Thermodynamic and Electronic and Electron Spin Resonance Spectroscopic Investigation of the Co-ordinating Properties of 4-Azaoctane-1,8-diamine (Spermidine) in Aqueous Solution

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The reason for the exchange of copper(II)-containing proteins with spermidine (4NH-od), according to a non-immunogenic mechanism for retardation of tumour growth, has been explored from a thermodynamic standpoint. Of the two complexes found in aqueous solution at physiological pH, $[Cu(H\text{-}4NH\text{-}od)]^{3+}$ and $[Cu(4NH\text{-}od)]^{2+}$, only the latter has the possibility of exchange. Its relatively high stability constant arises from a favourable enthalpy contribution, due to the full co-ordination of the three nitrogen atoms in a tetragonally distorted octahedral structure. This complex contains one seven-membered chelate ring fused to another six-membered ring. In the protonated spermidine complex the primary nitrogen atom of the tetramethylene chain is protonated. The spectroscopic (electronic and e.s.r.) properties of these two complexes have been determined. The protonation behaviour of spermidine has been also studied thermodynamically and the successive enthalpy and entropy values are explained on the basis of a tautomeric process which exists in symmetrical triamines.

IT has been demonstrated that the triamine spermidine (4-azaoctane-1,8-diamine; 4NH-od) and the tetra-amine spermine (4,9-diazadodecane-1,12-diamine; 4,9NH-ddd) are present in relatively high concentrations in some human cancer sera [1] stimulating growth in two tumour cell lines, whilst their copper(II) complexes inhibit such growth. We feel that the observed behaviour can be related to the thermodynamic properties in solution of the chelated copper(II) complexes which contain a system of condensed chelate rings one of which is seven-membered. The relation between the size of the chelate rings and the thermodynamic stability in solution of copper complexes of aliphatic tri- and tetra-amines has already been demonstrated and the relative contributions of the enthalpy and entropy changes to the stability have been evaluated.[2]

In the present paper we report values of the thermodynamic functions $\Delta H^\ominus$ and $\Delta S^\ominus$ associated with the formation of copper(II) complexes of spermidine in the range pH 5—9. These thermodynamic parameters are related to spectroscopic parameters obtained from e.s.r. and u.v.–visible measurements. Values of $\Delta H^\ominus$ and $\Delta S^\ominus$ for the successive stages in the protonation of spermidine have also been determined, and throw considerable light on the behaviour of this base towards the proton.

EXPERIMENTAL

Calorimetric Measurements.—The calorimetric measurements were carried out with an LKB 100700-I flow microcalorimeter using a mixing cell at 25 °C. The reagents were introduced into the calorimeter by means of two Mettler DV 105 burettes (50 cm^3). The rate of delivery from the two burettes was regulated by means of a resistance box and was varied from 3 to 7 µl s^{-1} according to the experiment. The error in the flow rate was always less than 0.3%. In order to measure the heat of protonation of the free base spermidine, solutions containing (*a*) the triamine hydrochloride and (*b*) sodium hydroxide were introduced into the calorimeter. The amount of triamine hydrochloride to be protonated was determined from the relative flow rates of the two burettes. The method of calculating the successive enthalpies of protonation from the microcalorimetric data (based on the method of mean squares) has already been described.[3] For the formation of the copper(II) complexes, one burette contained a $CuCl_2$ solution whilst the other contained a buffered solution of the triamine. The precise method of calculation of the enthalpies of formation of the complexes $[CuL]^{2+}$ and $[Cu(HL)]^{3+}$ (L = 4NH-od) used was that described previously.[3] The experimental details are in Tables 1 and 2.

