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Nuclear Magnetic Resonance and Potentiometric Studies of the Protonation Scheme of Two Tetraaza Tetraacetic Macrocycles

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The protonation constants of the macrocyclic ligands 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA) and 1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid (TETA) have been measured by potentiometry with the help of a hydrogen electrode. The extent of protonation of the various amino and carboxylate groups of DOTA and TETA has been determined in D_2O as a function of pD from the chemical shifts of the peaks exhibited by nonlabile protons. Shielding constants which account for the effect of the protonation of a given group on the chemical shift of adjacent methylenic moities were determined in an investigation of the NMR spectra of tetramethylated tetraaza macrocyles selected as model compounds. The results indicate that two amino groups of DOTA or TETA are protonated at high pD values. When the acidity is increased, protons are attached successively on the four carboxylate groups, thus leaving essentially unprotonated two amino groups in the internal cavity of the macrocycles. DOTA and TETA are the first polyaminopolycarboxylic acids featuring nitrogen atoms which are less basic than carboxylate groups.

Introduction

Interest in the complexation properties of macrocycles has been stimulated by reports of the remarkable ability of these ligands to selectivity bind certain cations.[1] Recently, new types of macrocycles featuring ionizable functions such as β-diketonate[2] or carboxylate groups[3,4] have been synthesized. It is hoped that more selective complexation will be achieved with these new ligands because of the combined effects of their internal cavity and of their pH-selective coordination groups. For instance, macrocyclic 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid, **1** (see structure in Figure 1), and 1,4,8,11-tetraazacyclotetradecane-*N,N',N'',N'''*-tetraacetic acid, **2** (see structure in Figure 2), designated respectively as DOTA and TETA, are complexones which exhibit several properties worthy of note. DOTA forms the most stable complexes known to date with calcium[3] and with the trivalent lanthanides.[5] These lanthanide complexes were shown by NMR spectroscopy to have unexpected conformational properties.[6] Also, TETA is a very strong complexing agent of strontium.[3]

The research work proposed herein was designed to determine the protonation constants and the protonation scheme of DOTA and TETA. Several NMR investigations[7-9] led to the inference that the sequence of protonation is the same for the anions of all noncyclic polyaminopolyacetic acids containing from two to six nitrogen atoms: the amino groups are successively protonated before a proton is attached on any of the carboxylate groups. A recent X-ray determination of the solid-state structure of ethylenediaminetetraacetic acid (EDTA)[10] entirely corroborates these spectroscopic findings. In the present paper, it is demonstrated by NMR that the tetrabasic anions of DOTA and TETA undergo protonation according to a different scheme. To help delineate the NMR spectra, we also conducted studies with the N-methylated tetraaza macrocycles **3** (see structure in Figure 3) and **4** (see structure in Figure 4). The protonation shifts exhibited by these cyclic amines were used for a quantitative assessment of the proton distribution at the various basic sites of DOTA and TETA.

Experimental Section

Reagents. 1,4,7,10-Tetraazacyclododecane tetrakis(hydrochloride) was synthesized according to the method of Atkins and co-workers.[11] The pure amine as obtained by dissolving the tetrakis(hydrochloride) in water and treating it with an excess of an aqueous solution of tetramethylammonium hydroxide. Water was eliminated by stirring with refluxing benzene and collecting the azeotrope in a Dean–Stark trap. After filtration, benzene was stripped off on a rotatory evaporator, and the remaining yellowish solid was sublimed under vacuum. 1,4,8,11-Tetraazacyclotetradecane was obtained by the template synthesis reported by Barefield et al.[12] Macrocycles **3** and **4** were then obtained by methylation with a mixture of formic acid and formaldehyde.[13]

The complexones DOTA and TETA were prepared as described elsewhere.[6] After the compounds were dried under vacuum, the purity was checked by pH titration. All other chemicals used in this research work were of reagent grade purity.

