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5-AMINO-6-HYDROXY-3,4,5,6-TETRAHYDROPYRAN-2-ONE (HAT): A STABLE, CYCLIC FORM OF GLUTAMATE 1-SEMIALDEHYDE, THE NATURAL PRECURSOR FOR TETRAPYRROLES

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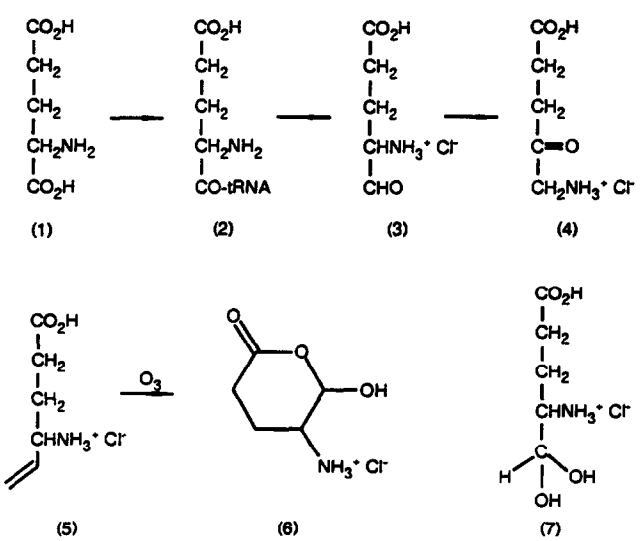
Abstract *Glutamate 1-semialdehyde is shown to exist in a cyclic form, 5-amino-6-hydroxy-3,4,5,6-tetrahydropyran-2-one (HAT), which explains its unexpectedly high stability for an α-aminoaldehyde. It is proposed that the cyclic form is the actual intermediate in the biosynthesis of tetrapyrroles.*

It is now firmly established that L-glutamate (1) is the precursor for 5-amino-laevulinic acid (4) in higher plants, algae and many bacteria[1,2]. Glutamate is first enzymically coupled to transfer-RNA_{glu}[3] and the resulting L-glutamyl-$tRNA_{glu}$ ester (2) is reduced by an NADPH-dependent reductase[4]. The product of this reaction has been proposed as L-glutamate 1-semialdehyde (3) which is finally transformed into 5-amino-laevulinic acid by a specific aminotransferase enzyme[5] (Scheme 1).

Scheme 1. Transformation of L-glutamic acid into 5-aminolaevulinic acid



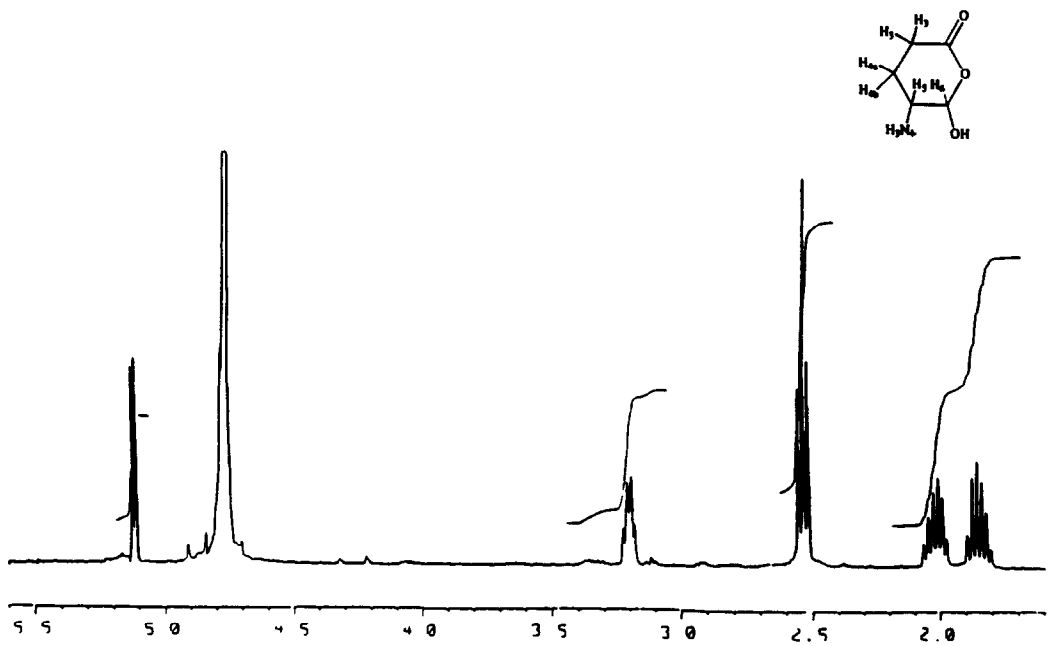
The involvement of L-glutamate 1-semialdehyde as an intermediate is unexpected since α-aminoaldehydes of the natural amino acids are notoriously unstable having long been sought, but never previously isolated[6]. This paper describes investigations which lead to the conclusion that L-glutamate 1-semialdehyde exists preferentially as a stable six-membered ring structure, 5-amino-6-hydroxy-3,4,5,6-tetrahydropyran-2-one (HAT) (6) rather than an open chain free α-aminoaldehyde (3), or its hydrated equivalent (7).

