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TETRAHEDRON

Synthesis and X-ray Crystal Structures of Schiff bases Prepared from Salicylaldehyde and the Diamino acids L-2-Amino-3-methylaminopropanoic acid, DL-2,4-Diamino-butanoic acid and DL-2,3-Diaminopropanoic acid

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Abstract: Three new Schiff bases have been synthesised from the reaction of salicylaldehyde with the plant amino acids L-2-amino-3-methylaminopropanoic acid (L-MeDAP), DL-2,4-diaminobutanoic acid (DL-DAB) and DL-2,3-diaminopropanoic acid (DL-DAP). The reaction of salicylaldehyde with L-MeDAP gave only the 2- (α-) product. The reaction of salicylaldehyde with DL-DAB or with DL-DAP produced, at equilibrium, predominately the 4- (ω-) or 3- (β-) species. X-ray single-crystal structures were determined for two compounds.

INTRODUCTION

A number of diamino acids found in plants, are neurotoxic to the mammalian central nervous system. The mechanisms by which this toxicity is expressed are of considerable interest because of the potential of these compounds as environmental neurotoxins. L-2-amino-3-methylaminopropanoic acid (L-MeDAP; synonym β-methylamino-L-alanine, L-BMAA), which was first isolated from seeds of *Cycas circinalis* (false sago palm),[1] is also present in seeds of other species of the genus *Cycas*.[2] The compound is one of a number of components that has been implicated in the complex human neurological disease amyotropic lateral sclerosis (motor neurone disease)-parkinsonism-dementia (ALS-PD) that is seen on the island of Guam in the western Pacific.[3-5] L-2,4-Diaminobutanoic acid, which is a component of *Vicia sativa* seed,[6] causes convulsions in rats. This effect may be mediated *via* inhibition of urea synthesis,[7] neurotoxicity would then result from the systemic accumulation of ammonium ions. However, other mechanisms have also been proposed.[8] L-2,3-Diaminopropanoic acid (DL-DAP) is a common plant metabolite, which may occurs free but also as the β-acetyl- or β-N-oxalyl-derivatives in some plants.[6] β-N-

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Oxalyl-L-α,β-diaminopropionic acid (β-ODAP, synonym β-oxalylaminopropionic acid, BOAA) has been implicated as the cause of human neurolathyrism,[9] an upper motor neurone disease that is endemic in parts of Ethiopia and the Indian sub-continent.

All three compounds are neurotoxic to cultured preparations of the murine central nervous system.[10-11] Such toxicity is bicarbonate-dependent[11] and it was proposed that the compounds reacted with bicarbonate/carbonate to form a complex of analogous structure to L-glutamate, the physiologically active neurotransmitter at these receptors. DL-DAP was the most potent of the three compounds and DL-DAB was least effective.[11] The neurotoxicity of L-MeDAP (and probably the other compounds also) were mediated mainly by the class of excitatory glutamate receptors activated by N-methyl-D-aspartate (NMDA).[11]

Under experimental conditions that approximated those used in *in vitro* systems, the amino groups of MeDAP, DAB and DAP react with bicarbonate to form carbamates.[12-13] The diamino acids form carbamates because the low pK_a values of their amino groups mean that the amino groups are uncharged at physiological pH values. All three amino acids have two free amino groups and it is possible for carbamate formation to occur at either, or both of these groups.[14] Thus MeDAP forms carbamates at both 2- (α-) and 3- (β-) positions with the former predominating;[13] DAB and DAP both form approximately equal amounts of carbamate at each amino group.[15] Although the reactivity of these groups are affected by the micro-constants for the ionization of the amino groups.

Amino acids containing uncharged amino groups, at physiological pH values, may also undergo Schiff base formation (i.e. condensation with aldehydes), which presents another potential mechanism for toxicity. Such compounds might be expected to react *in vivo* with pyridoxal phosphate, and other compounds containing an aldehyde group, and disturb normal metabolic processes. Under conditions that approximated those *in vivo* the diamino acids all formed Shiff bases with pyridoxal phosphate, pyridoxal and salicylaldehyde.[16] However, only the adducts formed with salicylaldehyde, which is a good model for pyridoxal phosphate-catalysed reactions,[17-18] were stable enough for structural studies.

