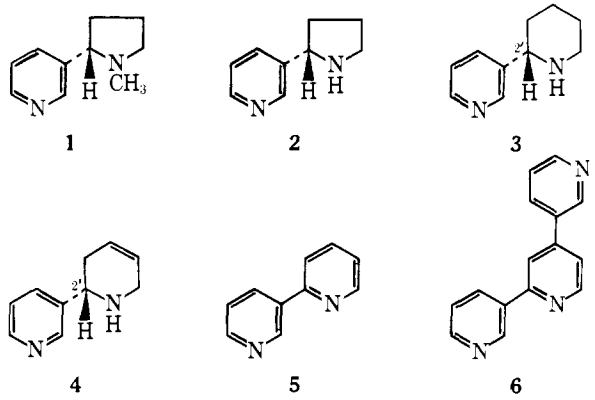
Incorporation of [2-^{14}C]- and [6-^{14}C]Nicotinic Acid into the Tobacco Alkaloids. Biosynthesis of Anatabine and α,β-Dipyridyl[1]

Edward Leete* and Sheila A. Slattery

Contribution No. 142 from the Natural Products Laboratory, School of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received March 11, 1976*

Abstract: The administration of [6-^{14}C]nicotinic acid to *Nicotiana glutinosa* plants yielded labeled anatabine which was subjected to a systematic degradation and found to have equal labeling at C-6 and C-6′. Similarly [2-^{14}C]nicotinic acid when fed to *Nicotiana tabacum* afforded [2,2′-^{14}C]anatabine. Since equal labeling was found in both rings of anatabine in several feeding experiments carried out under widely different conditions, it is suggested that the biosynthesis of anatabine involves the dimerization of a metabolite of nicotinic acid, possibly 2,5-dihydropyridine. The anabasine isolated from these feeding experiments was labeled only on its pyridine ring, indicating that anabasine is not formed by reduction of anatabine, in agreement with earlier observations. A different distribution of activity was found in the alkaloids isolated from fresh plants compared with plants which were allowed to dry in air for several weeks. In the species examined, *N. glutinosa* and *N. tabacum*, the most dramatic change was an increase in the level of activity in nornicotine compared with nicotine. In *N. tabacum* the amount of activity in α,β-dipyridyl was significantly greater in the air-dried plants. Degradations indicated that both rings of this alkaloid were labeled, indicating that it was not formed by the oxidation of anabasine, but could be derived from anatabine.

The general biosynthetic routes to the tobacco alkaloids nicotine (**1**), nornicotine (**2**), and anabasine (**3**) are now fairly well established.[2] However, no definitive work has been published on the biosynthesis of anatabine (**4**)[3], α,β-dipyridyl (**5**),[4] or nicotelline (**6**),[5] which are minor alkaloids found in various



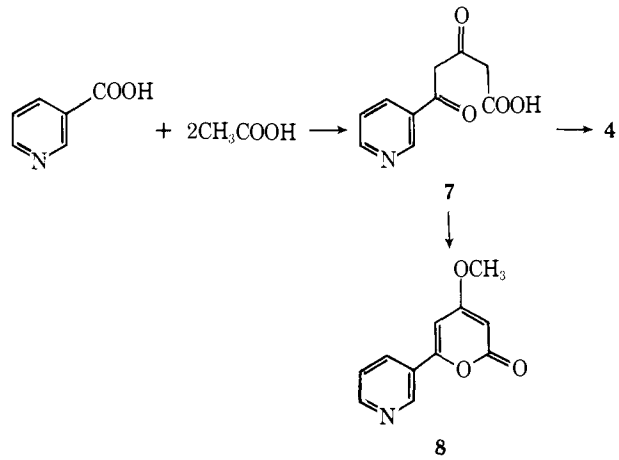
1 **2** **3** **4** **5** **6**

Nicotiana species. Tso[6] has examined the incorporation of $^{14}CO_2$, $^{3}H_2O$, and [^{15}N]nitrate into anabasine, anatabine, nicotine, and nornicotine in *Nicotiana rustica*. Significant activity was found in anatabine; however, the nature of these labeled precursors does not enable one to draw any conclusions about the biosynthetic origin of this alkaloid. In an experiment which involved the feeding of [^{15}N]anabasine to *N. rustica*, no significant incorporation of ^{15}N into anatabine was observed.[7] Alworth and Rapoport[8] studied the biosynthesis of the alkaloids of *Nicotiana glutinosa* by exposing the plants to $^{14}CO_2$ for varying lengths of time. In all cases the specific activity of the isolated anatabine was either equal to, or greater than, the specific activity of the anabasine. Since anatabine is about six times more abundant than anabasine in *N. glutinosa*, these results indicate that anabasine cannot be a precursor of anatabine.