TABLE 1

Experimental details of calorimetric measurements on the protonation reaction

v_H [a] / µmol s^{-1}	v_L [b] / µmol s^{-1}	R [c] / µl s^{-1}	ϕ [d] / mcal s^{-1}
(*i*) 0.147 7	0.049 2	10.035	0.106 3
(*ii*) 0.057 8			
(*i*) 0.194 0	0.064 7	10.089	0.099 7
(*ii*) 0.131 2			
(*i*) 0.171 0	0.057 0	9.808	0.103 0
(*ii*) 0.099 3			
(*i*) 0.164 2	0.054 7	10.251	0.110 1
(*ii*) 0.080 5			
(*i*) 0.113 9	0.038 0	9.977	0.089 2
(*ii*) 0.004 7			
(*i*) 0.089 1	0.029 7	9.982	0.070 3
(*ii*) −0.035 2			

[a] Total (or analytical) flow rate of H^+ ion (the minus sign refers to $[OH]^-$ ion) before (*i*) and after (*ii*) mixing. [b] Total flow rate of spermidine. [c] Flow rate, obtained as the sum of the flow rates of the two burettes. [d] Corrected for the heat flow of dilution of the two reactants in 0.1 mol dm^{-3} NaCl.

Experiments in which solutions containing (*a*) $CuCl_2$ and the triamine hydrochloride and (*b*) an alkali-metal hydroxide were introduced into the calorimeter gave results which could not be interpreted in terms of the set of constants [4] used in the other series of experiments. This could be explained by supposing that in solution (*a*) the copper(II) ion and the ligand, protonated to a greater or lesser extent, interact to give a complex species {possibly $[Cu(H_2L)]^{4+}$}. The presence of complex species under strongly acid conditions is also suggested by the e.s.r. results.

Spectroscopic Measurements.—The electronic spectra were determined at 25 °C using a Beckmann DK 2A instrument. The e.s.r. spectra were recorded on a Varian E9 spectro-

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[1] K. C. Tsou, M. Selzer, J. Weinstein, and D. Bender, *Excerpta Med. Internat. Congr. Ser.*, 1973, **275**, 300.

[2] P. Paoletti, L. Fabbrizzi, and R. Barbucci, *Inorg. Chim. Acta Rev.*, 1973, **7**, 43.

[3] C. Bianchini, L. Fabbrizzi, and P. Paoletti, *J.C.S. Dalton*, 1975, 1036.

[4] M. Gold and J. Powell, *J.C.S. Dalton*, 1976, 230.

TABLE 2

Experimental details of calorimetric measurements on the complex formation

v_M [a] / µmol s^{-1}	v_H [b] / µmol s^{-1}	v_L [c] / µmol s^{-1}	R [d] / µl s^{-1}	ϕ [e] / mcal s^{-1}
(*i*) 0.000 0	0.615 0	0.248 3	10.035	0.369
(*ii*) 0.032 14				
(*i*) 0.000 0	0.590 8	0.238 6	10.011	0.376
(*ii*) 0.033 2				
(*i*) 0.000 0	0.635 9	0.256 8	10.019	0.365
(*ii*) 0.030 9				
(*i*) 0.000 0	0.615 0	0.248 3	10.035	0.364
(*ii*) 0.032 14				
(*i*) 0.000 0	0.590 8	0.238 6	10.011	0.372
(*ii*) 0.033 2				
(*i*) 0.000 0	0.635 9	0.256 8	10.019	0.361
(*ii*) 0.030 9				
(*i*) 0.000 0	0.860 3	0.307 3	10.035	0.245
(*ii*) 0.032 1				
(*i*) 0.000 0	0.889 5	0.317 8	10.019	0.248
(*ii*) 0.030 9				
(*i*) 0.000 0	0.956 7	0.341 7	10.250	0.259
(*ii*) 0.029 9				

[a] Total (or analytical) flow rate of copper ion before (*i*) and after (*ii*) mixing. [b] Total flow rate of H$^+$ ion. [c] Total flow rate of spermidine. [d] Flow rate, obtained as the sum of the flow rates of the two burettes. [e] Corrected for the heat flow of dilution of the reactants in 0.1 mol dm^{-3} NaCl.