Potentiometric Measurements. The potentiometric titrations were carried out in a sealed vessel thermostated at 25 ± 0.01 or 80 ± 0.1 °C. The hydrogen ion activity was measured by using a digital Tekelec Airtronic TE 370 voltmeter fitted with a Ag/AgCl reference electrode and a hydrogen electrode. It is estimated that the error in the pH measurements does not exceed 0.001 pH unit. The titrations were performed by generating hydroxyl ions with a constant current coulometer. The apparatus used in this investigation is described elsewhere in detail.[14] The ionic strength was adjusted to 1.00 by the addition of NaCl. Typical concentrations of the CO_2-free solutions of the macrocycles were 0.01 M in DOTA and 0.005 M in the less soluble TETA. The protonation constants were computed with a least-squares best-fit computer program.[14].

Spectral Measurements. Fourier transform proton NMR spectra were recorded at 90 MHz and at probe temperature on a Brüker HFX-90 spectrometer equipped with a deuterium lock. All shifts were referenced either to *tert*-butyl alcohol or to sodium 3-(trimethylsilyl)propanesulfonate (TMS*). Resonance peaks were measured with a precision better than 0.02 ppm. The spectral data discussed below are all reported with respect to TMS*.

Solutions of the macrocycles (approximately 0.02 M) for NMR measurements were prepared in D_2O from the requisite amounts of

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(11) Atkins, T. J.; Richman, J. E.; Oettle, W. F. *Org. Synth.* **1978**, *58*, 86.
(12) Barefield, E. K.; Wagner, F.; Herlinger, A. W.; Dahl, A. R. *Inorg. Synth.* **1976**, *16*, 220.
(13) Barefield, E. K.; Wagner, F. *Inorg. Chem.* **1973**, *12*, 2435.
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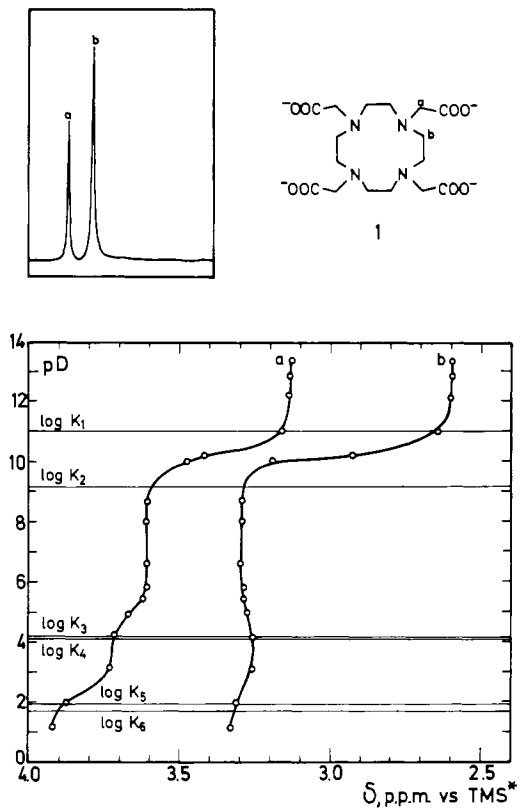


Figure 1. Proton NMR spectrum of DOTA at pD 10.16 and pD dependence of the chemical shifts of the peaks exhibited by this macrocycle.

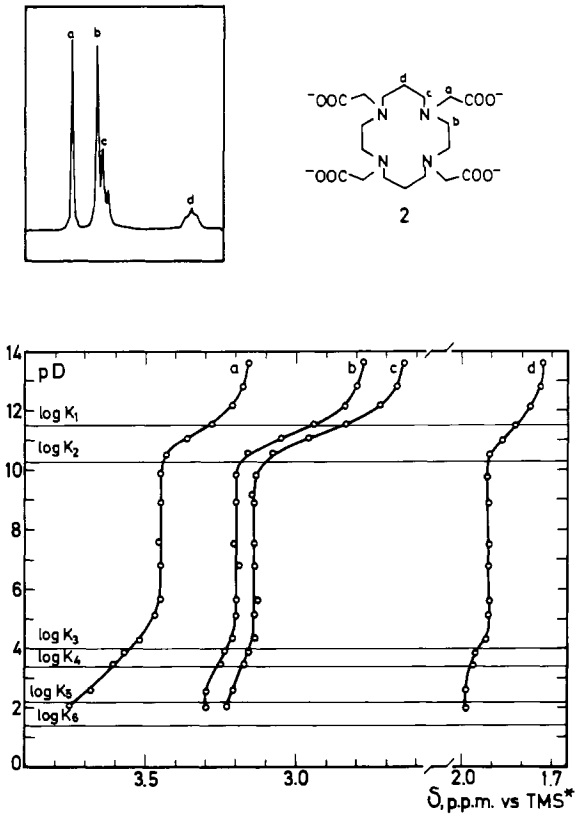


Figure 2. Proton NMR spectrum of TETA at pD 3.44 and pD dependence of the chemical shifts of the peaks exhibited by this macrocycle.