It is well established that the piperidine ring of anabasine is derived from lysine,[9] and it seemed reasonable to expect that the piperideine ring of anatabine might also be derived from this amino acid. However, the administration of DL-[2-^{14}C]lysine to *N. glutinosa* plants did not result in significant labeling of anatabine. Anabasine was labeled, and degradations[9a] indicated that all its activity was located at C-2′, in agreement with earlier studies in *Nicotiana glauca*.[9] Kisaki et al.[10] also failed to obtain labeled anatabine when either [2-^{14}C]lysine or [2-^{14}C]-4-hydroxylysine was fed to tobacco.

We then considered that perhaps the piperideine ring of anatabine was derived from acetate, the carboxyl group of nicotinic acid serving as a "starter unit" for a polyacetyl chain, as illustrated in Scheme I. Such a derivation of the piperideine

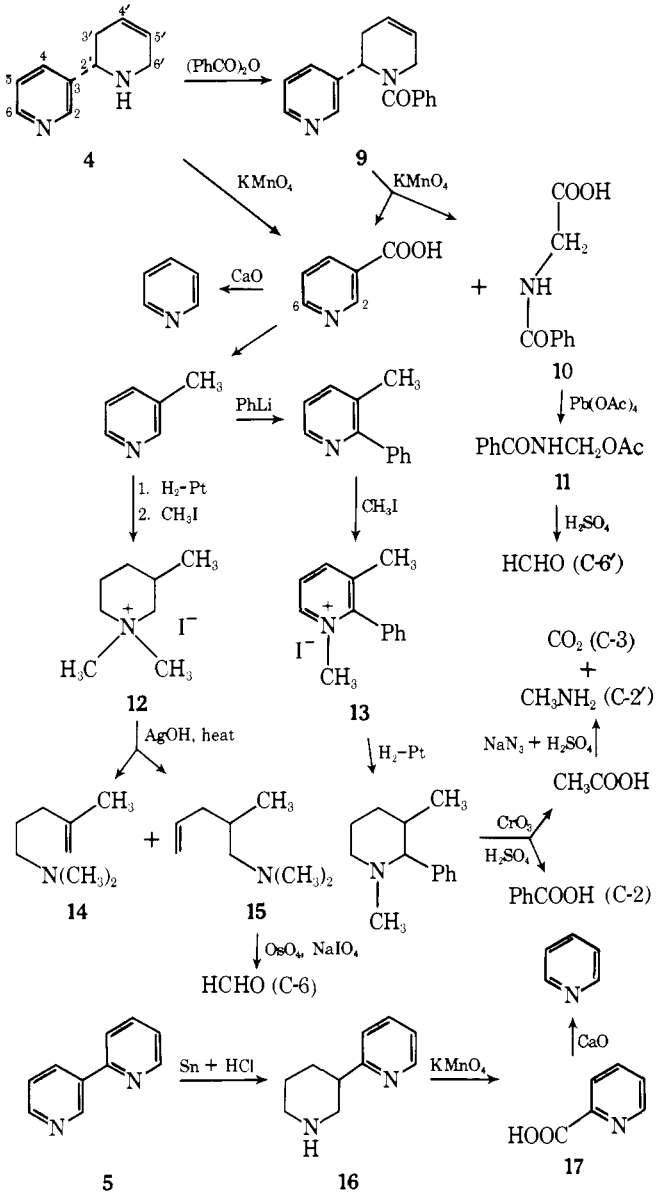
Scheme I. Hypothetical Biosynthesis of Anatabine and Anibine from Nicotinic Acid and Acetate



ring would have analogy in the formation of the hemlock alkaloids.[11] An intermediate such as **7** would also seem to be a probable precursor of the alkaloid anibine (**8**). 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*.

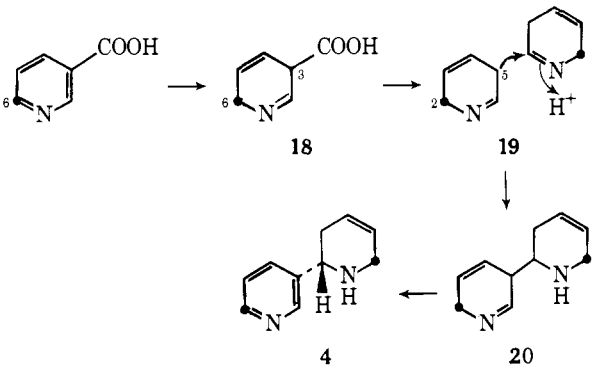
Since nicotinic acid is an established precursor of the pyridine ring of nicotine and anabasine, it seemed reasonable to assume that the pyridine ring of anatabine would also be derived from this precursor. We thus fed [6-^{14}C]nicotinic acid to *N. glutinosa* plants to determine whether anatabine was being synthesized in the 3-4-month-old plants which we were using in our experiments. To our delight labeled anatabine was obtained; indeed, the specific incorporation of activity into anatabine was higher than into any of the other alkaloids.[12] The labeled anatabine was degraded as illustrated in Scheme II. Benzoic anhydride in ether afforded *N*-benzoylanatabine

