BIOORGANIC CHEMISTRY 6, 273–286 (1977)

The Incorporation of [5,6-$^{13}C_2$]Nicotinic Acid into the Tobacco Alkaloids Examined by the Use of ^{13}C Nuclear Magnetic Resonance[1,2]

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Received April 13, 1977

[5,6-^{14}C,$^{13}C_2$]Nicotinic acid was prepared from [^{14}C,^{13}C]methyl iodide via nitromethane, 2-nitroacetaldehyde oxime, 3-nitroquinoline, 3-aminoquinoline, and quinoline in 20% overall yield. Administration of this material to *Nicotiana tabacum* and *N. glauca* afforded labeled anabasine, anatabine, nicotine, and nornicotine. Qualitative and quantitative incorporation (0.07–4.5% specific incorporation) was determined by radioactive assay and by examination of the ^{13}C NMR spectra of these alkaloids. Satellites due to spin–spin coupling of the incorporated contiguous ^{13}C atoms were observed at the resonances due to C-5 and C-6 in anabasine, nicotine, and nornicotine. In anatabine, satellites were found at C-5, C-6, C-5′, and C-6′.

In the last few years carbon-13 has been increasingly used as a tracer in biosynthetic studies (*1–4*), the location of the ^{13}C in the ultimate natural product being determined by examination of its proton noise-decoupled ^{13}C NMR spectrum. Enhancements of the ^{13}C signals above the natural abundance (1.1%) are observable when specific incorporations[4] of putative precursors greater than 0.3% are obtained. This technique, using singly labeled precursors, has been of most value in the study of the origin of microbial products, since high specific incorporations can often be realized. In higher plants, specific incorporations are usually low, and only three examples (illustrated in Fig. 1) involving the use of precursors singly labeled with ^{13}C have been reported (*5–7*). Much greater dilutions of administered precursors can be detected if one uses a doubly labeled ^{13}C compound, planning one's experiment so that the ultimate natural product contains contiguous ^{13}C atoms. These contiguous ^{13}C atoms give rise to satellite peaks in the ^{13}C NMR, located about central singlet peaks arising from natural abundance ^{13}C and the incorporation of singly labeled species. Since the natural abundance of contiguous ^{13}C atoms is only 0.01% (1.1 × 1.1%), quite low specific incorporations can be detected, especially as the satellite peaks usually occur at positions different from other

[1] This article is dedicated to the memory of S. Morris Kupchan, valued both as a scientific and social friend.

[2] Presented in part at the 30th Tobacco Chemists' Research Conference, Nashville, Tennessee, October 1976, and at the 173rd National Meeting of the American Chemical Society in New Orleans, March, 1977.

[3] Contribution No. 148 from this Laboratory.

[4] Specific incorporation = percentage of excess ^{13}C in the natural product/percentage of excess ^{13}C in the precursor.

