On the Mechanism of Protonation of Triamines

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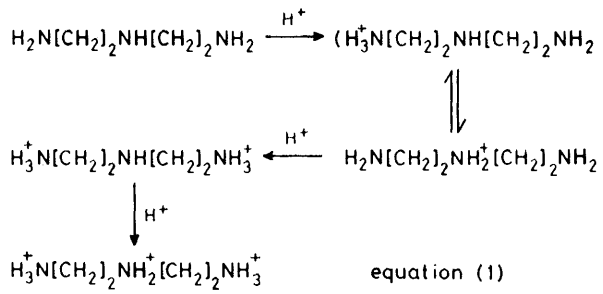
The protonation behaviour of several triamines having the general formula $H_2N[CH_2]_mNH[CH_2]_nNH_2$ with $m \leqslant n$ has been studied by ^{13}C and ^1H n.m.r. techniques. In all cases, in the first two protonation steps, only primary amino-groups are involved. The protonation of the secondary amino-group starts only after the protonation of the primary ones is complete.

In the case of symmetrical triamines, in the first step, the proton is shared by the two primary nitrogens to an equal extent. Unsymmetrical triamines having $m < 3$ are initially protonated on the primary nitrogen atom attached to the longer aliphatic chain. However the unsymmetrical triamine spermidine, having $m = 3$ and $n = 4$. behaves like the symmetrical ones.

UNTIL now, it has been generally accepted that during the protonation of polyamines containing non-equivalent nitrogen atoms, initially the proton is shared by all the basic nitrogens.[1] For instance, in the case of symmetrical triamines (*e.g.* 1,5-diamino-3-azapentane) a mechanism has been proposed, involving the following tautomeric equilibrium [2]:



equation (1)

According to this view, in the first step, the protonation percentage for each nitrogen is proportional to its relative microbasicity. In the second step, the charges are stabilized as far apart as possible, *i.e.* on the terminal nitrogens.[3] The inner nitrogen is protonated in the third step.

We have recently shown that ^{13}C n.m.r. spectroscopy is a powerful means to ascertain the order of protonation for basic compounds containing several amine nitrogen atoms.[4] Therefore, we performed a ^{13}C n.m.r. study on the protonation of triamines of general formula $H_2N[CH_2]_mNH[CH_2]_nNH_2$ [hereafter denoted by the symbol (m,n)-tri, where $m = 2$, 3, or 4 and $n = 2$ or 3].

EXPERIMENTAL

Materials.—The triamines were purchased from Fluka or Aldrich Co. Their purity was checked by ^{13}C or ^1H n.m.r. spectroscopy.

N.m.r. Spectroscopy.—^{13}C Spectra were run for 0.1M-solutions at room temperature on a Bruker XH 90 spectrometer operating at 22.63 MHz; pH measurements were performed as previously described.[4]

The spectral conditions for the ^{13}C n.m.r. spectra were as follows: pulse width 4 μs; points 16 K; width 3 000 Hz; acquisition time 2.720 s. The spectral conditions for the ^1H spectra were: pulse width 1 μs; points 4 K; width 300 Hz; acquisition time 6.800 s. The spectrometer operated at 90 MHz. All the chemical shifts were determined with an external reference and converted into the TSP (trimethylsilyl propionate). TSP at low pH values was corrected for its own pH dependence; no shift of the locking frequency with respect to the external reference was found.

RESULTS AND DISCUSSION

The triamines studied in this work are listed in the Table together with their basicity constants, previously determined by potentiometric techniques.

All triamines exhibit n.m.r. ^{13}C signals in three well defined ranges:[5] between 47 and 55 p.p.m. for CH_2 near an NH and another CH_2 group; between 38 and 44 p.p.m. for CH_2 near an NH_2 and a CH_2; between 25 and 35 p.p.m. for CH_2 in a β position with respect to an NH or an NH_2 group. Remembering that the β effect of a protonation is always the largest [6-9] the assignments of the signals and of the protonation sites are as follows.

Basicity constants of linear aliphatic triamines at 25 °C

Reaction	(2,2-Tri) [a]	(2,3-Tri) [b]	(3,3-Tri) [c]	(2,4-Tri) [d]	(3,4-Tri) [e]
$L + H^+ \rightleftharpoons LH^+$	9.79	10.437	10.65	10.669	10.889
$LH^+ + H^+ \rightleftharpoons LH_2^{2+}$	8.98	9.356	9.57	9.592	9.811
$LH_2^{2+} + H^+ \rightleftharpoons LH_3^{3+}$	4.25	6.374	7.72	6.984	8.345

[a] In 0.1M-KCl, M. Ciampolini and P. Paoletti, *J. Phys. Chem.*, 1961, **65**, 1224. [b] In 0.5M-KNO_3, R. Barbucci, L. Fabbrizzi, and P. Paoletti, *Inorg. Chem. Acta*, 1973, **7**, 157. [c] In 0.1M-KCl, A. Vacca, D. Arenare, and P. Paoletti, *Inorg. Chem.*, 1966, **5**, 1384. [d] In 0.5M-KNO_3, R. Barbucci, P. Paoletti, and A. Vacca, *Inorg. Chem.*, 1975, **14**, 302. [e] In 0.1M-NaCl, M. Gold and J. Powell, *J.C.S. Dalton*, 1976, 230.

