Protonation of Diaza Cyclic Complexones: ^{1}H NMR, Calorimetric and Molecular Mechanics Studies

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A detailed study of the sequences of protonation of three normal- and medium-ring cyclic diaminocarboxylate ligands (PIPDA, DACHDA and DACODA) has been carried out by ^{1}H NMR spectroscopy. Theoretical molecular mechanics calculations were carried out to predict the most stable conformations of their monoprotonated forms (HL^-) and to estimate relevant bond lengths and contributions to overall energy.

Determinations of the enthalpy changes were made by microcalorimetry, to confirm and relate the conclusions of these studies to the thermodynamic functions of the reactions of protonation.

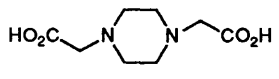
The results suggest that a strong NH · · · N hydrogen bond (bond length 2.02 Å) is formed in monoprotonated 1,5-diazacyclooctane-*N*,*N*′-diacetate (DACODA), the ring of which adopts a boat conformation, whereas monoprotonated 1,4-diazacycloheptane-*N*,*N*′-diacetate (DACHDA) adopts a semi-chair, and piperazine-*N*,*N*′-diacetate (PIPDA) adopts a *trans*-chair conformation for which NH · · · N hydrogen bonds are not possible. The doubly protonated species (H_2L) of these ligands seems to have both protons involved in intramolecular NH · · · $_2OC^-$ bonds to the acetate groups.

Hydrogen bonding and differences in ring inductive effects may explain the large discrepancies in the values of the protonation constants of the three ligands.

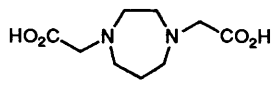
Polyamino carboxylic acids ('complexones') are well known ligands that have found wide application, not only in analytical chemistry but also in a number of other fields, from fundamental and applied medical studies, *e.g.* in detoxification or radiodiagnostics, to various agricultural and industrial uses.

The latest additions to a rather long list of common 'complexones' (although not so many are currently used) are those based in a more rigid macrocyclic skeleton. This may introduce unexpected effects, sometimes enhancing the stability of the metal complexes formed, or changing the selectivity ratios in more interesting directions. The group of *N*-acetate tetraaza macrocycles, of which 1,4,7,10-tetra-aza-cyclododecane-*N*,*N*′,*N*″,*N*‴-tetracetic acid (DOTA) and 1,4,8,11-tetra-aza-cyclotetradecane-*N*,*N*′,*N*″,*N*‴-tetracetic acid (TETA) are the best known members, already has a conspicuous place in the literature and is progressively receiving more attention as the applications of these compounds become more varied and appealing.

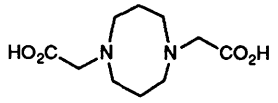
We have previously studied this[1] and other types of macrocyclic complexone, among which the simplest are the diaza cyclic complexones, piperazine-*N*,*N*′-diacetic acid (PIPDA, **1**), 1,4-diazacycloheptane-*N*,*N*′-diacetic acid (DACHDA, **2**) and 1,5-diazacyclooctane-*N*,*N*′-diacetic acid (DACODA, **3**).*



1, PIPDA



2, DACHDA



3, DACODA

Not unexpectedly these complexones differ considerably in their tendencies to complex metal ions,[2–4] but the large differences in their protonation constants and their apparently irregular order when the three compounds are compared cannot be explained in immediate and simple terms. Fully ionised DACODA is far more basic than either DACHDA or PIPDA (log K_1 for DACODA is 12.27, compared with 9.83 for DACHDA and 8.70 for PIPDA, but the value of log K_2 for DACHDA is 5.92, *cf.* 4.80 for DACODA and 4.41 for PIPDA (see Table 1).

To understand the reason for these differences and trends, and to provide a basis for the interpretation of the behaviour of each one of these simple compounds (and of the more complicated polyaza complexones) in their complexation reactions, we have undertaken a more detailed study of the sequence of protonation of the fully ionised ligands using ^{1}H NMR and calorimetric techniques. Theoretical molecular mechanics calculations were used to predict the most stable conformations of the molecules and to estimate some of their most relevant parameters, *i.e.* the distances and contributions to their overall energy.

Experimental

Reagents.—The diaza-diacetate cyclic complexones were obtained by carboxymethylation of the corresponding cyclic amines with chloroacetic or bromoacetic acid in an alkaline medium. The cyclic diamine 1,5-diazacyclooctane was synthesized as described in the literature.[5,6] The other cyclic amines

* The common acronyms of the complexones are used rather loosely to refer to the acid form LH_2 or the ionised forms LH^- or L^{2-} of the ligands. In the present paper they usually refer to the fully ionised form L^{2-} unless otherwise specified.

Table 1 Molecular mechanics parameters

(*a*) Atom	Mean charge from MOPAC
C (in CO_2^-)	0.28
C (adjacent to N)	0.11
N	−0.33
H (of NH^+)	0.30
O (in CO_2^-)	−0.54
C (adjacent to NH^+)	0.07
N (of NH^+)	0.00
H (of C)	0.00
(*b*) Bond	Bond dipole moment (μ/D)
C–O (in CO_2^-)	2.55
C–N	1.37
C–N (in NH^+)	0.22
N–H (in NH^+)	0.67
C–C (in $CH_2CO_2^-$)	0.64

Table 2 Protonation constants of ionized piperazine-*N*,*N*′-diacetic acid (PIPDA), 1,4-diazacycloheptane-*N*,*N*′-diacetic acid (DACHDA) and 1,6-diazacyclooctane-*N*,*N*′-diacetic acid (DACODA). T = 25.0 ± 0.1 °C; I = 0.1 mol dm^{-3} (KNO_3)

	PIPDA[a]	DACHDA[b]	DACODA[b]
log K_1	8.70	9.83	12.27
log K_2	4.41	5.92	4.70
log K_3	<2	2.04	1.84

[a] Ref. 2. [b] Ref. 4.

