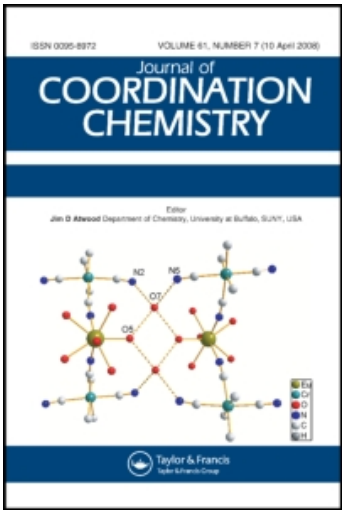

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Ampicillin acidity and formation constants with some metals and their thermodynamic parameters in different media. Crystal structures of two polymorphs isolated from the reaction of ampicillin with copper(II)

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The dissociation constants of ampicillin and the formation constants of complexes with cobalt(II), copper(II), nickel(II), iron(III), zinc(II), lead(II), mercury(II) and cadmium(II) were evaluated at different temperatures (20–45°C) by potentiometric measurements in the presence of different percentages of ethanol–water. Two polymorphs of the degradation product 3-oxo-2-phenyl-pyrazine-5-carboxylic acid ethyl ester have been isolated from the reaction of copper chloride with ampicillin in aqueous ethanol. In the crystal structure of both polymorphs, molecules related by a center of symmetry form a dimer by coupling of the N–H···O hydrogen bonds resulting in the $R_2^2(8)$ supramolecular synton. The π–π contacts indicate strong stacking interactions in both polymorphs.

Keywords: Ampicillin; Potentiometry; Formation constants; Acidity constants; Solvents effect; Polymorph

1. Introduction

Ampicillin **I** (figure 1) is among the most important antibiotics active against a wide range of gram-positive and gram-negative bacteria [1]. It has a special importance

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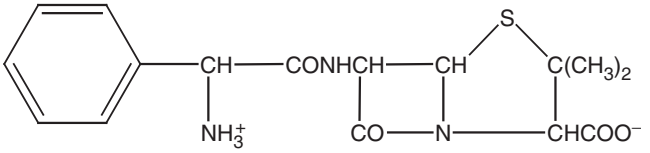


Figure 1. Structure of ampicillin **I**.

among β-lactam antibiotics because of its considerable acid stability due to the presence of the α-amino group. The latter plays an important role in its broader activity [1, 2]. Ampicillin is known to inhibit protein synthesis on a ribosome by causing misreading of the genetic codes [1]. The transfer of genetic information for the synthesis of a specific protein is influenced by metal ion, that may bind with the nucleotide fragments, enzyme, protein or the drug itself [3, 4]. Coordination of the drug with the enzyme-bound metal ions may cause selective denaturation of the enzyme protein [5]. The belief that antibiotic activity is related to the formation of complexes with metals has stimulated a great deal of investigation. The recently prepared complexes of ampicillin [6] and amoxycillin [7] with different metals are expected to increase their effectiveness for a broad antibacterial spectrum. Complexes of ampicillin have been synthesized with different metals such as Co, Ni, Cu, Zn, Cd, Mn, Al and Fe [6, 8–11] and their antibacterial activities have been evaluated [8–10]. Greater activity than ampicillin was observed for the complexes against certain bacteria. Many potentiometric studies using pH-metric techniques have been carried out to evaluate the acidity constant of ampicillin and its formation constants with different metals under physiological environments in a 0.1 M electrolyte in the temperature range 25–30°C [12–17]. The metals studied were Co, Ni, Cu, Zn [12–15], Cr, Cd [13], Mn, Fe and Zr [15]. Potentiometric studies involving the investigation of ternary complexes of ampicillin with a second ligand, e.g., nucleic acid bases [16], nitrilo and iminotriacetic acid, 2,2′-bipyridine and imidazole [17], aminoacids [18], 2,2′-bipyridine and 1,10 phenanthroline [13], dopamine and α-methyl L-dopa were reported [15]. Altough there are many reports in the literature of acidity and formation constants of ampicillin with various metals in water, little work has been carried out on the evaluation of the respective thermodynamic parameters. In order to obtain further information of the nature of the metal–drug interactions and solvent effects, we have evaluated the acidity constants of ampicillin **I** in different percentages of water–ethanol media and its formation constants with different metals Fe(III), Co(II), Ni(II), Cu(II), Zn(II), Cd(II), Hg(II), and Pb(II) in 25% ethanol–water. The corresponding thermodynamic parameters are evaluated and discussed.

We also report in the present work the isolation and crystal structures of two polymorphs of 3-oxo-2-phenyl-pyrazine-5-carboxylic acid ethyl ester **II**, a degradation product from the reaction of ampicillin with copper(II) (figure 2). The β-lactams of penicillins are susceptible to hydrolysis [19–22], aminolysis [23–25] and alcoholysis reactions [26, 27], and when transition metals are present they cause an increase in the rate of these reactions [19, 26–30]. It has been proposed that catalysis occurs by means of an intermediate 1 : 1 metal ion-antibiotic complex [28]. The role of the

metal ion is to establish the tetrahedral intermediate that is formed by addition of the nucleophile to the β-lactam carbonyl group [30].

2. Experimental

2.1. *Potentiometric materials and apparatus*

The chemicals used were Merck products and the solvents were of spectroscopic quality. All solutions were prepared in deionized water. Carbonate free sodium hydroxide solution (titrant) was prepared and checked by titration against standard potassium hydrogen phthalate solution. The potentiometric measurements were carried out using Denver Instrument Model 225 pH–Ion selective electrode meter fitted with a combined glass electrode (reading to ± 0.01 pH unit). The pH meter was calibrated from time to time at different temperatures using three standard buffers of variable pH values. A stream of purified nitrogen gas was passed during the entire titration. The cell compartment was kept constant at the desired temperature by using a thermostat Model Heto HMT 200 in the temperature range 20–45°C (± 0.1°C).

2.2. *Potentiometric procedure*

The acid dissociation constants of the anhydrous ampicillin were determined by introducing the appropriate volume of ligand (50.00 mL) into the titration cell in the presence of KCl ($\mu = 0.30$) and different percentages (V/V) of the organic solvent–water media. The titrations versus standard NaOH (1.00×10^{-2} M) were carried in a 150 mL thermostatic cell. The titration was stopped well before the second deprotonation of ampicillin from its amide group [16] which occurs at pH = 12. The correction factor, δ, for measuring the pH values in different percentages of organic solvent–water media were calculated as reported [31].

The acid–base titration technique was used in order to compute the formation constants of the complexes. The solution in the titration cell (50.00 mL) consisted of 5.00×10^{-4} M metal ions, 1.00×10^{-3} M ligand and KCl ($\mu = 0.30$). The pH readings were taken after each addition of 0.5 mL of 1.00×10^{-2} M NaOH solution. The calculations were restricted to the data obtained before pH = 7.3 to avoid precipitation and complications due to the hydrolysis of the complex species, and also to prevent the formation of ampicillin with deprotonated amide [12]. The model selected was that which gave the best statistical fit and proved consistent with the titration data without giving any systematic drifts in the magnitudes of various residuals.

2.3. *Instruments used for the characterization of the ampicillin degradation product*

IR spectra were recorded using a Schimadzu 8300 FTIR spectrophotometer using KBr pellets. The electronic absorption spectra were obtained using a Ciba Corning 2800 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a 300 MHz Bruker NMR. Elemental analysis of C, H, N and mass spectra were performed by the Microanalytical Service, Department of Chemistry, University of Surrey, Guildford, Surrey, GU2 5XH, UK.

