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STRUCTURAL EQUILIBRIUM AND RING-CHAIN TAUTOMERISM OF AQUEOUS SOLUTIONS OF 4-AMINOBUTYRALDEHYDE

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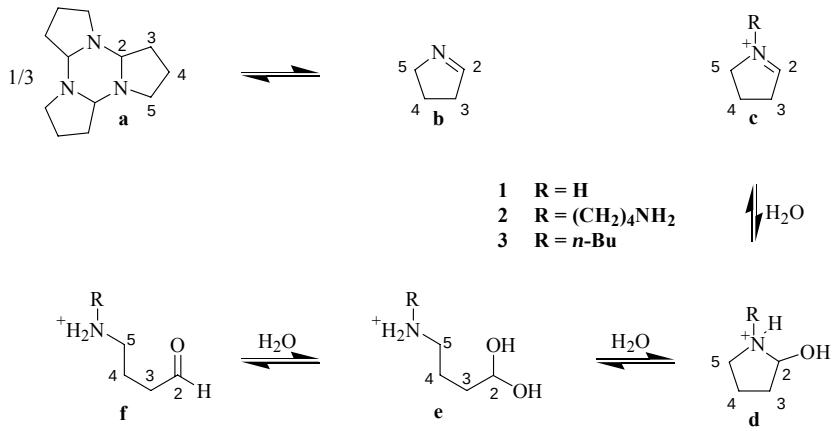
Abstract – NMR spectroscopy of aqueous solutions of 4-aminobutyraldehyde in the range 0 < pH < 13 established the occurrence of protonated and hydrated amino aldehydes in equilibrium with pyrrolines, and pyrrolinium salts.

INTRODUCTION

4-Aminobutyraldehyde (**1**) is potentially an important synthon. Most literature routes to **1** in aqueous solution involve hydrolysis of the diethyl acetal preferably with strong acids.[1] The crude product is usually used without further purification. However, the simultaneous occurrence of the amino and formyl function gives ample possibilities for the formation of by-products and the exact nature of the reacting species is not known. Biosynthetically, 4-aminobutyraldehyde (**1**) is an intermediate in the formation of several alkaloids.



Scheme 1 The equilibrium structures of 4-aminobutyraldehyde at different pD´s.

Thus, **1** (or the stable cyclic condensation product Δ^1-pyrroline (**1b**)) has been shown to be an ornithine-derived precursor of retronecine[2] and contributes to the production of hygrine and tropinone in *Hyoscamus albus*.[3] Moreover it emerges from important substrates, *e.g.* putrescine and spermine, by action of several types of mono- and polyamine oxidases containing topaquinone as well as flavin adenine dinucleotide as cofactors.[4] Directly or indirectly, **1** is a possible oxidation product of at least eight common metabolites derived from amino acids.[5] However, **1** is difficult to detect and quantify especially in aqueous solution due to the inherent reactivity. Usually **1** is converted to the stable and non-volatile 4-aminobutyric acid (GABA) by aldehyde dehydrogenase.[1f,6] Alternatively it may be isolated and characterized as a derivative[1b,1d] or the intramolecular condensation products monomer[1e,5a,7] (**1b**) or trimer[8] (**1a**) Δ^1-pyrroline easily available by synthesis.[9]

The possibility of ring-chain tautomerism of **1** in aqueous solution has never been investigated. Recently it was shown† that 4-(*N*-aminobutyl)aminobutyraldehyde (**2**) in aqueous solution at different pH gave rise to at least six species characterised by NMR spectroscopy. Moreover it is known that amino ketones in aqueous buffers exist in equilibrium with pyrrolinium forms while hemiaminals were absent.[10] The present paper describes the equilibria of **1** in aqueous solution as detected by ^{1}H NMR spectroscopy.

RESULTS AND DISCUSSION

Scheme 1 depicts the species involved in the case of **1** consistent with our results and with the results obtained from bimolecular formation of imines from aldehydes and amines.[11]