(1,4-diazacycloheptane and 1,4-piperazine) are commercially available.

The dimethylated amine *N*,*N*′-dimethyldiazacycloheptane (DMDACH) was prepared by refluxing the commercially available non-methylated product with formic acid and formaldehyde.[7] The pure amine was obtained by high-vacuum distillation. All the reaction products were characterized by the usual methods of analysis: m.p. IR and NMR spectra.

DACODA·HBr: m.p. 227–230 °C (decomp.) (Found: C, 38.65; H, 5.9; N, 8.75. $C_{10}H_{19}BrN_2O_4$ requires C, 38.60; H, 6.11; N, 9.01%).

DACHDA·HCl: m.p. 235 °C (decomp.) (Found: C, 42.55; H, 6.75; N, 10.85. $C_9H_{17}ClN_2O_4$ requires C, 42.77; H, 6.73; N, 11.90%).

PIPDA·HCl: m.p. 305 °C (decomp.) (Found: C, 39.9, H, 6.45, N, 11.55. $C_8H_{15}ClN_2O_4$ requires C, 40.25; H, 6.29; N, 11.74%).

DMDACH: oil (b.p. 75 °C, 0.6 mmHg (Found: C, 65.5, H, 12.55; N, 21.9. $C_7H_{16}N_2$ requires C, 65.62; H, 12.50; N, 21.88%).

NMR Measurements.—^{1}H NMR spectra were run at 100 MHz on a JEOL JMN 100 PFT spectrometer and at room temperature. Solutions of the cyclic complexones, concentration 2 × 10^{-2} mol dm^{-3} were prepared in D_2O with trimethylsilylpropanesulphonate as reference. The pD* (operational pD, since the pH meter was standardized with conventional buffers at pH 4 and 7) was varied by adding DCl or KOD (CO_2 free). The final pD* was monitored using a pH M63 Digital Radiometer instrument fitted with a combined Ingold 405-M3 microelectrode.

The percentages of protonation in each stage of the titrations were obtained as previously described.[1]

† 1 cal = 4.184 J.

Molecular Mechanics Calculations.—Molecular mechanics has been successfully applied to organic molecules for a number of years, using a variety of programs.[8] In our studies we have employed the MM2 (87) program,[9] which includes parameters developed recently by Allinger *et al.*[10] to take account of hydrogen bonding interactions.

We found it necessary to make some changes in the bond dipole-moment parameters. To estimate these, we used a method employed by several workers;[11,12] charges on the atoms for L^{2-} and LH^{-1} for both DACODA and DACHDA were calculated using MOPAC.[13] Values were then averaged and scaled, and bond dipole moments were calculated using the method of Damewood *et al.*[12] Details of the parametrization are listed in Table 1.

All other parameters were taken directly from the MM2(87) program.

Microcalorimetric Measurements.—The enthalpies of protonation of L^{2-} were determined with a Thermometric AB thermal activity monitor (Model 2277) equipped with Perfusion/Titration system and a Hamilton pump (model Microlab M). The calorimetric system as well as the Hamilton pump were controlled by an IBM Personal Computer (Personal System 2 Model 30) using the program AUTOTAM.[14] The enthalpy of ionization of water was determined by adding aqueous NaOH to a solution of HNO_3 contained in the calorimetric vessel. The measured value, −13.55(5) kcal mol^{-1},† was in good agreement with the accepted literature value.[15] The calorimetric ampoule was filled with 2 cm^3 of ligand (0.005 mol dm^{-3}). After equilibration, 10 or 20 mm^3 injections of titrant KOH or HNO_3 (0.04 mol dm^{-3}) were made using a 0.250 cm^3 gas-tight Hamilton syringe (model 1750 LT) attached to a Microlab M system. The timing and sequence of injections were regulated by means of a micro-computer which was also used for integration of the titration curves.[14] Entering the previously determined relevant stability constants, the amount of each species present at equilibrium before and after each addition was calculated together with the corresponding enthalpies of reaction, by means of the KK88 program.[16]

Results and Discussion

Protonation Studies by ^{1}H NMR.—The ^{1}H NMR spectra of the cyclic diaza ligands PIPDA, DACHDA, DACODA and DMDACH are quite simple and identification of the methylenic proton resonances is straightforward, taking into account the relative area and the spin–spin splitting of each resonance (see Figs. 1–4).

Figures 1–3 illustrate the NMR titration curves of the three diaza carboxylate ligands referred to above. Successive protonation of the stronger basic sites of the ligands results in two well defined inflexions in the titration curves. The first inflexion occurs in the range pD* 11.5–8.0 for PIPDA and DACHDA and from 14.0–10.5 for DACODA. The second inflexion occurs in the pD* range 6–3 for the three ligands. In both inflexions the central pD* correlates well with the values of log K_1 and log K_2 for these ligands (see Table 2), hence they probably correspond mainly to nitrogen protonation. On further decreasing the pD* a broader inflexion is observed in the titration curves, corresponding mainly to the protonation of the less basic carboxylate groups.