2.4. *Isolation of 3-oxo-2-phenyl-pyrazine-5-carboxylic acid ethyl ester II from the ampicillin degradation reaction with Cu(II) (figure 2)*

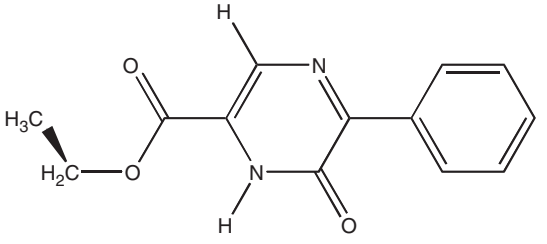


Figure 2. Ampicillin degradation product **II**.

2.5. *Isolation of polymorph IIA*

Anhydrous ampicillin (0.75 g, 2.15 mmol) and $CuCl_2 \cdot 2H_2O$ (0.37 g, 2.17 mmol) were dissolved in 100 mL ethanol–water (1 : 1). The greenish-blue mixture was stirred at room temperature for 3 h. The separated green leaflets were collected by filtration, washed with water, ethanol and then dried. Crystals for the X-ray analysis were chosen from these leaflets. Yield = 39%. Anal. Calcd (%) for $C_{13}H_{12}N_2O_3$ (%): C, 63.87; H, 4.91; N, 11.46. Found: C, 63.89; H, 5.05; N, 11.76. UV(DMSO) λ_{max}: 262, 345, 394, 497, 538, 576, 623 nm. ^{1}H NMR (DMSO): 1.33t, (7.10 Hz) (3H) CH_3, and 4.36q, (7.10 Hz) (2H) CH_2 ethyl; 12.78s, (1H)–NH, and 8.38s (1H) pyrazine; 8.26d, (2.07 Hz) (1H), 8.24d, (3.90 Hz) (1H), 7.51d, (3.57 Hz) (2H) and 7.48m (1H) phenyl. ^{13}C NMR (DMSO): 162.16; 156.17; 151.47; 135.49; 130.55; 129.29; 128.33; 62.02; 14.26. FTIR (KBr): 3168.8, 3082.0, 3041.5, 2966.3, 2900.7, 2856.4, 2779.2, 2727.2, 1722.3, 1647.1, 1616.2, 1591.2, 1514.0, 1487.0, 1456.2, 1442.7, 1386.7, 1369.4, 1296.1, 1272.9, 1247.9, 1174.6, 1161.1, 1091.6, 1072.3, 1020.3, 999.1, 977.8, 918.1, 862.1, 846.7, 810.0, 765.7, 752.2, 688.5, 642.3, 607.5, 576.7, 524.6, 472.5, 428.2 cm^{-1}.

2.6. *Isolation of polymorph IIB*

Anhydrous ampicillin (0.75 g, 2.15 mmol) and $CuCl_2 \cdot 2H_2O$ (0.37 g, 2.17 mmol) were disssolved in 100 mL ethanol–water (1 : 1). The greenish-blue mixture was stirred at room temperature for 3 h. The separated purple-black crystals were collected by filtration, washed with water, ethanol and then dried. Yield = 35%. Anal. Calcd (%) for $C_{13}H_{12}N_2O_3$ (%): C 63.87; H 4.91; N 11.46. Found: C 63.76; H 4.82; N 11.45. UV(DMSO) λ_{max}: 263, 344, 388, 504, 551, 613 nm. FTIR (KBr): similar to polymorph (IIA). ^{1}H NMR (DMSO): 1.33t, (7.08 Hz) (3H), $-CH_3$, and 4.36q, (7.11 Hz) (2H) $-CH_2$ ethyl; 12.77s, (1H), –NH, and 8.37s, (1H) pyrazine; 8.26d, (2.13 Hz) (1H), 8.24d, (3.99 Hz) (1H), 7.50d, (3.60 Hz) (2H), and 7.47m (1H) phenyl. ^{13}C NMR (DMSO): 162.18; 156.19; 135.50; 130.55; 129.30; 128.33; 73.79; 62.01; 14.27. MS (FAB): m/z ratio = 489.0, RA = 98 due to dimer of compound IIB; m/z = 245.1; RA = 99 due to molecular ion of compound IIB (molecular weight = 244.25).

2.7. *Isolation of [Cu(NH$_2$CH(COO)C(CH$_3$)$_2$(SH))(μ-Cl)]$_2$ · H$_2$O ID from the ampicillin degradation reaction with Cu(II)*

The filtrate obtained after the removal of **IIB** was left to stand and subsequently a blue-green powder of **ID** precipitated. The product was washed with water and dried. Yield = 45%. Anal. Calcd (%) for $C_{10}H_{22}O_5N_2S_2Cl_2Cu_2$ (%): C 23.44; H 4.30; N 5.47. Found: C 24.04, H 4.18, N 5.22. UV(DMSO) λ_{max}: 268, 362 nm. FTIR (KBr): 3570.0, 3280.7, 3222.8, 3136.0, 2987.5, 2966.3, 2927.7, 1635, 1590, 1558.4, 1456.2, 1375.2, 1342.4, 1282.6, 1244.0, 1191.9, 1132.1, 1080.1, 1055.0, 1006.8, 954.7, 925.8, 798.5, 711.7, 671.2, 601.7 cm^{-1}. MS (FAB): $m/z = 460.1$, RA = 4 due to compound ID with loss of Cl and H_2O; $m/z = 154$, RA = 100 due to $Cu(NH_2CHCO_2)(H_2O)$ fragment; $m/z = 137$, RA = 69 due to $Cu(NH_2CHCO_2)$ fragment.

2.8. *Single crystal X-ray studies*

X-ray diffraction measurements for polymorphs **IIA** and **IIB** were performed at 100(2) K on a Bruker-AXS SMART APEX/CCD diffractometer using Mo-Kα radiation ($\lambda = 0.7107$ Å). The data were corrected for Lorentz and polarization effects and for absorption using the SADABS program [32]. The structures were solved using direct methods and refined by full-matrix least-squares on F^2. Non-hydrogen atoms were refined anisotropically. The position of the hydrogen atom on N(7) was found from difference electron density synthesis and refined isotropically. The positions of carbon-bonded hydrogen atoms were calculated and were refined with temperature factors 1.2 times (or 1.5 for CH_3 group) those of their bonded atoms. All crystallographic calculations were conducted with the SHELXTL 6.1 program package [33]. A summary of the data collection, structure refinement is given in table 1.

3. Results and discussion

3.1. *Acidity constant studies*

Calculation of the acid dissociation constants of ampicillin by potentiometric measurements depends on evaluation of the average proton number associated with the ligand, n_A. Plots of pH versus n_A gave values of p*Ka* [34]. The point-wise method [35] was used to confirm the obtained p*Ka* values; the plots of $\log(n_A/1-n_A)$ versus pH give the required p*Ka*s at y = 0 for ampicillin in 25% ethanol (figure 3).

Protonated ampicillin ampH$_2^+$ contains –COOH and the –NH$_3^+$ groups which are successively deprotonated in the pH ranges 2–4 and 6–8, respectively. Ampicillin exists as a Zwitterion ampH$^{\pm}$, at neutral pH. Above pH 12, amp$^-$ tends to give a drawn out buffer region, obviously due to partial deprotonation of the amide (–CONH–) moiety [16]:

$$\begin{aligned} \text{ampH}_2^+ + \text{H}_2\text{O} &\rightleftharpoons \text{ampH}^{\pm} + \text{H}_3\text{O}^+ \qquad Ka_1 \\ \text{ampH}^{\pm} + \text{H}_2\text{O} &\rightleftharpoons \text{amp}^- + \text{H}_2\text{O}^+ \qquad Ka_2 \\ \text{amp}^- + \text{H}_2\text{O} &\rightleftharpoons (\text{H}_{-1}\text{amp})^{-2} + \text{H}_2\text{O}^+ \qquad Ka_3 \end{aligned}$$

Table 1. Crystal data and structure refinement for **IIA** and **IIB**.

