. The scans showed a peak at 322 nm where APE exhibits a maximum (APE stabilised with Cu^{2+}; BP 1968). This maximum exhibited an increase in its absorbance with time and eventually, a shift towards shorter wavelengths, thus showing the transformation of APE into another compound. The second singlet, at 8·25 ppm, was attributed to the penamaldic acid of ampicillin, which is a compound that has been proposed as an intermediate in the formation of HPP. This compound was detected with a maximum intensity after six days and HPP is formed just after this time.

Conclusions

Based on the sequence of formation of the different degradation products of ampicillin at pH 12, Scheme 1 is proposed to

illustrate the degradation pathways. The scheme can be described as follows:

5*R*-APC is the first ampicillin degradation product detected from ampicillin and it subsequently undergoes epimerization at C-5 to give 5*S*-APC. The alkaline conditions of the experiments undertaken here favoured the formation of APO (detected by HPLC) which was an unexpected result since previous reports indicated its formation under acidic conditions (Munro et al 1978; Suwarumpha & Freas 1989). This compound was isolated as a mixture of isomers and should proceed from the decarboxylation of 5*R*-APC. APE was detected in the reaction mixture by NMR spectroscopy. APE appeared after 20 min, when ampicillin has completely disappeared so this compound should also proceed from 5*R*-APC degradation.

Miyazaki et al (1974) and Lebelle et al (1979) reported that the cleavage of the β-lactam is essential for HPP formation which, in this case, was produced by the basic pH of the degradation mixture. The proposed mechanism to explain the formation of HPP under alkaline conditions includes the formation of APENAM which could correspond to some signals observed by NMR spectroscopy. APENAM seemed to be another degradation product of 5*R*-APC. No other additives, such as carbohydrates or polyalcohols, were present in the degradation mixture and, therefore, the formation of DKP was not observed in these experiments.

Finally, the formation of polymers depends on the concentration of the solution and is favoured by a basic pH. The results obtained in these experiments showed that polymer formation could be observed in the early stages of the reaction (4 h) and that dimers are formed first. Polymers of higher molecular weight could have formed but they were not monitored or recorded.

Acknowledgements

V. A. Robinson-Fuentes is grateful for financial support received from the Mexican Government through CONACYT. Thanks are expressed to Dr A. F. Casy for his valuable help with NMR spectra interpretation.

References

Akanni, A. O., Ayim, J. S. K. (1992) Determination of ampicillin in the presence of cloxacillin. J. Pharm. Biomed. Anal. 10: 43–47

Aki, H., Sawai, N., Yamamoto, K., Yamamoto, H. (1991) Structural confirmation of ampicillin polymers formed in aqueous solutions. Pharm. Res. 8: 119–122

Barbhaiya, R. H., Turner, P. (1976) Fluorimetric determination of cephalexin. J. Pharm. Pharmacol. 28: 791–792

Barbhaiya, R. H., Brown, R. C., Payling, D. L. O., Turner, P. (1978) Isolation and identification of a fluorescent degradation product of some β-lactam antibiotics. J. Pharm. Pharmacol. 30: 224–227

Bird, A. E., Cutmore, E. A., Jennings, K. R. Marshall, A. C. (1983) Structure re-assignment of a metabolite of ampicillin and amoxycillin and epimerization of their penicilloic acids. J. Pharm. Pharmacol. 35: 138–143

Blaha, J. M., Knevel, A. M., Kessler, D. P., Mincy, J. W., Hem, S. L. (1976) Kinetic analysis of penicillin degradation in acidic media. J. Pharm. Sci. 65: 1165–1170

Branch, S. K., Casy, A. F., Ominde, E. M. A. (1987) Application of ^{1}H nuclear magnetic resonance spectroscopy to the analysis of β-lactam antibiotics and their common degradation products. J. Pharm. Biomed. Anal. 5: 73–103

Bundgaard, H. (1976) Polymerization of penicillins: kinetics and mechanism of di- and polymerization of ampicillin in aqueous solution. Acta Pharm. Suec. 13: 9–26

Bundgaard, H. (1978) Impurities as a factor in therapeutic equivalence of drugs: analysis of ampicillin formulations for antigenic polimerization products. Arch. Pharm. Chem. Sci. Ed. 6: 63–68

Bundgaard, H., Larsen, C. (1977) Polymerization of penicillins IV. Separation, isolation and characterization of ampicillin polymers formed in aqueous solution. J. Chromatogr. 132: 51–59

Bundgaard, H., Larsen, C. (1979) Piperazinedione formation from reaction of ampicillin with carbohydrates and alcohols in aqueous solution. Int. J. Pharm. 3: 1–11

Connor, S. C., Everett, J.R., Jennings, K. R., Nicholson, J. K., Woodnutt, G. (1994) High resolution ^{1}H NMR spectroscopic studies of the metabolism and excretion of ampicillin in rats and amoxycillin in rats and man. J. Pharm. Pharmacol. 46: 128–134

Davies, A. M., Layland, N. J., Page, M. I., Martin, F., More O'Ferral, R. (1991) Thiazolidine ring opening in penicillin derivatives Part 2: enamine formation. J. Chem. Soc. Perkin Trans. II :1225–1229