This paper describes the preparation of crystalline products derived from reacting salicylaldehyde with L-2-amino-3-methylaminopropanoic acid, DL-2,4-diaminobutanoic acid and DL-2,3-diaminopropanoic acid; X-ray single-crystal structures of the Schiff bases thus formed are reported. The reaction of salicylaldehyde with L-MeDAP yielded only the 2- (α-) product where DL-DAB and DL-DAP produced predominately the 4- (ω-) or 3- (β-) species, respectively, and only small amounts of the 2- (α-) species of both compounds. To our knowledge these are the first X-ray crystal structures reported for Schiff bases derived from the reaction of salicylaldehyde with simple amino acids.

RESULTS AND DISCUSSION

X-ray crystal structures were determined using single crystals and were refined by least-square method to give final R_w = 4.28% for [3(methylamino)-2-[(hydroxybenzylidene) amino]-propanoic acid **(1)** and R_w = 7.85% for N-(2-hydroxy benzylidene)DL-2,4-diaminobutanoic acid **(2)**. The molecular structures of compound **(1)** and **(2)** are shown in Figures (1) and (2). A preliminary X-ray crystal structural study of N-(2-hydroxybenzylidene)-DL-2,3-diaminopropanoic acid **(3)** showed a structure similar to that of compound **(2)**.

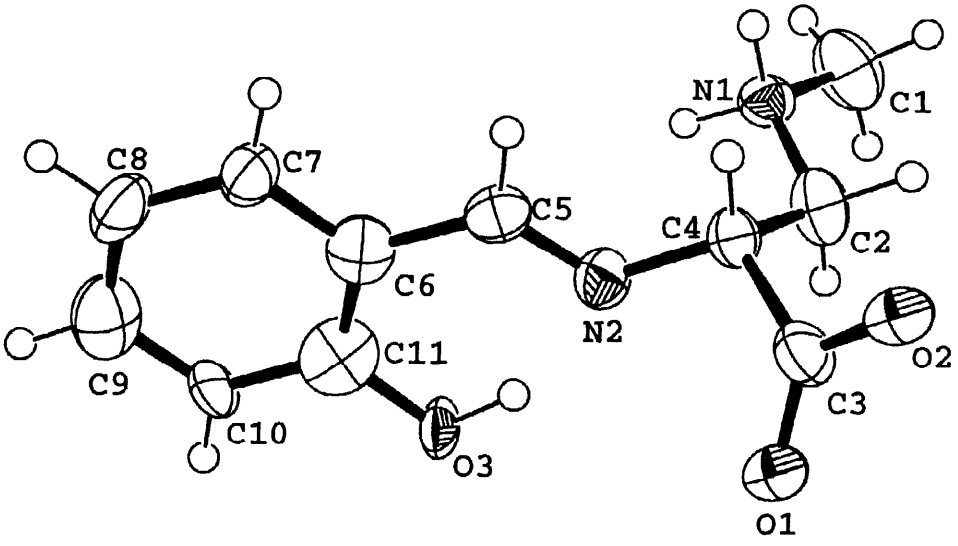

Figure1: Structure of [3(methylamino)-2-[(hydroxybenzylidene) amino]-propanoic acid **(1)**

Compounds **(1)** and **(2)** have similar bond lengths for C-C and C-N. The bond distance between H_3N^+-C (1.476 (3) Å) for **(1)** and (1.494 (5) Å) for **(2)** is close to that reported for L-2-amino-3-methylaminopropionic acid (1.491(4) Å),[15] L-2,4-diaminobutaroic acid (1.494(1) Å),[18] lysine (1.482 Å) and for ornithine (1.491) Å.[19]
The bond distances between C(1)-C(2) (1.513 (5) Å), C(2)-C(3) (1.511 (6) Å), and C(3)-C(4) (1.517 (7) Å) for compound **(2)** are almost identical to the C-C bond distances for lysine (1.522 Å), (1.524 Å) and for ornithine (1.526) Å, (1.530) Å, which are usually accepted as a standard. No significant change in C-CO_2 bond distance and angle is noticed. In compound **(1)** the bond distance of C(5)-N(2) (1.277(4) Å) is considerably shorter than those of N(1)-C(2) (1.476 (3) Å) and N(1)-C(1) (1.478 (4) Å) similarly in compound **(2)** the bond distance between N(2)-C(5) (1.246) (6) Å) is shorter than the distance between N(1)-C (2) (1.494) (5) Å) showing a double bond between C(5) and N(2).