The putative α-aminoaldehyde was prepared by the ozonolysis of racemic 4-amino-hex-5-enoic acid (5) followed by purification using Dowex 50W-X8 resin in dilute HCl[6]. A meringue-like solid was obtained, after freeze-drying the acidic solution of the purified material, that gave a single ninhydrin-positive spot (R_f = 0.5) on silica tlc using *n*-BuOH:AcOH:H_2O (6:2:2 v/v/v) as the solvent (R_f of glutamate = 0.25). The R_f indicated a much more non-polar compound than expected for an open chain hydrated α-aminoaldehyde of glutamic acid which had been proposed[6].

Ir spectroscopy (KBr disc) revealed no aldehyde or acid stretching frequencies but maxima at 3400-3000cm^{-1} (broad NH_3^+ and -OH stretchings); 1731cm^{-1} (6-membered cyclic ester C=O stretching similar to open chain ester); 1600 and 1500cm^{-1} ($-NH_3^+$ bending); 1202cm^{-1} (C-O stretching).

^{1}H Nmr spectroscopy (400MHz) in D_2O revealed a spectrum (figure 1) with H_6 δ=5.20 (1H;d;J=5Hz); H_5 δ=3.27 (1H;m); H_{4a} and H_{4b} δ=1.85 and δ2.05 (2H;m); H_3 δ=2.60 (2H; broad t).

Figure 1. The 400MHz ^{1}H nmr spectrum of the cyclic form of glutamate 1-semialdehyde 5-amino-6-hydroxy-3,4,5,6-tetrahydropyran-2-one (HAT) in D_2O