meter and were calibrated using diphenylpicrylhydrazyl (dpph) as a *g* marker. E.s.r. spectra of glasses were obtained at −140 °C and of solutions at room temperature. The solutions contained 10^{-2}—10^{-3} mol dm^{-3} Cu^{II} with ethylene glycol–water (1 : 3) as solvent. The procedure for identifying the species present in solution has already been described.[5] Starting from an acidic solution, the spectrum of the Cu^{2+}(aq) ion was replaced by a different spectrum in a pH region where potentiometric analysis does not indicate the presence of any such species. On increasing the pH this spectrum was replaced by that attributed to the [Cu(HL)]$^{3+}$ species. The low values of $g_{\parallel}$ and $A_{\parallel}$ calculated for the unknown spectrum seem to suggest a complex species in which only one nitrogen of the amine ligand is co-ordinated ($g_{\parallel}$ 2.351, $A_{\parallel}$ 143, and $g_{\perp}$ 2.08). Simulated spectra for a Lorentzian lineshape were obtained using Venable's program[6] and in each case it was possible to fit the experimental spectra extremely closely.

RESULTS AND DISCUSSION

Protonation.—The heat change due to the protonation of spermidine (4NH-od) decreases in successive steps, *i.e.* $|\Delta H_1| > |\Delta H_2| > \Delta H_3$. The same trend has previously been observed in the case of the triamines 3-azahexane-1,6-diamine (3NH-hxd)[7] and 3-azaheptane-1,7-diamine (3NH-hd)[8] (Table 3). Like spermidine these triamines are asymmetric, *i.e.* the aliphatic chains joining the central nitrogen atom to the two terminal nitrogens are of different lengths. In the case of the homologous symmetric triamines 3-azapentane-1,5-diamine (3NH-pd)[9] and 4-azaheptane-1,7-diamine (4NH-hd)[10] the trend in the successive enthalpies is different, *i.e.* $|\Delta H_1| < |\Delta H_2| > |\Delta H_3|$. This difference in behaviour will be discussed below.

The relatively high value of ΔH_1 for spermidine suggests that the nitrogen atom which is protonated in the first step is a primary nitrogen. Comparison with the other asymmetric triamines shows that the heat change increases with increasing length of the aliphatic chains joining the basic sites. ΔH_1 decreases in the order 4NH-od > 3NH-hd > 3NH-hxd. Also the first protonation step for spermidine is particularly exothermic, the value of $\Delta H^\ominus$ being practically equal to that for protonation of the limiting base, *i.e.* the hydroxide ion, in aqueous solution. The fact that [OH]$^-$ is a much stronger base is solely due to the much more favourable entropy contribution.[11]

TABLE 3

Thermodynamic functions for the reactions of linear aliphatic tri- and di-amines (L) with H$^+$ and Cu^{2+} ions in aqueous solution

	Spermidine (4NH-od)			3NH-hd			3NH-hxd		
Reaction	$-\Delta G^\ominus$ [a]	$-\Delta H^\ominus$ [a]	$\Delta S^\ominus$ [b]	$-\Delta G^\ominus$ [a]	$-\Delta H^\ominus$ [a]	$\Delta S^\ominus$ [b]	$-\Delta G^\ominus$ [a]	$-\Delta H^\ominus$ [a]	$\Delta S^\ominus$ [b]
$L + H^+ \rightleftharpoons [HL]^+$	14.85	13.39 (3)	4.9 (1)	14.55	12.86	5.7	14.23	12.18	6.89
$[HL]^+ + H^+ \rightleftharpoons [H_2L]^{2+}$	13.38	12.76 (5)	2.0 (2)	13.08	12.13	3.2	12.76	12.14	2.08
$[H_2L]^{2+} + H^+ \rightleftharpoons [H_3L]^{3+}$	11.38	11.69 (4)	−1.0 (1)	9.53	10.71	−4.0	8.69	9.95	−4.22
$[CuL]^{2+} + H^+ \rightleftharpoons [Cu(HL)]^{3+}$	8.34	10.41 (6)	−6.8(2.1)	9.29	9.26	0.1	3.89	4.46	−1.9
$[CuL_2]^{2+} + H^+ \rightleftharpoons [CuL(HL)]^{3+}$				14.25	14.03	0.7	13.18	11.98	4.09
$[CuL_2]^{2+} + 2H^+ \rightleftharpoons [Cu(HL)_2]^{4+}$				27.64	27.43	0.7			
$Cu^{2+} + [HL]^+ \rightleftharpoons [Cu(HL)]^{3+}$	9.32	11.11 (53)	−6.0 (1.8)	12.53	11.69	2.8	12.30	11.5	2.7
$Cu^{2+} + L \rightleftharpoons [CuL]^{2+}$	15.83	14.13 (9)	5.7 (3)	17.80	15.28	8.4	22.64	19.22	11.5
$Cu^{2+} + en \rightleftharpoons [Cu(en)]^{2+}$	14.43	12.6	6.3						
$Cu^{2+} + tn \rightleftharpoons [Cu(tn)]^{2+}$	13.86	11.0	9.6						