ligands under a nitrogen atmosphere. The pD was adjusted by the addition of DCl or CO_2-free KOD. The final pH was determined with a Radiometer Model 26 pH meter equipped with a combination

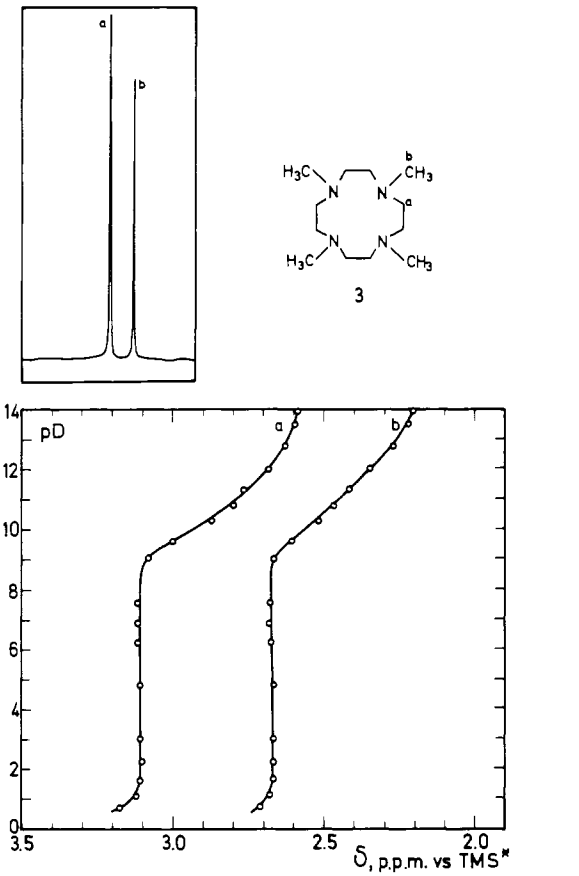


Figure 3. Proton NMR spectrum of **3** at pD 11.32 and pD dependence of the chemical shifts of the peaks exhibited by this macrocycle.

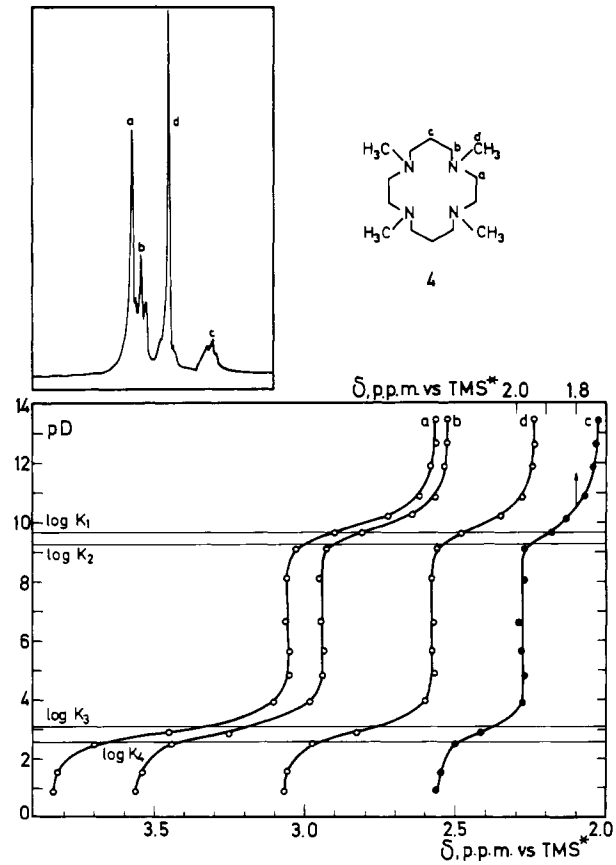


Figure 4. Proton NMR spectrum of **4** at pD 3.94 and pD dependence of the chemical shifts of the peaks exhibited by this macrocycle. The filled points are reported with respect to the upper δ scale.