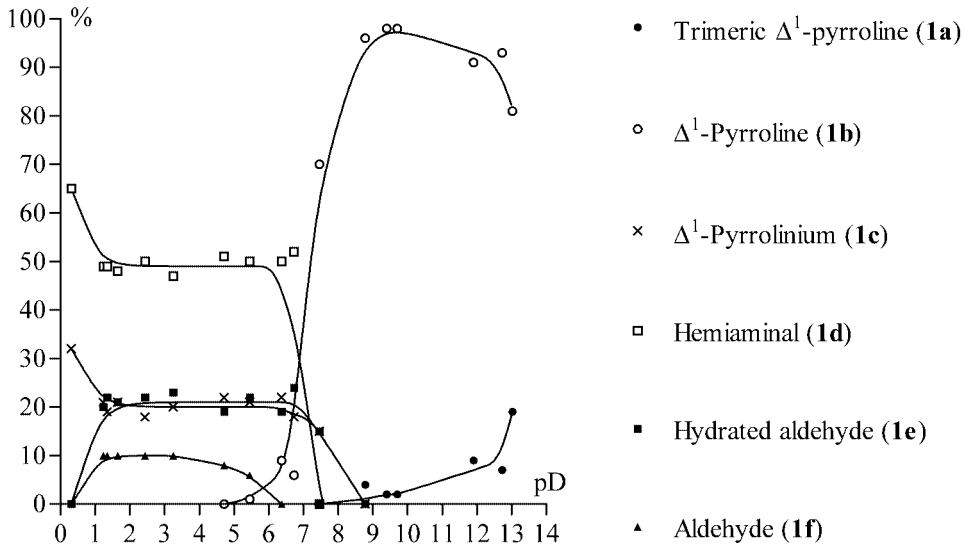


Figure 1 Product distribution at different pD´s.

The equilibrium involves two neutral (the pyrroline (**1b**) and the corresponding trimer (**1a**)) and four protonated species (pyrrolinium ion (**1c**), hemiaminal (**1d**), aldehyde (**1f**) and hydrate (**1e**)). The identification is primarily based on assignments of the ^{1}H NMR spectral data listed in Table 1 corroborated by spin decoupling, COSY experiments, etc.

When leaving 4-aminobutyraldehyde (**1**) for some time in basic solution (7 < pH < 13) an equilibrium is established between monomeric Δ^1-pyrroline (**1b**) together with small amounts of the trimeric (**1a**). Both **1a** and **1b** have been the subject of extensive investigations[8,9a] establishing the solvent dependence of the equilibrium, their structure and spectroscopic properties. Monomeric **1b** appear by dilution of solutions[12] or in gas phase.[7b,9b]

In the interval 6 < pH < 7 the intensity of signals from **1a** and **1b** disappear due to protonation of the imine functionality (pK_B = 7.3).[5b] Concomitantly, a complicated pattern of signals develop, which proves to originate from protonated species in the approximate ratio **1c**:**1d**:**1e**:**1f** = 20:50:20:10 as illustrated in figure 1. On the NMR spectral time-scale there is a sufficiently slow exchange and/or interconversion between the different species in the entire region 1 < pH < 6 to allow separate observation. The open-chain species (**1e**) and (**1f**) as well as **1d** and the conformationally rigid **1c**[13] gave satisfactory NMR spectra in this

Table 1 ^{1}H NMR Chemical Shifts.

Compound	H-2	H-3	H-4	H-5
1a[a]	**3.18**, 1H, t, *J* 6.4	**1.70**, 1H, m **1.95**, 1H, m	**1.70**, 1H, m **1.95**, 1H, m	**2.43**, 1H, dt, J_d 6.6, J_t 9.2 **2.91**, 1H, m
1b[a]	**7.67**, 1H, br s	**2.57**, 2H, br t, *J* 8.0 Hz	**1.80**, 2H, q, *J* 8.0	**3.74**, 2H, tq, J_t 7.5, J_q 2.2
1c	**8.81**, 1H, br s	**3.03**, 2H, dt, J_d 7.3, J_t 7.1	**2.29**, 2H, quintet, *J* 8.0	**4.13**, 2H, tq, J_t 8.0
1d	**5.49**, 1H, t, *J* 4.5	**1.96**, 1H, m **2.16**, 1H, m	**1.96**, 1H, m **2.16**, 1H, m	**3.29**, 1H, dt, J_d 11.7, J_t 7.1 **3.39**, 1H, dt, J_d 11.7, J_t 7.1
1e	**5.08**, 1H, t, *J* 5.3	**1.75**, 2H, m	**1.68**, 2H, m	**3.26**, 2H, br t, J_t 7.9
1f	**9.69**, 1H, s	**2.03**, ?H, m	**?**	**2.71**, 2H, t, *J* 7.1
2c[b]	**8.73**, 1H, m, *J* 1.5	**3.22**, 2H, br t, *J* 7.0	**2.35**, 2H, quintet, *J* 8.1	**4.21**, 2H, tq, J_t 8.1, J_q 1.8
3c[c]	**8.76**, 1H, m, *J* 1.4	**3.28**, 2H, br t, *J* 7.3	**2.42**, 2H, quintet, *J* 8.0	**4.27**, 2H, br t, *J* 7.3

[a]The carbon chemical shifts in $CDCl_3$ are: **1a** – 20.19 (C-4), 27.77 (C-3), 45.78 (C-5), 81.85 (C-2) and **1b** – 20.41 (C-4), 36.54 (C-3), 61.10 (C-5), 166.88 (C-2).

