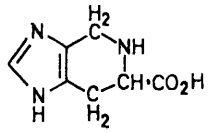
Acid–Base Properties of Spinaceamine and Spinacine and their Complexing Capacity with Divalent Metals

By A. Braibanti,* F. Dallavalle, E. Leporati, and G. Mori, Istituto di Chimica Generale, Università di Parma, Parma, Italy

The behaviour of spinaceamine (4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine) and spinacine (4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine-6-carboxylic acid) in aqueous solutions in equilibria with protons and divalent cations has been investigated potentiometrically. The compounds are cyclic homologues of histamine and histidine, respectively. The protonation constants at 25 °C, μ = 0·1M KCl for spinaceamine are log K_1 = 8·904(1), log K_2 = 4·895(2) and the protonation constants at the same conditions for spinacine are log K_1 = 8·663(4), log K_2 = 4·936(6), and log K_3 = 1·649(9). The thermodynamic functions ΔG, ΔH, ΔS for the protonation processes of both compounds have been calculated from potentiometric measurements of equilibria at temperatures between 5 and 35 °C. The values of ΔS for different association steps, as compared with those of histamine and histidine, are consistent with the rigid structure of the cyclic homologues. Spinaceamine does not form complexes in solution with divalent cations, Ni^{2+} and Cu^{2+}. Spinacine forms the complexes $HNiL^{2+}$, NiL^{+}, NiL_2 with nickel and the complexes $HCuL^{2+}$, $H_2CuL_2^{2+}$, $HCuL_2^{+}$ with copper; these complexes are very likely (*N,O*)-chelates of glycine-type. The proton of the hydrogen-complexes is probably bound to the tertiary nitrogen atom of the imidazole ring.

SPINACEAMINE (4,5,6,7-tetrahydro-1*H*-imidazo[4,5-*c*]pyridine) (I) and spinacine (4,5,6,7-tetrahydro-1*H*-imidazo-[4,5-*c*]pyridine-6-carboxylic acid) (II) synthesised by Vitali, Mossini, and Bertaccini,[1] are cyclic homologues of

(I)



(II)

histamine and histidine, respectively. They show neither histamine-like nor anti-histamine activity. The behaviour is probably related to the rigid structure of the cyclic compounds which prevent spinaceamine and spinacine from reacting with the appropriate sites of the protein molecules. The purpose of the present study is to examine the equilibrium between spinaceamine and spinacine and hydrogen ions, and metal cations in solution.

EXPERIMENTAL

Solutions were prepared as described previously;[2] the main steps of the procedure are repeated here for the sake of clarity. Twice distilled, freshly boiled, water was used throughout. Hydrochloric acid solutions (0·1—0·15M) were standardised against tris(hydroxymethyl)methylamine, and potassium hydroxide solutions (0·2—0·3M) against potassium hydrogen phthalate. Concentrations of stock solutions of divalent metal chlorides were determined by conventional analytical methods. Solutions to be titrated were prepared by adding successively exact volumes of standard solutions of the ligand, of hydrochloric acid and, when necessary, of metal chloride; then the required quantities of potassium chloride and water were added, to reach the total volume, v_o, which was 99·68 ± 0·01 or 99·62 ± 0·01 ml, depending on the vessel used. The titrant was added by a Metrohm piston burette, with a precision of 0·005 ml. Initial concentrations, pH and $\bar{n}$ intervals of the solutions employed are quoted in Tables 1, 2, and 3.

Potentiometric Measurements.—Potentiometric measurements, by digital potentiometer Radiometer PHM52, were carried out as described in a previous publication.[2] A nitrogen atmosphere was maintained in the cell. The glass electrode was standardised at the chosen ionic strength and at the different temperatures. The equivalence point, v_e, was determined following the principles of Gran [3] by a least squares method. The standard electrode potential, E^0, was obtained from

$$E^0 = E - b \ln [H^+]$$

where $b = RT/F$ and

$$[H^+] = \tfrac{1}{2}\frac{(v_e - v)N}{(v_o + v)} + \sqrt{\tfrac{1}{4}\left(\frac{v_e - v}{v_o + v}\right)^2 N^2 + K_w}$$

with v = volume of KOH added, v_o = initial volume, N = titre of KOH, K_w = ionic product of water. Several digits must be retained in the calculation. Further details are given in a previous publication.[2]

