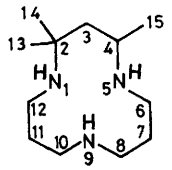
^{13}C Nuclear Magnetic Resonance Study of the Protonation of 2,2,4-Trimethyl-1,5,9-triazacyclododecane

Rajumati Bhula and David C. Weatherburn*
Chemistry Department, Victoria University of Wellington, P.O. Box 600, Wellington, New Zealand

^{13}C N.m.r. spectroscopy has been used to investigate the protonation of 2,2,4-trimethyl-1,5,9-triazacyclododecane. The chemical shifts which occur upon protonation are interpreted as indicating that each protonation step results in the protonation of only one nitrogen atom, and that the sequence is probably N-5, N-9, and then N-1. There is some evidence indicating that a hydrogen bond is formed between N-1 and N-9 during the second protonation step. Chemical shifts observed are due to protonation of the nitrogen atom and to conformational changes within the molecule. The significance of these results for the determination of the protonation sequence of aliphatic polyamines is discussed.

N.m.r. spectroscopy has been used to investigate the protonation behaviour of polyamines and amino carboxylates. Aliphatic diamines,[1–8] triamines,[7–14] tetra-amines,[7,8,12,14] penta-amines,[14] macrocyclic amines,[15–17] and amino carboxylates[16,18–20] have been investigated. *N*-(3-Aminopropyl)butane-1,4-diamine (spermidine) has been the subject of a number of studies and the results obtained have been interpreted differently by different groups. The earliest study[9] by ^{13}C n.m.r. concluded that the monoprotonated form of the amine had the primary nitrogen atoms (N-1 and -10) protonated 50% of the time and that protonation of the secondary amino group (N-5) did not occur until protonation of the primary amino groups was complete. Kimberly and Goldstein[10] concluded that in the monoprotonated form, N-10 was protonated 43% of the time, and each of the other two nitrogen atoms was protonated 32% of the time. It was also suggested that in the diprotonated form N-1, N-10, and N-5 are protonated 67, 86, and 42% of the time, respectively. From both ^{13}C and two-dimensional proton–carbon n.m.r. data Bunce and his co-workers[11–13] concluded that at the monoprotonation stage the fractions of the species protonated at N-10, N-5, and N-1 were 0.4, 0.35, and 0.22, respectively, in reasonable agreement with the results of Kimberly and Goldstein. Takeda *et al.*,[14] using both ^{15}N and ^{13}C n.m.r. data, concluded that the protonation sequence of spermidine is N-10, then N-1, and then N-5.

Reasons for the different conclusions reached in the various investigations have been discussed.[13] One of the major difficulties with studies of most polyamines and amino carboxylates is that, over the pH range of interest, significant amounts of all the protonated species and the free amine are present in solution. In order to interpret the chemical shifts, which are average values for all the species present, it is necessary to estimate chemical shifts of the various protonated species by using values of the shifts in model compounds and in the unprotonated and the fully protonated amine. Observed shifts are then fitted using these estimates and a protonation sequence is derived. The model compounds chosen are usually monamines and these may not provide a reliable guide because (among other reasons) the polyamines may form hydrogen-bonded species in solution.[1–4] Formation of hydrogen bonds can alter the protonation shifts experienced by carbon and nitrogen nuclei and could also result in conformational changes in the amine which would also affect the signals.[21–23] Recently Hague and his co-workers[7,8,24] developed empirical relationships ('amine shift parameters') which are derived only from n.m.r. shifts of polyamines. These parameters have been successfully applied in predicting the shifts of a number of related polyamines and to the study of the zinc complexes of these amines in solution.



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In this paper we report an investigation of the protonation of the triazamacrocycle 2,2,4-trimethyl-1,5,9-triazacyclododecane (**1**), using ^{13}C n.m.r. spectroscopy. The wide separation of the pK_a values of this amine (12.3, 7.34, and 2.51)[25] allows the preparation of solutions which contain essentially only one of the protonated forms. This amine was chosen also because its unsymmetrical nature means that the three nitrogen centres have different basicities, and this was expected to be useful in interpreting the results. It was hoped that the study would also provide a check on the shift parameters that have been used by other workers. The results obtained however suggest that conformational effects in a molecule such as (**1**) are very important in determining the chemical shift of a particular nucleus, and that direct comparisons with linear polyamines are not possible. Such a result is not unexpected but it does suggest that conformational effects in linear polyamines should not be ignored.