Compound	**IIA**	**IIB**
Empirical formula	$C_{13}H_{12}N_2O_3$	$C_{13}H_{12}N_2O_3$
Formula weight	244.25	244.25
Temperature (K)	100(2)	100(2)
Wavelength (Å)	0.71073	0.71073
Crystal system	Monoclinic	Monoclinic
Space group	$P2_1/n$	$P2_1/c$
a (Å)	12.790(2)	7.1625(12)
b (Å)	4.543(8)	19.473(3)
c (Å)	19.592(4)	8.3272(14)
β (°)	99.16(3)	101.828(3)
Volume (Å^3)	1124(3)	1136.8(3)
Z	4	4
Density (calc.) (Mg m^{-3})	1.443	1.427
Absorption coefficient (mm^{-1})	0.105	0.103
F(000)	512	512
Crystal size (mm^3)	0.20 × 0.15 × 0.15	0.40 × 0.10 × 0.07
θ range (°)	1.78 to 26.99	2.09 to 27.00
Index ranges	$-16 < h < 16$, $-5 < k < 5$, $-25 < l < 16$	$-8 < h < 9$, $-21 < k < 24$, $-9 < l < 10$
Reflections collected	6232	6936
Independent reflections [R(int)]	2422 [0.0552]	2470 [0.0451]
Completeness to $\theta = 26.99°$	98.8%	99.6%
Max. and min. transmission	0.9845 and 0.9794	0.9928 and 0.9598
Data/restraints/parameters	2422/0/167	2470/0/168
Goodness-of-fit on F^2	1.038	1.004
Final R indices [$I > 2\sigma(I)$]	$R_1 = 0.0590$, $wR_2 = 0.1446$	$R_1 = 0.0514$, $wR_2 = 0.1187$
R indices (all data)	$R_1 = 0.0776$, $wR_2 = 0.1536$	$R_1 = 0.0775$, $wR_2 = 0.1290$
Largest diff. peak and hole (e Å^{-3})	0.367 and −0.283	0.303 and −0.216

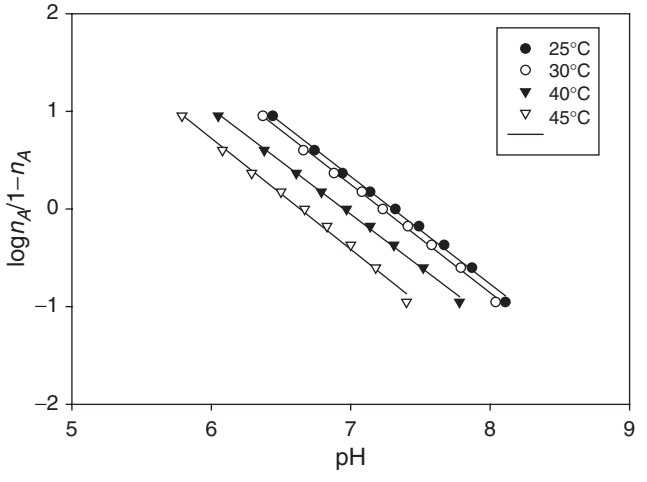


Figure 3. Point wise plot: $\log(n_A/1-n_A)$ *vs.* pH for ampicillin in 25% ethanol at different temperatures.

The acidity of ampicillin amp$H^{\pm}$ is thus caused by dissociation of a proton from the ammonium group with the ionization constant Ka_2 considered as Ka in this study. The pKa values of ampicillin in different percentages of ethanol–water are listed in table 2. These decrease with increasing temperatures, and depend on the proportion of organic

Table 2. pK values and thermodynamic parameters for ampicillin in different percentages of ethanol–water at (20–45°C).

Media	p$K \pm 0.03$						ΔG (kJ mol$^{-1} \pm 0.17$)	ΔH (kJ mol^{-1})	ΔS (J mol deg^{-1})
	20°C	25°C	30°C	35°C	40°C	45°C			
Aqueous[a]	7.37	7.30	7.16	6.95	6.88	6.78	41.67	44.81 ± 0.33	10.54 ± 1.13
25% (V/V)	7.35	7.28	7.14	6.93	6.83	6.67	41.55	50.29 ± 1.17	29.33 ± 3.93
35% (V/V)	7.33	7.20	7.11	6.87	6.78	6.62	41.09	51.25 ± 0.75	34.06 ± 2.51
50% (V/V)	7.29	7.18	7.02	6.84	6.71	6.52	40.96	55.14 ± 1.55	47.61 ± 5.19
75% (V/V)	7.27	7.14	6.97	6.83	6.63	6.42	40.75	60.00 ± 1.67	64.56 ± 5.61

[a]p$K = 7.05$ at 37°C, I = 0.1 M $NaNO_3$ [16].

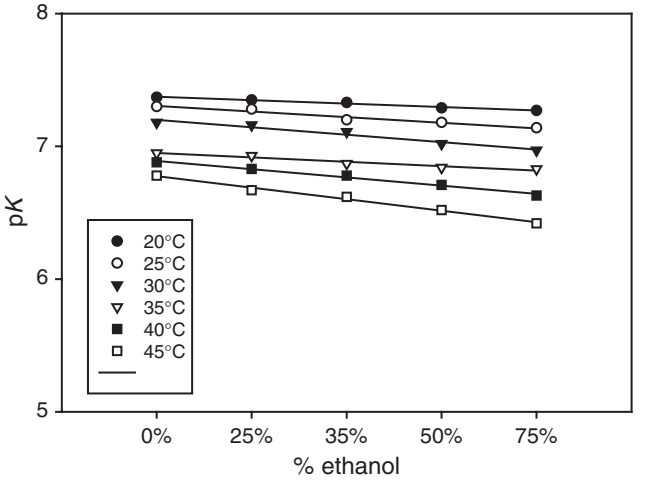


Figure 4. pK of ampicillin *vs.* % ethanol at different temperatures.

cosolvent [36]. Increase in % ethanol results in a decrease in pKa of ampicillin (table 2, figure 4).

The acidity constants in pure aqueous medium (Ka) can be related to those in water–organic solvent mixtures (Ka*) by the equation: $Ka = Ka * (\gamma_{H^+}\gamma_{A^-}/\gamma_{HA})$, where γ is the activity coefficient for the subscripted species in a partially aqueous medium to that in pure water [37]. In addition, the hydrogen-bonding interaction between the conjugate base (A^-) and the solvent molecules, the dispersion forces and proton–solvent interactions play vital roles in the ionization process of acids in the presence of organic solvent [37–39]. The difference in stabilization of the conjugate base ampicillinate (amp^-) played an important role for the decrease in the pK value as the amount of the organic co-solvent is increased (table 2, figure 4).

3.2. *Thermodynamic parameters of acid ionization*

Acid ionization was investigated in the temperature range 20–45°C, where the ΔG, ΔH and ΔS values are evaluated. On plotting the pK values versus $1/T$, straight lines are obtained with a slope of $\Delta H/2.3R$, from which the ΔH values (kcal mol^{-1}) are

computed (Van t'Hoff method) (table 2, figure 4). The free energy values ΔG (kJ mol^{-1}) are calculated at 25°C using the equation $\Delta G = 2.303RT\,pK$, whereas the entropy term ΔS (J mol deg^{-1}) is given as $\Delta S = 10^3(\Delta H - \Delta G)/T$ (table 2). The positive ΔH (kJ mol^{-1}) values for ampicillin in ethanol–water indicate that the weak acid dissociation is endothermic in different media [40].