ISSN 0045-2068

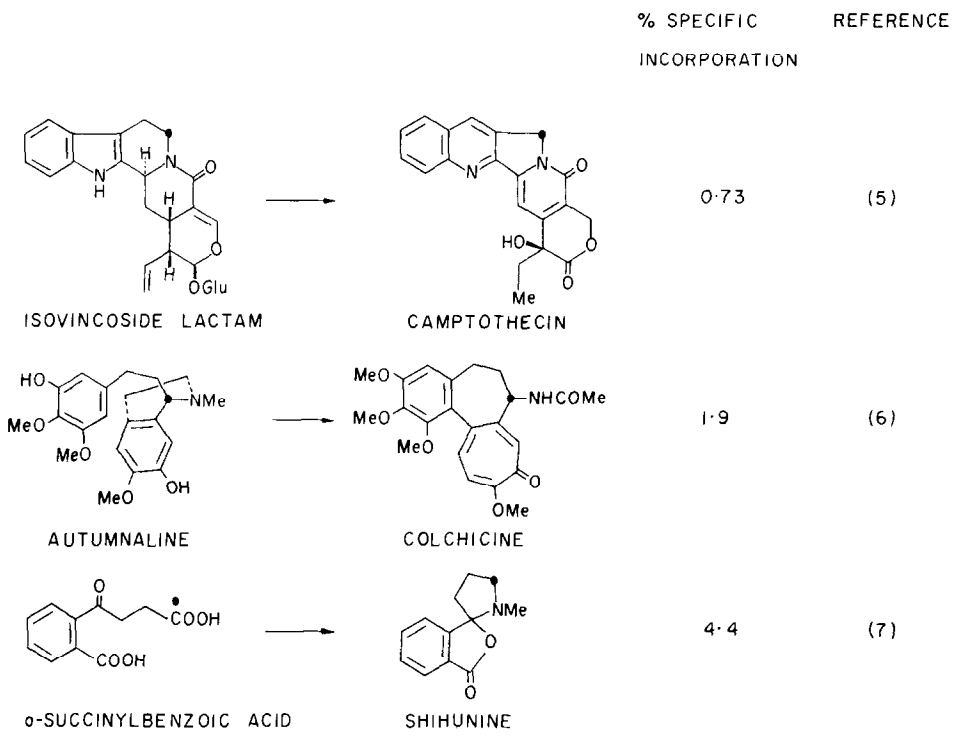

FIG. 1. Examples of the use of precursors labeled with ^{13}C (indicated with a heavy dot) for studying secondary natural products formed in higher plants.

resonances in the molecule. A large number of biosynthetic studies have been carried out with [1,2-$^{13}C_2$]acetic acid, the initial experiments being done by Seto and co-workers (*8*, *9*). By feeding [2,11-$^{13}C_2$]porphobilinogen it was shown that the rearrangement leading to the "switched" ring D in porphyrins is intramolecular (*10*). An intramolecular rearrangement of [1,3-$^{13}C_2$]phenylalanine yielded tropic acid (*11*) and tenellin (*12*) in which contiguous ^{13}C atoms were found. Doubly labeled mevalonate has been used in studies on the biosynthesis of terpenes (*13–15*) and cholesterol (*16*). The ^{13}C–^{13}C coupling which occurs in more complex systems containing more than two positions enriched with ^{13}C has been analyzed by London et al. (*17*). By this method Hutchinson et al. (*18*) have determined the ^{13}C enrichment at individual carbons of nicotine derived photosynthetically from $^{13}CO_2$.

The present article describes the use of [5,6-^{14}C,$^{13}C_2$]nicotinic acid in a study of the biosynthesis of the tobacco alkaloids. Dawson and co-workers (*19*, *20*) have shown that nicotinic acid (**1**) is incorporated into the pyridine rings of nicotine (**9**) and anabasine (**5**) with loss of its carboxyl group. It was also established that the point of attachment of the pyrrolidine ring of nicotine is at the site (C-3) from which the carboxyl group is lost (*21*, *22*). Nicotinic acid labeled around its pyridine ring with tritium or deuterium was fed to a culture of excised roots of *N. tabacum* for 4 weeks (*19*). The incorporation of [6-^{3}H]nicotinic acid was much lower (by a factor of 10) than that of the other labeled nicotinic acids. We have confirmed these results with intact tobacco plants (*23*), finding an even greater loss of hydrogen from C-6 of nicotinic acid during its conversion into

nicotine. Thus [6-^{14}C,^{3}H]nicotinic acid was incorporated into nicotine with a 98% loss of tritium relative to ^{14}C (E. Leete, unpublished observations). However, nicotinic acid recovered from the plant at the end of the feeding experiment had retained most of its tritium, indicating that the loss of tritium was not trivial, and is probably related to the activation of nicotinic acid which is required prior to its condensation with the *N*-methyl-Δ^1-pyrrolinium salt (**4**), the established precursor of the pyrrolidine ring of nicotine, being derived from ornithine and methionine (*24–26*).

