Stereochemistry of the Reduction of Nicotinic Acid when It Serves as a Precursor of Anatabine

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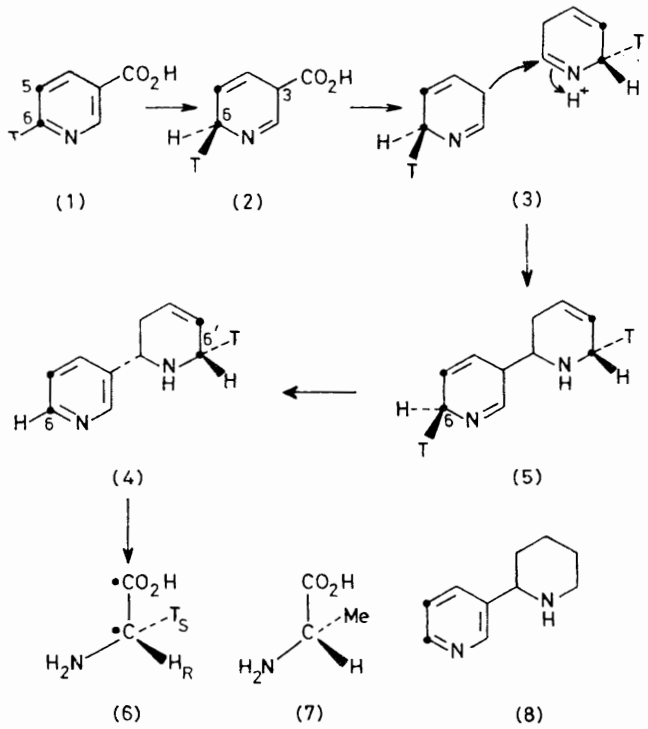
Summary Anatabine derived from [5,6-^{14}C,^{13}C,6-^{3}H]-nicotinic acid (which is a precursor of both rings of the alkaloid) was almost devoid of tritium in its pyridine nucleus, but retained 100% at C-6′; this tritium was in a *pro-S* position, indicating that nicotinic acid was reduced at C-6 by the introduction of a hydrogen in the *pro-R* position.

WE have previously established that both rings of (−)-anatabine (**4**) are derived from nicotinic acid,[1,2] and we have suggested that its biosynthesis, illustrated in the Scheme, involves the dimerization of 2,5-dihydropyridine (**3**), which is formed by the decarboxylation of the hypothetical intermediate, 3,6-dihydronicotinic acid (**2**). We considered that the stereochemistry of the reduction which occurs at C-6 of nicotinic acid could be probed by determination of the chirality at C-6′ of the anatabine which would be formed from [6-^{3}H]nicotinic acid.

Accordingly, a mixture of [5,6-^{14}C,$^{13}C_2$]nicotinic acid[2] and [6-^{3}H]nicotinic acid[3] (^{3}H/^{14}C, 7·0) was fed, by the wick method, to *Nicotiana glauca* plants for seven days. The resultant anatabine was labelled equally in both rings with ^{13}C (determined by ^{13}C n.m.r. spectroscopy[2]) and had ^{14}C activity: $1{\cdot}91 \times 10^{7}$ d.p.m. mmol^{-1} (3·8% specific incorporation), ^{3}H/^{14}C, 3·7 (53% retention of tritium). It was converted into *N*-benzoylanatabine which was oxidized with acidic potassium permanganate as previously described[1] to yield hippuric acid (^{14}C: $9{\cdot}6 \times 10^{7}$ d.p.m. mmol^{-1}, ^{3}H/^{14}C, 6·95, 100% retention of tritium) and nicotinic acid



SCHEME. (Heavy dots indicate position of ^{14}C and ^{13}C).

(^{14}C: $9{\cdot}7 \times 10^7$ d.p.m. mmol^{-1}, $^3H/^{14}C$, 0·52, 93% loss of tritium). The loss of tritium from C-6 of the pyridine ring of anatabine is in accord with previous work on the incorporation of [6-^{3}H]nicotinic acid into nicotine.[3,4] We have rationalized this loss by suggesting[2] that the final step in the biosynthesis of nicotine involves the stereospecific loss of the hydrogen which was originally present at C-6 of nicotinic acid. We presume that the final step in the biosynthesis of anatabine, namely the dehydrogenation of (**5**), is also stereospecific involving the loss of tritium from C-6. Anabasine (**8**), the major alkaloid of *N. glauca* was also isolated (^{14}C: $7{\cdot}33 \times 10^6$ d.p.m. mmol^{-1}, 1·98% specific incorporation), and had $^3H/^{14}C$, 0·37, representing a 95% loss of tritium from C-6.

The hippuric acid obtained from anatabine was hydrolysed with hydrochloric acid to afford glycine ($^3H/^{14}C$, 7·0). Control experiments with [2-^{14}C,^{3}H]hippuric acid indicated that its $^3H/^{14}C$ ratio remained unchanged in the course of the permanganate oxidation and subsequent hydrolysis to glycine. The chirality of the glycine was determined enzymatically.[5] When L-glutamic–pyruvic transaminase (E.C. No 2.6.1.2) which normally catalyses the reversible transfer of an amino group from L-alanine to 2-oxoglutarate, acts upon glycine in the absence of an amino group acceptor it is the *pro-R* hydrogen at C-2 of glycine, corresponding to the α-hydrogen of L-alanine (**7**) which is labilized. When the glycine derived from anatabine was incubated with this enzyme there was no loss of tritium ($^3H/^{14}C$, $6{\cdot}9 \pm 0{\cdot}1$). Non-chiral [2-^{14}C,^{3}H]glycine incubated with this enzyme under identical conditions lost $50 \pm 2\%$ of its tritium relative to the ^{14}C. This enzyme assay was carried out in triplicate. This result indicates that the glycine derived from anatabine was labelled with tritium in the *pro-S* position, as illustrated in structure (**6**). One can thus deduce that the initial reduction of nicotinic acid occurs by introduction of a hydrogen at C-6 in the *pro-R* position as indicated in structure (**2**).

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