[a] In kcal mol^{-1}. [b] In cal K^{-1} mol^{-1}.

For the second protonation step ΔH_2 decreases in the order 4NH-od > 3NH-hd ≃ 3NH-hxd. This trend can be rationalized if it is the second terminal primary nitrogen atom which is being protonated. Thus the highest value of ΔH_2 is observed for spermidine in which this nitrogen atom is at the end of a trimethylene chain.

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[5] R. Barbucci and M. J. M. Campbell, *Inorg. Chim. Acta*, 1976, **16**, 113.

[6] J. H. Venable, Ph.D. Thesis, 1965, Yale University Microfilm Inc. Arbor A., Michigan.

[7] R. Barbucci, L. Fabbrizzi, and P. Paoletti, *J.C.S. Dalton*, 1974, 2403.

[8] R. Barbucci and M. Budini, *J.C.S. Dalton*, 1976, 1321.

[9] M. Ciampolini, P. Paoletti, and L. Sacconi, *J. Chem. Soc.*, 1961, 2994.

[10] P. Paoletti, F. Nuzzi, and A. Vacca, *J. Chem. Soc. (A)*, 1966, 1385.

[11] R. M. Izatt and J. J. Christensen, *J. Phys. Chem.*, 1963, **67**, 2607 and refs. therein.

In both 3NH-hd and 3NH-hxd the second nitrogen atom to be protonated is attached to an ethylene chain and this explains why the enthalpy change is the same in both cases.

Addition of a third proton is probably affected by the electrostatic repulsion between the positive charges already present and it will be noted that spermidine, in which the two ammonium groups are separated from the secondary nitrogen by three and four carbon atoms respectively, has the most exothermic heat of protonation. The ΔH_3 value for 3NH-hd is appreciably greater than that for 3NH-hxd and this can be attributed to the fact that in the former compound one ammonium group is separated from the basic site by one more carbon atom than in the latter compound.

It is noteworthy that for spermidine the successive steps in the protonation are accompanied by decreasing entropy changes. In fact ΔS, positive in the first step, has become negative by the third step. Such a trend has previously been observed for homologous symmetric triamines.[9,10]

Table 4

Electronic and e.s.r. spectra of some copper(II)–polyamine complexes in aqueous solution

Complex	Electronic spectra [a] (10^3 cm^{-1})	E.s.r. spectra [b] $g_{\parallel}$	$g_{\perp}$	$g_{\perp}$ [c]	$g_{iso.}$	$A_{\parallel}$	$A_{\perp}$	$A_{\perp}$ [c]	$A_{iso.}$
$[Cu(H\text{-}4NH\text{-}od)]^{3+}$	d	2.307	2.071	2.043	2.131	170	15	23	72
$[Cu(H\text{-}3NH\text{-}hd)]^{3+}$	15.2 (47)	2.281	2.060	2.053	2.129	176	10	17	70
$[Cu(tn)]^{2+}$	15.8 (54)	2.276	2.070	2.058	2.131	175	14	11	66
$[Cu(en)]^{2+}$	15.7 (50)	2.272	2.070	2.074	2.140	177	17	14	68
$[Cu(4NH\text{-}od)]^{2+}$	16.4 (117)	2.217	2.042	2.067	2.117	166	12	8	50
$[Cu(4NH\text{-}hd)]^{2+}$	16.3 (93)	2.246	2.055	2.056	2.119	162	10	0	54
$[Cu(3NH\text{-}pd)]^{2+}$	16.6 (76)	2.226	2.042	2.050	2.109	190	20	16	74