Experimental

The amine (**1**) and its hydrobromide salt were prepared by a previously reported procedure.[25] ^{13}C N.m.r. spectra were recorded with a Varian FT80A spectrometer at 35 °C. Spectra were obtained for solutions in ^1H_2O in a tube which also contained a capillary of ^2H_2O and dioxane which provided the lock signal and the reference (δ_C 67.4). This arrangement meant that corrections to the pH measurements due to a deuterium effect were not necessary. Assignments of the carbon resonances were made on the basis of proton-decoupled and single frequency off-resonance decoupled (SFORD) spectra, obtained for seven solutions over the entire pH range studied. pH Values were measured with a Radiometer 80 pH meter at room temperature and are uncorrected. The lowest and highest pH values shown in the Figures were calculated from the known concentration of acid or alkali used to dissolve the amine or its

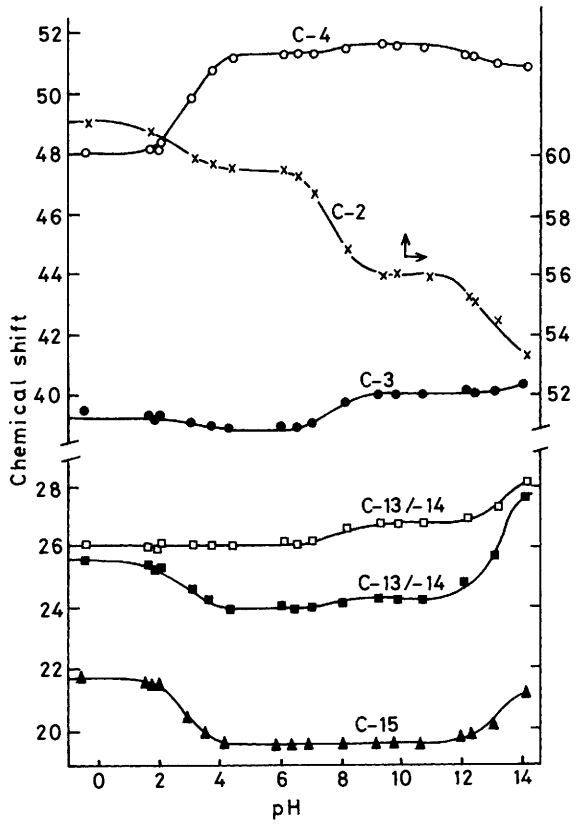

Figure 1. ^{13}C N.m.r. shift variation (in p.p.m.) for C-2, C-3, C-4, and the methyl carbon signals as a function of pH

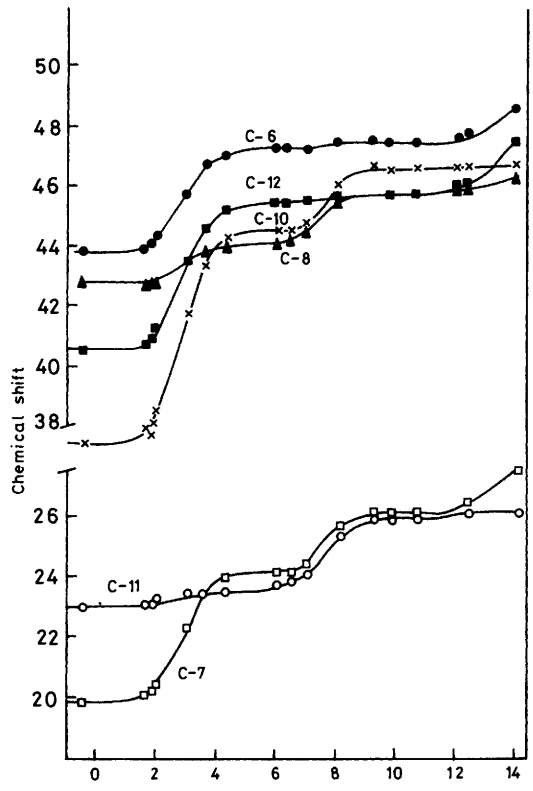

Figure 2. ^{13}C N.m.r. shift variation (in p.p.m.) for the remaining carbon signals as a function of pH

hydrobromide salt. Both these forms of the amine were used to prepare solutions; the concentration of the amine varied from 0.2 to 0.4 mol dm^{-3}. No attempt was made to control the ionic strength of the solutions.