There are several structures known for metal complexes[20-27] of Schiff bases. The C=N bond distance is longest in [{Cu(sal-L-phe)(H_2O}$_2$] (1.36(4) Å) and is shortest in [VO(salgly)(py)$_2$ (1.273 (8) Å)[28] which is close to those of compounds **(1)** (1.248 (6) Å), and **(2)** (1.277 (4) Å).

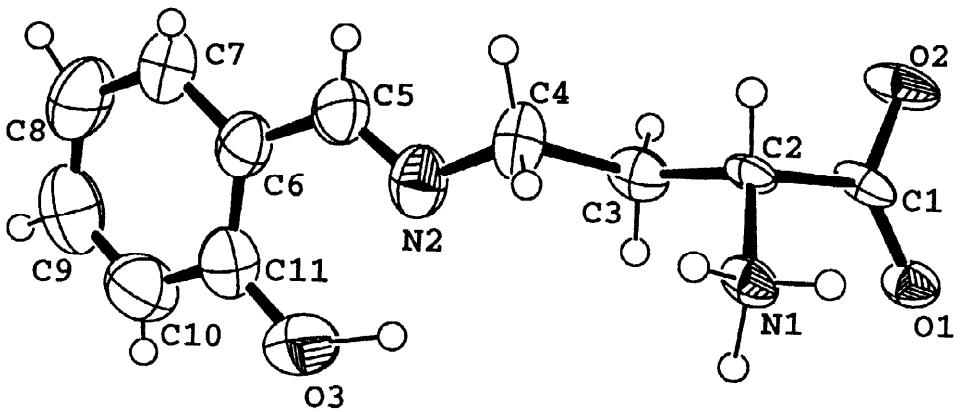

Figure 2: Structure of N-[2-(hydroxybenzylidene)]DL-2,4-diaminobutanoic acid **(2)**

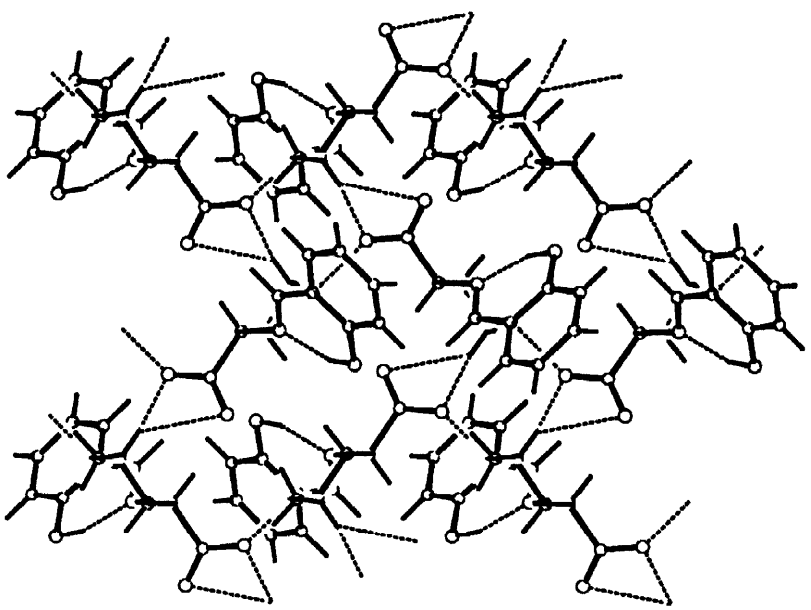

Figure 3: Packing diagram of [3(methylamino)-2-[(hydroxybenzylidene) amino]-propanoic acid. **(1)**

Packing diagrams for both compounds gave evidence for at least two types of H-bonding. The structure of compound **(1)** shows intramolecular hydrogen bonding through H(1) to N(2) (1.752 Å) and intermolecular

hydrogen bonding through H(1), H(2) to O(1), O(2) (1.912 and 1.772 Å). Figure (3) shows a packing diagram for compound **(1)**.