Scheme II. Degradation of Anatabine and α,β-Dipyridyl



(**9**)[3a] which was oxidized with permanganate, yielding a mixture of nicotinic acid and hippuric acid (**10**).[3a] The latter compound was oxidized with lead tetraacetate in acetic acid yielding 1-acetoxy-1-benzamidomethane (**11**) and carbon dioxide.[13] Distillation of this ester with dilute sulfuric acid yielded formaldehyde, collected and assayed as its dimedone derivative. The nicotinic acid was reduced to β-picoline as previously described.[14] Reduction and methylation then yielded 1,3-dimethylpiperidine methiodide (**12**), which was subjected to a Hofmann elimination affording a 1:19 mixture of 2-methyl-5-dimethylamino-1-pentene (**14**) and 4-methyl-5-dimethylamino-1-pentene (**15**) which was separated by gas chromatography.[15] The isomer **15** was oxidized to the 1,2-diol with osmium tetroxide and then cleaved with sodium metaperiodate affording C-6 as formaldehyde. The activity of anatabine and its degradation products is recorded in Table I (experiment 1), and it is apparent that all the activity of the alkaloid is located at C-6 and C-6′ and is equally divided between these positions.[16] This unexpected labeling of both rings led us to propose that the biosynthesis of anatabine involves the dimerization of 2,5-dihydropyridine (**19**) as illustrated in Scheme III. We suggest that nicotinic acid is reduced to 3,6-dihydronicotinic acid (**18**). This compound, being a β-imino acid, would be expected to readily decarboxylate yielding

Scheme III. Hypothetical Biosynthesis of Anatabine (the pattern of Labeling after Feeding [6-^{14}C] Nicotinic Acid Is Indicated)



19. Self-condensation of this dihydropyridine as illustrated would afford **20**, which on dehydrogenation yields anatabine. 2,5-Dihydropyridine is one of the five dihydropyridines which is theoretically capable of existence. So far only 1,2- and 1,4-dihydropyridine have been prepared.[17] However, molecular orbital calculations by the MINDO/3 method[18] indicate that the 2,5 isomer will have reasonable stability, and attempts are being made to synthesize it by unequivocal methods. This biosynthetic scheme for anatabine was conceived, bearing in mind that equal labeling of the two rings was obtained after feeding labeled nicotinic acid. We realized that this equal labeling may have been a consequence of the long feeding time (5 days) during which the various metabolic pools, which were a source of the two rings of anatabine, became labeled to the same level. One could thus imagine that anatabine is formed by a condensation between 3,6-dihydronicotinic acid and 2,5-dihydropyridine, followed by subsequent decarboxylation and dehydrogenation.[19] If this were the case a shorter feeding time, with different amounts of nicotinic acid, might result in unequal labeling of the two rings of anatabine. However, the anatabine isolated from *N. glutinosa* plants fed [6-^{14}C]nicotinic acid for only 20 h (experiment 2) was found to have equal labeling in the two rings. Radioactive anatabine was isolated from *N. tabacum* plants which had been fed [2-^{14}C]nicotinic acid (experiment 7). In this case activity was expected at C-2 and C-2′ of the alkaloid. Activity at C-2′ was determined by decarboxylation of the nicotinic acid obtained by the permanganate oxidation of anatabine. Activity at C-2 of the nicotinic acid was determined as illustrated in Scheme II, details of this degradation having been previously described.[14] Again, within experimental error, equal labeling of the two rings, in the expected positions, was observed (Table I). Our results thus remain consistent with the biosynthetic sequence shown in Scheme III.

It should be mentioned that the anabasine obtained from these feeding experiments was also degraded (see Experimental Section) and found to have negligible activity in its piperidine ring. Thus there is no significant formation of anabasine by the reduction of anatabine, in agreement with earlier observations.[7,8]

We then turned our attention to the origin of α,β-dipyridyl and nicotelline. The amounts present in the plants were not sufficient for direct isolation, so carrier amounts of these alkaloids were added at the time of extracting the plants. A significant amount of activity was detected in the reisolated α,β-dipyridyl (experiment 3, Table III); however, the activity in the nicotelline was negligible. It has been suggested[5b] that nicotelline is an artifact of isolation. One could imagine that nicotelline is a trimer of a dihydropyridine, perhaps even produced by nonenzymic reactions. We thus decided to examine the distribution of activity in the alkaloids isolated from fresh plants and to compare with the alkaloids obtained from plants which were allowed to dry at room temperature for