The extent to which nitrogen and carboxylate groups are protonated at medium and low pH can, in principle, be derived by using the approach of Sudmeier and Reilley.[17] In this method, the shielding contributions of the basic sites of the ligand to the shift δ_i of a nearby CH_2 group are considered to be additive, eqn. (1) where C_{ij} is the protonation shift constant

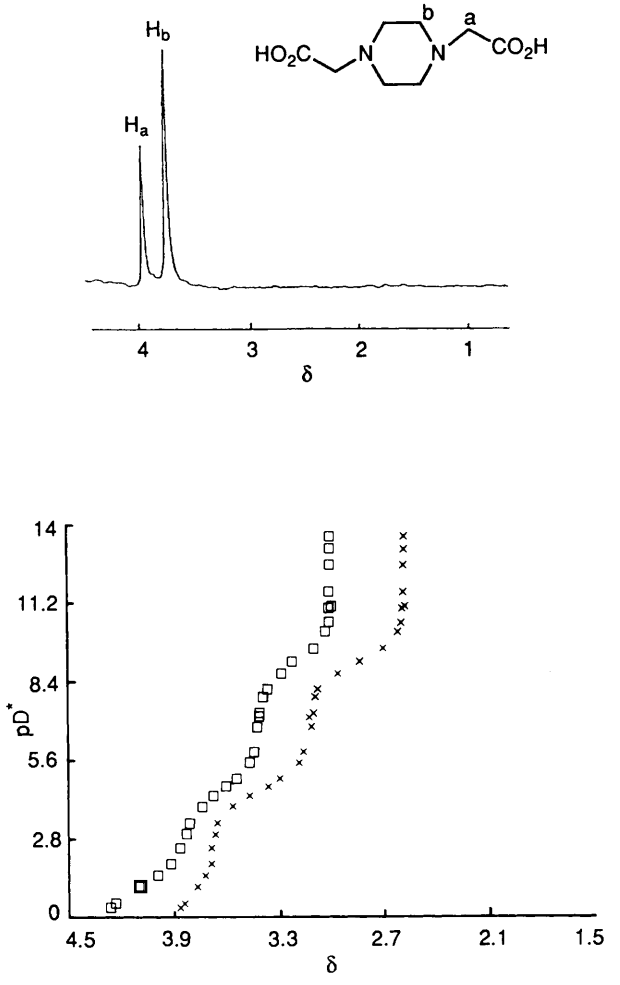


Fig. 1 ^{1}H NMR spectrum of PIPDA at pD* 1.92 and the titration curves, δ as a function of pD*.

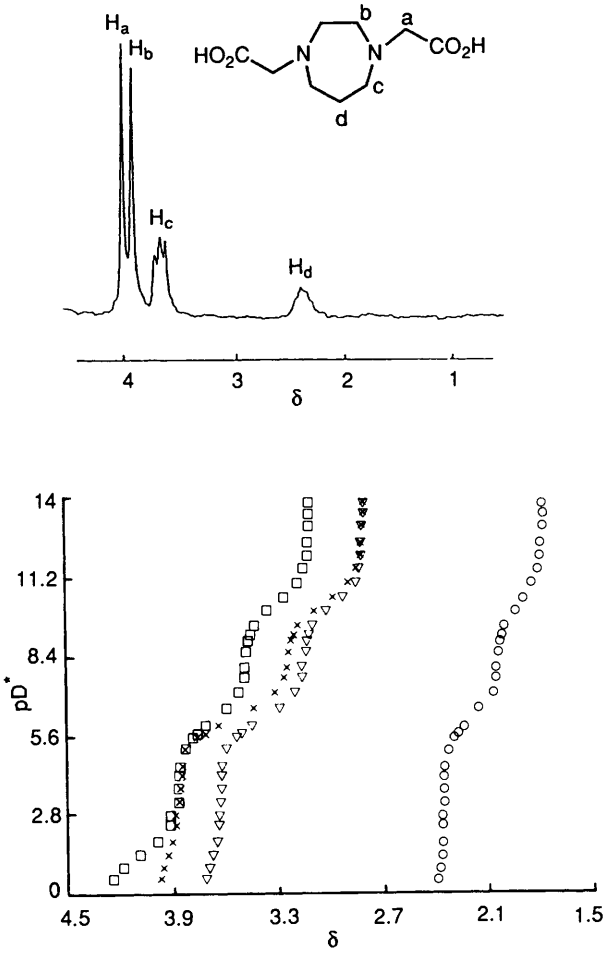


Fig. 2 ^{1}H NMR spectrum of DACHDA at pD* 1.85 and the titration curves, δ as a function of pD*.

Table 3 Comparison of C_N and $C_{N'}$ values (ppm) calculated from model amines

	C_N	$C_{N'}$	Ref.
Linear	0.75	0.35	17
Me_4[14]aneN_4	0.79 (1.01)	0.24 (0.26)	18
Me_4[15]aneN_4	0.86 (0.98)	0.26 (0.27)	1
DMDACH	0.84 (0.90)	0.22 (0.27)	This work

[a] Values for low pH in parentheses

$$\delta_i = \sum_{j=1}^{N} C_{ij} f_j \qquad (1)$$

of the i^{th} CH_2 group for the total protonation of the nearby j^{th} basic site and f_j the fraction of protonation of this site. Furthermore, on addition of n equivalents of acid, the following eqn. (2) holds, where α_j is the number of identical sites that correspond to j.