[b]The proton chemical shifts for the aminobutyl substituent of **2c** are: 1.74 (2 H, m, H-3'), 1.93 (2 H, m, H-2'), 3.06 (2 H, br t, *J* 7.7, H-4'), 3.98 (2 H, br t, *J* 7.5, H-1').

[c]The proton chemical shifts for the *n*-butyl substituent of **3c** are: 1.01 (3 H, t, J 7.3, H-4´), 1.45 (2 H, sextet, *J* 7.4, H-3´), 1.88 (2 H, quintet, *J* 7.5, H-2´), 3.99 (2 H, br t, J 7.3, H-1´).

pH range. Concentrated solutions were avoided due to the rapid emergence of acid catalyzed aldol condensation products easily recognized from signals in the range $5 < \delta < 6$ with allylic couplings from groupings such as -CH=CR-CH(OH)$_2$ or -CH=CR-CHOH-N$^+$HR^{1}R^2.

Similar results have been obtained in the intermolecular reaction between isobutyraldehyde and methylamine.[11a] Thus, in basic solutions, where neither amines nor imines are protonated, isobutyraldehyde is transformed largely (but not completely) to *N*-isobutylidenemethylimine by excess methylamine. In the case of **1** the tendency for formation of cyclic species increases due to the entropy factor and complete conversion to **1b/1a** is observed. The unprotonated hemiaminal is, as expected from similar reactions, only present in a very low steady-state concentration.[14] In acid solution, *N*-isobutylidenemethylimine is protonated and transformed by attack of water to a mixture of ammonium salt, isobutyraldehyde, and its hydrate *via* intermediate protonated carbinolamine. Again, in the case of **1** the entropy factor is responsible for the increased stability of the cyclic species (**1c**) and (**1d**) relative to the aldehyde (**1f**) and its hydrate (**1e**) as evidenced by the observation of signals from all 4 species in the NMR spectra. The unusual[15] dominance of protonated hemiaminal (**1d**) in the equilibrium mixture in acid solution can be attributed to the presence of the easily accessible and strongly electrophilic -CH=NH$^+$- group in **1c**.

It is interesting to compare these results with those reported for *N*-(4-aminobutyl)aminobutyraldehyde (**2**). In contrast to **1**, only signals from the quaternary pyrrolinium ion (**2c**) were observed, while the hemiaminal (**2d**) and the open chain forms (**2e**) and (**2f**) were absent. The *N*-(4-aminobutyl)-substituent in **2c** leads to increased steric hindrance for formation of **2d** by attack of water, and displaces the equilibrium towards **2c**. Provided the open-chain forms (**1e**) and (**1f**) are stabilised by intramolecular hydrogen bonding, *N*-substitution with the lipophilic aminobutyl group decreases the stabilisation and would explain the absence of **2e** and **2f**. This explanation is supported by the observation, that 4-butylaminobutyraldehyde (**3**) in acid solution exclusively exists as *N*-butylpyrrolinium ion (**3c**).

EXPERIMENTAL

General

All reagents and solvents were obtained from commercial suppliers and used without further purification. 4-Aminobutyraldehyde dimethyl acetal is 98 % pure from Tokyo Chemical Industry (TCI) America. NMR spectra are reported in δ units (ppm) relative to tetramethylsilane. The largest solvent peak is used as reference, except for spectra in D_2O where 0.5 mM DSS (2,2-dimethyl-2-silapentane-5-sulfonate) is used as reference. Values of coupling constants *J* are given in Hz. Mass spectra are recorded on a JEOL JMS-HX/HX 110A Tandem Mass spectrometer. Microanalyses were obtained on a Flash EA 1112 series elemental analyzer.