Table I. Activity of Anatabine and Its Degradation Products

	Expt 1 with [6-^{14}C]nicotinic acid		Expt 2 with [6-^{14}C]nicotinic acid		Expt 7 with [2-^{14}C]nicotinic acid	
	Spec act. dpm/mmol × 10^{-7}	Rel spec act.	Spec act. dpm/mmol × 10^{-6}	Rel spec act.	Spec act. dpm/mmol × 10^{-7}	Rel spec act.
Anatabine (**4**)	3.72	100	6.10	100	1.48	100
Anatabine dipicrate	3.80	102	6.15	101	1.48	100
N-Benzoylanatabine (**9**)	3.84	103				
Hippuric acid	1.91	51	3.05	50		
Formaldehyde dimedone (C-6′)	1.71	46	3.03	50		
Nicotinic acid	1.94	52	3.00	49	1.46	99
Pyridine picrate	1.93	52	3.00	49	0.76	51
(C-2′) by difference	0.01	<1			0.70	47
β-Picoline oxalate	1.90	51			1.55	105
1,3-Dimethylpiperidine methiodide (**12**)	1.89	51				
Formaldehyde dimedone (C-6)	1.64	44				
1,3-Dimethyl-2-phenylpyridinium iodide (**13**)					1.50	101
Benzoic acid (C-2)					0.80	54
N-Methylbenzamide (C-2′)					0.71	48
Barium carbonate (C-3)					0.02	1

Table II. Details of the Feeding Experiments to *Nicotiana* Plants

		Nicotinic acid fed							
Expt no.	*Nicotiana* species	[^{14}C] location	mmol	Spec act., dpm/mmol	Month fed[a]	Duration of feeding	Fresh wt of plants, g	Wt of air-dried plants, g	% abs incorpn into alkaloids[d]
1	*glutinosa*	6	0.0028	9.9×10^{10}	June	5 days	310		4.72
2	*glutinosa*	6	0.11	1.59×10^{9}	Oct	20 h	320		1.14
3	*glutinosa*	6	0.085	2.67×10^{9}	Dec	3 days	640		1.59
4	*glutinosa*	2	0.127	8.05×10^{8}	July	2 days	765		0.0052
5	*glutinosa*	2	0.127	8.05×10^{8}	July	2 days	765	96[b]	0.0015
6	*tabacum*	2	0.245	8.05×10^{8}	March	7 days	1130		2.40
7	*tabacum*	2	0.245	8.05×10^{8}	March	7 days	1150	95[c]	1.68
8	*glauca*	2	0.245	8.05×10^{8}	March	7 days	1145		14.9

[a] All experiments except 4 and 5 were carried out in a greenhouse. Experiments 4 and 5 were done on plants which had been growing out of doors for 2 months. All plants were 3–4 months old at the time of feeding. [b] Air-dried for 40 days. [c] Air-dried for 32 days. [d] This is defined as the total activity found in the isolated alkaloids divided by the total activity fed to the plants. In the preliminary experiments with *N. glutinosa*, the administration of DL-[2-^{14}C]lysine and [*carboxyl*-^{14}C]nicotinic acid resulted in absolute incorporations of 0.017 and 0.0014%, respectively.

Table III. Weights and Activities of the Alkaloids Isolated

	Nicotine			Nornicotine			Anabasine			Anatabine				
Expt no.	Wt, mg	Spec incorpn, %[a]	Abs incorpn, %	Wt, mg	Spec incorpn, %	Abs incorpn, %	Wt, mg	Spec incorpn, %	Abs incorpn, %	Wt, mg	Spec incorpn, %	Abs incorpn, %	α,β-Dipyridyl,[b] abs incorpn, %	Nicotelline,[b] abs incorpn, %
1	59	0.027	3.55	29	0.007	0.51	1.7	0.024	0.089	6.8	0.038	0.57		
2	65	0.25	0.93	28	0.023	0.039	1.9	0.43	0.046	5.9	0.38	0.13		
3	94	0.16	1.10	37	0.016	0.049	Not isolated			13	0.44	0.42	0.017	0.0006
4	149	0.063	0.0046	101	0.0061	0.00033	2.6	0.048	0.000075	6.3	0.070	0.00022		
5	7	0.11	0.00037	68	0.022	0.0008	1.7	0.061	0.000051	5.6	0.083	0.00023		
6	164	0.51	2.13	4.3	0.50	0.059	0.95	0.71	0.017	6.8	1.05	0.18	0.015	0.0021
7	47	0.81	0.96	24	0.61	0.40	0.80	0.91	0.018	5.3	1.83	0.25	0.050	0.0018
8	10	0.54	0.14	11	0.32	0.094	380	1.49	14.3	3.0	2.76	0.21	0.028	0.0004

[a] Specific incorporation is defined as the specific activity of the isolated alkaloid (dpm/mmol) divided by the specific activity of the administered precursor. [b] The absolute incorporation of these alkaloids is calculated on the amount of carrier (100 mg) added at the time of extraction.