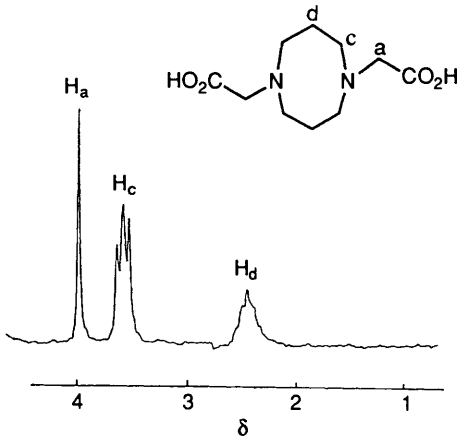
$$n = \sum_{j=1}^{N} \alpha_j f_j \qquad (2)$$

For linear polyamine carboxylates, eqn. (1) is valid throughout the whole pH range. However, when this is extended to the study of cyclic polyamino carboxylates, different values of the constants C_{ij} have to be used and a discontinuity in the values for high and low pH is observed. The possibility that these cyclic ligands adopt different conformations on protonation of the nitrogen atoms is normally accepted as being responsible for such a break.[18]

A strategy for interpreting the observed protonation shift of cyclic complexones has been the use of shielding constants C_{ij} determined from the titration curves of model cyclic amine compounds.[1,17] In this study shielding constants C_N for the fragment CH_2–NR_2 and $C_{N'}$ for CH_2–CH_2–NR_2 were determined from the titration curves of the model amine 1,4-dimethyl-1,4-diazacycloheptane (DMDACH).

Titration curves of DMDACH are shown in Fig. 4. They exhibit two well defined inflexions centred at *ca.* pD* 10 and pD* 6, corresponding to the successive protonation of the two nitrogens of the diaza ring. At *ca.* pD* 8.3, a well defined stage of protonation is reached and the protonation shifts of the CH_2 groups of the species LH^+ yields the shielding constants C_N 0.84 ppm and $C_{N'}$ 0.22 ppm. A second well defined stage of protonation occurs at very acid conditions (pD* ≈ 2.4) corresponding to the formation of the species LH_2^{2+}. The derived set of shielding constants is then C_N 0.90 ppm and $C_{N'}$ 0.27 ppm.

The previously calculated shielding constant values for DMDACH are different from those obtained for linear amines,[17] but are close to the values obtained by Desreux[18] for the amine Me_4[14]aneN_4, as well as to those calculated by us[4] for the amine Me_4[15]aneN_4 (see Table 3).



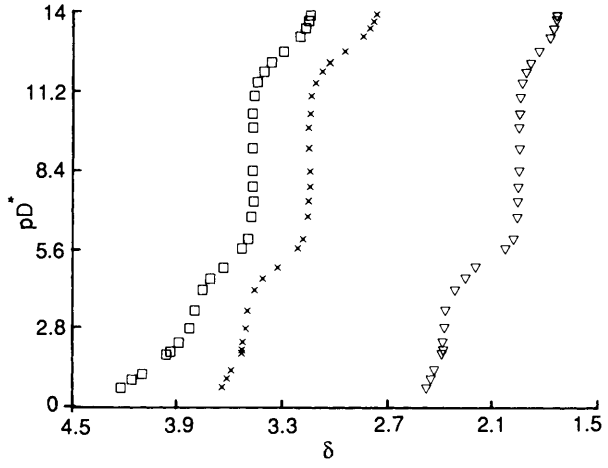
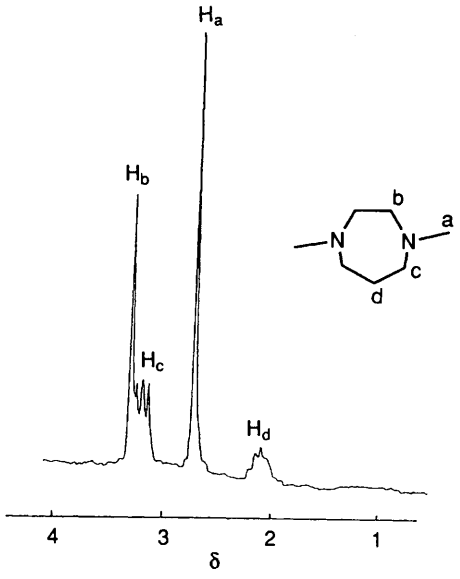


Fig. 3 ^{1}H NMR spectrum of DACODA at pD* 1.96 and the titration curves, δ as a function of pD*.



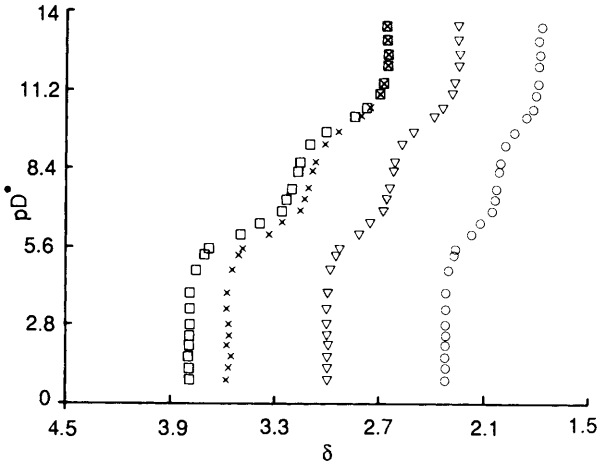


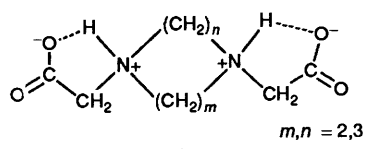
Fig. 4 ^{1}H NMR spectrum of DMDACH at pD* 6.89 and the titration curves, δ as a function of pD*.

