THE SYNTHESIS AND METAL ION CATALYSED HYDROLYSIS OF METHYL 4,5-DIAMINOPENTANOATE

A thesis
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by

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To my wife, Cynthia

ABSTRACT

The diamino acid ester, methyl 4,5-diaminopentanoate has been prepared for the first time. The successful five step synthesis starting from 4-hydroxybutanoic acid lactone, and two other possible pathways via ethyl 3-formylpropanoate, are described.

pK_a values for 4,5-diaminopentanoic acid dihydrochloride and its methyl ester dihydrochloride (determined by potentiometric titration at 25°C and I = 0.10molℓ^{-1}) are discussed in relation to those for other 1,2-diamines.

Stability constants of Cu(II) complexes of 4,5-diaminopentanoic acid and its methyl ester were evaluated from potentiometric titration data by the program SCOGS on a PDP 11/70 computer. Both compounds form high stability constant 1:1 and 1:2 complexes with Cu(II). (For 4,5-diaminopentanoic acid log K_1 = 10.94, log K_2 = 9.40 and for methyl 4,5-diaminopentanoate log K_1 = 9.84, log K_2 = 8.51, at 25°C and I = 0.10molℓ^{-1}). A variety of protonated complexes were also present at lower pH.

pH-stat and spectrophotometric investigations showed that in the alkaline hydrolysis of methyl 4,5-diaminopentanoate, the simple $B_{Ac}2$ reaction is slow and masked by a competing, extremely rapid, pair of intramolecular aminolysis (lactamisation) reactions. These involve competition between five and six membered lactam ring closure. Overall constants of the order $10^3 \ell mol^{-1}s^{-1}$ at 25°C and I = 0.10molℓ^{-1} are observed.

The Cu(II) catalysed hydrolysis of methyl 4,5-diaminopentanoate was studied at constant pH using a pH-stat. The kinetics of hydrolysis of solutions containing a 1:2 mole ratio of Cu(II): ester are consistent with the two consecutive second order (pseudo-first-order at constant pH) reactions (1) and (2) (where E is the ester and A^- the anion, of 4,5-diaminopentanoic acid).

$$CuE_2^{2+} + OH^- \longrightarrow CuEA^+ + CH_3OH \qquad 1$$

$$CuEA^+ + OH^- \longrightarrow CUA_2 + CH_3OH \qquad 2$$

The relative values for the two rate constants obtained ($k_{(CuE_2^{2+})}$ = 2.41$\ell mol^{-1}s^{-1}$ and $k_{(CuEA^+)}$ = 0.80$\ell mol^{-1}s^{-1}$ at 25°C and I = 0.10$mol\ell^{-1}$) can be explained by charge and statistical effects. The value of $k_{(CuEA^+)}$ was confirmed independently from studies on solutions containing a 2.5:1:4 mole ratio of Cu(II): ester: acid. The value of $k_{(CuEen^{2+})}$ = 0.96$\ell mol^{-1}s^{-1}$ obtained from solutions containing Cu(II): ester: 1,2-diaminoethane = 2.5:1:4 is approximately $k_{(CuE_2^{2+})}/2$ as expected statistically. 1:1 Solutions of Cu(II) and methyl 4,5-diaminopentanoate disproportionate, so no values for $k_{(CuE^{2+})}$ could be measured.

The absolute values for the Cu(II) catalysed rate constants are discussed in comparison with those for methyl 2,3-diaminopropanoate and other diamino acid esters. The results for methyl 4,5-diaminopentanoate can be explained by the attenuation of inductive and positive charge effects of the metal ion by the additional methylene groups of the methyl 4,5-diaminopentanoate alkyl chain. Direct methoxycarbonyl-metal ion interaction, presumably absent in methyl 4,5-diaminopentanoate must, therefore, be unimportant in the Cu(II) catalysed hydrolysis of methyl 2,3-diaminopropanoate.

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A^- (AH, AH_2^+, AH_2^{2+}) (di)amino acid and protonated forms.

E (EH^+, EH_2^{2+}) (di)aminoacid ester and protonated forms.

CuL_x Cu(II): ligand = 1:x complex. Charges omitted except when L = E or A^- e.g. CuE_2^{2+}, CuA_2
$CuEA^+$ is mixed Cu(II): ester: acid = 1:1:1 complex.

K_a^x Proton dissociation constant

x = T, thermodynamic (activity) constant,

x = M, mixed activity of hydrogen ions/concentration of other species constant.

K_n Stepwise stability constant of metal-ligand complex, n = 1, 2 ...

β_n Cumulative stability constant of metal-ligand complex, n = 1,2...
e.g. $\beta_1 = K_1$, $\beta_2 = K_1 \times K_2$.

k_x Rate constant for reaction of species x with OH^-
x = E, EH^+ etc or CuE^{2+}, CuE_2^{2+} etc.

I Ionic strength.

-I Negative inductive (electron withdrawing)effect.

T Temperature

Amino Acids

Common names are used for most of the amino acids referred to in this thesis. Where a compound is referred many times an abbreviation of the name is used. The abbreviations are initially introduced together with the unabbreviated form e.g. glycine (Gly). The following abbreviations are used (systematic IUPAC name is given in brackets, when not commonly used)

Glycine (2-amino acetic acid)	Gly
β-Alanine (3-aminopropanoic acid)	β-ala
4-Aminobutanoic acid	4-but
Glutamic acid (2-aminopentanedioic acid)	Glu
Arginine (2-amino-5-guanidopentanoic acid)	Arg
Tyrosine (2-amino-3(4-hydroxyphenyl) propanoic acid)	Tyr
Histidine (2-amino-3(4'-imidazole)propanoic acid	His

LIST OF ABBREVIATIONS (contd.)

2,3-diaminopropanoic acid	2,3-dap
2,4-diaminobutanoic acid	2,4-dab
3,4-diaminobutanoic acid	3,4-dab
Ornithine (2,5-diaminopentanoic acid	2,5-dape
4,5-diaminopentanoic acid	4,5-dape
Lysine (2,6-diaminohexanoic acid)	2,6-dah

The methyl ester of an amino acid is denoted by adding Me to the abbreviation, e.g. methyl histidinate, Me His. (Di)Hydrochlorides are denoted by adding (2)HCl to the abbreviation, e.g. 4,5-dape.2HCl.

1. INTRODUCTION

It is well established that many organic reactions including polymerisation, redox and hydrolytic reactions (e.g. hydrolysis of amino acid esters, phosphate esters, peptides and amides) may be catalysed by metal ions. The subject of metal ion catalysis of organic reactions is of wide interest in inorganic, organic and biological chemistry and has been extensively reviewed.[1-5]

The term 'catalysis' by metal ions is used in this thesis in its usual but not strictly correct context of 'rate acceleration'. In many cases metal ion 'promotion' would be more rigorous since, for the reactions discussed, the metal ion increases the rate of reaction by increasing the entropy of activation rather than by lowering the enthalpy of activation, and is often stochiometrically consumed.

A metal ion may influence the reactivity of a substrate in several ways. The coordination of a substrate to a metal ion introduces positive charges into the substrate which may facilitate the approach of a nucleophile. Alternatively coordination to a metal ion may change the electron distribution in the substrate through inductive effects. The resulting bond polarisation may again facilitate attack by a nucleophile. Because of their positive charge, metal ions catalyse many reactions also catalysed by hydrogen ions. A multipositive metal ion may be regarded as a superacid[6] since it carries more than one positive charge, can coordinate to more than one electron donating group and can exist in higher concentration in neutral or weakly basic solutions which is important for biological systems.

The reactivity of a coordinated substrate may also be increased by the metal ion acting as an 'electron sink' for electrons produced during the reaction or by stabilising a leaving group such as halide or thiolate ion and hence assisting in the loss of that group.

Also a metal ion can coordinate to two or more species, acting as a collecting point for components of a polymolecular reaction and thus increase the rate by increasing the entropy of activation[7]. The rate enhancement observed for metal ion catalysed hydrolytic reactions is considered to be primarily an entropy effect[8].

The roles metal ions play in catalysis of hydrolytic reactions are of considerable interest as models for some biological systems, e.g. studies on metal ion catalysis of amino acid esters have suggested ways in which the great enhancement of substrate reactivity can occur in metalloenzymes such as carboxypeptidase A. Evidence from such model systems assisted in the interpretation of the very complicated X-ray electron density maps of carboxypeptidase A containing a substrate. The active site map could be interpreted as involving direct peptide, carbonyl oxygen-metal ion interaction with a $-COO^-$ group nearby. Such direct interaction and $-COO^-$ neighbouring group participation has been shown by model system studies[9,10] to provide rate increases similar to those seen in the enzyme's hydrolysis of peptides and amino acid esters.

Carboxypeptidase A (CPA) cleaves the peptide bond at the carboxyl end of polypeptides and can also hydrolyse esters[11,12]. The enzyme consists of about 300 amino acid residues (the exact number depends on which of the four forms is considered) and has Zn^{2+} as a cofactor[13]. The enzyme is deactivated by the removal of Zn^{2+} and can be reactivated[14,15] with Co^{2+}, Ni^{2+}, Mn^{2+} and Fe^{2+} but not with Cu^{2+}. The Zn^{2+} is coordinated to His-69, Glu-72, His-196 (numbering from the N terminus) and a water ligand[11].

In the proposed mechanism[11] (Figure 1) the substrate replaces the zinc bound water molecule. Neighbouring enzyme groups Arg-145 and Tyr-248 interact with the substrate polarising the carbonyl bond and allowing nucleophilic attack by Glu-270 carboxyl oxygen (shown in Figure 1) or by H_2O, hydrogen bound to Glu-270 (general base catalysis)[11,12]

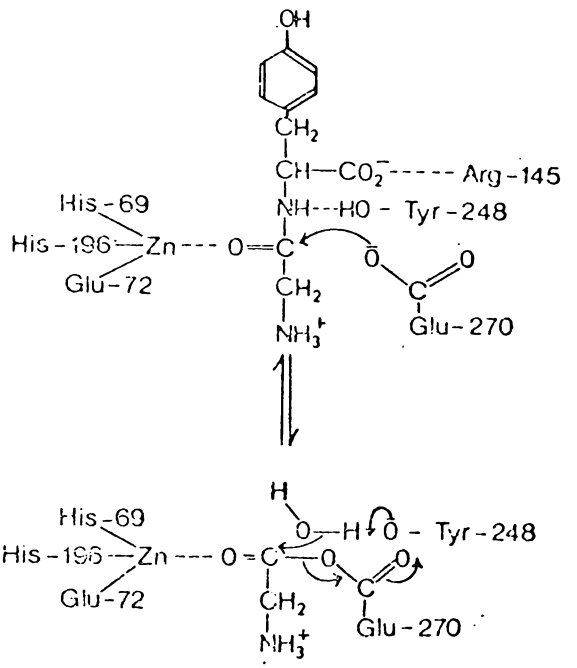


Figure 1: Possible mechanism for the carboxypeptidase A - catalysed hydrolysis of glycyl-L-tyrosine. (Lipscomb[11])

The X-ray studies[11] were necessarily carried out on a crystalline solid and any predictions from this structure as to the mode of action of the enzyme in solution must be considered with reservations. Also as the proposed mechanism is based on the determined structure of an only slightly reactive carboxypeptidase A-glycyl-L-tyrosine complex it is open to dispute. However the mode of binding of CPA with a reactive substrate is believed to be the same as for glycyl-L-tyrosine.

A great deal of work has been done on the metal ion catalysed hydrolysis of monoamino acid esters (mostly methyl or ethyl glycinate). The rate enhancements obtained have agreed in order of magnitude only[17-22] (see also reviews ref. 5 and 16). The problem is that because monoamino acid esters are essentially monodentate, their complexes with labile metal ions have only small stability constants. Hence a great variety of

complex species are present in solution for a given metal ion to ester ratio; e.g. a solution containing Cu(II): ester (E) = 1:1 will contain the complexes CuE^{2+}, CuE_2^{2+}, CuE_3^{2+} and CuE_4^{2+} as well as mixed complexes $CuEA^{+}$ and CuE_2A^{+} formed as the hydrolysis of E to A^{-} (the anion of the amino acid) occurs. It is thus difficult to know to which species the measured rate constant applies.

The rate accelerations observed, over the uncatalysed reactions, were large (ca. 10^5 times), and a mechanism involving direct interaction of the carbonyl oxygen with the metal ion was proposed[17,18] to account for this (Figure 2).

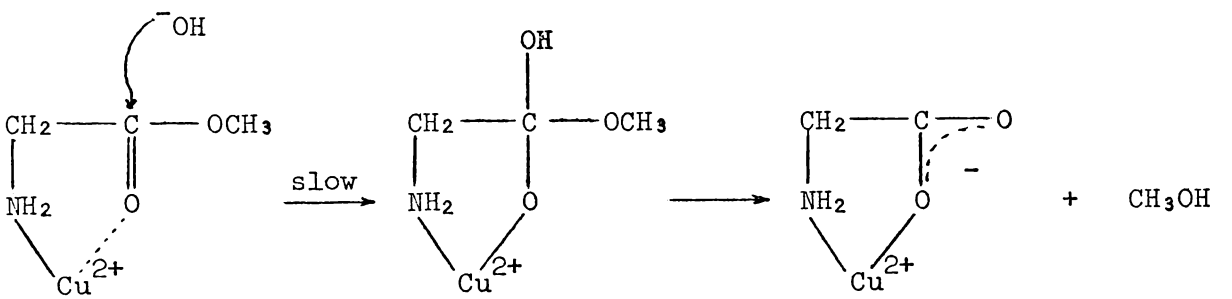
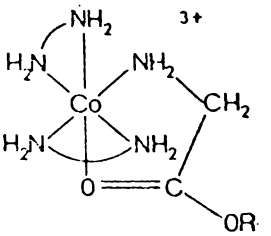


Figure 2: Proposed[17,18] mechanism for Cu^{2+} catalysed hydrolysis of methyl glycinate.

There is some evidence for direct interaction between the ester carbonyl oxygen and the metal ion in the solid state. Only a few metal ion complexes of monoamino acid esters have been isolated. Some of these appear to show coordination through both the amino nitrogen and the carbonyl oxygen (shown by a lowering in the IR carbonyl stretching frequency compared with the uncoordinated ligand[23,25]). However the presence of such an interaction in solution has been disputed[19].

This problem has been partially clarified by a study of non-labile Co(III)-amino acid ester systems. Following earlier work[26,27] on Hg^{2+} catalysed hydrolysis of Co(III) complexes of glycine esters in acid solutions, a complex containing chelated methyl glycinate was isolated[28]



and its presence confirmed in Hg^{2+} catalysed hydrolysis reactions[29].

An extensive study[10] has been made of the base hydrolysis of $[Co(en)_2XGlyOR]^{2+}$ ions (where X = Cl, Br; R = CH_3, C_2H_5, $(CH_3)_2CH$, $CH_3(CH_2)_3$, $(CH_3)_3C$ and $C_6H_5CH_2$) and a mechanism proposed which has two main pathways (Figure 3) from a 5-coordinate intermediate

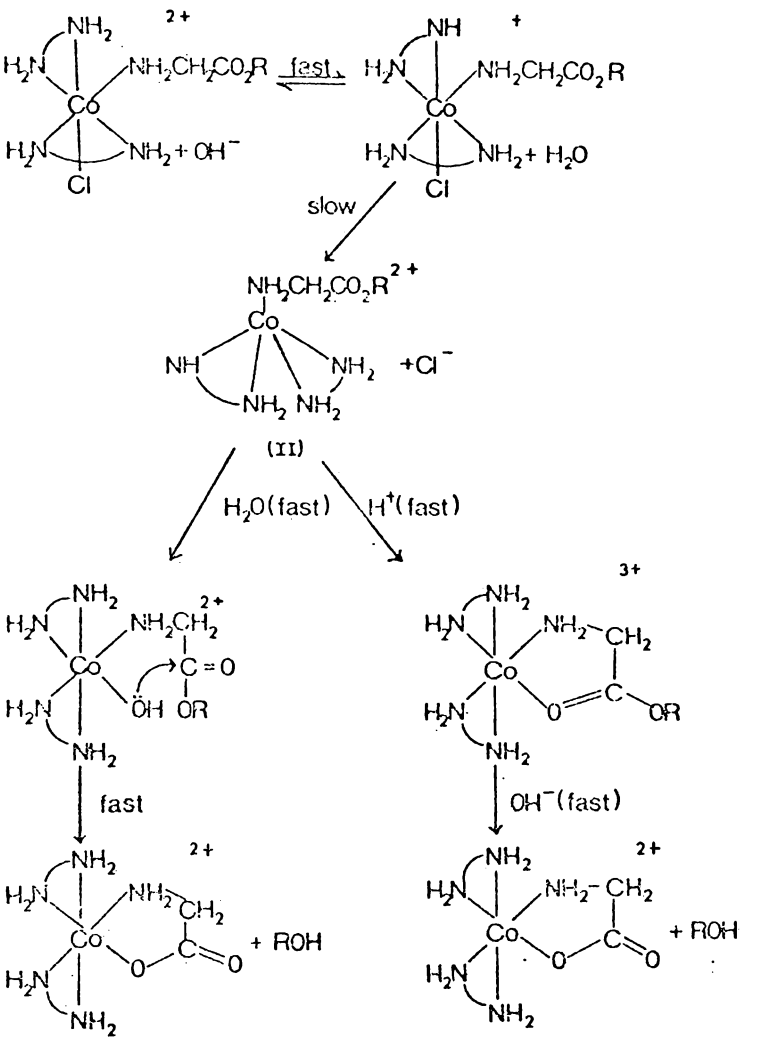


Figure 3: Proposed[10] mechanism for hydrolysis of $[Co(en)_2XGlyOR]^{2+}$

The two pathways are

(a) internal nucleophilic attack by bound hydroxide ion,

(b) Chelation of the ester by coordination of the carbonyl group.

The importance of internal nucleophilic attack has been confirmed[30] by the study of the hydrolysis of $[Co(NH_3)_5NH_2CH_2CO_2R]^{3+}$ where not only is the monodentate glycine complex $[Co(NH_3)_5(NH_2CH_2CO_2^-)]^{2+}$ formed but also the glycine amide complex $[Co(NH_3)_4(NH_2CH_2CONH)]^{2+}$. Formation of the amide can be rationalised by internal nucleophilic attack by one of the ammonia ligands (Figure 4).

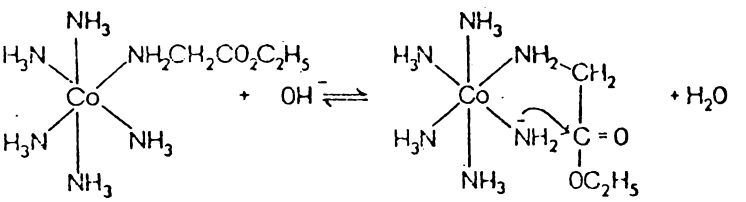


Figure 4: Internal nucleophilic attack by NH_2^-

The conclusion drawn from these studies is that attack by a coordinated hydroxide ion on a coordinated substrate leads to rate accelerations (over the base hydrolysis rate) of at least 10^7 times and possibly 10^{11} times, while external hydroxide ion attack on chelated species gives rate accelerations of about 10^6 times.

A more recent investigation[31] on the hydrolysis of cis-$[Co(en)_2Cl(NH_2(CH_2)_5CO_2CH_3)]^{2+}$, where intramolecular effects are not observed, show rate accelerations of only ca. 1.5 times. The coordination of the metal ion to the ester through only its amino group gives a similar increase in the rate of base hydrolysis to that expected for $^+NH_3(CH_2)_5CO_2CH_3$. The length of the alkyl chain considerably reduces the charge and inductive effects of the metal ion[31].

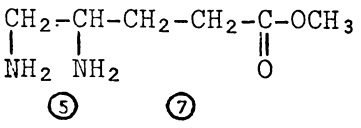
Hence, the studies on the non-labile (Co(III) complexes have shown that direct carbonyl oxygen-metal ion interaction provides similar rate

enhancement to that observed in Cu(II)-methyl glycinate systems. However, there are problems with the Co(III) work. Side reactions, e.g. the intramolecular OH^- attack in Figure 3, resulted in complicated kinetic data which was difficult to interpret; also Co(III) carries a very high charge (3+) on a very small ion, and this undoubtedly leads to unusual solvation effects and ion pairing, which are much smaller or absent in the labile systems studied, with their 2+ charged metal ions. As well as this the non-labile nature of the Co(III) complexes may result in them having quite unique and different behaviour to that of the labile metal ions or the enzyme systems.

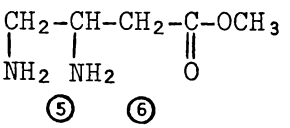
An alternative approach to the problem of the low stability constants of metal ion complexes of monoamino acid esters has been to use diamino acid esters. These are truly bidentate (cf. the essentially monodentate monoamino acid esters) and form very stable complexes with metal ions. E.g. in solutions containing methyl 2,3-diaminopropanoate (Me 2,3-dap)[32] or methyl histidinate (Me His)[33] to Cu(II) = 2:1, >99% of E is bound as CuE_2^{2+}. Rigorous interpretation of the kinetics is thus possible. However rate accelerations of only 10^3times over the uncatalysed reaction have been observed[34,35]. This presumably indicates that in these systems, direct metal ion-carbonyl oxygen interaction is unlikely (since most of the metal ion coordination sites are bound up most of the time by the bidentate ligand, or are sterically inaccessible). The larger (10^5times) rate enhancement observed for catalysed methyl glycinate hydrolysis presumably involves the extra carbonyl oxygen-metal ion interaction factor.

It may be argued that although the stability constants for bidentate diamino acid ester complexes with labile metal ions are high, these systems are labile and, for at least some of the time, the ester carbonyl oxygen may be bound to the metal ion. Thus the smaller rate

enhancement (10^3 times cf. 10^5 times for monoamino acid esters) for these bidentate esters may be due to the infrequent but extremely hydrolytically important, interaction of the carbonyl oxygen with the metal ion. One way to see whether such carbonyl oxygen-metal ion interaction is the major source of the activitation of these esters, or whether simple charge and inductive effects are responsible, is to study the hydrolysis of potentially bidentate amino acid esters where the ester group is remote from the site of metal ion bonding. Two possible esters of interest are methyl 4,5-diaminopentanoate (Me 4,5-dape) (1) and methyl 3,4-diaminobutanoate (Me 3,4-dab) (2)



1



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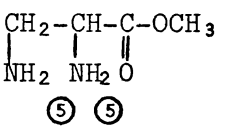


3

(the circled number indicates the size of the potential chelate rings)

For Me 2,3-dap (3), coordination to a metal ion through the two amino groups involves formation of a 5-membered chelate ring. Possible transient coordination through the α-NH_2 and ester carbonyl oxygen also involves a 5-membered ring. In going from (3) to (2) to (1), primary coordination remains as a 5-membered ring but the transient coordination changes to a less stable 6 then 7-membered ring. Consequently if carbonyl oxygen-metal ion interaction is important in the hydrolysis of diamino acid esters a greatly decreased rate enhancement might be expected to be observed with the change from (3) to (1). If carbonyl oxygen interaction is not important in any of the diamino acid esters, a smaller decrease in rate enhancement would be expected, associated with the attenuation of the inductive and positive charge effects of the metal ion by the additional methylene groups.

Accordingly studies of the uncatalysed and Cu(II) catalysed hydrolysis of Me 4,5-dape and Me 3,4-dab were to be carried out to complement the data previously obtained[34] for Me 2,3-dap. Me 4,5-dape and Me 3,4-dab have apparently not been prepared previously so new syntheses were attempted for these compounds. Me 2,3-dap was also prepared to check the existing literature results.

It was also necessary to measure the stability constants of complexes of these new esters with Cu(II) to check that they had sufficiently high values to allow rigorous interpretation of the hydrolysis kinetics. The values of the proton dissociation constants, needed in the stability constant calculations, were also determined.

Most of the kinetic studies in the present investigation were made using a pH-stat. This method has the following advantages:

(1) As no buffers are required no complexing of the metal ion with the buffer occurs. Also no general acid or base catalysis by buffers can occur.

(2) The pH is kept constant. This is essential as pH dependant 'rate constants' and equilibria were involved. As a range of pH values were to be studied attack by OH^- or H_2O could be distinguished.

The disadvantages of the method are the requirement for high stability constant metal ion complexes to prevent metal hydroxide precipitation and the increase in solution volume, owing to added alkali, as the reaction proceeds. This increase was kept to <2% experimentally and is not significant (linear kinetic plots were obtained for pseudo first-order reactions).

2. CHEMICALS AND APPARATUS

2.1 Amino Acids and Esters

Where available commercial samples (BDH or Sigma) of amino acids were used. These were specified as being chromatographically homogeneous or >99% pure. The diamino acids, 2,3-diaminopropanoic acid (2,3-dap) and 4,5-diaminopentanoic acid (4,5-dape) were synthesised as described in Section 3.

(Di)amino acid methyl esters (di)hydrochlorides were prepared as follows: (ref 36, Vol.2, p.925):

The (di)amino acid (20g, 0.1-0.2 mol) was refluxed for 3h in 'super dry' methanol (400ml) (ref 37, p.169), while a rapid stream of dry hydrogen chloride gas was passed through the solution. Hydrogen chloride was generated by adding concentrated sulphuric acid through a capillary into concentrated hydrochloric acid. The gas evolved was dried by passing it through concentrated sulphuric acid. Generally the amino acid dissolved after 0.5h and then the methyl ester (di)hydrochloride precipitated as the methanol became saturated with hydrogen chloride.

After 3h the reaction mixture was evaporated to dryness (rotary evaporator). Several small portions of 'super-dry' methanol and finally dry ether were added, the resulting slurry being reduced to dryness between each addition, in order to remove any traces of water produced by the esterification reaction. The entire esterification procedure was repeated and the resulting white solid recrystallised from 'super-dry' methanol/dry ether solutions. One further recrystallisation generally gave a satisfactory melting point.

Table 1 lists the prepared (di)amino acid ester (di)hydrochlorides.

Table 1: Amino acid methyl esters.

Amino Acid	Yield of Ester	m.p.°C (Lit)
Glycine	37%	177 (175)[a]
L-Serine	46%	166 (165-166)[a]
L-Cysteine	93%	200 (200-201)[a]
L-Histidine	87%	140 (140)[a]
D,L-2,3-diaminopropanoic acid	93%	167 (164-166)[b]
D,L-4,5-diaminopentanoic acid	91% (c)	146-147

(a) Ref. 36, Vol.2, p.925
(b) Ref. 38.
(c) Prepared from the lactam 5-(aminomethyl)-2-oxopyrrolidine hydrochloride.

2.2 Solutions

The following solutions were prepared for all potentiometric and kinetic measurements.

2.2.1. Water:

The water used was twice distilled in an all pyrex glass still. Immediately before use the water was thoroughly degassed (by boiling under vacuum at room temperature) to remove dissolved carbon dioxide. All solutions were prepared using this degassed distilled water.

2.2.2. Potassium Chloride:

A 1.000mol ℓ^{-1} potassium chloride solution was used to maintain an ionic strength of 0.10mol ℓ^{-1}. It was prepared using dried A.R. grade KCl.

2.2.3. Copper(II) Chloride:

0.1mol ℓ^{-1} solutions of A.R. $Cu(II)Cl_2$ were standardised by complexometric titration with EDTA using Fast Sulphon Black F as

indicator[39]. The concentration was adjusted to exactly 0.100mol ℓ^{-1} by addition of small amounts of concentrated $Cu(II)Cl_2$ solution or water.

2.2.4. Sodium Hydroxide:

Sodium hydroxide solutions were prepared using vials of concentrated solution (BDH. CVS). The solution was adjusted to exactly 1.000 mol ℓ^{-1} by addition of solid sodium hydroxide or water. The solution was standardised by titration against 0.05mol ℓ^{-1} potassium hydrogen phthalate buffer solution to an end point of pH = 8.5 on the pH meter. Carbonate contamination of the solution was minimised by use of concentrated solution vials and thoroughly degassed water. The diluted solution was protected from atmospheric CO_2 by a 'carbosorb' tube attached to the autoburette. The concentration of the solution was checked regularly.

2.2.5. Buffers:

2ℓ batches of 0.05 mol ℓ^{-1} potassium hydrogen phthalate and 0.01mol ℓ^{-1} borax buffer solutions were prepared as described by Bates[40]. The pH of these two N.B.S. buffers at 25°C are[40] KHPhthalate = 4.008 and borax = 9.180. Great care was taken in preparation of these solutions since they greatly affect the accuracy of the subsequent potentiometric measurements. The buffers were stored in tightly stoppered flasks and were replaced at least once a month.

2.3 General Experimental Methods

Microanalyses were carried out by Prof. A.D. Campbell at the University of Otago. Melting points were determined on a Reichert Kofler block and are uncorrected.

IR spectra were measured on a Perkin-Elmer 180 or a Shimadzu IR-27G spectrometer. A Carey 17 spectrophotometer was used to measure UV-visible

spectra and for the kinetic runs at fixed wavelength, with the sample space thermostated to (25 ± 0.05)°C.

NMR spectra were recorded on a Jeol C-60HL 60MHz spectrometer. The NMR data are expressed as parts per million (ppm) downfield shift (δ values) from tetramethylsilane (Tms) as the internal reference and are quoted as; position, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet and m = multiplet), coupling constant (J, Hz) and assignment. (The IUPAC numbering system was used).

Mass spectra (MS) were run by Dr. P.T. Holland (Ruakura Animal Research Station) using a Varian MAT CH5 mass spectrometer operating with a nominal ionising voltage of 70eV with a direct probe sample carrier and a source temperature of 200°C.

Silica gel for column chromatography was Silica Gel 60-100 mesh (BDH). Analytical thin layer chromatography (tlc) was conducted on plates of Kieselgel G nach Stahl (Merck) and chromatograms were developed with iodine vapour for neutral organic compounds and ninhydrin spray for amino acids. Preparative tlc was carried out on 1.5mm thick plates of Kieselgel $PF_{254+366}$ (Merck).

2.4 pH Assembly

All pH measurements were carried out using the cell

Glass electrode || Test solution | $KCl_{(satd.)}$, Calomel electrode.

i.e. $Ag_{(s)}$; $AgCl_{(s)}$, HCl || Test solution | $KCl_{(satd.)}$, $Hg_2Cl_{2(s)}$; $Hg_{(\ell)}$

The following Radiometer components were used:

pH meter, type PHM 26c

Autoburette, type ABU11 with 2.5ml burette

Titrator type, TTT 11b

Glass electrode, type G202B

Calomel electrode, type K401

The equipment was set up and checked in accordance with the instrument instruction manuals.

The pH-meter (PHM 26c) allows very precise pH-measurement. The 'ISO-pH' and 'electrode sensitivity controls' allow the instrument to be adjusted to an individual electrode pair. The precision scale expander built into the meter covers the full pH range (0-14pH) and gives a reproducibility of ±0.002pH. A routine check procedure described in the instruction manual checks that the 'temperature, ISO-pH, electrode sensitivity' and 'buffer adjustment' control potentiometers are providing the correct range of adjustment and that linearity exists between the 'normal' and 'expanded ×10' meter scale settings. Adjustments are provided to correct faults but were never needed.

2.4.1. Standardisation:

The pH meter was initially adjusted to the response of the electrode pair by standardisation against the two buffers, potassium hydrogen phthalate and borax. The following procedure was used. With the 'temperature' control set to the solution temperature (25°C, 'internal temperature control mode') and the 'ISO-pH' control set to the pH of the borax buffer (9.18), the electrodes were equilibrated for at least 1h in the borax buffer, to ensure stabilisation at 25°C. (Adjustment of the 'ISO-pH' potentiometer to the pH of the first buffer changes the electrical zero of the meter to that value. Subsequent setting of the 'electrode sensitivity' does not affect the initial setting of this first buffer.) The stirrer was stopped and the 'buffer adjustment' set to give exactly 9.180 pH on expanded scale. Turbulence caused by stirring of the solution caused an apparent lowering of pH. This was more noticeable in alkaline solution. (∿0.03pH for borax at 25°C) and at the low ionic strength of the buffers. In the titration or reaction solutions where ionic strength was 0.10 mol ℓ^{-1} the effect was usually negligible (∿0.003 pH).

The electrodes were then equilibrated to 25°C in KHphthalate buffer and the 'electrode sensitivity' set to give an expanded scale reading of exactly 4.008 pH. This adjustment allows for the sensitivity of the glass electrode normally falling between 98 and 100% of the theoretical value. As the sensitivity of the glass electrode decreases with time, the whole procedure outlined was repeated at least once a month. The electrodes were replaced if the sensitivity had fallen below 97%.

After this full standardisation the pH meter was standardised on only one buffer (KHphthalate) before each titration or kinetic run.

2.4.2. Electrodes:

The electrodes were prepared and stored in accordance with Radiometer instruction leaflets. The glass electrode used was of the high alkalinity type (pH 0-14, temp range 20-60°C) and no sodium or potassium ion error correction had to be made to meter pH. The electrode was replaced when its response became too slow or erratic, although its sensitivity was often still greater than 97%. The calomel electrode was very reliable and was only replaced when it became clogged with solid potassium chloride. This became apparent in fluctuating response of the pH meter.

2.4.3. End Point Control:

All hydrolysis kinetic runs were done using the pH assembly as a pH-stat. The injection of alkali into the reaction solution from the autoburette is controlled by the titrator 'end point' setting. For a kinetic run the 'end point' control was set so that the required pH was observed on the expanded scale of the pH meter. pH control was achieved by alteration of the titrator 'proportional band' setting and the autoburette 'speed' control. The 'proportional band' setting controls the length of time for which the plunger in the burette operates. The 'speed' control alters the amount of alkali delivered during this time.

Best control was obtained with the 'proportional band' set on 0.1 and the 'speed' control adjusted to give a rapid on-off action of the control switch. This provided an almost continuous addition of alkali, giving almost constant pH. Long 'on' periods of the switch were avoided as this raised the meter pH readings by as much as 0.02pH. As the kinetic run slowed down, the 'end point' setting was lowered to maintain a constant pH reading. Otherwise end point overshoot occurred.

2.4.4. Accuracy:

Radiometer claim for the pH meter 26 a reproducibility of ±0.002pH and a relative accuracy of ±0.007pH for measurement in a different range from the buffer standardisation. The pH meter 26 is very stable with a non-cumulative amplifier drift of only ±0.002pH per day.

It is thus the buffer standardisation which is the limiting factor for accuracy. Accuracy of standardisation depends on:

(1) the accuracy of the buffers pH values (±0.003pH)[40]

(2) the internal consistency of the NBS pH scale (±0.005-0.01pH)[40]

(3) the similarity of ionic strength (I) of the test solution to I of the calibrating buffer.

(A calculated value of the activity coefficient of Cl^- from the Debye-Hückel equation is required in the calculation of the buffer pH scale.)

Also the accuracy of pH-meter reading depends on the exact meaning of pH. It is generally assumed that:[40]

pH (meter reading) = -log (Hydrogen ion activity)

Overall experimental accuracy is probably about ±0.01pH (±2-3%).

2.4.5. Reaction Vessel:

All potentiometric and kinetic studies were carried out at (25±0.05)°C using a double walled glass vessel through which thermostatted water was pumped by a Braun Thermomix II.

The double walled vessel was closed with a tight fitting plastic coated cork through which passed the glass and calomel electrodes, alkali feed, thermometer and nitrogen gas inlet. The test solution was protected from atmospheric carbon dioxide and oxygen by passing a stream of oxygen free nitrogen over its surface. To prevent evaporation of the test solution the gas was first humidified by bubbling it through distilled water.

2.5 Activity Coefficients and Concentration of OH^-

As previously stated (Section 2.2.4) the pH meter reading was taken to be the negative log of hydrogen ion activity.

i.e. $pH = -\log \{H^+\}$

To calculate $[H^+]$ the activity coefficient of H^+, y_1, was estimated using the Davies equation[41] (see Appendix 1.1). The activity of OH^- ($\{OH^-\}$) was calculated from[42] $\{H^+\}\{OH^-\} = K_\omega^T = 10^{-13.9965}$ at 25°C. $[OH^-]$ is calculated from $\{OH^-\}$ using y_1 as before.

3. SYNTHESIS OF DIAMINO ACIDS AND THEIR ESTERS

3.1 2,3 Diaminopropanoic Acid (Method 1)

This naturally occurring diamino acid has been prepared by several methods (ref. 36, Vol.3, p.2463). Of the two used in this work, the most satisfactory was that of Hellmann and Haas[43]. Details of the six steps involved (Scheme 1) are given in the following sections.

3.1.1. N,N-dimethylaminomethylbenzamide (4)[43]

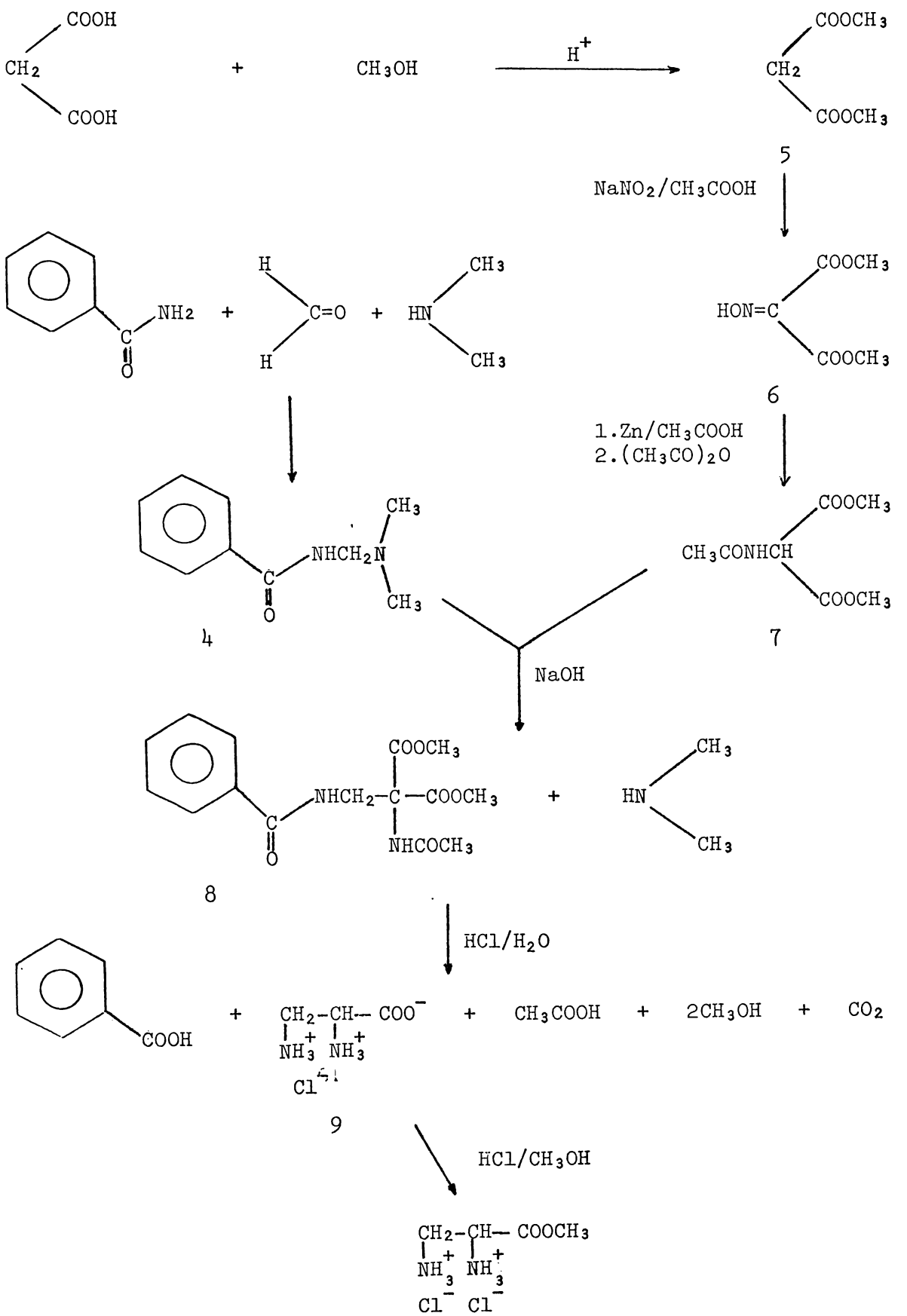
(4) was prepared by condensing together benzamide, formaldehyde and dimethylamine. The preparation was modified by adding ethanol to assist in dissolving the benzamide and thus prevent the loss of the very volatile dimethylamine (heat is otherwise required to dissolve the benzamide). Loss of dimethylamine resulted in recovery of unchanged benzamide. Benzamide (60g, 0.5mol) was gently warmed with 40% formaldehyde solution (37.5g, 0.5mol) and 33% dimethylamine solution (68.2g, 0.5mol) in ethanol (150ml) until all the benzamide had dissolved. After standing at 20°C for 3h saturated sodium carbonate solution (300ml) was added and the oily top layer which formed separated off. This oil was dried by heating under vacuum (rotary evaporator) and then solidified by prolonged refrigeration. The crude solid was purified by dissolving in cold benzene, filtering, then adding petroleum spirit (60-80°C) until cloudy, and refrigerating. An oil separated initially, which slowly crystallised. Filtration yielded N,N-dimethylaminomethylbenzamide (4) (69g, 78%) as deliquescent white plates m.p. 53-60°C (Lit[43] 57-59°C).

