Nuclear Magnetic Resonance Studies of the Protonation Sequence of Cyclic Tetra-azatetra-acetic Acids

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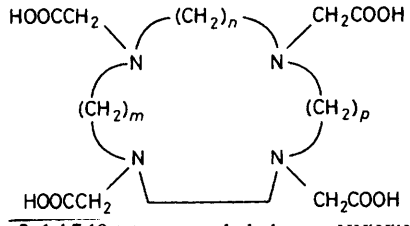
The protonation sequence of a series of cyclic tetra-azatetra-acetic acids (cDOTA, cTRITA, cTETA, and cPENTA) was studied by means of n.m.r. titration spectroscopy. The methods previously used for non-cyclic polyaminocarboxylates and for the most symmetrical members of the series, cDOTA and cTETA, did not prove adequate for the entire series. The shielding parameters C_N and $C_{N'}$ are different for each type of proton in the tetra-aza ring and acetate groups and vary with the pH of the medium. This behaviour can be attributed to changes in the conformation equilibria with the protonation of the various basic centres, in which electrostatic repulsions and hydrogen-bond formation play a role, and to the more restricted flexibility of these compounds compared to their non-cyclic analogues. C_N and $C_{N'}$, were estimated as functions of the probabilities of protonation of the carboxylate groups and used as such to derive protonation sequences of the cyclic polyaminocarboxylates by solving systems of linear equations relating chemical shifts to pH and to the number of equivalents of added acid. The results confirm that two nitrogens are protonated first in all four compounds and no protonation of the carboxylate groups occurs; further acidification results in preferential protonation of these groups from cDOTA to cTETA but not for cPENTA, which preferentially accepts a third proton in one nitrogen. cDOTA and cTRITA do not protonate further in their nitrogens but such a tendency can be observed in cTETA and clearly in cPENTA which can be fully protonated in their nitrogens and carboxylates. The sequence of protonation in this case lends support to the formation of hydrogen bonds between protonated nitrogens and non-protonated carboxylates.

The quantitative determination of the protonation sequence of polyaminocarboxylic acids provides important information for the study of complex equilibria of polybasic ligands and metal ions at variable pH. N.m.r. methods have been extensively used for this purpose, particularly in the case of linear polyaminocarboxylates with 2—6 nitrogen atoms and it has been shown that these atoms are all successively protonated before any of the carboxylate groups.[1–4] The study has recently been extended to some cyclic polyaminocarboxylates, which are of current interest due to their strong complexing properties and possible higher degree of selectivity for metals of the same family. Two such compounds, 1,4,7,10-tetra-azacyclododecane-*NN'N''N'''*-tetra-acetic acid and 1,4,8,11-tetra-azacyclotetradecane-*NN'N''N'''*-tetra-acetic acid, referred to respectively by cDOTA and cTETA, have been studied by Desreux *et al.*[5] who have found that electrostatic repulsion does not allow more than two protonated nitrogen atoms in the tetra-aza ring; the subsequent protons must be attached to the carboxylate groups, hence these two ligands are the first examples of complexones with nitrogen atoms which are effectively less basic than the carboxylates.

In our work on the properties of cyclic polyaminocarboxylates[6] these and two further members of the series have been synthesized: 1,4,7,10-tetra-azacyclotridecane-*NN'N''N'''*-tetra-acetic acid (cTRITA) and 1,4,8,12-tetra-azacyclopentadecane-*NN'N''N'''*-tetra-acetic acid (cPENTA). It seemed of interest to extend the study to the entire set of compounds to enable a comparison with the results obtained by previous authors and an appraisal of the underlying assumptions on which the application of the n.m.r. techniques to this purpose are based.



$m = n = p = 2$, 1,4,7,10-tetra-azacyclododecane-*NN'N''N'''*-tetra-acetic acid (cDOTA); $n = 3$, $m = p = 2$, 1,4,7,10-tetra-azacyclotridecane-*NN'N''N'''*-tetra-acetic acid (cTRITA); $n = 2$, $m = p = 3$, 1,4,8,11-tetra-azacyclotetradecane-*NN'N''N'''*-tetra-acetic acid (cTETA); $m = n = p = 3$, 1,4,8,12-tetra-azacyclopentadecane-*NN'N''N'''*-tetra-acetic acid (cPENTA)

Experimental

Reagents.—All cyclic polyaminocarboxylates (cDOTA, cTRITA, cTETA, and cPENTA) were synthesized in our laboratory.[6] For comparison we have also prepared the tetramethylated cyclic amine corresponding to cPENTA, *i.e.* N–Me_4[15]aneN_4, by refluxing the commercial non-methylated product (Strem Chemicals) with formic acid and formaldehyde for 24 h.[7] The pure product was obtained by vacuum distillation using a Kügelrohr distillator apparatus (80 °C at 0.05 mmHg). It is a viscous substance which does not crystallize.

All the other chemicals used in this work were of reagent grade quality.

Equipment.—Potentiometric measurements were made by using a Radiometer pHM4 instrument fitted with a Radiometer G202B glass electrode and a K401 saturated calomel electrode as reference. Titrations were carried out in a thermostatted cell at 25.0 ± 0.1 °C and the ionic strength of the solutions was kept at 0.10M with KNO_3 or $(Me)_4NNO_3$ (in the case of cDOTA which forms complexes with K^+).

Fourier-transform ^1H n.m.r. spectra were recorded at 100 MHz and probe temperature in a JEOL JNM 100 PTF spectrometer coupled to a JEOL 980A computer (cDOTA and cTETA). For cTRITA, cPENTA, and N–Me_4[15]aneN_4, the ^1H n.m.r.