Calculations.—Tentative protonation constants of spinaceamine and spinacine were assumed from analogous systems, and then refined by employing six different computer programmes, namely Map Z,[4] Gauss Z,[4] Ls Map G,[4] Gauss G,[4] Lgst,[5] and modified Scogs.[6] The programmes differ from one another either in the mathematical method of finding the minimum of the squares of the residuals, $U = \Sigma_i|\Delta_i|^2 = \Sigma_i(X_{c,i} - X_{o,i})^2$, (where $X_{c,i}$ and $X_{o,i}$ are calculated and observed quantities at point i), or in the quantity X employed. Programmes Map and Lgst search for the minimum of U by the ' pit mapping ' method described by Sillén; [7] the other programmes use the general least squares method. Apart from the different speed of convergence, the two mathematical methods give comparable results. Small differences in the results are found when different quantities X_i are employed. The quantity X_i taken to measure the agreement is the formation function, $X_i = \bar{n}$ in programmes Gauss Z and Map Z, the total acid concentration $X_i = H_i$ in programmes Gauss G and Ls Map G and the

[1] T. Vitali, F. Mossini, and G. Bertaccini, *Il Farmaco*, Ed. Sc., 1967, **22**, 821.

[2] A. Braibanti, G. Mori, F. Dallavalle, and E. Leporati, *Inorg. Chim. Acta*, 1972, **6**, 106.

[3] G. Gran, *Analyst*, 1952, **77**, 661.

[4] R. S. Tobias and M. Yasuda, *Inorg. Chem.*, 1963, **2**, 1307.

[5] A. Dei, P. Paoletti, and A. Vacca, *Inorg. Chem.*, 1968, **7**, 865.

[6] I. G. Sayce, *Talanta*, 1968, **15**, 1397; with statement number 150 of the Scogs programme amended.

[7] L. G. Sillén, *Acta Chem. Scand.*, 1962, **16**, 159; 1965, **18**, 1085.

TABLE 1

Protonation constant determinations. Initial concentrations (mM), $-\log[H^+]$, and $\bar{n}$ ranges for the titrations of spinaceamine

N	t °C	Spinaceamine	T_H	$-\log[H^+]$	$\bar{n}$
1	35	0·306306	0·978900	2·435—9·243	1·9—0·2
2		0·206968	0·658169	2·614—9·733	1·9—1·0
3		0·347352	0·998897	2·515—10·651	1·9—0·0
4		0·264681	0·748300	2·654—10·279	1·9—0·0
5	30	0·281430	0·805575	2·610—9·525	1·9—0·1
6		0·331099	0·965561	2·517—9·516	1·9—0·2
7		0·256389	0·854278	2·470—9·287	1·9—0·2
8		0·231596	0·731501	2·570—10·246	1·9—0·3
9	25	0·289391	0·578782	3·729—9·331	1·9—0·0
10		0·372229	1·096248	2·448—10·616	1·9—0·0
11		0·165591	0·381481	3·262—10·465	1·9—0·0
12		0·413937	1·230368	2·470—10·915	1·9—0·0
13	20	0·289722	0·824256	2·608—9·387	1·9—0·3
14		0·339392	0·960250	2·546—9·231	1·9—0·4
15		0·272973	0·802478	2·586—9·788	1·9—0·2
16		0·239888	0·675206	2·874—11·010	1·9—0·0
17	15	0·289722	0·836703	2·575—9·889	1·9—0·2
18		0·331099	0·907189	2·601—9·996	1·9—0·1
19		0·289474	0·823195	2·606—10·830	1·9—0·0
20		0·248097	0·740441	2·613—10·456	1·9—0·0
21	10	0·331099	0·931735	2·778—11·045	1·9—0·0
22		0·289722	0·824555	2·844—10·929	1·9—0·0
23		0·264681	0·809757	3·042—10·694	1·9—0·0
24		0·248097	0·740042	2·878—10·783	1·9—0·0
25	5	0·281430	0·782500	2·644—9·850	1·9—0·3
26		0·331099	0·906291	2·611—10·491	1·9—0·1
27		0·306058	0·881275	2·549—10·582	1·9—0·1
28		0·256389	0·769792	2·588—10·399	1·9—0·1

volume of the titrant added $X_i = v_i$ in Lgst and Scogs. Each programme has been completed by us with an analysis of the distribution of the residuals Δ_i as function of $X_{o,i}$. A straight line passing through the points Δ_i should have slope tending to zero (no correlation) and intercept near to zero, provided that no systematic error affects the data. The significance of the slope as variance test is analysed by the F-test.[8] Different quantities X_i introduce different weights for the points i and provide indications of parameters, such as concentrations, coefficient of the Nernst equation, *etc.*, which, if correlation is significant, might affect the data. A further check [9] is provided by the quantity R.

$$R = \sqrt{\frac{\Sigma_i(X_{o,i} - X_{c,i})^2}{\Sigma_i(X_{o,i})^2}}$$

The results obtained for spinaceamine with different calculation procedures are summarised in Table 4. The calculated F value has to be compared with $F_{5,1,159} = 3{\cdot}84$. The results show that there are small correlations between residuals and concentrations, but no correlation between residuals and formation function, $\bar{n}$, or volume, v. In any case, however, one must be cautious in handling the function F because it does not seem an absolute index of the reliability of the results.