Table I. Protonation Constants of the Macrocyclic Complexones DOTA and TETA and of the Corresponding Macrocyclic Amines 5 and 6

	DOTA (25 °C)[a]	DOTA (80 °C)[a]	DOTA (20 °C)[b]	5 (25 °C)	TETA (25 °C)[a]	TETA (80 °C)[a]	TETA (20 °C)[b]	6 (25 °C)
$\log K_1$	11.08 ± 0.07[d]	9.95 ± 0.04	11.36	10.7	11.56 ± 0.04	10.11 ± 0.06	11.07	11.58
$\log K_2$	9.23 ± 0.02	8.28 ± 0.07	9.73	9.7	10.18 ± 0.02	9.50 ± 0.32	9.75	10.62
$\log K_3$	4.24 ± 0.02	4.22 ± 0.03	4.54	1.73	4.05 ± 0.02	4.02 ± 0.02	4.31	1.61
$\log K_4$	4.18 ± 0.03	3.65 ± 0.02	4.41	0.94	3.38 ± 0.04	3.29 ± 0.04	3.46	2.41
$\log K_5$	1.88 ± 0.06	2.22 ± 0.03			2.17 ± 0.02	1.90 ± 0.15		
$\log K_6$	1.71 ± 0.07	1.30 ± 0.08			1.42 ± 0.06			

[a] Present work (1 M NaCl). [b] Taken from ref 16 (0.1 M KCl). [c] Data are taken from: Kodama, M.; Kimura, E. *Yuki Gosei Kagaku Kyokaishi* 1977, *35*, 632 (as cited in ref 17). [d] The errors given are the mean deviations of the protonation constants obtained from titrations carried out with samples originating from two different syntheses. [e] Taken from ref 16.

microelectrode. The pD was calculated as pH + 0.4.[15] Since the solubility of TETA is around 6×10^{-3} mol/L, no solutions with a pD lower than 1.5 were accessible to experiment.

Results and Discussion

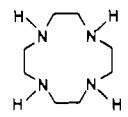
Ligand Protonation Constants. Potassium salts are often used to maintain a constant ionic strength during potentiometric titrations because of the small tendency of the cation K^+ to form metal complexes with a variety of ligands. This proved inconvenient in the present case because the tetraprotonated form of DOTA exhibits the unusual property of forming an insoluble complex with many potassium salts.[5] The ionic strength of the solutions investigated by potentiometry was therefore adjusted to 1.00 by the addition of sodium chloride. A correction for the complexation of sodium by DOTA and TETA was performed by assuming that the fully basic form of these ligands is the only species in solution which is able to complex sodium. The calculated values of the protonated constants are listed in Table I. The constants K_n refer to the equilibrium

$$H_{n-1}L + H^+ \rightleftharpoons H_nL \quad (1)$$

$$K_n = \frac{[H_nL]}{[H^+][H_{n-1}L]} \quad (2)$$

where L denotes a ligand and where the charges of the various protonated species were omitted. The hydrogen ion activities were converted into concentrations by taking into account the appropriate activity coefficients. As it appears from Table I, the protonation constants K_n of both ligands are grouped in three pairs of similar values. These constants are compared with the corresponding values reported by Stetter and Frank.[3] The available data compare reasonably well taking into account that Stetter and Frank's values were obtained at another ionic strength and at another temperature. Also included in Table I are the protonation constants log K_n obtained at 80 °C. These new data are necessary for an interpretation of the complexation of lanthanide ions, as will be reported elsewhere.[5] Nearly all protonation constants decrease when the temperature is increased. No value of log K_6 could be determined in the case of the ligand TETA at 80 °C, presumably because of the small value of this constant at elevated temperature. The stability constants of the sodium complexes as obtained by the least-squares treatment of the pH curves were found to be log $K_{Na} = 2.52 \pm 0.07$ in the case of DOTA and log $K_{Na} = 1.64 \pm 0.02$ in the case of the TETA at 25 °C. It is noteworthy that the hydrogen electrode used in the present work allows a reliable and accurate determination of the proton activity over an extended pH range.