Given that the j factor represents a solvent-transfer number characteristic of the tested chemical reaction which can be attributed to the transfer of the solvent, the following equation [41, 42] is tested:

$$j\log[S] - \log K = -\Delta G_t/(2.303RT) - W\log[H_2O]/[S] + j\log[H_2O]$$

$$\log[H_2O]/[S] = X, \qquad j\log[S] - \log K = Y$$

where $[S]$ and ΔG_t represent the solvent concentration and the free energy, respectively. The data are collected in table 3. Y is plotted against $-X$ for ampicillin (pKa) in ethanol–water media (figure 5). Trial values of $j = 1, 2, 3, 4$ were used to find values of W for the gradients of Y versus $-X$. The slopes of the Y–X relation give the values for W. The data obtained may throw light on the role of aquation and solvation during the dissociation. For pKa values of ampicillin in different ethanol–water media, and at temperatures 20–45°C, the W values are lowest for $j = 1$, i.e., aquation is predominant. At $j = 2$, 3 and 4, W values increased and hence the solvation process increases.

3.3. *Complex formation studies*

The acidic properties of ampicillin facilitate the investigation of coordinating behaviour towards cobalt(II), nickel(II), copper(II), iron(III), mercury(II), lead(II), zinc(II) and cadmium(II). Comparing the pH titration curves of the free ligands with that of the complex solutions reveals a drop in pH, indicating that the mechanism of complexation is based on hydrogen ion liberation, where the ligand is of stronger coordinating ability (figures 6 and 7).

Modified Bjerrum's method was adopted and the pH measurements during titration with NaOH of the solution of the chelating agent in the presence and absence of metal ions could be used to calculate the free ligand exponent (pA) and the degree of formation of the system n (equations (1) and (2)). C_A is the total concentration of ligand, $[A^-]$ is the concentration of free ligand anion and a is the number of moles of base added per mole of ligand present. n is the average number of moles of ligand bound per mole of metal ion, and K_1 is the acid dissociaton constant of ligand:

$$[A^{-2}] = \frac{(2-a)C_A - [H^+] + [OH^-]}{2[H^+]/K_1} \tag{1}$$

$$\bar{n} = \frac{1}{C_M}\left(C_A - \left[\frac{[H^+]}{K_1}\right][A^-]\right) \tag{2}$$

By plotting n values versus pA, the log K_{f1}, and log K_{f2} values are obtained at pA values equivalent to 0.5 and 1.5, respectively [38, 43, 44]. The data are collected in table 4.

Table 3. X–Y data for ampicillin in different percentages of ethanol–water and at different temperatures.

			Y, 20°C				*Y*, 25°C				*Y*, 30°C			
[*S*] %	−log[*S*]	–X	*J* = 1	*J* = 2	*J* = 3	*J* = 4	*J* = 1	*J* = 2	*J* = 3	*J* = 4	*J* = 1	*J* = 2	*J* = 3	*J* = 4
25	0.602	−0.477	6.75	6.15	5.54	4.94	6.68	6.08	5.47	4.87	6.60	6.00	5.39	4.79
35	0.456	−0.269	6.87	6.42	5.96	5.51	6.74	6.29	5.83	5.38	6.65	6.20	5.74	5.29
50	0.301	Zero	6.99	6.69	6.39	6.09	6.88	6.58	6.28	5.98	6.72	6.42	6.12	5.82
75	0.125	0.477	7.14	7.02	6.90	6.77	7.02	6.89	6.77	6.64	6.84	6.72	6.60	6.47
			Y, 35°C				*Y*, 40°C				*Y*, 45°C			
25	0.602	−0.477	6.33	5.73	5.12	4.52	6.23	5.63	5.02	4.42	6.07	5.47	4.86	4.26
35	0.456	−0.269	6.41	5.96	5.50	5.05	6.32	5.87	5.41	4.96	6.16	5.71	5.25	4.80
50	0.301	zero	6.54	6.24	5.94	5.64	6.41	6.11	5.81	5.51	6.22	5.92	5.62	5.32
75	0.125	0.477	6.71	6.58	6.46	6.33	6.50	6.38	6.26	6.13	6.30	6.17	6.04	5.92

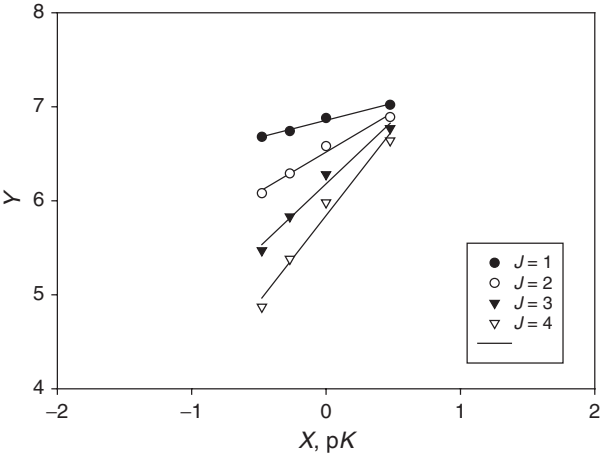


Figure 5. *X–Y* relationship for p*K* of ampicillin in different % ethanol–water media at 25°C.

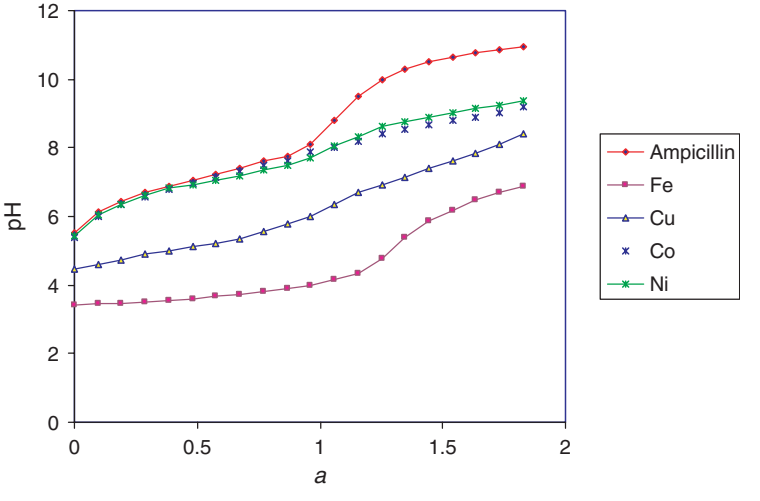


Figure 6. Titration curve of ampicillin: pH *vs. a* (moles of base added per moles of acid) with the metals Fe(III), Cu(II), Co(II), and Ni(II) in 25% ethanol–water at 25°C.

Hydrolysis of metal cations is ignored due to the pH of the studies and the low concentration used in 25% ethanol–water medium. The pH ranges of points used in calculation of $\log K_{f1}$ is (6.0–7.3) for Co(II), Ni(II), Hg(II), Pb(II), Zn(II), and Cd(II), (3.30–3.70) for Fe(III), and (4.75–5.40) for Cu(II). The pH ranges for $\log K_{f2}$ calculation is (5.5–6.4) for Fe(III), and (6.75–7.75) for Cu(II) (figures 6 and 7).