The protonation constants for spinaceamine are quoted in Table 5, all referring to the same data set, refined with different programmes. Here none of the calculations provides an F value (to be compared with $F_{5,1,247} = 3{\cdot}88$) below the statistical level of significance. This means that some factors, which we have not been able to identify, affect the data. Some improvements have been achieved by changing titres or, at temperatures $t \neq 25$ °C, by changing the Nernst coefficient for the electrode. Those corrections have been confirmed independently by applying Gran's method to acid–base titrations at the same temperatures. Values completely unbiased by systematic errors cannot be obtained, nevertheless the constants can be accepted with confidence

TABLE 2

Protonation constant determinations. Initial concentrations (mM), $-\log[H^+]$, and $\bar{n}$ ranges for the titrations of spinacine

N	t °C	Spinacine	T_H	$-\log[H^+]$	$\bar{n}$
1	35	0·296706	1·198584	2·262—10·013	2·2—0·0
2		0·280195	1·131885	2·311—9·386	2·2—0·1
3		0·263519	1·064519	2·303—10·705	2·2—0·0
4		0·247008	0·997820	2·331—10·778	2·2—0·0
5	30	0·288451	1·165235	2·264—9·621	2·2—0·1
6		0·247255	0·998820	2·423—10·868	2·2—0·1
7		0·263519	1·064519	2·303—10·190	2·2—0·0
8		0·300586	1·214259	2·251—9·202	2·2—0·2
9	25	0·288451	1·165235	2·268—10·967	2·2—0·0
10		0·329398	1·330649	2·221—9·887	2·2—0·1
11		0·263519	1·064519	2·309—10·710	2·2—0·0
12		0·247255	0·998820	2·331—10·298	2·2—0·0
13	20	0·304962	1·231934	2·431—10·388	2·2—0·0
14		0·263766	1·065519	2·330—10·166	2·2—0·0
15		0·337571	1·363665	2·505—10·205	2·1—0·0
16		0·247008	0·997820	2·336—10·283	2·2—0·0
17	15	0·255511	1·032170	2·449—10·061	2·1—0·1
18		0·304714	1·230934	2·419—9·910	2·1—0·1
19		0·329646	1·331649	2·319—10·096	2·1—0·1
20		0·288451	1·165235	2·262—9·685	2·1—0·1
21	10	0·288203	1·164235	2·339—9·806	2·2—0·1
22		0·263519	1·064519	2·410—10·101	2·1—0·1
23		0·329646	1·331649	2·332—10·228	2·2—0·1
24		0·304962	1·231934	2·250—10·064	2·2—0·1
25	5	0·288203	1·164235	2·287—10·462	2·1—0·0
26		0·304714	1·230934	3·249—10·149	2·0—0·1
27		0·272022	1·098869	3·434—10·176	2·0—0·1
28		0·329646	1·331649	2·207—10·422	2·1—0·0

TABLE 3

Complex formation constant determinations. Initial concentrations (mM) and $-\log[H^+]$ ranges for the titrations of spinacine with divalent metals

N	Ion	T_M	T_L	T_H	$-\log[H^+]$
1	Cu^{2+}	0·083163	0·412119	1·664811	2·143—7·181
2		0·092614	0·370841	1·498063	2·182—6·547
3		0·095946	0·288202	1·164233	2·255—6·518
4		0·082330	0·329398	1·330648	2·203—6·704
5	Ni^{2+}	0·079195	0·412119	1·664812	2·407—8·513
6		0·088610	0·370593	1·497063	2·160—8·579
7		0·110776	0·329398	1·330649	2·218—8·548
8		0·093450	0·288450	1·165234	2·270—8·613

because the results for spinaceamine have shown that in any case the constants do not change appreciably even if some systematic errors affect the data.

Programme Scogs [6] was used in the calculation of the equilibria involving metal complexes.

All the calculations were performed on the computer CDC 6600 of 'Centro di Calcolo Interuniversitario dell' Italia Nord-Orientale', Bologna. A complete list of the

[8] G. J. Brookes, I. G. Betteley, and S. M. Loxston, 'Mathematics and Statistics for Chemists,' Wiley, London, 1966, p. 352.
[9] A. Vacca, personal communication.