several weeks. Feedings were carried out with *N. glutinosa* (experiment 4 and 5) and *N. tabacum* (experiment 6 and 7), details of these experiments being recorded in Tables II and III. In both species the amount of nicotine in the air-dried plants was much lower than in the freshly harvested plants. Some of this loss is probably due to vaporization fom the leaves; however, another route for the disappearance of nicotine is by demethylation to nornicotine, a well-established reaction which occurs during drying.[20] Our current experiments certainly confirm this, the ratio of nicotine to nornicotine decreasing dramatically in the air-dried plants. The absolute incorporation of activity into the alkaloids of *N. glutinosa* plants which were grown out of doors (experiments 4 and 5) was surprisingly low compared with the previous experiments on this species, where feedings were carried out in a greenhouse. Thus, no significant activity was detected in the α,β-dipyridyl or nicotelline. We are unable to offer an explanation for the low incorporation of activity observed in these experiments. The plants growing out of doors were healthy, feedings were carried out during warm sunny weather, and the rate of growth seemed to be

similar to plants which were growing in a greenhouse. This dramatic difference in apparent rate of alkaloid formation is certainly worthy of further investigation, especially as the vast majority of tracer feeding experiments, carried out by us and others, has been done in a greenhouse environment.

In *N. tabacum* there was a significant increase in the amount of activity found in the α,β-dipyridyl from the air-dried plants compared with the freshly harvested material (0.015 → 0.050% absolute incorporation). The activity found in the nicotelline was again negligible. It is of interest to note that the specific activity of the nicotine, nornicotine, anatabine, and anabasine increased in the air-dried *N. tabacum* plants. This result indicates that alkaloid formation was still taking place in the drying plants.

The α,β-dipyridyl obtained from the air-dried *N. tabacum* plants, which had been fed [2-^{14}C]nicotinic acid, was sufficiently active to carry out degradations, which are illustrated in Scheme II. Reduction with tin and hydrochloric acid yielded 2,3′-piperidylpyridine (**16**)[21], which was oxidized with permanganate affording α-picolinic acid (**17**) having 47% the specific activity of the α,β-dipyridyl. Decarboxylation of the α-picolinic acid, by heating with calcium oxide, yielded pyridine also having 47% the activity of the alkaloid. The α,β-dipyridyl was thus labeled in both rings, indicating that it could not be derived from anabasine. It plausibly could be formed by the oxidation of anatabine[22] or perhaps directly from two molecules of the labeled nicotinic acid or its metabolites. The metabolism of labeled anatabine in *Nicotiana* species is being examined.

A final feeding experiment (no. 8) was carried out with *N. glauca*, a species which contains anabasine as its major alkaloid. The incorporation of activity from [2-^{14}C]nicotinic acid into anabasine and anatabine was excellent (specific incorporations of 1.49 and 2.76%, respectively). Some activity was detected in α,β-dipyridyl, but again negligible activity in nicotelline. We are thus led to the conclusion that nicotelline is not formed from nicotinic acid. Alternatively, if nicotinic acid is indeed a precursor, we must conclude that no nicotelline synthesis was taking place during the course of any of our experiments.

Experimental Section[23]

Labeled Precursors. The following were obtained from the indicated commercial sources: DL-[2-^{14}C]lysine (ICN, Calif.), [*carboxyl*-^{14}C]nicotinic acid (Mallinckrodt), [6-^{14}C]nicotinic acid (Amersham-Searle). [2-^{14}C]Nicotinic acid was prepared in 71% yield from [1-^{14}C]aniline[24] (ICN).

Administration of the Precursors to Nicotiana Species and Isolation of the Alkaloids. The precursors were dissolved in water and fed by the wick method. Activity not absorbed by the plants at the termination of the feeding experiment was <0.02% in all cases. Details of the weights and activities of the ring-labeled nicotinic acid which was fed to the tobacco plants are recorded in Table II. In the preliminary experiments DL-[2-^{14}C]lysine (0.127 mmol, 2.3 × 10^8 dpm) was fed to *N. glutinosa* plants for 5 days (in June). The fresh plants (540 g) afforded anabasine (1.9 mg, 8.8 × 10^5 dpm/mmol), anatabine (10.6 mg, 2.5 × 10^4 dpm/mmol), nicotine (115 mg, 2.8 × 10^4 dpm/mmol), and nornicotine (41 mg, 2.5 × 10^4 dpm/mmol). [*carboxyl*-^{14}C]-Nicotinic acid (0.056 mmol, 7.4 × 10^8 dpm) was fed to *N. glutinosa* plants for 5 days (in August). The fresh plants (430 g) afforded anabasine (1.2 mg, 8.0 × 10^4 dpm/mmol), anatabine (9.7 mg, 1.1 × 10^5 dpm/mmol), nicotine (137 mg, 2.1 × 10^4 dpm/mmol), and nornicotine (70 mg, 3.0 × 10^4 dpm/mmol).