A satisfactory sample for analysis could not be prepared for this compound, which was used without further purification.

3.1.2. Dimethyl malonate (5)

Commercially available samples of (5) were usually used but when not available, it was prepared by esterification (ref. 37, p.386) of malonic acid with absolute methanol (ref. 37, p.169) using concentrated sulphuric

Scheme 1: Synthesis of 2,3-dap.HCl (9) and Me 2,3-dap.2HCl (3a)



acid as the catalyst and dry benzene as the solvent. The reaction was performed on a 1 mole scale, dimethyl malonate (5) (69.3g, 42%) being collected at 179-183°C, n_D^{20} 1.4139 (Lit[44] 181.4°C, n_D^{20} 1.41348).

3.1.3. Dimethyl isonitrosomalonate (6)[45]

This unstable compound, prepared by treating dimethylmalonate with nitrous acid, was not isolated but was used directly in the next reaction.

Dimethyl malonate (41.2g, 0.31mol) was cooled and a mixture of glacial acetic acid (56.6g, 0.94mol), and water (81g) added. A solution of sodium nitrite (65g, 0.94mol) in water (50g) was added dropwise with stirring over 1.5h, the solution being kept at 5°C.

The reaction mixture was then stirred at 20°C for a further 4h. The solution was extracted with ether (2 × 100ml) and the ether solution used directly in the next reaction.

3.1.4. Dimethyl acetamidomalonate (7)[45]

Zinc/Acetic acid reduction of (6) followed by acetylation of the product gives dimethyl acetamidomalonate (7).

Acetic anhydride (86g, 0.84mol.) and glacial acetic acid (225ml, 3.95mol) were added to the ethereal solution of (6). Zinc powder (78.5g, 1.20mol) was added with vigorous stirring over 1.5h, the solution temperature being kept between 40 and 50 C. Stirring was continued for 0.5h, then the solution was filtered and the residue washed with glacial acetic acid (2 × 200ml). The combined filtrate and washings were concentrated (rotary evaporator) to yield a yellow oil. Water (100ml) was added, the solution warmed to allow complete mixing and then refrigerated. Dimethyl acetamidomalonate (7) (25g, 43%) crystallised as fine white needles, m.p. 128-129°C (Lit[46] 127°C).

Found	C, 44.38;	H, 5.75;	N, 7.21%
$C_7H_{11}NO_5$ requires	C, 44.40;	H, 5.86;	N, 7.40%

The yield obtained is considerably less than that reported[45] (77%). This is probably because zinc powder was used rather than the finer zinc dust which was unavailable. A different, more active brand of zinc powder improved the yield to 68%.

3.1.5. Dimethylbenzamidomethyl-acetamidomalonate (8)[43]

(4) and (7) can be condensed together in the presence of a small amount of powdered sodium hydroxide to produce dimethylbenzamidomethyl-acetamidomalonate (8).

(4) (22.25g, 0.125mol.) and (7) (23.63g, 0.125mol) were added to a mixture of dry toluene (375ml) and finely powdered sodium hydroxide (0.62g). The mixture was refluxed and a stream of nitrogen bubbled through the solution until no more dimethylamine issued from the top of the reflux condenser (10-11h). The reaction mixture was cooled and the precipitate of crude (8) which formed was filtered, washed with water to remove sodium hydroxide, then dried under vacuum. The crude product was recrystallised from toluene to yield dimethylbenzamidomethyl-acetamidomalonate (8) (25.5g, 63.3%) m.p. 173°C (Lit[43] 151°C).

Found	C, 56.14;	H, 5.84;	N, 8.03%
$C_{15}H_{18}N_2O_6$ requires	C, 55.90;	H, 5.63;	N, 8.69%

3.1.6. D,L - 2,3-diaminopropanoic acid monohydrochloride (9)[43]

Hydrolysis of (8) with constant boiling point hydrochloric acid solution gave 2,3-diaminopropanoic acid monohydrochloride (9). (8)(24.52g, 0.076mol) was gently refluxed with constant boiling point hydrochloric acid (125ml, b.p. 110°C; 20.24% HCl, 79.26% H_2O ω/ω), for 9h. (after about 2h oily droplets of benzoic acid formed in the refluxing solution).

The mixture was allowed to cool and the benzoic acid which crystallised out was filtered off and washed with water. To the combined filtrate and washings ethanol (400ml) was added and the resulting solution refrigerated until crystallisation was complete. Filtration yielded 2,3-diaminopropanoic acid monohydrochloride (9) (9.74g, 91%) m.p. 225°C (decomp.) (Lit[47] 239°C).

Found	C, 25.64;	H, 6.41;	N, 19.92;	Cl, 24.93%
$C_3H_9N_2O_2Cl$ requires	C, 25.63	H, 6.45;	N, 19.93;	Cl, 25.21%

3.1.7. Methyl D,L-2,3-diaminopropanoate dihydrochloride (3a)

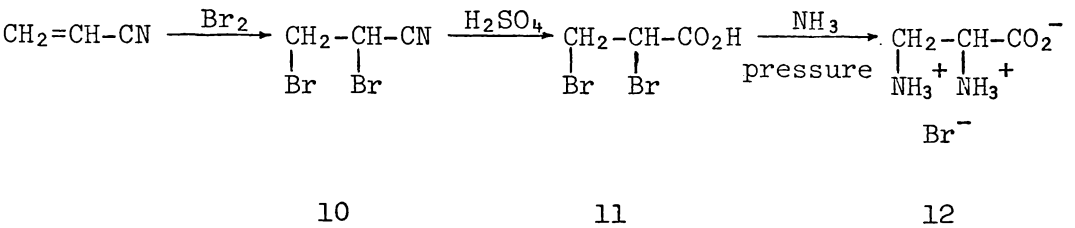
The methyl ester of (9) was prepared by the general esterification procedure described previously (Section 2.1). This gave Me 2,3-dap 2HCl (3a) (25.82g, 93%, 0.14mol scale) m.p. 167°C (Lit[38] 164-166°C)

Found	C, 25.14;	H, 6.31;	N, 14.62;	Cl, 36.61%
$C_4H_{12}N_2O_2Cl_2$ requires	C, 25.15;	H, 6.33;	N, 14.66;	Cl, 37.11%

3.2 2,3-Diaminopropanoic Acid (Method 2)

This method has the advantage of only three steps but offers an overall yield of only 18%, cf. 40% for method 1 (Section 3.1). This is due to the low yield (40%) of the third step. As Greenstein and Winitz (ref.36, Vol.3, p.2463) have noted, amination of 2,3-dibromopropanoic acid must be carried out under pressure or a side reaction produces considerable amounts of 3-amino-2-hydroxypropanoic acid (isoserine) and a diminished yield of 2,3-diaminopropanoic acid. The three steps of this method (Scheme 2) are:

Scheme 2: Synthesis of 2,3-dap. HBr (12)



10 11 12

3.2.1. 2,3-Dibromopropanonitrile (10)[48]

Bromine (160g, 1mol) was added dropwise over a period of 8h to a stirred solution of freshly distilled acrylonitrile (53g, 1mol) (b.p. 77.3°C) and dimethyl formamide (5g, 0.023mol), the temperature being maintained between 20 and 25°C. The product 2,3-dibromopropanonitrile (10) (131g, 61%) is very corrosive and lachrymatory and was hydrolysed directly without purification.

3.2.2. 2,3-Dibromopropanoic acid (11)[48]

A mixture of sulphuric acid (72.5g, 0.74mol) and water (11g, 0.62mol) was added dropwise with stirring to 2,3-dibromopropanonitrile (10) (131g, 0.62mol) at 20°C. The stirring was continued overnight. Water was added to the resulting thick syrup yielding a white precipitate. Recrystallisation from water gave 2,3-dibromopropanoic acid (11) (150g, 73%) m.p. 129°C (Lit[48] 127°C).

3.2.3. 2,3-Diaminopropropanoic acid monohydrobromide (12) (ref36,V3,p2465)

A solution of 2,3-dibromopropanoic acid (11) (40g) in water (400ml) was saturated with ammonia at 0°C. The solution was maintained at 100°C and 2 atmospheres pressure (pressure cooker) for 4h. Evaporation to dryness (rotary evaporator) under nitrogen yielded an orange residue. This was taken up in a minimum volume of boiling water and refrigerated. Crystals of 2,3-diaminopropanoic acid monohydrobromide (12) were obtained (16g, 40%) m.p. 240°C decomp. (Lit 230°C, ref.36, Vol.3, p.2465). This was converted to the hydrochloride (9) by bubbling HCl gas into a solution of (12) in ethanol. The resulting product gave identical m.p., tlc, and IR spectra to the authentic compound.

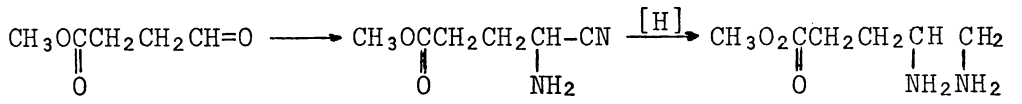
3.3 4,5-Diaminopentanoic Acid

The preparation of this compound has not been reported in the literature. The separation of the amino and carboxylic groups means that syntheses of the types reported for 2,3-diaminopropanoic acid[36, 43] cannot be used. Arndt-Eistert "ascent of the homologous series" reaction from 2,3-diaminopropanoic acid was rejected because synthesis of the starting material involves six steps, and its conversion into 4,5-dape a further three steps with consequently a very low overall yield.

Hence it was decided to build up the required functional groups on an appropriate C-skeleton in a stepwise fashion.

The 1,2-diamine grouping is accessible from α-aminonitriles which can be prepared from the corresponding aldehydes[49]. It appeared therefore that Me 4,5-dape (1) could be prepared from methyl 3-formylpropanoate (13) via methyl 4-amino-4-cyanobutanoate (14) (Scheme 3).

Scheme 3: Proposed synthesis of Me 4,5-dape (1).

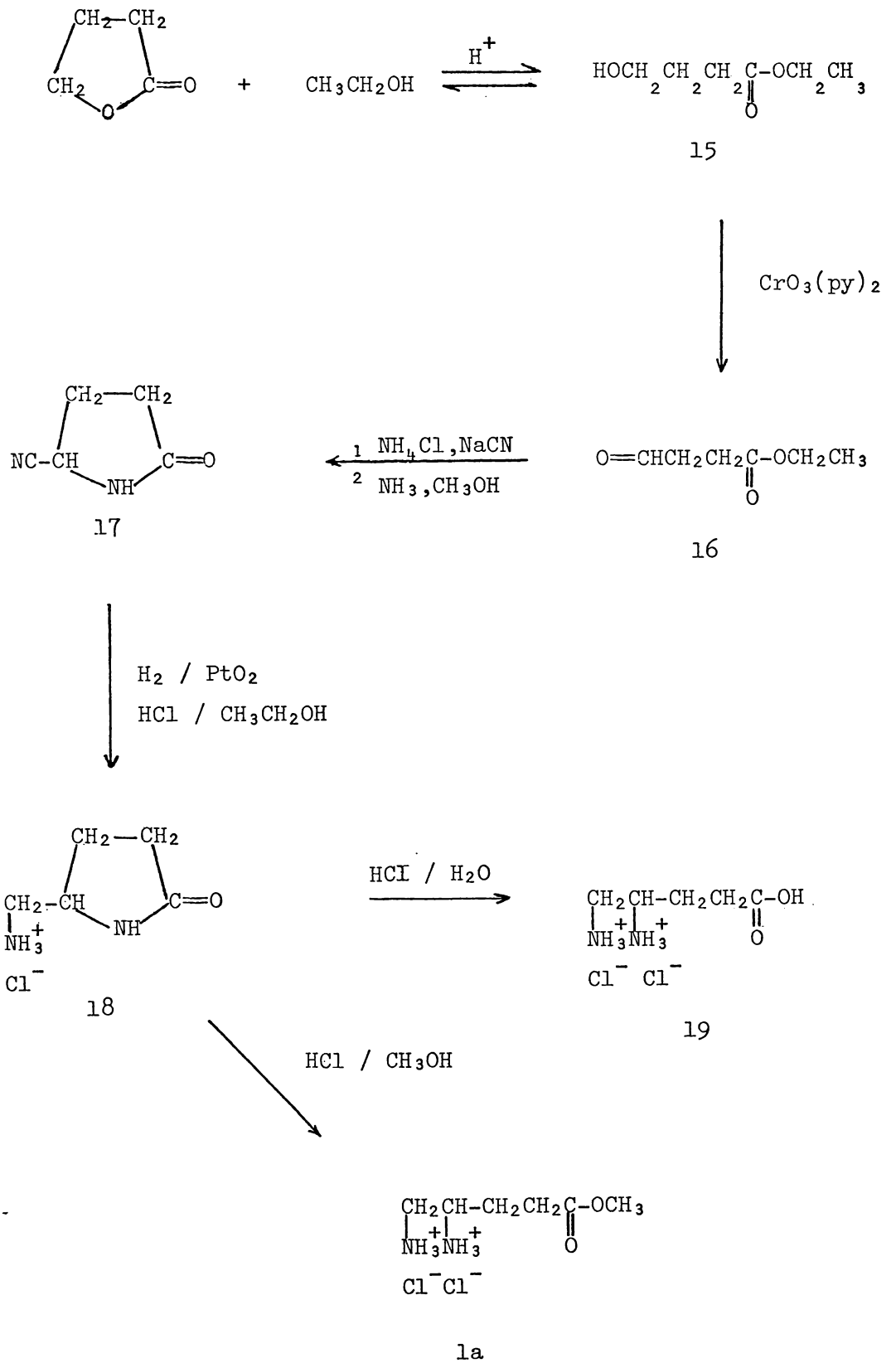


13 14 1

Methyl 3-formylpropanoate (13) was not available commercially and also had to be prepared.

The eventual synthesis of the Me 4,5-dape, although following the same reaction sequence, produced very different compounds (Scheme 4). The five steps of this synthesis are discussed in the following subsections.

Scheme 4: Synthesis of Me 4,5-dape.2HCl(1a) and 4,5-dape.2HCl(19).

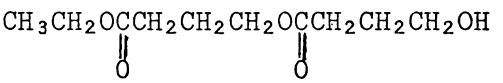


3.3.1. Ethyl 4-hydroxybutanoate (15)

This was prepared using a modified version of the Brown and Keblys[50] synthesis of 4-hydroxybutanoic acid esters. The preparation is complicated because the reaction is reversible with a small equilibrium constant for ester formation; consequently a large excess of the alcohol must be used. Also the catalyst (H_2SO_4) is difficult to neutralise completely; traces remaining result in reversal of the equilibrium on removal of the alcohol, and attempted fractional distillation gives the lower boiling point lactone as the only product. An initial flash distillation to remove the last traces of H_2SO_4 prevents this. Even so recovery of the ester from the equilibrium mixture is poor with the ethyl ester giving the highest yield (53%)[50]. In compensation, recovered lactone can be recycled. Early attempts at preparing the methyl ester gave even lower yields than those reported[50] (13%).

4-Hydroxybutanoic acid lactone (258g, 3mol) (Sigma Chemical Co., purified by distillation b.p. 39° at 0.4mm) was added to a stirred mixture of "superdry" ethanol (1380g, 30mol) (ref.3, p.167) and concentrated sulphuric acid (6g) in a flask protected by a calcium chloride drying tube. Ethanol freshly distilled from magnesium ethylate (ref.3, p.167) was found to be essential as slight traces of water greatly decreased the yield. Stirring was continued for 24h; longer stirring did not improve the yield. Finely powdered calcium carbonate (30g) was added and the mixture stirred for a further 2h at least. The excess calcium carbonate was vacuum filtered off, the ethanol removed (rotary evaporator) keeping the temperature below 50°C and the residue taken up in ether (1300ml). (Better yields were obtained when not quite all the ethanol was removed.) The ether solution was thoroughly washed with 5% Na_2CO_3 solution (200ml), water (200ml) and saturated NaCl solution (200ml) and dried over magnesium sulphate. Thorough washing (vigorous shaking for 10min) was essential to

remove most of the sulphuric acid catalyst. Even so not all was removed as evidenced by charring of the residue of the flash distillation. Ether was removed (rotary evaporator) and the residual oil flash distilled at about 3mm pressure using a splash head and an ice cooled receiver. This removed the last traces of H_2SO_4 and gave a crude fractionation. The first fraction (in a typical reaction ca. 3g, b.p. up to 45°C at 3mm) contained ethanol and traces of lactone; the second (typically about 120g, b.p. 50-120°C at 2mm) was a mixture of lactone and the ester product, while the third fraction (about 30g, b.p. >120°C at 1mm) was a dimeric product 3-carbethoxypropyl 4-hydroxybutanoate (20).



20

The products were compared by tlc.

The combined aqueous washings were extracted with ether (1 ℓ) in a continuous liquid-liquid extractor for at least 12h. The ether extract was dried over magnesium sulphate, the ether removed and the residue flash distilled as before. Typically the first fraction gave about 3g, the second about 90g and the third about 4g.

The second fractions from each of the flash distillations were combined and carefully fractionated using an 8cm Vigreux column. Usually this gave five fractions:

			Typical Yields
(a)	b.p. 33-40°C, 0.3mm;	lactone and traces of ester	100g
(b)	b.p. 40-48°C, 0.2mm;	lactone and ester	38g
(c)	b.p. 48-51°C, 0.2mm;	ester and traces of lactone	10g
(d)	b.p. 51-52°C, 0.2mm;	pure ester	60g
	Residue (not distilled);	dimer (20)	2g
			210g

Refractionation of (b) gave lactone (28g) and ester (10g) while (c) gave lactone (1g) and ester (9g).

Hence, a typical reaction yielded ethyl 4-hydroxybutanoate (15) (79g, 20% overall yield ignoring recovered lactone; 42% if this is considered), recovered 4-hydroxybutanoic acid lactone (135g) and crude dimer (20) (36g). Further refractionation of the crude dimer generally gave a further 12g each of ester, lactone and pure dimer.

Ethyl 4-hydroxybutanoate (15)

Found C, 54.51; H, 9.56%

$C_6H_{12}O_3$ requires C, 54.53; H, 9.15%

IR ν_{max} ($CHCl_3$) 3470 (O-H), 2960 (C-H), 1735 (C=O), 1250, 1160, 1060cm^{-1} (C-O, ester and hydroxyl groups).

NMR $\delta(CDCl_3)$ 1.23 (t, 3, J=7Hz, ester group methyl protons), 1.87 (m, 2, C_3 methylene protons), 2.33 (m, 2, C_2 methylene protons), 3,57 (m, 2, C_4 methylene protons becomes t, 2, J=6Hz with D_2O exchange), 3.80 (t, 1, J=6Hz OH proton exchanged with D_2O), 4.08 ppm (q, 2, J=7Hz, Ester group methylene protons).

3-Carbethoxypropyl 4-hydroxybutanoate (20)

Found C, 55.30; H, 8.24%

$C_{10}H_{18}O_5$ requires C, 55.03; H, 8.31%

IR as for ethyl 4-hydroxybutanoate (15)

NMR $\delta(CDCl_3)$ 1.23 (t, 3, J=7Hz, ester group methyl protons), 1.85 (m, 4, 2×C_3 methylene protons), 2.32 (m, 4, 2×C_2 methylene protons), 3.57 (t, 2, C_4 methylene protons), 3.70 (s, 1, OH proton exchanged with D_2O), 4.08ppm (q, 4, ester group methylene protons).

3.3.2. Ethyl 3-formylpropanoate (16) (Method 1)

This compound was most efficiently prepared by the partial oxidation of ethyl 4-hydroxybutanoate (15). Two literature methods[51,52] also investigated are discussed in Sections 3.4 and 3.5. Only one of these was successful but proved less satisfactory than the new method discussed in this section. Another alternative method of preparing aldehydes (the "Oxo" reaction)[53] by high temperature (90-200°C) and high pressure (125-200 atm) addition of carbon monoxide to alkenes using cobalt catalysts was considered beyond the capabilities of our laboratory. The oxidation of alcohols was usually accomplished by the use of chromium (VI), in the form of chromium trioxide or dichromate salts in acid solution. The preparation of the complex, dipyridine-chromium (VI) oxide[54], allows the oxidation of alcohols in basic medium[55]. Originally the complex was prepared in an excess of pyridine, isolated and then reacted in pyridine or acetone solutions[56]. The complex was found[57] to be soluble in polar chlorocarbons, dichloromethane being the most suitable. Oxidations carried out in this solvent gave higher yields because isolation of the products was simplified[57]. The tendancy of the complex to ignite during preparation, and its extremely hygroscopic nature were the disadvantages in preparing a pure sample. The refinement[58] of preparing and reacting the complex directly in dichloromethane solution neatly overcame these problems.

More recently a complex 3,5-dimethylpyrazole-chromium (VI) oxide has been prepared and a series of oxidations carried out[59]. This complex was prepared and reacted in dichloromethane solution, in the same way as for the pyridine complex.

For the oxidation of ethyl 4-hydroxybutanoate (15) to ethyl 3-formylpropanoate (16), the 3,5-dimethylpyrazole-chromium (VI) oxide appeared initially to have two advantages:

(1) Less reagent is required. A 2.5:1 mole ratio of complex to substrate is sufficient whereas the pyridine complex is used with a 6:1 mole ratio.

(2) The pyrazole complex is more soluble in dichloromethane allowing more concentrated solutions to be used.

These advantages become obvious when, to oxidise 0.1 mole of alcohol the 3,5-dimethylpyrazole complex requires only 250ml dichloromethane while the pyridine complex needs 1500ml.

However, it was found experimentally that the increased solubility of the pyrazole complex resulted in difficulty in isolating the product from the complex, and yields were inconsistent and low ranging from 0 to 20%. Use of the pyridine complex, although more laborious, gave a consistent 60% yield. A discussion of both methods follows.

Oxidation with dipyridine-chromium (VI) oxide complex.

Analytical grade chromium trioxide was stored in a vacuum desiccator over phosphorous pentoxide for at least two days before use. Anhydrous pyridine was prepared by distillation of reagent grade material from barium oxide and was stored over 4A molecular seives. Reagent grade dichloromethane was purified by shaking with concentrated sulphuric acid, 5% NaOH solution, water and saturated NaCl solution. After drying with calcium chloride, the dichloromethane was distilled and stored over 4A molecular seives. Solvent recovered from reaction solutions needed only to be dried and distilled before reuse.

In a typical oxidation, a mechanically stirred solution of pyridine (94.9g, 1.2mol) in dichloromethane (1500ml) in a flask protected by a calcium chloride drying tube was cooled (ice-bath) to 15°C. Chromium trioxide (60g, 0.6mol) was added and immediately a fine yellow suspension was formed. This dissolved to give the deep burgundy coloured solution

of the complex; the temperature rose to 30°C. Stirring was continued for 15min; the ice-bath was removed when the temperature had returned to 20°C. At the end of this period, ethyl 4-hydroxybutanoate (15) (13.2g, 0.1mol) in dichloromethane (50ml) was added in one portion. A tarry black deposit separated immediately. After stirring for a further 15min at 20°C, the solution was decanted from the residue which was washed with ether (1ℓ). (This residue was best removed from glassware by washing with strong NaOH solution.) The dichloromethane solution was concentrated (rotary evaporator) and the residue shaken with the ether washings. The resulting brown ether solution, which sometimes required filtering to remove solid residue, was washed with 5% $NaHCO_3$ solution (250ml), 5% hydrochloric acid (250ml), 5% $NaHCO_3$ solution (200ml) and saturated NaCl solution (100ml). The now colourless ether solution was dried ($MgSO_4$) and concentrated (rotary evaporator) to give an oil (generally about 6g) which was mainly (tlc) ethyl 3-formylpropanoate (16).

The washing solutions were combined, the resulting solution adjusted to pH3 and extracted with ether (500ml) on a continuous liquid-liquid extractor (5h was sufficient to extract all the product). After drying ($MgSO_4$) the ether solution was concentrated to give more oil as before (generally about 8g).

The crude products were combined and stored in ether until 12 reactions had been done, when the total product was vacuum distilled. This yielded pure ethyl 3-formylpropanoate (16) (94g, 60%) b.p. 34°C, 0.5mm (Lit[52] 84-85°C, 12mm).

Found C, 55.00; H, 7.31%

$C_6H_{10}O_3$ requires C, 55.37; H, 7.75%

IR ν_{max} (Thin film) 3000, 2870, 2760 (C-H); 1735 (C=O, aldehyde and ester groups); 1200, 1035 cm^{-1} (C-O).

NMR $\delta(CDCl_3)$ 1.23 (t, 3, J=7HZ, ester group methyl protons), 2.65 (m, 4, C_2, C_3 methylene protons), 4.09 (q, 2, J=7Hz, ester group methylene protons), 9.7ppm (s, 1, aldehyde proton).

Oxidation with 3,5-dimethylpyrazole-chromium (VI) oxide

3,5-Dimethylpyrazole was prepared from acetylacetone and hydrazine (ref.37, p.842). The chromium trioxide and dichloromethane used were treated as described for the pyridine complex.

3,5-Dimethylpyrazole (24g, 0.25mol) was added to a suspension of chromium trioxide (25g, 0.25mol) in dichloromethane (250ml) and the mixture stirred at 20°C under nitrogen for 15min, yielding a dark red solution. Ethyl 4-hydroxybutanoate (15) (13.2g, 0.1mol) in dichloromethane (50ml) was then added over a period of 30min. After stirring for a further 30min the dichloromethane was removed (rotary evaporator) and the residue subjected to one of two treatments[59].

(1) Extraction with pentane followed by filtration through a short silica gel column. Generally it was found that pentane did not extract the product from the residue. Although easily extracted with ether, the product could not be separated from the complex by filtration through silica gel.

(2) Extraction with ether; the resulting ether solution was stirred over finely powdered $NaHSO_4$ (prepared by precipitating the salt from an aqueous solution by the addition of an excess of acetone).

The brown ether solution was stirred over $NaHSO_4$ usually for 12h by which time decolourisation, if successful had occurred. Variable results were obtained experimentally. The 3,5-dimethylpyrazole complex is possibly very sensitive to the presence of traces of water as decolourisation of the ether solution was not always possible. The ether solution, when decolourisation had occurred, was concentrated to give an oil (usually about 7g) which was a mixture (tlc) of ethyl 3-formylpropanoate (16), a second (more polar) component and traces of 3,5-dimethylpyrazole. The combined product (42.2g) of 6 such reactions was vacuum distilled to give ethyl 3-formylpropanoate (16) (8.6g, 11%) b.p. 32-35°C, 0.5mm, contaminated with the second component. This appeared (tlc) to be 4-hydroxybutanoic acid lactone and could not be removed by vacuum distillation.

When decolourisation did not occur, the ether solution was flash distilled at 0.5mm until crystals of 3,5-dimethylpyrazole formed in the condenser. An ethereal solution of the distillate was then washed with dilute hydrochloric acid, $NaHCO_3$ solution and saturated NaCl solution. The combined ether solutions of three such reactions were concentrated to give an oil (7g) which was identical (tlc) to that obtained from reactions where decolourisation occurred.

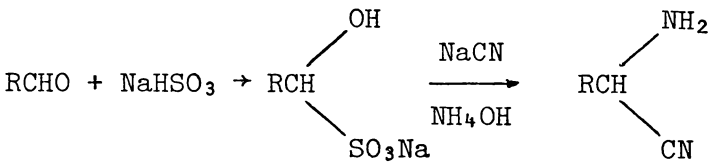
However, because of the difficulties in handling the 3,5-dimethylpyrazole-chromium (VI) oxide complex and the generally low yields obtained this method was abandoned in favour of the pyridine complex.

3.3.3. 5-Cyano-2-oxopyrrolidine (17)

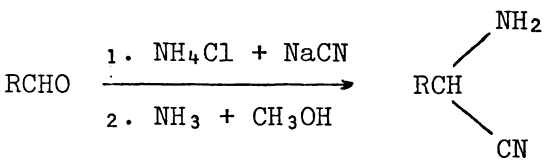
The first step of the Strecker[60] synthesis for α-amino acids is α-aminonitrile formation,

e.g. $RCHO + NH_3 + HCN \rightarrow R\text{-}\underset{\displaystyle NH_2}{\underset{|}{CH}}\text{-}C{\equiv}N \rightarrow R\underset{\displaystyle NH_2}{\underset{|}{CH}}COOH$

Two standard procedures based on this synthesis were investigated in attempts to prepare the α-aminonitrile (14) (Scheme 3). These were the methods of Knoevenagel[61] (equation 3.1) and Tiemann[62] (equation 3.2).



3.1



3.2

The Knoevenagel[61] reaction was carried out on both methyl 3-formylpropanoate (13) and ethyl 3-formylpropanoate (16), but in both cases produced dimeric products (Section 3.6).

5-Cyano-2-oxopyrrolidine (17), prepared unexpectedly by the Tiemann[62] reaction on ethyl 3-formylpropanoate (16) has also been prepared by a similar reaction from ethyl 4,4-dimethoxybutanoate[63] and from methyl 4-cyano-4-hydroxybutanoate[64].

Ethyl 3-formylpropanoate (16) (20g, 0.154mol) in ether (50ml) was added to a cooled (5°C) solution of ammonium chloride (10.17g, 0.19mol) in water (50ml). A solution of sodium cyanide (8.04g, 0.164mol) in water (35ml) was added so that the temperature did not exceed 10°C (15min) The mixture was stirred for 3h at 5°C, then extracted with ether on a continuous liquid-liquid extractor (12h). The ether extract was concentrated to give a red oil (23g), which was taken up in methanol (200ml) and saturated with ammonia at 0°C. The flask was sealed and allowed to stand for 2 days at 20°C. The excess ammonia was expelled by a rapid current of air and the solution concentrated to a red oil. Purification was achieved by filtering a chloroform solution of this oil

through a short column of silica gel. The resulting solid was recrystallised from dichloromethane to give 5-cyano-2-oxopyrrolidine (17) (8.1g, 48%) m.p. 99°C (Lit[63] 92-93°C)

Found C, 54.33; H, 5.50; N, 25.70%

$C_5H_6N_2O$ requires C, 54.54; H, 5.49; N, 25.44%

IR $\nu_{max}(CHCl_3)$ 3420, 3220 (N-H); 3020(C-H), 2240 (very weak CN), 1710 (C=O), 1225 cm^{-1} (complex amide III band).

NMR $\delta(CDCl_3)$ 2.47 (m, 4, C_3 and C_4 methylene protons), 4.50 ppm (m, 1, C_5 methyne proton).

3.3.4. 5-(Aminomethyl)-2-oxopyrrolidine hydrochloride (18)

Catalytic hydrogenation[49] of 5-cyano-2-oxopyrrolidine (17) over platinum dioxide in ethanolic hydrogen chloride gave 5-(aminomethyl)-2-oxopyrrolidine hydrochloride (18) (Scheme 4).

A solution of (17) (5g, 0.046mol) in superdry ethanol (ref.3, p.167) (140ml) in a Paar Hydrogenator bottle was cooled below 10°C and 18.5% alcoholic hydrogen chloride (40ml, 0.2mol) added slowly. Platinum dioxide (0.2g) was added and the mixture hydrogenated at 60 psi (414 kPa) for 8h. A quantitative uptake of hydrogen was observed (6 psi (41kPa) pressure drop). The solution was filtered and concentrated to give an oil (6g). Treatment with absolute ethanol yielded a white solid (1.8g). The residue of the reaction, which consisted of product and catalyst, was washed with water and the resulting solution concentrated to give further solid (3.24g). Recrystallisation of the combined product from ethanol/water gave 5-(aminomethyl)-2-oxopyrrolidine hydrochloride (18) (4.2g, 60%) m.p. 176-177°C.

Found C, 39.72; H, 7.46; N, 18.85; Cl, 23.24%

$C_5H_{11}N_2OCl$ requires C, 39.87; H, 7.36; N, 18.60; Cl, 23.54%

IR ν_{max} (KBr disc) 3220 (lactam N-H), 3000, 2900 (N-H of NH_3^+ and C-H), 1690 (C=O), 1618 (N-H), 1260 cm^{-1} (C-N).

NMR $\delta(D_2O)$ 2.90 (m, 4, C_3 and C_4 methylene protons), 3.67 (d, 2, J=5Hz, $CH_2NH_3^+$ methylene protons), 4.60 ppm (m, 1, C_5 methyne proton).

Although not shown by the analytical figures, tlc of this compound showed traces of ammonium chloride. The acidic medium required for successful hydrogenation of α-aminonitriles[49] was apparently causing slight decomposition. Such ammonium chloride impurities also appeared in samples of methyl 4,5-diaminopentanoate dihydrochloride (1a) (Section 3.3.5) and were difficult to remove.

3.3.5. Methyl 4,5-diaminopentanoate dihydrochloride

The general esterification technique previously described (Section 2.1) was used to prepare Me 4,5-dape.2HCl (1a) from the lactam (18) (Scheme 4).

The lactam (18) (5g) in absolute methanol (200ml) (ref.37, p.169) was treated with dry HCl gas for 2h. Concentration (rotary evaporator) of the reaction mixture yielded an oil which solidified only after prolonged drying under vacuum over phosphorous pentoxide. A small portion of this extremely hygroscopic material was retained for seed crystals, the remainder being taken up in the minimum of methanol. Seeding produced non hygroscopic plate crystals of methyl 4,5-diaminopentanoate dihydrochloride (1a) (6.6g, 91%) m.p. 146-147°C.

Found C, 32.98; H, 7.19; N, 12.77; Cl, 32.14%

$C_6H_{16}N_2O_2Cl_2$ requires C, 32.89; H, 7.36; N, 12.79; Cl, 32.36%

IR ν_{max} (KBr disc) 3700-2200 (broad N-H of NH_3^+ and C-H), 1950 (broad NH_3^+), 1720 (C=O), 1575, 1520 (NH_3^+) 1210, 1030cm^{-1}(C-O).

NMR $\delta(D_2O)$ 2.66 (m, 2, C_2 methylene protons), 3.16 (m, 2, C_3 methylene protons), 3.87 (m, 2, C_5 methylene protons), 4.22 ppm (m, 4, C_4 methyne and ester group methyl protons).

Traces of ammonium chloride were also apparent in tlc of Me 4,5-dape.2HCl. Titration of the ester in the presence of 0.5mole% Cu(II)/mole of ester (Section 5.2.1) indicated 98% purity. Repeated recrystallisation (5 times) from methanol/ether removed this impurity (titrimetrically 100% pure).

3.3.6. 4,5-Diaminopentanoic acid dihydrochloride (19)

This compound was prepared by acid hydrolysis of 5-(aminomethyl)-2-oxopyrrolidine hydrochloride (18).

The lactam (18) (12g) was refluxed in constant boiling point hydrochloric acid (40ml) for 2h. Concentration of the reaction mixture gave an oil from which crystals were obtained following treatment with 15% alcoholic hydrogen chloride. The solid (4g) was filtered off. The mother liquors were concentrated then refluxed again in hydrochloric acid, yielding further crystals (2.7g). Recrystallisation from methanol/ether was found to be unsatisfactory because lactamisation occurred. Hence, recrystallisation was carried out at pH<1 to keep the carboxylate group in protonated form (pKa (COOH) = 3.24, Section 4.3). However, even though recrystallisation was carried out in 15% alcoholic hydrogen chloride (pH<<1) traces of the lactam (18) were always formed. The resulting product, 4,5-diaminopentanoic acid dihydrochloride (19) (6.7g, 60%) m.p. 175-176°C, was later shown to be usually about 98% pure (Sections

4.3, 5.2.1). The impurity, lactam (18), could not be removed by recrystallisation.

Found C, 29.30; H, 6.97; N, 13.29; Cl, 34.23%

$C_5H_{14}N_2O_2Cl_2$ requires C, 29.28; H, 6.88; N, 13.66; Cl, 34.57%

IR ν_{max} (KBr disc) 3600-2300 (NH_3^+, COOH and C-H), 1705 (C=O), 1600-1500 (4 bands, NH_3^+), 1410, 1205cm^{-1} (COOH).

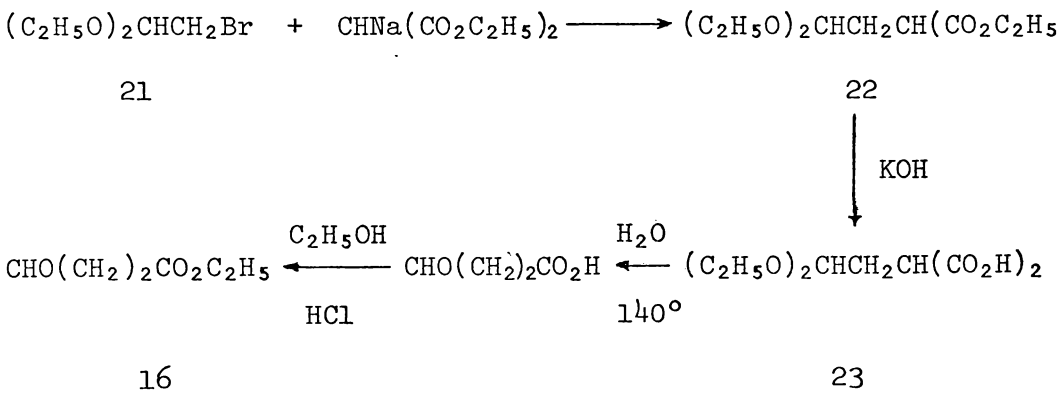
NMR $\delta(D_2O)$ 2,56 (m, 2, C_2 methylene protons), 3.10 (m, 2, C_3 methylene protons), 3.85 (m, 2, C_5 methylene protons) 4.20 ppm (m, 1, C_4 methyne proton).

3.4 Ethyl 3-formylpropanoate (Method 2)

(See also Sections 3.3.2 and 3.5)

This method (Scheme 5) for ethyl 3-formylpropanoate (16) was developed by Perkin and Sprankling[51]. The second step (the hydrolysis of diethyl acetalmalonate (22) to acetal malonic acid (23) is difficult; it was found[51] that if hydrolysis with alcoholic KOH is prolonged then elimination of the group $CH_2CH(OC_2H_5)_2$ occurs and malonic acid is formed. In the present work attempts to purify diethyl acetalmalonate by vacuum distillation resulted in decomposition and the recovery of diethylmalonate.

Scheme 5: Synthesis of Ethyl 3-formylpropanoate (16) (Method 2)[51]



3.4.1 Bromoacetal (21)

Bromoacetal was prepared as outlined in "Organic Syntheses" (ref.65). Yield 51%, b.p. 79°C, 34mm.

3.4.2 Diethyl acetalmalonate (22)[51]

Sodium (3.5g) was reacted with "superdry" ethanol (100ml) (ref.37, p.167). The resulting solution of sodium ethoxide was cooled to 30°C and freshly distilled diethyl malonate (24.8g) added slowly with swirling. Bromoacetal (38.8g) was added dropwise over 15min with occasional swirling.

The now red solution was refluxed for 5.5h after which time some white solid had formed. The excess ethanol was removed (rotary evaporator) and water (100ml) added. The white solid dissolved and a dense red oil separated out as a bottom layer. The mixture was extracted with ether, the ether extracts washed with water and dried ($MgSO_4$). The ether was removed (rotary evaporator) and the residue vacuum distilled. The largest fraction (30ml) (66-145°C, 16mm) was shown (tlc) to be unreacted diethylmalonate and bromoacetal. A second fraction (10ml) (145-146°C, 16mm) appeared (tlc) to have some diethylmalonate present but was mostly the desired product. The unchanged reactants were recycled three times through the process to react most of the bromoacetal. This gave 27ml of crude product with the following NMR data:

$\delta(CDCl_3)$ 1.2 (q, 12, 4×methyl groups), 2.07 (m, 2, C_2 protons), 3.5 (m, 5, C_1 and C_3 protons), 4.1 ppm (m, 8, 4×methylene groups).

An attempt to fractionate this product by vacuum distillation resulted in decomposition and recovery of diethyl malonate. At this point this method was abandoned in favour of the more direct method 1 (Section 3.3.2).