Figures 6 and 7 show the titration curves of ampicillin (pH *vs. a* (moles of base added per mole of acid)) with the metals (Fe(III), Cu(II), Co(II), Ni(II)) and (Zn(II), Cd(II), Pb(II), and Hg(II)), respectively. The lowering of metal curves compared to free ampicillin indicates complexation of metal cations:

$$M^{+2} + \text{ampH}^{\pm} \rightleftharpoons \text{Mamp}^{+} + \text{H}^{+} \quad K_{f1}$$

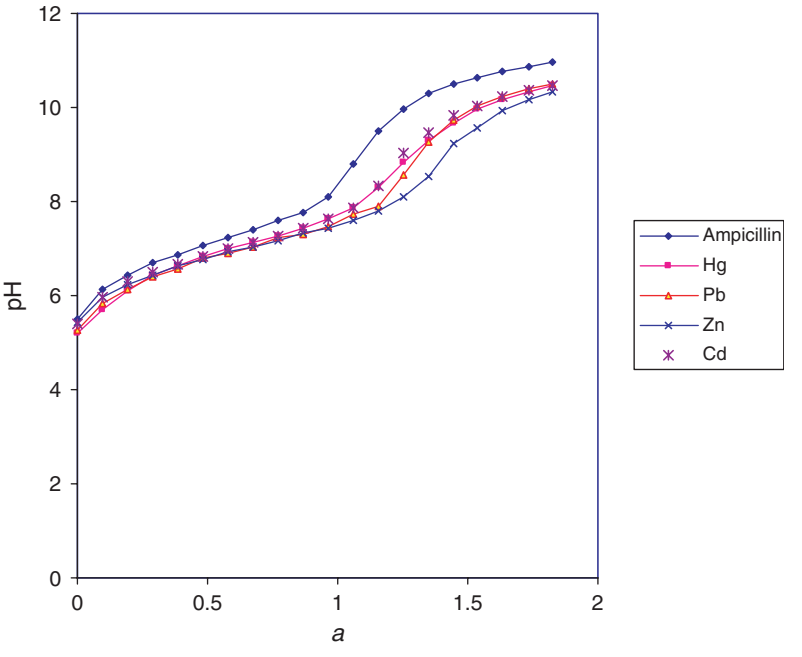


Figure 7. Titration curve of ampicillin: pH *vs.* *a* (moles of base added per moles of acid) with the metals Hg(II), Pb(II), Zn(II), and Cd(II) in 25% ethanol–water at 25°C.

Table 4. Log K_f and thermodynamic parameters for metal complexes with ampicillin in 25% ethanol at 20–35°C.

Metal ion	log $K_f \pm 0.05$ 20°C	25°C	30°C	35°C	ΔG (kJ mol^{-1} ± 0.29)	ΔH (kJ mol^{-1})	ΔS (J mol deg^{-1})
Cu(II)	5.10	5.11	5.12	5.13	−29.16	3.51 ± 0.25	109.54 ± 0.25
	(3.37)	(3.35)	(3.33)	(3.30)	(−19.12)	(−8.08) ± 0.50	(37.03) ± 0.71
Co(II)	3.31	3.25	3.22	3.15	−18.58	−17.95 ± 0.34	2.09 ± 0.29
Ni(II)	3.31	3.28	3.26	3.22	−18.74	−10.17 ± 0.88	28.79 ± 1.97
Fe(III)	6.78	6.87	6.94	7.00	−39.20	25.69 ± 1.09	216.73 ± 2.68
	(4.58)	(4.56)	(4.54)	(4.52)	(−26.02)	(−7.03) ± 0.34	(63.72) ± 0.63
Hg(II)	3.15	3.16	3.18	3.19	−18.03	4.90 ± 0.25	76.90 ± 0.29
Pb(II)	3.27	3.28	3.29	3.30	−18.74	3.51 ± 0.25	74.64 ± 0.29
Zn(II)	3.38	3.32	3.27	3.18	−18.95	−22.80 ± 1.05	−12.93 ± 2.51
Cd(II)	3.22	3.13	3.05	2.99	−17.87	−27.07 ± 1.59	−30.88 ± 4.35

Values in ( ) are log K_{f2}.

In the case of Fe(III) and Cu(II), the lowering is much greater indicating a much higher stability and thus greater log K_{f1} values. This also permits calculation of log K_{f2} according to the equilibrium:

$$\mathrm{Mamp}^{+} + \mathrm{ampH}^{\pm} \rightleftharpoons \mathrm{M(amp)_2} + \mathrm{H}^{+} \quad K_{f2}$$

The above mechanism is supported by a study of pH dependences of absorption maxima (λ_{max}) of binary M(II)–ampicillin mixture and correlation with the geometry of amide (–CONH–) bonded metal(II) complexes which reveals (N,O) bidentate

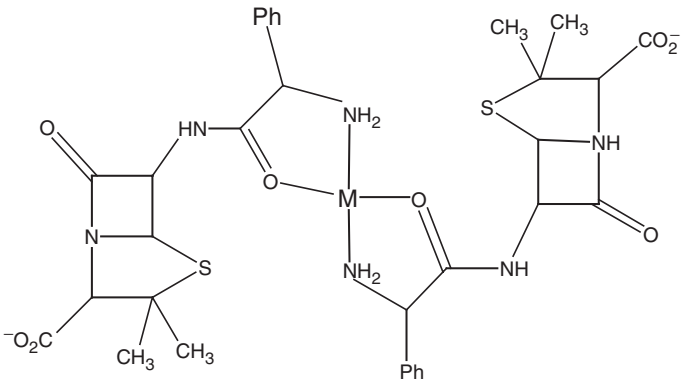


Figure 8. Structure of $M(amp)_2$ **III**.

chelation by the ligand Amp^- in $Mamp^+$ and $M(amp)_2$ complexes using the amino N– and amide carbonyl O– atoms. The complexes M(A) appear as the predominant species at pH 3–7 for M = (Co, Ni, Cu and Zn), and $M(A)_2$ for Cu(II) at pH less than 7.7 [12, 17, 18].

The (N,O) coordinated amp^- undergoes amide deprotonation at pH 8 and the resulting $H_{-1}amp^{2-}$ ion coordinates as an (N, N, S) tridendate ligand using the amino nitrogen, deprotonated amide nitrogen, and the thiazolidine S atoms [12, 16]. Characterization of isolated solid complexes $[M(amp)Cl]_2 \cdot nH_2O$ (M = Co(II), Ni(II), Cu(II), Zn(II) and Cd(II) ions), indicates a dinuclear structure where each ampicillin acts as a bidentate ligand to one metal centre through the amino nitrogen and the amide carbonyl oxygen atoms. It also acts as a bidentate ligand to a second metal centre through the carboxylate and the β-lactamic carbonyl groups [6, 8]. Structure III has been proposed for the $M(amp)_2$ complex, figure 8 [12].

Studies of metal ampicillin complexation in 25% ethanol at 25°C table 4, reveal the following trends:

$$\text{for } \log K_{f1}\text{: } Fe^{3+} > Cu^{2+} > Zn^{2+} > Ni^{2+},\ Pb^{2+} > Co^{2+} > Hg^{2+} > Cd^{2+}$$
$$\text{and for } \log k_{f2}\text{: } Fe^{3+} > Cu^{2+}$$

The decrease in stability constants with concomitant increase in the atomic number, Z, of the metals is not fully obeyed by all metals as expected for the Irving–Williams order [38]. The average radii of the metal ion in the complexes increases in the following order: $Fe^{3+} < Ni^{2+} < Cu^{2+} < Co^{2+} < Zn^{2+} < Cd^{2+} < Hg^{2+} < Pb^{2+}$.

Thus, the stability constants K_{f1} of complexes decrease with an increase in the radii of the metal ion except for Ni^{2+}, Zn^{2+} and Pb^{2+}. Log K_{f1} are compared with literature values in table 5.

3.4. *Thermodynamic parameters of complex formation*

The enthalpy of complexation ΔH (kcal mol^{-1}), was determined from the plot of log K_f versus $1/T$. Straight lines were obtained with a slope of $-\Delta H/2.303R$. The free energy

Table 5. Comparison of values of metal ampicillin formation constants $\log K_{f1}$ and ($\log K_{f2}$) with literature values.

Co	Ni	Cu	Zn	Cd	Media	T (°C)	Reference
3.12	3.66	4.79	2.98		Water[a]	37	[12, 16, 17]
3.15	3.22	5.13	3.18	2.99	25% Ethanol–water[b]	35	This work
3.12	3.78	6.38	3.17	2.79	Water[a]	25	[13]
3.25	3.28	5.11	3.32	3.13	25% Ethanol–water[b]	25	This work

[a] 0.1 M $NaNO_3$; [b] 0.3 M KCl.