In several papers,[7-9] including one recently published,[8] it is demonstrated that the protonation of linear polyaminopolyacetic ligands proceeds by the successive addition of protons on all the nitrogen atoms while the carboxylate groups are negligibly protonated. Besides the NMR data which are in favor of this hypothesis, it has been pointed out[8] that a fairly good correlation exists between the protonation constants of polyaminopolyacetic acids and the corresponding polyamines. This correlation has been put forward in support of the high basicity of the amino groups of ethylenediaminetetraacetic acid (EDTA) and of its higher analogues. In Table I, the protonation constants of DOTA and TETA are compared with the corresponding constants reported for the macrocyclic polyamines 1,4,7,10-tetraazacyclododecane, **5**, and 1,4,8,11-tetraazacyclotetradecane, **6**. There is an obvious correlation



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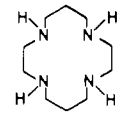

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between the two highest protonation constants of DOTA or TETA and of macrocycles **5** and **6**, respectively. However, this is not at all the case for log K_3 and log K_4, these constants being markedly higher for the polycarboxylic acids than for the polyamines. It thus seems that the protonation scheme of DOTA and TETA could differ from that of their classical noncyclic analogues such as EDTA.

NMR Studies. Several authors[7-9] reported that the protonation of a basic site leads to a deshielding of the adjacent methylene groups and thus to a shift toward low fields in the ^1H NMR spectrum. Sudmeier and Reilley[7] have measured the protonation shift exhibited by a wide variety of compounds. These authors calculated mean protonation shifts or shielding constants, designated here as C_i, for the italicized methylene groups in the moieties CH_2COO^- (C_O = 0.20 ppm), CH_2NR_2 (C_N = 0.75 ppm), and $CH_2CH_2NR_2$ ($C_{N'}$ = 0.35 ppm). If it is assumed that contributions from the protonation of N basic sites located in the vicinity of a methylene group are additive, then the chemical shift δ of the NMR peak of that methylene group is related to the parameters C_i by

$$\Delta\delta = \sum_{i=1}^{N} C_i f_i \quad (3)$$

where f_i is the fraction of time during which the ith basic site is protonated. The factors f_i are often expressed as percentages of protonation. Furthermore, if n equivalents of acid have been added to a ligand featuring N basic sites, it is obvious that

$$\sum_{i=1}^{N} \alpha_i f_i = n \quad (4)$$

where α_i is the number of equivalent sites of type i. The combination of eq 4 with m equations such as 3 obtained for the m NMR peaks exhibited by the ligand leads to a set of simultaneous linear equations which is overdetermined and which can be solved for the percent protonation f_i by standard least-squares techniques provided the shielding constants are known. In the present study, the shielding constant C_O of a methylene moiety adjacent to a carboxylate group was set equal to 0.20 ppm as suggested by Sudmeier and Reilley.[7]

(15) Mikkelsen, K.; Nielsen, S. O. *J. Phys. Chem.* **1960**, *64*, 632.

These authors reported that the additivity rule which is implied in eq 3 holds better if modified constants C_N and $C_{N'}$ are relied on in the interpretation of the NMR spectra of linear polyamines such as triethylenetetramine. Indeed, these amines, when protonated can adopt one or several preferred spatial arrangements of reduced coulombic repulsion energy which exhibit shielding constants different from those of simpler monofunctional compounds. It is thus likely that new parameters C_N and $C_{N'}$ should be used in the assessment of the protonation sites of the cyclic DOTA and TETA. These parameters are determined below in the investigation of the protonation of macrocycles **3** and **4** which are considered as models for the analysis of NMR spectra of the corresponding polycarboxylic compounds.

The equilibrium constants listed in Table I were used in the calculation of the percent protonation f_i. This could lead to small inconsistencies because the NMR measurements were carried out at a different temperature (around 30 °C), in D_2O and with no special attempt to control the ionic strength.