NMR studies were carried out on all three compounds. The ^{1}H NMR spectrum of (3-methylamino)-2-[(hydroxybenzylidene)amino]-propanoic acid **(1)** gave a clear spectrum for the presence of the 2- (α-) species, the only Schiff base (Figure 4). The evidence for the 2- (α-) species can also be obtained from the large change in chemical shift of the 2- (α-) proton (δ 3.82 for pure amino acid and δ 4.25 in Schiff base). The N-methyl group gives a singlet at high field. The N-methylene group gives two sets of doublets of doublets due to the non-equivalent protons which couple with each other and with the neighbouring 2- (α-) protons. The 2- (α-) proton appears as a triplet at low field as expected. The signals for salicylaldehyde appear in the range 6.9 to 7.8 ppm. The spectrum showed the presence of some free amino acid and aldehyde, which may be the result of dissociation of the compound. The spectra repeated after 24hr showed no change, but after heating at 50 °C for 1hr the signals in the aromatic region were considerably depressed due to the evaporation of the dissociated aldehyde.

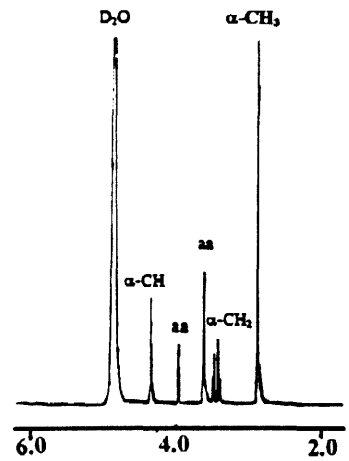

Figure 4: ^{1}H NMR spectrum of [3(methylamino)-2-[(hydroxybenzylidene) amino]-propanoic acid. in D_2O. Section of the spectrum given is that associated with the amino acid moiety aa = amino acid.

The ^{1}H NMR spectra of the crude products from the reaction of DL-2,4-diaminobutanoic acid, or DL-2,3-diaminopropanoic acid, with salicylaldehyde showed the presence of 2- (α-) and 4- (ω-) or 2- (α-) and 3- (β-) species, respectively, with the 4- (ω-) or 3- (β-) species predominating. The ^{1}H NMR were repeated for each sample after 2hr, 3hr, and 24hr at room temperature. Both diamino acids showed similar trends in their