TABLE 4

Stepwise and total protonation constants of spinaceamine at 25 °C and ionic strength μ = 0·1M KCl. Results with different refinement programmes

Computer programme	Minimised function	log K_1 (σ)	log β_2 (σ)	log K_2 (σ)	N	F	R
Gauss Z	$\Sigma(\bar{n}_c - \bar{n}_o)^2$	8·904(1)	13·799(2)	4·895(2)	159	1·55	0·003
Map Z	$\Sigma(\bar{n}_c - \bar{n}_o)^2$	8·903(1)	13·798(2)	4·895(2)	159	0·82	0·003
Gauss G	$\Sigma(H_c - H_o)^2$	8·904(1)	13·800(2)	4·896(2)	159	6·35	0·003
Ls Map G	$\Sigma(H_c - H_o)^2$	8·902(1)	13·799(2)	4·897(2)	159	4·58	0·003
Lgst	$\Sigma(v_c - v_o)^2$	8·903(1)	13·799(2)	4·896(2)	159	1·38	0·002
Scogs	$\Sigma(v_c - v_o)^2$	8·902(1)	13·799(2)	4·897(2)	159	0·07	0·002

H = Total acid equivalents; v = volume of KOH added; $\bar{n}$ = formation function, subscript o, c, indicate calculated and observed, respectively; R = factor ratio test; N = number of experimental points.

TABLE 5

Stepwise and total protonation constants for spinacine at 25 °C and ionic strength μ = 0·1M KCl. Results with different refinement programmes

Computer programme	Minimised function	log K_1(σ)	log β_2(σ)	log K_2(σ)	log β_3(σ)	log K_3(σ)	N	F	R
Gauss Z	$\Sigma(\bar{n}_c - \bar{n}_o)^2$	8·663(4)	13·599(5)	4·936(6)	15·249(7)	1·649(9)	247	16·04	0·009
Map Z	$\Sigma(\bar{n}_c - \bar{n}_o)^2$	8·652(4)	13·585(6)	4·933(7)	15·226(8)	1·641(10)	247	22·08	0·009
Gauss G	$\Sigma(H_c - H_o)^2$	8·664(4)	13·601(5)	4·937(6)	15·252(7)	1·651(9)	247	19·33	0·007
LS Map G	$\Sigma(H_c - H_o)^2$	8·653(4)	13·587(5)	4·934(6)	15·228(7)	1·641(9)	247	22·35	0·007
Lgst	$\Sigma(v_c - v_o)^2$	8·654(4)	13·588(5)	4·934(6)	15·232(7)	1·645(9)	247	34·37	0·005
Scogs	$\Sigma(v_c - v_o)^2$	8·653(4)	13·587(6)	4·934(7)	15·230(8)	1·643(10)	247	33·89	0·005

H = Total acid equivalents; v = volume of KOH added; $\bar{n}$ = formation function, subscripts o, c, indicate calculated and observed, respectively; R = factor ratio test; N = number of experimental points.

experimental data is available in Supplementary Publication No. 20546 (61 pp., 2 microfiches).*

DISCUSSION

Protonation Equilibria.—Sites of the spinaceamine molecule suitable for protonation are >NH of the piperidine ring and ≥N of the imidazole ring, respectively. The formation constants for these equilibria at 25 °C and ionic strength μ = 0·1M KCl are log K_1 = 8·904(1) and log K_2 = 4·895(2), respectively. These two constants can be compared with constants of 4-aminomethylimidazole (log K_1 = 9·37, log K_2 = 4·71 [10]) and 4-(2′-aminoethyl)imidazole (histamine) (log K_1 = 9·80, log K_2 = 5·94 [10]), and with those of 1,2-diaminoethane (log K_1 = 9·93, log K_2 = 6·85 [11]) and 1,3-diaminopropane (log K_1 = 10·30, log K_2 = 8·29 [12]). The comparison shows that spinaceamine resembles those compounds with two carbon atoms between the basic sites and particularly 4-aminomethylimidazole; this is consistent with the assignment of >NH in the imidazole ring to position (1) and ≥N to position (3), in agreement with structural determinations.[13,14] The protonation constants of the α-amino-acid spinacine, log K_1 = 8·663(4), log K_2 = 4·963(6), log K_3 = 1·649(9), follow the same trend as spinaceamine, with very small influence of the carboxylic group associated with K_3. The differences between constants of spinaceamine and spinacine are practically parallel to those of histamine [10] and histidine (for histidine, log K_1 = 9·17, log K_2 = 6·12, log K_3 = 1·96 [15]). This interpretation of successive protonation steps based upon relationships between log K (or ΔG) and structure seems, on the whole, satisfactory. It follows the opinions of Ritchie and Sager,[16] King,[17] Larson and Hepler,[18] and Bolton and Hepler [19] that differences in ΔG along series of analogous compounds are related to variations of the molecular structure.