ΔG (kJ mol^{-1}) was calculated at 25°C from the relation $\Delta G = -2.303RT\log K_f$. The entropy ΔS (J mol deg^{-1}) is given as $\Delta S = 10^3(\Delta H - \Delta G)/T$ [38]. The negative values of ΔG_1 and ΔG_2 for the formation of 1 : 1 and 1 : 2 metal–ligand complexes indicate thermodynamically favoured processes (table 4). Stabilities of complexes are influenced by the size of cation, the type of donor atom of the chelate, the flexibility of the ligand and solvation effects. The ΔH (kJ mol^{-1}) values for K_{f1} of ampicillin complexes with Cu(II), Fe(III), Hg(II) and Pb(II) are less negative than those of Co(II), Ni(II), Cd(II) and Zn(II) due to strong solvation interactions of these metal ions in water–ethanol medium that also cause the entropies to be highly positive (table 4).

3.5. *Discussion on the degradation of ampicillin in aqueous alcohol in the presence of the copper(II) ion*

Ampicillin belongs to the β-lactam penicillins. Their biological activity is caused by the chemical reactivity of their four member rings [45]. Instability of the β-lactam ring is due to its tension that results from 90° angles of bonding between the atoms, which are much smaller than 109.47° in sp^3 hybridization, or 120° in sp^2 hybrdization. This structural characteristic of the β-lactams makes them susceptible to hydrolysis, aminolysis and alcoholysis reactions. Studies of these degradation reactions can explain the mechanism by which some bacteria resist penicillins. Bacteria produce β-lactamase enzyme that assists in opening of the β-lactam ring of penicillin by the serine hydroxy group to give an ester of penicilloic acid [20, 46, 47].

The common mechanism of hydrolysis has been described by different authors [19–22]. The α-amino penicillins undergo autoaminolysis in an aqueous solution by means of nucleophilic attack of the amino group of the lateral chain of a molecule on the β-lactam bond of a second molecule of antibiotic [25, 28]. The metal acts as a catalyst in hydrolysis, aminolysis and alcoholysis reactions. By forming the 1 : 1 (metal : antibiotic) complex, it can assist in establishing the tetrahedral intermediate formed by addition of the nucleophilic group to the β-lactam carbonyl group [26–30].

There have been numerous theoretical and experimental studies that describe decomposition of ampicillin in alcoholic media [48–50] and catalyzed by transition metals (Cd, Zn, ... etc.) [26, 27]. The mechanism is explained by the initial formation of intact ampicillin metal complex which decomposes due to the nucleophilic attack of methanol on the β-lactam carbonyl leading to ester formation. The final product obtained consists of two molecules of penamaldate derivative of ampicllin and metal ion [26, 27].

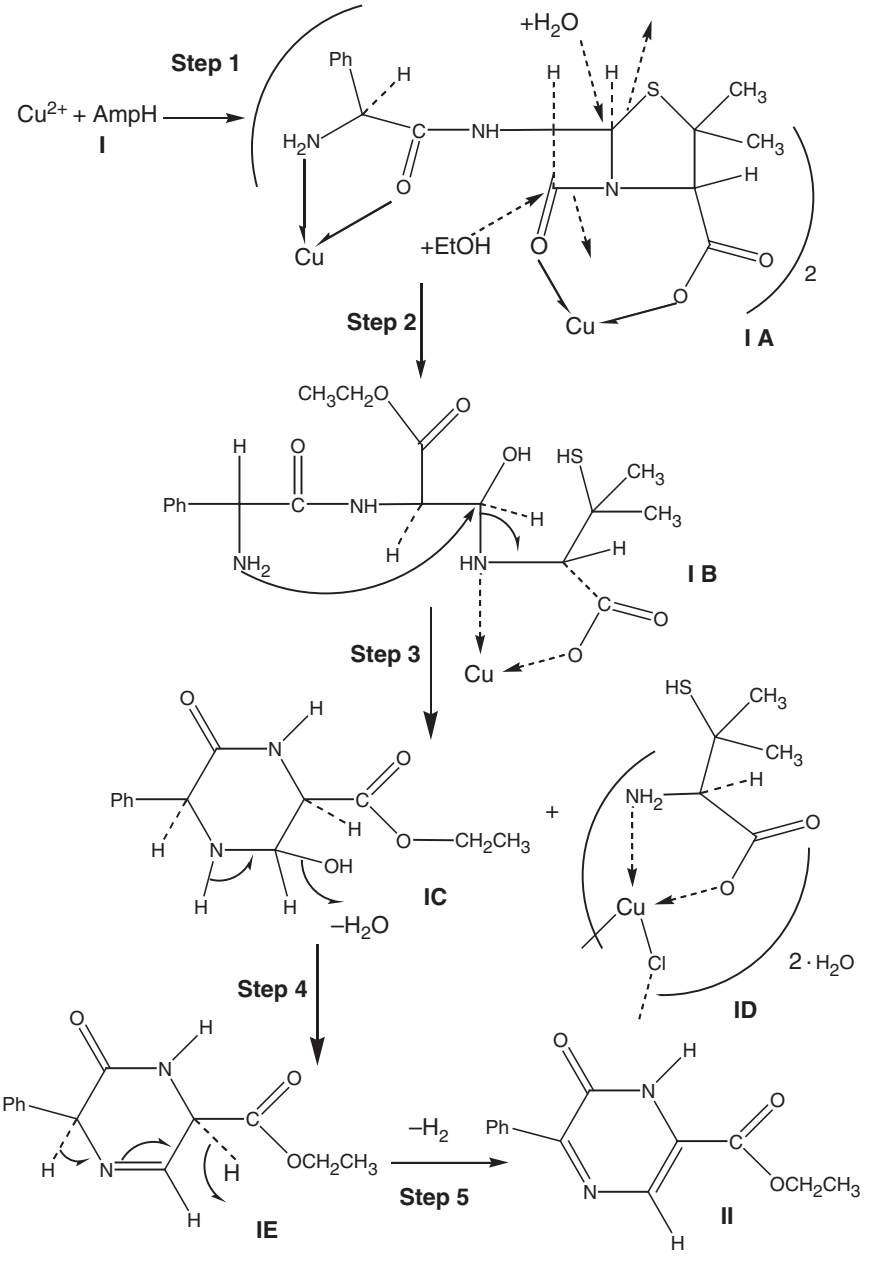
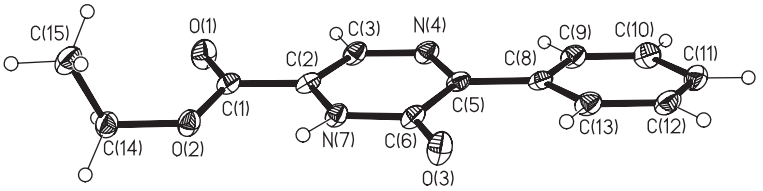
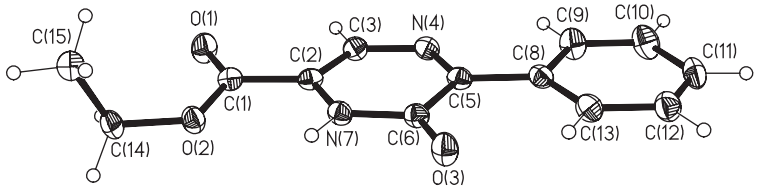


Figure 9. Scheme 1: degradation of ampicillin.

In this work, ethanolysis of ampicillin in the presence of copper(II) leads to isolation of two polymorphs of 3-oxo-2-phenyl-pyrazine-5-carboxylic acid ethyl ester **II** (figure 2). A copper ampicillin metal intermediate **IA** first rearranges into the ampicilloyl ester metal complex **IB** as a result of attack by water and ethanol as nucleophiles on the ampicillinate ligand. This is followed by attack of the α-amino



IIA



IIB

Figure 10. Perspective view of **IIA** and **IIB,** showing the 50% thermal elipsoids.

group on the β-carbon of ester, leading eventually to the formation of **II** and a dimeric copper complex **ID** with the thiol fragment of ampicillin, as suggested in scheme 1 (figure 9).