3.5 Ethyl 3-formylpropanoate (Method 3)

(See also Sections 3.3.2 and 3.4)

Diethyl formylsuccinate (24), prepared by condensation of diethyl succinate and ethyl formate, can be hydrolysed to give 3-formylpropanoic acid (25) (Scheme 6)[52]. Although treatment with ethanol and hydrogen chloride gives the desired ester, ethyl 3-formylpropanoate (16) (Scheme 6), a side reaction also occurs giving the acetal (26)

$(CH_3CH_2O)_2CHCH_2CH_2CO_2H$

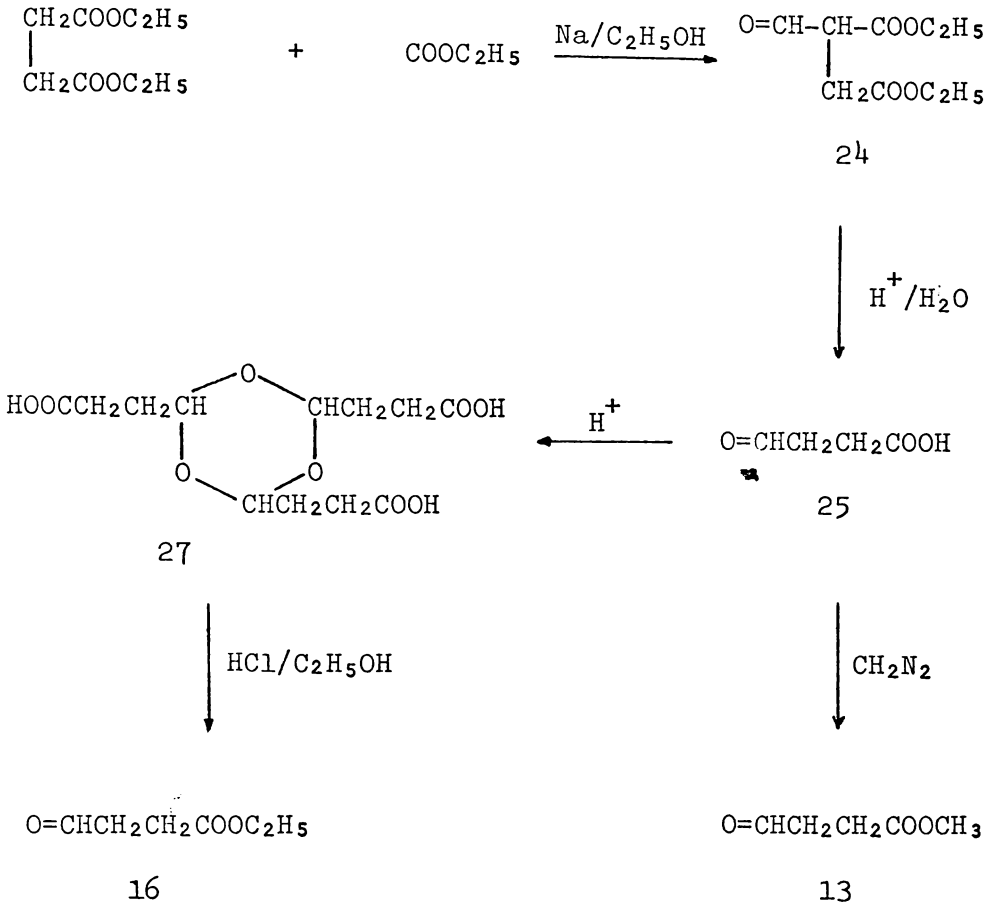
26

The free 3-formylpropanoic acid (25) spontaneously trimerises to give the solid (27), a process catalysed by added mineral acid. It was found[52] that esterification of the trimer (27) gave only ethyl 3-formylpropanoate (16) and none of the acetal (26). Clearly the trimer (27) is stable under the esterification conditions, preventing acetal formation, but decomposes during distillation to give the aldehyde. The four steps of this method (Scheme 6) are discussed in the following sections.

3.5.1 Diethyl formylsuccinate (24)

Sodium (40.5g, 1.76 mol) was finely granulated by melting in boiling toluene, followed by violent agitation while cooling. The toluene was then decanted off and the resulting sodium "sand" suspended in dry ether (850ml). The flask was cooled in ice and "superdry" ethanol (81g, 1.76 mol) (ref.37, p.167) added with stirring. Stirring and cooling were continued while a mixture of diethylsuccinate (255g, 1.47mol) and ethyl formate (130g, 1.76mol) was added over 0.5h. The reaction mixture, which had become orange, was stirred for 2.5days at 5°C, during which time a large amount of white solid was precipitated. The suspension was poured into a mixture of concentrated sulphuric acid (173g) and ice (400g),

Scheme 6: Synthesis of Ethyl 3-formylpropanoate (16) (Method 3)[52]



the ether layer separated off and the aqueous layer extracted with ether (3×400ml). The ether solutions were combined and washed with 5% $NaHCO_3$ solution (500ml) and saturated NaCl solution (500ml). After drying over $MgSO_4$ the ether was removed (rotary evaporator) and the residue flash distilled at 0.5mm. The resulting distillate (252 .2g) was fractionated under reduced pressure using a 120mm Vigreux column. Three fractions were collected. The first fraction (60g) (59-70°C, 0.6mm) was shown (tlc) to contain a large amount of unreacted diethyl succinate. The second fraction (74.7g) (70-73°C, o.5mm) contained a small amount of diethyl succinate while the final fraction (94.8g) (73°C, 0.5mm) was pure diethyl formylsuccinate (24). Several refractionations of the mixtures gave further pure (24) (61.6g). Diethyl formylsuccinate (156.4g, 53%)

prepared by this method was a colourless liquid b.p. 73°C, 0.5mm (Lit[44] 114-9°C, 5mm, Aldo form).

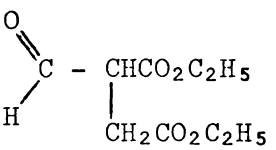
Found C, 53.12; H, 7.09%

$C_9H_{14}O_5$ requires C, 53.46; H, 6.98%

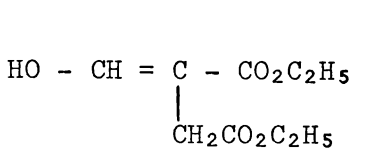
IR ν_{max} (thin film) 3300 (O-H enol group), 3000 (C-H), 1735 (C=O aldehyde and ester groups), 1680 (C=C enol form), 1170 (C-O ester group), 1100, 1030cm^{-1} (O-H, C-O enol form)

NMR $\delta(CDCl_3)$ 1.23 (t, 6, J=6Hz ester group methyl protons), 3.0 (m, 2, C_3 methylene protons), 3.9 (m, 1, C_2 proton), 4.2 (q, 4, ester group methylene protons), 6.92, 7.13 (2s, 1, enol proton, possibly cis and trans forms, collapses to 1 with D_2O exchange), 10.66ppm (s, 1, aldehyde proton.

Carrière[52] reports two boiling points for diethyl formylsuccinate, 128-135°C at 15mm and 142-148°C at 15mm. Diethyl formylsuccinate can occur in both aldo (24a) and enol (24b) forms



24a



24b

The lower boiling point corresponds to a mixture containing 30% enol form while the higher value corresponds to a mixture containing 60% enol form[52]. In the present work, although only one boiling point was observed the spectral data suggest that both forms are present in the resulting sample.

3.5.2 3-Formylpropanoic Acid (25)[52]

Diethyl formyl succinate (24) (100g, 0.5mol) was added to a gently refluxing solution of oxalic acid (15g, 0.12mol) in water (500ml).

Every 0.5h during the reaction alcohol which was formed from the reaction

$$\begin{array}{l} OCH\text{-}CHCO_2C_2H_5 \\ \quad\quad\quad | \\ \quad\quad CH_2CO_2C_2H_5 \end{array} + 2H_2O \longrightarrow OCHCH_2CH_2CO_2H + 2C_2H_5OH + CO_2$$

was distilled off for 10min. This greatly increases the rate of reaction[52] which was complete after 6h. Calcium acetate (30g) in water (100ml) was added to precipitate the oxalic acid as calcium oxalate. This was removed by centrifuging and the solution concentrated (rotary evaporator)to remove water and acetic acid. The residue was taken up in water and the solution extracted with ether on a liquid-liquid extractor overnight. The resulting ethereal solution was dried ($MgSO_4$) and evaporated, leaving 43g of residue. This was vacuum distilled to give 3-formylpropanoic acid (25) (15.6g, 31%) b.p. 89-90°C, 0.5mm (Lit[52] 142-143°C, 15mm). The crude product may be methylated directly with diazomethane but it was usually distilled and then polymerised to the trimer (27).

3.5.3 Polymerisation of 3-formylpropanoic acid

Sulphuric acid (0.2g) was added to 3-formylpropanoic acid (16g). The reaction mixture became solid within 1h. Recrystallisation from water yielded white needles of the trimer 2,4,6-tri(2'-carboxyethyl) trioxane (27) (7g, 44%) m.p. 155-156 (Lit[52] 167°C). A further 9.6g impure solid were obtained by concentrating the filtrate.

Found C, 47.04; H, 5.92%

$C_{12}H_{18}O_9$ requires C, 47.06; H, 5.92%

IR ν_{max} (KBr disc) 2900 (broad COOH and C-H), 1700, 1420, 1250, 920 (COOH), 1120, 1160cm^{-1}(C-O).

NMR $\delta(D_2O)$ 2.45, 2.93 (m, 6, C_2, C_3, methylene protons), 5.62ppm (t, 3, C_2, C_4, C_6 methyne protons).

3.5.4 Methyl 3-formylpropanoate (13)

(13) can be prepared directly from crude 3-formylpropanoic acid by methylation with diazomethane (Scheme 6). An ethereal solution of diazomethane (~22g, 0.5mol in 300ml ether) was prepared from N-nitrosomethylurea (80g) (ref.37, p.969). This solution was added in small portions to a cooled solution of crude 3-formylpropanoic acid (46g, 0.45mol) in ether (100ml) until an excess of diazomethane was present (ref.37, p.969) (ca 90% of the diazomethane solution was required). The ether was distilled off and the residue vacuum distilled to give methyl 3-formylpropanoate (13) (23.3g, 40%) b.p. 34°C, 0.65mm (Lit[47] 69-71°C, 15mm).

Found C, 51.41; H, 6.86%

$C_5H_8O_3$ requires C, 51.72; H, 6.94%

IR ν_{max}(thin film) 2975, 2850, 2725 (C-H), 1735 (C=O) 1200cm^{-1} (C-O).

NMR $\delta(CDCl_3)$ 2.70 (m, 4, C_2, C_3 methylene protons), 3.63 (s, 3, ester group methyl protons), 9.72ppm (s, 1, aldehyde proton).

3.5.5 Ethyl 3-formylpropanoate (16)

The trimer (27) (16.6g) was stirred for 44h in "superdry" ethanol (30g) (ref.37, p.167) containing 4% hydrogen chloride. The reaction mixture was poured into iced water (100ml) and the lower phase (crude

ester) separated off. The aqueous phase was extracted with ether (3×50ml), and these extracts combined with the ester phase. The ethereal solution was washed with 5% sodium bicarbonate solution (50ml) and water (50ml), dried over magnesium sulphate and concentrated (rotary evaporator). Vacuum distillation of the residue yielded a clear liquid (18g, b.p. 79-85°C, 10mm) which was shown (tlc) to be a mixture of ethyl 3-formylpropanoate and a second compound. Further vacuum fractionation using a 200mm Vigreux column failed to separate these two components. The fraction (b.p. 103-105°C at 8.5mm) was apparently (tlc) rich in the second compound; it had an NMR spectrum consistent with the acetal (26). A one proton triplet at δ = 4.50ppm and a four proton multiplet at δ = 3.55ppm were assigned to the acetal (26), C_4 proton and ethyl group methylene protons respectively. Similar chemical shifts of δ = 4.50ppm and δ = 3.22ppm respectively are observed for the equivalent protons of diethyl acetal $CH_3CH(OCH_2CH_3)_2$. The remaining peaks in the spectrum are assigned to both ethyl 3-formylpropanoate (16) and the acetal (26).

NMR $\delta(CDCl_3)$ 1.15 (2t, 9, ester and acetal group methyl protons), 1.90 (m, 2, C_3 methylene protons), 2.28 (m, 2, C_2 methylene protons), 3.55 (m, 4, acetal group methylene protons), 4.07 (q, 2, ester group methylene protons), 4.50 (t, 1, C_4 acetal proton), 10.1ppm (s, 1, C_4 aldehyde proton).

Carrière[52] was able to separate ethyl 3-formylpropanoate (16) and the acetal (26) by fractional vacuum distillation and quotes boiling points 84-85°C at 12mm and 101-102°C at 12mm respectively. In the present work complete separation could not be achieved.

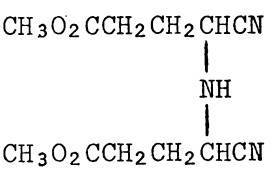
Although a pure sample of methyl 3-formylpropanoate (13) was obtained the overall yield was only 6% which does not compare favourably with method 1 (section 3.3.2), for ethyl 3-formylpropanoate (16) (overall yield 25%).

3.6 Attempted Synthesis of α-aminonitriles by the Knoevenagel[61] reaction

In attempts to prepare the α-aminonitrile (14) (Scheme 3), the Knoevenagel[61] reaction (see Section 3.3.3) was carried out on both methyl and ethyl 3-formylpropanoate. The reactions are discussed in the following sections.

3.6.1 Knoevenagel[61] Reaction on Methyl 3-formylpropanoate

Methyl 3-formylpropanoate (45g, 0.39mol) was added to a stirred solution of sodium metabisulphite (36.9g, 0.20mol) in water (65ml), the temperature rising to 50°C. Ammonia solution (35ml, 0.890s.g.) was added and the mixture stirred at 55-60°C for 1h. The solution was cooled to 6°C and a solution of sodium cyanide (19g, 0.39mol) in water (60ml) added dropwise so that the temperature remained below 10°C. Stirring was continued for an additional 0.5h at 6°C and then 1h at 30°C. Water (30ml) was then added and the resulting solution extracted with ether (2×250ml). The dried ($MgSO_4$) ether solution was concentrated to give an oil (5.5g). which on treatment with ether yielded a white crystalline solid. Recrystallisation from ether gave a compound whose NMR and mass spectra are consistent with di(3-carbomethoxy-1-cyanopropyl)amine (28) (2g, 2%) m.p. 66°C.



28

The NMR spectrum is what is expected for the carbon skeleton $CH_3O_2CCH_2CH_2CH$ A very small MS molecular ion peak at $^m/e$ 267, consistent with structure (28), suggests a dimeric structure. A base beak of $^m/e$ 27 (HCN) and peaks at $^m/e$ 240 (loss of HCN) and $^m/e$ 213 (loss of 2HCN) confirm the presence

of two cyanide groups. Confirmation of two ester groups comes from peaks at $^{m}/e$ 276 (loss of CH_3O), $^{m}/e$ 204 (loss of two CH_3O from M^+-1) and $^{m}/e$ 149 (loss of $2CH_3O_2C$). That the molecule is dimeric through the amine function is suggested by the peaks $^{m}/e$ 141 ($[CH_3O_2CCH_2CH_2CH(CN)NH]^{+\bullet}$) and $^{m}/e$ 154 ($[CH_3O_2CCH_2CH_2CH(CN)NHCH]^{+\bullet}$).

Found C, 54.20; H, 6.60; N, 15.57%

$C_{12}H_{17}N_3O_4$ requires C, 53.92; H, 6.41; N, 15.72%

MS $^{m}/e$ 267 ($M^{+\bullet}$), 240(<1%), 236(<1%), 213 (14%), 204(23%), 182(48%), 177(38%), 154(76%), 149(72%), 135(59%), 122(66%) 94(62%), 74(41%), 27(100%).

IR ν_{max} ($CHCl_3$) 3320 (NH), 3020, 2950, 2840 (C-H), 1730 (C=O), 1435, 1370 (CH), 1250-1130cm^{-1} (5 bands C-O and C-N).

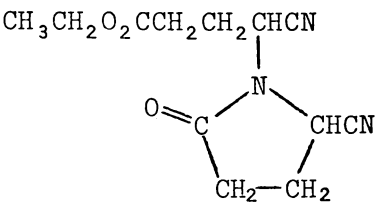
NMR $\delta(CDCl_3)$ 2.13 (m, 4, 2×C_3methylene protons), 2.50 (m, 4, 2×C_2 methylene protons), 3.70 (s, 6, ester group methyl protons) 3.83 ppm (m, 2×C_1methyne protons).

3.6.2 Knoevenagel[61] Reaction on Ethyl 3-formylpropanoate

Ethyl 3-formylpropanoate (26g, 0.2mol) was reacted with sodium metabisulphite (21g, 0.2mol), ammonia (12.15g, 0.890s.g.) and sodium cyanide (9.8g, 0.2mol) in the same manner as described for methyl 3-formylpropanoate (Section 3.6.1). The final reaction mixture was extracted with ether on a continuous liquid-liquid extracter for 12h. The extract was dried ($MgSO_4$) and concentrated to give a yellow oil (14g) containing four major components (tlc). A small portion (1.5g) of this oil was separated into its components by preparative layer chromatography. The compounds, listed as bands in decreasing order of polarity were identified as follows:

Band 1: 5-cyano-2-oxopyrrolidine (17) (0.14g, 14%) having identical tlc and NMR to an authentic sample (Section 3.3.3).

Band 2: Tentatively identified as N-3'-carbethoxy-1'-cyanopropyl-5-cyano-2-oxopyrrolidine (29) (0.33g, 33%).



29

The mass spectrum shows a molecular ion ($^{m}/e$ 249) consistent with this structure. Other peaks, $^{m}/e$ 222 (loss of HCN), $^{m}/e$ 203 (loss of C_2H_5OH), $^{m}/e$ 176 (loss of $C_2H_5O_2C$) and $^{m}/e$ 148 (loss of $C_2H_5O_2CCH_2CH_2$) suggest a partial structure $C_2H_5O_2CCH_2CH_2CH(CN)$-. A peak at $^{m}/e$ 110 corresponds to 5-cyano-2-oxopyrrolidine. The analysis figures and NMR data also fit this assignment best.

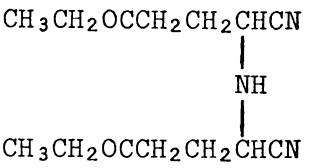
Found	C, 58.01;	H, 6.47;	N, 15.57%
$C_{12}H_{15}N_3O_4$ requires	C, 57.82;	H, 6.07;	N, 16.86%

IR ν_{max} ($CHCl_3$) 3025-2855 (C-H 4 bands), 1730 (C=O), 1230, 1200 (C-O and C-N).

NMR $\delta(CDCl_3)$ 1.27 (t, 3, J=7Hz, ester group methyl protons), 2.47 (m, 6, C_2, C_3, $C_{2'}$, $C_{3'}$ (methylene protons), 4.17 (q, 2, ester group methylene protons), 4.66 (m, 1, C_5 methyne proton), 5.27 ppm (m, 1, $C_{5'}$, methyne proton)

MS 249 ($M^{+\cdot}$), 222(28%), 204(92%), 203(100%), 176(70%), 161(50%), 149(64%), 148(69%), 110(36%).

Band 3: By analogy to the compound obtained from methyl 3-formylpropanoate (Section 3.6.1) this compound was assigned the structure di(3-carbethoxy-1-cyanopropyl)amine (30) (0.39g, 39%)



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A very small molecular ion ($^{m}/e$ 295) is observed in the mass spectrum of this compound. Peaks, $^{m}/e$ 296 ($m^{+\bullet}+1$), $^{m}/e$ 297 ($m^{+\bullet}+2$), $^{m}/e$ 294 ($m^{+\bullet}-1$) and $^{m}/e$ 293 ($m^{+\bullet}-2$) are all much more intense than $m^{+\bullet}$. This phenomena is common in aliphatic cyanides[66]. A similar fragmentation to the analogous methyl ester compound (Section 3.6.1) is observed.

IR ν_{max} ($CHCl_3$) 3320 (N-H), 3020-2940 (C-H), 1730 (C=O), 1200 (C-O, C-N), 1025cm^{-1} (C-N).

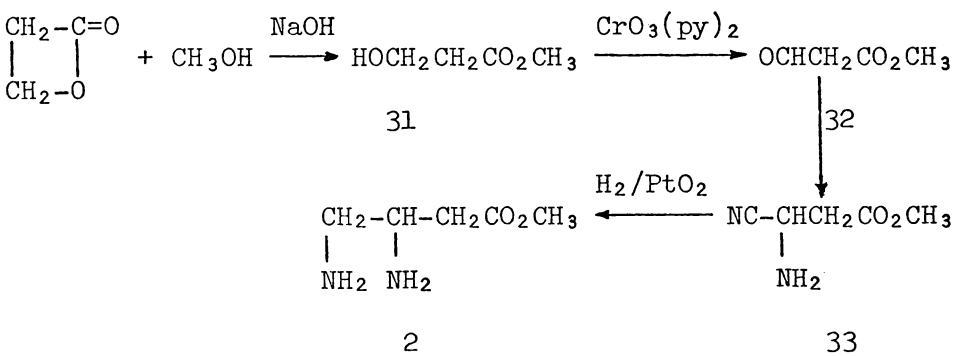
NMR δ($CDCl_3$) 1.27 (t, 6, J=7Hz ester group methyl protons), 2.13 (m, 4, 2×C_3 methylene protons), 2.50 (m, 4, 2×C_2 methylene protons), 4.10 (q, 4, J=7Hz, ester group methylene protons).

MS $^{m}/e$ 296 ($M^{+\bullet}$, $C_{14}H_{22}N_3O_4$), 268 (9%), 224 (44%), 204 (78%), 196 (44%), 195 (41%), 155 (85%), 149 (78%), 148 (70%), 109 (100%).

3.7 Attempted synthesis of 3,4-diaminobutanoic acid

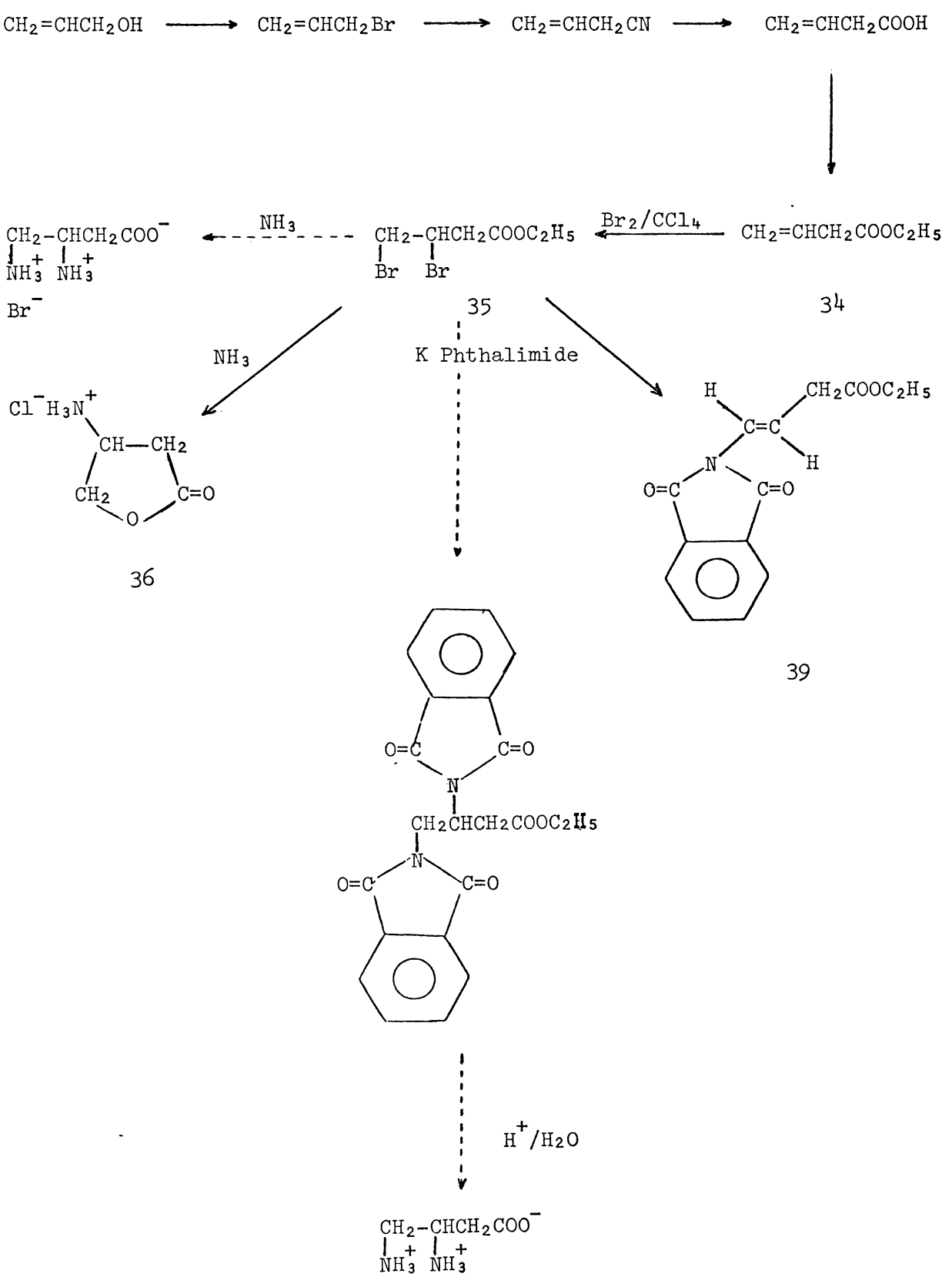
The first approach to the synthesis of 3,4-diaminobutanoic acid was to build up the required functional groups on the appropriate C-skeleton in the same way as for the synthesis of 4,5-diaminopentanoic acid (Section 3.3). It was hoped that catalytic hydrogenation of the α-aminonitrile (33) prepared from methyl 2-formylacetate (32) would yield the desired methyl 3,4-diaminobutanoate (2) (Scheme 7). Methyl 3-hydroxypropanoate (31) was readily prepared[67] from 3-hydroxypropanoic acid lactone which was available commercially (Scheme 7). It was hoped that methyl 2-formylacetate (32) could be prepared by $CrO_3(py)_2$ oxidation (Section 3.3.2) of methyl 3-hydroxpropanoate (31) (Scheme 7). However no product could be isolated from the oxidation reaction mixture. Apparently[47] both 2-formylacetic acid and its methyl or ethyl esters are extremely unstable and have never been isolated.

Scheme 7: Proposed synthesis of methyl 3,4-diaminobutanoate (2)



Because of the problems associated with high pressure NH_3 addition to dibromo compounds (Section 3.2), difficulty was expected with the second approach to 3,4-diaminobutanoic acid. Ethyl 3,4-dibromo-butanoate (35) was reacted with ammonia under pressure in sealed tubes (Scheme 8) An alternative procedure[68] tried, was the addition of potassium phthalimide to the dibromo compound (35), to be followed by hydrolysis of the

Scheme 8: Proposed synthesis of 3,4-diaminobutanoic acid (Method 2).



phthalimide groups[68] (Scheme 8). The intended reactions are indicated by dotted arrows (Scheme 8). while the reactions which occurred are shown by solid arrows. Although 3,4-diaminobutanoic acid was not obtained, it is possible that modification of the phthalimide reaction (Section 3.7.3) could lead to the desired product.

Allyl alcohol was converted through the sequence, allyl bromide, allyl cyanide, vinylacetic acid to ethyl vinylacetate (34) (Scheme 8) by the standard procedures described by Vogel[37]. The remaining reactions (Scheme 8) carried out are described in the following sections.

3.7.1 Ethyl 3,4-dibromobutanoate (35)

Bromine (28.2g, 0.176mol) was added dropwise (30min) to ethyl vinylacetate (20g, 0.176mol) in carbon tetrachloride (100ml) at -10°C. The reaction mixture was vacuum distilled to give ethyl 3,4-dibromobutanoate (35) (41.91g, 87%) b.p. 71°C at 0.6mm.

Found C, 25.98; H, 3.69; Br, 58.56%

$C_6H_{10}O_2Br_2$ requires C, 26.31; H, 3.68; Br, 58.34%

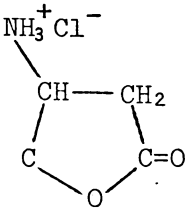
IR ν_{max} ($CHCl_3$) 2980 (C-H), 1730 (C=O), 1300, 1200, 1140 (C-O), 535cm^{-1} (C-Br).

NMR δ($CDCl_3$) 1.27 (t, 3, J=7Hz, ester group methyl protons), 3.00 (m, 2, C_2 methylene protons), 3.8 (m, 2, C_4 methylene protons), 4.17 (q, 2, J = 7Hz, ester group methylene protons), 4.5ppm (m, 1, C_3 methyne proton).

3.7.2 Reaction of ethyl 3,4-dibromobutanoate with NH_3

Ethyl 3,4-dibromobutanoate (10g) was dissolved in aqueous ammonia (30ml, s.g. 0.890) which was cooled below 0°C and saturated with ammonia gas. The resulting solution was sealed in heavy walled glass tubes and heated at 145°C for 24h. The tubes were cooled and opened and the reaction mixture concentrated to a red solid. This was dissolved in the minimum

of boiling water and refrigerated for several days. No crystallisation occurred; tlc showed a large number of products present in this solution. HCl gas was then bubbled through the solution and the solid which formed filtered off. Analysis of this compound yielded an empirical formula $C_4H_8NO_2Cl$ consistent with structures (36) (Scheme 8), (37) and (38)



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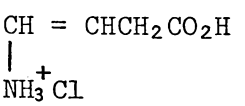

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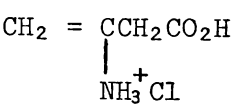

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The IR spectrum does not show a band for $\nu_{c=c}$ and the NMR (D_2O) shows three resonances. Structure (38) would show only 2 resonances and both (37) and (38) should show an IR band for $\nu_{c=c}$. The integral signal of the NMR spectrum shows that two of the complex doublets correspond to two protons and the remaining doublet to one proton, which is consistent only with structure (36). Hence the solid was identified as 3-amino-4-hydroxybutanoic acid lactone hydrochloride (36). It was obtained in 14% yield (0.75g) m.p. 270°C (decomp.).

Found C, 34.08; H, 5.76; N, 10.51; Cl, 24.68%

$C_4H_8NO_2Cl$ requires C, 34.92; H, 5.86; N, 10.18; Cl, 25.77%

IR ν_{max} (KBr disc) 3600-2200 (NH_3^+ and CH), 1740 (C=O), 1550 (NH_3^+), 1200cm^{-1}(CO).

NMR $\delta(D_2O)$ 3.35 (d, 2, J=6Hz, C_2 methylene protons) 3.83 (d, 1, J=14Hz, C_3 methyne proton), 4.28 ppm (d, 2, J=14Hz, C_4 methylene protons).

3.7.3 Reaction of ethyl 3,4-dibromobutanoate with potassium phthalimide[68]

Potassium phthalimide (14.9g, 0.08mol) was added to a stirred solution of ethyl 3,4-dibromobutanoate (35) (10g, 0.04mol) in dimethylformamide (40ml). The temperature rose to 52°C but not all the

phthalimiade dissolved. Stirring was continued for 30min; chloroform (60ml) was then added and the reaction mixture poured into water (200ml) The white solid formed was filtered off and identified (comparative tlc) as phthalimide. The chloroform layer was separated and the aqueous layer extracted with chloroform (2×20ml). The combined chloroform solutions were washed with 5% sodium hydroxide solution (100ml) and water (2×100ml), dried over $CaSO_4$ and concentrated to give a white solid (6.3g). Recrystallisation gave ethyl trans-4-phthalimido-but-3-enoate (39) (4.08g, 43%) m.p. 94-95°C. The analysis figures suggest $C_{14}H_{13}NO_4$, consistent with the structure (39) (Scheme 8). The integral signal of the NMR spectrum shows a 1:1 ratio of ethyl ester to aromatic protons suggesting only one phthalimide group. The remainder of the spectrum is an ABX pattern. A doublet (J=16Hz) at 5.85ppm splitting an adjacent proton at 6.87ppm into a doublet (J=16Hz). This second doublet is further split into triplets (J=5Hz) by two adjacent protons which form a doublet (J=5Hz) at 4.42ppm. This is consistent with the structure (39). The trans configuration is indicated by the large spin-spin coupling (J=16Hz, c.f. J=6-14Hz for cis).

Found C, 64.79; H, 5.05; N, 5.31%

$C_{14}H_{13}NO_4$ requires C, 64.86; H, 5.05; N, 5.40%

IR ν_{max} ($CHCl_3$) 3030, 2980 (C-H), 1770 (phthalimide C=O), 1715 (ester C=O), 1715 (ester C=O), 1660 (C=O) 1610, 1470, 1180, 1040, 775, 720 (σ subst. phenyl), 1275, 1115cm^{-1} (ester C-O).

NMR $\delta(CDCl_3)$ 1.25 (t, 3, J=7Hz ester methyl protons), 4.13 (q, 2, J=7Hz ester methylene protons), 4.42 (d, 2, J=5Hz, C_2 methylene protons), 5.85 (d, 1, J=16Hz, C_4 methyne proton), 6.87 (doublet of triplets, 1, J_{AB}=16Hz, J_{AX}=5Hz, C_3 methyne proton), 7.78 ppm (4 aromatic protons).

4. PROTON DISSOCIATION CONSTANTS

4.1 Introduction

The thermodynamic proton dissociation constants K_a^T's, of 4,5-diaminopentanoic acid dihydrochloride (4,5-dape. 2HCl) and its methyl ester dihydrochloride (Me 4,5-dape. 2HCl) were determined by potentiometric titration using a Radiometer, pH meter and autoburette. As a check on experimental technique, accuracy of equipment and method of calculation the K_a^T values for methyl L-histidinate dihydrochloride (Me L-His. 2HCl) were determined and compared with literature values.

Diamino acid hydrochlorides and their methyl ester dihydrochlorides behave as dibasic acids in aqueous solution e.g. (omitting (aq) subscripts)

$$CH_2CH(CH_2)_2COO^- + H_2O \rightleftharpoons CH_2CH(CH_2)_2COO^- + H_3O^+$$
$$\quad\;\; | \quad | \qquad\qquad\qquad\qquad\quad | \quad |$$
$$\quad NH_3^+ NH_3^+ \qquad\qquad\qquad\quad NH_3^+ NH_2$$

$$CH_2CH(CH_2)_2COO^- + H_2O \rightleftharpoons CH_2CH(CH_2)_2COO^- + H_3O^+$$
$$\quad\;\; | \quad | \qquad\qquad\qquad\qquad\quad | \quad |$$
$$\quad NH_3^+ NH_2 \qquad\qquad\qquad\quad NH_2 NH_2$$

and

$$CH_2CH(CH_2)_2COOCH_3 + H_2O \rightleftharpoons CH_2CH(CH_2)_2COOCH_3 + H_3O^+$$
$$\quad\;\; | \quad | \qquad\qquad\qquad\qquad\quad | \quad |$$
$$\quad NH_3^+ NH_3^+ \qquad\qquad\qquad\quad NH_3^+ NH_2$$

$$CH_2CH(CH_2)_2COOCH_3 + H_2O \rightleftharpoons CH_2CH(CH_2)_2COOCH_3 + H_3O^+$$
$$\quad\;\; | \quad | \qquad\qquad\qquad\qquad\quad | \quad |$$
$$\quad NH_3^+ NH_2 \qquad\qquad\qquad\quad NH_2 NH_2$$

These ionisations can be more conveniently represented:

$$AH_2^+ \overset{K_{a1}^T}{\rightleftharpoons} AH + H^+$$

$$AH \overset{K_{a2}^T}{\rightleftharpoons} A^- + H^+$$

$$K_{a1}^T = \frac{\{AH\}\{H^+\}}{\{AH_2^+\}} \tag{4.1a}$$

$$= \frac{[AH]\{H^+\}}{[AH_2^+].y_1} = K_{a1}^M \cdot \frac{1}{y_1} \tag{4.1b}$$

$$K_{a2}^T = \frac{\{A^-\}\{H^+\}}{\{AH\}} \tag{4.2a}$$

$$= \frac{[A^-].y_1.\{H^+\}}{[AH]} = K_{a2}^M \cdot y_1 \tag{4.2b}$$

and $$EH_2^{2+} \overset{K_{a1}^T}{\rightleftharpoons} EH^+ + H^+$$

$$EH^+ \overset{K_{a2}^T}{\rightleftharpoons} E + H^+$$

$$K_{a1}^T = \frac{\{EH^+\}\{H^+\}}{\{EH_2^{2+}\}} \tag{4.3a}$$

$$= \frac{[EH^+].y_1.\{H^+\}}{[EH_2^{2+}].y_2} = K_{a1}^M \cdot \frac{y_1}{y_2} \tag{4.3b}$$

$$K_{a2}^T = \frac{\{E\}\{H^+\}}{\{EH^+\}} \tag{4.4a}$$

$$= \frac{[E]\{H^+\}}{[EH^+].y_1} = K_{a2}^M \cdot \frac{1}{y_1} \tag{4.4b}$$

Where y_1 and y_2 are the molar activity coefficients of univalent and bivalent ions respectively, {} represents activity ($\{H^+\}$ is the activity of hydrogen ions as measured by the glass electrode, $pH = -\log\{H^+\}$), [] represents concentration and K_a^M is a mixed proton dissociation

constant (see Appendix 1.1).

Two calculation methods (Noyes[69] and Speakman[70]) were used to separate and evaluate the overlapping pKa values obtained from potentiometric data. Both methods and the computer programs used to perform the calculations are discussed in Appendix 1. The Speakman method uses a least squares refinement procedure[71b] to give the best values of the thermodynamic dissociation constants, for the experimental data. It is preferred to the algebraic calculation of the Noyes[71a] method which does not allow for errors in the experimental points. The Noyes method was retained for comparison, and because it calculates mixed dissociation constants only (Appendix 1.1) it can be applied to systems which the activity coefficient corrections of the Speakman method do not cover.

4.2 Methyl 4,5-diaminopentanoate dihydrochloride (Me 4,5-dape. 2HCl)

The ester (0.21911g, 10^{-3}mol) was weighed out and transferred to the uncorked reaction vessel. Volumes of water and 1.000mol ℓ^{-1} potassium chloride solution were added such that when the solution was half neutralised (10^{-3}mol NaOH ≡ 1.000ml of 1.000mol ℓ^{-1} NaOH) the ionic strength was exactly 0.1 mol ℓ^{-1} and the total solution volume was exactly 100ml. The reaction vessel was assembled and the solution allowed to come to equilibrium (about 0.5h). 1.000mol ℓ^{-1} sodium hydroxide was added in small aliquots to the solution from the autoburette. After each addition the pH was allowed to stabilise, then the stirrer was stopped (see section 2.4.1) and the pH recorded when the reading had become steady. After half neutralisation, ester hydrolysis became increasingly significant causing downscale pH drift. This effect was minimised by taking pH readings rapidly without stopping the stirrer. Even so, the pK_{a2}^{T} values obtained were initially nearly constant, then decreased rapidly. The hydrolysis lowered values were ignored in

calculating a mean pK_{a2}^{T}.

Table 2 lists the average of the pK_a^T values calculated by the Speakman method computer program, PKDI.

The test titration of Me L-His. 2HCl using the same techniques and analysis of data yielded results in good agreement with the literature[33] (Table 2). Slower hydrolysis past half neutralisation resulted in a smaller error in pK_{a2}^{T}.

Table 2: pK_a^T values at T = (25 ± 0.05)°C and I = 0.1M(KCl)

	pK_{a1}^{T}	pK_{a2}^{T}
Me 4,5-dape. 2HCl	5.888 ± 0.005	8.78* ± 0.02
Me L-His. 2HCl	5.006 ± 0.005 (5.007 ± 0.006)	7.236 ± 0.004 (7.230 ± 0.010)
4,5-dape. 2HCl	6.653 ± 0.015	9.920 ± 0.010
	pK_a^T(COOH) = 3.243 ± 0.022	

Values in brackets are from reference 33.
Errors are estimated standard deviations.
* accuracy reduced by hydrolysis.

4.3 4,5-Diaminopentanoic acid dihydrochloride (4,5-dape. 2HCl)

This compound differs from other diamino acids as it can be isolated only as a dihydrochloride (Section 3.3.6). When titrated, three ionisations steps occur:

1. $$\underset{\substack{|\\ NH_3^+}}{\overset{|}{CH_2CH(CH_2)_2COOH}}\underset{\substack{|\\ NH_3^+}}{\overset{|}{}} + H_2O \overset{K_a^T(COOH)}{\rightleftharpoons} \underset{\substack{|\\ NH_3^+}}{\overset{|}{CH_2CH(CH_2)_2COO^-}}\underset{\substack{|\\ NH_3^+}}{\overset{|}{}} + H_3O^+$$

i.e. $$AH_3^{2+} \overset{K_a^T(COOH)}{\rightleftharpoons} AH_2^+ + H^+$$

2. $$AH_2^+ \overset{K_{a1}^T}{\rightleftharpoons} AH + H^+$$

3. $$AH \overset{K_{a2}^T}{\rightleftharpoons} A^- + H^+$$

} as before

where $$K_a^T(COOH) = \frac{\{AH_2^+\}\{H^+\}}{\{AH_3^{2+}\}} \qquad (4.5a)$$

$$= \frac{[AH_2^+].y_1.\{H^+\}}{[AH_3^{2+}].y_2} = K_a^M(COOH) \cdot \frac{y_1}{y_2} \qquad (4.5b)$$

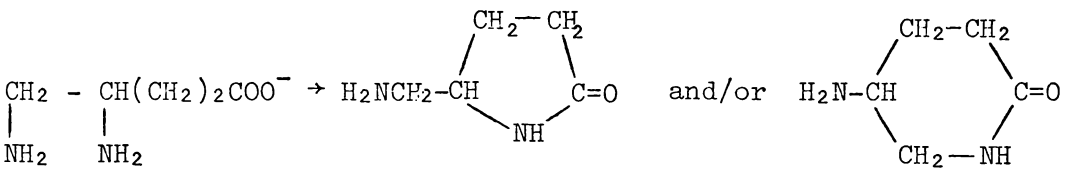
and K_{a1}^T and K_{a2}^T are as in equations (4.1) and (4.2).