[a] Values in parentheses are the molar absorption coefficients (ε/dm^3 mol^{-1} cm^{-1}). [b] All the hyperfine coupling constants are in units of 10^4 cm^{-1}. [c] Calculated from $g_{iso.} = \frac{1}{3}(g_{\parallel} + 2g_{\perp})$ or $A_{iso.} = \frac{1}{3}(A_{\parallel} + 2A_{\perp})$. [d] Not measured due to the low concentration of this complex in solution.

The different thermodynamic behaviour of the asymmetric triamines (4NH-od, 3NH-hd, and 3NH-hxd) and the symmetric triamines (3NH-pd and 4NH-hd) merits comment. It has been proposed that in the case of the symmetric triamines the first protonation step involves both the terminal primary nitrogen atoms and the secondary central nitrogen atom.[12] In other words the monoammonium ion has to be considered as a tautomeric equilibrium between two forms. On addition of a

$$\overset{+}{H_3N}\text{—}NH\text{—}NH_2 \rightleftharpoons NH_2\text{—}\overset{+}{N}H_2\text{—}NH_2$$

second proton the electrostatic repulsion of the charged amine group ensures that it is the two terminal nitrogens which are protonated. Thus the second step involves transfer of a proton from a secondary to a primary nitrogen atom. The fact that the net heat change is greater for protonation of a primary than a secondary nitrogen atom explains the experimental observation of $|\Delta H_2| > |\Delta H_1|$. The sequence for the asymmetric triamines, *i.e.* $|\Delta H_1| > |\Delta H_2| > |\Delta H_3|$, appears to exclude a tautomeric mechanism for protonation.[8] A difference in behaviour between symmetric and asymmetric triamines is also found when ΔH_1 values are plotted against the corresponding ΔS_1 values. Thus, for the asymmetric triamines the points lie on a straight line while for the symmetric triamines they manifestly do not (Figure 1).

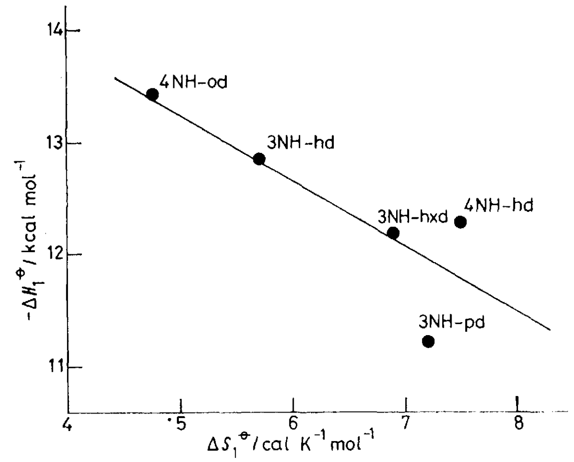

Figure 1 Relation between the enthalpies and the entropies of the first protonation for linear aliphatic triamines

A decrease in ΔH with an increase in ΔS can be explained by the fact that both quantities depend on the number of water molecules liberated in the protonation step. The larger the number of water molecules released the larger is the endothermic effect of desolvation and the larger the positive contribution to the translational entropy. The fact that for the asymmetric bases there is a linear dependence between the two thermodynamic quantities suggests that there is a unique mechanism with protonation occurring on the primary nitrogen atom attached to the longer aliphatic chain, as suggested above.

