Biosynthesis of Anatabine and Anabasine in *Nicotiana glutinosa*

By EDWARD LEETE

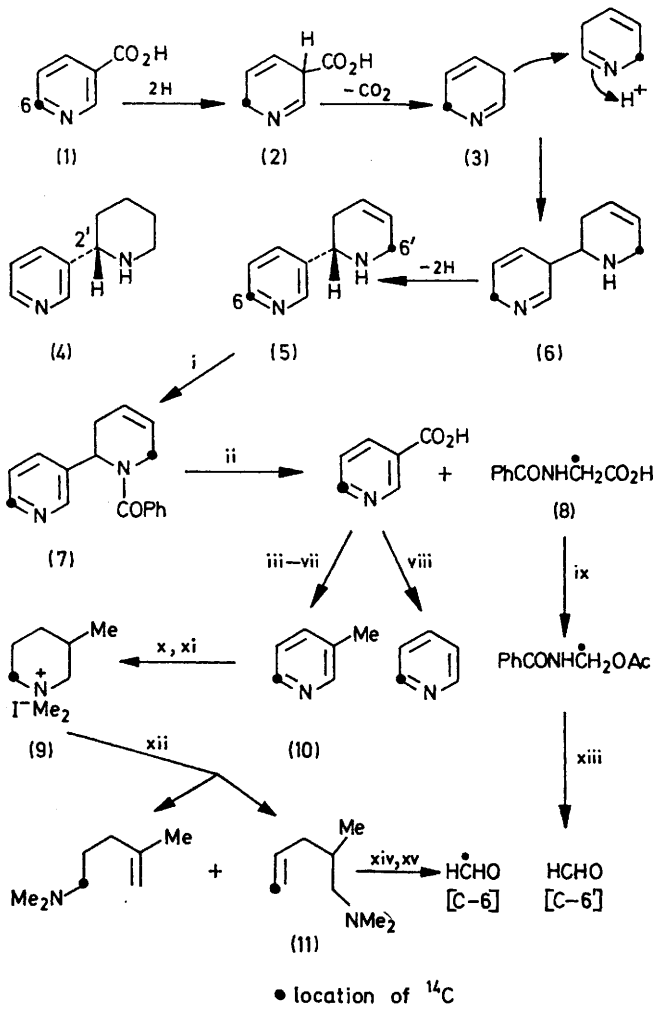
(*Natural Products Laboratory, School of Chemistry, University of Minnesota, Minneapolis, Minnesota*, 55455)

Summary Feeding experiments in *Nicotiana glutinosa* have shown that both [6-^{14}C]nicotinic acid and [2-^{14}C]-lysine are incorporated into anabasine (**4**) whereas only [6-^{14}C]nicotinic acid is incorporated into anatabine (**5**), the activity being equally distributed between the two heterocyclic rings at C-6 and C-6′.

(−)-ANATABINE (**5**) is one of the minor alkaloids of various *Nicotiana* species[1]. Since (**5**) is $\Delta^{4'}$-dehydroanabasine it seemed likely that it would be formed by a biosynthetic route similar to that for anabasine (**4**). Nicotinic acid[2] and lysine[3] are the precursors of the pyridine and piperidine rings respectively of (**4**). When [2-^{14}C]lysine was fed to *N. glutinosa* plants† only anabasine had appreciable radioactivity, and degradation[3] indicated that all the activity was located at C-2′, in agreement with earlier studies in *N. glauca*.[3] The failure of [2-^{14}C]lysine to yield labelled anatabine is consistent with the earlier observations of Kisaki and coworkers.[4] Also the relative specific activities of anabasine and anatabine obtained from *N. glutinosa* plants which had been subjected to short-term $^{14}CO_2$ feeding indicated that anabasine was not a precursor of anatabine.[5] We considered that the piperideine ring of anatabine could be derived from acetate, nicotinic acid serving as a starter unit for a poly-acetyl chain. However the administration of [carboxyl-^{14}C]nicotinic acid, which would be expected to label C-2′ of anatabine, if this hypothesis were correct, failed to label significantly any of the alkaloids of *N. glutinosa*. Since it is well established that the pyridine ring of nicotine and anabasine is derived from the pyridine ring of nicotinic acid, [6-^{14}C]nicotinic acid (**1**)‡ was fed to 3-month-old *N. glutinosa* plants in order to

† 3-month-old specimens of this species contained nicotine, nornicotine, anatabine, and anabasine in the ratio, by wt. of 100:49-:12:2·5.

‡ Commercially available from Amersham-Searle.



SCHEME

i, $(PhCO)_2O$; ii, $KMnO_4$; iii, $SOCl_2$; iv, MeOH; v, $LiAlH_4$; vi, $SOCl_2$; vii, H_2–Pd on $CaCO_3$; viii, CaO, heat; ix, $Pb(OAc)_4$; x, Ht_2–P, dil. HCl; xi, MeI, $NaHCO_3$; xii, AgOH, heat, separate by g.l.c.; xiii, 2N H_2SO_4, distil; xiv, OsO_4, Na_2SO_3; xv, $NaIO_4$.

determine whether the pyridine ring of anatabine was being synthesized from this precursor in plants of this age. After 5 days it was found that all the alkaloids were labelled.§

The radioactive anatabine was diluted with (±)-anatabine[6] and degraded[7] (Scheme). The activities of the degradation products are recorded in the Table, and it is apparent that all the activity of the anatabine is equally divided between C-6 and C-6'.[8] These results are consistent with the biosynthetic pathway illustrated in the Scheme. The pyridine ring of nicotine is derived from nicotinic acid and it is generally agreed[9] that a dihydronicotinic acid is the immediate precursor of the pyridine ring. We now propose that 3,6-dihydronicotinic acid (**2**) is this precursor. Decarboxylation affords 2,5-dihydropyridine (**3**) which can condense with the *N*-methyl-Δ^1-pyrrolinium salt[10] to yield nicotine, or undergo self-condensation to yield a dihydroanatabine (**6**). Dehydrogenation then affords anatabine.

TABLE

Activity of anatabine (derived from [6-^{14}C]nicotinic acid) and its degradation products

	Specific activity/ dpm mM^{-1} × 10^{-7}	Relative specific activity
Anatabine (**5**)	3·72	100
Anatabine dipicrate	3·80	102
N-Benzoylanatabine (**7**)	3·84	103
Hippuric acid (**8**)	1·91	51
Formaldehyde dimedone [C-6']	1·71	46
Nicotinic acid	1·94	52
Pyridine picrate	1·93	52
β-Picoline (**10**)	1·90	51
1,3-Dimethylpiperidine methiodide (**9**)	1·89	51
Formaldehyde dimedone [C-6]	1·64	44

The anabasine (2·34 × 10^7 dpm mM^{-1}) obtained from the [6-^{14}C]nicotinic acid was oxidized with permanganate yielding nicotinic acid (2·36 × 10^7 dpm mM^{-1}), which on decarboxylation yielded pyridine (2·30 × 10^7 dpm mM^{-1}). These results indicate that there is no significant formation of anabasine by the reduction of the labelled anatabine.

It is suggested that anatalline (2,4-di(3-pyridyl)-piperidine)[4] and nicotelline (2,4-di(3-pyridyl)-pyridine)[11] are trimers of (**3**). These compounds may be artifacts produced by non-enzymic reactions which could occur during the harvesting of tobacco. The lack of optical activity in the isolated anatalline[4] supports this hypothesis.

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§ The ratio of the specific activities of the isolated anatabine, nicotine, anabasine, and nornicotine were 100 : 73 : 63 : 20 respectively.

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