Tetraaza Macrocycles 3 and 4. The proton NMR spectrum of macrocyclic 1,4,7,10-tetramethyl-1,4,7,10-tetraazacyclododecane, **3**, is reproduced in Figure 3. The two singlets with areas in approximately a 4:3 ratio are ascribed respectively to the methylene groups a and the methyl groups b. Figure 3 also shows a plot of the chemical shift values of the two NMR peaks as a function of pD. For both hydrogens a and b, two breaks appear in these curves: one of them is observed around pD 9 while the other starts at very acidic pDs. The protonation constants of macrocycle **3** are unknown, but by analogy to the data available[16] in the case of macrocycle **4**, the break at basic pD values can be attributed to the formation of the cation H_2L^{2+}. Furthermore, as no protonation shifts are observed between pD 9 and pD 1, it is concluded that the fully protonated form of compound **3**, H_4L^{4+}, exists only in very acidic media.

No detailed analysis of the protonation shifts of the methyl groups was undertaken because their shielding constant is of no use in the interpretation of the NMR spectra of DOTA and TETA. The shift toward low fields of the methylene peak a arises from the combined effects of the protonation of nitrogen atoms one bond (C_N) and two bonds ($C_{N'}$) away. From the position of the methylene peak at pD 14 and in the extended pD range where the chemical shifts are constant, only the sum of the shielding constants $C_N + C_{N'}$ can be deduced. Indeed, the small number of NMR peaks exhibited by ligand **3** precludes the possibility of computing each parameter separately. The value of $C_N + C_{N'}$ is estimated to be 1.02 ppm. No shielding constants can be obtained for the tetraprotonated form of **3** since, even at pD 0.5, only a small fraction of this species appears to be formed.

More information can be inferred from the protonation shifts of the macrocycle 1,4,8,11-tetramethyl-1,4,8,11-tetraazacyclotetradecane, **4**. Figure 4 shows the structural formula of **4**, a representative proton spectrum, and the dependence of the chemical shifts of the various NMR peaks upon pD. The assignment of the peaks from both their relative areas and their splitting pattern is straightforward. The methylene groups a appear as a singlet because of the symmetry of the molecule while the methylene groups b occur as a triplet partially overlapping peak a. The poorly resolved quintuplet is due to hydrogens c, and the intense singlet is of course attributed to the methyl groups d.

Each of the two breaks in the δ vs. pD curves can be ascribed to the nearly simultaneous protonation of two amino groups of macrocycle **4**. The protonation constants log K_n indicated in Figure 4 were reported by Micheloni et al.[16] (log K_1 = 9.70, log K_2 = 9.31, log K_3 = 3.09, log K_4 = 2.63). As three methylene NMR peaks occur in the spectrum, the value of the two shielding constants C_N and $C_{N'}$ can be assessed by a least-squares treatment. From the data obtained at pD 14 (unprotonated form) and between pD 5 and 8 (diprotonated form), it is concluded that the values of C_N and $C_{N'}$ are 0.79 ± 0.03 and 0.24 ± 0.02 ppm, respectively. The sum of these shielding constants is identical with the value determined for macrocycle **3** within the limits of the experimental error. The constant C_N agrees favorably with the value deduced by Sudmeier and Reilley[7] from the study of a large number of noncyclic model compounds. On the other hand, the shielding constant $C_{N'}$ is lower than the value previously reported by these authors. A similiar least-squares treatment of the NMR data obtained at low acidity yields new shielding constants C_N and $C_{N'}$ of 1.01 ± 0.02 and 0.26 ± 0.01 ppm, respectively. The effect of the protonation of an amino group on the NMR shift of an adjacent methylene moiety is thus larger in the tetraprotonated form H_4L^{4+} of the cyclic polyamine **4** than in the diprotonated form H_2L^{2+}. This difference may arise from the conformational behavior of **4** which is probably affected by the number of protonated nitrogen atoms. Small cycles indeed have unusual conformational properties,[6,18] and it seems reasonable to anticipate that these properties will be altered to various degrees by the protonation of amino groups. The observation that the band width of the methylene peaks of **3** and **4** varies with pD lends support to this hypothesis. In the following discussion, the values of the parameter C_N will be designated as "low" or "high" according to the fact that they have been computed from the protonation shifts of the diprotonated or the tetraprotonated forms of **3** and **4**.