Degelaen, J. P., Loukas, S. L., Feeney, J., Roberts, G. C. K., Burgen, A. S. V. (1979) A nuclear magnetic resonance study of the degradation of penicillin G in acidic solution. J. Chem. Soc. Perkins Trans. II :86–90

Fogg, A. G., Fayad, N. M. (1980) Differential pulse polarographic study of the degradation of ampicillin. Anal. Chim. Acta 113: 91–96

Ghebre-Sellasie, I., Helm, S. L., Knevel, A. M. (1984) Epimerization of benzylpenicilloic acid in alkaline media. J. Pharm. Sci. 73: 125–128

Haginaka, J., Wakai, J. (1986) Liquid chromatographic determination of penicillins by post-column alkaline degradation with sodium hypochlorite. Anal. Chem. 58: 1896–1898

Hou, J. P., Poole, J. W. (1971) β-Lactam antibiotics: their physicochemical properties and biological activities in relation to structure. J. Pharm. Sci. 60: 503–533

James, M. J., Riley, C. M. (1985) Stability of intravenous admixtures of aztreonam and ampicillin. Am. J. Hosp. Pharm. 42: 1095–1100

Jusko, W. J. (1971) Fluorometric analysis of ampicillin in biological fluids. J. Pharm. Sci. 60: 728–732

Kessler, D. P., Cushman, M., Ghebre-Sellasie, I., Knevel, A. M., Helm, S. L. (1983) Investigation of a proposed penicillin G acidic degradation scheme using high pressure liquid chromatography and optimization techniques and mechanistic considerations. J. Chem. Soc. Perkins Trans. II: 1699–1703

Kiener, P. A., Waley, S. G. (1978) Reversible inhibitors of penicillinases. Biochem. J. 169: 197–204

Kuchinskas, E. J., Levy, G. N. (1972) Comparative stabilities of ampicillin and hetacillin in aqueous solution. J. Pharm. Sci. 61: 727–729

Larsen, C., Johansen, M. (1982) Separation and semi-quantitative determination of ampicillin polymers in ampicillin bulk preparations by means of thin layer chromatography. J. Chromatogr. 246: 360–362

Lebelle, M. J., Vilim, A., Wilson, W. L. (1979) Isolation and identification of a fluorophore from ampicillin degradation. J. Pharm. Pharmacol. 31: 441–443

Lipczynski, A. M. (1988) The Degradation of Benzylpenicillin in Aqueous Solution. PhD Thesis, University of Bath, UK

Masada, M., Kuroda, T., Nakagawa, T., Uno, T. (1980) Structural investigation of new metabolites of amino-penicillins excreted in human urine. Chem. Pharm. Bull. 28: 3527–3536

Miyazaki, K., Ogino, O., Arita, T. (1974) Fluorometric determination of ampicillin. Chem. Pharm. Bull. 22: 1910–1916

Munro, A. C., Chainey, M. G., Woroniecki, S. R. (1978) Preparation and immunological cross-reactions of penicilloic and penilloic acids. J. Pharm. Sci. 67: 1197–1204

Roets, E., De Pourcq, P., Toppet, S., Hoogmartens, J., Vanderhaegue, H., Williams, D. H., Smith, R. J. (1984) Isolation and structure elucidation of ampicillin and amoxycillin oligomers. J. Chromatogr. 303: 117–129

Schwartz, M. A. (1969) Chemical aspects of penicillin allergy. J. Pharm. Sci. 58: 643–661

Stanfield, M. K., Butcher, B. T., Stewart, G. T. (1978) Spectroscopic analysis of polymers of benzylpenicillin and ampicillin. Anal. Biochem. 89: 1–13

Suwanrumpha, S., Freas, R. B. (1989) Identification of metabolites of ampicillin using LC/thermospray MS and FAB tandem MS. Biomed. Env. Mass Spec. 18: 983–994

Takagi, S., Nobuhara, Y., Nakanishi, Y. (1983) Formation of penicillin polymers and determination of molecular weight. J. Chromatogr. 258: 262–266

Tomlinson, E., Notari, R. E., Byron, P. R. (1980) Simultaneous partitioning and hydrolysis kinetics of amoxycillin and ampicillin. J. Pharm. Sci. 69: 655–658

Tsuji, A., Nakashima, E., Hamano, S., Yamana, T. (1978) Physicochemical properties of amphoteric β-lactam antibiotics I: stability, solubility and dissolution behaviour of aminopenicillins as a function of pH. J. Pharm. Sci. 7: 1059–1060

Ueno, H., Nishikawa, M., Muranaka, M., Horiuchi, Y. (1981) High-speed gel filtration chromatography of polymers formed by β-lactam antibiotics. J. Chromatogr. 207: 425–429

Uno, T., Masada, M., Kuroda, Y., Nakagawa, T. (1981) Isolation and structural investigation of the fluorescent degradation products of ampicillin. Chem. Pharm. Bull. 29: 1344–1354

Urleb, U., Krbavcic, A., Cop, A., Rotar, A. (1985) Determination of some impurities in capsulated ampicillin trihydrate. Acta Pharm. Jugosl. 35: 53–60

Van Krimpen, P. C., Van Bennekom, W. P., Bult, A. (1987) Penicillins and cephalosporins: physicochemical properties and analysis in pharmaceuticals and biological matrices. Pharmaceutisch Weekblad Scientific Edition 9: 1–23