Particularly relevant is the large increase in the values of $C_{N'}$ for DMDACH, from low to high pD*, when compared with the slight increase in the values of $C_{N'}$ for the other two cyclic amines.

The percentages of protonation, f_N for nitrogen atoms and $f_{CO_2^-}$ for carboxylate groups, obtained with C_N and $C_{N'}$ values for DMDACH (see Table 4) and using Reilley's value $C_{CO_2^-}$ 0.20 ppm for carboxylate groups, are presented in Table 3. These values show that the first proton becomes attached to one nitrogen atom, while the other nitrogen atom of the diaza ring is protonated only after addition of a second equivalent of acid. The carboxylate groups, being less basic, are protonated at lower pD* but slight competition between the carboxylate groups for the proton seems to occur in DACODA and DACHDA when the second proton is added. However, the carboxylate groups are never protonated above 47% for DACHDA, even at pD* as low as 0.3.

The present results for the protonation sequences of these three cyclic diaza complexones are similar to those of linear complexones such as EDTA or EDDA,[17] for which full protonation of the nitrogens before that of the carboxylates is also observed, but they are in contrast with those of the cyclic tetraazatetraacetic ligands.[1] In this case the protonation occurs firstly in two opposite nitrogen atoms of the ring. The other nitrogens, being less basic, either remain essentially deprotonated or, in a few cases, are protonated together with the carboxylate groups.

The different behaviour of the diaza cyclic complexones may arise from the involvement of the two carboxylate groups in intramolecular hydrogen bonds with the protons attached to nitrogens in the neutral species **4**, which is not possible for all the carboxylates in the tetra-aza complexones.[18]



m,n = 2,3

4

The special stability of species **4** is supported by the rather low log K_3 values found for the three complexones (see Table 2) and is associated with the possibility of the diaza-ring being locked in stable conformations with the two ammonium ions being

Table 4 Percentage of protonation of amino and carboxylate groups in the complexones PIPDA, DACHDA and DACODA[a]

	PIPDA		DACHDA		DACODA	
n	f_N	$f_{CO_2^-}$	f_N	$f_{CO_2^-}$	f_N	$f_{CO_2^-}$
1	49 ± 1	1 ± 1	43 ± 2	7 ± 2	42 ± 3	9 ± 3
2	95 ± 0	5 ± 0	89 ± 3	11 ± 3	82 ± 9	18 ± 9
3	117 ± 6	33 ± 6	103 ± 3	47 ± 3	108 ± 7	42 ± 7

[a] Using the values of C_N and $C_{N'}$ for DACHDM in Table 2 and $C_{CO_2^-}$ 0.20 ppm.

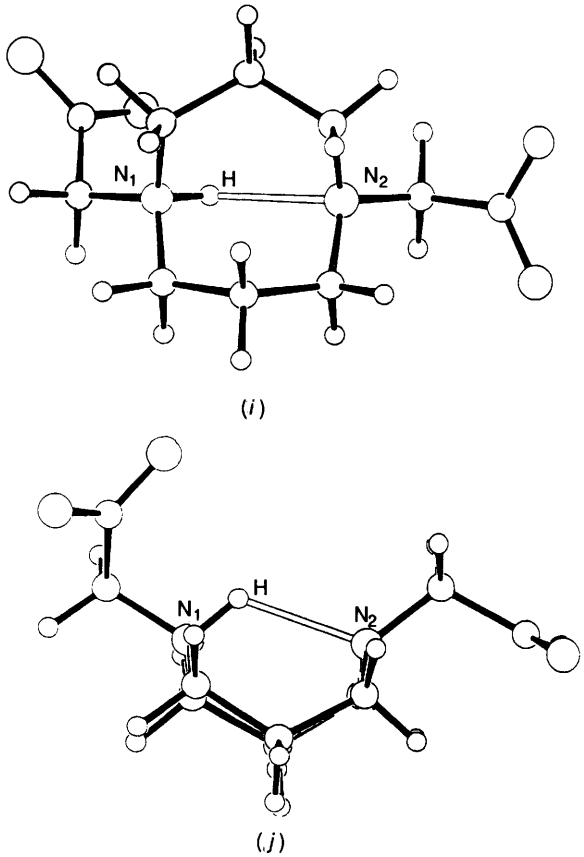


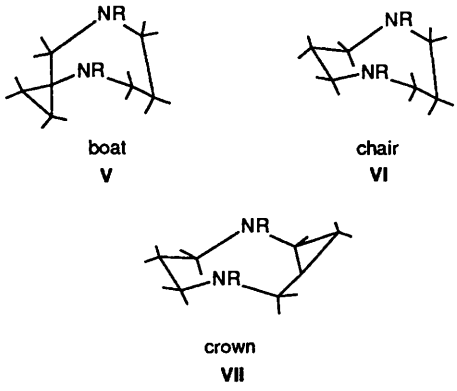
Fig. 5 Plot of two conformations calculated for $DACODAH^-$: (*i*) chair (view from bottom); (*j*) boat (side view). Hydrogen bonds shown as unshaded lines. The weak NH–O bond is not represented.

sufficiently well separated to reduce coulombic repulsions. Conformations like the *trans*-chair proposed earlier by Irving and Pettit [2] for PIPDA fulfil this aim.