2,2-*Tri* ($H_2N[CH_2]_2NH[CH_2]_2NH_2$).—The ^{13}C n.m.r. spectrum of this compound consists of two signals whose chemical shifts as a function of pH are shown in Figure 1 together with the signal assignments. From this Figure it is clear that in the first and second step the protonation sites are the external primary amine groups with no protonation of the secondary inner one. During the

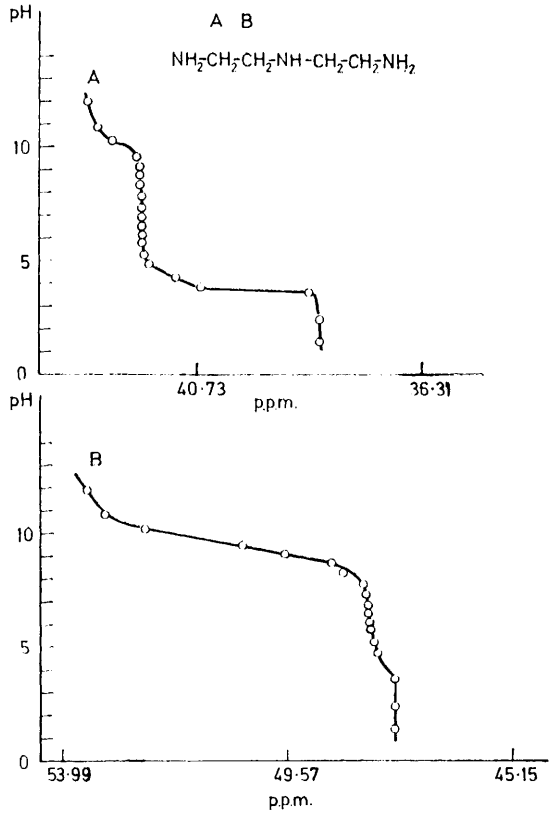


FIGURE 1 ^{13}C N.m.r. shift variation in p.p.m. as a function of pH value for 2,2-tri

first protonation step a tautomeric equilibrium exists in which the proton is shared between the two external amine groups. The second protonation involves again the external primary amine groups with no protonation on the inner secondary nitrogen atom. The last site protonates only at much lower pH values. In order to get pK values from Figure B we assumed that the β effect is proportional to the effective charge. This hypothesis means that β contribution in the first and second protonation is the same; this is well supported by literature data [5] and by theoretical calculations.[8,9]

The obtained pK values, $pK_1 = 10.1 \pm 0.2$, $pK_2 = 9.1 \pm 0.2$, and $pK_3 = 3.9 \pm 0.2$, are in reasonable agreement with potentiometric data (see Table).

This amine was previously studied by Sudmeier and Reilley by ^1H n.m.r. spectroscopy.[10] However the instrument conditions allowed no clear interpretation because of extensive overlap of the signals.

2,3-*Tri* ($H_2N[CH_2]_2NH[CH_2]_3NH_2$).—The ^{13}C n.m.r. spectrum of 2,3-tri consists of 5 signals whose chemical shifts as function of pH are shown in Figure 2. Signal assignment was made by comparing the chemical-shift values with the corresponding ones of 1,5-diamino-3-azapentane (2,2-tri) and 1,7-diamino-4-azaheptane (3,3-tri).

From these results it appears that the initial protonation is on the amine nitrogen in position 1; the second protonation involves nitrogens 1 and 8, while the third protonation involves the inner secondary nitrogen.