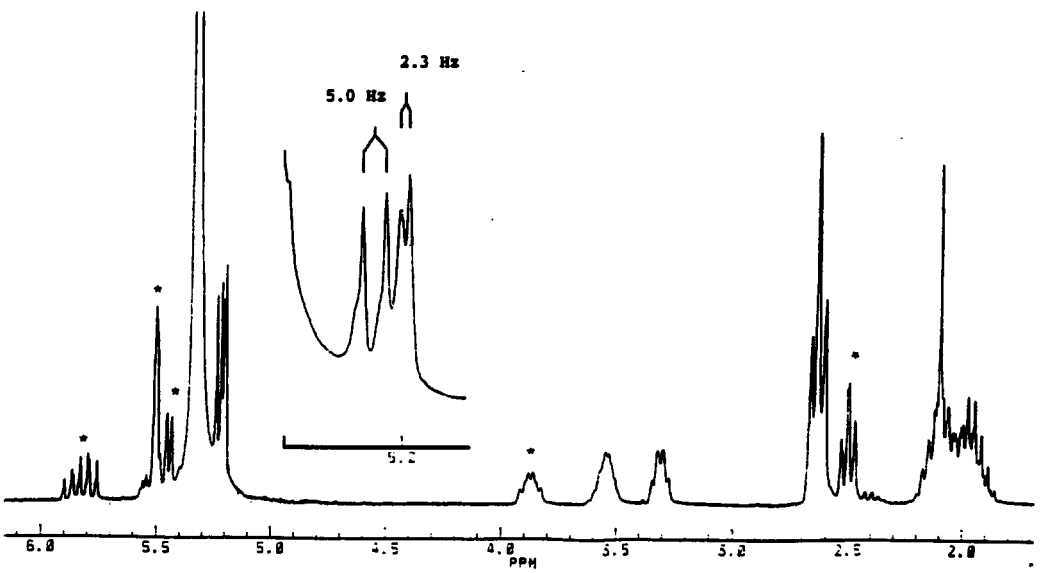


The signals of the H_4 protons, which are at widely different chemical shifts indicate a degree of asymmetry expected for a restricted conformation such as a ring structure (6). It is interesting that each of the two methylene hydrogens adjacent to the chiral centre in glutamate anhydride, also a six membered ring structure, possess chemical shifts similar to those of (6) above (data not shown).

^{13}C Nmr at natural abundance in D_2O (62.89 MHz) revealed signals at δ=179.971 (C=O); δ=91.079 (-OCHOH); δ=58.110 ($-CHNH_2$); δ=32.756 ($-CH_2-$) and δ=26.207 ($-CH_2-$) also consistent with the cyclic structure (6). The chemical shifts around δ=90 are in the region of those found for C1 of glucosamine, a cyclic α-aminoaldehyde stabilised as a pyranose ring.

Additional evidence for a cyclic tetrahydropyranone structure (6) was obtained from an *in situ* ^1H n.m.r. study (*ca.* 0.1M DCl/D_2O) that indicated the presence of two sets of peaks immediately after ozonolysis. One of the sets (for H_5) is at δ=3.54 (broad quartet) and δ=3.30 (broad peaks) and the other (for H_6) is at δ=5.23 (doublet; J=5Hz) and δ 5.20 (doublet;J=2.3Hz) as shown in figure 2.

Figure 2. *In situ* 250MHz ^1H nmr measured during the ozonolysis of 4-aminohex-5-enoic acid to the anomers of 5-amino-6-hydroxy-3,4,5,6-tetrahydropyran-2-one in 0.1M DCl/D_2O. The nmr signals from the starting material are indicated *.



The two sets of signals shown in figure 2 are attributed to the presence of a pair of anomers for each of the D- and L-enantiomers, with *5R,6R* and *5R,6S* configurations and *5S,6S* and *5S,6R* configurations respectively. The coupling constants indicate that the protons (H_5 and H_6) occupy both *cis* and *trans* configurations in the initial mixture of products. Had the structure been a non-cyclic hydrated aldehyde with a single chiral

centre at $C5^6$, no such spectrum would have been observed. After storage for several days at 0°C, the sample gave the spectrum shown in figure 1, in which the H_6 is made up exclusively of the doublet with the coupling constant of J=5Hz, consistent with a single anomeric cyclic species.

The cyclic stucture of glutamate 1-semialdehyde was finally confirmed by electro-spray mass spectrometry in aqueous solution which indicated a species with a mass of 132 (data not shown). This mass corresponds either to the free α-aminoaldehyde (3) or the cyclic structure (6). Since no nmr evidence for the presence of the free aldehyde was obtained, it follows that the species of mass 132 represents the cyclic form (6).

The cyclic structure (6) satisfactorily explains the unexpectedly high stability of glutamate 1-semialdehyde. It is interesting that glucosamine, another naturally occurring α-aminoaldehyde, is stabilised in a cyclic form in which the reactive aldehyde is masked as a hemiacetal. The existence of a cyclic form of glutamate 1-semialdehyde suggests that (6) may be the product of glutamate-tRNA reductase and the substrate for glutamate 1-semialdehyde aminotransferase (1,2-aminomutase). Thus the involvement of the highly reactive ring-opened compound in solution could be avoided. Whilst the data above provide strong evidence for the existence of the cyclic form of glutamate 1-semialdehyde, it does not allow the stereochemistry of the hydrogen atoms at the 5- and 6-positions to be determined with certainty and further work is under way to resolve this matter.

Acknowledgements

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