Table 1. Protonation constants of cyclic tetra-azatetra-acetic acids and of the amine N-Me_4[15]aneN_4. *T* 25.0 ± 0.1 °C; μ 0.10M(KNO_3 or $Me_4N{\cdot}NO_3$)

	cDOTA[a]	cTRITA[a]	cTETA[a]	cPENTA[b]	N-Me_4[15]aneN_4[b]
log K_1	12.09 ± 0.04	11.35 ± 0.05	10.682 ± 0.005	10.896 ± 0.009	9.62 ± 0.01
log K_2	9.680 ± 0.001	9.734 ± 0.002	10.136 ± 0.002	9.651 ± 0.009	8.541 ± 0.003
log K_3	4.548 ± 0.003	4.157 ± 0.006	4.091 ± 0.004	5.52 ± 0.02	5.54 ± 0.02
log K_4	4.130 ± 0.003	3.323 ± 0.004	3.347 ± 0.004	3.52 ± 0.02	2.815 ± 0.004
log K_5	1.88 ± 0.06[c]		2.17 ± 0.02[c]	2.18 ± 0.05	
log K_6	1.71 ± 0.07[c]		1.42 ± 0.06[c]		

[a] Ref. 6. [b] Present work. [c] Ref. 5.

spectra were recorded at 300 MHz and at probe temperature in a Bruker CXP 300 spectrometer coupled to an ASPECT 2000 computer. In all cases, sodium 3-(trimethylsilyl)propanesulphonate (TMS*) was used as internal reference. Solutions of the cyclic complexones of concentration 10mM (except in the case of cTETA for which 5 mM solutions were used due to solubility restrictions) were prepared in D_2O and the pD* (operational pD, since the pH meter was standardized with conventional buffers) was adjusted by adding DCl or KOD (CO_2 free). The final pD* was determined using a pH M63 Digital Radiometer instrument fitted with a combined Ingold 405 M3 microelectrode and calibrated with two buffers at pH 4 and 7.

Calculations.—The protonation constants for N-Me_4[15]aneN_4 and cPENTA were obtained as described for the other compounds in a previous paper[6] using the Miniquad Program and a IBM 360 computer.

In the general approach to the problem using single C_N, $C_{N'}$, and C_{COO^-} values, the protonation fractions were obtained from the experimental data (n.m.r. titrations) using a multiple linear regression program developed by one of us (J. R. A.).

In the other cases, the resulting systems of linear equations were solved by standard procedures.

Results and Discussion

The protonation constants obtained for the amine N-Me_4[15]aneN_4 and for cPENTA are presented in Table 1 together with the corresponding values for the other ligands used in the present work reported in a previous paper.[6] Both the amine and cPENTA differ from the previous set in having higher values of log K_3; furthermore, it was possible to obtain for cPENTA a fifth protonation constant $-\log K_5$.

Being a measure of the 'macroscopic' basicity of the polybasic ligands, these constants by themselves do not indicate the sequence of protonation, which is necessary for the correct interpretation of the complexation reactions at the microscopic level.

This can be achieved by n.m.r. techniques, following the chemical shifts of the methylene protons of the ligands as a function of pD*. Protonation increases the deshielding effect of the basic groups and the frequencies of absorption of the methylene protons are shifted downfield.

Based on the results obtained with a large number of linear polyaminocarboxylates Sudmeier and Reilley[1] demonstrated an approximate relationship between the shift of the *i* proton of a methylene group and the probability of protonation f_j of the various neighbouring basic groups *j* (average time of protonation of the *j* basic groups). The contributions of these groups are considered to be additive and the relationship has the form $\Delta\delta_i = \sum_{j=1}^{N} C_{ij} f_j$. The proportionality constants C_{ij} represent the chemical shift δ_i of the methylene proton *i* caused by full protonation of a given basic centre relative to the value obtained when this centre is entirely deprotonated. Their values are determined by various factors, such as the type of basic centre considered, the distance to the methylene group and eventual changes in conformation of the compound when the pH is varied.

For linear polyaminocarboxylates, Sudmeier and Reilley used the following set of 'shielding constants' C_{ij}: $-CH_2-COO^-$ 0.20 p.p.m. (C_{COO^-}); $-CH_2-NR_2$ 0.75 p.p.m. (C_N); $-CH_2-CH_2-NR_2$ 0.35 p.p.m. ($C_{N'}$). However, Desreux *et al.*[5] found that these values of C_N and $C_{N'}$ were not adequate for cyclic polyaminocarboxylates and determined two new sets of constants using as model compounds the tetramethylated amines N-Me_4[12]aneN_4 and N-Me_4[14]aneN_4, one set valid for values of pD < 4 and the other for values of pD > 5: pD < 4, C_N 1.01 ± 0.02 p.p.m., $C_{N'}$ 0.26 ± 0.01 p.p.m., pD > 5, C_N 0.79 ± 0.03 p.p.m., $C_{N'}$ 0.24 ± 0.02 p.p.m.

The reason for the differences (mainly in the value of C_N at pD < 4) was attributed by these authors to changes in conformation of the tetra-aza rings to minimize repulsions when the nitrogen atoms are protonated.

When the values obtained by Desreux *et al.* were used with our own data (Figures 1—5) the results summarized in Table 2 were obtained by multiple linear regression analysis (only for cTRITA and cPENTA)*.

Various anomalies are apparent in Table 2: very large limits of error for f_{COO^-} with several negative values, irregular trends, and unlikely results, *e.g.* values of f_j well over 100%.

Since Desreux' values were obtained using the methylated amines as model compounds, the reason for the discrepancies might be the inadequacy of these models for our less symmetric ligands. A study was then carried out using the tetramethylated amine N-Me_4[15]aneN_4, which is more closely related to our ligands and allows a more detailed analysis of the effects of protonation since it has several methylene protons in different conditions.

Before discussing the results obtained we will briefly describe the n.m.r. spectra of the different compounds studied in the present work and the assignment of the different absorptions in each case.

N.m.r. Spectra.—N-Me_4[15]aneN_4. The n.m.r. spectrum of this amine at pD* 6.0 and the titration curves δ (p.p.m.) of the various peaks as a function of pD* are shown in Figure 1.

The identification of the peaks is straightforward taking into account the area ratio and the pattern of each absorption. Hence, the protons of the methyl groups, *a* and *b*, give two

* The relation between the values of f_j and the number of moles of acid added per mole of polyaminocarboxylate is $\sum_{j=1}^{n} \alpha_j f_j = n$, where α_j is the number of equivalent sites of protonation of type *j*.