Molecular Mechanics Calculations on DACODA and DACHDA.—Molecular mechanics calculations were carried out for the various conformations of DACODA and DACHDA to provide a basis from which to explain the differences in their protonation abilities, namely the high value for log K_1 and low value for log K_2 of DACODA relative to DACHDA.

We followed the technique of Alder [19] who used molecular mechanics to calculate the steric energies of amines and their protonated ions and related the difference between the two energies to pK values. We therefore studied the structures of both L^{2-} and LH^- species and were particularly interested in the effect of internal hydrogen bonding on the total strain energy of the ligands.

For the simulation of DACODA we considered three of the most common conformations, derived by Allinger [10] for eight-membered rings, namely boat–boat, boat–chair and chair–chair, that we decided to call boat (**5**), chair (**6**) and crown (**7**), respectively.



We began by modelling the species LH^-. For each ring conformation of this species, the effect of four different orientations of the acetate arms on the total strain energy and on the possibility of hydrogen-bond formation was considered. The four orientations [(*a*)–(*d*)] are the following: (*a*) both carboxylate groups oriented towards the protonated nitrogen N_1; (*b*) only the carboxylate group attached to N_1 oriented towards the proton (internal ring); (*c*) only the carboxylate group on the non-protonated nitrogen N_2 oriented towards the proton (external ring); (*d*) both carboxylate groups oriented away from the proton in N_1

Table 5 summarizes the results of our calculations on DACODA (LH^- form). From the total energy values, it is clear that the arrangement of the acetate groups makes little difference to the overall steric energy for all conformations: E_{boat} (mean 122.8 kJ mol^{-1}) < E_{chair} (mean 134.2 kJ mol^{-1}) ≪ E_{crown} (mean 161.3 kJ mol^{-1}). The major difference between the energy values is caused by the torsion-angle terms which are much larger for the chair and the crown conformations than for the boat.

In all the conformations but one, the calculations suggest a N–H • • • N hydrogen bond (Fig. 5). The exception is the chair form in orientation (*a*), in which, presumably, the hydrogen bond is destabilized by the large dipole interaction. It is interesting to note that the three conformations have different calculated N–H • • • N hydrogen bond lengths in the order boat (2.10 Å) < chair (2.19 Å) < crown (2.24 Å).

The general preference for the boat conformation relative to the chair conformation in monoprotonated DACODA is in contrast with what has been found for cyclooctane.[8] This can be explained not only by the possible N–H • • • N interaction, but also by the presence of the acetate groups.

The study of DACHDA was restricted to just one ring conformation, 'semi-chair', of the LH^- species (Fig. 6). The various orientations of the acetate arms, relative to the proton attached to the nitrogen atom, were the same as those considered for DACODA.

Theoretical modelling of monoprotonated DACHDA (see Table 6 and Fig. 6) proved that in order for the ring skeleton to have *gauche* C–C torsion angles the N • • • N distance must be higher than the corresponding values found for monoprotonated $DACODAH^-$ in all of its three most stable ring conformations. In two of the orientations of the acetate arms [(*a*), (*c*) in Table 6], there is the possibility of formation of strong N–H • • • O hydrogen bonds. These are much shorter than is possible for DACODA which may be due to a longer N • • • N distance for DACHDA. It should also be noted that the two conformations with no hydrogen bond, namely (*b*) and (*d*), have an energy *ca.*

Table 5 Results of molecular mechanics calculations of $DACODAH^-$. (Energies in kJ mol^{-1}: distances in Å)[a,c]

	Boat				Chair				Crown			
	(*a*)	(*b*)	(*c*)	(*d*)	(*a*)	(*b*)	(*c*)	(*d*)	(*a*)	(*b*)	(*c*)	(*d*)
Total[a]	121.6	124.6	121.2	127.1	158.8	136.3	131.7	139.2	158.0	163.4	158.4	165.1
Stretch	6.7	7.1	6.3	6.3	6.3	5.9	5.9	5.9	7.1	6.7	6.7	6.7
Bending	40.1	41.0	42.6	43.9	38.9	28.0	28.8	30.9	25.5	27.17	28.4	29.3
St-bend	3.3	2.9	3.3	3.3	3.3	2.9	2.9	3.3	2.9	3.3	2.9	3.3
VdW 1,4	44.7	42.6	43.5	42.2	49.7	46.8	46.8	46.0	51.4	50.2	50.2	49.3
Other	1.3	1.7	2.9	3.8	−4.6	−7.1	−5.4	−4.2	−5.9	−5.4	−4.2	−3.3
Torsion	14.2	14.2	14.2	13.4	43.1	44.3	43.9	43.1	66.0	65.6	66.0	64.8
Dipole	11.3	14.6	8.4	13.4	22.6	15.5	8.4	14.2	10.8	15.9	8.8	14.6
Distances[e]												
[b]O(1)–HN(1)	3.50	5.26	3.42	5.28	3.80	5.35	3.55	5.38	3.60	5.35	3.56	3.58
[b]O(2)–HN(1)	2.65	2.67	4.11	4.12	2.48	2.62	4.12	4.14	2.76	2.59	4.07	4.07
N(2)–HN(1)	2.10	2.10	2.09	2.10	3.55	2.18	2.18	2.20	2.25	2.23	2.22	2.26
N(2)–N(1)	2.76	2.76	2.77	2.77	3.74	2.83	2.84	2.86	2.85	2.85	2.84	2.87
Energy of L^{2-}	166.8	176.0	175.6	182.7	182.7	183.1	187.7	194.0	202.7	208.2	209.8	211.9
Difference between E of L^{2-} and LH^-	46.4	51.4	54.3	54.8	23.4	46.8	56.0	54.8	44.7	44.7	51.4	46.8