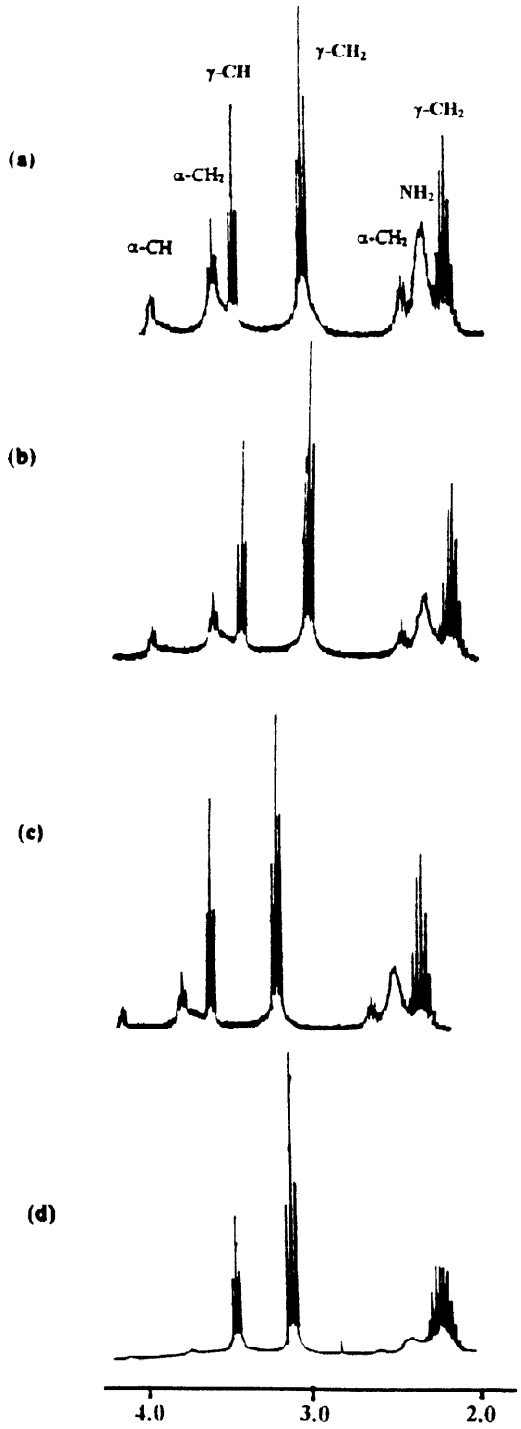

Figure 5: ^{1}H NMR spectra of N-(2-hydroxybenzylidene)-DL-2,4-diaminobutanoic acid crude products in D_2O (a) 0 hr, (b) 2 hr, (c) 3 hr, (d) 24 hr. All four show the section of the spectrum associated with the amino acid protons.

behaviour. N-(2-hydroxy benzylidine)-DL-2,3-diaminopropanoic acid **(3)**, at zero time contained 2- (α-) and 3- (β-) species in the ratio of 1:10, but after two hours the amount of the 2- (α-) species was reduced one half (2- (α-) : 3- (β-) ratio 1:20), after another hour the concentration of the 2- (α-) species was further reduced and the ratio 2- (α-) : 3- (β-) was 1:30; after 24hr only traces of the 2- (α-) species were observed. After heating at 50 °C for 1h the spectrum showed a slight decrease in intensity for the signals in the aromatic region, indicating the evaporation of free aldehyde. The same phenomena were observed for N-(2-hydroxybenzylidine)-DL-2,4-diaminobutanoic acid **(2)** (Figure 5 (a), (b), (c) and (d)).

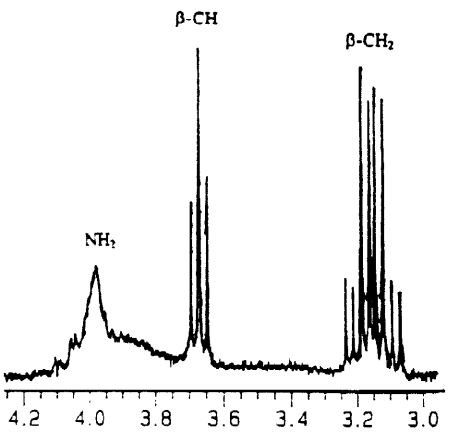

Figure 6: ^{1}H NMR spectrum of N-(2-hydroxy benzylidine)-DL-2,3-diaminopropanoic acid recrystallized product in D_2O. Section of the spectrum given is that associated with the amino acid moiety.

The ^{1}H NMR spectra of the recrystallized compounds showed the presence of only one species. Compound **(2)** gave a multiplet for the 3- (β-) methylene protons, a triplet for the 4- (ω-) methylene protons and a triplet for the 2- (α-) proton. Compound **(3)** showed two sets of doublets of doublets for N-methylene protons at the high field, a triplet for the methyne proton and a broad signal for the amino protons (Figure 6). The spectra of a pure samples after 24hr showed no change, but after heating at 50 °C for 1hr showed, in both cases, a slight dissociation of compounds similar to that observed for the crude products.

