Conservation of the Carbon–Nitrogen Bond of Aspartic Acid in the Biosynthesis of 3-Nitropropanoic Acid

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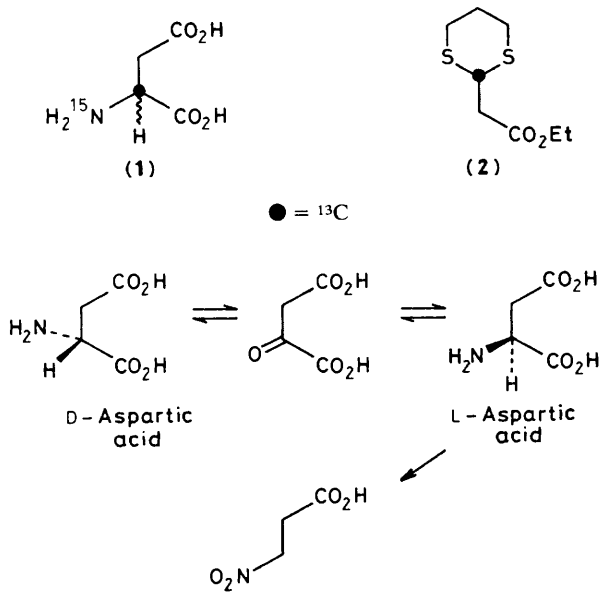
[2-^{13}C,^{15}N]Aspartic acid, prepared by a modified Strecker procedure from ethyl 3-[3-^{13}C]oxopropanoate, was incorporated into 3-nitropropanoic acid by *Penicillium atrovenetum* with retention of the integrity of the ^{13}C–^{15}N bond.

3-Nitropropanoic acid is a toxic metabolite produced by plants of the family *Fabaceae*[1] and by a number of fungi.[2] The compound has been shown to be a potent inhibitor of several mammalian enzymes.[3] Despite the structural simplicity of the metabolite various aspects of its biosynthesis are uncertain. Early investigations using ^{14}C-labelled precursors[4—7] indicated that in *Penicillium atrovenetum* the 2, 3, and 4 carbon atoms of L-aspartic acid, but not of the D-isomer,[7] were directly incorporated into positions 3, 2, and 1 of the propionate skeleton. The suggestion by Birch and his co-workers[4] that the nitro group arises by *in situ* oxidation of the amino group of L-aspartate remains controversial however in



Scheme 1

view of the pivotal position occupied by aspartate in nitrogen metabolism. While labelled nitrogen from [^{15}N]aspartate is incorporated into the nitro group of 3-nitropropanoic acid,[5,7] administered ammonium ion is also efficiently incorporated.[7] On the basis of these results it is not possible to distinguish between *intact* incorporation of the nitrogen of aspartate, or *indirect* incorporation in which aspartate acts either as a specific amino group donor or merely as an intracellular source of ammonium ions. In this communication we describe the labelling of 3-nitropropanoic acid derived from [2-^{13}C,^{15}N]-aspartic acid which unambiguously demonstrates the conservation of the C–N bond of aspartic acid during biosynthesis.

DL-[2-^{13}C,^{15}N]aspartic acid (**1**) was synthesised by a route starting with [^{13}C]paraformaldehyde which was converted *via* [2-^{13}C]-1,3-dithiane[8] into ethyl [2-^{13}C]-1,3-dithian-2-ylacetate (**2**) using established procedures.[9] Although the corresponding free aldehyde, ethyl 3-oxopropanoate, rapidly polymerises under mildly basic conditions and was thus an unsuitable substrate for amino acid synthesis under normal Strecker conditions[10] it can be readily trapped as its cyanohydrin by treatment with aqueous NaCN at pH 4 and 0 °C. Thus (**2**) was deprotected by treatment with *N*-bromosuccinimide,[11] the product was converted into its cyanohydrin, and this was immediately treated, without purification, with $^{15}NH_4Cl$ in 2 M NaOH. Acid-catalysed hydrolysis of the resultant ^{13}C,^{15}N-enriched aminonitrile followed by cation-exchange chromatography afforded (**1**) [δ_C(D_2O–DCl; pH 1) 49.2 (d, $^1J_{CN}$ 7.0 Hz, C-2)].