Copper(II) *Complex Formation*.—Of the species present in aqueous solution[4] we have only determined the spectroscopic and thermodynamic parameters of the complexes $[Cu(HL)]^{3+}$ and $[CuL]^{2+}$. The hydroxo-complex $[Cu(OH)L]^{+}$ which is also formed is only present in concentrations which might allow it to be characterized at very high pH values. The e.s.r. and electronic parameters are shown in the Table 4 together with those of other copper(II)–polyamine species. The parameters

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[12] M. Ciampolini and P. Paoletti, *J. Phys. Chem.*, 1961, **65**, 1224.

of $[Cu(HL)]^{3+}$ (L = 4NH-od) are typical of a tetragonally distorted octahedral complex with a $d_{x^2-y^2}$ ground state and are essentially the same as those found for other complexes having diamine ligands.[5] This protonated complex has a higher value of $g_\parallel$ than the complex of protonated 3NH-hd whose structure involves a five-membered chelate ring.[8] The formation of a larger chelate ring is known to lead to an increase in $g_\parallel$.[13] However, the difference in this case is much larger than that between the $g_\parallel$ values of $[Cu(tn)]^{2+}$ (tn = 1,3-diaminopropane) and $[Cu(en)]^{2+}$ (en = ethylenediamine) which have respectively a six- and a five-membered chelate ring (Table 4). The presence of a positive charge on the protonated nitrogen atom can be expected to have an effect on the e.s.r. parameters. Thus the $g_\parallel$ value of $[Cu(HL)]^{3+}$ (L = 3NH-hd) is markedly larger than that of $[Cu(en)]^{2+}$. In the case of $[Cu(HL)]^{3+}$ (L = 4NH-od), weaker in-plane bonding leads to stronger axial bonding with solvent molecules, as is borne out by the lower value of $A_\parallel$ (the result of an increased 4*s* population in the Cu^{2+} ion). The heat of formation of the protonated spermidine complex is equal to that [14] of $[Cu(tn)]^{2+}$ and markedly smaller than those of analogous complexes having five-membered chelate rings, *i.e.* $[Cu(HL)]^{3+}$ (L = 3NH-hd or 3NH-hxd) and $[Cu(en)]^{2+}$ (Table 3). This confirms the above hypothesis that a six-membered chelate ring is present in $[Cu(HL)]^{3+}$ (L = 4NH-od) and that it is the most basic nitrogen which is protonated, *i.e.* that attached to the tetramethylene chain. The entropy change is the lowest yet found for a protonated copper(II) complex and this can be compared with the low value of $A_\parallel$ which is found. Both quantities are probably the result of a strong interaction with solvent molecules.