[a] Mean energy: boat 122.8, chair 134.2 (excl. a), crown 161.3 kJ mol^{-1}. [b] (1) is defined as the closest oxygen to N(1) of the two making up the acetate group in the other ring to N(1). [c] (2) is defined as the oxygen atom that is closest to N(1) of the two making up the acetate group in the same ring as N(1). [d] (*a*), (*b*), (*c*), (*d*) refer to the four different orientations of the acetate groups relative to the ammonium proton. [e] Mean distance: boat 2.10, chair 2.19 (excl. a), crown 2.24.

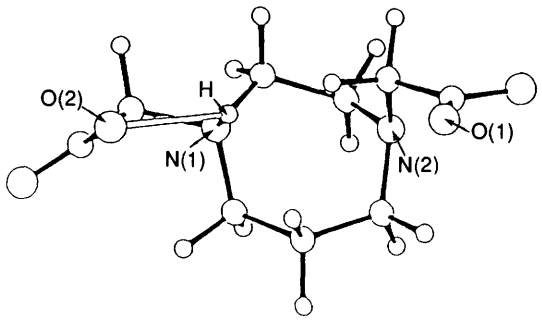


Fig. 6 Plot of the most stable conformation calculated for $DACHDAH^-$ [conformation (*b*)]. Hydrogen bonds shown as unshaded lines.

21 kJ mol^{-1} lower than that of the (*a*) and (*c*) conformations. This is primarily caused by an increase in the bending and torsion angle terms that are only slightly compensated by the possibility of formation of the hydrogen bond.

The energies of the $DACHDA^{2-}$ species were also calculated and on average, are only 20.5 kJ mol^{-1} greater than the protonated species. This contrasts with a difference of 49.7 kJ mol^{-1} for DACODA. These results are, of course, dependent upon the parametrization of the force field and in particular of the bond dipole moments. We would not argue that our values are ideal, but we did find that quite large changes in the values of the moments did not significantly alter the results. These energy differences fit well with the experimental data.

The first protonation constant log K_1 of DACODA is shifted towards higher values relative to that of the linear DACHDA or of EDDA.[20] For the same reason, the second protonation constants log K_2 are shifted to values below that of DACHDA or EDDA. In the two ligands, the observed differences in the first and second protonation constants are larger for DACODA than for DACHDA, in agreement with the estimated length of the intramolecular N ··· HN hydrogen bonds, which are 2.10 Å for the first ligand and are ≥2.99 Å for the second one, if any.

The previous calculations also predict a more significant participation in hydrogen bonds of the carboxylate groups in the LH^- species of DACHDA than in the LH^- species of DACODA. As a result, the carboxylate groups of DACODA can be more easily protonated after addition of the second equivalent of acid ($f_{CO_2^-} = 18 \pm 9$ for DACODA as compared with $f_{CO_2^-} = 11 \pm 3$ for DACHDA, see above).

For the six-membered-ring compound (PIPDA) only preliminary studies were made which showed that the diaza-ring should adopt an essentially chair conformation as happens for cyclohexane.[5] This is due to the fact that the boat or even twist boat conformation have non-ideal torsion angles and the corresponding overall energy is too high to be compensated by intramolecular NH ·· N hydrogen bonding. It is therefore clear that both the LH^- and LH_2 species of PIPDA must adopt a *trans*-chair (**8**) conformation in order to minimize repulsive forces. The calculated N ··· N distance is *ca.* 2.86 Å.

The lack of the NH ··· N hydrogen bond interaction for the chair conformation of the monoprotonated species together with stronger inductive effects of the nitrogen atoms justifies the much lower values found for log K_1 (8.70) as compared with that value for DACODA (12.27) or, to some extent, for DACHDA (9.83). The lowering of the log K_2 value of PIPDA relative to DACHDA is justified by the difference in the length of the $N_1 \cdots N_2$ carbon chains in the two compounds through which the inductive effect of the $-\overset{+}{N}H<$ group formed when the first proton is added is transmitted, causing the addition of the second proton to be more difficult in the case of PIPDA.

The present results provide a rational basis upon which to explain the apparent anomalies in the protonation constants of the three diaza cyclic complexones DACODA, DACHDA and PIPDA, and to stress the importance of both intramolecular hydrogen bonds and Coulombic repulsions in the determination of the low-energy conformations of protonated small ring cyclic amino-carboxylate ligands. It also supports the suggestion made by Desreux[18] for the break in shielding

Table 6 Results of molecular mechanics calculations of $DACHDAH^-$. (Energies in kJ mol^{-1}; distances in Å)

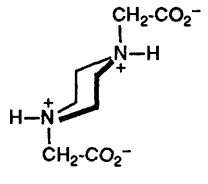
	Semi-chair			
	(*a*)	(*b*)	(*c*)	(*d*)
E_{total}	143.8	121.6	144.2	123.7
Stretch	3.8	4.2	3.8	4.2
Bending	53.9	38.9	55.8	41.8
St-Bend	2.9	2.5	2.9	2.5
VdW 1,4	41.8	42.2	40.1	40.5
Other	−11.3	−10.0	−10.0	−7.1
Torsion	30.1	28.0	30.1	26.3
Dipole	22.2	15.9	20.1	15.0
Distances				
[a] O(1)–HN(1)	2.02	4.37	2.02	4.35
[a] O(2)–HN(1)	2.87	2.49	4.06	4.06
N(2)–HN(1)	3.10	3.00	3.06	2.99
N(2)···N(1)	3.10	3.05	3.09	3.06
$E(L^{2-})$	159.3	141.7	166.8	150.5
E_{diff} [b]	15.5	20.1	22.6	26.8

[a] O(1) is defined as the closest oxygen to N(1) of the two making up the acetate group in the other ring to N(1). [a] O(2) is defined as the closest oxygen to N(1) of the two making up the acetate group in the same ring as N(1). [b] Mean E_{diff} 20.5.