TABLE 6

Protonation constants of spinaceamine at different temperatures (μ = 0·1M KCl)

t °C		log K_1 (σ)	log β_2 (σ)	log K_2 (σ)	N	F
5	obs	9·445(2)	14·669(3)	5·224(4)	196	9·13
	calc *	9·456		5·245		
10	obs	9·308(4)	14·450(6)	5·142(7)	192	5·71
	calc *	9·308		5·150		
15	obs	9·167(2)	14·230(2)	5·063(3)	204	1·32
	calc *	9·168		5·062		
20	obs	9·043(1)	14·025(1)	4·982(1)	173	0·01
	calc *	9·035		4·979		
25	obs	8·904(1)	13·799(2)	4·895(2)	159	1·55
	calc *	8·909		4·901		
30	obs	8·803(3)	13·646(4)	4·843(5)	180	4·12
	calc *	8·789		4·828		
35	obs	8·668(2)	13·417(2)	4·750(3)	199	5·04
	calc *	8·676		4·760		

* Values calculated by the equations:

$$\log K_1 = \frac{4770{\cdot}7}{T} - 56{\cdot}581 + 20 \log T$$

and

$$\log K_2 = \frac{3926{\cdot}3}{T} - 57{\cdot}757 + 20 \log T$$

The protonation constants both for spinaceamine (Table 6) and spinacine (Table 7) have been determined at

* For details of Supplementary Publications see Notice to Authors No. 7 in *J. Chem. Soc.* (*A*), 1970, Issue No. 20.

[10] F. Holmes and F. Jones, *J. Chem. Soc.*, 1960, 2398.

[11] D. H. Everett and B. R. W. Pinsent, *Proc. Roy. Soc.*, 1952, *A* **215**, 416.

[12] A. Gero, *J. Amer. Chem. Soc.*, 1954, **76**, 5148.

[13] A. Chiesi Villa, G. Gaetani Manfredotti, M. Nardelli, and G. Pelizzi, *J. Cryst. Mol. Struct.*, 1971, **1**, 123.

[14] G. D. Andreetti, L. Cavalca, and P. Sgarabotto, *Gazzetta*, 1971, **101**, 625.

[15] A. C. Andrews and D. M. Zebolsky, *J. Chem. Soc.*, 1965, 742.

[16] C. D. Ritchie and W. F. Sager, *Progr. Phys. Org. Chem.*, 1964, **2**, 323.

[17] E. J. King, 'Acid–base Equilibria,' Macmillan, New York, 1965, p. 140.

[18] J. W. Larson and L. G. Hepler, 'Solute–solvent Interactions,' eds. J. F. Coetzee and C. D. Ritchie, Dekker, New York, 1969, p. 39.

[19] P. D. Bolton and L. G. Hepler, *Quart. Rev.*, 1971, 521.

different temperatures between 5 and 35 °C at ionic strength $\mu = 0{\cdot}1$M KCl and thermodynamic functions have been calculated. The data can be correlated by Pitzer's expression [20]

$$\log K_n = \frac{A_n}{T} - B_n + 20 \log T$$

By differentiating this equation, values of ΔH and ΔS, at 25 °C and ionic strength $\mu = 0{\cdot}1$M KCl, can be obtained followed by tails of solvent molecules. Protonation of the first amino-group, with gain in positive charge, diminishes the rigidity of the environment, because it destroys oriented hydrogen bonds. For all the compounds, ΔS_1 of the process $H_2N{-}X{-}NH_2 + H^+ \rightleftharpoons H_2N{-}X{-}NH_3^+$ is positive, the only exception being histamine for which $\Delta S_1 = 0$, probably because of the various possibilities of folding of the chain, in the neutral molecule, over $N(1)$ of the imidazole ring. On the other

TABLE 7

Protonation constants of spinacine at different temperatures ($\mu = 0{\cdot}1$M KCl)

t °C		log K_1 (σ)	log β_2 (σ)	log K_2 (σ)	log β_3 (σ)	log K_3 (σ)	N	F
5	obs	9·099(5)	14·321(6)	5·222(7)	15·809(13)	1·488(14)	174	1·47
	calc *	9·107		5·231		1·548		
10	obs	8·984(3)	14·135(4)	5·151(5)	15·762(7)	1·627(8)	244	1·33
	calc *	8·982		5·148		1·560		
15	obs	8·861(4)	13·930(5)	5·069(6)	15·432(8)	1·502(9)	205	1·57
	calc *	8·865		5·070		1·574		
20	obs	8·755(4)	13·760(6)	5·005(7)	15·331(9)	1·571(11)	209	3·10
	calc *	8·753		4·998		1·590		
25	obs	8·663(4)	13·599(5)	4·936(6)	15·249(7)	1·649(9)	247	16·04
	calc *	8·649		4·931		1·608		
30	obs	8·543(4)	13·406(5)	4·863(6)	15·009(7)	1·603(9)	172	1·63
	calc *	8·550		4·868		1·628		
35	obs	8·450(5)	13·254(7)	4·804(9)	14·919(9)	1·665(11)	226	26·07
	calc *	8·456		4·809		1·650		