Because of instability of 4,5-dape (Section 3.3.6) initial temperature equilibration of the titration solution was carried out at pH $\sim$ 1 by adding a known amount of hydrochloric acid. The following procedure was adopted.

The acid (0.20509g, 10^{-3}mol) was weighed out and transferred to the reaction vessel. 20.00ml 0.201mol ℓ^{-1} HCl were added and then volumes of water and 1.000mol ℓ^{-1} KCl solution such that at the half neutralisation point between pK_{a1}^T and pK_{a2}^T (after 6.014ml 1.002 mol ℓ^{-1} NaOH have been added) the ionic strength was exactly 0.1mol ℓ^{-1} and the volume exactly 100ml. The solution was allowed to equilibrate to 25°C and 1.002mol ℓ^{-1} NaOH (4.014ml) added from the autoburette, to

neutralise the excess hydrochloric acid. The solution was then titrated as in Section 4.2.

A check was made for cyclisation (lactamisation) by returning the solution to the starting pH with hydrochloric acid and repeating the titration. When plotted, the two titration curves were superimposable. Any significant cyclisation:



would have resulted in loss of the basic $-COO^-$, and one of the $-NH_2$ groups giving only one neutralisation point in the titration curve. Clearly no cyclisation occurs in the short time required for a titration ($\sim$0.5h) but does occur during recrystallisation (Section 3.3.6). Rigorous purification of the acid by crystallisation was impossible as traces of lactam from the above reactions were always present. The difference between successive titration end points at 0 and 1, and 1 and 2 moles OH^-/mole of amino acid was 0.980ml 1.002mol ℓ^{-1} NaOH (theoretically 0.998ml) indicating 98% purity. The end point at 3 moles OH^-/mole of amino acid was not observed as the pH was too high for the graph to show an inflexion. This unavoidable impurity (allowed for in calculating the pKa values) may be responsible for the trends in pKa values observed in Tables 3 and 4. The calculated pKa values must therefore be regarded as approximate within the quoted error. Assuming the first ionisation (COOH) does not overlap with the subsequent ammonium ionisations it may be represented by:

$$RCOOH \overset{K_a(COOH)}{\rightleftharpoons} RCOO^- + H^+ \qquad (4.6)$$

$$K_a^M(COOH) = \frac{[RCOO^-]\{H^+\}}{[RCOOH]}$$

A solution of total acid concentration C_A is titrated with a base, concentration $[Na^+]$. Then

$$C_A = [RCOOH] + [RCOO^-] \tag{4.7}$$

and electroneutrality requires that

$$[H^+] + [Na^+] = [OH^-] + [RCOO^-] \tag{4.8}$$

Also $$[H^+] \times [OH^-] = K_\omega^c \tag{4.9}$$

Substituting for $[OH^-]$ in (4.8)

$$[H^+] + [Na^+] = \frac{K_\omega^c}{[H^+]} + [RCOO^-]$$

i.e. $$[RCOO^-] = [H^+] + [Na^+] - \frac{K_\omega^c}{[H^+]}$$

From (4.7) $$[RCOOH] = C_A - [RCOO^-]$$

$$= C_A - [H^+] - [Na^+] + \frac{K_\omega^c}{[H^+]}$$

Substituting in (4.6)

$$K_a^M(COOH) = \frac{\left[[H^+] + [Na^+] - \frac{K_\omega^c}{[H^+]}\right]\{H^+\}}{C_A - [H^+] - [Na^+] + \frac{K_\omega^c}{[H^+]}}$$

$\frac{K_\omega^c}{[H^+]}$ is negligible at pH = 3,

$$\therefore \quad K_a^M(COOH) = \frac{([H^+] + [Na^+])\{H^+\}}{C_A - [H^+] - [Na^+]} \tag{4.10}$$

Table 3 lists the $pK_a^M(COOH)$ values calculated using equation (4.10).

Table 3: $pK_a^M(COOH)$ values for 4,5-dape. 2HCl calculated from equation (4.10) T = (25 ± 0.05)°C I = 0.10mol ℓ^{-1} (KCl)

Titre (ml)	pH	$10^4\{H^+\}$	$10^4[H^+]$	$10^3[Na^+]$	10^2C_A	$10^4K_a^M(COOH)$	$pK_a^M(COOH)$
0.155	3.053	8.851	11.47	1.611	1.019	3.285	3.484
0.255	3.230	5.888	7.632	2.647	1.018	2.966	3.528
0.355	3.399	3.990	5.172	3.682	1.017	2.806	3.552
0.455	3.568	2.704	3.505	4.714	1.016	2.688	3.571
0.555	3.740	1.820	2.359	5.744	1.015	2.610	3.583
0.655	3.924	1.194	1.548	6.772	1.014	2.574	3.589
0.755	4.140	0.724	0.939	7.798	1.013	2.554	3.593

Mean $pK_a^M(COOH)$ = 3.56 ± 0.04 $\therefore$ $pK_a^T(COOH)$ = 3.23

The trend in $pK_a(COOH)$ suggests overlap between $K_a(COOH)$ and K_{a1} may be important. Consequently calculations assuming overlapping pK_a values were done using both the Noyes method (PKN) and the Speakman method (PKDI) computer programs (Appendix 1). Since a combination of equations (4.5) and (4.1) is similar to equations (4.3) and (4.4) (in activity coefficient terms) the program PKDI was applied as for a diamino acid ester dihydrochloride. The result for the Noyes method (Table 4) shows a smaller trend in $pK_a^M(COOH)$ values. $pK_a^T(COOH)$ was calculated from the average $pK_a^M(COOH)$ by equation (4.5b) using y_1 and y_2 values from the Davies equation (Appendix 1.1).

Table 4: $pK_a^M(COOH)$ and $pK_{a_1}^M$ for 4,5-dape. 2HCl by Noyes Method (PKN);
T = (25 ± 0.05)°C I = 0.10mol ℓ^{-1}(KCl)

Titre (ml)	pH	$pK_a^M(COOH)$	$pK_{a_1}^M$
0.155	3.053	3.542	
1.755	7.343		6.767
0.255	3.230	3.562	
1.655	7.108		6.764
0.355	3.399	3.573	
1.555	6.910		6.758
0.455	3.568	3.585	
1.455	6.728		6.755
0.555	3.740	3.594	
1.355	6.540		6.747
0.655	3.923	3.599	
1.255	6.337		6.743
0.755	4.140	3.603	
1.155	6.080		6.734

Mean: $pK_a^M(COOH) = 3.580 \pm 0.022$ $\therefore pK_a^T(COOH) = 3.243$
$pK_{a_1}^M = 6.753 \pm 0.012$ $\therefore pK_{a_1}^T = 6.640$

The same answers ($pK_a^T(COOH) = 3.251 \pm 0.062$ and $pK_{a_1}^T = 6.650 \pm 0.009$) were obtained from the Speakman (PKDI) calculation but a greater error was estimated for $pK_a(COOH)$ (±0.062).

The Speakman method (PKDI) as applied to an ampholyte, i.e. a compound containing both acidic and basic groups, was used to calcualte $pK_{a_1}^T$ and $pK_{a_2}^T$. The volume of the first end point (0.980ml) being subtracted from subsequent volumes to give suitable data for the computer. The output of the program is given in Table 5. The same value for $pK_{a_1}^T = 6.65 \pm 0.01$ was obtained from both sets ($pK_a^T(COOH)$ and $pK_{a_1}^T$,

pK_{a1}^{T} and pK_{a2}^{T}) of overlapping constants.

The average of pK_{a}^{T}(COOH), pK_{a1}^{T} and pK_{a2}^{T} values obtained for 4,5-dape. 2HCl are summarised in Table 2.

Table 5: pK_{a1}^{T} and pK_{a2}^{T} values for 4,5-dape. 2HCl by Speakman Method (PKDI); T = (25 ± 0.05)°C I = 0.1molℓ^{-1}(KCl)

Titre (ml)	pH	pK_{a1}^{T}	pK_{a2}^{T}
0.175	6.080	6.636	
0.275	6.337	6.640	
0.375	6.540	6.643	
0.475	6.728	6.651	
0.575	6.910	6.656	
0.675	7.108	6.665	
0.775	7.343	6.678	
1.175	9.197		9.905
1.275	9.440		9.915
1.375	9.640		9.924
1.475	9.821		9.929
1.575	9.997		9.931
1.675	10.177		9.924
1.775	10.381		9.910

Mean pK_{a1}^{T} = 6.653 ± 0.015

pK_{a2}^{T} = 9.920 ± 0.010

4.4 Discussion:

Clark and Perrin[72] have developed a method for predicting approximate pK_a values of organic bases in water, at 20°C. The pK_a is calculated by subtracting the base weakening effects ($-\Delta pK_a$) (Table 6) of the substituents of the substituted amine from the pK_a of the appropriate unsubstituted amine (typical primary amine pK_a = 10.8[72]).

Table 6: Base Weakening Effects of Substituents in Aliphatic Amines

Substituent	$-\Delta pK_a$	
	(a)	(b)
COO^-	+0.8	-0.2
NH	-	+0.8
COOR	+3.0	+1.3
NH_3^+	_	+3.6

(a) 1 carbon atom between substituent and basic group.

(b) 2 carbon atoms between substituent and basic group.

The effect should be halved for each further additional carbon atom.

4,5-Diaminopentanoic acid and its methyl ester may be considered substituted 1,2-diaminoethanes and a comparison between the pK_a's of these compounds, 1,2-diaminoethane (en) and 2,3-diaminopropanoic acid (2,3-dap) and its methyl ester (Me 2,3-dap) is interesting.

The pKa values for these substituted 1,2-diaminoethanes were calculated by the Clark and Perrin treatment (Table 7), e.g. for Me 4,5-dape. 2HCl:

The ammonium ion on C_4 will deprotonate first since it experiences the combined base weakening effect of the $COOCH_3$ and an adjacent NH_3^+.

pK_{a1} ($(\omega-1)$-NH_3^+) = pK_a of typical primary amine - effect of $COOCH_3$ 3 carbon atoms distant - effect of NH_3^+ 2 carbon atoms distant

= 10.8 - 0.65 - 3.6 = 6.55.

pK_{a2}(ω-NH_3^+) = pK_a of typical primary amine - effect of CO_2CH_3 4 carbon atoms distant - effect of NH_2 2 carbon atoms distant

= 10.8 - 0.3 - 0.8 = 9.7.

The method shows a weakness in the case of 4,5-dape. HCl. The effect of COO^- (Table 5) is base weakening ($-\Delta pK_a$ = 0.8) when on the carbon next to the basic centre but becomes base strengthening ($-\Delta pK_a$ = -0.2) for 2 or more carbon atoms between COO^- and the basic centre. These empirical values are not easily explained. Clark and Perrin[72] explain the base weakening effect of the COO^- in α-amino acids by hydrogen bonding but offer no reason for the base strengthening effect observed in β-amino acids. An interplay between -I inductive effects and the entropy change with ionisation clearly exists. The result is that the theory cannot predict whether the ω-NH_3^+ or the $(\omega-1)$-NH_3^+ deprotonates first. The predicted pK_a values are 7.3 for $(\omega-1)$-NH_3^+ and 7.25 for ω-NH_3^+. However, regardless of which process occurs first, the theory predicts $\Delta pK_a = pK_{a2} - pK_{a1}$ to be the observed $\sim 3pK_a$ units.

With the exceptions discussed below, the agreement between calculated and observed values is generally reasonable. The calculated values predict the observed trend to higher pK_a values (i.e. more 1,2-diaminoethane like) as the base weakening -COOH or -COOR group is withdrawn from the amino groups by adding methylene groups.

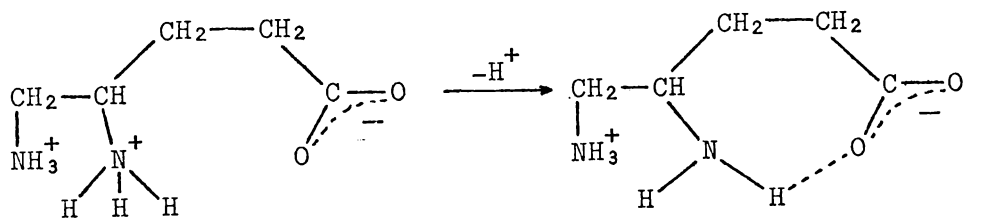
Table 7: Clark and Perrin[72] Calculated pK_a^T values, 20°C

	$pK_{a_1}((\omega\text{-}1)NH_3^+)$		$pK_{a_2}(\omega\text{-}NH_3^+)$	
	Calc	Obs*	Calc	Obs*
en. 2HCL[a]	7.2	6.859	10.3	9.943
4,5-dape. HCl	7.3	6.653	10.1	9.920
Me 4,5-dape. 2HCl	6.55	5.888	9.7	8.78
2,3-dap. HCl[b]	6.4	6.674	10.2	9.623
Me 2,3-dap.2HCl[b]	4.2	4.412	8.7	8.250

*Observed values at 25°C (a) from ref 73

(b) from ref 32

The observed pK_{a_1} values for 4,5-dape. HCl and Me 4,5-dape. 2HCl are considerably lower than predicted, i.e. these species deprotonate more readily than expected on simple inductive effects. A possible explanation is added stabilisation, by hydrogen bonding, of the product species over the reactant, e.g. the zwitterion (41) over the monocation (40)



40 41

However, the observed effect may well be an entropy difference, and in the absence of thermodynamic data no firm conclusions can be drawn.

A parallel effect is observed for pK_{a_2} values of Me 4,5-dape. 2HCl, 2,3-dap. HCl and Me 2,3-dap. 2HCl. Although, for 2,3-dap. HCl and Me 2,3-dap. 2HCl, entropy and enthalpy changes for deprotonation of the β-NH_3^+ (K_{a_2}) are similar[32] to those observed for simple α-amino acids and esters, the result is a greater tendancy to deprotonate (lower pK value) than is calculated on simple inductive effects. The large difference $pK_{a2}(calc) - pK_{a2}(obs) = 0.92$ for Me 4,5-dape. 2HCl may be due in part to hydrolysis lowering of the observed value (Section 4.2).

5. STABILITY CONSTANTS

5.1 Introduction

An understanding of the types of species, and their concentrations, in metal ion-ester solutions is essential to any interpretation of the kinetics of metal ion catalysed ester hydrolysis. For this reason the concentration stability constants (as cumulative constants, β values) of Cu(II) complexes of Me 4,5-dape and 4,5-dape were determined at 25°C and 0.1 mol ℓ^{-1} ionic strength.

In addition, stability constants of the species present in Cu^{2+}: Me 4,5-dape: 4,5-dape = 1:1:1 solutions were measured, so the extent of disproportionation of the hydrolysis intermediate $CuEA^{+}$ to CuE_2^{2+} and CuA_2 could be estimated.

Since the Cu(II) catalysed hydrolysis of Me 4,5-dape was also effected in the presence of 1,2-diaminoethane (en), the stability constants of Cu(II)-en complexes and mixed Cu(II)-E-en complexes were determined.

The stability constants were determined by the Bjerrum potentiometric titration method[74] of examining the competition between a proton and a metal ion for a basic ligand. From the resulting pH and titre values the stability constants were evaluated by the computer program SCOGS[75] (Appendix 2.1). Because the calculated values of stability constants may depend on the method used, a complete summary of titration data is listed in Appendix 2.2.

5.2 Potentiometric Titrations

Titrations were carried out on solutions containing the metal ion Cu(II) and a ligand in the mole ratios of 1:1 and 1:2, and on solutions of Cu(II) and two ligands in the ratio of 1:1:1. The method used is discussed for each system.

5.2.1 1:2 Solutions

Me 4,5-diaminopentanoate dihydrochloride or 4,5-diaminopentanoic acid dihydrochloride (10^{-3}mol) was weighed out and transferred to the uncorked reaction vessel. For 1,2-diaminoethane an aliquot of a standardised solution (0.995.molℓ^{-1}) and sufficient hydrochloric acid (9.955ml 0.201molℓ^{-1}) to form the dihydrochloride was used. Copper (II) chloride (5.00×10^{-4}mol) was added as an aliquot of a standardised (0.100molℓ^{-1}) solution, together with volumes of water and 1.000molℓ^{-1} potassium chloride solution such that when the solution was half neutralised (1.00×10^{-3}mol NaOH ≡ 1.000ml 1.000molℓ^{-1}NaOH) the ionic strength was o.10molℓ^{-1} and the total solution volume 100.00ml. Since 4,5-dape was added as the dihydrochloride an extra mole of sodium hydroxide/mole of acid was required. Thus I = 0.10molℓ^{-1} and the total volume = 100.00ml after 2.000ml 1.000molℓ^{-1}NaOH had been added. The reaction vessel was assembled and the potentiometric titration carried out as described for the determination of proton dissociation constants (Section 4.2.1).

As alkali was added, the solution colour changed from pale blue-green, through blue to finally a deep purple. No precipitation occurred during any of these titrations and hydrolysis of the ester (see 4.2.1) was insignificant until after the end point of the titration (pH ≃ 8.2).

The titration end points were good purity criteria of the synthesised compounds. With methyl 4,5-diaminopentanoate dihydrochloride the presence of traces of ammonium chloride (see Section 3.3.5) appeared as a slight inflexion in the end point, the neutralisation of the ester occurring first (pK_a's ∿5.9 and 8.8) followed immediately by neutralisation of the ammonium ion (pKa ∿9.3). Repeated recrystallisation removed the impurity.

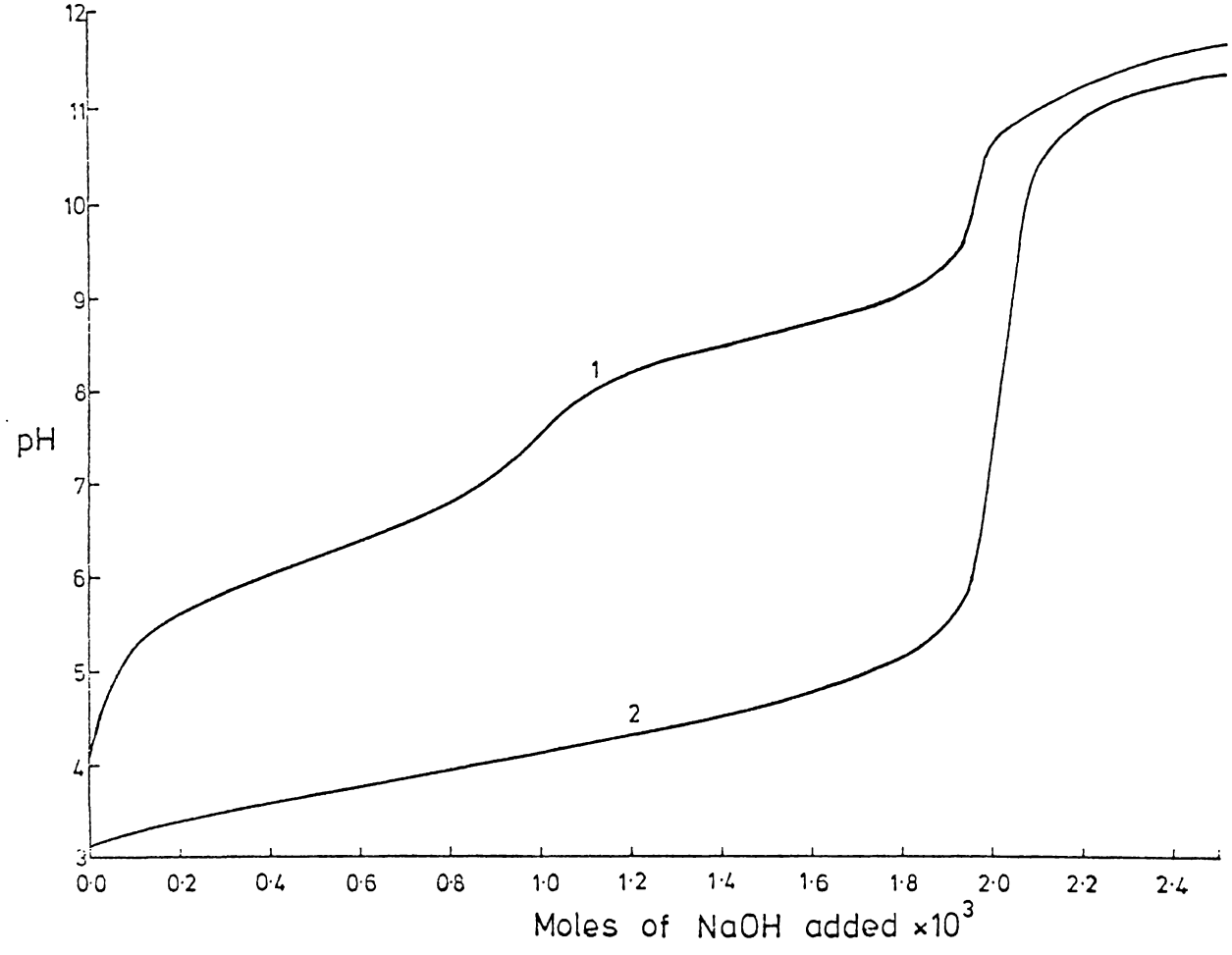


Figure 5: Titration curves for Me 4,5-dape.2HCl; T = 25°C, I = 0.10molℓ^{-1}(KCl) [Me 4,5-dape] = 10^{-2}molℓ^{-1} at half neutralisation.

1. Me 4,5-dape.2HCl alone.

2. Me 4,5-dape.2HCl:$CuCl_2$ = 2:1.

In 4,5-dape, 98% purity (Section 3.3.6) was shown by a low end point. The impurity, shown by comparative tlc to be 5(aminomethyl)-2-oxopyrrolidine could not be removed by recrystallisation (Sections 3.3.6 and 4.3). The problem was partially overcome by weighing out a slight excess of the acid.

Figure 5 shows typical titration curves obtained for Me 4,5-dape in the absence of and in the presence of half a mole of Cu(II)/mole of ester. Similar curves are obtained for en and 4,5-dape. The titration curve of the ligand is lowered in the presence of a metal ion which displaces ligand protons. The extent of this lowering is a measure of the stability of the Cu(II)-ligand complex.

The titration data used to calculate the overall stability constants is summarised in Appendix 2.2. The following input information was required by the program SCOGS[75].

1. The activity coefficient of hydrogen ions (taken as 0.7715, see Appendix 1.1 and the pK_ω value of water at 25°C (taken as 13.997)
2. Acid association constants for the ligands. pK_a^m values were required and were taken from the results of Section 4. For en literature values[73] were used.
3. Initial estimates of the formation constants and a description of each species. Estimates were made by interpolation from literature values for related systems[32,33,76,77].

For the 1:2 solutions possible species are: CuL, CuL_2 and protonated species CuLH, $Cu(LH)_2$, CuL(LH), where L is any of the diamine ligands studied. Hydroxy species of the types $Cu_n(OH)_{2n-2}^{2+}$ (ref 78) or CuL(OH) (ref 79) were not expected to form in the pH range (ca. pH 3-5) of these titrations, and this was verified by failure of the program to converge constants for these species (see Appendix 2.1).

In addition the constant for the species $Cu(LH)_2$ was not converged in any of the systems studied, indicating that $\%Cu(LH)_2$ is always very small.

The stability constants obtained are summarised in Table 8. The constants listed with their respective complexes refer to the following equilibria (charges omitted, Cu = $Cu^{2+}_{(aq)}$, H = $H^{+}_{(aq)}$)

$Cu + L$	$\rightleftharpoons CuL$	β_1
$Cu + 2L$	$\rightleftharpoons CuL_2$	β_2
$Cu + H + L$	$\rightleftharpoons Cu(LH)$	β_1'
$Cu + 2H + 2L$	$\rightleftharpoons Cu(LH)_2$	β_2'
$Cu + H + 2L$	$\rightleftharpoons CuL(LH)$	β_m
$Cu + L$	$\rightleftharpoons CuL(OH) + H$	β_1^*
$2Cu + 2L$	$\rightleftharpoons Cu_2L_2(OH)_2 + 2H$	β_2^*

For comparison values of constants for complexes of Cu(II) with histidine (His) and its methyl ester (Me His), 2,3-diaminopropanoic acid (2,3-dap) and its methyl ester (Me 2,3-dap) and 2,4-diaminobutanoic acid (2,4-dab) are also listed in Table 8. Constants for Me 2,3-dap and Me His at 25°C were calculated by SCOGS from published titration data[73]. Literature values are given for His at 25°C[33] and 37°C[77], 2,3-dap at 25°C[80] and 2,4-dab at 20°C[80].

Where stepwise stability constants were published[77,80], they were converted to overall stability constants. The errors quoted are computed standard deviation in the constants and the estimated standard deviation (ESD) in titre (see Appendix 2.1).

5.2.2 1:1 Solutions:

A solution containing Me 4,5-dape. 2HCl (10^{-3}mol) and Cu(II) ions (10^{-3}mol) at 25°C (with I = 0.1mol ℓ^{-1}(KCl) at half neutralisation) was titrated with 1.000molℓ^{-1} NaOH in the manner described for 1:2 solutions.

Table 8: Stability Constants of Cu(II) complexes of 1,2-diamines: T = (25 ± 0.05)°C, I = 0.10mol ℓ^{-1}(KCl)

Ligand	Complex Species and its Stability Constant (β value)							Titre ESD (ml)
	CuL $\log_{10}\beta_1$	CuL_2 $\log_{10}\beta_2$	Cu(LH) $\log_{10}\beta_1'$	$Cu(LH)_2$ $\log_{10}\beta_2'$	CuL(LH) $\log_{10}\beta_m$	CuL(OH) $\log_{10}\beta_1$*	$Cu_2L_2(OH)_2$ $\log_{10}\beta_2$*	
en	10.56±0.01	19.66±0.01	-	-	23.19±0.29	-	-	0.002
4,5-dape	10.94±0.01	20.34±0.01	14.99±0.01	-	24.80±0.02	-	-	0.002
Me 4,5-dape	9.84±0.01	18.35±0.01	12.60±0.08	-	22.17±0.07	-	-	0.003
2,3-dap [a]	11.46±0.01	19.95±0.01	15.82±0.01	-	25.25±0.02	-	-	0.005*
Me 2,3-dap [b]	8.95±0.01	16.77±0.01	11.62±0.05	-	20.20±0.07	-	-	0.002*
2,4-dab(20°C) [a]	~10.4	19.48±0.03	17.59±0.02	33.88±0.03	27.66±0.04	-	-	0.005*
His (25°C) [c]	10.22±0.02	18.20±0.02	14.35±0.03	-	24.05±0.02	-	-	0.007*
His (37°C) [d]	9.79±0.05	17.41±0.05	14.03±0.08	-	23.05±0.06	2.40±0.06	7.43±0.14	-
Me His [b]	8.43±0.01	14.40±0.01	10.32±0.13	-	18.51±0.08	-	5.36±0.03	0.008*

- denotes concentration of species concerned is too small to allow estimation of stability constant.

a: Ref 80; b: Calculated from data, ref 73; c: Ref 33; d: Ref 77.

* Adjusted from [NaOH] = 0.100mol ℓ^{-1} to [NaOH] = 1.000mol ℓ^{-1}.

These titrations were different from the 1:2 solutions, two end points were observed, one at two moles of OH^-/mole of ester and one at 3 moles of OH^-/mole of ester. Also, precipitation occurred immediately after the first end point. This precipitate did not dissolve at high pH (pH ∿12) values.

The colour of the 1:1 solution changed from a pale blue-green to blue at 2 moles of OH^-/mole of ester. After this point a blue-green precipitate progressively formed and the supernatent solution became violet. Hydrolysis of the ester was insignificant until near the second end point (pH ∿9). Fig 6 shows a typical titration curve. This behaviour of 1:1 solutions of Cu(II) and Me 4,5-dape. is different from that reported for Me His and Me 2,3-dap[32,33], where no precipitation occurs until some ester hydrolysis has taken place and the precipitate formed dissolves at high pH (pH ∿11).

To find out why these similar systems behaved in different ways a spectrophotometric study was made on 1:1 solutions of Cu(II) and various diamine ligands.

A 1:1 Cu^{2+}:ligand solution was prepared for each of the ligands: en.2HCl, 2,3-dap. HCl, Me 2,3-dap.2HCl, 4,5-dape.2HCl, Me 4,5-dape2HCl, His.HCl and Me His.2HCl. The pH of the solution was raised (1 mol ℓ^{-1}NaOH) to complete neutralisation of the ligand protons (2moles OH^-/mole of ligand). The visible spectrum of the resulting blue solution was run, then the pH was raised progressively and spectra run at intervals to 3 moles OH^- added/mole of ligand. Any precipitate formed was "millipore" filtered off (Millipore Corporation, Bedford, Massachusetts, USA) before running the spectrum. The pH was finally raised to about pH 11.5 to see of any of the precipitates dissolved.

1:2 solutions of Cu(II) and the various ligands were also prepared and titrated to complete neutralisation of the ligand protons

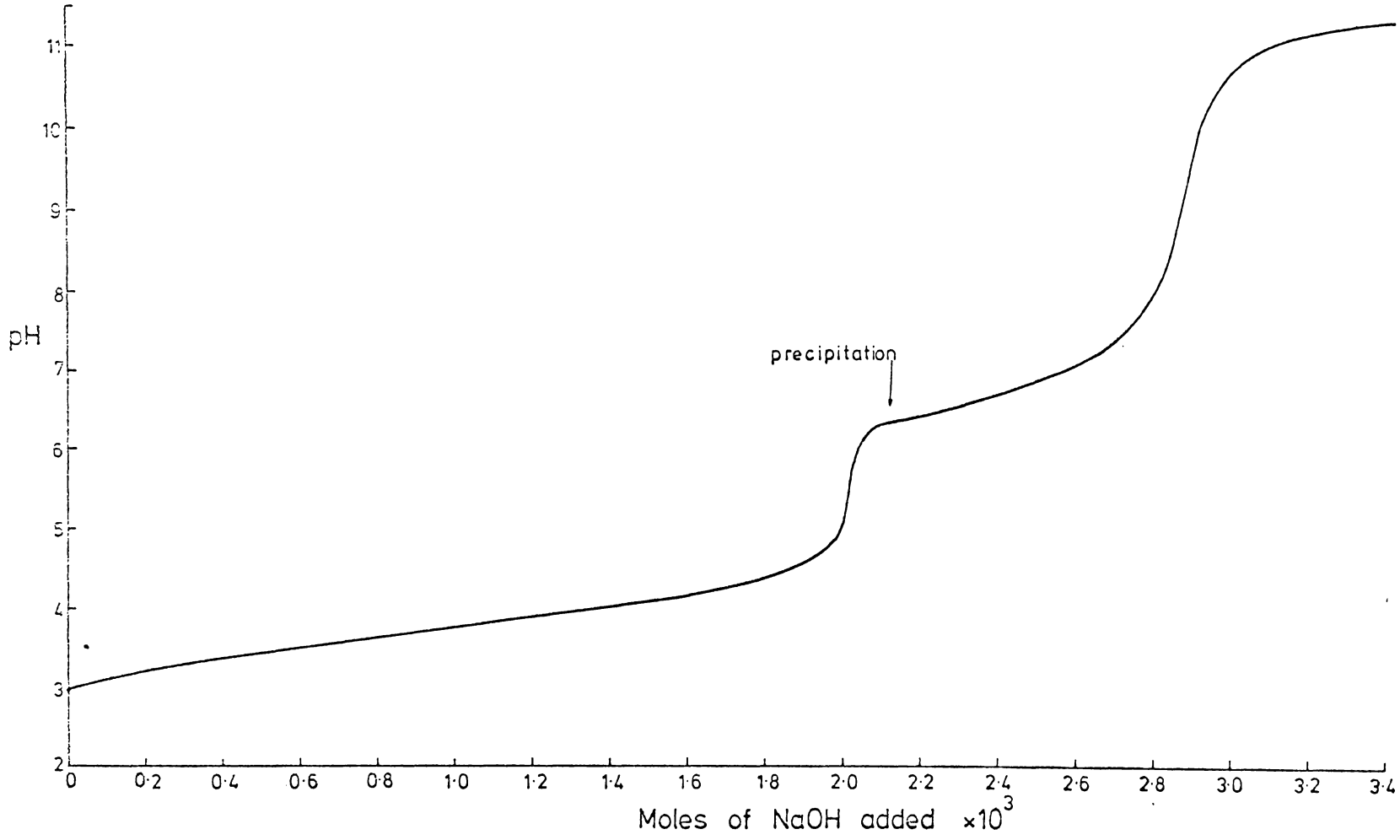


Figure 6: Titration curve for an equimolar mixture of Me 4,5-dape.2HCl and $CuCl_2$; T = 25°C, I = 0.10molℓ^{-1} (KCl) [Me 4,5-dape] = 10^{-2}molℓ^{-1} at half neutralisation.

(2 moles OH^-/mole of ligand). A sample was withdrawn and a visible spectrum run for comparison with the 1:1 system. Rapid hydrolysis of Me 2,3-dap meant that spectra could not be run for either 1:1 or 1:2 solutions.

The results obtained are summarised in Table 9.

Table 9: Visible Absorption Spectra of Copper (II) complexes of various ligands. T = 25°C I = 0.10mol ℓ^{-1}

Ligand	1:2 Solution			1:1 Solution					
	2mol OH^-/mol ligand			2mol OH^-/mol ligand			3mol OH^-/mol ligand		
	pH	λ_{max}(nm)	ε	pH	λ_{max}(nm)	ε	pH	λ_{max}(nm)	O.D.
en .2HCl	9.2	547	63.2	6.0	656	31.5	9.2	547	0.325 $[CuL_2]=5\times10^{-3}$mol ℓ^{-1}
Me 4,5-dape .2HCl	9.0	547	61.6	6.05	645	28.0	9.2	547	0.305 $[CuL_2]=5\times10^{-3}$
4,5-dape . HCl	9.0	547	62	6.0	676	24.6	11.4	547	0.205 $[CuL_2]=3\times10^{-3}$
2,3-dap .HCl	9.0	547	51.2	6.3	640	21.3	9.0	547	0.25 $[CuL_2]=5\times10^{-3}$
His .HCl	9.15	640	86	6.13	673	34.5	9.16	616	0.325
Me His.2HCl	7.65	625	92	5.75	683	36.5	8.90	611	0.430

$[CuL_2]$ calculated using corresponding ε value.

For en, 2,3-dap, 4,5-dape and Me 4,5-dape the solutions at 2moles OH^-/mole of ligand have varying λ_{max} but all are blue. The λ_{max} values suggest a mixture of N and O donor atoms [(N + 3O) to (3N + O) about the Cu^{2+} but not Cu(4N) (λ_{max}=547nm)[81]] and can be explained by the presence of CuL and varying minor amounts of complexes such as CuL(LH), CuL(OH). The ε values in Table 9 have been calculated assuming 100% CuL .

For the 1,2-diamine ligands a blue-green precipitate formed at 2moles OH^-/mole of ligand and the filtered solutions at 3moles OH^-/mole of ligand were violet with λ_{max}=547nm. The precipitates did not dissolve at high pH. Violet solutions with the same λ_{max} and ε values were obtained for 1:2 solutions of these ligands at 2 moles OH^-/mole of ligand. No precipitation occurred in the 1:2 solutions. The violet colour of these solutions indicates Cu(II)(4N),[81] which agrees with the computed stability constant result that such solutions contain 100% of CuL_2. (see 5.4 and figs 7, 8 and 9).

The violet solutions in the 1:1 systems at pH$\sim$9 have the same λ_{max} as CuL_2 and from ε_{CuL_2} evidently contain 5×10^{-3}mol ℓ^{-1} of CuL_2 i.e. 50% of the Cu(II) is as CuL_2 with the remainder presumably a $Cu(OH)_2$ precipitate. The result for 4,5-dape appears anomalous. Because of the difficulty of obtaining this ligand in a pure state, the solutions involved were prepared by hydrolysing the corresponding Cu(II)-Me 4,5-dape solutions and some decomposition may have occurred associated with the hydrolysis reaction time.

Such disproportionation of CuL to an equimolar mixture of CuL_2 and $Cu(OH)_2$ has been observed previously[79] and is due to the similarity in the stability constants for the addition of the first and second ligands to the metal ion. Thus during titration, as more ligand is neutralised, there is a tendancy for (charges omitted, Cu = $Cu^{2+}_{(aq)}$).

$$CuL + L \rightarrow CuL_2$$

to occur in competition with

$$Cu + L \rightarrow CuL$$

This leaves free $Cu^{2+}_{(aq)}$ at high pH which precipitates as $Cu(OH)_2$ which further promotes the formation of CuL_2, until all the ligand is bound as CuL_2. As a further check, the precipitate was prepared in a larger quantity and analysed. A ten times more concentrated solution of Cu:(en) = 1:1 was prepared and sodium hydroxide added until precipitation was complete. The very fine blue-green precipitate was "millipore" filtered off (to leave a violet supernatent solution) and analysed for copper by complexometric titration[39]. A microanalysis for C, H and N was also obtained.

Found	Cu, 54.7;	C,	1.77;	H,	2.08;	N,	1.04%
$Cu(OH)_2$ requires	Cu, 65.13;			H,	2.07%		
$Cu(OH)_2H_2O$ requires	Cu, 63.81;			H,	4.05		
$Cu(OH)_2$en requires	Cu, 40.30;	C,	15.24;	H,	6.39;	N,	17.77%

The precipitate is evidently a very non-stochiometric copper (II) hydroxide[82] with traces of CO_3^{2-} (from CO_2 absorption) and en (adsorbed). There seemed little point in trying to purify the precipitate as it was obviously not a Cu(en)(OH) type of complex as has been observed[33] for His.

L-His and Me His behaved very differently from the substituted 1,2-diaminoethanes (Table 9). With Me His, precipitation did not occur in the 1:1 solution until after the second end point (3moles OH^-/mole of ligand, pH >10) and after appreciable hydrolysis had occurred. With His precipitation occurred at pH 7.2. In both cases the precipitate dissolved at pH ∿11. This precipitate has previously been shown[33] to be the neutral complex Cu(His)(OH)(H_2O). Also in both His and Me His solutions, λ_{max} for the 1:2 solution (royal blue) is quite different from

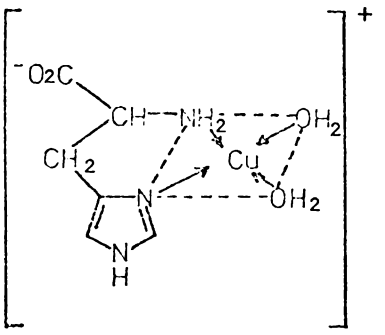
Table 10: Change in visible absorption of Cu(II) complexes of histidine and histamine with pH. T = 25°C I = 0.10mol ℓ^{-1} (KCl).

moles OH^- added / moles ligand	pH	Solution Colour	λ_{max}(nm)	Optical Density	ε*
(a) Histidine					
2.00	6.13	green-blue	673	0.345	34.5
2.25	6.76	"	658	0.348	34.8
2.50	7.19	"	641	0.340	34.0
2.75	7.88	"	628	0.333	33.3
3.00	10.47	"	616	0.363	36.3
3.25	10.70	Royal blue	611	0.503	50.3
3.50	11.03	"	610	0.543	54.3
3.75	11.20	"	609	0.565	56.5
4.00	11.30	dark blue	606	0.595	59.5
>>4	11.50	"	606	0.605	60.5
(b) Histamine					
2.00	6.02	green blue	678	0.348	34.8
2.25	6.76	"	658	0.350	35.0
2.50	7.10	blue	636	0.360	36.0
2.75	7.51	"	615	0.383	38.3
3.00	9.00	"	598	0.440	44.0
3.25	10.71	"	593	0.493	49.3
3.50	11.15	Royal blue	592	0.540	54.0
3.75	11.41	"	590	0.570	57.0
4.00	11.60	Dark blue	590	0.585	58.5
>>4	12.00	"	590	0.600	60.0

* Calculated assuming all Cu(II) present as 1:1 complex (1cm cell).

λ_{max} for the 1:1 solution (green-blue). The values for Me His (Table 9) are approximate because of rapid ester hydrolysis in these solutions.