The general procedure for isolation of the alkaloids was as follows. The fresh or air-dried plants were macerated in a Waring Blendor with chloroform and concentrated NH_3. In experiments 3-8 carrier alkaloids [α,β-dipyridyl[25] (100 mg) and nicotelline[26] (100 mg)] were added at this stage of the extraction. The filtered chloroform solution was evaporated in the presence of 2 N HCl. The aqueous solution was filtered from some black tar, made basic with concentrated NH_3, and extracted with chloroform. The dried ($MgSO_4$) extract on evaporation yielded the crude alkaloids which were separated by preparative TLC on silica gel PF-254 (Merck), developing with a mixture of ether–2-propanol–concentrated NH_3 (80:10:3). In this system α,β-dipyridyl had the highest R_f, followed by nicotine, nicotelline, anatabine, anabasine, and nornicotine. Anatabine and anabasine were best separated by extracting these zones with methanol and then rechromatographing on silica gel PF-254 with chloroform–methanol–concentrated NH_3 (90:10:1), anatabine having the higher R_f. The recovery of the added α,β-dipyridyl was 60–80%. The nicotelline, which was purified by sublimation and crystallization from water, was recovered to the extent of 40–60%. Alkaloids present in small amounts were distilled into a U tube cooled in dry ice and assayed by uv spectroscopy. Dilutions were then carried out with nonradioactive alkaloids prior to the preparation of picrates or perchlorates which were then crystallized to constant activity. The anatabine isolated from *N. glutinosa* had $[\alpha]^{20}D$ −89° (*c* 0.4, 80% MeOH) and was diluted with *dl*-anatabine[27] prior to degradation. The optical purity of the isolated anatabine is uncertain. For the neat alkaloid, Späth and Kesztler[3a] reported $[\alpha]^{17}D$ −177.8°. Wada et al.[3b] reported $[\alpha]^{19}D$ −98.15° (MeOH).

Degradation of the Anatabine Derived from [6-^{14}C]Nicotinic Acid. *N*-Benzoylanatabine (9). Benzoic anhydride (120 mg) was added to a solution of anatabine (83 mg) in ether (5 ml). After being stirred overnight the solution was evaporated to dryness and the residue subjected to TLC on silica gel PF-254, developing with a mixture of chloroform–methanol–concentrated NH_3 (90:10:1). The zone corresponding to *N*-benzoylanatabine (R_f 0.9) was extracted with chloroform in a Soxhlet extractor. The residue obtained on evaporation of the extract was distilled [140 °C (0.01 mm)] yielding *N*-benzoylanatabine as a colorless viscous oil (117 mg).

Oxidation of *N*-Benzoylanatabine. A solution of potassium permanganate (160 mg) in water (10 ml) was added slowly to a solution of *N*-benzoylanatabine (115 mg) in water (30 ml) containing concentrated H_2SO_4 (0.3 ml). After being stirred for 2 h, the solution was adjusted to pH 3 with NaOH and extracted with ether overnight. The white residue obtained on evaporation of the extract was subjected to fractional sublimation under reduced pressure (0.02 mm) in an oil bath at 140 °C. Two distinct zones were obtained, the more volatile being nicotinic acid (26 mg) and the less volatile hippuric acid (16 mg).

Oxidation of Hippuric Acid with Lead Tetraacetate. Hippuric acid (54 mg) and lead tetraacetate (160 mg, containing 10% acetic acid) were heated in acetic acid (1 ml) on a steam bath for 4 h. The solution was evaporated to dryness in vacuo, and the oily residue distilled with 2 N sulfuric acid (20 ml), water being replenished in the reaction flask until 50 ml of water had distilled. Dimedone (80 mg) dissolved in water was added to the distillate, resulting in the formation of formaldehyde dimedone (25 mg), identical with an authentic specimen.

Degradation of Nicotinic Acid to Determine Activity at C-6. Nicotinic acid was converted to β-picoline as previously described.[14]

1,3-Dimethylpiperidine Methiodide (12). β-Picoline (120 mg) dissolved in ethanol (30 ml) containing concentrated HCl (1 ml) was hydrogenated at 2-atm pressure in the presence of Adams catalyst (0.2 g) for 4 h. The filtered solution was evaporated to dryness and the residue refluxed overnight with a mixture of ethanol (50 ml), methyl iodide (5 ml), and sodium bicarbonate (1 g). The reaction mixture was evaporated to dryness and the residue extracted with boiling chloroform. The residue obtained on evaporation of the extract was crystallized from a mixture of ethanol, ethyl acetate, and ether to afford colorless plates of 1,3-dimethylpiperidine methiodide (260 mg, 79%), mp 203–204 °C (lit.[28] mp 196–197 °C).

Hofmann Degradation of 1,3-Dimethylpiperidine Methiodide. The methiodide **12** (255 mg) was dissolved in water (10 ml) and shaken with silver hydroxide (from 0.4 g of $AgNO_3$) for 30 min. The filtered solution was lyophilized and the residue heated [140 °C (0.01 mm)]. The distillate, collected in a cooled U tube, was subjected to GLC on a 20 ft × ⅜ in. Carbowax 20M column, at 70 °C with a He flow rate of 20 ml/min. 4-Methyl-5-dimethylamino-1-pentene (94 mg), retention time 15 min, and 2-methyl-5-dimethylamino-1-pentene (5 mg), retention time 11 min, were thus obtained. In a cold run the former compound was converted to its methiodide: colorless plates from ethanol–ethyl acetate; mp 142–144 °C (lit.[29] mp 134–135 °C). Anal. ($C_9H_{20}NI$) C, H, N.