* Values calculated by the equations:

$$\log K_1 = \frac{4401{\cdot}0}{T} - 55{\cdot}601 + 20 \log T,$$

$$\log K_2 = \frac{3747{\cdot}0}{T} - 57{\cdot}125 + 20 \log T, \quad \text{and}$$

$$\log K_3 = \frac{2252{\cdot}3}{T} - 55{\cdot}435 + 20 \log T.$$

TABLE 8

Thermodynamic functions [a] ΔG (kJ mol^{-1}), ΔH [b] (kJ mol^{-1}), and ΔS [b] (J K^{-1} mol^{-1}) for protonation of spinaceamine and spinacine

	$L + H^+ \rightleftharpoons HL$			$HL + H^+ \rightleftharpoons H_2L$			$H_2L + H^+ \rightleftharpoons H_3L$		
L	ΔG_1	ΔH_1	ΔS_1	ΔG_2	ΔH_2	ΔS_2	ΔG_3	ΔH_3	ΔS_3
Spinaceamine	−50·80(2)	−41·7(4)	30·4(13)	−27·93(3)	−25·6(5)	7·9(18)			
(Spinacine)$^-$	−49·43(2)	−34·7(3)	49·5(10)	−28·16(1)	−22·2(2)	20·2(8)	−9·41(10)	6·5(17)	53·2(56)
Histamine [c]	−56·07	−56·1	0	−33·97	−42·3	−29·3			
Histidine [d]	−51·76	−43·6	27·2	−26·02	−29·3	−13·0			
Imidazole [e]				−39·87	−36·7	10·5			
NH_3 [f]	−52·76	−51·9	2·9						
$H_2N[CH_2]_2NH_2$ [g]	−56·57	−50·0	22·2	−40·67	−46·1	−18·4			
$H_2N[CH_2]_2NH_2$ [h]	−56·94	−50·9	20·1	−41·51	−45·6	−13·8			
$H_2NCH_2COO^-$	−55·81 [i]	−44·2 [g]	38·9 [g]				−13·47 [i]	−4·1 [j]	31·4 [j]
CH_3COO^- [f]							−27·1	0·5	92·5

[a] E.s.d.'s in parentheses, in units of the last digit. [b] Number of variables: 5. [c] W. C. Nicholas and W. C. Fernelius, *J. Phys. Chem.*, 1961, **65**, 1047. [d] J. J. Christensen, R. M. Izatt, D. P. Wrathall, and L. D. Hansen, *J. Chem. Soc.* (*A*), 1969, 1212. [e] J. J. Christensen, D. P. Wrathall, and R. M. Izatt, *Analyt. Chem.*, 1968, **40**, 175. [f] R. P. Bell, 'The Proton in Chemistry,' Methuen, 1959, London, p. 65. [g] J. A. Partridge J. J. Christensen, and R. M. Izatt, *J. Amer. Chem. Soc.*, 1966, **88**, 1649. [h] A. Vacca and D. Arenare, *J. Phys. Chem.*, 1967, **71**, 1495. [i] E. J. King, *J. Amer. Chem. Soc.*, 1951, **73**, 155. [j] J. J. Christensen, R. M. Izatt, and L. D. Hansen, *J. Amer. Chem. Soc.*, 1967, **89**, 213.

for the two compounds. These are compared with values obtained for other compounds (Table 8). The ΔS values are particularly significant because they confirm the correctness of the chosen correspondence of protonation sites and protonation constants. Changes in the ΔS's can be attributed to changes in the order of environmental solvent molecules; these changes are a consequence both of changes in solute–solvent interactions and of modifications in the rigidity of the solute molecules

[20] K. S. Pitzer, *J. Amer. Chem. Soc.*, 1937, **56**, 2365.

hand ΔS_2 is negative for 1,2-diaminoethane, 1,3-diaminopropane, histamine, and histidine, whereas ΔS_2 is positive for spinaceamine and spinacine. It is reasonable to think that in non-cyclic compounds the process $H_2N{-}X{-}NH_3^+ + H^+ \rightleftharpoons {}^+H_3N{-}X{-}NH_3^+$ contributes to increasing the 'stiffening' of the molecules because of the higher repulsion between positive charges. The repulsion is not effective in spinaceamine and spinacine because these molecules are 'stiff' in themselves; then the loss of order of the solvent molecules, due to changes in solute–solvent

interactions, could become the outstanding process determining the increase of entropy.

Metal Complexes.—Potentiometric titrations failed to detect any complexing between spinaceamine and the ions, Cu^{2+} and Ni^{2+}, which is in contrast with imidazole and histamine.[21] Spinacine, however, forms complexes with Ni^{2+} and Cu^{2+}, as shown by the titration curves (Figure 1).