The His 1:1 systems are evidently unique and so a similar spectral study of these solutions was made to see why. A 1:1 solution of Cu(II): His was titrated and samples drawn off every 0.25moles OH^-/mole of ligand from 2 to 4 moles OH^-/mole of ligand. The pH was noted and a visible spectrum run. If necessary the solution was "millipore" filtered. It was returned to the titration vessel after the spectrum had been run. The results are summarised in Table 10. At 2moles OH^-/mole of ligand the solution contains mostly the 1:1 complex $Cu(His)(H_2O)_2^+$(42) (λ_{max} = 673nm, $Cu(N_{Im}, NH_2, 20))$[81] although computer analysis of titration data[77] shows some $Cu(His)(OH)(H_2O)$(43) is also present. From 2 to 3 moles OH^-/mole of ligand a steady large, shift in λ_{max} is observed (673 to 616nm) which is consistent with deprotonation of one of the water ligands of (42) to form (43). From 3 to 4 moles OH^-/mole of ligand a further but smaller change in λ_{max} occurs (616nm to 606nm) with a gradual intensification of the blue colour. This small change in ligand field strength is consistent with titration of the imidazole nitrogen proton to give $Cu(HisH_{-1})(OH)^-$ (44). The alternative interpretation of deprotonation of a second water ligand to give $Cu(His)(OH)_2^-$ would be expected to give a larger λ_{max} shift. Formation of the anion (44) causes the precipitate to dissolve. A related species $Cu(HisH_{-1})(His)$ was observed by Kruck and Sarkar[83] at high pH (>10) in solutions of Cu(II) and His at ratios of 1:2, 1:4 and 1:8.



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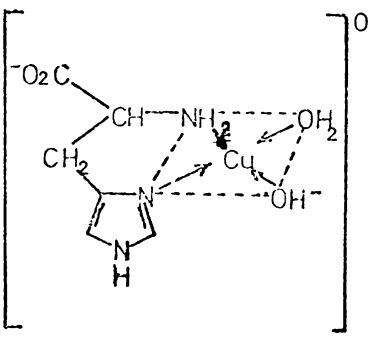

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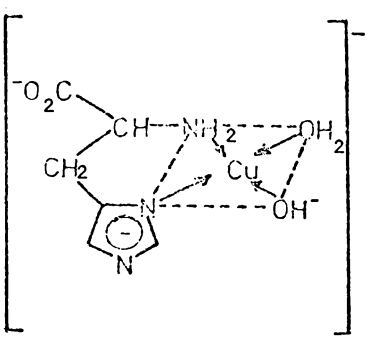
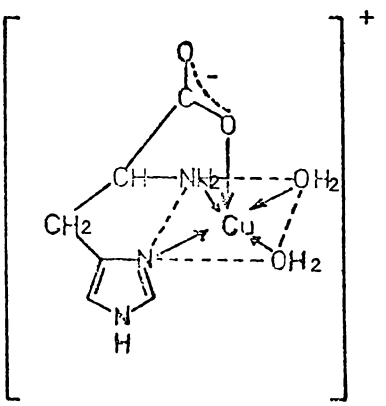


44

Hence in the His systems, no disproportionation occurs. This is because stability constants for CuL and CuL_2 (from CuL) are not the same (log K_1 - log K_2 ∿2.0) cf. the substituted 1,2-diamines. The reason for the additional stability of the 1:1 complex over the 1:2 for His may be that His is essentially tridentate in CuL[84] (45).



45.

However an even larger difference in relative stabilities of CuL and CuL_2 exists in Histamine (log K_1 - log K_2 = 9.76 - 6.71 = 3.05)[79] where no carboxylate oxygen is present. A spectrophotometric study of 1:1 solutions of Cu(II) and histamine was carried out in the manner described for histidine. Similar results (Table 10) were obtained except that for histamine no precipitation occurred at any pH. This is presumably because histamine is a neutral ligand, so the species are charged (cf. 43 with His system) except for the final species (cf. 44 for His system) which exists only at high pH and is apparently soluble.

Clearly the source of the unique behaviour of His and histamine compared with the 1,2-diamines is the imidazole ring.

Computer (SCOGS, Appendix 2.1) investigation of species present in 1:1 Cu(II):4,5-dape systems failed to yield satisfactory stability constants for hydroxy-complexes because of the disproportionation problem. Precipitation of $Cu(OH)_2$ occurs before appreciable amounts of CuL(OH) can be formed. Similar results have been reported for en[79].

However, for 1:1 Cu(II):Me His solutions[33], satisfactory convergence was obtained including hydroxy complexes. The best technique was found to be to use the values for the stability constants of CuL, CuL_2, Cu(HL) and CuL(HL) from the 1:2 titration data[33]. These were held constant while initial stability constant values were obtained for CuL(OH) and $Cu_2L_2(OH)_2$ from the 1:1 titration data[73]. Only the species $Cu_2L_2(OH)_2$ gave a satisfactory constant (5.36 ± 0.03) with titre ESD = 0.21ml. It was expected that better values for the constants of CuL and Cu(LH) would be obtained from this 1:1 titration because concentrations of these species would be higher than in the 1:2 solution. Accordingly the constants for the species CuL Cu(LH) and $Cu_2L_2(OH)_2$ were varied and the constants for CuL_2 and CuL(LH) held constant using the 1:1 titration data[73]. The refined values for CuL (8.43 ± 0.01), Cu(LH) 10.32 ± 0.13) with titre ESD = 0.04ml were then held constant with data for the 1:2 titration[33] to give new values for CuL_2 and CuL(LH) which were expected to have greatest concentrations in this solution. The final results CuL (8.43 ± 0.01), CuL_2 (14.40 ± 0.01), Cu(LH) (10.32 ± 0.13) and CuL(LH) (18.51 ± 0.08) with titre ESD = 0.08ml compare favourably with those obtained directly from the 1:2 titration (8.48, 14.46, 10.88, 18.40 respectively with ESDT = 0.02ml) for the major species CuL and CuL_2. Agreement for the species Cu(LH) and CuL(LH) is not good, but these species never attain

concentrations greater than 10% of the total copper (II) and major changes in their constants have little effect on their concentrations. These results are summarised in Table 8. In Table 8 the titre ESD's have been adjusted to [NaOH] = 1.000mol ℓ^{-1}, to allow meaningful comparison The titrations taken from the literature for 2,3-dap, Me2,3-dap, His and Me His at 25°C and 2,4-dab at 20°C were all carried out with [NaOH] = 0.100mol ℓ^{-1}.

Thus for L = Me His, the species $Cu_2L_2(OH)_2$ is predominant over CuL(OH). The reverse occurs[77] for L = His, due to hydrogen bonding interaction between the hydroxylic hydrogen atom of CuL(OH) and one of the carboxylate oxygens, which stabilises CuL(OH). No such stabilisation is possible in the binuclear diolate complex $Cu_2L_2(OH)_2$. Replacement of the $-COO^-$ by $-COOCH_3$ also prevents this stabilisation of CuL(OH), and Me His then behaves like histamine[79].

5.2.3 1:1:1 Solutions

The stability constants for the mixed species Cu(II) (4,5-dape) (Me 4,5-dape) and Cu(II) (en) (Me 4,5-dape) were required for kinetic studies. Solutions containing 5×10^{-4} mol of each of Cu(II), Me 4,5-dape and 4,5-dape and solutions containing 5×10^{-4} mol of each of Cu(II), en and Me 4,5-dape such that I = 0.10mol ℓ^{-1} and total volume = 100.00ml at half neutralisation were titrated as previously described (Section 5.2.1).

The pH-titration data obtained was analysed by SCOGS program (Appendix 2.1). Previously obtained stability constants for complexes of the single ligands were held constant while constants of mixed species e.g. $CuEA^+$ or protonated mixed species were refined. Initial estimates of the stability constants for the mixed species CuEL were the mean values for CuE_2 and CuL_2 (L = en or 4,5-dape). Inclusion of hydroxy

complexes resulted in a failure to converge in all cases. The results are summarised in Table 11 and the titration data in Appendix 2.2. The constants listed refer to the following equilibria (charges omitted):

$$Cu + E + L \rightleftharpoons CuEL \qquad \beta_{111}$$

$$Cu + E + L + H \rightleftharpoons CuELH \qquad \beta_{111}'$$

$$Cu + E + L + 2H \rightleftharpoons Cu(EH)(LH) \qquad \beta_{111}^{m}$$

where L = en or 4,5-dape

Cu = $Cu^{2+}_{(aq)}$, H = $H^{+}_{(aq)}$

Table 11: Stability Constants of Mixed Complexes
$T = 25°C \quad I = 0.10 mol\,\ell^{-1}$.

Ligands	Complex Species and log (constant)			Titre ESD
	CuEL*	CuE(LH)	Cu(EH)(LH)	
Me 4,5-dape + 4,5-dape	19.68 ± 0.01	23.99 ± 0.03	27.21 ± 0.47	0.004ml
Me 4,5-dape + en	19.30 ± 0.01	23.60 ± 0.07	-	0.004ml
Me 2,3-dap[(a)] + 2,3-dap	18.77 ± 0.02	23.91 ± 0.02	-	-

* L = 4,5-dape, en or 2,3-dap

(a) Ref 32.

5.3 Discussion

Figures 7-12 show the effect of pH on the composition of various solutions, computed using their respective stability constants (Table 8) The unique nature of the Cu(II)-His and Cu(II)-Me His systems has been discussed (Section 5.2.2) and will not be considered further.

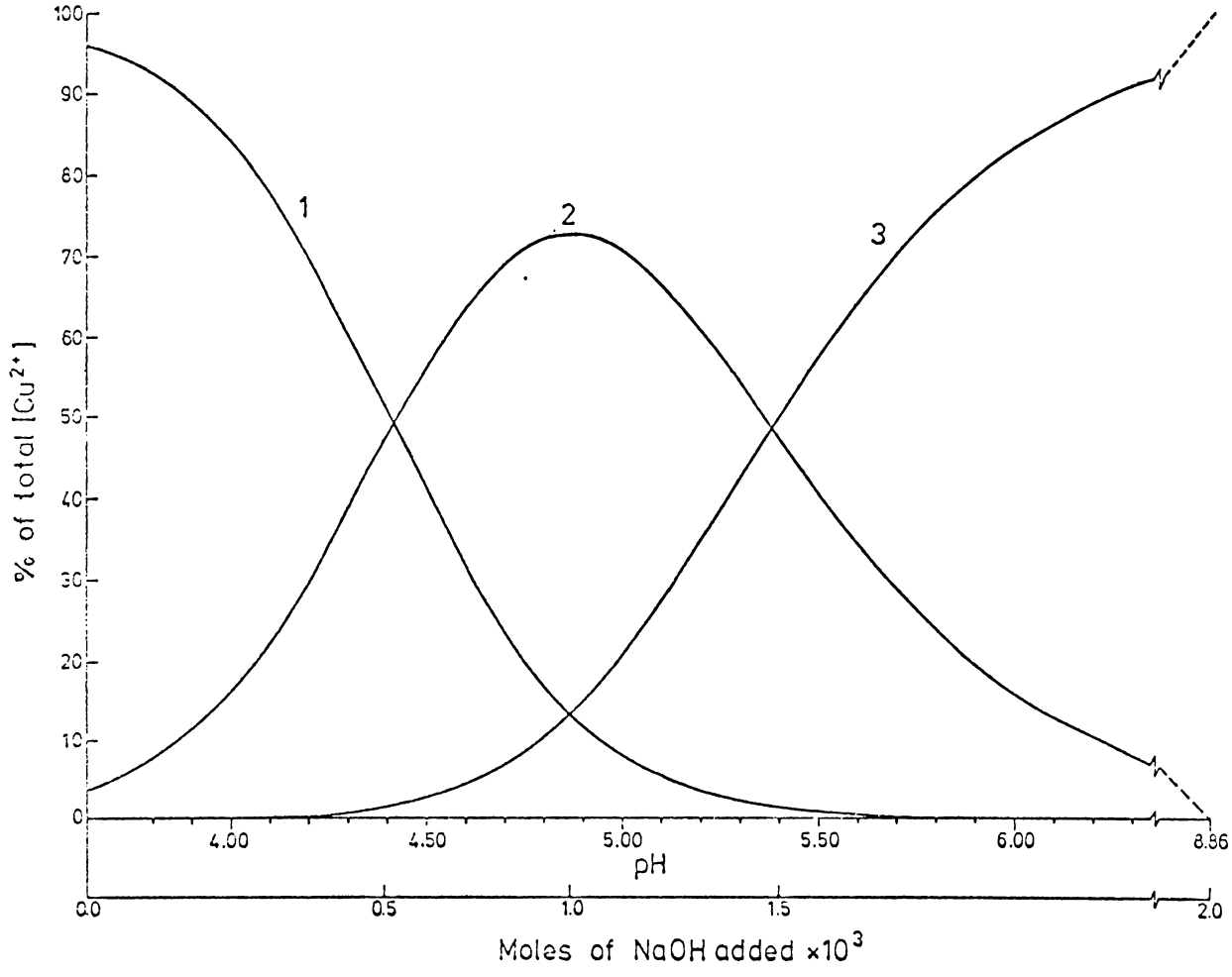


Figure 7: Effect of pH on species present in Cu(II):en = 1:2 solutions; T = 25°C I = 0.10molℓ^{-1}(KCl). $[Cu^{2+}] = 5 \times 10^{-3}mol\ell^{-1}$; $[en] = 10^{-2}$molℓ^{-1}.

1. free Cu^{2+} 2. $Cu(en)^{2+}$ 3. $Cu(en)_2^{2+}$

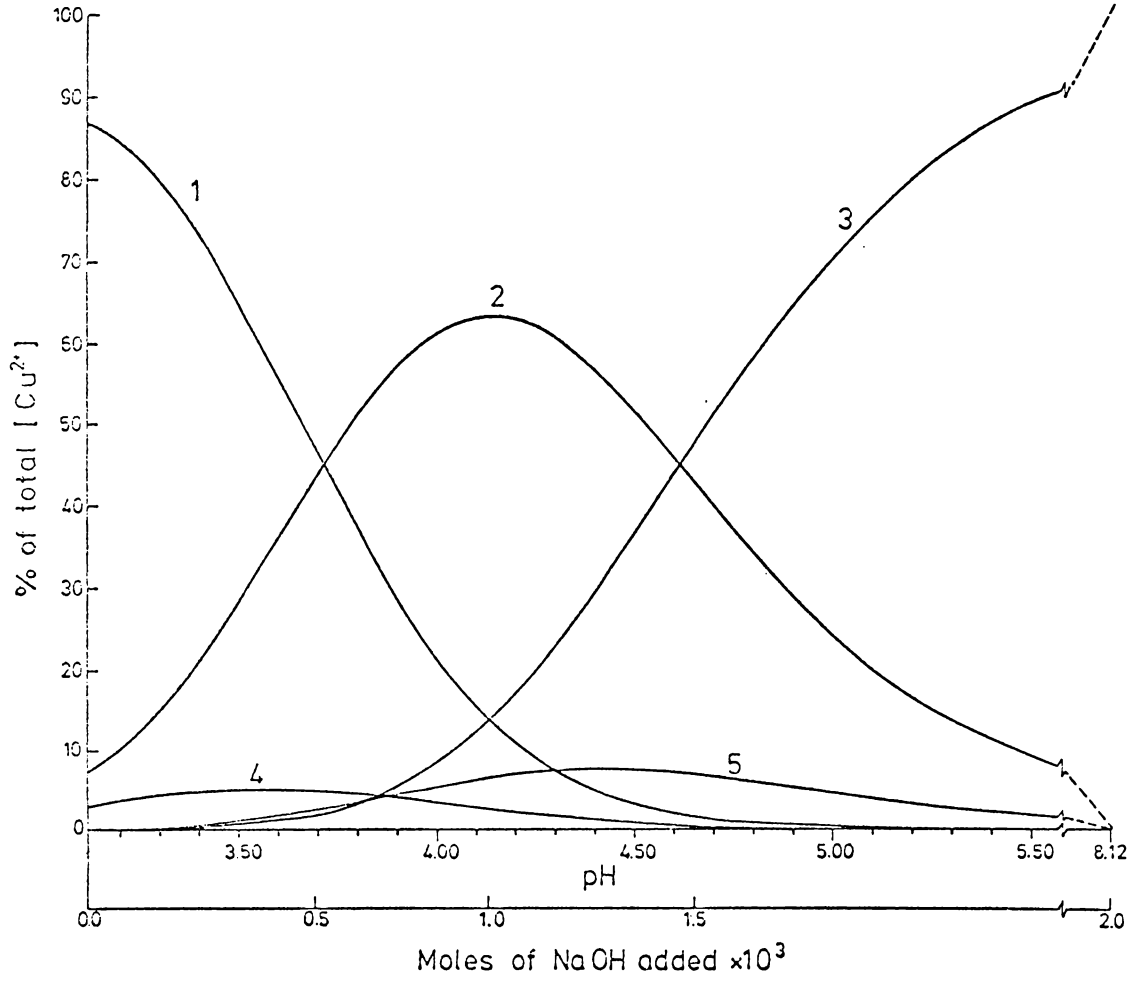


Figure 8: Effect of pH on species present in Cu(II): Me 4,5-dape = 1:2 solutions; T = 25°C I = 0.10molℓ^{-1} (KCl). $[Cu^{2+}] = 5 \times 10^{-3}mol\ell^{-1}$; [Me 4,5-dape] = 10^{-2}molℓ^{-1}.

1. Free Cu^{2+}
2. CuE^{2+}
3. CuE_2^{2+}
4. $Cu(EH)^{3+}$
5. $CuE(EH)^{3+}$

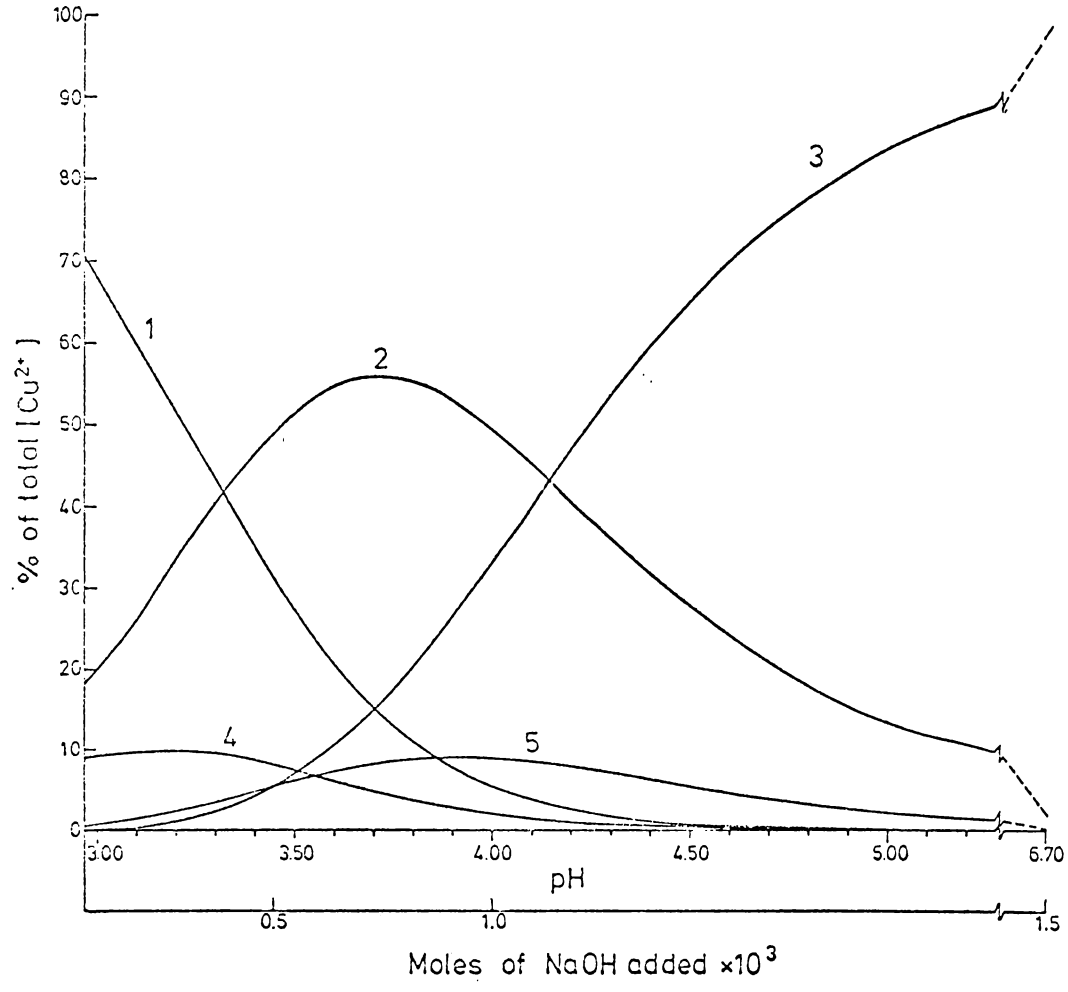


Figure 9: Effect of pH on species present in Cu(II): Me 2,3-dap = 1:2 solutions; T = 25°C I = 0.10molℓ^{-1}(KCl) $[Cu^{2+}] = 5 \times 10^{-3}mol\ell^{-1}$; [Me 2,3-dap] = 10^{-2}molℓ^{-1}.

1. Free Cu^{2+}
2. CuE^{2+}
3. CuE_2^{2+}
4. $Cu(EH)^{3+}$
5. $CuE(EH)^{3+}$

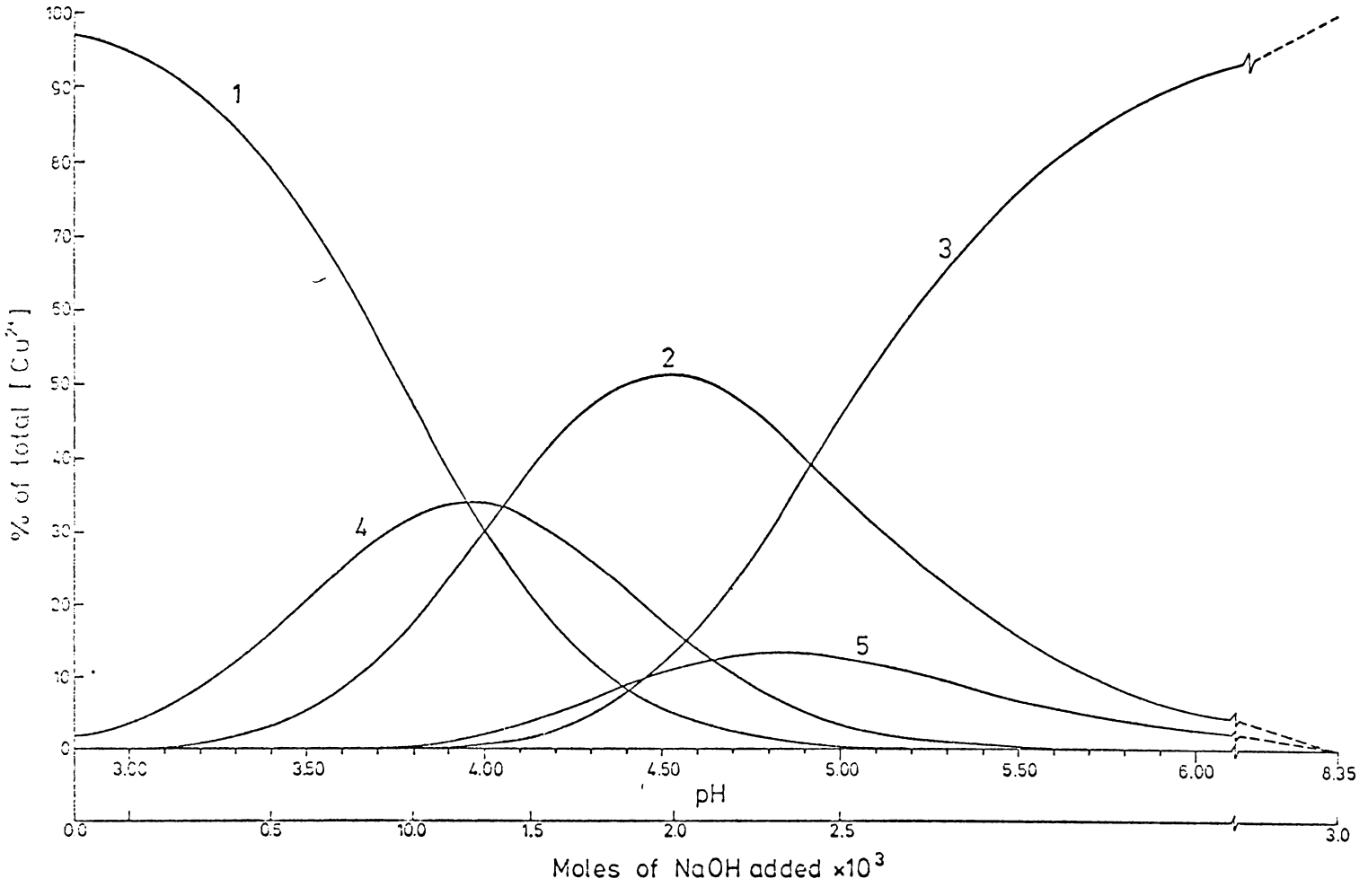


Figure 10: Effect of pH on species present in Cu(II): 4,5-dape = 1:2 solutions; T = 25°C I = 0.10molℓ^{-1} (KCl).
$[Cu^{2+}] = 5 \times 10^{-3}mol\ell^{-1}$; [4,5-dape] = 10^{-2}molℓ^{-1}.

1. Free Cu^{2+}
2. CuA^{+}
3. CuA_2
4. $Cu(AH)^{2+}$
5. $Cu(AH)A^{+}$

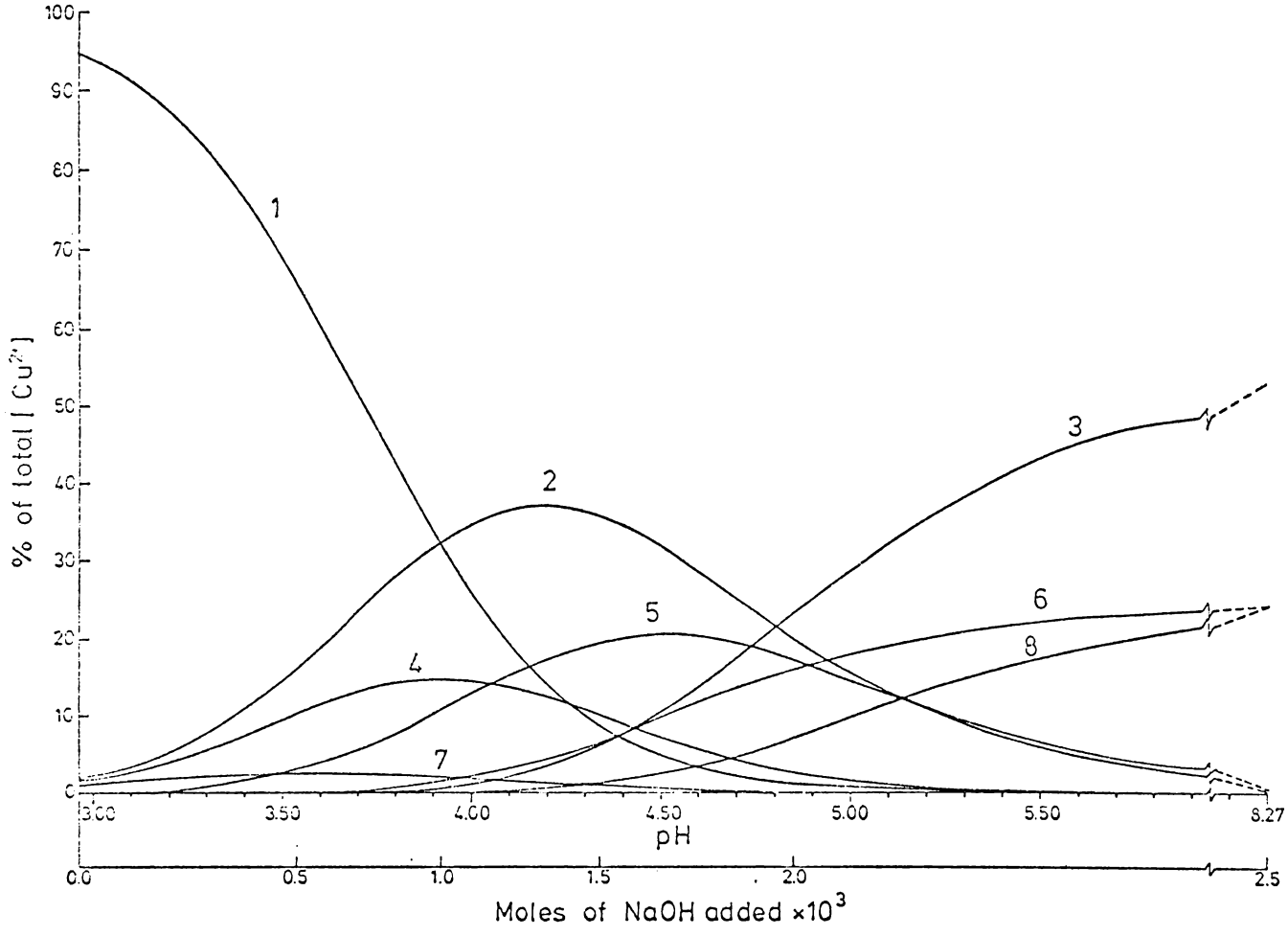


Figure 11: Effect of pH on species present in Cu(II): Me 4,5-dape: 4,5-dape = 1:1:1 solutions; T = 25°C
I = 0.10molℓ^{-1} (KCl). [Cu^{2+}] = [Me 4,5-dape] = [4,5-dape] = 5 × 10^{-3}molℓ^{-1}.

1. Free Cu^{2+}
2. CuE^{2+}
3. $CuEA^{+}$
4. $Cu(AH)^{+}$
5. CuA^{+}
6. CuE_2^{2+}
7. $Cu(EH)^{3+}$
8. CuA_2

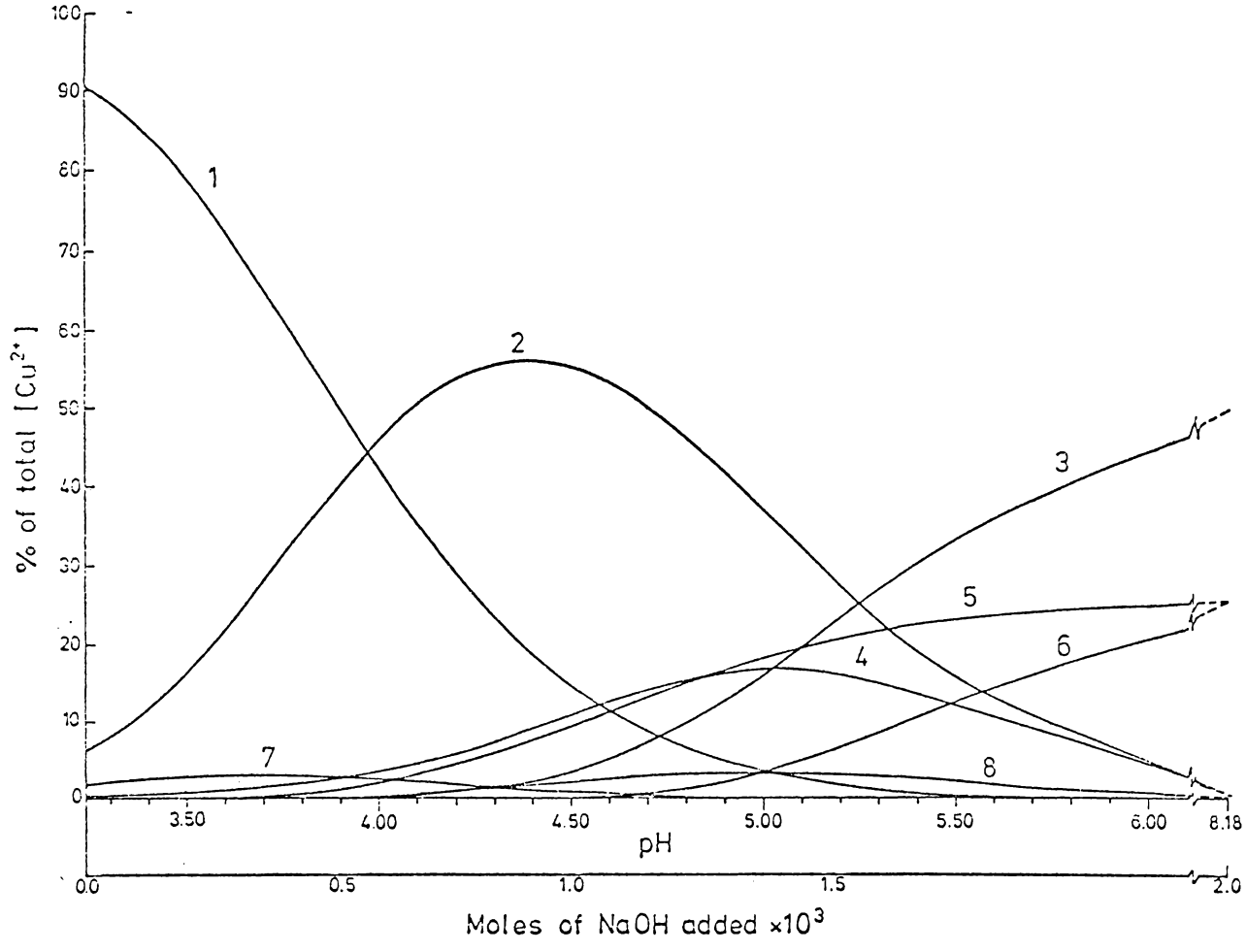
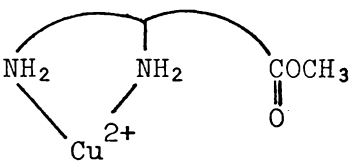


Figure 12: Effect of pH on species present in Cu(II): Me 4,5-dape: en = 1:1:1 solutions; T = 25°C I = 0.10molℓ^{-1}(KCl). [Cu^{2+}] = [Me 4,5-dape] = [en] = 5×10^{-3}molℓ^{-1}.

1. Free Cu^{2+}
2. CuE^{2+}
3. $CuE(en)^{2+}$
4. $Cu(en)^{2+}$
5. CuE_2^{2+}
6. $Cu(en)_2^{2+}$
7. $Cu(EH)^{3+}$
8. $Cu(EH)(en)^{3+}$

5.3.1 Ester Complexes

Coordination between the carbonyl oxygen of α-amino acid esters and a metal ion has been shown[25] to be very weak or absent. The esters generally act as monodentate ligands with metal ion-nitrogen bonding only. Thus the diamino acid esters listed in Table 7 presumably form chelate complexes bonding through the two amino N's with the ester group "dangling" (46) and are monodentate in protonated complexes with dangling ammonium and ester groups (47)



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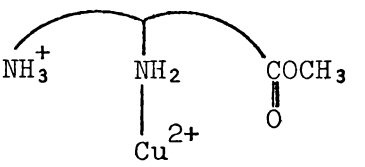

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Considering Me 4,5-dape and Me 2,3-dap as substituted 1,2-diamino-ethanes, Figures 7-9 show an increase in the variety and amounts (% of total $[Cu^{2+}]$) of protonated complexes in the order

en < Me 4,5-dape < Me 2,3-dap.

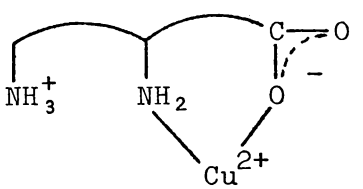
The reverse order is observed in the pKa values as the base weakening $-COOCH_3$ is moved closer to the NH_2's (Section 4.4 and Table 7). En has the highest pKa's (lowest Ka's), i.e. binds more strongly to H^+ and consequently binds most strongly to Cu^{2+}. Consequently en shows the greatest tendancy to form chelate complexes and the least tendancy to form protonated complexes. In fact for en the percentage of the species $Cu(enH)^{3+}$ is too small to allow convergence of its stability constant. On the other hand, Me 2,3-dap has the lowest pKa's (highest Ka's) and hence shows the least tendency to chelate and thus can be monodentate with a dangling ammonium group.

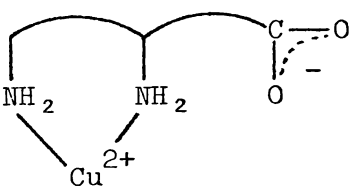
A parallel trend is observed between a decrease in pKa values for en, Me 4,5-dape and Me 2,3-dap (Table 7) and an increase in the extent of protonated complex formation (Figures 7-9). Also the usual parallel between decrease in pKa values (Table 7) and decrease in log β (Table 8) is observed for both simple and protonated complexes in the order

en > Me 4,5-dape > Me 2,3-dap.

5.3.2 Diamino Acid Complexes

The diamino acid ligands have three potential coordination sites, the two amino groups and a carboxylate oxygen. Visible spectra of some Cu(II)-diaminoacid systems[81] show a small shift to longer wavelength of the absorption bands compared with only bidentate (2N) coordination, which it is claimed indicates apical carboxylate interaction as a third coordination site. Generally, however, it is considered[32,80] that the preference of Cu(II) for tetragonal (distorted square planar) coordination means that Cu(II) cannot coordinate simultaneously to all three donor atoms of a tridentate ligand such as a diamino acid (2N's, 1 O). Consequently, as the pH of a solution containing Cu(II) and a diamino acid is raised, the initial coordination (pH <6) through $-COO^-$ and the adjacent NH_2 (with dangling $-NH_3^+$) changes to coordination through the two amino groups at higher pH.

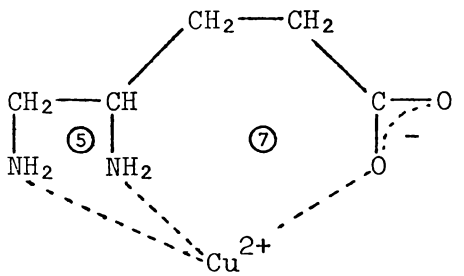




The presence of chelation at lower pH is responsible for the greatly increased stability constants of protonated complexes of diamino acids over their respective esters or en (Table 8). The effect is apparently not due to a decrease in pKa values as for diamino acid esters (Section 5.3.1) since these values are very similar to those for en (Table 7).

The result is an increased percentage of the protonated complexes $Cu(AH)^{2+}$ and $Cu(AH)A^{+}$ and a corresponding decreased percentage of CuA^{+} for 4,5-dape (Figure 10) compared with the analogous complexes for Me 4,5-dape (Figure 8) and en (Figure 7).

The relationship between stability of a complex and the size of the chelate ring formed is reflected in the amounts of the various complexes of Cu(II) and the ligands 4,5-dape (48), 2,3-dap (49) and 2,4-dab (50) (the circled number indicates the size of the potential chelate ring).



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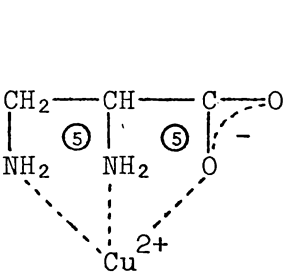

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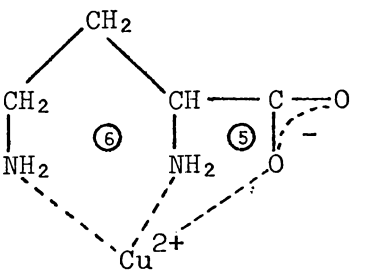

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Table 12 lists the maximum percentage of total $[Cu^{2+}]$ reached by the various complexes of these ligands at the indicated pH.

4,5-Dape (7 membered chelate ring) forms less $Cu(AH)^{+}$ (N and O donor atoms) than 2,3-dap or 2,4-dab (both 5 membered chelate rings.) However less CuA^{+} (2N donor atoms) is formed for 2,4-dab (6-ring) than for 4,5-dape or 2,3-dap (both 5-rings).

Another factor which must also be considered in the protonated complexes is the electron withdrawing and metal ion repelling effect of the dangling ammonium group. In 4,5-dape and 2,3-dap this group is

Table 12: Percentages of protonated and non protonated complexes of Cu(II) and the ligands 4,5-dape, 2,3-dap and 2,4-dab.

Species	Maximum % Total $[Cu^{2+}]$					
	4,5-dape[a]		2,3-dap[b]		2,4-dab[b]	
	%	pH	%	pH	%	pH
$Cu(AH)^{2+}$	35	3.95	69	3.6	67	4.1
$Cu(AH)_2^{2+}$					54	5.5
CuA^{+}	52.5	4.55	52	4.7	1.2	6.8
$Cu(AH)A^{+}$	14	4.8	26	5.3	71	7.25
CuA_2	% increase steadily as pH raised.					

(a) This work (b) Reference 80

adjacent to the sites of metal ion bonding, whereas in 2,4-dab it is separated by a further methylene group. A lowering of the value of log β_1' and log β_m, by two log units, for 4,5-dape and 2,3-dap compared with 2,4-dab (Table 8) may be attributed to this factor.

The combined effect of the relative separation of the dangling ammonium group from the metal ion binding site and the most favourable chelate ring size results in $Cu(AH)_2^{2+}$ being a major species for 2,4-dab (Table 11) while, for 4,5-dape and 2,3-dap, its concentration is too small (<0.5%) to allow convergence of its stability constant. The concentration of CuA^{+} is correspondingly reduced in 2,4-dab. The amount of $Cu(AH)A^{+}$ increases in the order 4,5-dape (5-ring and 7-ring), 2,3-dap (2 5-rings), 2,4-dab (5-ring and 6-ring) and reflects a combination of these two effects.