Table II. Percent Protonation of the Amino Groups f_N and of the Carboxylate Groups f_O of the Macrocycles DOTA and TETA

n	f_N	f_O
	DOTA	
1	28 ± 16	−6 ± 12
2	51 ± 10	3 ± 5
4	52 ± 2	48 ± 2
6	58 ± 7	92 ± 6
	TETA	
1	27 ± 1	−2 ± 2
2	45 ± 6	5 ± 6
3	41 ± 5	34 ± 4
4	46 ± 5	54 ± 4
5	48 ± 6	77 ± 4

DOTA. A typical spectrum of the polyaminopolycarboxylic acid DOTA is presented in Figure 1. The peaks are easily assigned by relying on their respective areas. The calculation based on eq 3 and 4 were performed with protonation shifts measured for a number n of protons added to the ligand equal to 1, 2, 4, and 6 but not for n equal to 3 or 5 because of the close proximity of the values of log K_3 and log K_4 or of log K_5 and log K_6. When the parameter n is 1 or 2, the low value of the shielding constant C_N as determined above for the diprotonated amines **3** and **4** was used in the calculations. When n is greater than 2, the protonation percentages were obtained by introducing in eq 3 the high value of C_N deduced from the study of the tetraprotonated amines. The protonation percentages of the nitrogen atoms f_N and of the carboxylic oxygen atoms f_O are listed in Table II. The first two equivalents of acid added to ligand **1** become attached to two amino groups located probably as far apart as possible in order

(16) Micheloni, M.; Sabatini, A.; Paoletti, P. *J. Chem. Soc., Perkin Trans. 2* **1978**, 828.

(17) Leugger, A. P.; Hertli, L.; Kaden, T. A. *Helv. Chim. Acta* **1978**, *61*, 229.

(18) Anet, F. A. L.; Anet, R. In "Dynamic Nuclear Magnetic Resonance Spectroscopy"; Jackman, L. M., Cotton, F. A., Eds.; Academic Press: New York, 1975; p 543 ff and references cited therein.

to reduce the electrostatic repulsion. The addition of 4 or 6 equiv of acid leads to the protonation of two or four carboxylate groups, two nitrogen atoms remaining virtually unprotonated. One could argue that, in this case, the low value of the shielding constant C_N, i.e., the value obtained in the investigation of the diprotonated macrocycles **3** and **4**, should be used in the computations at low pDs rather than the high value of C_N. This would yield the parameters $f_N = 65 \pm 2$ and $f_O = 34 \pm 2$ for $n = 4$ and $f_N = 74 \pm 4$ and $f_O = 76 \pm 4$ for $n = 6$. The situation is then paradoxical in that, if three amino groups are protonated when $n = 6$, the high value of C_N should be selected for the calculations, which is precisely what is done in Table II. A mean value of the shielding constant C_N yields the parameters $f_N = 57 \pm 1$ and $f_O = 42 \pm 1$ for $n = 4$ as well as $f_N = 65 \pm 2$ and $f_O = 85 \pm 2$ for $n = 6$. As a matter of fact, the lack of protonation of two nitrogen atoms is readily seen by simply examining the curves reproduced in Figure 1: the protonation shift of the methylene groups b is nearly constant between pD 9 and pD 1 while the acetate peak a continuously moves downfield when pD decreases. It is thus concluded that, within the limits of the errors encountered in the mathematical treatment, the first two equivalents of protons prefer to be situated on two nitrogen atoms and that the following equivalents of protons are populating the four carboxylate groups, thus leaving two nitrogen atoms negligibly protonated. This scheme is observed for the first time: in the case of EDTA and its higher analogues, all amino groups protonate more readily than the carboxylate groups.[7-9].

Around pD 4, the peak attributed to the methylene protons b is slightly shifted upfield. Letkeman and Martell[8] recently reported the same phenomenon for EDTA although at much lower pH. According to these authors, the protonated carboxylate groups in the H_4L form of EDTA are free to spin in the immediate vicinity of the ethylene backbone of the ligand and can introduce a small positive shielding because of the magnetic anisotropy of their carbonyl moiety. A similar effect could occur in the case of DOTA since the upfield shift of hydrogens b is observed precisely when it is postulated that the ligand has two protonated carboxylic functions.