TABLE 9

Equilibria and formation constants of complexes between spinacine and divalent ions, Cu^{2+} and Ni^{2+}

	Equilibrium	Constant
(A)	$Cu^{2+} + H^+ + L^- \rightleftharpoons HCuL^{2+}$	$\log \beta_{111} = 13{\cdot}378(26)$
(B)	$Cu^{2+} + 2H^+ + 2L^- \rightleftharpoons H_2CuL_2^{2+}$	$\log \beta_{212} = 25{\cdot}725(40)$
(C)	$Cu^{2+} + H^+ + 2L^- \rightleftharpoons HCuL_2^+$	$\log \beta_{112} = 20{\cdot}312(43)$
(D)	$HCuL^{2+} + L^- \rightleftharpoons HCuL_2^+$	$\log K^{L}_{HCuL_2} = 6{\cdot}93(5)$
(E)	$HCuL_2^+ + H^+ \rightleftharpoons H_2CuL_2^{2+}$	$\log K^{H}_{H_2CuL_2} = 5{\cdot}41(4)$
(F)	$Ni^{2+} + H^+ + L^- \rightleftharpoons HNiL^{2+}$	$\log \beta_{111} = 11{\cdot}611(27)$
(G)	$Ni^{2+} + L^- \rightleftharpoons NiL^+$	$\log \beta_{011} = 5{\cdot}330(28)$
(H)	$Ni^{2+} + 2L^- \rightleftharpoons NiL_2$	$\log \beta_{012} = 9{\cdot}320(28)$
(I)	$NiL^+ + L^- \rightleftharpoons NiL_2$	$\log K^{L}_{NiL_2} = 3{\cdot}99(3)$
(J)	$NiL^+ + H^+ \rightleftharpoons HNiL^{2+}$	$\log K^{H}_{HNiL} = 6{\cdot}28(3)$

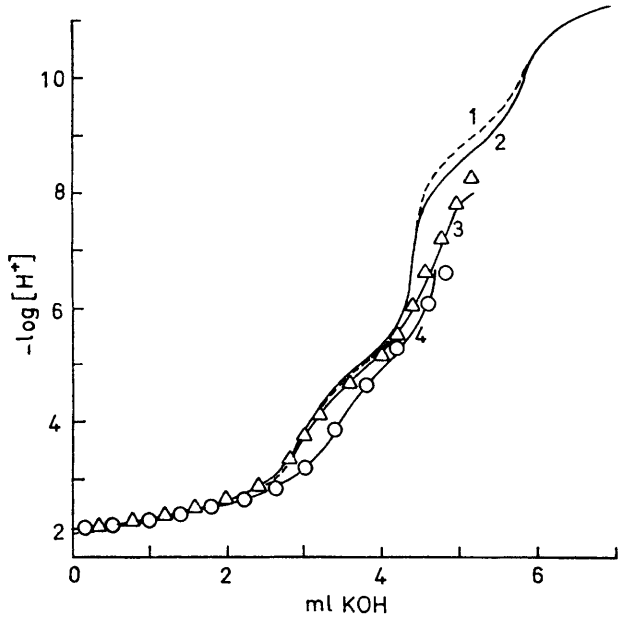


FIGURE 1 Titration curves calculated by Haltafall programme [22] (1) spinaceamine, (2) spinacine, (3) spinacine–Ni^{2+} (T_M = 0·00079 M), Δ = experimental points, (4) spinacine–Cu^{2+} (T_M = 0·00083 M), ○ = experimental points; for each curve: T_H = 0·01671 M, T_L = 0·00413 M

For the interpretation of the equilibria in spinacine–metal solutions, after a wide range Scogs searching including hydroxo-complexes, the choice was restricted to three main sets,

(1) HML^{2+}, $H_2ML_2^{2+}$, HML_2^+
(2) HML^{2+}, ML^+, $H_2ML_2^{2+}$
(3) HML^{2+}, ML^+, ML_2

It is worth noting that species ML^+, ML_2, HML_2^+ would be equivalent for the computer programme to species $HML(OH)^+$, $HML_2(OH)$, and $H_2ML(OH)^+$, respectively. Their existence, however, does not seem likely, at least in the light of the interpretative scheme given below. The calculations show that for Ni^{2+} set (3) is unequivocally the best, whereas for Cu^{2+} both sets (1) and (2) give good agreements. Set (2) for copper has been excluded on the grounds that the formation of unprotonated species intermediate between $HCuL^{2+}$ and $H_2CuL_2^{2+}$ is unlikely. On the other hand, the choice of set (1) for copper and of set (3) for nickel is consistent with the experimental observation that copper complexes are formed in solutions at pH 3—6 and nickel complexes in solutions at pH 6—8; this explains how hydrogen complexes only are formed by copper and some complexes without protons by nickel. At pH >6·8 for copper solutions and at pH >8 for nickel solutions precipitation occurs, probably due to formation of hydroxo-complexes.

