

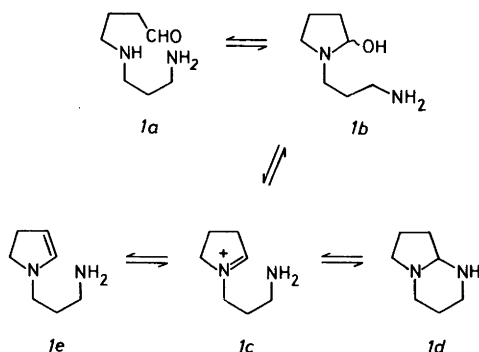
## Ring-Chain Tautomerism of an *N,N*-Acetal Formed by Enzymatic Oxidation of Spermine or Spermidine

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Enzymatic oxidation of spermine<sup>1,2</sup> or spermidine<sup>1</sup> gives a metabolite to which the enamine structure *1e* was originally ascribed.<sup>1-3</sup> Other conceivable forms of the metabolite are *1a-d* (Scheme 1). Compound *1* is the likely intermediate in the metabolism of spermidine to *N*-(3-aminopropyl)-2-pyrrolidinone<sup>4</sup> in mice and this raises the question of which form of *1* is being oxidized to the lactam. While Seiler *et al.*<sup>4</sup> favoured the carbinolamine *1b*, results from our studies of the metabolism of nicotine suggest that the iminium form *1c* may be the substrate for the oxidizing enzyme involved.<sup>5</sup> Knowledge of the structure of *1* under physiological conditions is obviously relevant to this question.

It was recently reported that the metabolite has the *N,N*-acetal (aminal)<sup>6</sup> structure *1d* and not the enamine structure *1e*.<sup>7</sup> This conclusion was based on a comparison (<sup>1</sup>H and <sup>13</sup>C NMR, IR, GLC, MS) between the metabolite and *1d* synthesized from 1,3-diaminopropane and 4-chlorobutanal. Although the solvents used were not specified in



**Scheme 1.** Five of the conceivable forms of the metabolite *1*. For sake of brevity, *N*-protonated forms have been omitted. Nine-membered ring forms are also possible but less likely (see text) and have been omitted.

the communication,<sup>7</sup> it seems likely that aqueous solutions were not studied and that the results therefore have little biological relevance. The *N,N*-acetal *1d* is expected to predominate in *e.g.* CDCl<sub>3</sub> by analogy with the base form of the alkaloid elaeocarpidine which exists at the closely related *N,N*-acetal *2*<sup>8</sup> in this solvent.

In this communication we describe structural studies of *1* in aqueous solutions at various pH's. Octahydropyrrolo[1,2-*a*]pyrimidine (1,5-diazabicyclo[4.3.0]nonane, *1d*) was prepared by reduction of the commercially available amidine 2,3,4,6,7,8-hexahydropyrrolo[1,2-*a*]pyrimidine (1,5-diazabicyclo[4.3.0]non-5-ene) with lithium aluminium hydride. The product was purified by distillation and the <sup>1</sup>H and <sup>13</sup>C NMR (CDCl<sub>3</sub>) and mass spectra established the *N,N*-acetal structure *1d*. Treatment of *1d* with perchloric acid yielded the hydrodiperchlorate of *1c*. The structural study was performed by dissolving either *1d* or the salt of *1c* in D<sub>2</sub>O, adjusting the pD<sub>c</sub> (pD<sub>c</sub>=pH meter reading+0.40<sup>9</sup>) and recording the <sup>1</sup>H and <sup>13</sup>C NMR spectra of the solutions.

<sup>1</sup>H NMR spectra of acidic solutions, pD<sub>c</sub>≤4.5, displayed a signal at δ 8.80 which must be ascribed to the iminium form *1c*. Under the recording conditions used, the integral of the signal indicated only 0.8–0.9 H, but the <sup>13</sup>C NMR spectra showed no signals from any minor form. The iminium carbon resonated at 183.2 ppm and the signals from the iminium group were thus similar to those obtained from 3,4-dihydro-1-methyl-(2*S*)-(3'-pyridinyl)-2*H*-pyrrolium (3)<sup>10</sup> (δ 8.93 and 186.8 ppm).

Solutions having a pD<sub>c</sub> value between 4 and 7 gave rise to broadened NMR signals. The iminium proton resonance at δ 8.80, and therefore also *1c*, gradually decreased and finally disappeared as the pD<sub>c</sub> value was changed from 4 to 7 (Fig. 1). Owing to the instability of these solutions (*cf.* Ref. 10 and references therein), <sup>13</sup>C NMR spectra provided no further structural information. A broad multiplet at δ 4.4–3.8 given by the four hydrogen atoms adjacent to the iminium nitrogen atom also disappeared during the change from acidic to neutral conditions but a triplet (*ca.* 1 H) remained at δ≈4.20. A <sup>13</sup>C NMR spectrum recorded at pD<sub>c</sub> 7.2 displayed a characteristic signal at 80.1 ppm. These <sup>1</sup>H and <sup>13</sup>C resonances were ascribed to the methine group of the monoprotonated *N,N*-acetal *1d*. The signal from the acetal carbon atom in *2* is at 83.7 ppm.<sup>11</sup> We assume that the secondary amino group is the most favoured protonation site in *1d* but we have not studied this. It was not possible to acquire clear cut NMR evidence for *1d* below pD<sub>c</sub>≈6 but it seems likely that the decrease in *1c* shown in Fig. 1 is related to a corresponding increase in *1d*