Both the e.s.r. and electronic spectral parameters found for the simple complex $[CuL]^{2+}$ (L = 4NH-od) are shown in Table 4. These data are similar to those obtained for copper(II) complexes with homologous triamines for which co-ordination *via* all three atoms of nitrogen has been proven beyond doubt.[5] Thus it is clear that the spermidine complex with a six-membered chelate ring fused to a seven-membered chelate ring also involves three nitrogen donor atoms. The thermodynamic data (see Table 3) confirm this view. Thus the enthalpy of formation of this complex is *ca.* 3 kcal greater than that for the formation of the protonated complex, and is also larger than the heat of formation of $[Cu(en)]^{2+}$ which only has two nitrogen donor atoms,[15] although it is the lowest value yet found for a complex involving three nitrogen donor atoms.* This relatively low value of $-\Delta H^\ominus$ can be related to the difficulty encountered by the spermidine ligand in embracing three co-ordination sites. The tetramethylene chain in particular is ill adapted to the configuration required if a bond is to be formed between the terminal nitrogen atom and the Cu^{2+} ion. A recent *X*-ray structure of the copper(II) complex of spermine (4,9NH-ddd), which also involves a seven-membered chelate ring, shows that the Cu–N distances are comparable to those found in other copper(II)–polyamine complexes, but that the torsion angle between two of the carbons in the seven-membered ring is anomalous.[16] The spectroscopic parameters depending mainly on the chromophore CuO_3N_3 would not be much affected by such an anomaly, and thus are similar to those found for other CuO_3N_3 chromophores (Table 4). The enthalpy on the other hand is expected to be very sensitive to such strain. The entropy of formation of $[CuL]^{2+}$ (L = 4NH-od) is also the lowest found for a copper(II)–triamine complex and this must be a reflection of the greater loss of entropy by the ligand. If we calculate the partial molar entropy of all the previously studied aliphatic triamine ligands by means of Cobble's formula [17] and plot them against the partial molar entropies of the corresponding complexes from $S^\ominus(CuL) = \Delta S^\ominus - S^\ominus(L) - S^\ominus(H_2O)$ we obtain a straight line (Figure 2). This shows that the entropy of each complex depends mainly on the conformational contribution of the aliphatic chains involved in the chelate rings, and that the five complexes all have the same structure in solution. A triamine ligand could co-ordinate either facially or meridionally but it has been shown that in the first case the apical nitrogen will give rise to a lower value of $A_\parallel$,[5] so that it can be assumed that all three nitrogen atoms for each of the five ligands are in the equatorial plane. The reaction $[CuL]^{2+} + H \rightleftharpoons [Cu(HL)]^{3+}$, which is regarded as a good test for the presence or absence of free (unco-ordinated) nitrogen atoms, confirms this hypothesis.[18] The enthalpy change

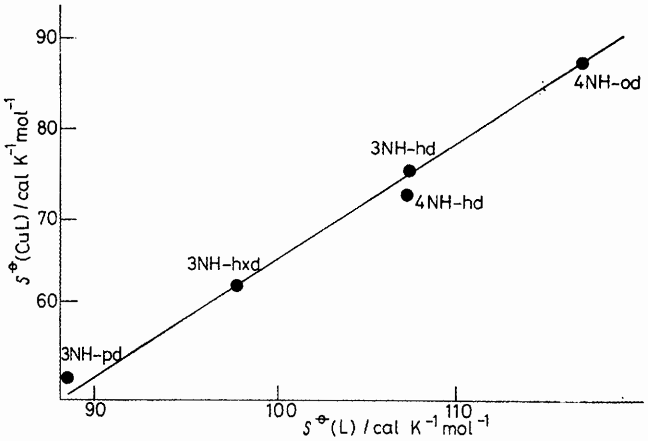

FIGURE 2 Plot of standard molar entropies for copper(II) complex ions against the standard molar entropies for the polyamines in aqueous solution

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* Throughout this paper: 1 cal = 4.184 J.

[13] R. Barbucci, P. Paoletti, and M. J. M. Campbell, *Inorg. Chim. Acta*, 1974, **10**, 69.

[14] F. Holmes and D. R. Williams, *J. Chem. Soc.* (*A*), 1967, 1702.

[15] R. Barbucci, L. Fabbrizzi, P. Paoletti, and A. Vacca, *J.C.S. Dalton*, 1972, 740.

[16] R. Boggs and J. Donohue, *Acta Cryst.*, 1975, **B31**, 320.

[17] J. W. Cobble, *J. Chem. Phys.*, 1953, **21**, 1451.

[18] R. Barbucci, L. Fabbrizzi, and P. Paoletti, *Inorg. Chim. Acta*, 1973, **7**, 157.

for this reaction is *ca.* 3 kcal lower than that for the first stage of protonation of spermidine and this difference is the same as that between the enthalpies of protonation of free and co-ordinated 3NH-hd. Both quantities are lower than the values found for 3NH-hxd (Table 3). This indicates that (*i*) protonation of spermidine and 3NH-hd occurs on equivalent basic sites, (*ii*) the nitrogen atom protonated is terminal and is at the end of a tetramethylene aliphatic chain, and (*iii*) the Cu–NH_2 bond in the seven-membered chelate ring is the same both in spermidine and 3NH-hd and is thus independent of the dimensions of the other chelate ring to which it is fused.

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