Table 2. Probability of protonation of nitrogen atoms and carboxylate groups in complexones cTRITA and cPENTA (using the values of C_N and $C_{N'}$ obtained in ref. 5).

	cTRITA				cPENTA			
n	$f_{N(1)}$	$f_{N(2)}$	$f_{COO(3)^-}$	$f_{COO(4)^-}$	$f_{N(1)}$	$f_{N(2)}$	$f_{COO(3)^-}$	$f_{COO(4)^-}$
1	25 ± 4	47 ± 5	−38 ± 25	17 ± 25	10 ± 6	24 ± 6	3 ± 34	14 ± 33
2	45 ± 6	71 ± 7	−49 ± 36	33 ± 36	46 ± 6	57 ± 6	43 ± 35	−46 ± 35
3	41 ± 4	58 ± 4	−13 ± 29	64 ± 29	39 ± 9	78 ± 10	−31 ± 67	66 ± 67
4	45 ± 3	59 ± 4	11 ± 24	85 ± 24	40 ± 9	81 ± 10	−22 ± 65	101 ± 65
5	48 ± 3	61 ± 3	34 ± 23	108 ± 23	45 ± 7	86 ± 8	5 ± 52	115 ± 51
6	52 ± 4	72 ± 4	57 ± 29	120 ± 29	47 ± 7	87 ± 7	37 ± 51	130 ± 51
7					61 ± 7	92 ± 7	71 ± 48	126 ± 48
8					101 ± 6	103 ± 7	111 ± 46	85 ± 45

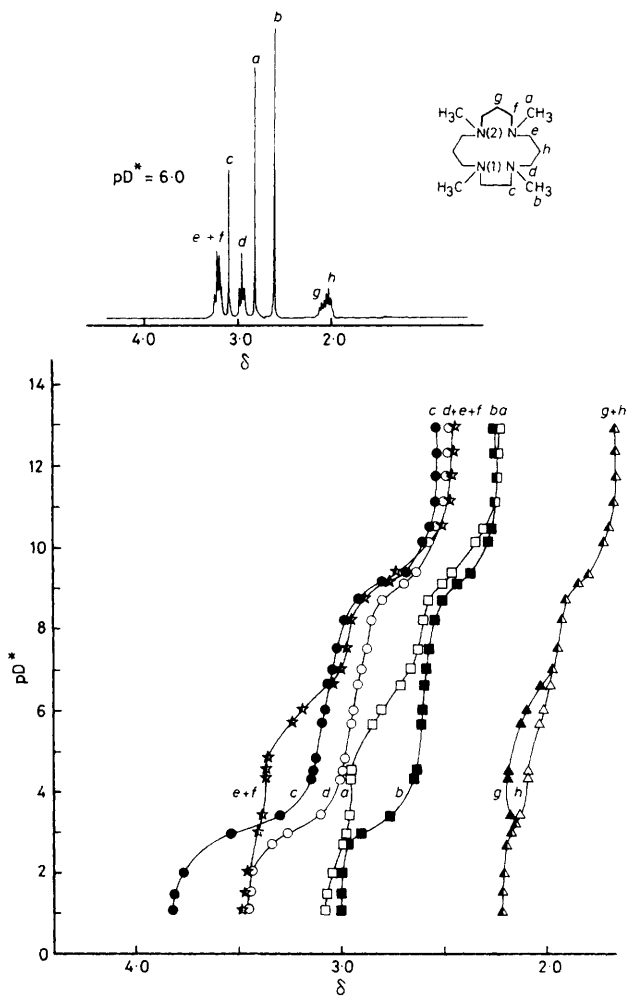


Figure 1. ^{1}H N.m.r. spectrum of N-Me$_4$[15]aneN$_4$ at pD* 6.0 and the titration curves δ as a function of pD*

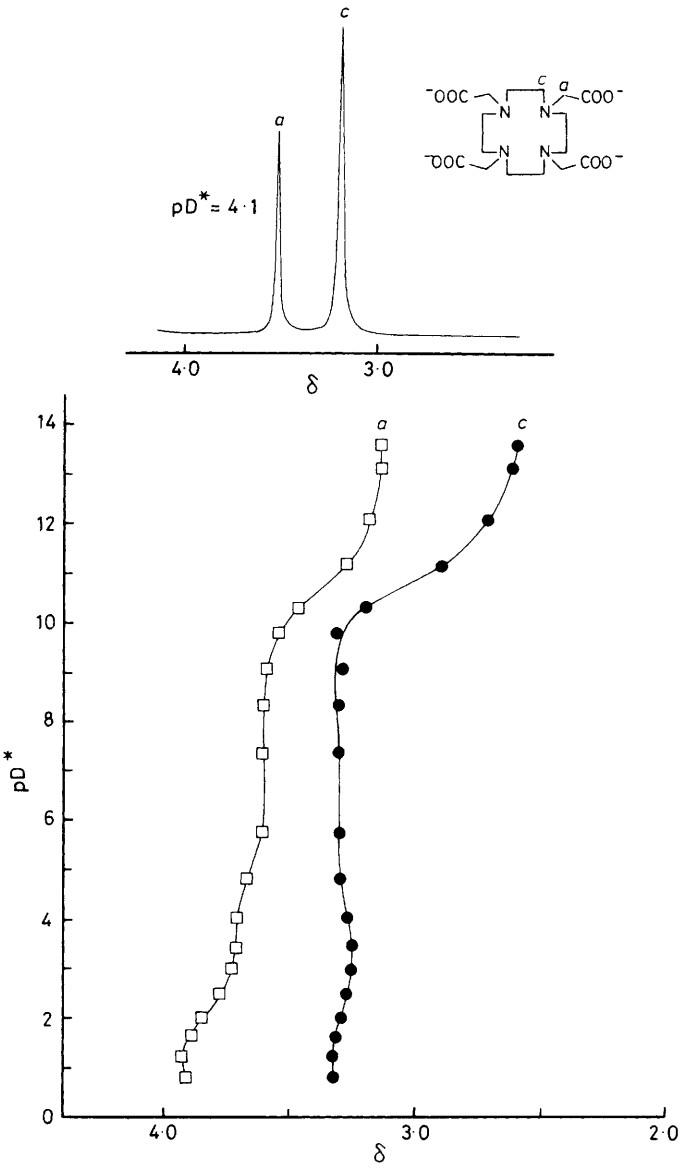


Figure 2. ^{1}H N.m.r. spectrum of cDOTA at pD* 4.1 and the titration curves δ as a function of pD*

intense singlets and the same happens with the methylene proton *c* due to the symmetry of the molecule.