Spectra of (**1**) usually contained 12 signals, although accidental degeneracies occurred at some pH values. Above pH 13 additional signals appeared in the spectra of solutions where sodium hydroxide was used to control the pH. These signals were absent when Me_4NOH was used and all signals due to the amine in these solutions were present in the sodium hydroxide solutions. We suggest therefore that (**1**) forms a sodium complex which gives rise to the additional signals.

Results and Discussion

Figures 1 and 2 show the dependence of the chemical shifts on the pH of the solution.

Protonation constants of (**1**) have been determined previously at constant ionic strength and at a different temperature from this study;[25] nevertheless qualitative agreement between the reported protonation constants and the present results is evident from the Figures. The five high-field signals are assigned to the three methyl carbon atoms C-13, C-14, and C-15 and the methylene carbon atoms C-7 and C-11. Geminal methyl signals are expected to occur downfield of the C-15 signal, so the two methyl signals at about δ 28 in the spectrum of the free amine are assigned to the geminal methyl carbon atoms. This expectation is based on the assignment of the methyl signals of 1,1,3-trimethylcyclohexane,[26] methyl-substituted piperidines,[27] and triazamacrocycles[28] and is consistent with previous assignments for metal complexes of 2-methylpentane-2,4-diamine.[29] The C-15 signal occurs at about δ 21 in the spectrum of the free amine. Signals due to the methylene carbon atoms C-7 and C-11 display different pH-dependent behaviour, and the assignment shown in Figure 1 is based on the protonation sequence discussed later.

In the low-field region of the spectrum, seven signals are usually observed, and the quaternary C-2 and tertiary C-4 give signals which are readily distinguished, on the basis of SFORD spectra, from the five methylene signals. In the case of C-2, the downfield protonaton shifts are also indicative of a quaternary carbon. The C-3 signal is expected at higher field in the spectrum of the free amine than the signals due to C-6, C-8, C-10, and C-12, so the signal at δ *ca.* 40 is assigned to C-3. The pH-dependence of this signal is different from that of the other four signals, consistent with this assignment. The remaining four signals have not been assigned to particular carbon atoms as the signals occur within 3 p.p.m. of one another over most of the pH range studied, and protonation of the amine causes 'crossovers' and degeneracies which make firm assignments impossible.

Figures 1 and 2 show that the signals undergo very variable shifts as the amine is protonated, and that some carbon signals are either unaffected or shifted only a little by a particular protonation. Shifts observed upon addition of the third proton are usually larger and are sometimes in the direction opposite to those observed during the first two protonations. Only the C-2 signal and one of the high-field methylene carbon signals (C-7) are affected significantly by all three protonations. The simplest explanation for this observation is that each protonation of the amine occurs predominantly at just one nitrogen atom. The structure of (**1**) is such that if protonation occurred at more than one nitrogen atom to a significant extent, then no carbon atom would be more than four atoms away from a protonation centre. Explanations for the unusual behaviour of C-2 and C-7 will be suggested later. In the discussion that follows, signals which do not shift on protonation are emphasised because these

carbons must be remote from the protonaton centre, which is thus identified.

During the first protonation one of the high-field methylene carbon signals (C-7 or C-11) does not shift, and one of the low-field methylene signals is also unaffected. A second low-field methylene signal experiences only a small shift. This behaviour is consistent with either N-1 or N-5 but not N-9 as the protonation site. Shifts experienced by the methyl resonances should in principle allow a distinction between N-1 and N-5 as the protonation site; a methyl group β to the protonation site is expected to experience a larger protonation shift than a methyl group δ to such a site. However, the magnitudes of the observed shifts (3 and 1.3 p.p.m. for the geminal methyl groups and 1.6 p.p.m. for C-15) do not allow this distinction to be made. It could be argued that the shifts observed for the C-2 signal (2.6 p.p.m.) and the C-4 signal (0.6 p.p.m.) suggest that N-1 is the protonation site, but in view of the different natures of these carbons the magnitudes of the shifts should not be compared directly. There is indirect evidence (discussed later) that N-5 is the first protonation site.