The C=N bands of all compounds were difficult to identify in the i.r. spectra due to large variations in the intensities of the bands. These bands were found near to 1639cm^{-1} - 1640cm^{-1} (C=N). However, the Raman spectra showed sharp and very intense bands at 1640cm^{-1}, 1642cm^{-1} (C=N) for compound **(1)** and **(2)** and 1641cm^{-1} (C=N) for compound **(3)**. Two bands near 2500 - 2000cm^{-1} (-NH_3^+) were found. The strong sharp band due to ionised carboxyl groups at 1620, 1430cm^{-1} (-CO_2^-) and symmetrical CH_3 stretching frequency at 1354cm^{-1} (CH_3) were also found.

CONCLUSION

Our observations on the reactivity of these diamino acids with salicylaldehyde allow us to make some interesting comments on the reactivity of the (α-) and (ω-) positions of such compounds. The results of previous workers[29-30] show a preference for the formation of ω- derivatives. However, our studies of freshly prepared solutions of salicylaldehyde with DL-DAB and DL-DAP show clearly the initial formation of considerable quantity of Schiff base at the 2- (α-) position, which re-equlibrates and forms an equilibrium mixture containing predominantly the 4- (ω-) isomer. L-MeDAP can form a Schiff base only at the 2- (α-) position (Schiff's bases cannot be formed at secondary amine functions). However in our study of the related phenomenon of carbamate formation by L-MeDAP we noted an initial predominance of the 2- (α-) isomer,[12] with such solution containing at equilibrium a small predominance of the 2- (α-) isomer. These observations are readily explained by the initial product of the reaction of, e.g. CO_2 with L-MeDAP or salicylaldehyde with DL-DAP, being those of kinetic control (reflecting the availability of the deprotonated amine functions at neutral pH values) with a slow redistribution to an equilibrium mixture of 2- (α-) and 3- (β-) isomers. From our results with DL-DAB the 4- (ω-) isomer, and with DL-DAP the 3- (β-) isomer, of the Schiff base clearly predominate, whereas for the carbamate of L-MeDAP the 2- (α-) isomer, which originally is formed in large excess, yields an equilibrium mixture in which the 2- (α-) isomer is present in a small excess.

Our studies of these non-protein amino acids show that they readily form Schiff bases. The reactions of diamino acids at neutral pH, to form carbamates or Schiff bases, may be important factors in the exclusion of such amino acids for normal mammalian metabolism and an especially important factor in understanding the toxicity of the plant amino acids in animals.

EXPERIMENTAL

Analytical Methods: Infrared spectra were recorded as in Nujol mulls between KBr plates using a Mattson polaris FTIR. Raman spectra were recorded using a Perkin-Elmer 760 x FT-IR instrument fitted with a 700 x NIR FT-Raman accessory. NMR spectra were recorded on a AM 250 pulsed Fourier Transform NMR instrument, using D_2O as the solvent. Microanalysis (CHN) were performed by University College and Imperial College London Microanalytical Services.

Starting Materials: Salicylaldehyde was obtained from Aldrich; L-2-amino-3-methylaminopropanoic acid monohydrochloride (L-MeDAP) was synthesised.[1] DL-2,4-diaminobutanoic acid dihydrochloride (DL-DAB) and DL-2,3-diaminopropanoic acid monohydrochloride (DL-DAP) were obtained from Sigma.

The X-ray structures of compounds **(1), (2)** were determined using single crystals, in glass capillaries. The intensity data were collected by CAD4 diffractometer using $\omega/2\theta$ scan mode with graphite-monochromated M_o K_α radiation. The unit cell parameters were determined by a least-squares refinement on diffractometer angles for automatically centred 25 reflections ($10 \leq \theta \leq 13°$). The structures were solved by direct method, using SHELXS-93 program[31] and refined anisotropically (all non-hydrogen atoms) on F^2, using SHELXL-93 program.[31] Some hydrogen atoms were positioned geometrically but all refined freely without any restrain. The molecules were drawn using the ORTEP-3 program[32]. Positional parameters, hydrogen atom co-ordinates, a full list of bond lengths and angles, anisotropic displacement factor coefficients, and F_o/F_c values are included in the supplementary material. Atomic co-ordinates, bond lengths, bond angles and thermal parameters have been deposited at the Cambridge Crystallographic Data Centre.