The methylene protons *d*—*f* give three triplets, two of them superimposed at lower field, corresponding to protons *e* and *f*. The assignment of the triplet corresponding to proton *d*, at higher field, was made by double-resonance experiments at pD* 4.3. Finally, protons *g* and *h* give two quintuplets at high field, with an area ratio of 1:2, which are superimposed for values of pD* out of the range 3.2—7.0.

cDOTA *and* cTETA. The n.m.r. spectra of cDOTA and cTETA at pD* 4.1 and 6.9 (and their respective titration curves) are shown in Figures 2 and 3. The high symmetry of these molecules gives rise to simple spectra; for cDOTA (Figure 2)

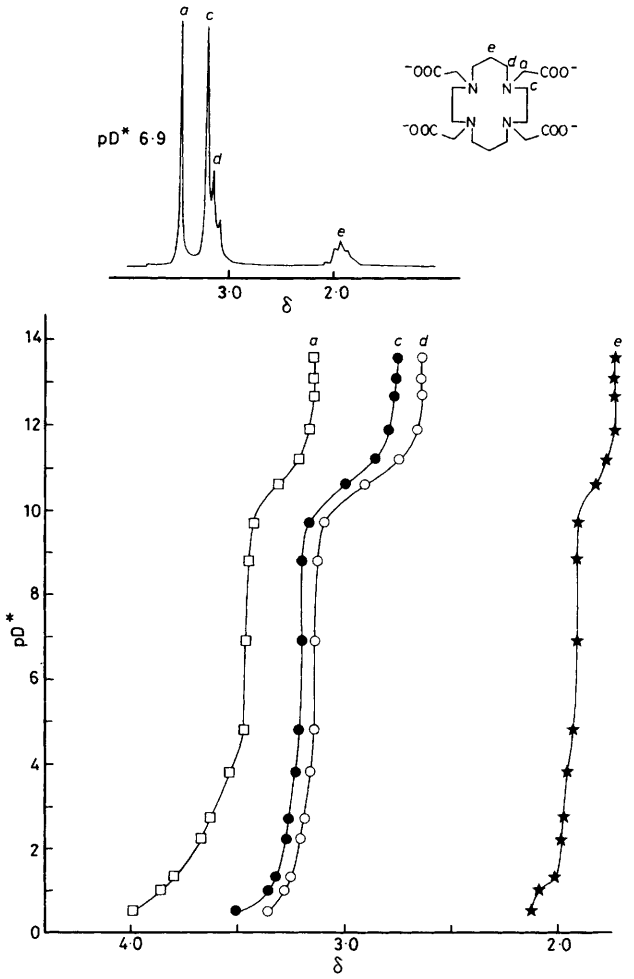


Figure 3. ^{1}H N.m.r. spectrum of cTETA at pD* 6.9 and the titration curves δ as a function of pD*

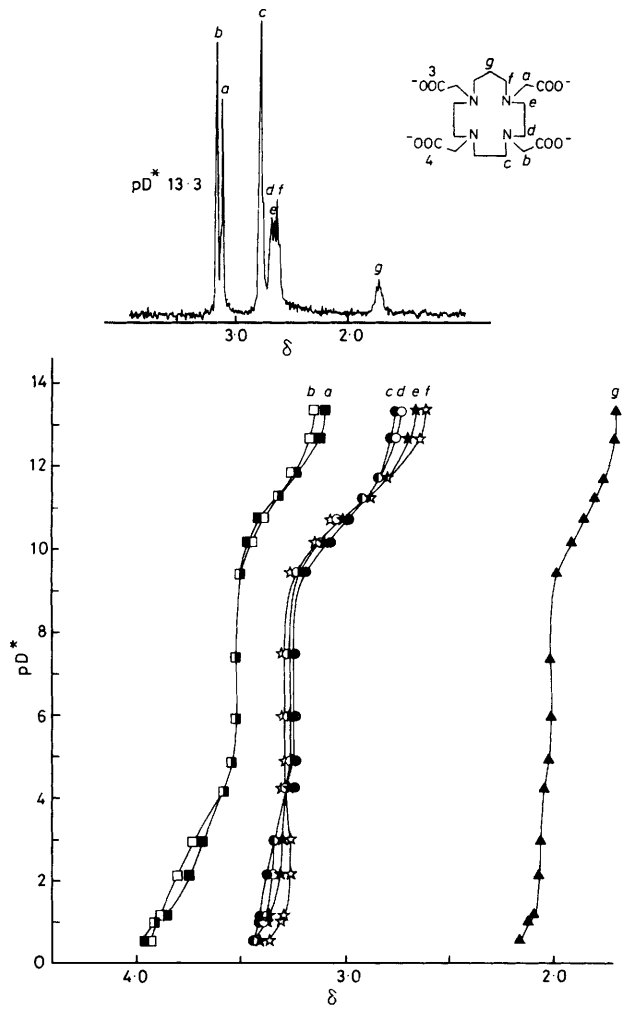


Figure 4. ^{1}H N.m.r. spectrum of cTRITA at pD* 13.3 and the titration curves δ as a function of pD*

only two singlets are seen at the entire range of pD*, with an area ratio of 1:2, that at lower field corresponding to the methylene protons of the acetate groups; the spectrum of cTETA has four peaks, a singlet at lower field corresponding to the eight methylene protons of the acetate groups *a*, a poorly resolved quintuplet at higher field corresponding to the four methylene protons *e*, an intense singlet corresponding to the eight methylene protons *c*, and a triplet, sometimes partially superimposed with the singlet *c*, corresponding to the eight methylene protons *d*.

cTRITA *and* cPENTA. The lower symmetry of these complexones originates spectra with a higher number of resonance peaks. The spectrum of cTRITA (at 300 MHz and pD* 13.3) (Figure 4) shows a poorly resolved quintuplet at high field (δ 1.70), corresponding to the *g* methylene protons, a triplet at δ 2.61, due to the coupling of the *f* and *g* methylene protons, a singlet at δ 2.76 due to the *c* methylene protons and an A_2B_2 pattern corresponding to the methylene protons *d* and *e* (the *d* protons should occur at lower field, δ −2.73, than the *e* protons, δ −2.66, due to the increasing trend, at this pD*, of the chemical shifts of CH_2 groups as one goes from the larger side of the tetra-aza ring to the narrower side). Finally, there are two singlets at δ 3.15 and 3.10 corresponding to the methylene protons of the acetate groups, which were assigned to protons *b* and *a* by observing the nuclear Overhauser effect (NOE) in these peaks by irradiation at the resonance frequence of the *c* and *d* protons at pD* 13.3. When these resonances are saturated, the increase of intensity of the peak at δ 3.15 is much more pronounced than the increase of intensity of the peak at δ 3.10. Since the NOE depends on the inverse of the sixth power of the average distance between the proton whose resonance has been saturated and the proton whose resonance is affected, the peak at δ 3.15 must be due to the *b* protons and the peak at δ 3.10 to the *a* protons.