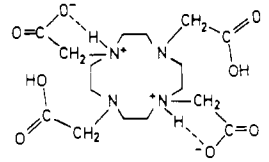
TETA. The complexone TETA and macrocycle **4** have the same structure except for the acetate groups which replace the methyl groups. Hence the NMR spectrum of TETA is identical with that of **4** except for the singlet at low fields which is assigned to the methylene groups a (see Figure 2). The protonation percentages were computed as described above for n varying from 1 to 5. Because of the small difference between log K_3 and log K_4 or between log K_5 and log K_6, the concentration of the species H_3L^- or H_5L^+ never exceed 60% of the total amount of ligand. Some caution must thus be advised in accepting the parameters f_i determined for $n = 3$ and $n = 5$. The protonation percentages of TETA are presented in Table II. As in the case of DOTA, virtually complete protonation of two nitrogen atoms takes place at high pDs. When the acidity is increased, protons are attached successively on the carboxylate groups. Of course, one is also confronted here with the problem of selecting the appropriate shielding constant C_N. If the low value of C_N is used in the calculations, the protonation percentages become $f_N = 51 \pm 6$ and $f_O = 24 \pm 5$ for $n = 3$, $f_N = 57 \pm 6$ and $f_O = 43 \pm 5$ for $n = 4$, and $f_N = 59 \pm 7$ and $f_O = 65 \pm 6$ for $n = 5$. With the limitations in the computation procedure taken into account, it is thus concluded that two nitrogen atoms belonging to the internal cavity of macrocycle TETA are essentially unprotonated in acidic media.

Concluding Remarks

An aspect of particular interest in the present NMR investigation of the macrocycles DOTA and TETA is that these compounds represent the first examples for polyaminopolycarboxylic acids which contain nitrogen atoms less basic than carboxylate groups.

It has recently been reported[16,17] that tetraaza macrocycles such as **3–6** form fully protonated species only at low pH values (see Table I). The small tendency to protonate of the nitrogen atoms has been attributed by Kaden et al.[17] to the electrical repulsion between the positive charges of the NH groups. This is contrasted with the report of Micheloni et al.,[16] who consider that the low values of log K_3 and log K_4 of polyamine **6** are due to the formation of hydrogen bonds between protonated and unprotonated nitrogen atoms in the macrocycle. However, a crystallographic analysis[19] of the dihydroperchlorate of **6** does not permit conclusions to be drawn as to whether such intramolecular hydrogen bonds are really present. Hydrogen bonding between trimethylamine and the trimethylammonium ion has been observed in acetonitrile,[20] but this association is weak and very sensitive to steric effects. It is likely that hydrogen bonding is only of minor importance in the case of DOTA and TETA. On the other hand, electrostatic repulsion between the protonated amino groups is probably the main factor which could account for the behavior of these complexones. The influence of electrostatic repulsion has already been noted in the case of diethylenetriaminepentaacetic acid (DTPA).[7,8] The first equivalent of acid is attached to the central nitrogen atom of this compound. When a second equivalent of acid is added, the central nitrogen atom loses its positive charge while the two terminal nitrogen atoms are protonated, thus minimizing the electrostatic repulsion energy. Such effects are most probably prevailing for macrocyclic compounds because the charged groups are maintained close to each other in the internal cavity of these ligands.

A better insight into the acidic properties of DOTA and TETA is now feasible. The constants log K_1 and log K_2 are relative to the protonation of two nitrogen atoms located trans to each other. The two following constants log K_3 and log K_4 are related to the protonation of the two carboxylate moieties which are not adjacent to a nitrogen atom bearing a proton. Because there is no charge in the vicinity of these carboxylate groups, their protonation constants are near to the one of acetic acid (log $K_1 = 4.8$) and are markedly higher than in the case of EDTA (log $K_3 = 2.60$, log $K_4 = 2.00$). The neutral form H_4L of DOTA is thus believed to have structure **7**. The



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hydrogen bonds between the carboxylate and the NH^+ groups are indicated only tentatively, but they are in keeping with the X-ray structure analysis of EDTA.[10] Finally, the constants log K_5 and log K_6 are associated with the protonation of the two remaining carboxylate groups. Because these groups are attached to protonated nitrogen atoms, they exhibit a fairly low basicity, and hence, their protonation constants are comparable to those of EDTA.

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