5.3.3 Mixed Complexes

Figures 11 and 12 show the effect of pH on the computed composition of mixed ligand species in solutions of Cu(II), 4,5-dape, Me 4,5-dape and Cu(II), en, Me 4,5-dape = 1:1:1. Only major species are shown, minor species reach maximum concentrations (% total $[Cu^{2+}]$) at the following pH values; $Cu(AH)A^{+}$, pH 4.85, 3%; $Cu(AH)E^{2+}$, pH 4.63, 7.2%; $Cu(EH)E^{3+}$, pH = 4.4, 2.1%; $Cu(EH)(AH)^{3+}$, pH 4.16, 0.5%. For the en system; $Cu(enH)(en)^{3+}$, pH 5.32, 0.15%.

From the arguments of Sections 5.3.1 and 5.3.2 the species $CuEAH^{2+}$ has the composition $Cu(AH)E^{2+}$ while $CuEenH^{3+}$ is probably $Cu(en)(EH)^{3+}$

It has been observed[85] that the stability constant for the addition of a neutral bidentate ligand to a 1:1 complex of Cu(II) and an ionic bidentate ligand is similar in magnitude to the value for addition of that ligand to $Cu^{2+}_{(aq)}$. The converse has also been observed[85] but if both ligands are anionic or neutral the constant for the addition of the second ligand is smaller. A statistical correction (0.60 = log 4) should be subtracted from the constant for the addition of a ligand to $Cu^{2+}_{(aq)}$ since there are 4 ways pairs of H_2O ligands can be displaced from $Cu^{2+}_{(aq)}$ by a bidentate ligand, whereas in a 1:1 complex there is only one way.

In the present work:- (E = Me 4,5-dape, A = 4,5-dape)

$\log \beta_1(CuE^{2+}) = 9.84 - 0.60 = 9.24$

$\log \beta\ (CuA^{+}+E) = \log \beta_{111}(CuEA) - [\log \beta_1(CuA) - 0.60]$

$= 19.68 - 10.34$

$= 9.34$

i.e. a difference in log β of 0.1.

For the converse process the difference is

$\log \beta(CuE^{2+}+A) - \log \beta\ (CuA^{+}) = 10.44 - 10.34$

$= 0.10.$

For the Cu(II):E:en = 1:1:1 system where both ligands are neutral, the

same differences (0.10) are observed, i.e. the constant for the addition of the second neutral ligand is similar to that observed for the anionic 4,5-dape. Clearly, although 4,5-dape is negatively charged, this charge is sufficiently removed from the binding site to make this ligand behave similarly to both Me 4,5-dape and en.

In both the mixed systems studied, the species CuEL (L = 4,5-dape or en) has a stability constant intermediate between that of CuE_2 and CuL_2 which is the result expected statistically. A similar result is also seen for $CuEA^+$ in MeHis, His[33] and Me 2,3-dap, 2,3-dap[32] systems. In some mixed bidentate amino acid systems such as the Cu(II)-histidine-threonine[86] and the Cu(II) - histidine-serine[87] systems stabilisation of the mixed complexes over the statistically expected values have been observed. Similar stabilisations are seen in mixed Cu(II) complexes of histamine and serine[85] , and histamine and a variety of α-amino acids[88] The increased stabilisation apparently arises[88] from entropy and enthalpy effects (favouring mixed species) which are presumably absent in the present work.

5.4 Summary

Tables 8 and 11 summarise the stability constants obtained by potentiometric titration. The following points are useful in interpreting the kinetic data:

1. In 1:2 solutions of Cu(II) and Me 4,5-dape only CuE_2^{2+} is present (100% of total $[Cu^{2+}]$) at pH values where hydrolysis is measurable (pH >9).

2. In 1:1 solutions of Cu(II) and Me 4,5-dape, disproportionation of the complex CuE^{2+} occurs to give free Cu^{2+} (which precipitates as $Cu(OH)_2$) and CuE_2^{2+}, at pH values where hydrolysis can be sutdied (pH >8.5).

3. In mixed 1:1:1 solutions of Cu(II), Me 4,5-dape and 4,5-dape or en, the mixed complexes CuEL (L = 4,5-dape or en) are the major species but there are significant percentages of the 1:2 complexes. The percentages are for Cu^{2+}: Me 4,5-dape: 4,5-dape, $CuEA^{+}$(52.15%), CuE_2^{2+} (23.83%), CuA_2(23.81%) and for Cu^{2+}: Me 4,5-dape:en, $CuE(en)^{2+}$ (49.34%) CuE_2^{2+} (25.21%) and $Cu(en)_2^{2+}$ (25.15%). Consequently, during hydrolysis of CuE_2^{2+}, disproportionation of the reaction product $CuEA^{+}$ to CuE_2^{2+} and CuA_2 cannot be ignored. Clearly, disproportionation can be repressed by addition of excess CuA_2 which drives the equilibrium

$$2\ CuEA^{+} \rightleftharpoons CuE_2^{2+} + CuA_2$$

to the left.

6. UNCATALYSED HYDROLYSIS OF METHYL 4,5-DIAMINOPENTANOATE

6.1 Introduction

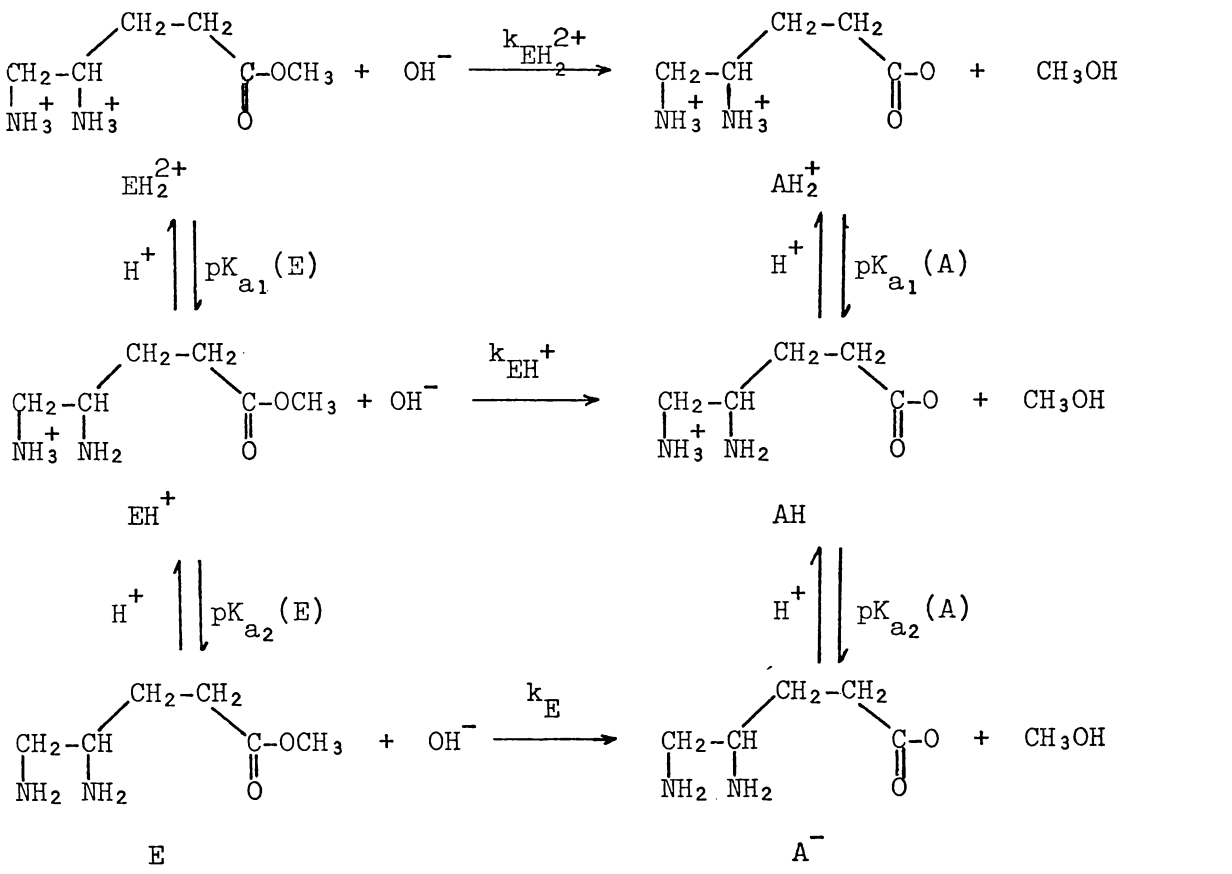
The rate constant (k_E) for the base hydrolysis (i.e. for the uncatalysed reaction) of the neutral form of Me 4,5-dape is needed so that a comparison can be made with the rate constants for the metal ion catalysed reactions, to give the catalytic effect of the metal ion.

It was apparent from titrations for pK_a determination (Section 4.2) that hydrolysis of Me 4,5-dape occurs extremely rapidly at low pH. Also, attempts to follow this hydrolysis by pH-stat resulted in very little uptake of NaOH. Examination (tlc) of the products, after hydrolysis was complete, suggested that both the free acid 4,5-dape and the lactam 5-(aminomethyl)-2-oxopyrrolidine had been formed. Clearly, intramolecular aminolysis (lactamisation) is occurring in competition with base hydrolysis. A similar result has been observed[89] for methyl 4-aminobutanoate, methyl 2,4-diaminobutanoate and methyl 2,5-diaminopentanoate (methyl ornithinate)

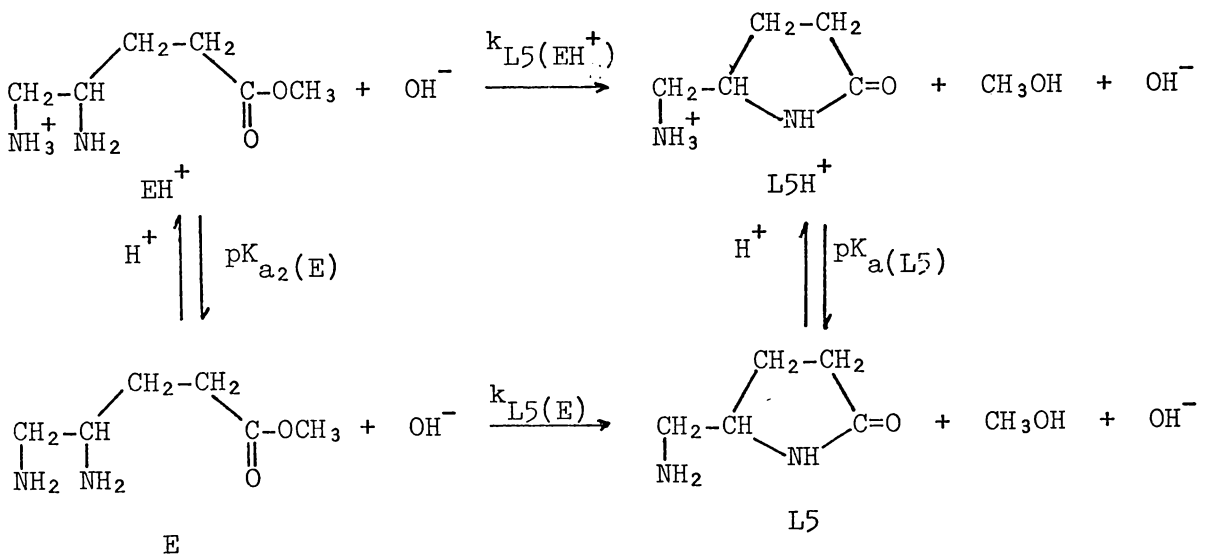
The hydrolysis of the Me 4,5-dape is complicated since it is possible for the neutral ester (E) to cyclise to either a 5-membered ring lactam, 5-(aminomethyl)-2-oxopyrrolidine (L5) or a 6-membered ring lactam 5-amino-2-oxopiperidine (L6) (see Scheme 9). The monocation (EH^+) can cyclise only to L5 while EH_2^{2+} cannot lactamise. Also, the diamino acid product of base hydrolysis can cyclise to either of the two lactams. However this process was shown to be very slow in comparison to the rate of ester hydrolysis (Section 6.4). These lactamisation reactions involve no nett consumption of NaOH. Scheme 9 summarises the ionisation equilibria and hydrolytic reactions possible for Me 4,5-dape.

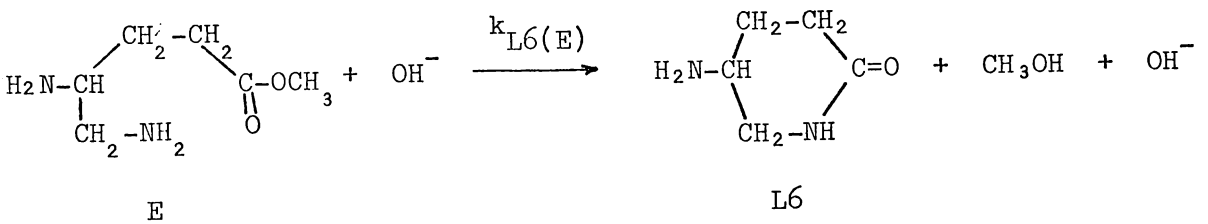
Since the lactamisation reactions are very fast and out compete simple base hydrolysis, k_E cannot be measured experimentally. Therefore an estimate was made from comparison with known rate constants for related

Scheme 9: Ionisation equilibria and hydrolytic reactions for Me 4,5-dape



Also





systems where lactamisation does not occur.

As the amino and ester functions are separated by more methylene groups, the rate of hydrolysis decreases rapidly; methyl β-alaninate hydrolyses much more slowly than methyl glycinate (Table 13). No further decrease in rate is observed in going to methyl 6-aminohexanoate. In fact these esters hydrolyse at a similar rate to methyl acetate.

Table 13: Effect of additional methylene groups on ester hydrolysis rate. (T = 25°C and I = 0.10mol ℓ^{-1}).

Ester	Structure	k_E (ℓ $mol^{-1}s^{-1}$)
Methyl glycinate[a]	$CH_2CO_2CH_3$ | NH_2	1.28
Methyl β-alaninate[a]	$CH_2CH_2CO_2CH_3$ | NH_2	0.14
Methyl 6-aminohexanoate[b]	$CH_2(CH_2)_4CO_2CH_3$ | NH_2	0.15
Methyl acetate[c]	$CH_3CO_2CH_3$	0.14

(a) Ref 90 (b) Ref 31 (c) Ref 91

Assuming that the decrease in hydrolysis rate is due solely to the decrease in -I effect exerted by the amino group, and that entropy effects are fairly similar throughout the series, k_E for methyl 4,5-diaminopentanoate can be estimated as about 0.14 ℓ $mol^{-1}s^{-1}$, at 25°C.

In addition, the rate constants for the lactamisation reactions and base hydrolysis of EH_2^{2+} and EH^+ are of interest. The lactamisation rate constants can be compared with literature values for other cases of intramolecular aminolysis, to assist in understanding the factors which

affect the very high rate constants for these reactions. The lactamisation reactions of E provide an interesting competition between 5 and 6-membered ring closure involving an amino group. The rate constants for base hydrolysis of EH^{+} and EH_2^{2+} would provide a comparison of the catalytic effects of a proton which may be compared with the effect of a metal ion.

The hydrolysis of Me 4,5-dape was followed both by pH-stat and spectrophotometric methods. The pH-stat method suffered from the low base consumptions involved (down to 10% of the theoretical amount) due to the lactamisation reaction. As a check on the pH-stat rate constants, they were also measured by following the appearance of the lactam -NHCO- band in the UV, using buffers to control pH. This method suffers from the usual problems of buffer catalysis (general base in this case) and the limited accuracy associated with working on the side of a charge transfer band.

Both methods and an analysis of the data obtained are discussed in the following sections.

6.2 pH-stat method

The standardisation and operation of the pH assembly as a pH-stat has been described (Section 2.4). A solution of Me 4,5-dape (10^{-3}mol), such that I = 0.10 mol ℓ^{-1} at half neutralisation (Section 4.2) was prepared and allowed to equilibrate to 25°C. Sodium hydroxide (1.000 mol ℓ^{-1}) was added slowly to bring the solution to the required pH. The titrator was started and the reaction followed for at least 10 half-lives. The pH was maintained to within ±0.005pH of the desired value by manually adjusting the "end point" control.

These reactions were pseudo-first-order at constant pH ("infinity" plots covering at least 4 half-lives were plotted and were linear), and

the volume of NaOH versus time data was analysed by a non-linear least squares computer program[92] run on a PDP 11/05 computer. The results obtained are summarised in Table 14.

The pH range studied was restricted by the calibration stability and electrode response time of the pH-stat. Below pH6.3 (half life >100min) the reactions were too slow to be followed because of slow electrode calibration drift, and above pH8.4 (half life <1min) the reaction times were too short compared with the response time of the electrodes. These difficulties were compounded by the low volumes of NaOH consumed at infinite time (V_∞). Because of the competing lactamisation reaction the infinity values (Table 14) were generally small (1.000ml 1.000molℓ^{-1} NaOH expected theoretically) and pass through a minimum at pH7.3 corresponding to the maximum concentration of EH^+ (Figure 14) which will be near the maximum extent of lactamisation.

As a check on technique and calibaration of the pH-stat, methyl serinate was hydrolysed at pH 10.60, 10.80 and 11.00. The mean value obtained (k_E = 1.00 ± 0.03$\ell mol^{-1}s^{-1}$) agrees well with the literature value of 0.99$\ell mol^{-1}s^{-1}$ (25°C, I = 0.10molℓ^{-1})[90].

6.3 Spectrophotometric method

The rate of lactamisation of Me 4,5-dape was followed by increase in UV absorption of the reaction solution at 210nm. Although on the side of an intense charge transfer band the reactions were followed at this wavelength for two reasons. The λ_{max} values for the lactam products lie in the region of absorption of the solvent, water, and air (<200nm). Also the extinction coefficients of the products are very high. Therefore to allow concentrations as similar as possible to the pH-stat reactions to be used but at the same time to observe a large total absorption change the reactions were followed at 210nm. Figure 13 shows the UV spectrum of the reaction solution before and after reaction at pH 7.3.

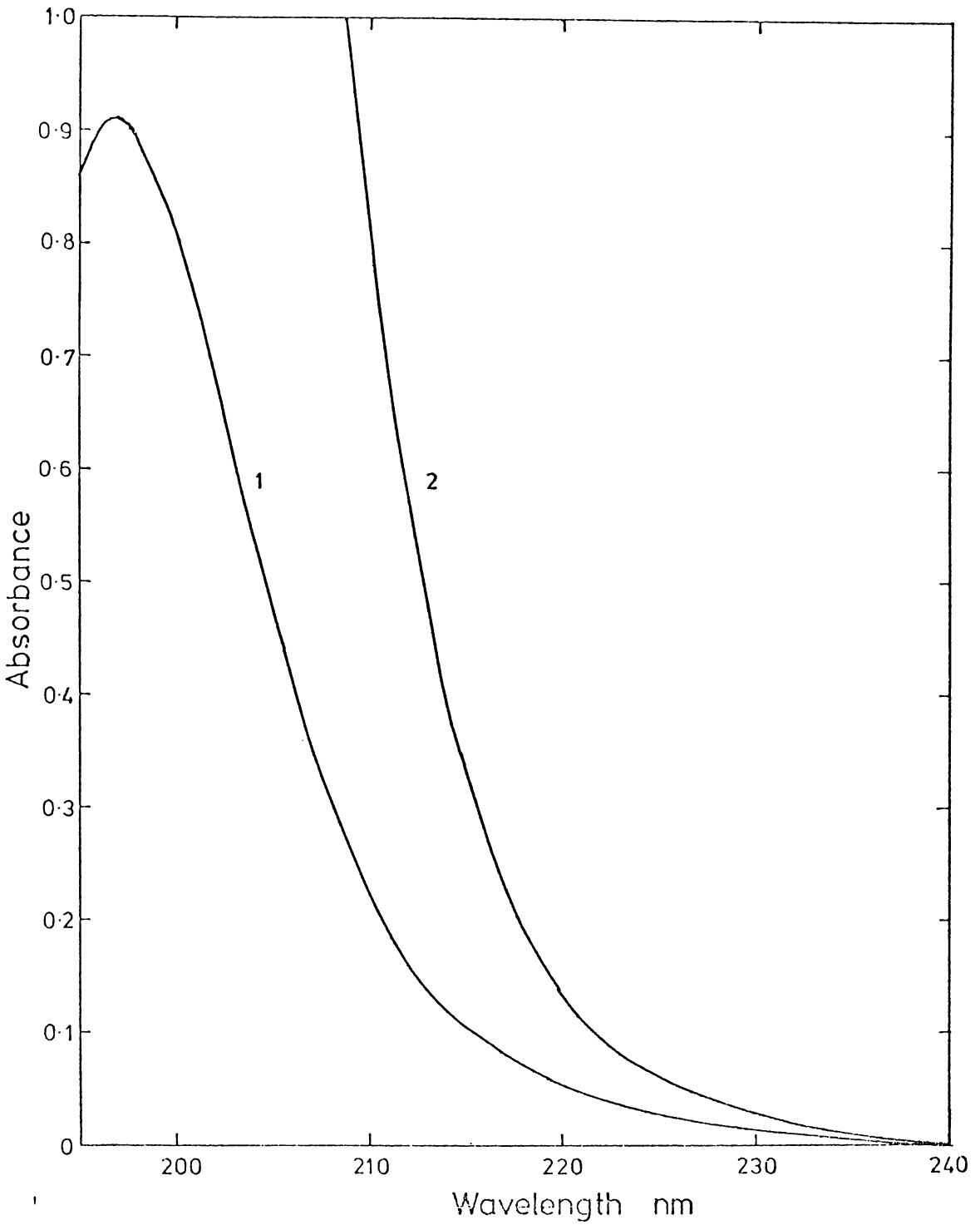


Figure 13: UV spectrum of reactant and products before and after reaction at pH 7.3 .
1. Me 4,5-dape 2. Lactam products.

Table 14: Observed rate constants for base hydrolysis of Me 4,5-dape; T = (25 ± 0.05)°C I = 0.10mol ℓ^{-1}(KCl)

pH-stat Method				Spectrophotometric Method		
pH	$10^3 \times k_{obs}$ (s^{-1})	$10^{-3} \times k_{obs}/[OH^-]$ ($\ell mol^{-1} s^{-1}$)	V_∞ (ml)	pH	$10^3 \times k_{obs}$ (s^{-1})	$10^{-3} \times k_{obs}/[OH^-]$ ($\ell mol^{-1} s^{-1}$)
6.30	0.12	4.45	0.452	6.31	0.12	4.43
6.50	0.20	4.80	0.351	6.51	0.25	5.80
6.70	0.35	5.30	0.249	6.72	0.41	5.95
7.00	0.69	5.27	0.179	7.01	0.81	6.02
7.30	1.23	4.72	0.148	7.31	1.68	6.28
7.50	1.85	4.47	0.166	7.50	2.66	6.43
7.60	2.35	4.52	0.162	7.66	2.80	4.69
7.80	3.53	4.28	0.221			
8.00	5.10	3.90	0.222	8.04	6.02	4.20
8.20	8.04	3.87	0.264			
8.40	11.41	3.47	0.301	8.31	10.82	4.05
				8.86	35.79	3.78
				9.31	62.10	2.33
				9.50	75.19	1.82

The pHs of the reaction solutions were controlled by the buffers: phosphate (pH 6.0-7.5), trishydroxymethylaminomethane (tris, pH 7.6-8.9) and borate (pH 9.3, 9.5). These were made up[96] to 0.02 mol ℓ^{-1}, the minimum strength possible to give sufficient buffer capacity to maintain constant pH. The ionic strength was brought up to 0.1 mol ℓ^{-1} by added 1 mol ℓ^{-1} KCl solution.

The reactions were studied by the following technique. Two cuvettes filled with buffer, were equilibrated to 25°C in the sample and reference chambers of the spectrophotometer. The recorder pen was zeroed, the sample cuvette lifted out and ester solution (2.5μℓ, 4.56×10^{-1} mol ℓ^{-1} in CH_3OH)

injected into the buffer. The cuvette was rapidly shaken, replaced in the spectrophotometer and the reaction recorded for at least 10 half-lives. The concentration of ester in these reaction solutions (5×10^{-4}mol ℓ^{-1}) is 1/20 of that used in the pH-stat reactions. Higher concentrations of ester exceeded the buffer capacity of the solution (purposely restricted to minimise buffer catalysis and absorption problems) and a constant pH could not be maintained.

The pH of the reaction solution was checked when hydrolysis was complete. Also parallel reactions were run, monitored by the pH meter, for each buffer to check that pH remained constant throughout the reaction.

The reactions were pseudo-first-order at constant pH and the rate data was analysed by computer in the same way as for the pH-stat reactions. The results obtained are summarised in Table 14.

The following control reactions were also carried out.

1. The reaction was followed at pH 7.0 at 218nm and 210nm to check what effect the wavelength used had on the rate constant. The observed rate constants agreed within experimental error (±5%) ($k_{obs}^{210nm} = 8.05 \times 10^{-4}s^{-1}$, $k_{obs}^{218nm} = 8.27 \times 10^{-4}s^{-1}$).

2. Each buffer was checked for buffer catalysis effects by following reactions at various buffer concentrations (0.02, 0.04, 0.06 and 0.08 mol ℓ^{-1}) at constant pH. No buffer catalysis was observed for phosphate or tris but a large effect was seen with borate. Borate buffers often show considerable effects over and above general base catalysis (e.g. see Ref 4, p.30). The rate constants summarised in Table 14 are all for buffer concentrations = 0.02mol ℓ^{-1}. However, it became clear from comparison with the pH-stat results, that some buffer catalysis occurred for phosphate, even although the measured rate constants were independent of the phosphate concentration.

The reactions were studied to a much higher pH than was possible for the pH-stat reactions; the technique used allowed reactions up to pH 9.50 (half-life 9s) to be followed.

6.4 Discussion

6.4.1 Products

The reaction products were difficult to establish unequivocally, because the diamino acid formed tends to lactamise very readily during concentration of the reaction solutions. Three products appear (tlc) to be formed at all pH values studied. These are 4,5-dape, 5-(aminomethyl)-2-oxopyrrolidine (L5) and a third compound with similar R_f to L5. This is probably 5-amino-2-oxopiperidine (L6). Because of the equivocal nature of the product analysis and the difficulties of chromatographic separation (very similar R_f values) isolation of the products was not attempted.

6.4.2 Spectrophotometric Results

The $k_{obs}/[OH^-]$ values observed in phosphate and tris buffers are consistently higher than those from the pH-stat for a given pH (Table 14). Apparently general base catalysis by the buffer is occurring although this effect could not be measured by varying the buffer concentration. A buffer effect was observed for the two runs in borate buffer (pH 9.3 and 9.5) but the actual extent of this is uncertain since these reactions were too fast for the pH-stat. It has been observed for borate buffers[93] that the catalysis increases initially with increasing buffer concentration but becomes constant at higher buffer concentrations (>0.08mol ℓ^{-1}). Presumably the minimum buffer concentration (0.02mol ℓ^{-1}) required to maintain a constant pH for phosphate and tris buffers lies in such a plateau region so that no further catalysis is observed with higher buffer concentrations for these buffers.

There were additional difficulties with the spectrophotometric method. The reactions had to be followed on the side of the charge transfer absorption band (Section 6.3) which gave poor reproducibility in total absorbance. Also two lactams were formed of which only 5-(aminomethyl)-2-oxopyrrolidine was available as a pure sample. No ε_{max} values for the products could, therefore, be obtained and hence the percentage of lactamisation could not be determined. However the very low consumption of NaOH in the range pH 7-8 in the pH-stat reactions suggests that the extent of lactamisation is large in comparison with the base hydrolysis reaction.

The rate of lactamisation of the product diamino acid, 4,5-dape, in the various buffers, was checked spectrophotometrically. It was found to be very slow at all pH values and so does not interfere with the ester reaction.

The spectrophotometric data was not analysed because of the buffer catalysis problems; it was used only to check that the pH-stat rate constants measured under conditions of low base consumption were 'sensible' (particularly pHs $\sim$7.5)

6.4.3 pH-stat Results

The effect of pH on the concentrations of the ester species EH_2^{2+}, EH^+ and E was found, using the computer program SCOGS[75] (Appendix 2.1), from the pH-titration data for Me 4,5-dape. 2HCl. The result (Figure 14) is useful in analysing the observed pH/rate profile. This can be divided into three regions.

1. pH $\leqslant$ 7: The hydrolysis of only EH_2^{2+} and EH^+ is presumably important (since [E] is very low) and the following rate equation can be written (refer to Scheme 9)

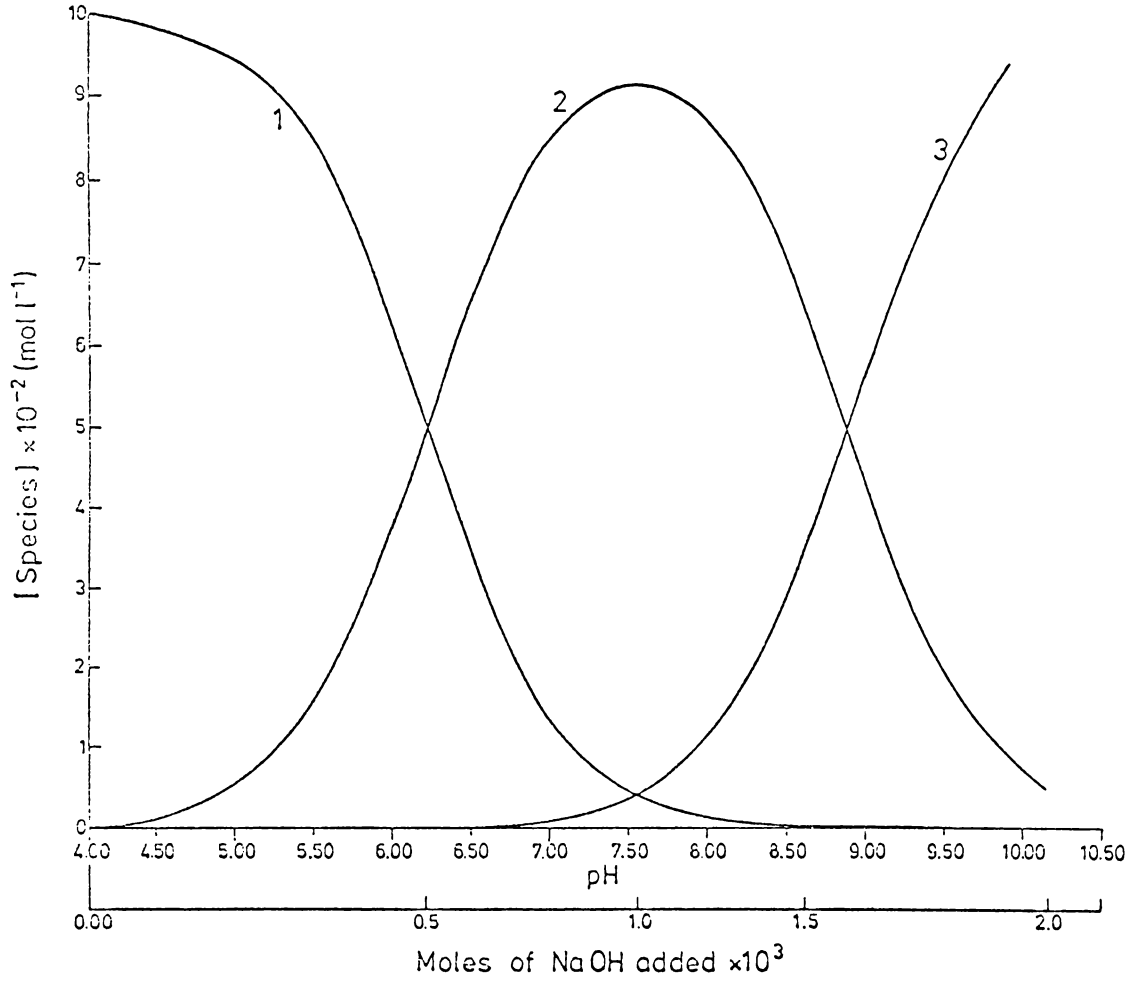


Figure 14: Effect of pH on species present in Me 4,5-dape solutions; T = 25°C I = 0.10molℓ^{-1}(KCl)
[Me 4,5-dape] = 10^{-2}molℓ^{-1} at half neutralisation.

1. EH_2^{2+} 2. EH^{+} 3. E

$$-\frac{d}{dt}[\text{Ester}]_T = k_{EH_2^{2+}}[EH_2^{2+}][OH^-] + k_{EH^+}[EH^+][OH^-] + k_{L5(EH^+)}[EH^+][OH^-]$$

$$= k_{obs}([EH_2^{2+}] + [EH^+])$$

But $$[EH_2^{2+}] = \frac{y_1^2 \cdot [EH^+][H^+]}{y_2 \cdot K_{a1}^T} \quad \text{(from equation(4.3b))}$$

$$\therefore -\frac{d}{dt}[\text{Ester}]_T = k_{EH_2^{2+}} \cdot y_1^2 \cdot \frac{[EH^+][H^+]}{y_2 \cdot K_{a1}^T} \cdot [OH^-] + k_{EH^+}[EH^+][OH^-] + k_{L5(EH^+)}[EH^+][OH^-]$$

$$= k_{obs}\left(y_1^2 \cdot \frac{[EH^+][H^+]}{y_2 \cdot K_{a1}^T} + [EH^+]\right)$$

$$\therefore \frac{k_{obs}}{[OH^-]}\left(\frac{y_1^2 \cdot [H^+]}{y_2 \cdot K_{a1}^T} + 1\right) = k_{EH_2^{2+}} \cdot \frac{y_1^2 \cdot [H^+]}{y_2 \cdot K_a^T} + k_{EH^+} + k_{L5(EH^+)}$$

Thus a plot of $y = \frac{k_{obs}}{[OH^-]}\left(\frac{y_1^2 \cdot [H^+]}{y_2 \cdot K_{a1}^T} + 1\right)$ versus $x = [H^+]$ should be

linear with slope $= k_{EH_2^{2+}} \cdot \frac{y_1^2}{y_2 \cdot K_{a1}^T}$ and y-intercept $= (k_{EH^+} + k_{L5(EH^+)})$

Experimentally such a plot of the pH-stat results, was found to be linear between pH 6.3 and 6.7

$$k_{EH_2^{2+}} = 2.37 \times 10^3 \ell\ \text{mol}^{-1}\text{s}^{-1} \quad \text{(from slope)}$$

$$\left(k_{EH^+} + k_{L5(EH^+)}\right) = 6.20 \times 10^{-3} \ell\ \text{mol}^{-1}\text{s}^{-1} \quad \text{(from y-axis intercept)}$$

$$= k_{EH^+}\ \text{(total)}$$

The spectrophotometric results for pH < 7 give a steeper slope, reflecting the general base catalysis by phosphate buffer. The same y-intercept value is obtained however, suggesting the catalysis is more important for the hydrolysis of EH_2^{2+} than for EH^+.

2. pH 7-8: Although $[EH^+]$ > 85% of the total ester concentration in this pH range, hydrolysis of EH_2^{2+} and E is expected to make an important contribution to the observed rate. All six possible rate constants (Scheme 9) are involved and consequently interpretation of the data is very complex and was not attempted.

3. pH ≥ 8: Hydrolysis of only EH^+ and E is important; however analysis of the results is complicated by an uncertain value for pK_{a2}^T (an accurate value is difficult to obtain because of hydrolysis, see Section 4.2). Consequently the rate constants obtained may be somewhat inaccurate. The following rate equation can be written (refer to Scheme 9)

$$-\frac{d}{dt}[\text{Ester}]_T = k_{EH^+}[EH^+][OH^-] + k_{L5(EH^+)}[EH^+][OH^-] + k_E[E][OH^-] + k_{L5(E)}[E][OH^-] + k_{L6(E)}[E][OH^-]$$

$$= k_{obs}([EH^+] + [E])$$

But $$[EH^+] = \frac{[E][H^+]}{K_{a2}^T} \qquad \text{(from equation 4.46)}$$

Hence $$\frac{k_{obs}}{[OH^-]}\left(\frac{[H^+]}{K_{a2}^T} + 1\right) = (k_{EH^+} + k_{L5(EH^+)})\frac{[H^+]}{K_{a_2}^T} + (k_E + k_{L5(E)} + k_{L6(E)})$$

Thus a plot of $y = \frac{k_{obs}}{[OH^-]} \left(\frac{[H^+]}{K_{a2}^T} + 1 \right)$ versus $x = [H^+]$ should be linear of slope $= \frac{(k_{EH^+} + k_{L5(EH^+)})}{K_{a2}^T}$ and intercept $= k_E + k_{L5(E)} + k_{L6(E)}$

When plotted the pH-stat results gave a linear plot between pH 8.00 and 8.40 which yielded:

$$k_{EH^+} + k_{L5(EH^+)} = 4.28 \times 10^3 \text{ℓ mol}^{-1}\text{s}^{-1} = k_{EH^+} \text{ (total)}$$

and $$k_E + k_{L5(E)} + k_{L6(E)} = 1.63 \times 10^3 \text{ℓ mol}^{-1}\text{s}^{-1} = k_E \text{ (total)}$$

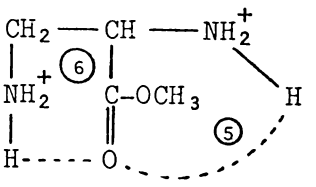
The spectrophotometric results for this pH range had a parallel slope reflecting a lower buffer catalysis effect by tris buffer. The result for $k_{EH^+} + k_{L5(EH^+)}$ (4.28×10^3) is in poor agreement with that obtained for the intercept in pH <7 range (6.20×10^3). Presumably this is due to the inaccurate K_{a2}^T value. The conclusions which may be drawn are as follows:

1. Maximum rate of lactamisation of EH^+ (5-membered ring closure $= 6.20 \times 10^3 \text{ℓ mol}^{-1}\text{s}^{-1}$.
2. Maximum rate of lactamisation of E (5 or 6-ring closure) $= 1.63 \times 10^3 \text{ℓ mol}^{-1}\text{s}^{-1}$

6.4.4. Interpretation of rate constants

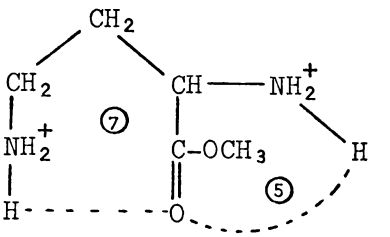
Figures 15, 16 and 17 show the structures of the various forms of diamino acid esters, along with their respective rate constants, which are useful in discussing the observed rate constants for Me 4,5-dape.

$k_{EH_2^{2+}}$: The only literature $k_{EH_2^{2+}}$ values available are those for methyl ornithinate (Me 2,5-dape) and methyl lysinate (Me 2,6-dah) (see Figure 15). The value for Me 2,6-dah ($73.5\text{ℓ mol}^{-1}\text{s}^{-1}$) is about that expected on the basis of k_{EH^+} for α-amino acid esters[90] (all k_{EH^+} ~41.7 - $83.3\text{ℓ mol}^{-1}\text{s}^{-1}$ except Me gly, $= 28.3\text{ℓ mol}^{-1}\text{s}^{-1}$). The major effect is the 1+ charge in



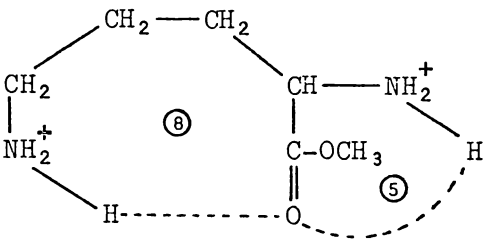
Me 2,3-dap

$k_{EH_2}^{2+}$?



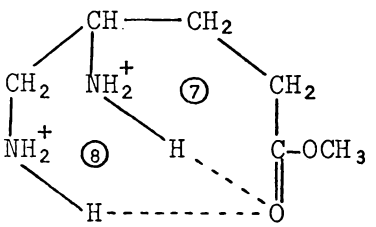
Me 2,4-dab

?