The spectrum of cPENTA at pD* 1.6 (and the titration curves of the different peaks) are shown in Figure 5. These titrations curves are analogous to those of the parent amine, particularly at high pD*, and this helps the assignment of the different resonances. Hence, two poorly defined quintuplets appear at higher field and that of smaller area corresponds to the methylene protons *g* and that of greater area to the methylene protons *h*; the triplet at δ 3.17 corresponds to the methylene protons *d*, the singlet at δ 3.29 to the methylene protons *c*, and the pattern at δ 3.48 to the triplets resulting from the methylene protons *e* and *f*. The assignment of the peaks corresponding to the methylene protons of the acetate groups was made, as in the case of cTRITA, by irradiating the triplet of protons *d* at δ 3.17 (pD* 1.6). The NOE was obtained only for the peak of the acetate groups at δ 3.80 which must correspond to the methylene protons *b*, and by exclusion the peak at δ 4.02 corresponds to the methylene protons *a*.

Table 3. Average values of C_N and $C_{N'}$ calculated from the titration curves of the model amine N–Me_4[15]aneN_4

Number of equiv. of acid added	pD* range used	Assumptions	Calc. C_N	Calc. $C_{N'}$
4	<1.5	$f_{N(1)} = f_{N(2)} = 1$	0.98 ± 0.01	0.27 ± 0.01
3	3.8—4.3	$f_{N(1)} = 0.5; f_{N(2)} = 1$	0.88 ± 0.08	0.28 ± 0.02
2	7.5—8.2	$f_{N(1)} = f_{N(2)} = 0.5$	0.86 ± 0.17	0.26 ± 0.01

Table 4. Probabilities of protonation of nitrogen atoms and carboxylate groups in complexones cDOTA and cTETA (using the values of C_N and $C_{N'}$ obtained from the study of the amine N–Me_4[15]aneN_4 as 'model compound' and C_{COO^-} 0.20 p.p.m.).

	cDOTA		cTETA	
n	f_N	f_{COO^-}	f_N	f_{COO^-}
1	25 ± 5	0 ± 6	25 ± 3	0 ± 3
2	55 ± 3	0 ± 3	39 ± 4	11 ± 5
3	54 ± 2	21 ± 2	43 ± 4	32 ± 4
4	53 ± 1	47 ± 1	46 ± 4	54 ± 4
5	59 ± 1	66 ± 2	45 ± 6	80 ± 6
6	61 ± 1	89 ± 1	70 ± 3	81 ± 3

Titration Curves.—The n.m.r. titration curves of the amine N–Me_4[15]aneN_4 shows the effect of the successive protonation of the various basic centres of the molecule; the first inflexion at high pD* (8.5—10.5) corresponds to that obtained in the potentiometric titration of this compound and is analogous to that observed by Desreux *et al.*[5] for the amines N–Me_4[12]aneN_4 and N–Me_4[14]aneN_4. All the methylene protons exhibit the same behaviour, hence it corresponds to the simultaneous protonation of two nitrogen atoms of the macrocyclic, certainly in opposite positions of the ring to minimize electrostatic repulsions.

Since the protons *g* and *h* have the same chemical shift in this range of pD*, both N(1) and N(2) atoms (see Figure 1) have equal probability of being protonated.

The second inflexion of the curve occurs at lower pD* (5.0—7.0) and it can be observed in protons *e*—*g* and also in the protons of the methyl group *a*; it must then correspond to the total protonation of the N(2), since no significative change in the resonance frequence of these protons occurs below pD* 4.

At pD* 2.5—3.5, a third inflexion can be seen in the n.m.r. titration curves of N–Me_4[15]aneN_4, for protons *c*, *d*, and *h* and those of the methyl group *b*; it must then correspond to the protonation of N(1).

It is therefore possible, in this amine, to protonate all four nitrogen atoms, and it is to be expected that the conformation of the molecule is altered to accommodate the four positive charges and minimize the necessarily higher electrostatic repulsions between the protonated nitrogens.

When one applies Sudmeier and Reilley's method of calculation to our results with the 'model' amine N–Me_4[15]aneN_4, variable values for C_N but different from those of Desreux *et al.* are obtained in the various pD* ranges (see Table 3).

As it can be seen from Table 3, the values calculated for $C_{N'}$ are practically constant over the entire range of pD* and are only slightly higher than those of Desreux *et al.* The values of C_N decrease with the increase of pD* but are closer than those calculated by the previous workers, hence a single value may well be used over the entire range as if no change in conformation does take place or doesn't affect, appreciably at least, the shielding constants.

We have therefore selected the values C_N 0.91 and $C_{N'}$ 0.27 p.p.m. and adopted Sudmeier and Reilley's value of C_{COO^-} 0.20 p.p.m. for the estimation of the protonation probabilities f_j of the cyclic complexones cDOTA, cTRITA, cTETA, and cPENTA (Tables 4 and 5).