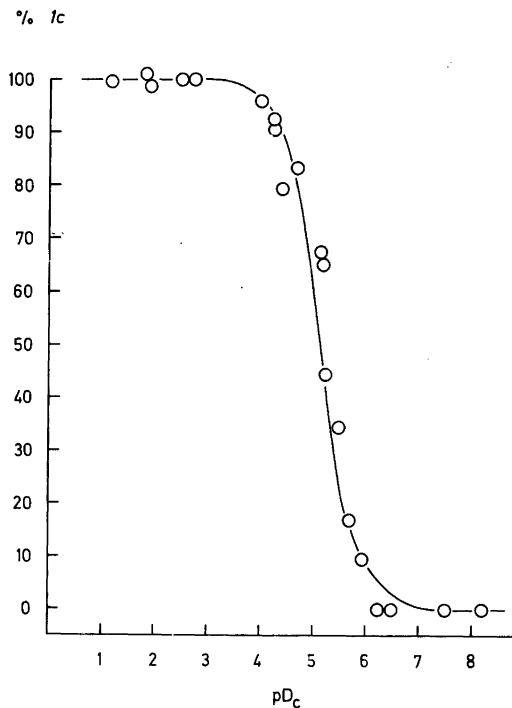
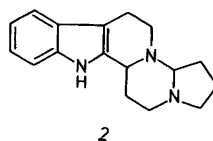


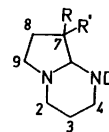
Fig. 1. Relative content of iminium form *Ic* as a function of the acidity of the solution of *I* (0.1 M) in  $D_2O$ . The figures are based on integration of the  $\delta$  8.80 signal in  $^1H$  NMR spectra and are normalized to 100 % for the strongly acidic solutions.

and that other molecular species are below the NMR detection limit. As found by altered chemical shifts, monoprotonated *Id* is deprotonated mainly between  $pD_c$  9 and 11; at  $pD_c$  11.6 the  $^{13}C$  signal from the methine group was at 77.5 ppm whereas the  $^1H$  signal was among the unresolved multiplets below  $\delta$  3.2. The chemical shifts expected for *Ib* are close to those obtained for a carbinolamine in the nicotine series,<sup>10</sup> i.e.  $\delta$  4.70 and 90.2 ppm. There are only small differences between the  $^{13}C$  NMR spectrum of distilled *Id* in  $CDCl_3$  and that in  $D_2O/NaOD$  ( $0.2 \leq \Delta\delta \leq 1.5$  ppm) and this further supports the conclusion that *Id* is the principal form present in strongly alkaline aqueous solutions.

To investigate the possibility that the iminium form observed was the nine-membered ring isomer of *Ic*, we reduced *I* with sodium cyanoborohydride in  $H_2O$ ,  $pH \approx 7$ . The only reduction product detected (GLC) was indistinguishable ( $^{13}C$  NMR, MS) from authentic *N*-(3-amino-propyl)-pyrrolidine, prepared in two steps from

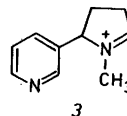


2



4: R=H, R'=D

5: R=R'=D



3

pyrrolidine and acrylonitrile. Therefore, the major iminium ion should be *Ic* and there is no evidence of the nine-membered ring iminium ion.

Scheme 1 is based upon mechanistic studies of the condensation of amines with carbonyl compounds.<sup>12</sup> As a consequence of the interconversion of the *Ic* and *Ie* forms, *I* should incorporate deuterium in  $D_2O$  solution to produce *4* and *5*; this was borne out by experiments. A solution of *I* which showed  $pD_c$  9.3 was kept at 23 °C and the deuterium incorporation was followed by  $^{13}C$  NMR spectroscopy. After 5 h, the signal at 29.7 ppm (C-7) had decreased to about 40 % of its initial height and was accompanied by a weak 1:1:1 triplet centered 0.32 ppm upfield which must be due to C-7 in *4*. There was also a doublet splitting (0.10 ppm) of the signal at 20.9 ppm which we interpret as being due to a deuterium isotope effect on C-8 in *4*. After 27 h, the signal at 29.7 ppm had decreased to about 10 % of its initial height and the doublet at 20.9 ppm had moved towards higher field. The splitting in the doublet was again 0.10 ppm, but this time it should be due to the difference between C-8 in *4* and *5*. A rough study of the effect of  $pD_c$  on the rate of deuterium incorporation showed that it should display a rate maximum near the middle of the  $pD_c$  scale. Thus, no incorporation was seen at  $pD_c$  10.7 after 4 h, at  $pD_c$  14.3 after 5 d, nor at  $pD_c \approx 1$  after 30 d (23 °C). On the other hand, the incorporation was faster at  $pD_c$  7.8 than at  $pD_c$  9.3; 50 % of *4* had been formed after 2 h. Deuterium incorporation into an *N,N*-acetal was first described by Janné and Ahlberg.<sup>13</sup>