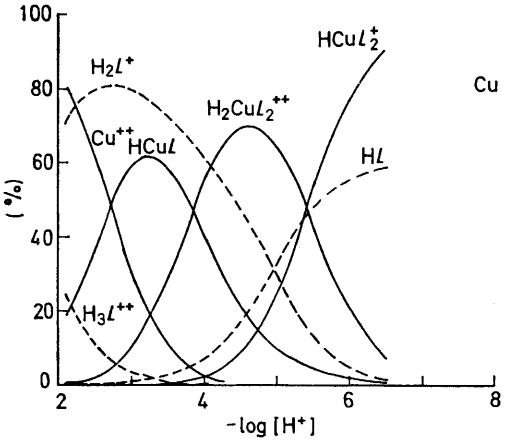


FIGURE 2 Typical distribution diagram for the system spinacine–Cu^{2+}. The percentages have been calculated from the data of titration no. 1 in Table 3 by Haltafall programme.[22] Broken lines show species not containing metal in percent of total ligand; continuous lines show species containing metal in percent of total metal

The sets of formation constants with corresponding equilibria and some relevant stepwise formation constants are reported in Table 9. Typical distribution diagrams are shown in Figures 2 and 3.

We think that the complexes of spinacine are chelates with pentatomic rings formed by the α-amino-acid residue;

[21] A. Chakravorty and F. A. Cotton, *J. Phys. Chem.*, 1963, **67**, 2878.

[22] N. Ingri, W. Kakolowicz, L. G. Sillén, and B. Warnqvist, *Talanta*, 1967, **14**, 1261.

the proton of the hydrogen complexes should be attached to N(3) of the imidazole ring. The glycine-type chelate with *N,O* donor atoms is confirmed by comparison of the

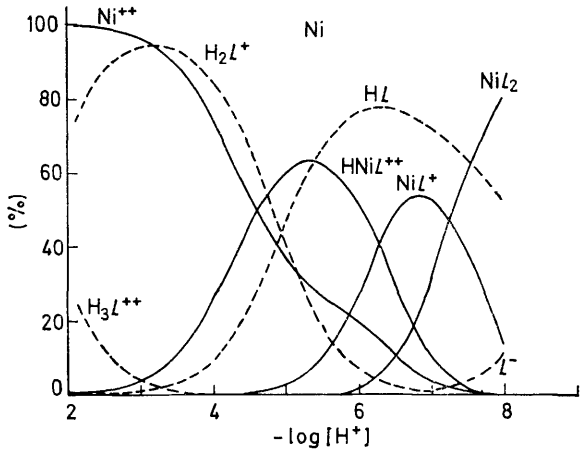


FIGURE 3 Typical distribution diagram for the system spinacine–Ni^{2+}. The percentages have been calculated from the data of titration no. 5 in Table 3 by the Haltafall programme.[22] Broken lines show species not containing metal in percent of total ligand; continuous lines show species containing metal in percent of total metal

formation constants of equilibrium D (*cf.* Table 9) with log K_2 = 6·83,[23] 7·09,[24] for $Cu(gly)_2$ and of the formation constants of equilibria G and I with log K_1 = 5·94,[23] for $Ni(gly)^+$ and log K_2 = 4·84 [23] for $Ni(gly)_2$, respectively.

In the imidazole ring, *N*(3) becomes a stronger base in complexes than in the free ligand spinacine, as shown by equilibria E and J when compared with log K_2 = 4·94 (present work).

CONCLUSION

Spinaceamine and spinacine present two and three protonation constants, respectively. They can be reasonably assigned to the protonation of amino-nitrogen (log K_1), tertiary nitrogen of the imidazole ring (log K_2) and, in spinacine, carboxylic group (log K_3). The trend of log K_n, although shifted toward lower values, follows that of their non-cyclic homologues, histamine and histidine having the same basic sites. With respect to histamine and histidine, however, they present relevant differences in the entropy change, ΔS_2, associated with the protonation of the imidazole ring, and in the complexing capacity with the divalent ions, Cu^{2+} and Ni^{2+}. The properties of spinaceamine and spinacine can be related to their ' stiff ' molecular structure.

We thank Professor M. Nardelli for research facilities, Professor T. Vitali for samples of spinaceamine dihydrochloride and spinacine monohydrate, and the CNR (Rome) for support.

[2/1211 *Received*, 31*st May*, 1972]

[23] V. S. Sharma, H. B. Mathur, and P. S. Kulkarni, *Indian J. Chem.*, 1965, **3**, 146, 475.

[24] K. P. Anderson, W. O. Greenhalgh, and R. M. Izatt, *Inorg. Chem.*, 1966, **5**, 2106.