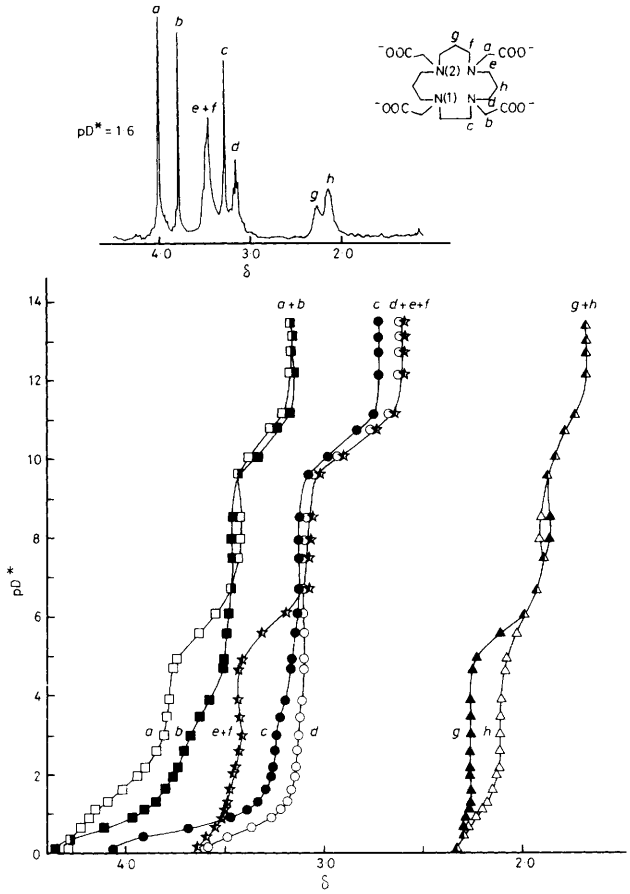


Figure 5. ^{1}H N.m.r. spectrum of cPENTA at pD* 1.6 and the titration curves δ as a function of pD*

As can be seen from Tables 4 and 5, the results obtained for cDOTA and cTETA compare with those obtained by Desreux *et al.* with errors of the same order of magnitude, showing that there is no need to consider two sets of shielding constants. It can be seen that, in both cases, two nitrogen atoms of the ring are protonated before any of the carboxylates, *i.e.* the two remaining non-protonated nitrogens are less basic than the carboxylates, as found by the previous authors.

For cTRITA and cPENTA, however, the results are difficult to interpret and one is confronted with discrepancies identical with those found when Desreux's values are used. Hence, none of the sets of shielding constants is satisfactory for these cases, *i.e.* the cyclic amines are not sufficiently good models for their derived polyaminocarboxylic acids.

To get a deeper insight into the reasons for the discrepancies

Table 5. Probabilities of protonation of nitrogen atoms and carboxylate groups in complexones cTRITA and cPENTA (using the values of C_N and $C_{N'}$ obtained from the study of the amine N–Me_4[15]aneN_4 as 'model compound' and C_{COO^-} 0.20 p.p.m.).

	cTRITA				cPENTA			
n	$f_{N(1)}$	$f_{N(2)}$	$f_{COO(3)^-}$	$f_{COO(4)^-}$	$f_{N(1)}$	$f_{N(2)}$	$f_{COO(3)^-}$	$f_{COO(4)^-}$
1	21 ± 3	40 ± 3	−33 ± 20	23 ± 20	9 ± 3	21 ± 3	−12 ± 3	32 ± 22
2	37 ± 5	60 ± 5	−40 ± 35	43 ± 34	38 ± 4	48 ± 4	51 ± 27	−38 ± 27
3	43 ± 4	62 ± 4	−16 ± 25	61 ± 25	39 ± 7	83 ± 7	−34 ± 48	62 ± 48
4	47 ± 3	63 ± 3	8 ± 20	82 ± 20	43 ± 7	87 ± 7	−26 ± 46	96 ± 46
5	50 ± 3	65 ± 3	31 ± 20	105 ± 20	48 ± 5	91 ± 5	0 ± 33	110 ± 33
6	54 ± 4	76 ± 4	53 ± 24	117 ± 24	50 ± 5	93 ± 5	33 ± 33	125 ± 33
7					65 ± 4	98 ± 4	66 ± 28	121 ± 28
8					108 ± 3	110 ± 3	102 ± 22	80 ± 22

Table 6. Values of C_N and $C_{N'}$ calculated for various cyclic polyaminocarboxylic acids at $n = 2$ ($f_{N(1)} = f_{N(2)}$ 0.5 and f_{COO^-} 0) and at low pD* (in parentheses) for cPENTA ($n = 8$)

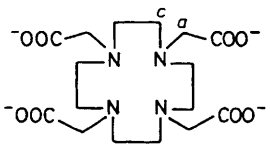
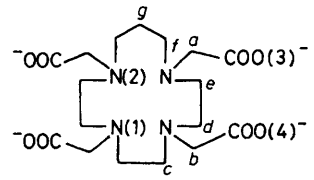
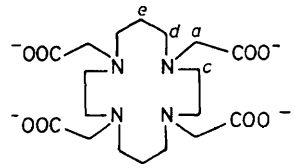
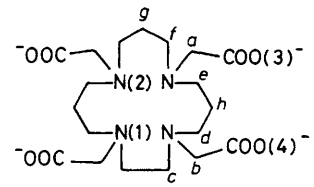
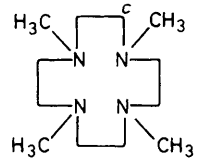
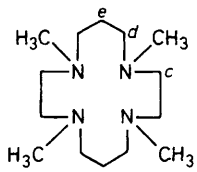
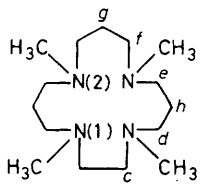
Molecule	Proton type	C_N	$C_{N'}$
	*a*	0.92	
	c	0.92	
	*a*	0.82	
	b	0.73	
	c	0.73	0.26
	d	0.73	0.34
	e	0.82	0.40
	f	1.37	
	g		0.30
	*a*	0.62	
	c	0.62	0.26
	d	1.00	
	e		0.18
	*a*	0.60 (0.86)	
	b	0.60 (0.93)	
	c	0.60 (0.99)	0.20 (0.32)
	d	0.96 (0.97)	
	e	0.96 (1.0)	
	f	0.96 (1.0)	
	g		0.20 (0.32)
	h		0.20 (0.32)

Table 7. Values of C_N and $C_{N'}$ calculated for various cyclic tetramethylpolyamines at $n = 2$ ($f_{N(1)} = f_{N(2)}$ 0.5 and f_{COO^-} 0) and at low pD* (in parentheses)

Molecule	Proton type	$C_N + C_{N'}$	C_N	$C_{N'}$
 †	*c*	1.02 (1.22)		
 †	*c*	0.90 (1.23)	0.66 (0.97)*	
	d		0.88 (1.06)	
	e			0.24 (0.26)
 ‡	*c*	0.90 (1.26)	0.64 (0.99)*	
	d		0.80 (0.98)	
	e		1.00 (0.98)	
	f		1.00 (0.98)	
	g			0.26 (0.27)
	h			0.26 (0.27)

* Calculated from $C_N + C_{N'}$ and $C_{N'}$. † Estimated from ref. 5. ‡ Present work.

found in the protonation sequency we have used the four complexones to calculate shielding constants in well defined protonation stages, such as that corresponding to $n = 2$, when the two protons have equal residence times in two opposite nitrogen atoms of the tetra-aza ring ($f_{N(1)} = f_{N(2)} = 0.5$ and $f_{COO^-} = 0$) and at low pD*, when no more inflexions are observed, and final protonation situations may reasonably be inferred from the values of *n* (Table 6).