Table 7 Thermodynamic functions for the successive protonation reactions (stepwise) of ionized piperazine-*N*,*N*′-diacetic acid (PIPDA), 1,4-diazacycloheptane-*N*,*N*′-diacetic acid (DACHDA) and 1,5-diazacyclooctane-*N*,*N*′-diacetic acid (DACODA). T = 25.0 ± 0.1 °C; I = 0.1 mol dm^{-3} (KNO_3 or $[NME_4]NO_3$); ΔG, ΔH kJ mol^{-1}; ΔS J K^{-1} mol^{-1}

	PIPDA	DACHDA	DACODA
ΔG_1	−49.5	−56.1	−69.9
ΔG_2	−25.5	−33.7	−26.8
ΔG_3		−11.6	−10.5
ΔH_1	−10.2	−26.7	−54.3
ΔH_2	−10.5	−23.8	−12.6
ΔH_3		−4.6	−1.0
ΔS_1	98	98	52
ΔS_2	43	39	48
ΔS_3		23	28

Standard deviation: ΔG ± 0.1; ΔH ± 0.3; ΔS ± 2.



8

trans–Chair conformation for PIPDA (LH_2 form)

constants C_N and $C_{N'}$ from high to low pH values as being due to conformation changes.

Calorimetric Determinations.—To confirm and relate the conclusions above to the thermodynamic parameters of the reactions of protonation of the three ligands, direct calorimetric determinations of the enthalpy changes were carried out as described in the Experimental section. The values of the entropy changes were estimated from the relationship $\Delta G° = \Delta H° - T\Delta S°$ and the results are shown in Table 7.

As shown in this table, the protonation of the first basic centre of the three ligands, a nitrogen atom, is more than twice as exothermic in fully ionised DACODA than in PIPDA or DACHDA. In principle this is to be expected, although these nitrogen atoms are all analogous (tertiary), since the negative inductive effect of the charged $-\overset{+}{N}H<$ group on the other nitrogen atom decreases with the length of the carbon chains between the two and the positive contribution of the $-CH_2$ groups is small. However, the difference in the ΔH_1 values is too large (compare PIPDA and DACHDA) and confirms that the protonation processes are not identical.

The calculations above suggest the formation of a rather strong NH··N bond in monoprotonated DACODA as the main factor that should account for the difference. An NH···N bond in monoprotonated DACHDA, if formed, must be very weak and such a bond is not possible in monoprotonated PIPDA; in the last two ligands the NH···$^-{}_2$OC– bonds predominate and the protonation reactions are probably analogous (as supported by identical ΔS_1 values).

On addition of the second proton to monoprotonated DACODA, the NH···N bond is disrupted and replaced by two NH···$^-{}_2$OC bonds, whereas on addition of a further proton to monoprotonated PIPDA only a second NH···$^-{}_2$OC bond is formed. Comparison of the values of ΔH_2 for PIPDA and DACODA extrapolated to take into account the differences in basicity suggest that the NH···N hydrogen bond in the monoprotonated form of this last ligand may account for an extra stabilization of *ca.* 20 kJ mol^{-1}, which is not unreasonable for the energy of such a bond at an NH···N distance.[21,22]

The ΔH_2 value for the protonation of DACHDA is higher than expected but the inductive effect of the charged $-\overset{+}{N}H<$ group is smaller than in the case of PIPDA, hence the value of ΔH_2 must be higher in the first case whereas the ΔS_2 values ought not to be very different. In fact, the value of ΔH_2 for DACHDA is normal; it is the corresponding ΔH_2 value for DACODA that is too low due to the breaking of the NH···N bond on addition of the second proton. If this bond were not formed, ΔH_2 for DACODA would be *ca.* −36 kJ mol^{-1} and log K_2 *ca.* 8.5, comparable to log K_2 for propanediamine (log K_2 = 8.74) and for propane-1,3-diamine tetraacetate (log K_2 = 8.02).

The thermodynamic parameters are therefore in good agreement with the conformations of the protonated ligands suggested by the molecular mechanics study, and provide an adequate explanation for the apparently anomalous values of the protonation constants of the three *N*-acetate diaza cyclic complexones.

A final comment is perhaps in order regarding the physical reality of the postulated NH···N and NH···$^-{}_2$OC– hdyrogen bonds in the protonated forms of the ligands. As happens in other cases, the actual existence of these bonds can only be considered as demonstrated when independent evidence is provided, since the changes in the thermodynamic functions may alternatively be interpreted in terms of effects on the hydration shells of the charged species involved, on solvent exclusion and reforming around the protonated ligands, *etc.* This is a well known question in studies of acid–base behaviour in water and its discussion is not relevant for the present problem.[23,24]

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