In the radioactive run the 4-methyl-5-dimethylamino-1-pentene was dissolved in ether (20 ml) containing a drop of pyridine. Osmium tetroxide (254 mg) was added and the mixture allowed to stand

overnight at room temperature. The residue obtained on evaporation of the reaction mixture was refluxed with aqueous methanol containing sodium sulfite (1 g) for 1 h. The filtered solution was evaporated to dryness, and the residue was dissolved in water and extracted with ether (3 × 50 ml). The residue obtained on evaporation of the ether extract was dissolved in water (30 ml), and the pH adjusted to 5 with acetic acid. Sodium metaperiodate (220 mg) was added, and after 30 min the mixture was distilled into a solution of dimedone (200 mg) in water (100 ml). After standing overnight the dimedone derivative of formaldehyde separated (136 mg).

Degradation of the Anatabine Derived from [2-^{14}C]Nicotinic Acid. Anatabine dipicrate (101 mg) was dissolved in dilute HCl, and the solution was extracted with ether to remove picric acid. The aqueous solution was made basic with NaOH and extracted with ether. The residue obtained on evaporation of the ether was dissolved in water (10 ml), made basic with 1 ml of 10% NaOH, and then stirred with potassium permanganate (0.2 g) for 3 h at room temperature. The solution was then refluxed for 1 h, cooled, decolorized with SO_2, and extracted with ether overnight. The residue obtained on evaporation of the extract was sublimed [140 °C (0.01 mm)] affording nicotinic acid (13.2 mg, 67%). This nicotinic acid was diluted and converted to 1,3-dimethyl-2-phenylpyridinium iodide (**13**) as previously described.[14] Reduction afforded 1,3-dimethyl-2-phenylpiperidine which yielded benzoic and acetic acids by a Kuhn-Roth oxidation. A Schmidt reaction on the acetic acid yielded methylamine, collected as *N*-methylbenzamide, and CO_2 collected and assayed as $BaCO_3$.

Degradation of the Anabasine Derived from [6-^{14}C]Nicotinic Acid. Anabasine (from experiment 1, 2.34×10^7 dpm/mmol) was oxidized with potassium permanganate yielding nicotinic acid[14] (2.36×10^7 dpm/mmol). Heating an intimate mixture of the nicotinic acid and calcium oxide yielded pyridine, collected as its picrate (2.30×10^7 dpm/mmol).

Degradation of the α,β-Dipyridyl Derived from [2-^{14}C]Nicotinic Acid. α,β-Dipyridyl dipicrate (from experiment 7, 300 mg, 1.58×10^5 dpm/mmol) was dissolved in hot 2 N HCl, cooled, and extracted with ether until a colorless aqueous solution was obtained. Evaporation yielded α,β-dipyridyl hydrochloride which was degraded as previously described[21] yielding α-picolinic acid (45 mg, 7.4×10^4 dpm/mmol) which on heating with calcium oxide yielded pyridine assayed as its picrate (7.4×10^4 dpm/mmol).

Acknowledgment. This investigation was supported by Research Grant GM-13246 from the National Institutes of Health, U.S. Public Health Service.

References and Notes

(1) A preliminary account of part of this work has appeared: E. Leete, *J. Chem. Soc., Chem. Commun.*, 9–10 (1975); also presented at the 29th Tobacco Chemists Research Conference, University of Maryland, Oct 1975.

(2) Reviewed: (a) E. Leete, *Adv. Enzymol.*, **32**, 373–422 (1969); (b) *Philip Morris Sci. Symp., Proc., 1st, 1973*, 92–103 (1973).

(3) (a) Anatabine was first isolated from *N. tabacum* by E. Späth and F. Kesztler, *Chem. Ber.*, **70**, 239–243, 704–709, 2450–2454 (1937); (b) it was later found in *N. glutinosa* by E. Wada, T. Kisaki, and M. Ihida, *Arch. Biochem. Biophys.*, **80**, 258–267 (1959).

(4) α,β-Dipyridyl was isolated from *N. tabacum* by E. Späth and E. Zajic, *Chem. Ber.*, **69**, 2448–2452 (1936).

(5) (a) Nicotelline was isolated by A. Pictet and A. Rotschy, *Chem. Ber.*, **34**, 696–708 (1901); (b) its structure was determined by F. Kuffner and E. Kalser, *Monatsh. Chem.*, **85**, 896–905 (1954).

(6) (a) T. C. Tso, *Arch. Biochem. Biophys.*, **92**, 248–252 (1961); (b) T. C. Tso and R. N. Jeffrey, *ibid.*, **97**, 4–8 (1962); (c) T. C. Tso, *Phytochemistry*, **5**, 287–292 (1966).