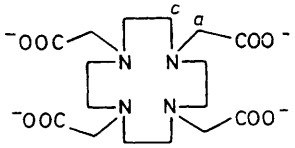
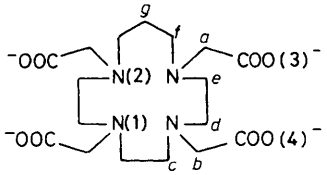
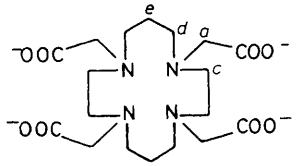
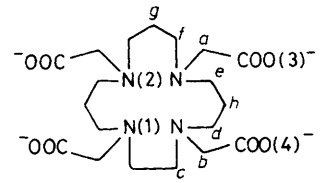
For comparison, the same was done for the parent methylated amines N–Me_4[12]aneN_4, N–Me_4[14]aneN_4, and N–Me_4[15]aneN_4 (Table 7).

As can be seen from Tables 6 and 7, the calculated C_N and $C_{N'}$ values depend on the type of proton considered but some trends are apparent for both the acids and the amines. (a) There is a general decrease in the shielding constants when the size (and flexibility) of the tetra-aza ring increases, but the differences become smaller for analogous protons in the larger rings. (b) The value of C_N for the neighbouring methylene groups is the same for the acetate group and for the ethane chain, but it is considerably higher for the methylene groups of the propane chains. (c) For the amines, the values of C_N calculated at low pD* are close to 1.00 in all cases and for all protons.

One can rationalize these observations in terms of the effects that the increase in size of the macrocyclic ring has on the flexibility of the corresponding molecule and on the likelihood that those effects are reflected on the conformation equilibria in solution. Although little is known on these equilibria for tetra-azatetra-acetic acids, there are considerable data on other tetra-aza macrocyclic ligands which show that the number of stereochemical arrangements of the donor atoms increases with the increase in size of the ring.[8]

Still other effects are obviously operative, deriving from the protonation of the nitrogens and carboxylates and from the probable occurrence of hydrogen bonding between protonated nitrogens and carboxylates and between protonated carboxylates and non-protonated nitrogens. All these effects may slow down the rates of nitrogen inversion and may also force the

Table 8. Values of C_N and $C_{N'}$ as functions of f_{COO^-} for the various types of protons in the cyclic tetra-azatetra-acetic acids

Ligand	Type of proton	C_N	$C_{N'}$
	*a*	$0.92 + 0.3\,f_{COO^-}$	
	c	$0.92 + 0.1\,f_{COO^-}$	$0.48 + 0.1\,f_{COO^-}$
	*a*	$0.82 + 0.3\,f_{COO(3)^-}$	
	b	$0.73 + 0.3\,f_{COO(4)^-}$	
	c	$0.73 + 0.1\,f_{COO(4)^-}$	$0.26 + 0.1\,f_{COO(4)^-}$
	d	$0.73 + 0.1\,f_{COO(4)^-}$	$0.34 + 0.1\,f_{COO(3)^-}$
	e	$0.82 + 0.1\,f_{COO(3)^-}$*	$0.40 + 0.1\,f_{COO(4)^-}$*
	f	$1.37 + 0.05\,f_{COO(3)^-}$	
	g		$0.30 + 0.05\,f_{COO(3)^-}$*
	*a,b*	$0.62 + 0.3\,f_{COO^-}$	
	c	$0.62 + 0.1\,f_{COO^-}$	$0.26 + 0.1\,f_{COO^-}$
	d	$1.00 + 0.05\,f_{COO^-}$	
	e		$0.18 + 0.05\,f_{COO^-}$
	*a*	$0.60 + 0.3\,f_{COO(3)^-}$	
	b	$0.60 + 0.3\,f_{COO(4)^-}$	
	c	$0.60 + 0.1\,f_{COO(4)^-}$	$0.20 + 0.15\,f_{N(1)} + 0.1\,f_{COO(4)^-}$
	d	$0.96 + 0.05\,f_{COO(4)^-}$	
	e,f	$0.96 + 0.05\,f_{COO(3)^-}$	
	g		$0.20 + 0.15\,f_{N(2)} + 0.05\,f_{COO(3)^-}$
	h		$0.20 + 0.15\,f_{N(1)} + 0.05\,f_{COO(4)^-}$

* Only after $n = 5$.

tetra-aza ring to adopt a non-planar arrangement to minimize electrostatic repulsions between protonated nitrogens.

The overall results will differ from ligand to ligand, particularly when the protonation sequences are diverse, and cannot be anticipated from what occurs in the parent amines and even less in the case of the more flexible non-cyclic polyaminocarboxylate ligands. Furthermore, since the shielding constants are not identical for all protons in each ligand, no single average values of C_N and $C_{N'}$ can be used to derive the sequences of protonation unless discrepant values are excluded (such as those of the methylene groups of propane chains).

On the other hand, one cannot have a unique set of shielding parameters for the entire range of the titration curves since C_N and, to a less extent, $C_{N'}$, change with the values of pD*, but it may be possible to express both C_N and $C_{N'}$ as functions of pD*. Indeed, by comparing values obtained at the two definite protonation situations, taking N–Me_4[15]aneN_4 and cPENTA as model compounds, approximate relationships can be established between C_N or $C_{N'}$, and the probability of protonation of the carboxylates, which is itself a function of pD* and is also related to the degree of hydrogen-bond formation above $n = 2$. This corresponds to admitting that, *e.g.* for C_N, one can have: $C_N(\text{acid}) = C_N(\text{basic}) + \alpha f_{COO^-}$.