When an equimolar amount of ampicillin (ampH) and copper(II) chloride are stirred in aqueous alcoholic solution at room temperature, ampicillin readily loses H^+ and coordinates to copper(II) forming a CuAmp complex possibly Cu_2Amp_2 **IA** (step 1, scheme 1) [6]. The formation of 1:1 Cu(Amp) or Cu_2Amp_2 has $\log K_{f1} = 5.11$ with $\Delta G = -29.16\,\text{kJ mol}^{-1}$, $\Delta H = 3.51\,\text{kJ mol}^{-1}$, $\Delta S = 109.54\,\text{J mol deg}^{-1}$. The formation of $Cu(Amp)_2$ is also favoured thermodynamically $\log K_{f2} = 3.35$, $\Delta G = -19.12\,\text{kJ mol}^{-1}$, $\Delta H = -8.08\,\text{kJ mol}^{-1}$, $\Delta S = 37.03\,\text{J mol deg}^{-1}$ (table 4). Both Cu(amp) and $Cu(Amp)_2$ are intermediates toward formation of penamaldate as supported by kinetic studies of degradation of ampicillin in methanol catalyzed by cadmium and zinc [26, 27]. The coordination mode of copper to amp^{-1} in $Cu_2(amp)_2$ involves: (i) at the first copper center (a) binding from oxygen of the β-lactamic carbonyl group since the IR spectrum of solid $[Cu_2(amp)_2Cl_2(H_2O)_2]$ shows β-lactamic ν(C=O) well below ν(C=O) of free sodium ampicillin ($1777\,\text{cm}^{-1}$), and (b) monodentate binding from the carboxylate group since the separation value ($\nu_{asym} - \nu_{sym}$) COO in the solid is $240\,\text{cm}^{-1}$ which is $>200\,\text{cm}^{-1}$ and also different from $\Delta\nu(188\,\text{cm}^{-1})$ of Na(Amp); (ii) at the second copper center (a) amino group binding since the NH_2 stretch in the complex is $3231\,\text{cm}^{-1}$ while it is $3335\,\text{cm}^{-1}$ in Na(amp), and (b) amide C=O binding where the IR stretch drops from $1692\,\text{cm}^{-1}$ in the sodium salt to $1656\,\text{cm}^{-1}$ in the copper complex [6, 8].

Step 2 involves nucleophilic attack of ethanol on the β-lactamic carbonyl group, break of the β-lactamic bond and subsequent migration of hydroxylic hydrogen to the nitrogen of β-lactam amide giving an ester of penicilloic acid [26, 27, 48–50]. The opening of the β-lactam ring induces rearrangement in the coordination mode of copper. Copper binds to the lactamic nitrogen in place of the carbonyl oxygen of the lactam ring forming [Cu–N–C–(C=O)–O] five-membered ring in the complex $CuL(H_2O))(Cl)$ **IB**. Also included in step 2 is nucleophilic attack on the five membered

Table 6. Bond lengths [Å] and angles [°] for **IIA** and **IIB**.

	IIA	**IIB**
O(1)–C(1)	1.209(3)	1.205(2)
O(2)–C(1)	1.344(3)	1.331(2)
O(2)–C(14)	1.462(3)	1.454(2)
O(3)–C(6)	1.241(3)	1.234(2)
N(4)–C(3)	1.354(3)	1.356(2)
N(4)–C(5)	1.315(3)	1.315(2)
N(7)–C(2)	1.371(3)	1.362(2)
N(7)–C(6)	1.365(3)	1.369(2)
C(1)–C(2)	1.482(3)	1.490(2)
C(2)–C(3)	1.362(3)	1.355(2)
C(5)–C(6)	1.493(3)	1.478(2)
C(5)–C(8)	1.479(3)	1.483(2)
C(8)–C(9)	1.415(3)	1.400(2)
C(8)–C(13)	1.401(3)	1.397(2)
C(9)–C(10)	1.382(3)	1.378(3)
C(10)–C(11)	1.388(3)	1.377(3)
C(11)–C(12)	1.397(3)	1.385(3)
C(12)–C(13)	1.381(3)	1.387(3)
C(14)–C(15)	1.507(3)	1.503(2)
C(1)–O(2)–C(14)	117.57(15)	117.79(14)
C(3)–N(4)–C(5)	120.09(18)	119.79(16)
C(2)–N(7)–C(6)	123.11(17)	123.30(16)
O(1)–C(1)–O(2)	125.4(2)	126.01(17)
O(1)–C(1)–C(2)	123.11(19)	122.98(17)
O(2)–C(1)–C(2)	111.48(17)	111.01(14)
C(3)–C(2)–N(7)	118.4(2)	118.60(16)
C(1)–C(2)–C(3)	121.82(18)	121.88(16)
N(7)–C(2)–C(1)	119.74(18)	119.52(16)
N(4)–C(3)–C(2)	122.57(19)	122.41(16)
N(4)–C(5)–C(6)	121.0(2)	121.52(16)
N(4)–C(5)–C(8)	117.07(18)	116.82(16)
C(6)–C(5)–C(8)	121.93(17)	121.65(15)
O(3)–C(6)–N(7)	120.04(18)	120.56(16)
O(3)–C(6)–C(5)	125.2(2)	125.05(16)
N(7)–C(6)–C(5)	114.77(17)	114.38(15)
C(5)–C(8)–C(9)	118.04(17)	118.38(16)
C(5)–C(8)–C(13)	123.72(19)	123.72(17)
C(9)–C(8)–C(13)	118.2(2)	117.89(17)
C(10)–C(9)–C(8)	120.90(19)	121.22(18)
C(9)–C(10)–C(11)	120.1(2)	120.46(19)
C(10)–C(11)–C(12)	119.6(2)	119.28(17)
C(13)–C(12)–C(11)	120.70(19)	120.77(18)
C(12)–C(13)–C(8)	120.4(2)	120.32(18)
O(2)–C(14)–C(15)	109.1(2)	109.79(14)

thio ring by water causing ring breakage with a pendent SH. There is also loss of the copper centre coordinated to the amide group. The ligand L in complex **IB** is closely related to the α–β unsaturated ester ligand "penamaldate derivative" [26, 27]. The related $Cd(L')_2(H_2O)_4$ (L′: penamaldate ligand) has been isolated by degradation of sodium ampicillinate with cadmium in methanol. The complex was characterized by elemental analysis, NMR and mass spectra [26].

Six-membered ring cyclization occurred in step 3, where the NH_2 group attacks the carbon bearing the hydroxyl group causing the formation of **IC** and complex **ID**, characterized by elemental analysis, MS, UV and IR spectroscopy. In comparison,

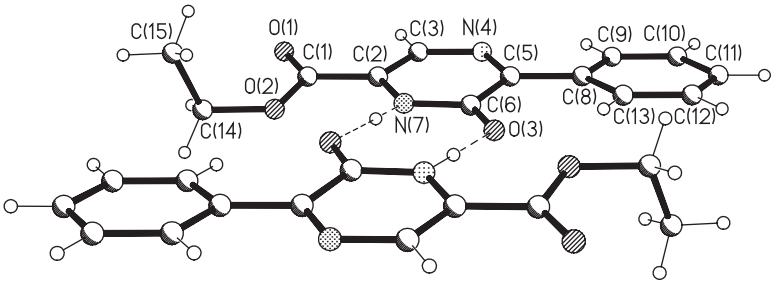


Figure 11. View of centrosymmetric dimer in the structure of polymorphs **IIA** and **IIB**.