Protonation of (**1**) at around pH 7.5 occurs predominantly at N-9. Both C-7 and C-11 signals shift (2.2 and 2.5 p.p.m.), and this is consistent only with protonation of N-9. The C-15 signal does not shift, and the two geminal methyl groups experience only small (< 1.0 p.p.m.) shifts, consistent with groups remote from the protonation site. The relatively large shifts experienced by C-2 and C-3 signals due to this protonation are surprising. A simple explanation is that partial protonation of N-1 occurs or that a hydrogen bond is formed between N-9 and N-1. This would explain the shifts observed for C-2 and the three carbon atoms β to N-1 (C-3 and the two geminal methyl groups), and the slightly larger shift observed for C-11 as compared with C-7. The effect of this partial protonation of N-1 is attenuated sufficiently that C-4 experiences only a small shift and C-15 is unaffected. If this explanation is correct then N-5 must be the first protonation site.

Protonation at *ca.* pH 2 results in large shifts in all signals except for those of C-3 (small) and one of the geminal methyl groups (zero). Two methyl signals and the C-3 signal shift in the opposite direction from that expected for a protonation shift. In addition the magnitude of the shifts experienced by many of the carbon signals is much greater than expected for a protonation shift. These changes suggest that the third protonation results in a large conformational change and the shifts observed are a combination of protonation and conformational effects. A large conformational change due to charge repulsion is not unexpected. This conformational change also explains why the signal assigned to C-7 which shifts on protonation of N-1 is thus affected by all three protonations.

It has been suggested[25] that the reason for the very high pK_1 value of (**1**) in comparison with linear polyamines is due to the ability of the first proton to form hydrogen bonds to all three nitrogen atoms. Hydrogen bonds between nitrogen atoms of a protonated triaza-macrocycle have been observed in the solid state.[30] The present results however, are not in accord with this suggestion as the shifts observed are consistent with either N-1 or N-5 being protonated, but not both. An alternative explanation could be that the high pK_a value is a manifestation of the same phenomena which give rise to the increase in stability of the metal complexes of macrocyclic ligands: the macrocyclic effect. Factors which contribute to the macrocyclic effect have been considered in detail by Hancock and need not be enumerated here.[31]

One of the objects of this study was to investigate whether protonation shifts measured for monoamines and diamines can be used to determine the protonation sequence of triamines like spermidine. Results obtained suggest that considerable caution is necessary. Kimberly and Goldstein[10] used a protonation shift of 2.66 p.p.m. downfield for carbon atoms in the β-position to a secondary amino group in their analysis of the protonation sequence of spermidine. Dagnall *et al.*[7,8] determined an amine shift parameter of 3.0 p.p.m. for such a carbon atom in linear polyamines. Shifts experienced in each protonation step by the C-7 and C-11 signals of (**1**) are 1.5, 2.6, and 0.75 for one and 0, 2.1, and 4.25 for the other. If the shifts due to the third protonation step are excluded because of the large conformational effects associated with that protonation, the agreement between the observations and the expected shifts is still not encouraging. While it is true that conformational effects in a macrocyclic amine are likely to be much more important than in linear polyamines, such effects are unlikely to be absent in the latter and should be taken into account. Empirical parameters such as the amine shift parameter,[7,8] which are derived from a study of the shifts determined with linear polyamines, are more likely to give reliable results when used to study other polyamines and their metal complexes than are shifts derived from monoamines, because conformational effects in the polyamines are likely to be similar. This study has shown, however, that these 'amine shift parameters' cannot be used to predict the shifts in the macrocycle (**1**).