The protons most affected are those of the acetate groups, for which α *ca.* 0.3, and the least affected are the methylene protons of propane chains next to the nitrogens, for which α *ca.* 0.05; all the others are affected to approximately the same extent with α *ca.* 0.1.*

The values of $C_{N'}$ for the *c*, *g*, and *h* protons of cPENTA seem to depend on the probability of protonation of the nitrogens and this can be taken into consideration by adding a further term βf_N with an estimated β *ca.* 0.15.

These equations can be generalized to the other macrocycles and Table 8 summarizes the results obtained.

Although the procedure may be considered redundant to a certain extent (since two stages of protonation had to be considered to obtain C_N and $C_{N'}$ as functions of f_{COO^-} in the model compounds), these functions may be used to estimate the entire sequence of protonation of the four ligands by solving systems of linear equations relating chemical shifts to the number of equivalents of added acid and pD*. The results obtained are shown in Tables 9 and 10.

Table 9. Probability of protonation of nitrogen atoms and carboxylate groups in complexones cDOTA and cTETA (using the values of C_N and $C_{N'}$ of Table 8 and C_{COO^-} 0.20 p.p.m.)

	cDOTA		cTETA	
n	f_N	f_{COO^-}	f_N	f_{COO^-}
1	25 ± 4		25 ± 7	
2	50 ± 1		50 ± 1	
3	45 ± 2	30 ± 5	50 ± 2	25 ± 3
4	48 ± 5	52 ± 6	50 ± 3	50 ± 5
5	50 ± 6	75 ± 2	50 ± 4	75 ± 6
6	50 ± 5	100 ± 10	56 ± 3	94 ± 4

* For cTRITA and cPENTA we have used C_{COO^-} 0.24 p.p.m.[2] instead of C_{COO^-} 0.20, proposed by Sudmeier and Reilley,[1] since the first value was found to give better agreement with our experimental data for these asymmetric compounds, namely a constant value for α.

Table 10. Probability of protonation of nitrogen atoms and carboxylate groups in complexones cTRITA and cPENTA (using the values of C_N and $C_{N'}$ of Table 8 and C_{COO^-} 0.24 p.p.m.)

	cTRITA				cPENTA			
n	$f_{N(1)}$	$f_{N(2)}$	$f_{COO(3)^-}$	$f_{COO(4)^-}$	$f_{N(1)}$	$f_{N(2)}$	$f_{COO(3)^-}$	$f_{COO(4)^-}$
1	24 ± 5	26 ± 6			23 ± 2	27 ± 4		
2	50 ± 1	50 ± 1			50 ± 1	50 ± 1		
3	49 ± 1	50 ± 2	23 ± 3	28 ± 2	50 ± 1	82 ± 3	15 ± 4	3 ± 2
4	48 ± 3	50 ± 5	45 ± 3	57 ± 3	50 ± 1	81 ± 2	25 ± 2	44 ± 1
5	49 ± 3	50 ± 2	66 ± 5	85 ± 4	52 ± 1	82 ± 3	52 ± 3	64 ± 3
6	50 ± 3	53 ± 4	97 ± 3	100 ± 3	56 ± 1	83 ± 2	81 ± 4	80 ± 2
7					67 ± 5	86 ± 3	100 ± 5	97 ± 4
8					100 ± 10	100 ± 3	100 ± 7	100 ± 5

It is interesting to see that the protonation probabilities are now coherent, the deviations small, and the trends easily interpreted.

Hence, it is found for all the ligands that two nitrogen atoms are protonated first and no protonation of the acetate groups occurs; further acidification results in preferential protonation of the carboxylate groups from cDOTA to cTETA but not for cPENTA, which accepts preferentially a third proton on N(2) although the carboxylate groups also begin to be protonated.

For $n = 6$, two nitrogens and all four carboxylate groups of cDOTA, cTRITA, and cTETA are protonated; a slight tendency for further protonation of the nitrogen can be observed in cTETA and is clear in cPENTA which seems to be practically protonated in its four nitrogens and carboxylates for $n = 8$ (actually, values below 100% should be obtained since the compounds will be partially ionized even at the low pD* considered, but this is the result of the method of derivation of the C_N and $C_{N'}$, functions in which this stage was considered as one of the references).

Nitrogens N(1) and N(2) appear to behave similarly in cTRITA, but N(2) is clearly preferred in cPENTA; the same seems to happen with the carboxylate group (4) at $n = 4$ and 5 for the two compounds; this is in satisfactory agreement with the postulated formation of hydrogen bonds between protonated nitrogens and non-protonated carboxylates or *vice versa*, implying that in this asymmetrical molecule protonation of these groups should take place in opposite sites, as is indeed found.

Conclusions.—The determination of the protonation sequences of tetra-azatetra-acetates and analogous ligands by Sudmeier and Reilley's method [1] is complicated by the unusual conformation behaviour of these cyclic compounds, when compared with their linear polyaminocarboxylate analogues. Indeed, the shielding constants C_N and $C_{N'}$ of the methylene protons of the tetra-aza ring and of the acetate groups exhibit a continuous increase with pD* and marked differences for each type of proton in their respective molecules.

This behaviour can be attributed to changes in the conformation equilibria with protonation of the various groups (in which electrostatic repulsive and hydrogen-bond formation play a role) and to the restricted flexibility of these ligands compared with the related non-cyclic compounds.

For these reasons it is not adequate to use single values of C_N and $C_{N'}$ for each macrocycle and less still a unique set for the entire series of ligands. The use of two sets, one for the basic and another for the acid zones, still leads to several discrepancies which can be attenuated by using empirical relations of C_N and $C_{N'}$ with the probabilities of protonation of the carboxylate groups, accounting for both the changes with increasing acidity and the formation of hydrogen bonds.

The restrictions decrease as the tetra-aza ring of the ligands increases in size, thus approaching the conditions of the non-cyclic polyaminocarboxylates extensively studied by various authors.

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Received 11*th July* 1984; *Paper* 4/1208