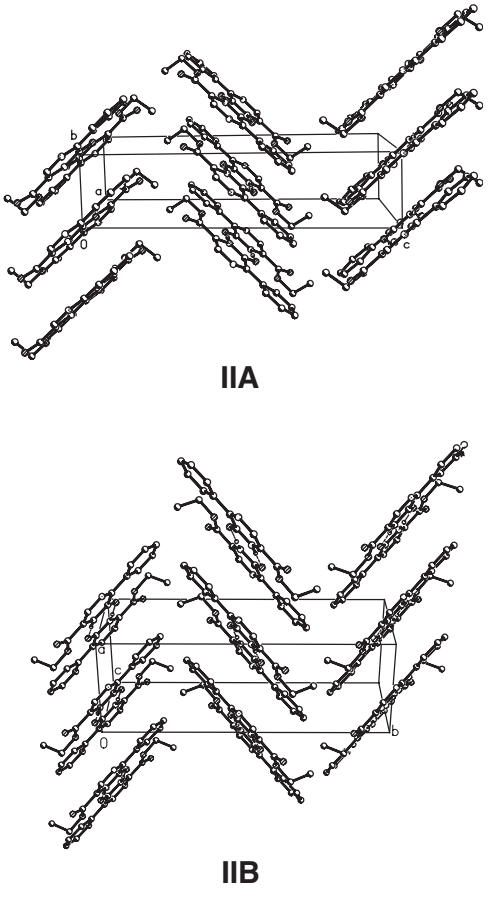


Figure 12. The packing diagram in **IIA** and **IIB**; hydrogen atoms on carbon atoms are omitted for clarity.

Garcia *et al*. [26] interpreted their mass spectrum where the observed peak at an m/z ratio of 465 is due to the binding of two fragments (penamaldic derivatives) $m/z = 233$ [Ph–C(H)(NH_2)–C(O)–NH–C(R)=CH] where R = CH_3–O–(C=O)–.

Loss of H^+ and OH^- from adjacent carbons in the ring of **IC** forms a carbon nitrogen double bond and eventually leads to formation of **IE** (step 4).

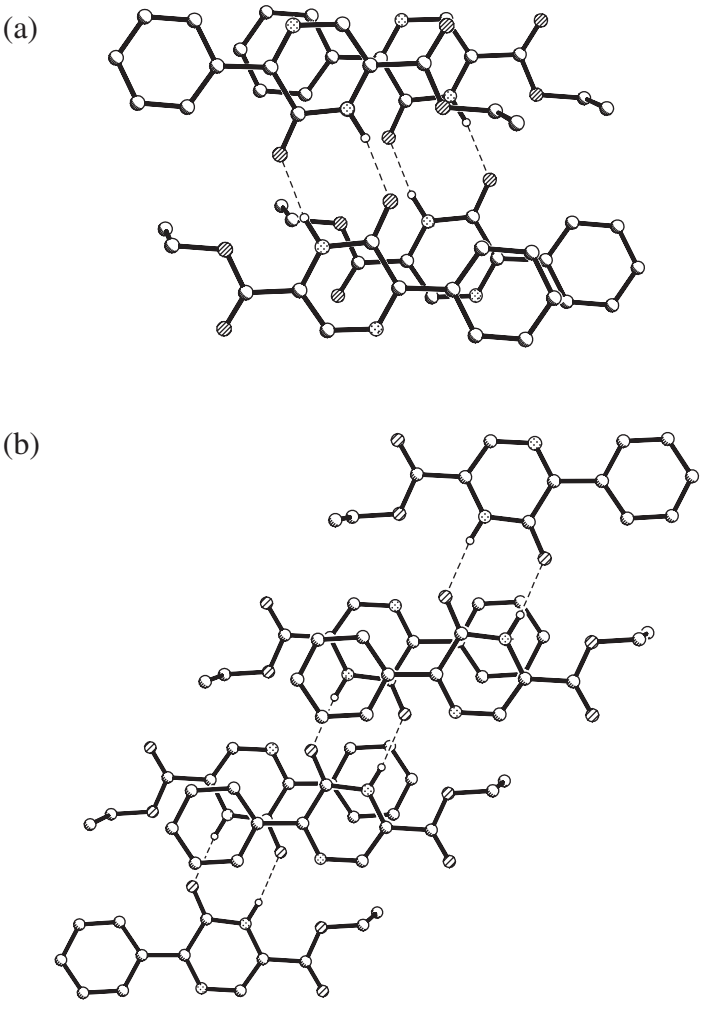


Figure 13. The manner of stacking interaction in the structure of **IIA** (a) and **IIB** (b); carbon-bonded hydrogen atoms are omitted for sake of clarity.

Step 5 involves loss of two hydrogen atoms from the ring and formation of **II** the final degradation product of ampicillin, 3-oxo-2-phenyl-pyrazine-5-carboxylic acid ethyl ester, which is fully characterized by single crystal X-ray diffraction method, UV-visible, IR, NMR, MS and elemental analysis.

3.6. *Crystal structure of 3-oxo-2-phenyl-pyrazine-5-caroxylic acid ethylester II*

Two polymorph crystals of the degradation products **IIA** and **IIB** 3-oxo-2-phenyl-pyrazine-5-caroxylic acid ethylester were analyzed by single crystal X-ray methods.

The overall molecular structure (figure 10), and the bond lengths and the bond angles (table 6) in the molecules **IIA** and **IIB** are very similar.

The phenyl and pyrazine rings are almost coplanar and form a dihedral angle of 8.0(1) and 2.5(1)° for **IIA** and **IIB**, respectively. The torsion angle C(1)–O(2)–C(14)–C(15) within the ethyl acetate group equals 92.1(2) and 93.6(2)° in the **IIA** and **IIB**. In the crystal structure of both polymorphs, molecules related by a center of symmetry form a dimer by coupling of the N–H···O hydrogen bonds [N(7)···O(3) = 2.828(5), N(7)–H = 0.94(2), H···O(3) = 1.90(2) Å, ∠N(7)–H···O(3) = 167(2)° for **IIA** and (N(7)···O(3) = 2.778(2), N(7)–H = 0.88(2), H···O(3) = 1.91(2) Å, ∠N(7)–H···O(3) = 172(2)° for **IIB**], resulting in the $R_2^2(8)$ supramolecular synton (figure 11) [51, 52].

The dimer and core-building unit in the crystal structures of **IIA** and **IIB** are invaried and their packing determines the difference of the polymorphs. The packing diagrams (figure 12) indicate the similarity in crystal structures of **IIA** and **IIB**.

In both structures the dimers are packed in infinite piles by π–π stacking interactions between the pyrazine and phenyl rings, but the mutual arrangement of dimers in the piles is different for **IIA** and **IIB**. Molecules in the crystal structure of **IIA** are parallel, with pyrazine ring of one molecule overlapping the phenyl ring of an adjacent molecule. The interplanar spacing of aromatic rings is 3.364 Å, and the centroid–centroid distance is 3.656 Å (figure 13a). Each molecule is stacked with two adjacent molecules in the pile, so both sides of each molecule participate in the stacking interactions.

In the crystal structure of polymorph **IIB**, the centro-symmetric related antiparallel molecules take part in the stacking interaction. Pyrazine and phenyl rings of one molecule overlap the phenyl and pyrazine rings of adjacent molecules (figure 13b). Only couples of molecules are involved in the stacking interaction and only one side of the molecules participate. The interplanar spacing between the best plane of aromatic rings and pyrazine ring–phenyl ring centroid–centroid distance in the stack are equal to 3.337 and 3.700 Å, respectively. The π–π contacts indicate strong stacking interactions [53] for both polymorphs.

Supplementary data

Crystallographic data for the structures reported in this article have been deposited with the Cambridge Crystallographic Data Centre as Supplementary Publication Nos. CCDC-246327, 246328. Copies of the data can be obtained free of charge from the CCDC (12 Union Road, Cambridge CB2 1EZ, UK; Tel.: +44-1223-336-408; Fax: +44-1223-336-003; E-mail: deposit@ccdc.cam.ac.uk; www:http://ccdc.cam.ac.uk).

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