In summary, *Id* strongly predominates in a neutral aqueous solution of *I*. The results of the treatment of *I* with sodium cyanoborohydride (and possibly also the NMR studies) demonstrates the presence of *Ic* in such solutions; the incorporation of deuterium into *Id* demonstrates the presence of *Ie*.

**Experimental.** General methods have been described before.<sup>14</sup>

*1,5-Diazabicyclo[4.3.0]nonane*. (1d). 1,5-Diazabicyclo[4.3.0]non-5-ene (Fluka, 4.0 g, 0.032 mol) was reduced with lithium aluminium hydride (1.23 g, 0.032 mol) in diethyl ether (50 ml, reflux, 2 h). Hydrolysis was accomplished with 0.1 M sodium hydroxide (25 ml, 25 °C, 10 min) and the reduction product was extracted with methylene chloride (5×100 ml). The combined organic phases were dried over MgSO<sub>4</sub>, filtered and concentrated. Distillation afforded a colourless product (purity >95 %, <sup>13</sup>C NMR) which was collected and stored over NaOH (s); b.p. 60 °C (2 kPa); yield 2.75 g (68 %); <sup>13</sup>C NMR (CDCl<sub>3</sub>): 78.7, 51.8, 51.5, 45.6, 30.1, 26.1 and 19.1 ppm. MS (*m/z*, rel. abundance): M<sup>+</sup>=126 (75 %), 125 (100), 98 (97), 97 (39), 70 (63), 69 (38), 42 (40), 41 (69). <sup>13</sup>C NMR (D<sub>2</sub>O, pD<sub>c</sub> 11.6): 77.5, 50.8, 50.7, 44.7, 29.7, 24.6 and 19.3 ppm. <sup>1</sup>H NMR (D<sub>2</sub>O, pD<sub>c</sub> 8.25, monoprotonated 1d): δ 4.18 (t, *J*≈6 Hz, 1 H), 3.7–2.7 (m, 6 H), 2.3–1.7 (m, 6 H).

*1-(3'-Aminopropyl)-3,4-dihydro-2H-pyrrolium* (1c) *hydrodiperchlorate*. *N,N*-Acetal 1d (0.50 g) was dissolved in ether (10 ml) and the solution was added dropwise to a stirred solution of excess perchloric acid in methylene chloride<sup>10</sup> (50 ml). The precipitated salt was filtered off and recrystallized from absolute ethanol to give 1.2 g (83 %) of small rods m.p. 120–122 °C. <sup>13</sup>C NMR (D<sub>2</sub>O, pD<sub>c</sub> 3.4): 183.2, 60.1, 51.8, 37.6, 36.9, 25.3 and 20.2 ppm. <sup>1</sup>H NMR (D<sub>2</sub>O, pD<sub>c</sub> 1.1): δ 8.80 (s, 1 H), 4.4–4.0 (m, 4 H), 3.5–3.0 (m, 4 H), 2.6–2.1 (m, 4 H).

*Reduction of 1 with sodium cyanoborohydride*. *N,N*-Acetal 1d (0.25 g, 2.0 mmol) in water (25 ml), adjusted with dilute hydrochloric acid to ca. pH 7, was treated with sodium cyanoborohydride (0.25 g, 4.0 mmol) under stirring at 23 °C (16 h). Aqueous alkali was added to pH 10–11 and the organic compounds were extracted with methylene chloride (5×10 ml). Drying (Na<sub>2</sub>SO<sub>4</sub>) and concentration of the combined organic phases gave a residue (0.20 g) of pure (<sup>13</sup>C NMR) *N*-(3-aminopropyl)-pyrrolidine; <sup>13</sup>C NMR (D<sub>2</sub>O, pD<sub>c</sub> 1.0): 56.9, 54.2, 39.2, 26.2, and 25.2 ppm.

*N*-(3-aminopropyl)-pyrrolidine was prepared by reduction of 3-(pyrrolidino)-propanenitrile<sup>15</sup> (3.5 g, 28 mmol) with lithium aluminium hydride (1.0 g, 26 mmol) in refluxing tetrahydrofuran (16 h). Hydrolysis with aqueous sodium hydroxide (4 M, 25 ml, 23 °C, 1.5 h), separation of the organic phase, extraction of the aqueous phase with ether (4×50 ml), combination of the organic phases, drying (Na<sub>2</sub>SO<sub>4</sub>), and concentration gave a crude product (2.5 g) which was indistinguishable (<sup>13</sup>C NMR) from the product obtained in the above reduction of 1.

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