The hypothesis that in the first protonation step the

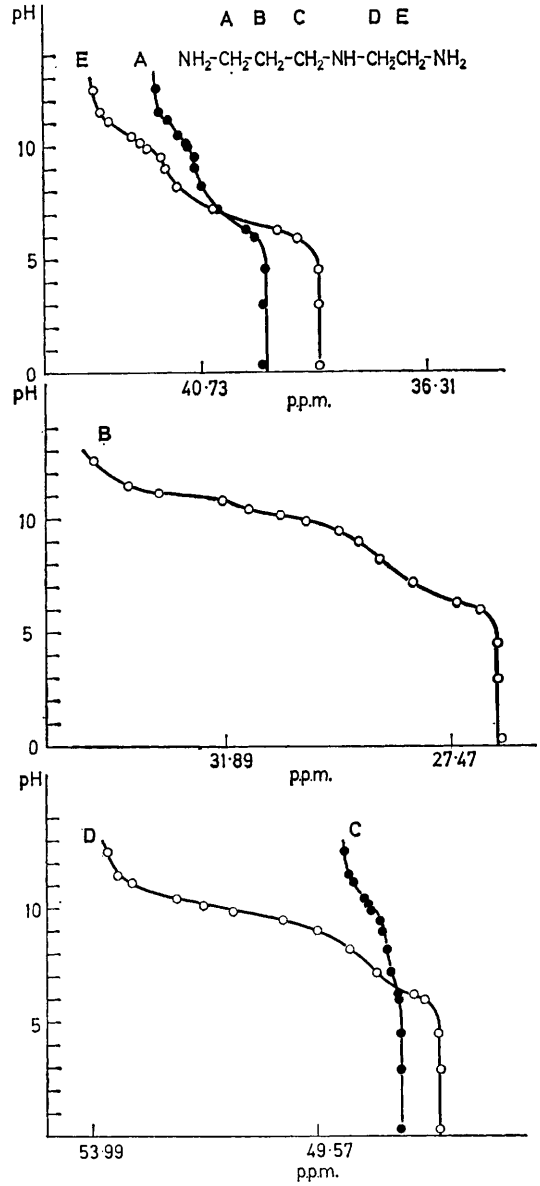


FIGURE 2 ^{13}C N.m.r. shift variation in p.p.m. as a function of pH value for 2,3-tri

proton is shared between all the three basic sites [as in mechanism (1)] must be discarded, because ^{13}C n.m.r. signals due to group E and B indicate that the protonation of the inner secondary amine group starts only at pH values lower than 7. On the other hand, if in the first step the proton was shared by both the primary amino-groups, a coincidence between the protonation constants measured on carbons B and D (both in a β

position with respect to the basic sites) should occur. This condition is not fulfilled.

In fact, by supposing equal contribution of the charge with respect to the induced shift, the pK values measured on B carbon atom are p$K_1 \simeq 11$ and p$K_2 \simeq 10.2$ while the pK values measured on D carbon atom are p$K_1 \simeq 10.0$ and p$K_2 \simeq 9.0$.

This consistent disagreement suggests that the first protonation begins on the NH_2 in position 1, while the NH_2 in position 8 is mostly protonated in the second step. On the other hand, pK_3 values, as measured on E and B carbon atoms in the third protonation, are consistent (6.7 and 6.8 respectively), confirming that the secondary amine group is protonated only in the third step.

3,3-*Tri* ($H_2N[CH_2]_3NH[CH_2]_3NH_2$).—The ^{13}C n.m.r. spectrum of compound (III) consists of three signals,* but because the central carbon atom is in a β position with respect to both NH and NH_2 groups, it is not possible to determine which group is protonated first. However, reasonable pK values can be obtained: p$K_1 \simeq 10.9$, p$K_2 \simeq 9.7$, and p$K_3 \simeq 8.2$. In order to check which basic site is protonated first, ^1H n.m.r. spectroscopy was used. At 90 MHz chemically different groups give quite well separated spectra, so that it is possible to obtain chemical-shift plots as a function of pH. Our results (Figure 3) clearly show that a tauto-

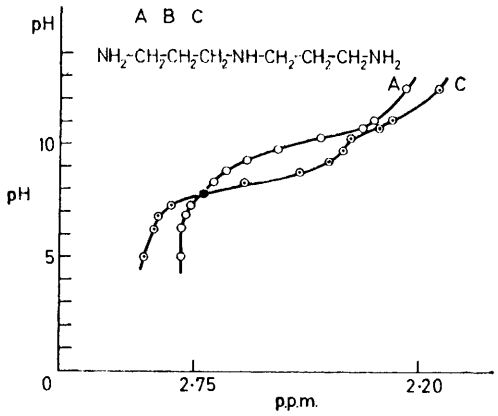


FIGURE 3 ^1H N.m.r. shift variation in p.p.m. as a function of pH value for 3,3-tri

meric equilibrium of all three amine nitrogens in the first protonation step must be excluded. In fact the inner secondary nitrogen is protonated only in the third step, with pK = 8.2. The pK values obtained from ^1H n.m.r. spectra as measured on H_A are p$K_1 \simeq 10.8$ and p$K_2 \simeq 9.4$, in good agreement with the ^{13}C n.m.r. and potentiometric results.