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References

1 J. Hine and W.-S. Li, *J. Org. Chem.*, 1975, **40**, 1795.
2 D. J. Cralk, G. C. Levy, and A. Lombardo, *J. Phys. Chem.*, 1982, **86**, 3893.
3 V. Barone, F. Lelj, O. Nicolaus, G. Abbate, and R. Barbucci, *Gazz. Chim. Ital.*, 1984, **114**, 249.
4 C. Yu and G. C. Levy, *Org. Magn. Reson.*, 1984, **22**, 131.
5 J. Schaubroeck, C. T. Huys, and A. M. Goeminne, *Spectrochim. Acta, Part A*, 1984, **40**, 303.
6 J. E. Sarneski, H. L. Surprenant, F. K. Molen, and C. N. Reilley, *Anal. Chem.*, 1975, **47**, 2116.
7 S. P. Dagnall, D. N. Hague, and M. E. McAdam, *J. Chem. Soc., Perkin Trans. 2*, 1984, 435, 111.
8 S. P. Dagnall, D. N. Hague, M. E. McAdam, and A. D. Moreton, *J. Chem. Soc., Faraday Trans. 1*, 1985, **81**, 1483.
9 M. Delfini, A. L. Segre, F. Conti, R. Barbucci, V. Barone, and P. Ferruti, *J. Chem. Soc., Perkin Trans. 2*, 1980, 900.
10 M. M. Kimberly and J. H. Goldstein, *Anal. Chem.*, 1981, **53**, 789.
11 D. A. Aikens, S. C. Bunce, O. F. Onasch, H. M. Schwartz, and C. Hurwitz, *J. Chem. Soc., Chem. Commun.*, 1983, 43.
12 D. Aikens, S. Bunce, F. Onasch, R. Parker III, C. Hurwitz, and S. Clemans, *Biophys. Chem.*, 1983, **17**, 67.
13 F. Onasch, D. Aikens, S. Bunce, H. Schwartz, D. Nairn, and C. Hurwitz, *Biophys. Chem.*, 1984, **19**, 245.
14 Y. Takeda, K. Samejima, K. Nagano, M. Watanabe, H. Sugeta, and Y. Kyogoku, *Eur. J. Biochem.*, 1983, **130**, 383.
15 M. Ciampolini, M. Micheloni, N. Nardi, P. Paoletti, P. Dapporto, and F. Zanobini, *J. Chem. Soc., Dalton Trans.*, 1984, 1357.
16 C. F. G. C. Geraldes, M. C. Alpoim, M. P. M. Marques, A. D. Sherry, and M. Singh, *Inorg. Chem.*, 1985, **24**, 3876.
17 M. Ciampolini, M. Micheloni, F. Vizza, F. Zanobini, S. Chimichi, and P. Dapporto, *J. Chem. Soc., Dalton Trans.*, 1986, 505.
18 J. R. Ascenso, R. Delgado, and J. J. R. F. da Silva, *J. Chem. Soc., Perkin Trans. 2*, 1985, 781.
19 C. H. Taliaferro and A. E. Martell, *Inorg. Chem.*, 1985, **24**, 2408.
20 J. F. Desreux, E. Merciny, and M. F. Loncin, *Inorg. Chem.*, 1981, **20**, 987.
21 I. Morishima, K. Yoshikawa, K. Okada, T. Yonezawa, and K. Goto, *J. Am. Chem. Soc.*, 1973, **95**, 165.
22 R. O. Duthaler and J. D. Roberts, *J. Am. Chem. Soc.*, 1978, **100**, 3889.
23 J. G. Batchelor, *J. Chem. Soc., Perkin Trans. 2*, 1976, 1585; *J. Magn. Reson.*, 1977, **28**, 123.

24 S. P. Dagnall, D. N. Hague, M. E. McAdam, and A. D. Moreton, *J. Chem. Soc., Dalton Trans.*, 1985, 2381; S. P. Dagnall, D. N. Hague, and A. D. Moreton, *ibid.*, 1986, 1499, 1505.
25 R. W. Renfrew, R. S. Jamison, and D. C. Weatherburn, *Inorg. Chem.*, 1979, **18**, 1584.
26 T. P. Forrest and J. Thiel, *Can. J. Chem.*, 1981, **59**, 2870.
27 C. G. Beguin, M.-N. Deschamps, V. Boubel, and J.-J. Delpuech, *Org. Magn. Reson.*, 1978, **11**, 418.
28 P. G. Graham and D. C. Weatherburn, *Aust. J. Chem.*, 1984, **37**, 2243.
29 C. J. Hawkins, R. Holm, J. A. Palmer, and D. D. Traficante, *Aust. J. Chem.*, 1982, **35**, 1815.
30 T. W. Bell, H.-J. Choi, and W. Harte, *J. Am. Chem. Soc.*, 1986, **108**, 7427.
31 V. J. Thöm, J. C. A. Boeyens, G. J. McDougall, and R. D. Hancock, *J. Am. Chem. Soc.*, 1984, **106**, 3198.

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