Δ^1-Pyrroline (1b)

4-Aminobutyraldehyde dimethyl acetal (13.33 g, 0.100 mol) is cooled to 0 °C, before addition of ice-cold 2 M HCl (200 mL, 0.400 mol). The reaction mixture is agitated by a magnetic stirrer for 25 min at 0 °C before slowly adding a solution of 2.67 M K_2CO_3 (300 mL, 0.800 mol) through a dropping funnel. The alkaline mixture is allowed to return to rt, before extraction with CH_2Cl_2 (3 × 200 mL). The combined extracts were dried ($MgSO_4$) and evaporated under reduced pressure to leave *the cyclized aminoaldehyde* **1b** (5.93 g, 86 %) as a light yellow oil. Destillation of the crude product (bp 91-105 °C) yields a colourless stable oil (4.70 g, 68 %). The destilled product contains approximately 10 moles of water pr. mol Δ^1-pyrroline (^{1}H NMR), and about 5 % CH_2Cl_2 (^{1}H NMR). The CH_2Cl_2 can be removed by flushing the destilled oil with nitrogen, while placed in an ultrasonic waterbath at 45 °C for 1 h. The water can be removed by addition of 6 molecular sieves (4 Å) to the oil followed by treatment in an ultrasonic waterbath for 48 h. Δ^1-Pyrroline may be used without further purification or re-destilled mixed with powdered KOH to yield analytically pure Δ^1-pyrroline. In our hands purification of the product by eluting the oil with ether through a column of neutral alumina as proposed by Nomura *et al.*,[8] yielded the product (**1b**) and an impurity of 15 % ether upon evaporation. (Found: C, 69.2; H, 10.1; N, 20.2. C_4H_7N requires C, 69.5; H, 10.2; N, 20.3 %); δ_H(400 MHz, D_2O) and δ_C(100 MHz, $CDCl_3$) see Table 1. NMR assignments of monomeric (**1b**) and trimeric (**1a**) pyrroline have been further substantiated by COSY, HETCOR, DEPT and spin decoupling experiments; *m/z* (EI) 69 (M^+, 89%), 68 (31), 67 (3), 54 (6), 42 (42, M - HCN), 41 (100, M - C_2H_4), 40 (15), 39 (28). *m/z* (FAB, 3-nitrobenzyl alcohol + 1 % TFA) 70.1 ($[M + H]^+$), 206.1 (trimeric pyrroline, $[M + H - H_2]^+$).

Purification of Δ^1-pyrroline from its bis(Δ^1-pyrroline)diiodozinc complex proved unsuccessful. Liberation of Δ^1-pyrroline from the complex was not possible, which was ascribed to the low vapour pressure of complex. The *zinc-complex* was prepared from purified Δ^1-pyrroline (**1b**) by a method reported by Baxter *et al.*.[16]

ZnI_2 (1.192 g, 3.73 mmol) is added to a solution of Δ^1-pyrroline (**1b**) (0.517 g, 7.48 mmol) in dry ether (100 mL), and stirred for 2 h at 30 °C, before being cooled to 0 °C. The beige precipitate was filtered off (1.32 g, 40 %), washed with ether and recrystalized from $CHCl_3$/Heptane to give the pyrroline complex as colourless crystals mp 203–205 °C (from $CHCl_3$/*n*-C_7H_{16}); (Found: C, 21.05; H, 2.9; N, 5.9. $C_8H_{14}N_2ZnI_2$ requires C, 21.0; H, 3.1; N, 6.1 %); δ_H(400 MHz, $CDCl_3$) 2.08 (4 H, quintet, *J* 7.9, H-4), 2.88 (4 H, br t, *J* 7.9, H-3), 4.02 (4 H, br t, *J* 6.6, H-5), 8.29 (2 H, br s, H-2); δ_C(100 MHz, $CDCl_3$) 20.1 (C-4), 36.7 (C-3), 59.2 (C-5), 177.5 (C-2).