[3(methylamino)-2-[(hydroxybenzylidene) amino]-propanoic acid **(1)** was prepared by adding a solution of L-2-amino-3-methylaminopropanoic acid monohydrochloride (0.46g, 3 mmol neutralised with 2M NaOH) in water ($10cm^3$) to a solution of salicylaldehyde (0.36g, 3 mmol) in ethanol ($15cm^3$) at room temperature which gave a deep yellow colour. The solution was allowed to evaporate at room temperature and, after two days had changed colour from yellow to red and deposited yellow, transparent, crystals. The crystals were filtered off and dried at room temperature (0.48g, 2.1 mmol yield 72%). (found: C, 59.13; H, 5.77; N, 12.64. $C_{11}H_{14}N_2O_3$ required: C, 59.44; H, 6.34; N, 12.60%.)

CH_3-NH-CH_2-CH(COO^-)-N=CH-C_6H_4-OH-(*o*) **(1)**

^{1}H NMR (D_2O) pD = 7.4 δ = 2.70 (3H, s, $\underline{H_3C}$-NH), δ = 3.41 (2H, m , H_3C-NH-$\underline{CH_2}$), δ = 4.25 (1H, t, H_3C-NH-CH_2-$\underline{CH}$), δ = 6.85-7.65, m, (C_6H_4)

N-(2-hydroxybenzylidene)DL-2,4-diaminobutanoic acid **(2)** was synthesised by adding a solution of salicylaldehyde (0.36g, 3 mmol) in aqueous ethanol ($15cm^3$) dropwise into a solution of DL-2,4-diaminobutanoic acid dihydrochloride (0.57g, 3 mmol, neutralised with 2M NaOH) in water ($10cm^3$) at room temperature. A yellow crystalline precipitate was formed, which was separated by filtration and dried at room temperature (0.49g, 2.2 mmol yield 73%) was obtained. The solid product was recrystallised from aqueous ethanol (1:5 v/v) yielding yellow transparent thin hexagonal crystals. (found: C, 59.68; H, 6.25; N, 12.53. $C_{11}H_{14}N_2O_3$ required: C, 59.44; H, 6.34; N, 12.60%.)

(*o*)-HOC_6H_4CH=NCH_2CH_2CH(NH^+_3)COO^- **(2)**

^{1}H NMR in (D_2O) pD = 7.4 δ = 2.15 (2H, m, N-CH_2-$\underline{CH_2}$), δ = 3.1 5 (2H, t, N-$\underline{CH_2}$-CH_2),

δ = 3.7 (1H, t, N-CH_2-CH_2-$\underline{CH}$), δ = 4.3 (1H, t, N-CH_2-CH_2-$\underline{CH}$), 2- (α-) isomer δ = 2.29 (2H broad, $\underline{H_2N}$-CH), δ = 6.65-8.40, m, (C_6H_4)

The preparation of N-(2-hydroxybenzylidene)DL-2,3-diaminopropanoic acid **(3)** was similar to that of compound (**2**). A yellow crystalline product (0.50g, 2.4 mmol yield 80%) was obtained. The solid product was

recrystallised from aqueous ethanol (1:5v/v) to give yellow transparent hexagonal crystals. (found: C, 57.43; H, 5.58; N, 13.27. $C_{10}H_{12}N_2O_3$ required: C, 57.69; H, 5.81; N, 13.45%.)

(*o*)-$HOC_6H_4CH{=}NCH_2CH(NH^+_3)COO^-$ **(3)**

^{1}H NMR (D_2O) pD = 7.34 δ = 3.20 (2H, qq, N-$\underline{CH_2}$), δ = 3.7 (1H, t, N-$\underline{CH}$), δ = 4.57 (1H, m, N-$\underline{CH}$), 2- (α-) isomer δ = 3.98 (2H, broad, ($\underline{H_2}$N-CH) δ = 6.8- 8.60 (m, C_6H_4).

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