Surface cultures of *P. atrovenetum* grown in an ammonium-rich medium (95 mM NH_4^+)[12] were fed with a mixture of the sodium salts of DL-[2-^{13}C,^{15}N]aspartic acid (16 mg) and L-[U-^{14}C]aspartic acid (0.58 μCi, 225 mCi/mmol) which was administered in equal aliquots 48, 60, and 72 h after inoculation.

In the ^{1}H-decoupled ^{13}C n.m.r. spectrum of the isolated 3-nitropropanoic acid (specific activity 3.0 × 10^5 d.p.m./mmol) the signal corresponding to the C-3 methylene (δ_C 69.3) was observed as a composite of a broad singlet ($w_{\frac{1}{2}}$ 1.8 Hz, ^{13}C–^{14}N) and a sharp doublet ($^1J_{CN}$ 8.7 Hz, ^{13}C–^{15}N) (Figure 1). The observation of the doublet, offset to lower

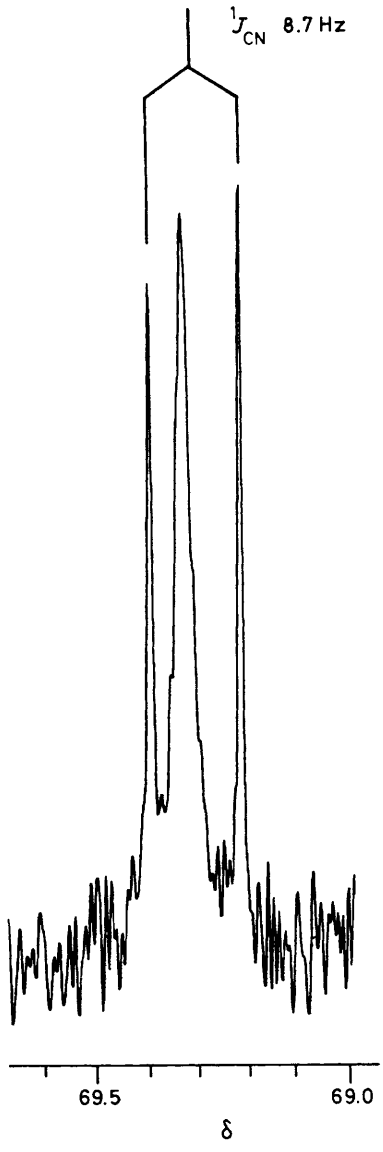


Figure 1. The C-3 methylene region of the 50 MHz ^{13}C{^{1}H} n.m.r. spectrum of 3-nitropropanoic acid biosynthetically derived from DL-[2-^{13}C,^{15}N]aspartic acid (**1**): spectral width 1000 Hz, acquisition time 8.2 s, 668 transients. No weighting factor was applied to the free induction decay prior to Fourier transformation.

frequency as a result of the ^{15}N isotope shift, is entirely consistent with intact incorporation of the 'labelled' ^{13}C–^{15}N bond of enriched aspartate (Figure 1). Correlation of the doublet–singlet integral ratio observed for the C-3 methylene with the 58-fold dilution[13] of ^{14}C from administered L-(U-^{14}C]aspartate indicates an upper limit for exchange of ^{15}N with ^{14}N by transamination prior to incorporation of *ca.* 20%. This figure must also include any contribution for incorporation of ^{13}C from D-[2-^{13}C,^{15}N)aspartate *via* [2-^{13}C]oxaloacetate (Scheme 1). Furthermore, the lack of significant enhancement of the C-2 (δ_C 30.7) or C-1 (δ_C 174.0) signals over natural abundance eliminates the possibility of either equilibration of enrichment of the C-2 and C-3 positions of oxaloacetate through malate dehydrogenase and fumarase activities or randomisation through the tricarboxylic acid cycle.

This result shows unequivocally that in the biosynthesis of 3-nitropropanoic acid from L-aspartate the amino group of

L-aspartate is *directly* incorporated and oxidised *in situ*. The nature of the decarboxylation and oxidation processes are, however, unkown and are receiving attention.[14]

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