2,4-*Tri* ($H_2N[CH_2]_2NH[CH_2]_4NH_2$).—The ^{13}C n.m.r. spectrum of compound (IV) consists of six signals relative to the number of different carbon atoms in the molecule: *

$$\underset{A}{H_2N}\underset{B}{CH_2}\underset{C}{CH_2}\underset{D}{CH_2}\underset{E}{NH}\underset{F}{CH_2}CH_2NH_2$$

* Experimental details, together with the n.m.r. tracings, are available from the authors on request.

From our results, the hypothesis that in the first step the proton is shared between all the basic sites, as in mechanism (1), must be discarded, since the chemical-shift variation of methylene E indicates clearly that the protonation of the inner secondary amine group occurs between pH 6 and 8, with p$K_3 = 7.0$. On the other hand, if in the first step the proton was shared to an equal extent by the two primary amine nitrogens, a coincidence between the protonation constants, as measured on carbon atoms B and E, should occur. This is not found. In fact, by supposing equal contributions of the charges with respect to the β induced shift, the pK values measured on the curve for the B carbon atom are p$K_1 = 10.8$ and p$K_2 = 9.8$, while the curve for the E carbon atom gives an average value (pK_1 + pK_2)/2 ≃ 9.7, clearly not consistent with the previous data.

As a consequence, we can conclude that the protonation process starts on the amine nitrogen in position 1.

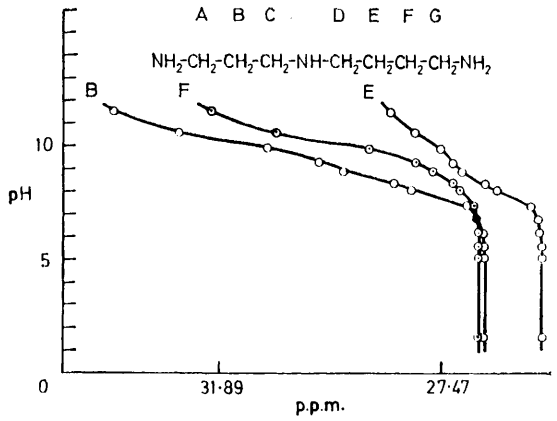


FIGURE 4 ^{13}C N.m.r. shift variation in p.p.m. as a function of pH value for 3,4-tri

As regards the third protonation, a consistent value, p$K_3 = 7.0$, is obtained from both the curves for the C and F carbon atoms; hence, we can assess that the third protonation step involves only the inner secondary amine group.

3,4-*Tri* ($H_2N[CH_2]_3NH[CH_2]_4NH_2$).—The ^{13}C n.m.r. spectrum of compound (V) consists of seven signals, of these the chemical shifts of B, E, and F carbon atoms as a function of pH are shown in Figure 4 together with the signal assignments. It follows from these curves that in the first stage the proton is shared to an equal extent by the two primary amine groups. In fact, pK_1 constants as measured on the curves for both B and F carbon atoms consistently give a value of 10.5. The second protonation occurs again on the primary amine groups; in fact the values p$K_2 = 9.4$ (measured on B) and p$K_2 = 9.3$ (measured on F) are consistent. The inner secondary amine group is protonated only after the complete protonation of the external primary basic sites. For the third protonation constant, consistent values are obtained; p$K_3 = 8.1$ measured on the plot relative to carbon B and p$K_3 = 8.2$ measured on the plot relative to carbon E.

We wish to point out that the behaviour of compound (V) (asymmetric) is similar to that of compound (III) (symmetric), and not to that of compounds (II) and (IV) (asymmetric).

Conclusion.—The hypothesis that, in the case of triamines, in the first protonation step there is a tautomeric equilibrium between species protonated on the primary nitrogens and species protonated on the secondary one, has not been confirmed. In fact, our results indicate that in all the triamines examined the protonation of the inner nitrogen occurs only after the protonation of the two external nitrogens is complete.

Moreover, for the symmetrical triamines (I) and (III), as expected, a fast proton exchange between the two identical primary nitrogens occurs in the first step. In the case of the asymmetric triamines (II) and (IV), the protonation of the nitrogen attached to the longer aliphatic chain starts first. However, the protonation of the nitrogen atom attached to the shorter aliphatic chain starts before the protonation of the other one is complete.

Finally, in the case of the triamine (V), both primary nitrogens are initially protonated at the same time, although they are attached to aliphatic chains of different length. Their microbasicity constants, as obtained by ^{13}C n.m.r. shifts, are equal. This means that the effect of the number of methylenes of the chains become negligible after a certain length. This result agrees well with the observed trend in the basicity constants of linear aliphatic polymethylenediamines.[11] The biggest change occurs between ethylenediamine and trimethylenediamine, while for longer aliphatic chains the addition of a methylene group has a negligible effect on the basicity.

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