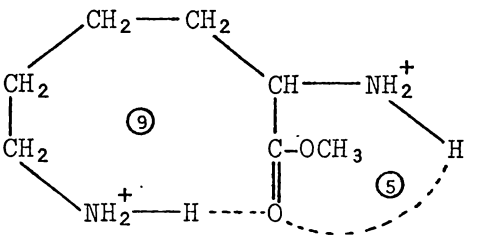
Me 2,5-dape

$k_{EH_2}^{2+} = 2083 \ell mol^{-1}s^{-1}$



Me 4,5-dape

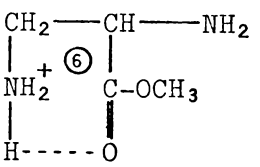
$k_{EH_2}^{2+} = 2367$



Me 2,6-dah

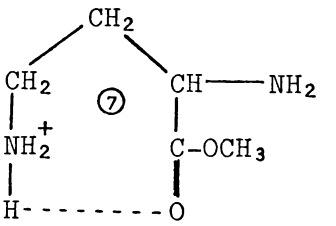
$73.5 \ell \ mol^{-1}s^{-1}$

Figure 15: Structures and base hydrolysis rate constants for EH_2^{2+} forms of diamino acid esters.
(circled numbers indicate possible H-bonding ring sizes)



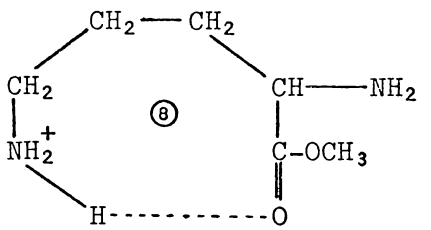
Me 2,3-dap

k_{EH}^{+} = 57.3



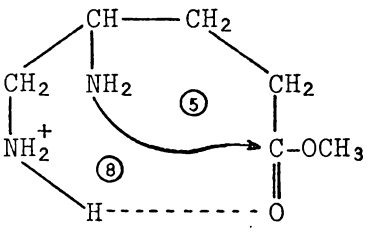
Me 2,4-dab

456ℓ $mol^{-1}s^{-1}$



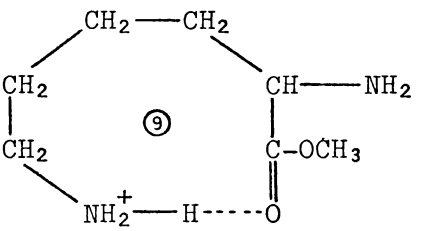
Me 2,5-dape

k_{EH}^{+} = 2300ℓ $mol^{-1}s^{-1}$



Me 4,5-dape

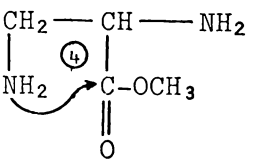
k_{EH}^{+} = 6200 (lactamisation possible)



Me 2,6-dah

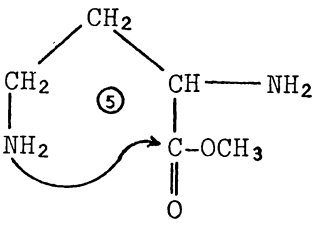
1.27ℓ $mol^{-1}s^{-1}$

Figure 16: Structures and base hydrolysis rate constants for EH^{+} forms of diamino acid esters.
(circled numbers indicate possible ring sizes; H-bonding, dotted lines; lactamisation, solid arrow)



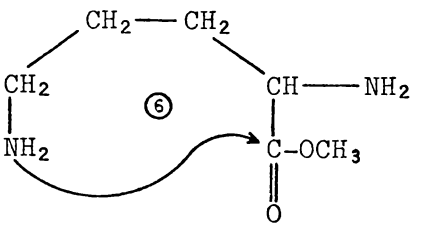
Me 2,3-dap

k_E = 0.73
(no lactamisation)



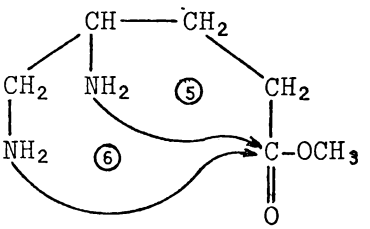
Me 2,4-dab

422 ℓ $mol^{-1}s^{-1}$
(lactamisation)



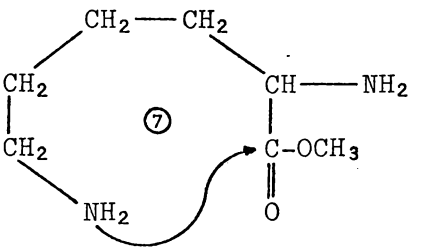
Me 2,5-dape

k_E = 3183 ℓ $mol^{-1}s^{-1}$
(lactamisation)



Me 4,5-dape

k_E = 1630
(lactamisation)



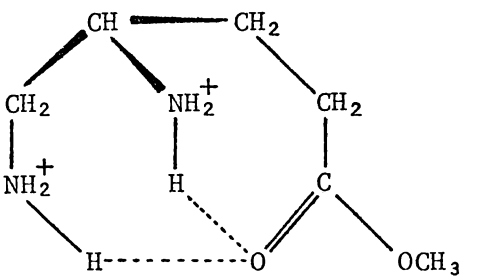
Me 2,6-dah

0.49 ℓ $mol^{-1}s^{-1}$
(no lactamisation)

Figure 17: Structures and base hydrolysis rate constants for E forms of diamino acid esters.
(circled numbers indicate possible lactam ring sizes).

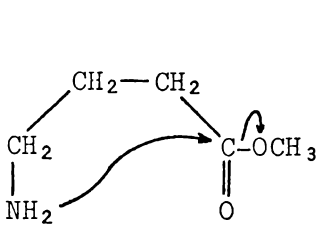
the 2-position; introducing the second NH_3^+ group in the 6-position, being remote from the reaction centre, is expected to have very little effect on the rate of OH^- attack. Therefore, on an electrostatic basis the value of $k_{EH_2}2+$ for Me 4,5-dape would be expected to be $<<73.5\ell\ mol^{-1}s^{-1}$. The effect of moving the NH_3^+ two methylenes should be to greatly decrease the rate constant; in fact the rate constant would be similar to $k_{EH}+$ for methyl 4-aminobutanoate ($2.83\ell mol^{-1}s^{-1}$)[89], the adding of a second NH_3^+ at C_5 having little additional effect on the rate. Hence, $k_{EH_2}2+$ for Me 4,5-dape is expected to be ca. $1.67\text{-}16.7\ell mol^{-1}s^{-1}$; the observed value ($2.37\times10^3\ell mol^{-1}s^{-1}$) indicates considerable assistance (at least 142 times) presumably via intramolecular H-bonding assistance. This assists OH^- attack by lowering electron density on the ester carbonyl carbon (51). A similar effect is observed in $k_{EH_2}2+$ for Me 2,5-dape ($2.08\times10^3\ell mol^{-1}s^{-1}$), but is absent in Me 2,6-dah ($73.5\ell\ mol^{-1}s^{-1}$). Hence optimum ring sizes for intramolecular H-bonding assistance appear to be 7 or 8 (not 5 or 9 , although 6 may be suitable) (see Figure 15).

The value of $k_{EH_2}2+$ for Me 4,5-dape (2.37×10^3) is perhaps unexpectedly high compared to $k_{EH_2}2+$ for Me 2,5-dape (2.08×10^3). The NH_3^+ group at C_2 is expected to accelerate OH^- attack more efficiently than one at C_4. This indicates either 7-membered ring H-bonding is greatly favoured (unlikely, see discussion on $k_{EH}+$ below), or that in Me 4,5-dape there is simultaneous double H-bonding occurring (51).

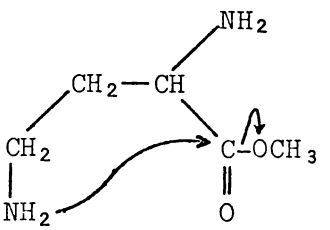


k_{EH}^+: (See Figure 16). In going along the series Me 2,3-dap, Me 2,4-dab, Me 2,5-dape, Me 4,5-dape, Me 2,6-dah, lactamisation is possible only for Me 4,5-dape. Hence k_{EH}^+ (total) for this ester (6.20×10^3ℓ $mol^{-1}s^{-1}$) undoubtedly involves a large component from lactamisation ($k_{L5(EH^+)}$). The contribution made by k_{EH}^+ can be estimated from the k_{EH}^+ values for the other diamino acid esters. The k_{EH}^+ values for Me 2,3-dap (57.3ℓ$mol^{-1}s^{-1}$)[34] and Me 2,6-dah (1.27ℓ$mol^{-1}s^{-1}$)[89] are about those expected on known electrostatic effects. Thus k_{EH}^+ for Me 2,4-dab (456ℓ$mol^{-1}s^{-1}$) (amended result from ref 73) suggests assistance by intramolecular H-bonding (7-membered ring) and that for Me 2,5-dape (2.30×10^3ℓ$mol^{-1}s^{-1}$)[73] suggests that 8-membered ring intramolecular H-bonding, is even more rate accelerating. Thus k_{EH}^+ for Me 4,5-dape, which involves possible 8-membered ring H-bonding assistance, may have a value as high as 2.3×10^3ℓ$mol^{-1}s^{-1}$; however removal of the NH_2 group from C_2 to C_4 is expected to lower this. A further estimate of k_{EH}^+ for Me 4,5-dape can be made from $k_{EH_2}^{2+}$ for that ester; the latter (2.37×10^3ℓ$mol^{-1}s^{-1}$) apparently involves both 7 and 8-membered ring H-bonding. Removal of the H^+ from the C_4 amino group decreases the overall charge by +1 and destroys 7-membered ring H-bonding assistance, both of which should decrease the rate of OH^- attack, making $k_{EH}^+ << 2.37 \times 10^3$ℓ $mol^{-1}s^{-1}$. In a 'normal' situation, removal of a C_4 - NH_3^+ positive charge results in a decrease in rate constant of at least 10 times (methyl 4-aminobutanoate, $k_{EH}^+ = 2.83$ℓ $mol^{-1}s^{-1}$; k_E estimated ~0.14ℓ $mol^{-1}s^{-1}$). Hence, k_{EH}^+ for Me 4,5-dape is probably about 237ℓ $mol^{-1}s^{-1}$ leaving the rate constant for the lactamisation reaction, $k_{L5(EH^+)}$ $\sim5.96 \times 10^3$ℓ $mol^{-1}s^{-1}$.

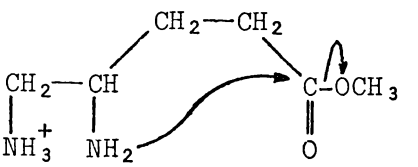
Comparing $k_{L5(EH^+)}$ for Me 4,5-dape with the rates for other 5-membered ring lactams[89]:



Me 4-aminobutanoate



Me 2,4-dab



Me 4,5-dape

k_{L5} 29.3 422 5960ℓ $mol^{-1}s^{-1}$

For methyl 4-aminobutanoate, simple intramolecular aminolysis occurs with no additional neighbouring group effects. For Me 2,4-dab the -I effect of the C_2 - NH_2 gives a large rate increase, while for Me 4,5-dape the C_5 - NH_3^+ is presumably providing an even larger -I and electrostatic assistance for the synchronous OH^- attack involved in the lactamisation mechanism[89].

k_E: The rate constants for the hydrolysis of the E forms of the diamino acid esters (Figure 17) show that lactamisation is only possible where 5 or 6 membered rings can be formed (i.e. Me 2,4-dab, Me 2,5-dape, Me 4,5-dape). Lactamisation of Me 2,4-dab involves 5-membered ring formation with assistance by the C_2-amino group. Increasing the ring size to 6(Me 2,5-dape) results in about an eightfold increase in the lactamisation rate constant. Removing the C_2-amino group to C_4 (Me 2,5-dape to Me 4,5-dape) results in about a two fold decrease in rate, as expected.

The more rapid rate of 6-membered ring formation compared with 5-membered ring formation is also observed[93] where ethyl 5-aminopentanoate (k_{L6} = 216.7ℓ $mol^{-1}s^{-1}$, 25°C, I = 0.2mol $ℓ^{-1}$) lactamises 25 times more rapidly than ethyl 4-aminobutanoate (k_{L5} = 8.83ℓ $mol^{-1}s^{-1}$, 25°C, I = 0.2mol $ℓ^{-1}$).

It is therefore suggested that the major pathway for lactamisation of Me 4,5-dape involves 6-membered ring formation. Lactamisation of Me 4,5-dape as EH^+ ($k_{L5(EH^+)}$, involves 5-membered ring formation with (1+) charged species) is greater, as expected, than E ($k_{L6(E)}$, involves 6-membered ring formation with a neutral species) by 3.6 times due to an electrostatic effect reduced by the less rapid 5-membered ring versus 6-membered ring formation.

6.5 Summary

The following rate constants have been calculated or estimated for the base hydrolysis of Me 4,5-dape (refer to Scheme 9): T = (25 ± 0.05) °C and I = 0.10 mol ℓ^{-1}(KCl)

$$k_{EH_2^{2+}} = 2.37 \times 10^3\ \ell\ mol^{-1}s^{-1}$$

$$k_{EH^+} \simeq 237\ell\ mol^{-1}s^{-1}$$

$$k_E \simeq 0.14\ell\ mol^{-1}s^{-1}$$

$$k_{L5(EH^+)} \simeq 5.96 \times 10^3\ \ell\ mol^{-1}s^{-1}$$

$$k_{L5(E)} + k_{L6(E)} = 1.63 \times 10^3\ell\ mol^{-1}s^{-1}$$

7. COPPER (II) CATALYSED HYDROLYSIS

7.1 Introduction

The alkaline hydrolysis of Me 4,5-dape was studied at 25°C, by the pH-stat technique, at 1:1 and 1:2 mole ratios of Cu(II) to Me 4,5-dape and at a 2.5:1:4 mole ratio of Cu(II):Me 4,5-dape:4,5-dape or en. As a check on calibration and operation of the pH-stat, the hydrolysis of Me His was studied, at 25°C, at pH 8.40, at a 1:2 mole ratio of Cu(II) to Me His.

The kinetic data obtained for hydrolysis of bis complexes CuE_2^{2+} (E = Me 2,3-dap (ref. 34) or Me His (ref 35)) is consistent with two second-order (pseudo-first-order at constant pH) reactions:[34,35]

$$CuE_2^{2+} + OH^- \xrightarrow{k_{(CuE_2^{2+})}} CuEA^+ + CH_3OH$$

$$CuEA^+ + OH^- \xrightarrow{k_{(CuEA^+)}} CuA_2 + CH_3OH$$

Initial hydrolysis of CuE_2^{2+} (expected to be rapid for electrostatic and statistical reasons) is followed by slower hydrolysis of $CuEA^+$. An "infinity" plot ($\log_{10}(V_\infty - V_t)$ versus time where V_∞ is volume of NaOH consumed at "infinite" time and V_t the volume at time t) of such a system shows initial concave curvature followed by a linear region at high values of t where only $CuEA^+$ is hydrolysing. A "half-infinity" plot ($\log_{10}(V_\infty/2 - V_t)$ versus time), which covers the initial stage of the reaction (hydrolysis of CuE_2^{2+} only) shows an initial linear portion curving away convexly at higher t values as the slower hydrolysis of $CuEA^+$ becomes significant. Accordingly, approximate values of the two pseudo-first-order rate constants at constant pH can be obtained from the initial slope of the "half-infinity" plot ($= -k_{obs(CuE_2^{2+})}/2.303$) and the final slope of the "infinity" plot ($= -k_{obs(CuEA^+)}/2.303$).

These consecutive rate constants can be found more precisely by the Swain "time-ratio" method[94] as described by Frost and Pearson[95].

Table 15: Time-ratio evaluation of $k_{(CuE_2^{2+})}$ and $k_{(CuEA^{+})}$ for Cu(II) catalysed hydrolysis of Me His at 25°C and I = 0.10molℓ^{-1}. Cu(II):ester = 1:2 pH = 8.40.

(a) Time (t_x min) required to reach x% reaction.

t_{10}	t_{20}	t_{30}	t_{40}	t_{50}	t_{60}	t_{70}	t_{80}	t_{90}
3.32	7.56	13.14	20.65	31.00	49.00	76.00	121.42	201.20

(b) Selected Time-ratios:

	$t_{90}:t_{60}$	$t_{80}:t_{50}$	$t_{70}:t_{40}$	$t_{70}:t_{20}$	$t_{60}:t_{30}$	$t_{60}:t_{10}$
Found	4.106	3.917	3.680	10.05	3.729	14.76
Calculated (κ = 0.14)	4.138	3.879	3.718	9.855	3.658	13.857

(c) Values of $10^2\tau/t(min^{-1})$ at the stated reaction percentages using κ=0.14

10%	20%	30%	40%	50%	60%	70%	80%	90%	Mean
(6.605)*	6.454	6.321	6.262	6.345	6.201	6.326	6.284	6.249	6.305

*(excluded in determining mean since obtained from back extrapolated part of V versus t curve.)

(d) Calculation of $k_{(CuE_2^{2+})}$ and $k_{(CuEA^{+})}$

$$10\, k_{(CuE_2^{2+})}[OH^-] = 10^2\tau/t = 6.305 min^{-1}$$

$k_{(CuE_2^{2+})} = 19.21\times10^3 \ell mol^{-1}min^{-1}$; $k_{(CuEA^{+})} = \kappa k_{(CuE_2^{2+})} = 2.69\times10^3 \ell mol^{-1}min^{-1}$

(e) Comparison of Time-ratio, graphical and literature values

	$k_{(CuE_2^{2+})}\ell mol^{-1}s^{-1}$ ($\ell mol^{-1}min^{-1}$)	$k_{(CuEA^{+})}\ell mol^{-1}s^{-1}$ ($\ell mol^{-1}min^{-1}$)
Time Ratio	320.2 (19,210)	44.83 (2,690)
Graphical	343.5 (20,610)	43.7 (2,622)
Literature[35]	(19,690)	(2,560)

Evaluation of the rate constants $k_{(ME_2^{2+})}$ and $k_{(MEA^+)}$ involves comparing the time taken for the experimental reaction to reach a given reaction percentage, with the times expected for given (theoretical) ratios of the two rate constants. Table 15 gives the result obtained for Cu(II) hydrolysis of Me His in this work.

The approximate values of the rate constants obtained by the graphical method differ slightly from those obtained by the time ratio method (Table 15). This is because of additional contributions to the observed rate by $CuEA^+$ hydrolysis at the start of the reaction and by CuE_2^{2+} hydrolysis near the end of the reaction.

The present results agree well with the literature[35] values for hydrolysis of $Cu(Me\ His)_2^{2+}$ (Table 15).

Two difficulties arise in applying the time-ratio method. First, the entire reaction must be observed: i.e. the reaction must be caught at its start (t_0). This is impractical with the pH-stat technique because of hydrolysis while the solution in brought up to the desired pH and the titrator is adjusted to hold this pH. Consequently the experimental V_∞ value is always less than the theoretical value. This problem is largely overcome by following the reaction to infinity (at least 10 half-lives), then extrapolating the titre(V) versus time (t) curve back in time to allow for the missed OH^- consumption at the start of the reaction, i.e. $\Delta V = (V_\infty(theor.) - V_\infty(exptl.))$. The V vs t curve is then extrapolated back by the amount ΔV to get the true t_0, and $\Delta t = t_0(true) - t_0(exptl)$. A new table of values $V = V(exptl) + \Delta V$ and $t = t(exptl) + \Delta t$ is constructed from which the selected time ratios can be calculated.

The second difficulty is that the time-ratio method ignores the possible disproportionation of $CuEA^+$:

$$2CuEA^+ \rightleftharpoons CuE_2^{2+} + CuA_2$$

Stability constant calculations for the histidine system[33] show that at complete neutralisation of the ligand protons (pH∿6.7) a Cu(II):Me His: His = 1:1:1 solution contains $CuEA^+$ (66.6%) and its disproportionation products, CuE_2^{2+} and CuA_2 (16.7% each). However, disproportionation in hydrolysis reactions will be somewhat less than this[35]. At the start of the reaction there is an excess of CuE_2^{2+} while at the end of the reaction there is an excess of CuA_2, both of which repress disproportionation of $CuEA^+$.

Having achieved satisfactory results for the Cu(II) - Me His system studies were begun on the Cu(II) catalysed hydrolysis of Me 4,5-dape.

7.2 Hydrolysis at Cu(II):Me 4,5-dape = 1:2 Mole Ratio

The Cu(II) catalysed hydrolysis of Me 4,5-dape was studied in solutions containing Me 4,5-dape. 2HCl (1×10^{-3}mol) and $CuCl_2$ (5×10^{-4}mol) with I = 0.10 mol ℓ^{-1} (made up with 1 molℓ^{-1} KCl) and total volume = 100.00ml at complete neutralisation of the ligand protons. Hydrolysis (at 25°C) was followed on the pH-stat using essentially the same method as described for the uncatalysed hydrolysis (Section 6.2). The titre versus time results were adjusted to give true t_0 (Section 7.1) and were initially analysed by "half-infinity" plots (Figure 18) and "infinity" plots (Figure 19). Experimental infinity values ranged from 0.82ml to 0.96ml (1.00ml theoretical). As the reaction rate increased (higher pH), the V_∞ values generally decreased (more of the reaction was missed) necessitating more approximate extrapolation. The pH dependance of the observed graphical rate constants is summarised in Table 16.

The time-ratio method[94,95] should also enable separation and evaluation of the rate constants for the two processes. However, the method proved unsatisfactory. At any given pH, the K values obtained were not constant throughout the run (Table 17 shows a typical result).

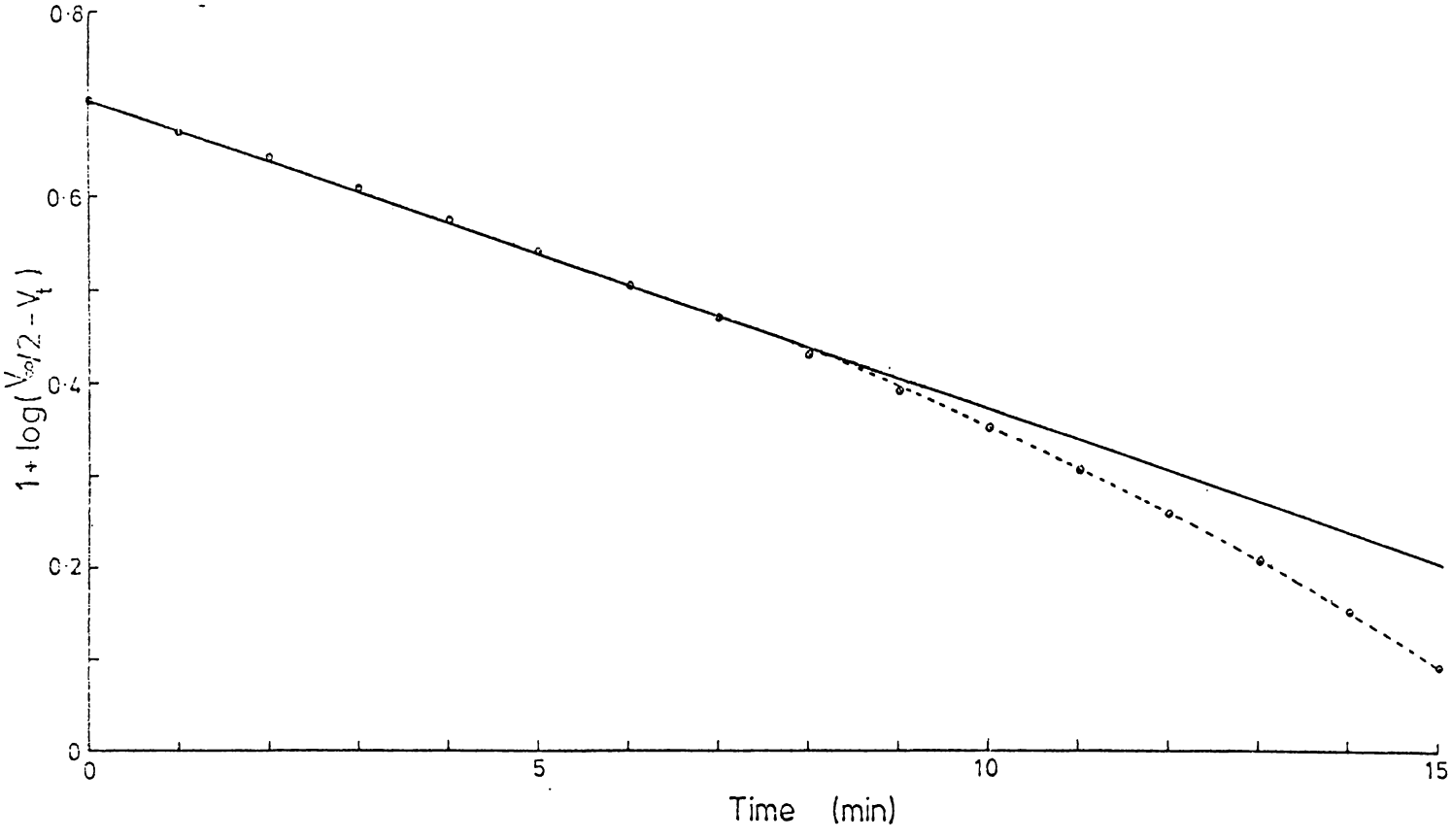


Figure 18: Half-infinity plot for Cu(II) catalysed hydrolysis of Me 4,5-dape; T = 25°C I = 0.10molℓ^{-1}(KCl) Cu(II): Me 4,5-dape = 1:2, pH 10.60.

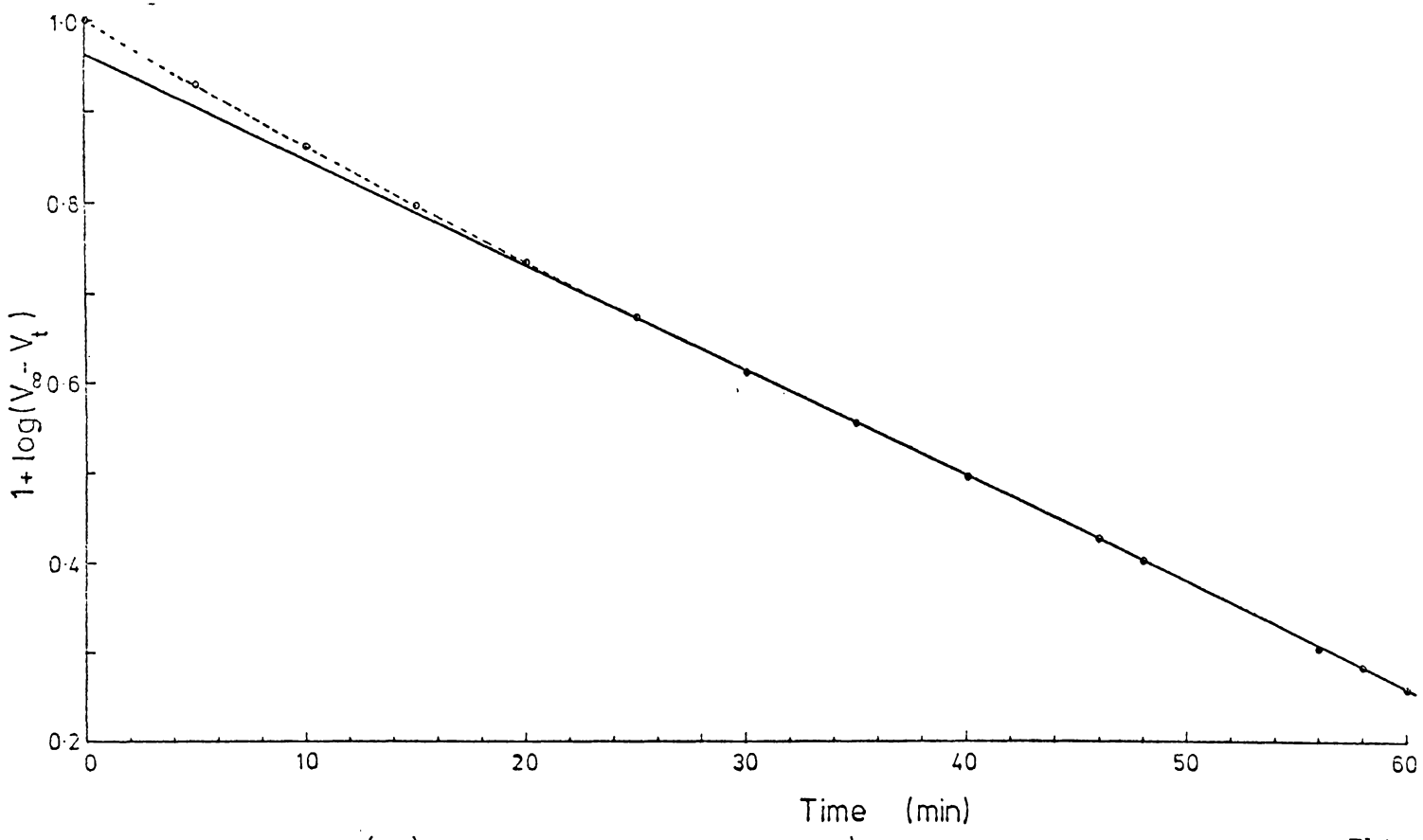


Figure 19: Infinity plot for Cu(II) catalysed hydrolysis of Me 4,5-dape; T = 25 C I = 0.10molℓ^{-1}(KCl) Cu(II): Me 4,5-dape = 1:2, pH 10.60.

Table 16: Rate constants for Cu(II) catalysed hydrolysis of Me 4,5-dape. Cu(II): E = 1:2, T = (25 ± 0.05)°C I = 0.10molℓ^{-1}.

pH	$10^4 k_{obs(CuE_2^{2+})}$ (s^{-1})	$k_{obs(CuE_2^{2+})}/[OH^-]$ $\ell mol^{-1}s^{-1}$	$10^4 k_{obs(CuEA^+)}$ s^{-1}	$k_{obs(CuEA^+)}/[OH^-]$ $\ell mol^{-1}s^{-1}$
10.10	3.947	2.400	1.344	0.817
10.20	5.105	2.458	1.712	0.827
10.30	6.167	2.367	2.017	0.775
10.40	7.683	2.338	2.350	0.718
10.50	10.108	2.447	3.272	0.792
10.60	12.872	2.475	4.502	0.866

Mean: $k_{(CuE_2^{2+})}$ = 2.414$\ell mol^{-1}s^{-1}$ (145$\ell mol^{-1}min^{-1}$)

$k_{(CuEA^+)}$ = 0.799$\ell mol^{-1}s^{-1}$ (48.0$\ell mol^{-1}min^{-1}$).

Table 17: Example of attempted time-ratio evaluation of rate constants for Cu(II) catalysed hydrolysis of Me 4,5-dape. Cu(II):E = 1:2, T = (25 ± 0.05)°C and I = 0.10molℓ^{-1}. pH = 10.20.

(a) Time (t_x min) required to reach x% reaction:

t_{10}	t_{20}	t_{30}	t_{40}	t_{50}	t_{60}	t_{70}	t_{80}	t_{90}
7.33	16.81	28.50	42.59	59.69	81.20	109.44	149.06	214.34

(b) Selected time-ratios:

	$t_{90}:t_{60}$	$t_{80}:t_{50}$	$t_{70}:t_{40}$	$t_{70}:t_{20}$	$t_{60}:t_{30}$	$t_{60}:t_{10}$
	2.640	2.497	2.570	6.510	2.849	11.08
K*	0.42	0.38	0.35	0.30	0.31	0.24

* K value which gives time ratio value closest to the experimental ratio.

The reason for failure of the time ratio analysis is not clear. The problem is apparently a fairly small one since the "half infinity" and "infinity" plots each give a set of $k_{obs}/[OH^-]$ values which are essentially independent of pH. (Table 16). The time ratio method is fairly sensitive to small changes in times taken to reach a particular reaction percentage. Clearly the problem is not due to apparatus or technique since the results for Me His (Table 15) agree well with the literature values. As an apparently 100% pure sample of Me 4,5-dape was prepared (Section 3.3.6) purity of the ester used is not the source of the problem.

One potential difficulty in these hydrolysis studies on labile metal ion: diamino acid ester = 1:2 systems is disproportionation of the intermediate $CuEA^+$ (Section 7.1). However examination of the stability constants involved (Tables 8 and 11) shows that disproportionation will be only slightly worse for the 4,5-dape system cf. 2,3-dap. In a 1:1:1 mixture of Cu(II) to ester to amino acid the percentages of $CuEA^+$ and its disproportionation products are:

	4,5-dape	2,3-dap	His
$\%CuEA^+$	52.2	56.8	66.6
$\%CuE_2^{2+}$	23.8	21.6	16.7
$\%CuA_2$	23.8	21.6	16.7

The time-ratio method works equally well for both His and 2,3-dap systems (in fact at 3 temperatures)[34, 35] and the 2,3-dap system shows a much greater extent of disproportionation than the His system. Hence it would seem unlikely that disproportionation is the source of the failure of the method for 4,5-dape.

However, in spite of the extents of disproportionation being similar in the 4,5-dape and 2,3-dap systems, it could still be the source of the problem depending on the relative values of the rate constant for

hydrolysis of CuE_2^{2+} and $CuEA^+$ for E = Me 4,5-dape. If $k_{(CuE_2^{2+})}$ was much larger than $k_{(CuEA^+)}$, then E would prefer the kinetic route

$$CuE_2^{2+} \longrightarrow CuEA^+ \rightleftharpoons CuA_2 + CuE_2$$

and very little would hydrolyse as $CuEA^+$. However this does not appear to be the case; the value of $k_{(CuE_2^{2+})}$ for Me 4,5-dape is only 3 times that for $k_{(CuEA^+)}$. A similar factor (3.5 times) is observed for the 2,3-dap system. There appears little likelihood that the rate constants obtained in the 4,5-dape system are incorrect. The value of $k_{(CuEA^+)}$ is confirmed independently (Section 7.4) by studies on Cu(II):E:A = 1:1:1 systems and the value of $k_{(CuE_2^{2+})}$ by $k_{(CuEen^{2+})}$ (which is slightly less than the statistically expected $\frac{1}{2} \times k_{(CuE_2^{2+})}$; a similar result has been obtained[34] for 2,3-dap).

The features which distinguish the 4,5-dape system from 2,3-dap are:

1. Rates of Cu(II) catalysed hydrolysis are much slower (ca. 120 times) for 4,5-dape. Therefore studies must be made at higher pH values (range used pH 10.10-10.60 cf. 2,3-dap, pH 8.20-8.60) to obtain reasonable reaction rates.

2. For 4,5-dape, both the ester and the free acid show a tendancy to undergo cyclisation and form lactams. Such a side reaction is unavailable to 2,3-dap.

It is debatable whether either of these features can account for the failure of the time ratio analysis. The higher pH may lead to decomposition in the 4,5-dape system although no unexpected products could be detected (see below). Any lactamisation would similarly interfere with the kinetics. However, in the dilute reaction solutions, lacatamisation of 4,5-dape is slow (Section 6.4.2); lactamisation of the free ester is extremely rapid (Section 6.4.4; at pH 10.60, $t_{\frac{1}{2}}$ is ca. 0.8s), and this side reaction would result in a lowered V_∞ value (since it involves no nett consumption of base).

The experimental V_∞ values obtained (0.820-0.912 ml) are less than the theoretical 1.000ml. Most of this difference is due to the reaction missed at the beginning (Section 7.1) but it is possible that there is a contribution from the cyclisation side-reaction. This contribution must be small (< ~5%) since the experimental V_∞'s are similar to those observed insystems where no lactamisation is possible (e.g. Me 2,3-dap, Me His)[73] Such a reaction produces a stable lactam, however this was not present in the reaction products to within the detection limits (ca. 5%).

It was important, because of the rapid rate of lactamisation, to show that the presence of Cu(II) coordinated to the amino groups prevented intramolecular aminolysis, within the experimental reaction times. The following investigation of the products of the hydrolysis reaction was carried out. The reaction solutions, after t_∞, were brought to pH1 (conc. HCl), concentrated to dryness (rotary evaporator) and dried under vacuum over phosphorous pentoxide. IR spectra (KBr disc) and NMR spectra (D_2O solution) were run and compared with spectra for authentic samples of 4,5-dape.2HCl and the lactam 5-(aminomethyl)-2-oxopyrrolidine (18). The NMR spectrum of 4,5-dape.2HCl has two multiplets at δ = 2.65 and 3.20ppm corresponding to C_3 and C_4 methylene protons. In the lactam (18) (Section 3.3.4) the corresponding methylene protons form a single complex multiplet at δ = 2.90ppm. The product of uncatalysed hydrolysis of Me 4,5-dape (Section 6) shows this single multiplet. The Cu(II) catalysed hydrolysis product shows two multiplet signals at δ = 2.6 and 3.2ppm. This spectrum is poorly defined due to diamagnetic Cu(II) line broadening but suggest that the free acid 4,5-dape is formed rather than the lactam (18).

The IR spectrum of the lactam has bands at 3220, 1690, 1618 and 1400cm^{-1}. To more closely duplicate experimental conditions, the lactam was contaminated with Cu(II) and KCl. The resulting spectrum had lost the

distinctive band at 3220cm^{-1} and showed small shifts in other bands. 4,5-dape.2HCl has bands at 1710, 1600 and 1410cm^{-1} and although different in appearance to the lactam bands, the presence of Cu(II) and KCl makes them similar. However, 4,5-dape.2HCl has a strong band at 1205cm^{-1} lacking in the lactam (18). The Cu(II) catalysed hydrolysis products all have this band. Attempts to remove Cu(II) from the reaction product, so spectra could be run in the absence of Cu(II), resulted in extensive lactamisation of the free 4,5-dape during the reaction product workup. It is clear that any lactamisation which occurs during the Cu(II) catalysed hydrolysis must be small and ca. <5%.

In conclusion, failure of the time-ratio analysis is presumably due to a small (<5%) amount of lactamisation of the ester occurring concurrently with its Cu(II) catalysed hydrolysis. This will not significantly affect the slope of the "infinity" and "half-infinity" plots. However, the time ratio analysis which depends on the time taken to reach a given reaction extent, will be more sensitive to the progressively increasing extent of lactamisation. Because of the failure of the time-ratio method the data from Cu(II):Ester = 1:2 hydrolysis was analysed only by graphical methods (Table 16). Clearly the results are somewhat approximate although they are in good agreement with the independent results from other systems studied (Section 7.4).

7.3 Hydrolysis at Cu(II): Me 4,5-dape = 1:1 Mole Ratio

The pH-stat was used to study the hydrolysis of Me 4,5-dape in solutions containing equimolar amounts (10^{-3}mol) of Cu(II) and the ester, at 25°C (with I = 0.10molℓ^{-1} and volume = 100ml at complete neutralisation of ligand protons) over a range of pH values.

Spectrophotometric data from 1:1 solutions of Cu(II) and Me 4,5-dape (Section 5.2.2) suggests the disproportionation reaction occurs:

$$2CuE^{2+} \rightleftharpoons CuE_2^{2+} + Cu^{2+}_{(aq)}.$$

The free Cu^{2+} precipitates as $Cu(OH)_2$ leaving effectively a 1:2 Cu(II): ester solution. This precipitation caused several difficulties in analysis of the kinetic data obtained. High infinity values were observed in these hydrolysis reactions, (ca. 1.5ml 1.0molℓ^{-1} NaOH cf. 1.0ml theoretically). Presumably further precipitation of free $Cu^{2+}_{(aq)}$ consumes the extra base. The theoretical infinity value (1.0ml 1.0molℓ^{-1} NaOH $\equiv$ 1×10^{-3}mol) was used to prepare "infinity" plots which showed an initial linear portion followed by a concave curve. Curvature was probably caused by trapping of ester complexes in the $Cu(OH)_2$ precipitate giving a lower observed rate. Rate constants were calculated from the initial slopes of the infinity plots (Table 18). The $k_{obs}/[OH^-]$ values show a decrease with decreasing pH. This is a reflection of the amount of disproportionation. At lower pH's (<pH9) disproportionation is incomplete and slower hydrolysis of CuE^{2+} (or $CuE(OH)^+$) is observed. Because of the curvature of these infinity plots, k_{obs} values are not related to the $k_{obs(CuEA^+)}$ values obtained for true 1:2 solutions. However an estimation of $k_{(CuE_2^{2+})}$ can be obtained from the initial slope of the "half-infinity" plot. The values obtained (Table 18) are of the same order but somewhat less than those observed with true 1:2 solutions (Table 16), probably because of the heterogeneous nature of the solution. The result however confirms that disproportionation of CuE^{2+} to CuE_2^{2+} and $Cu^{2+}_{(aq)}$ occurs in 1:1 solutions of Cu(II) and Me 4,5-dape.

Table 18: Base hydrolysis of Me 4,5-dape in 1:1 Cu(II) to ester solutions at 25°C and I = 0.10molℓ^{-1}.

(a) Infinity Plot.

pH	$10^5 k_{obs}(s^{-1})$	k_{obs}/ OH^- $\ell mol^{-1}s^{-1}$
8.50	0.192	0.465
8.80	0.475	0.580
9.00	0.761	0.582
9.20	1.700	0.820
9.40	2.727	0.831
10.20	17.233	0.832
10.40	27.483	0.837
10.60	43.583	0.838

(b) 'Half' infinity plot.

pH	$10^4 k_{obs}(s^{-1})$	k_{obs}/ OH^-	$= k_{(CuE_2^{2+})}$ $\ell mol^{-1}s^{-1}$
10.2	3.910		1.888
10.4	6.042		1.842
10.6	9.795		1.883

Similar disproportionation was observed in 1:1 solutions of Cu(II) and 2,3-dap and was apparent in 1:1 solutions of Cu(II) and Me 2,3-dap, although hydrolysis of the ester was too rapid to allow the relevant spectra to be run (Section 5.2.2). In 1:1 solutions of metal ion: ester, the hydrolysis of Me 2,3-dap has been interpreted[34] in the same way as for Me His where such disproportionation does not occur[35] (Section 5.2.2). The results have been rationalised in terms of the reactions[34]:

$$\begin{array}{ccccc} CuE^{2+} & + & OH^- & \xrightarrow{k_{(CuE^{2+})}} & CuA^+ + CH_3OH \\ H^+ \rightleftharpoons K_a(CuE^{2+}) & & & & H^+ \rightleftharpoons K_a(CuA^+) \\ CuE(OH)^+ & + & OH^- & \xrightarrow{k_{(CuE(OH)^+)}} & CuA(OH) + CH_3OH \end{array}$$

The infinity plots for these systems are initially linear then show concave curvature. $k_{obs}/\ OH^-$ values obtained decrease with increasing pH due to the conversion of CuE^{2+} into the less reactive $CuEOH^+$. Clearly the hydrolysis of Cu(Me 2,3-dap)$^{2+}$ must take place rapidly ($k_{(CuE^{2+})}$ = $3.71 \times 10^3 \ell mol^{-1} min^{-1}$)[34] and at sufficiently low pH (max pH studied = 8.8)[34] such that the initial rate can be observed before disproportionation is the problem it is in the 4,5-dape system.