_N_-Butyl-4-aminobutyraldehyde diethyl acetal

A solution of 4-chlorobutyraldehyde diethyl acetal (4.60 g, 0.025 mol) in butylamine (40 mL, 0.4 mol) is refluxed (77 °C) for 24 h. Cyclohexane (200 mL) is added, and excess butylamine is removed as the azeotrope with cyclohexane (60/40) until the bp of the mixture rises to 81 °C (after 150 - 200 mL destillate has been collected). After cooling, the resulting slurry of precipitated hydrochloride in cyclohexane is added 2.5 M NaOH (20 mL) and extracted with cyclohexane (2 × 25 mL). The combined organic extracts are washed successively with 5 % $NaHCO_3$ (2 × 25 mL), brine (2 × 25 mL) and finally dried over $MgSO_4$. Evaporation of the solvent followed by destillation of the residue (75 °C / 0.4 mbar) yields the desired product (4.83 g, 87 %) as a colourless oil (Found: C, 66.0; H, 12.4; N, 6.5. $C_{12}H_{27}NO_2$ requires C, 66.3; H, 12.5; N, 6.4 %); δ_H(400 MHz, $CDCl_3$) 0.84 (3 H, *J* 7.3, H-9), 1.13 (6 H, t, *J* 7.1, CH_3), 1.22 (1 H, br s, N*H*), 1.27 (2 H, m, *J* 7.4, H-8), 1.40 (2 H, m, H-7), 1.50 (2 H, m, H-3), 1.57 (2 H, m, H-2), 2.53 (2 H, t, *J* 7.1, H-6), 2.55 (2 H, t, *J* 7.1, H-4), 3.42 and 3.57 (4 H, dq, *J* 9.5 and 7.1, C*H*$_2$), 4.42 (1 H, t, *J* 5.5, H-1); δ_C(100 MHz, $CDCl_3$) 13.8 (C-9), 15.1 (CH_3), 20.3 (C-8), 25.2 (C-3), 31.3 (C-2), 32.1 (C-7), 49.5 and 49.6 (C-6 and C-4), 60.7 (CH_2), 102.6 (C-1); *m/z* (FAB, 3-nitrobenzyl alcohol) 218.2 ($M + H^+$); *m/z* (EI) 188 (95%, $M - C_2H_5$), 172 (28, $M - C_2H_5O$), 126 (86), 103 (25), 99 (30), 86 (73, $M - CH_2CH_2CH(OEt)_2$), 84 (100), 71 (35).

_N_-Butyl-4-aminobutyraldehyde hydrochloride (3)

N-Butyl-4-aminobutyraldehyde diethyl acetal (0.505 g, 2.32 mmol) was hydrolysed in water (20 mL), with 1 M HCl (2.53 mL, 2.53 mmol, 9 % excess) for 25 min at rt, before the entire content was lyophilized for two days to give hygroscopic white amorphous crystals, which were used without further purification. The *aminoaldehyde* analysed as $C_8H_{16}NCl$ with varying amounts of water. (Found: C, 57.4; H, 10.1; N, 8.4. $C_8H_{16}NCl$ + 3.5 % H_2O requires C, 57.35; H, 10.0; N, 8.4%); δ_H(400 MHz, D_2O) see entry **3c** in table 1; δ_C(100 MHz, D_2O): 12.5 (C-4′), 18.7 and 19.1 (C-4 and C-3′), 27.9 (C-2′), 36.4-(C-3), 53.6 (C-1′), 58.6 (C-5), 180.2 (C-2); *m/z* (FAB, 3-nitrobenzyl alcohol) 126.1 (M^+).

The pH equilibrium of Δ^1-pyrroline investigated by ^{1}H NMR spectroscopy

The samples (0.6 mL) were prepared in NMR tubes using the following procedure, which ensures uniformity. A stock solution was prepared by dissolving Δ^1-pyrroline (30 mg, 0.434 mmol) in D_2O (9.7 mL) and adding DSS in D_2O (50 mM, 0.1 mL). Half of the sample (4.9 mL) is transferred to a beaker fitted with the pH electrode and DCl is added (20 %, 0.1 mL) to give a total volume of 5.0 mL. Next, the pD (close to 1) is measured accurately, 0.6 mL is placed in a NMR tube, and the ^{1}H NMR spectrum recorded. D_2O (0.1 mL) is added to the remaining stock solution to a total volume of 5.0 mL. Transferring 0.6 mL of the stock solution to the beaker leaves the concentration unhanged and restores the volume to

5.0 mL. The pD can now be adjusted to a higher value with 12 M NaOD in D_2O using capillary tubes without changing the volume significantly. Again, 0.6 mL is placed in a NMR tube, and the ^{1}H NMR spectrum recorded. By repeating this procedure, ^{1}H NMR spectra covering the pD range 1-12 may be recorded. ^{1}H NMR spectra of the samples were recorded within 3 hours after preparation using 40 scans per spectrum. DSS (0.5 mM) is used as internal standard and the largest peak arising from DSS is referenced at 0.00 ppm. DSS is preferred to the readily available solvent peak HDO because of the narrow linewidth and chemical shift insensitivity to pH in the range 2 – 11.[17] pD values are calculated by adding 0.40 units[18] to the pH meter reading, obtained from a standard pH-meter calibrated to measure pH in aqueous solutions. One of the alkaline samples was titrated to an acidic pD. The spectrum proved identical to that recorded for the acidic range demonstrating that the equilibrium is reversible.

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