(7) T. C. Tso and R. N. Jeffrey, *Arch. Biochem. Biophys.*, **80**, 46–56 (1959).

(8) W. L. Alworth and H. Rapoport, *Arch. Biochem. Biophys.*, **112**, 45–53 (1965).

(9) (a) E. Leete, *J. Am. Chem. Soc.*, **78**, 3520–3523 (1956); (b) *ibid.*, **80**, 4393–4394 (1958); (c) E. Leete, E. G. Gros, and T. J. Gilbertson, *ibid.*, **86**, 3907 (1964); (d) M. L. Solt, R. F. Dawson, and D. R. Christman, *Plant Physiol.*, **35**, 887–894 (1960).

(10) T. Kisaki, S. Mizusaki, and E. Tamaki, *Phytochemistry*, **7**, 323–327 (1968).

(11) E. Leete and J. O. Olson, *J. Am. Chem. Soc.*, **94**, 5472–5477 (1972).

(12) This was also found to be the case in most of the subsequent feeding experiments; cf. Table III.

(13) O. Süs, *Justus Liebigs Ann. Chem.*, **564**, 137–140 (1949).

(14) A. R. Friedman and E. Leete, *J. Am. Chem. Soc.*, **85**, 2141–2144 (1963).

(15) This Hofmann degradation and separation of the resultant alkenes was first carried out by Y.-Y. Liu, Ph.D. Thesis, University of Minnesota, 1972.

(16) The specific activity of the formaldehyde derived from C-6 of nicotinic acid was only 85% that of the nicotinic acid. It is probable that this discrepancy is due to the formation of some inactive formaldehyde by the oxidation of the *N*-methyl groups of **15**. When this degradation was carried out with authentic [6-^{14}C]nicotinic acid a similar discrepancy in activity was noticed. This behavior of *N*-methyl groups has been previously observed: A. R. Battersby, R. B. Bradbury, R. B. Herbert, M. H. G. Munro, and R. Ramage, *J. Chem. Soc., Perkin Trans. 1*, 1394–1399 (1974); E. Leete, *J. Chem. Soc., Chem. Commun.*, 1524–1525 (1971).

(17) (a) N. C. Cook and J. E. Lyons, *J. Am. Chem. Soc.*, **88**, 3396–3403 (1966); (b) F. W. Fowler, *J. Org. Chem.*, **37**, 1321–1323 (1972).

(18) M. J. S. Dewar, S. Kirschner, and E. Leete, unpublished work.

(19) The biosynthesis of morphine is an analogous example. [2-^{14}C]Tyrosine was shown to be a precursor of the two halves of the alkaloid, equal labeling being observed at two positions in morphine: A. R. Battersby and B. J. T. Harper, *Chem. Ind. (London)*, 364 (1958); E. Leete, *J. Am. Chem. Soc.*, **81**, 3948–3951 (1959). However, it was later established that tyrosine serves as a precursor of two different compounds, dopamine and 3,4-dihydroxyphenylpyruvic acid, which are the actual precursors of morphine: M. L. Wilson and C. J. Coscia, *J. Am. Chem. Soc.*, **97**, 431–432 (1975); A. R. Battersby, R. C. F. Jones, and R. Kazlauskas, *Tetrahedron Lett.*, 1873–1876 (1975).

(20) (a) T. C. Tso and R. N. Jeffrey, *Plant Physiol.*, **31**, 433–440 (1956); **32**, 86–92 (1957); (b) E. Wada, *Arch. Biochem. Biophys.*, **62**, 471–475 (1956).

(21) E. Leete, *J. Am. Chem. Soc.*, **91**, 1697–1700 (1969).

(22) We have observed that a small amount of α,β-dipyridyl is produced on prolonged exposure of anatabine to air.

(23) Melting points are corrected. Radioactivity measurements were carried out in a Nuclear Chicago liquid scintillation Mark II counter, using as solvent dioxane–ethanol with the usual scintillators.[T4] Elementary analyses were carried out by the Clark Microanalytical Laboratory, Ill., and agree with the calculated values within ±0.4%.

(24) D. Gros, A. Feige, and H. R. Schutte, *Z. Chem.*, **5**, 21 (1965).

(25) C. R. Smith, *J. Am. Chem. Soc.*, **52**, 397–403 (1930).

(26) F. Krohnke and W. Zecher, *Angew. Chem., Int. Ed. Engl.*, 1, 626–632 (1962).

(27) We are indebted to John C. Lechleiter, a Lando Fellow, University of Minnesota, summer 1974, for the preparation of *dl*-anatabine by the method of P. M. Quan, T. K. B. Karns, and L. D. Quin, *J. Org. Chem.*, **30**, 2769–2772 (1965).

(28) W. Jacobi and G. Merling, *Justus Liebigs Ann. Chem.*, **278**, 1–20 (1894).

(29) M. Ferles and J. Beran, *Collect. Czech. Chem. Commun.*, **32**, 2998–3003 (1967).