7.4 1:1:1 Solutions:

A check on the value of $k_{(CuEA^+)}$ obtained from 1:2 Cu(II) to ester solutions can be made by studying solutions containing equimolar amounts of Cu(II), ester and amino acid. The infinity plots prepared from data of 1:1:1 solutions of Cu(II), Me 2,3-dap and 2,3-dap showed slight initial curvature but were linear after about one half-life[34]. Disproportionation of the initial $CuEA^+$ complex, means that more rapid hydrolysis of the CuE_2^{2+} formed is superimposed on the main reaction. Formation of CuA_2, as the reaction proceeds, represses disproportionation so that hydrolysis of $CuEA^+$ alone is observed. Adding an excess of CuA_2 decreases initial curvature but has little effect on the final slope of the plot[34].

Accordingly kinetic measurements (pH-stat) were carried out on solutions containing Cu(II) (1.25×10^{-3}mol), Me 4,5-dape.2HCl (5×10^{-4}mol) and 4,5-dape.2HCl (2×10^{-3}mol) i.e. a ratio of Cu:E:A = 2.5:1:4, the maximum possible if ionic strength is to be kept at $0.10 mol \ell^{-1}$. The infinity plots showed no initial curvature and were linear for more than four half-lives. The titre versus time data was therefore analysed by a non-linear least squares computer program[92] and the results are summarised in Table 19. The value obtained for $k_{(CuEA^+)}$ (= $k_{obs}/\ OH^-$) is in good agreement with the value obtained from 1:2 solutions of Cu(II) and Me 4,5-dape (Table 16).

Table 19: Rate constants for hydrolysis of mixed ligand complexes of Me 4,5-dape at 25°C and I = 0.10molℓ^{-1}.

(a) Cu: Me 4,5-dape: 4,5-dape = 2.5:1:4.

pH	$10^4 k_{obs}$ s^{-1}	$k_{obs}/[OH^-]$ $\ell mol^{-1}s^{-1}$
10.20	1.583	0.764
10.40	2.583	0.785
10.60	4.533	0.872

Mean: $k_{obs}/[OH^-] = k_{(CuEA^+)} = 0.807\ell mol^{-1}s^{-1}$ $(48.42\ell mol^{-1}min^{-1})$

(b) Cu: Me 4,5-dape: en = 2.5:1:4.

pH	$10^4 k_{obs}$ s^{-1}	$k_{obs}/[OH^-]$ $\ell mol^{-1}s^{-1}$
10.20	1.967	0.946
10.40	3.283	1.001
10.50	3.533	0.854
10.60	5.383	1.035

Mean: $k_{obs}/[OH^-] = k_{(CuEen^{2+})} = 0.959\ell mol^{-1}s^{-1}$ $(57.54\ell mol^{-1}min^{-1})$

Kinetic measurements were also made on 2.5:1:4 solutions of Cu(II) Me 4,5-dape.2HCl, and en.2HCl. As $k_{(CuE^{2+})}$ could not be obtained and because en is a neutral ligand with similar properties to Me 4,5-dape (Section 5), the hydrolysis of $CuE(en)^{2+}$ should provide some estimate of $k_{(CuE^{2+})}$. Linear infinity plots were also obtained with this system and the data was analysed by the computer program. The results are also summarised in Table 19.

7.5 Summary:

The rate constants determined for base hydrolysis, at 25°C and I = 0.10molℓ^{-1}, of the various Cu(II) chelates of Me 4,5-dape are summarised in Table 20.

Table 20: Summary of rate constants for Cu(II) catalysed hydrolysis of Me 4,5-dape. ($T = 25°C$ and $I = 0.10 mol\ell^{-1}$)

Species	$k\ell mol^{-1}s^{-1}$
CuE_2^{2+}	2.414
$CuEA^{+}$	0.803
$CuE(en)^{2+}$	0.959

Two effects on the hydrolysis rate introduced by the presence of the metal ion are apparent from Table 20.

1. Charge effect: The rate of hydrolysis is increased as the positive charge near the reaction centre is increased. The charge effect is measured by the ratio $k_{(CuEen^{2+})}/k_{(CuEA^{+})} = 1.2$. This effect is smaller than for Me His ($k_{(CuE^{2+})}/k_{(CuEA^{+})} = 4.1$)[35] and for Me 2,3-dap ($k_{(CuE^{2+})}/k_{(CuEA^{+})} = 7$)[34]. This reflects the greater distance between the positive charge, i.e. metal ion, binding site (4,5-diamino group pair) and the ester function, in Me 4,5-dape.
2. Statistical effect: The rate of hydrolysis of CuE_2^{2+} would be expected to be twice that for CuE^{2+} (or $CuE(en)^{2+}$) on a simple statistical basis. The ratio $k_{(CuE_2^{2+})}/k_{(CuEen^{2+})} = 2.5$ should be similar to that for $k_{(CuE_2^{2+})}/k_{(CuE^{2+})}$, and is approximately what is expected statistically.

8. CONCLUSIONS

Extensive literature searches, and laboratory work indicate that there is no general synthetic route to ω(ω-1)-diamino acids. Each individual diamino acid requires a specialised synthesis, such as has been developed for 4,5-diaminopentanoic acid in this work, and has been used for 2,3-diaminopropanoic acid[43]. Similarly an individual synthesis has been partially developed for 3,4-diaminobutanoic acid.

The relative values of the rate constants for Cu(II) catalysed hydrolysis of Me 4,5-dape can be explained by a combination of charge and statistical effects (Section 7.5).

The actual rates of the Cu(II) catalysed hydrolysis of Me 4,5-dape, as the various complexes studied, are very slow as was expected from the separation of the metal ion binding site and ester functions. Rate accelerations over the uncatalysed reaction of only about 8 times are observed, compared with the 10^3 times seen for Me 2,3-dap and the 10^6 times observed in Co(III) systems involving direct carbonyl oxygen-metal ion interaction.

One of the kinetic objectives of the present studies was to gain an insight into whether direct carbonyl oxygen-metal ion interaction is a significant factor in the rate accelerations observed in the Cu(II) - Me 2,3-dap system. As discussed in the Introduction, it may be argued that the observed rate acceleration factors of about 10^3 times for the Me 2,3-dap system contain an important contribution from a species involving direct carbonyl oxygen-metal ion coordination; this species although of very low concentration would be kinetically extremely important. In going from Me 2,3-dap to Me 4,5-dape, the diamine binding site for Cu(II) remains unchanged (forming a 5-membered chelate ring), but the binding of Cu(II) to the carbonyl oxygen and nearest amino group becomes extremely unlikely (a 5-membered chelate ring is replaced by a 7-membered one).

Hence, if direct carbonyl oxygen-metal interaction is important in Me 2,3-dap then, in going from Me 2,3-dap to Me 4,5-dape, a larger decrease in rate acceleration would be seen than that expected solely from attenuation of charge and inductive effects. The problem is to establish the decrease in rate expected solely on attenuation grounds.

The experimentally observed decrease in rate of the Cu(II) catalysed reaction in going from Me 2,3-dap to Me 4,5-dape can be seen in Table 21. The rate acceleration factors ($F = k_x/k_E$), produced by Cu(II) (relative to the uncatalysed rate), are listed for the two esters. The last column gives the ratio of the rate acceleration for Me 2,3-dap cf. Me 4,5-dape.

Table 21: Comparison of rate constants obtained for Cu(II) catalysed hydrolysis of Me 2,3-dap and Me 4,5-dape.
$T = 25°C$ $I = 0.10 mol \ell^{-1}$.

x	Me 2,3-dap[34]		Me 4,5-dape		Relative rate acceleration
	$k_x(\ell mol^{-1}s^{-1})$	$F=k_x/k_E$	$k'_x(\ell mol^{-1}s^{-1})$	$F'=k'_x/k'_E$	F/F'
CuE_2^{2+}	305	418	2.414	17	24
$CuE(en)^{2+}$	125	171	0.959	7	25
$CuEA^{+}$	88.7	122	0.803	6	21
E	0.73		∿0.14		

Notes:

1. k_E value for Me 4,5-dape estimated since rapid intramolecular aminolysis competes with base hydrolysis.
2. For comparison of F values for a given ester, the value for x = CuE_2^{2+} should be divided by a statistical factor of two.
3. $k_{CuE^{2+}}$ not measurable for Me 4,5-dape. $k_{CuE^{2+}} = 618 \ell mol^{-1}s^{-1}$ for Me 2,3-dap[34] and gives F value = 850, i.e. $\sim 10^3$ which seems anomalously large.

Hence for any (metal ion-ester) species (x) the rate acceleration factor for Me 2,3-dap averages about 23 times that for Me 4,5-dape.

The theoretically expected decrease in rate acceleration in going from Me 2,3-dap to Me 4,5-dape, due solely to attenuation of charge and inductive effects of the coordinated Cu^{2+} by methylene groups, is uncertain. No information is available from Co(III) systems where the absence of direct carbonyl oxygen-metal ion interaction can be rigorously established. The Cu(II) ion may be considered similar to some positively charged substituent group on the ester. A rough estimate of attenuation effects can thus be obtained from the effect, on the rate of alkaline hydrolysis of esters, of moving this positively charged group away from the ester function. Direct interaction effects between a (1+) charged group and the ester carbonyl oxygen should be small compared to those for Cu(II). The only suitable group, which has been studied, is NH_3^+.

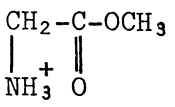
Thus the attenuation of charge and inductive effects of coordinated Cu(II), in going from Me 2,3-dap to Me 4,5-dape should be similar to the change observed in $k_{EH_2}^{2+}$ for these esters. However, there are problems. $k_{EH_2}^{2+}$ for Me 4,5-dape involves considerable assistance from intramolecular H-bonding and $k_{EH_2}^{2+}$ for Me 2,3-dap has not been measured. Hence values for k_{EH}^{+} must be used.

Looking at the series of diamino acid esters Me 2,3-dap, Me 2,4-dab, Me 2,5-dape, Me 2,6-dah (Figure 16, Section 6), k_{EH}^{+} values for Me 2,4-dab and Me 2,5-dape are again enhanced by intramolecular H-bonding (7 and 8 membered rings respectively). However in going from Me 2,3-dap to Me 2,6-dah, the 3 additional methylene groups between the ammonium group and the reaction centre in Me 2,6-dah (EH^+ form) gives rise to a decrease in rate of 45-times. In the series of monoamino acid esters, methyl 2-aminoacetate (methyl glycinate, Me Gly) methyl 3-aminopropanoate (methyl β-alaninate, Me β-ala), methyl 4-aminobutanoate (Me 4-but), and methyl 5-aminopentanoate (Me 5-pent), an H-bonding enhancement of the rate constant k_{EH}^{+} would similarly be expected for Me 4-but, since the same 7-membered ring

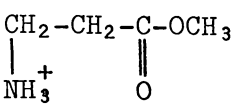
structure as for Me 2,4-dab is possible. However the value (k_{EH}^+ = 2.08ℓmol^{-1}s^{-1} cf. 456ℓmol^{-1}s^{-1} for Me 2,4-dab) suggests that such enhancement is not occurring.

Apparently a second amino group is requried to force the conformation required for H-bonding. A similar argument would presumably apply for Me 5-pent but k_{EH}^+ for this ester has not been measured.

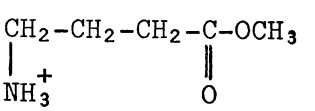
Hence the only currently available measure for the attenuation of charge and inductive effects by methylene groups, free from the influence of H-bonding, is given by the series:



Me Gly



Me β-ala



Me 4-but

k_{EH}^+ = 28.3 6.87 2.08ℓ mol^{-1}s^{-1}

Moving one, (1+) charged, ammonium group from C_2 to C_3 results in a 4.1 times decrease in the rate of hydrolysis with a further 3.3 times decrease in going from C_3 to C_4 (i.e. a 14 fold decrease from C_2 to C_4).

This 14 fold decrease in k_{EH}^+ is presumably similar to the decrease which would be observed in $k_{EH_2}^{2+}$ in going from Me 2,3-dap to Me 4,5-dape (in the absence of H-bonding effects). The decrease in the rate acceleration factor F produced by Cu(II) (Table 21), in going from Me 2,3-dap to Me 4,5-dape, is about 23 times. This is very similar to the 14 fold decrease expected from attenuation of charge and inductive effects alone (it is acknowledged that the comparison of Cu^{2+} to two NH_3^+ groups is obviously fairly crude). Hence, it is suggested, that in the present state of our knowledge of charge and inductive effects, the decrease in rate acceleration by Cu^{2+} in going from Me 2,3-dap to Me 4,5-dape can be explained solely by attentuation of these effects. The more dramatic decrease expected from the collapse of direct carbonyl oxygen-metal ion interaction in Me 2,3-dap is not observed.

APPENDIX I: CALCULATION OF PROTON DISSOCIATION CONSTANTS

A1.1 The Noyes Method[69]:

This calculates overlapping mixed proton dissociation constants (Ka^M's) of dibasic acids rather than thermodynamic values, i.e. values obtained using $\{H^+\}$ (pH-meter) but concentrations of all other species. The advantage of Ka^M is that no activity coefficient assumption is required. However Ka^M is not independent of the concentration of the acid concerned.

The method used was essentially that outlined by Albert and Serjeant[71a]. For a diamino acid ester dihydrochloride of total concentration C_E

$$C_E = [EH_2^{2+}] + [EH^+] + [E] \qquad (A1.1)$$

The ester solution is titrated with a strong monoacidic base to give a molar concentration C_t. Allowance must be made for dilution of the solution by the added base.

Hence

$$C_E = \frac{W.10^3}{M(V + V_t)} \qquad (A1.2)$$

and

$$C_t = \frac{V_t.N}{(V + V_t)} \qquad (A1.3)$$

where

W = weight of ester taken

M = molecular weight of the ester

V = initial solution volume

V_t = volume of titrant

N = concentration of titrant

Define $$X = \{H^+\}(C_t - C_E + \{H^+\}) \quad (A1.3)$$

$$Y = 2C_E - (C_t + \{H^+\}) \quad (A1.4)$$

$$Z = \{H^+\}^2(C_t + \{H^+\}) \quad (A1.5)$$

If X_1, Y_1, Z_1 refer to X, Y, Z values calculated from points when less than one equivalent of titrant has been added and X_2, Y_2, Z_2 to values from points when more than one equivalent has been added, then,

$$K_{a_1}^M = \frac{Y_1Z_2 - Y_2Z_1}{X_1Y_2 - X_2Y_1} \quad (A1.6)$$

$$K_{a_2}^M = \frac{X_1Z_2 - X_2Z_1}{Y_1Z_2 - Y_2Z_1} \quad (A1.7)$$

The pairs of points are usually selected symmetrically about one equivalent of titrant.

To simplifiy calculation a computer program, PKN, was written in ORACL (ORtec Analytical Computer Language). A listing of this is given in Table 22. The program, run on a PDP 11/05 computer calculates $pK_{a_1}^M$ and $pK_{a_2}^M$ for each pair of points.

The pK_a^T values can be calculated from the average pK_a^M values by equations (4.3b) and (4.4b). Values for molar activity coefficients y_1 and y_2 were calculated from the Davies equation[41]

$$-\log y_i = Az_i^2\left(\frac{\sqrt{I}}{1+\sqrt{I}} - 0.2I\right) \quad (A1.8)$$

where A = Debye-Hückel parameter[42]

z_i = charge on species i

I = ionic strength

At 25°C in a solution with I = 0.1molℓ^{-1} exactly $y_1 = 0.7715$ and $y_2 = 0.3545$.

The program PKN yeilds pK_a^M values for any dibasic acid. Evaluation of pK_a^T values requires activity coefficient corrections which depend on

Table 22: PKN

```
    1 REMARK CALCULATES OVERLAPPING MIXED PK VALUES OF BIFUNCTIONAL
    2 REMARK COMPOUNDS FROM POTENTIOMETRIC TITRATION DATA BY
    3 REMARK THE NOYES METHOD.
    5 ASK "NUMBER ",N,"HALF-NEUTRAL POINTS ",N2,!
   10 ASK "CONC BASE ",CB," WEIGHT OF COMPOUND ",EW,!
   15 ASK "INITIAL VOLUME ",VI,"MOL. WT. ",MW,!
   20 ASK "DATA IN? ",F,!
   25 IF(F).EQ.('YES);COMMON PH,VOL;GOTO 50
   30 COMMON PH,VOL;ERASE PH,VOL;DIMENS COMMON PH(N),VOL(N)
   35 TYPE !!"ENTER DATA"!"VOL"!
   40 FOR I=1,N2;ASK VOL(I);NEXT I
   41 FOR I=N,-1,N2+1;ASK VOL(I);NEXT I;TYPE !"PH"!
   45 FOR I=1,N2;ASK PH(I);NEXT I
   46 FOR I=N,-1,N2+1;ASK PH(I);NEXT I;TYPE !
   50 COMMON X,Y,Z,B,C,KM1,KM2,HA,PKM1,PKM2;ERASE X,Y,Z,B,C,KM1,KM2,HA,P
KM1,PKM2
   55 DIMENS COMMON X(N),Y(N),Z(N),B(N),C(N),KM1(N2),KM2(N2),HA(N),PKM1(
N2),PKM2(N2)
   60 FOR I=1,N
   65 B(I)=CB*VOL(I)/(VOL(I)+VI);C(I)=EW*1000/MW*(VOL(I)+VI)
   70 HA(I)=EXP(-PH(I)*LOG(10));X(I)=HA(I)*(B(I)-C(I)+HA(I))
   75 Y(I)=2*C(I)-(B(I)+HA(I));Z(I)=(HA(I)^2)*(B(I)+HA(I))
   80 NEXT I
   85 FOR I=1,N2
   90 KM1(I)=(Y(I)*Z(N2+I)-Y(N2+I)*Z(I))/(X(I)*Y(N2+I)-X(N2+I)*Y(I))
   95 KM2(I)=(X(I)*Z(N2+I)-X(N2+I)*Z(I))/(Y(I)*Z(N2+I)-Y(N2+I)*Z(I))
  100 PKM1(I)=-LOG(KM1(I))/LOG(10);S1=S1+PKM1(I)
  105 PKM2(I)=-LOG(KM2(I))/LOG(10);S2=S2+PKM2(I)
  110 NEXT I
  115 TYPE !!!!"VOL NAOH      PH          PKM1          PKM2" !
  120 TYPE,%3.02,CB,"M"!!
  125 FOR I=1,N2
  130 TYPE %5.03,VOL(I),"   ",PH(I),"      ",PKM1(I),!
  135 TYPE VOL(N2+I),"   ",PH(N2+I),"                    ",PKM2(I),!
  140 NEXT I
  145 FOR I=1,N2
  150 SD1=SD1+(PKM1(I)-S1/N2)^2;SD2=SD2+(PKM2(I)-S2/N2)^2
  155 NEXT I
  160 SD1=SQRT(SD1/(N2-1));SD2=SQRT(SD2/(N2-1))
  165 TYPE !!"PKM1 =   ",S1/N2," + -",SD1,!
  170 TYPE "PKM2 =   ",S2/N2," + -",SD2,!!
```

*

the nature of the dibasic acid e.g. for a diamino acid hydrochloride, the equations needed are 4.1b and 4.2b.

A1.2: The Speakman Method[70]:

As applied to a diamino acid ester dihydrochloride this method is as follows[71b]. If C_E and C_t are given by equations (A1.1), (A1.2) and (A1.3), electroneutrality of the solution requires that

$$C_t + [H^+] + [EH_2^{2+}] + [EH^+] = [Cl^-] + [OH^-] = 2C_E + [OH^-] \qquad (A1.9)$$

Since the ester is a dihydrochloride, $[Cl^-] = 2C_E$. Substituting equation (A1.1) in (A1.9)

$$C_t + [H^+] = [EH^+] + 2C_E + [OH^-] \qquad (A1.10)$$

$$\text{Define} \quad F = \frac{C_t + \{H^+\} - \{OH^-\}}{C_E} \qquad (A1.11)$$

The Speakman equation[70] for overlapping pK_a values can be written in the form

$$\frac{1}{K_{a1}^M} \cdot \frac{\{H^+\}^2 . F}{(2-F)} - K_{a2}^M = \{H^+\} \cdot \frac{1-F}{2-F} \qquad (A1.12)$$

The literature program, PKDI[71b] available for solving equation (A1.12) uses the Debye-Huckel equation[42] for activity coefficients

$$-\log y_i = \frac{Az_i^2 \sqrt{I}}{1 + B a_i \sqrt{I}} \qquad (A1.13)$$

At 25°C $A = 0.5115 mol^{-\frac{1}{2}} \ell^{\frac{1}{2}}$, $B = 0.3291 \times 10^8 cm^{-1} mol^{\frac{1}{2}} \ell^{\frac{1}{2}}$ and $a_i = 5 \times 10^{-8} cm$. This equation (A1.13) was retained for the PKDI calculation of pK_{a1}^T and pK_{a2}^T although elsewhere in this thesis the usually more accurate, empirical, Davies equation (A1.8) has been used. The two equations yield essentially identical pK_a values.

Substituting for B and a_i in equation (A1.13)

$$-\log y_i = \frac{A z_i^2 \sqrt{I}}{1 + 1.6\sqrt{I}} \qquad \text{(A1.14)}$$

Define $$FS = \frac{\sqrt{I}}{1 + 1.6\sqrt{I}} \qquad \text{(A1.15)}$$

Hence $$y_i = \frac{1}{10^{(Az_i^2\ FS)}} \qquad \text{(A1.16)}$$

i.e. $$y_1 = \frac{1}{10^{(0.5115\ FS)}} \qquad y_2 = \frac{1}{10^{(2.046\ FS)}}$$

Substituting in equations (4.3b) and (4.4b) equation (A1.12) becomes

$$\frac{1}{K_{a_1}^T} \cdot \frac{\{H^+\}^2.F}{(2-F)} \quad \frac{10^{(2.046\ FS)}}{10^{(0.5115\ FS)}} - K_{a_2}^T \cdot \frac{1}{10^{(0.5115\ FS)}} = \{H^+\} \cdot \frac{(1-F)}{(2-F)}$$

Let $X = \frac{\{H^+\}^2.F}{(2-F)}$ and $Y = \frac{\{H^+\}.1-F}{2-F}$

Thus $$\frac{1}{K_{a_1}^T} \cdot X \cdot 10^{(2.046\ FS)} - K_{a_2}^T = Y \cdot 10^{(0.5115\ FS)} \qquad \text{(A1.17)}$$

The thermodynamic constants are calculated from equations (A1.12) and (A1.17) by the following procedure:

1. Equation (A1.12) is solved by the least squares method[71b] to give approximate values of $K_{a_1}^M$ and $K_{a_2}^M$.

2. The concentrations of ionised species are calculated using these values of $K_{a_1}^M$ and $K_{a_2}^M$ in the equations

$$[EH_2^{2+}] = \frac{\{H^+\}^2.C_E}{D} \qquad \text{(A1.18)}$$

$$[EH^+] = \frac{K_{a_1}^M.\{H^+\}.C_E}{D} \qquad \text{(A1.19)}$$

where $D = \{H^+\}^2 + K_{a1}^M\{H^+\} + K_{a_1}^M \cdot K_{a_2}^M$ (A1.20)

3. These concentrations are used to calculate the ionic strength which is given by

$$I = 0.5([Cl^-] + C_t + 4[EH_2^{2+}] + [EH^+] + 3\{H^+\}) \quad (A1.21)$$

From this the activity functions (FS, equation A1.15) are determined.

4. The hydrogen activity is converted to concentration by $[H^+] = \{H^+\} \cdot 10^{(0.5115\ FS)}$ and this term and $[OH^-]$ calculated from it are used to recalculate F (A1.11). The activity corrections to be applied in equation (A1.17) are also calculated.

5. Equations (A1.12) and (A1.17) are solved by least squares and $K_{a_1}^M$ and $K_{a_2}^M$ from (A1.12) used to calculate $[EH_2^{2+}]$ and $[EH^+]$ as before. These concentrations and $[H^+]$ are then used to refine the ionic strength values.

6. The sequence of steps 4 and 5 are repeated until successive values of $K_{a_1}^T$, and of $K_{a_2}^T$, are constant.

For a diamino acid hydrochloride (i.e. an ampholyte) equation (A1.17) becomes

$$\frac{1}{K_{a_1}^T} \cdot X - K_{a_2}^T = \frac{Y}{10^{(0.5115\ FS)}}$$

and $I = 0.5\ ([Cl^-] + C_t + [AH_2^+] + [A^-] + 3[H^+])$

where $[AH_2^+] = \dfrac{\{H^+\}^2 . C_E}{D}$

(D is defined in equation (A1.20).

The PKDI program[71b] in FORTRAN IV was translated into ORACL (Table 23) and run on a PDP 11/05 computer. This program is applicable to dibasic acids, ampholytes (compounds with both acidic and basic groups) and diacidic

bases, for a given temperature and A and B activity coefficient parameters.

Table 23: PKDI

```
  5 REMARK  CALCULATES OVERLAPPING PK VALUES OF BIFUNCTIONAL
 10 REMARK  COMPOUNDS FROM POTENTIOMETRIC DATA AT ANY TEMPERATURE.
 15 ASK !!"NUMBER ",N," NUMBER PTS IN 1ST EQUIVALENT ",K
 20 ASK !"MOLECULAR WEIGHT ",SL," WEIGHT IN VOLUME ",WT
 25 ASK !"INITIAL VOLUME ",AL," CONC TITRANT ",AC," TEMP ",T
 30 ASK !"K TYPE, NEG-ACID, 0-AMPHOLYTE, POS-BASE ",KTYPE
 35 ASK !"PKW AT TEMP ",KW," MOLES ACID IN DIACIDIC BASE ",MA
 37 ASK !"CONC KCL ",KM," VOL ADDED ",VK
 40 ASK !"PARAMETERS A & B AT TEMP ",D1,D2
 41 ASK !"DATA IN MEMORY ? ",WQ,I
 45 SET D2=D2*5*10^(-3)
 50 COMMON PH,VA,NSUBS
 55 IF(W Q).NE.(QYES);GOSUB 400
 60 DIMENS X(N),Y(N),H(N),OH(N),ACT(N),ACT1(N),ACT2(N)
 65 FOR I=1,N
 70 H(I)=10^(-PH(I));OH(I)=10^(-KW+PH(I))
 75 ACT(I)=1,ACT1(I)=1,ACT2(I)=1
 80 NEXT I
100 REMARK  SOLVE EQUATIONS  1 & 2 BY LEAST SQUARES TO OBTAIN
101 REMARK  MIXED CONSTANTS, CK1 & CK2, AND THERMODYNAMIC CONSTANTS
102 REMARK  TK1 & TK2.
105 TK1=0;TK2=0
110 SUMX=0,SUMY=0,SUMXY=0,SUMA=0,SUMX2=0,SUMB=0,SUMAB=0,SUMA2=0
112 TK1A=TK1,TK2A=TK2
115 FOR I=1,N
120 F=(VA(I)*AC/(AL+VA(I))+H(I)-OH(I))*SL*(AL+VA(I))/WT*1000
125 A=((10^(-PH(I)))^2)*F/(2-F);B=(10^(-PH(I)))*(1-F)/(2-F)
130 IF(KTYPE)135,140,145
135 X(I)=A/ACT2(I);Y(I)=B/ACT1(I);GOTO 150
140 X(I)=A;Y(I)=B/ACT(I);GOTO 150
145 X(I)=A*ACT2(I);Y(I)=B*ACT(I)
150 SUMY=SUMY+Y(I);SUMB=SUMB+B;SUMX=SUMX+X(I);SUMA=SUMA+A
155 SUMXY=SUMXY+X(I)*Y(I);SUMX2=SUMX2+X(I)^2;SUMA2=SUMA2+A^2
160 SUMAB=SUMAB+A*B
165 NEXT I
170 D11=N*SUMX2-SUMX^2;D12=N*SUMA2-SUMA^2
175 TK1=D11/(N*SUMXY-SUMX*SUMY)
180 CK1=D12/(N*SUMAB-SUMA*SUMB)
185 TK2=ABS((SUMX2*SUMY-SUMX*SUMXY)/D11)
190 CK2=ABS((SUMA2*SUMB-SUMA*SUMAB)/D12)
200 REMARK  IONIC STRENGTH AND ACTIVITY FUNCTIONS COMPUTED AT TEMP T-
205 FOR I=1,N
207 P=10^(-PH(I))
210 D1=(P^2+CK1*P+CK1*CK2)*SL*(AL+VA(I))/WT*1000
215 HA=CK1*P/D1;AA=CK1*CK2/D1;H2A=P^2/D1
220 CL=MA*1000/(AL+VA(I));KC=VK*KM/(AL+VA(I))
225 IF(KTYPE)230,235,240
230 ST=.5*((VA(I)*AC/(AL+VA(I)))+HA+4*AA)+1.5*H(I)+KC;GOTO 245
235 ST=.5*((VA(I)*AC/(AL+VA(I)))+CL+H2A+AA)+1.5*H(I)+KC;GOTO 245
240 ST=.5*((VA(I)*AC/(AL+VA(I)))+CL+H2A*4+HA)+1.5*H(I)+KC
245 FS=SQRT(ST)/(1+D2*SQRT(ST))
250 ACT(I)=10^(D1*FS);ACT1(I)=10^(3*D1*FS)
255 ACT2(I)=10^(4*D1*FS);H(I)=P*ACT(I)
260 OH(I)=(10^(-14))/H(I)
265 NEXT I
270 REMARK  CHECK CONVERGENCE OF SUCCESSIVE VALUES  FOR CONSTANTS
275 EPA=ABS(TK1*10^(-5));EPB=ABS(TK2*10^(-5))
280 DA=ABS(TK1A-TK1);DB=ABS(TK2A-TK2)
285 IF(DA).GT.(EPA);GOTO 110
290 IF(DB).GT.(EPB);GOTO 110
295 REMARK  CALCULATE PK1 AND PK2
300 SUM=0,SUM2=0
```

Continued ...

Table 23: (continued)

```
305 TYPE !!!"SUBSTANCE:   ",NSUBS," = ",%6.03,SL,!
310 TYPE %3.01,"TEMPERATURE:   ",T,!!
315 TYPE "   TITRANT";AC,"M KOH"!"    (ML)      PH        PK1          PK2"!!
320 FOR I=1,K
325 PK=X(I)/(Y(I)+TK2);PK=LOG(1/PK)/LOG(10);SUM=SUM+PK;SUM2=SUM2+PK*PK

330 TYPE %5.03,VA(I),"   ",PH(I),"  ",PK,!
335 NEXT I
340 AV=SUM/K;SD=SQRT((SUM2-SUM*SUM/K)/(K-1));SUM=0,SUM2=0
345 FOR I=K+1,N
350 PK=X(I)/TK1-Y(I);PK=LOG(1/PK)/LOG(10);SUM=SUM+PK,SUM2=SUM2+PK*PK
355 TYPE VA(I),"   ",PH(I),"              ",PK,!
360 NEXT I
365 TYPE !"MEAN & ST. DEV.:  PK1 = ",AV,"    ",SD,!
366 SD2=SQRT((SUM2-SUM*SUM/(N-K))/(N-K-1))
367 TYPE "                    PK2 = ",SUM/(N-K),"    ",SD2,!
370 GOTO 440
400 REMARK  DATA ENTRY
405 ERASE PH,VA,NSUBS;STRING COMMON NSUBS(30)
410 DIMENS COMMON PH(N),VA(N)
415 ASK !"NAME OF COMPOUND: ",NSUBS
420 TYPE !"ENTER VOL-PH DATA:"!
425 FOR I=1,N;ASK VA(I),PH(I);NEXT I;TYPE !
430 RETURN
```

*

APPENDIX II: CALCULATION OF STABILITY CONSTANTS

A2.1 SCOGS[75] (Stability Constants Of Generalised Species)

This computer program can calculate formation constants, either together or individually, of any complex species where the degree of complex formation is pH dependent, in systems containing up to two metals and two ligands. Complexes include protonated ligand and hydrolysed metal ion species. The program written in FORTRAN IV was run in the published form[75] on a PDP 11/70 computer.

The program refines overall practical formation constants (β's) defined in terms of the concentrations of metal ions and ligands and the activity of hydrogen ions. E.g. if M is a metal and L a ligand, for the equation

$$M + L + H \rightleftharpoons M(LH)$$

$$\beta = \frac{[M(LH)]}{[M][L]\{H\}}$$

For complexes containing hydrolysed metal ion species, the constant for the equation

$$M + L \rightleftharpoons ML(OH) + H$$

is

$$\beta = \frac{[ML(OH)]\{H\}}{[M][L]}$$

$$= \frac{[ML(OH)] \cdot K_{\omega}}{[M][L]\{OH\}}$$

(Charges on all species have been omitted.)

This interpretation of the constants differs from the published description of the program[75] but describes more exactly the form required by the program. The program refines such constants by a Gauss non-linear

least squares method which minimises the sum of squares of residuals. (Residuals are the differences between experimental and calculated titres for each experimental point of a potentiometric titration.)

In order to do this, estimates of free ligand and free metal concentrates are required. For the first point the free metal is assumed to be the total metal concentration and the free ligand is calculated using the acid association constants of the ligand assuming no complex formation. Subsequently the final values of the (n-1)th point are used as initial estimates of the nth point. These estimates are refined by a Newton-Raphson iteration until they are satisfactory roots to simultaneous equations for total metal and total ligand concentrations (the convergence limit is set at 10^{-5}% of the free concentrations).

The free metal and free ligand concentrations obtained and the input estimates of the formation constants are used to calculate the concentrations of each species and hence the analytical hydrogen ion concentration. This is then used to calculate the titre and thus the residual for each point.

The program progresses through each experimental point setting up and solving by matrix inversion the least squares equations, and giving shifts forthe estimated formation constants. The improved values of the constants are then used to repeat the cycle. Usually only five such cycles are required to minimise the residuals and give zero shifts for the constants.

When calculation is complete the computer prints out the following information. The initial concentrations of metals, ligands and titrant base and the initial volume are listed along with a table showing the composition of the proposed complexes and the estimates of their constants as logarithms. Following this, for each cycle, the improved constants are

printed along with the shift value. A measure of precision can be gauged from the computed standard deviation which is given for each constant. The estimated standard deviation in titre is also given which shows the ability of the refined values to fit the experimental data. Finally, after the allowed number of cycles, a table is printed showing for each experimental point, the pH, experimental titre, residual, the total concentrations of each metal and ligand, the concentrations of each free metal and free ligand and the concentration of each complex species. These quantities are all calculated from the final set of constants.

The program requires, in the input, initial estimates of the cumulative stability constants. These estimates were made from published data for related systems, if necessary by combining the relevant stepwise stability constants.

In this work, the acid association constants established previously (Section 4) were kept constant and the stability constants of the possible complex species were allowed to vary. Possible species included the simple complexes (ML, ML_2, etc.), protonated complexes (M(LH), ML(LH), etc.) and hydroxy complexes (ML(OH), etc.). If a species was an insignificant component of the mixture (<0.5%) its stability constant failed to converge. This appeared as a steady diminishing of the value or as a "hunting" of the value about the initial estimate . The technique used was to initially converge only simple complexes and then progressively introduce protonated species and hydroxy species. If the additional species were important and their constants converged a lowering of the estimated standard deviation in the titre occurred. Introduction of constants for insignificant species tended to raise the estimated standard deviation in titre.

A2.2 pH-Titration data for Cu(II) complexes of various ligands

All concentrations are in molℓ^{-1} and all volumes in ml. Temperature = (25 ± 0.05)°C and ionic strength = 0.10 molℓ^{-1}(KCl) in all cases (see also Section 5).

1. Cu(II): en = 1:2

Initial conditions: [Cu^{2+}] = 0.00505051; [en.2HCl] = 0.01010101; [Titrant NaOH] = 1.008; Volume = 99.00

Titre	pH	Titre	pH	Titre	pH
0.000	3.630	0.700	4.570	1.400	5.282
0.100	3.915	0.800	4.663	1.500	5.400
0.200	4.075	0.900	4.762	1.600	5.532
0.300	4.192	1.000	4.862	1.700	5.690
0.400	4.295	1.100	4.968	1.800	5.899
0.500	4.390	1.200	5.070	1.900	6.232
0.600	4.480	1.300	5.173	2.000	8.662

2. Cu(II): Me 4,5-dape = 1:2

Initial conditions: [Cu^{2+}] = 0.00505051; [Me 4,5-dape.2HCl] = 0.01010101; [Titrant NaOH] = 1.000; Volume = 99.00.

Titre	pH	Titre	pH	Titre	pH
0.000	3.118	0.700	3.855	1.400	4.536
0.100	3.274	0.800	3.941	1.500	4.652
0.200	3.399	0.900	4.032	1.600	4.784
0.300	3.501	1.000	4.126	1.700	4.943
0.400	3.595	1.100	4.223	1.800	5.157
0.500	3.684	1.200	4.323	1.900	5.532
0.600	3.769	1.300	4.427	2.000	8.119

3. Cu(II): 4,5-dape = 1:2

Initial conditions: $[Cu^{2+}]$ = 0.00510204; [4,5-dape.2HCl] = 0.01020410; [Titrant NaOH] = 1.0016; Volume = 98.00

Titre	pH	Titre	pH	Titre	pH
0.000	2.845	1.100	3.847	2.100	4.620
0.100	2.978	1.200	3.915	2.200	4.712
0.200	3.105	1.300	3.985	2.300	4.805
0.300	3.213	1.400	4.057	2.400	4.909
0.400	3.315	1.500	4.130	2.500	5.020
0.500	3.403	1.600	4.205	2.600	5.148
0.600	3.487	1.700	4.287	2.700	5.303
0.700	3.563	1.800	4.367	2.800	5.512
0.800	3.638	1.900	4.449	2.900	5.865
0.900	3.709	2.000	4.533	3.000	8.353
1.000	3.779				

4. Cu(II): Me 4,5-dape = 1:1

Initial conditions: $[Cu^{2+}]$ = 0.01010101; [Me 4,5-dape.2HCl] = 0.01010101; [Titrant NaOH] = 0.9954; Volume = 99.00

Titre	pH	Titre	pH	Titre	pH
0.000	3.000	1.100	3.810	2.100	6.285
0.100	3.138	1.200	3.877	2.200	6.39 *
0.200	3.233	1.300	3.941	2.300	6.51
0.300	3.317	1.400	4.005	2.400	6.66
0.400	3.390	1.500	4.080	2.500	6.83
0.500	3.457	1.600	4.163	2.600	6.98
0.600	3.518	1.700	4.266	2.700	7.36
0.700	3.579	1.800	4.382	2.800	7.95
0.800	3.637	1.900	4.560	2.900	9.82
0.900	3.693	2.000	5.099	3.000	10.66
1.000	3.751				

* precipitation occurs

5. Cu(II): Me 4,5-dape: 4,5-dape = 1:1:1

Initial conditions: $[Cu^{2+}]$ = [Me 4,5-dape.2HCl] = [4,5-dape.2HCl]= 0.00507614; [Titrant NaOH] = 1.0016; Volume = 98.50.

Titre	pH	Titre	pH	Titre	pH
0.000	2.959	0.900	3.845	1.800	4.630
0.100	3.109	1.000	3.921	1.900	4.738
0.200	3.241	1.100	4.000	2.000	4.853
0.300	3.350	1.200	4.080	2.100	4.988
0.400	3.448	1.300	4.163	2.200	5.151
0.500	3.537	1.400	4.250	2.300	5.372
0.600	3.617	1.500	4.340	2.400	5.778
0.700	3.693	1.600	4.432	2.500	8.268
0.800	3.769	1.700	4.530		

6. Cu(II): Me 4,5-dape: en = 1:1:1

Initial conditions: $[Cu^{2+}]$ = [Me 4,5-dape.2HCl] = [en.2HCl] = 0.00505051; [Titrant NaOH] = 1.0008; Volume = 99.00.

Titre	pH	Titre	pH	Titre	pH
0.000	3.233	0.700	4.119	1.400	5.032
0.100	3.415	0.800	4.237	1.500	5.175
0.200	3.567	0.900	4.363	1.600	5.323
0.300	3.685	1.000	4.498	1.700	5.500
0.400	3.795	1.100	4.632	1.800	5.721
0.500	3.902	1.200	4.769	1.900	6.088
0.600	4.005	1.300	4.903	2.000	8.180

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