Activation at the 3-position of nicotinic acid can be achieved by reduction to a dihydro derivative. Reduction at the 1,4-positions is of course a common phenomenon

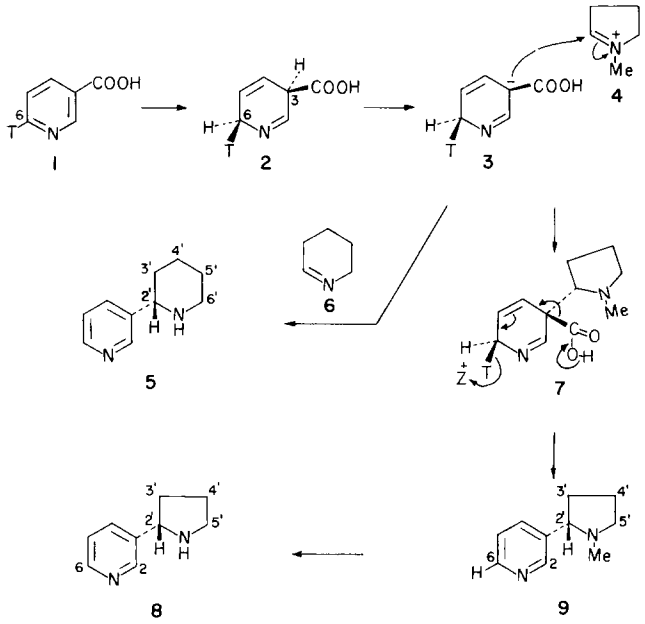

FIG. 2. Biosynthesis of anabasine (**5**), nicotine (**9**), and nornicotine (**8**).

with the nicotinamide nucleotides (NAD, NADP). However, the presence of NAD or NADP in all species is probably a good reason for rejecting them as precursors of nicotine, since this alkaloid is certainly not ubiquitous in nature. Although presently unknown, we favor 3,6-dihydronicotinic acid (**2**) as the activated form of nicotinic acid.[5] A biochemical reduction of nicotinic acid would be expected to be stereospecific. It is thus suggested that the hydrogen which is added at C-6 during this reduction is the one which is retained in the final nicotine; i.e., the terminal dehydrogenation which occurs at C-6 eliminates the hydrogen which was originally present at C-6 in nicotinic acid. The utilization of [6-^{3}H]nicotinic acid according to this scheme is illustrated in Fig. 2. The stereochemistry of the initial reduction depicted in this scheme is arbitrary. The 3,6-dihydronicotinic acid is a β-imino acid and, like a β-keto acid, would be expected to readily loose a proton to yield the carbanion (**3**). Reaction of **3** with the *N*-methyl-Δ^1-

[5] This same intermediate has also been considered by Dawson (*27, 28*) to be the most likely one between nicotinic acid and nicotine.

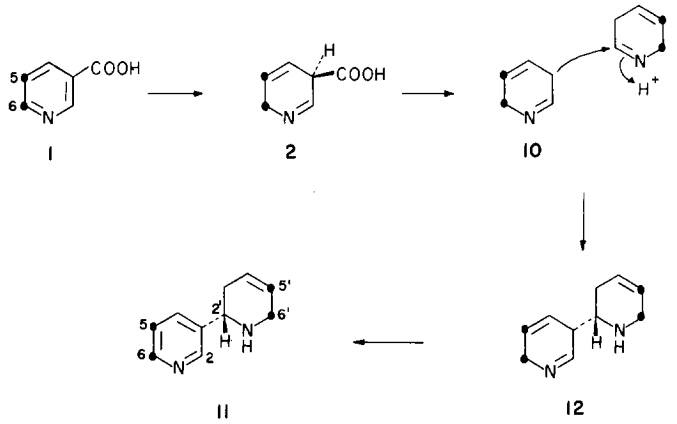

FIG. 3. Biosynthesis of anatabine (**11**). The pattern of labeling expected from [5,6-$^{13}C_2$]nicotinic acid indicated with heavy dots.

pyrrolinium salt affords (**7**), which then undergoes a concerted decarboxylation and loss of tritium at C-6, the tritium being removed by Z^+, a hydride acceptor such as NAD^+. The recently discovered biosynthetic route to anatabine (**11**) (*29*, *30*) can also be rationalized by invoking the intermediacy of 3,6-dihydronicotinic acid. It is proposed that this β-imino acid decarboxylates to afford 2,5-dihydropyridine (**10**) which then dimerizes, as illustrated in Fig. 3, to yield 3,6-dihydroanatabine (**12**), which on dehydrogenation affords anatabine. In our previous work (*30*) on the biosynthesis of anatabine we found that [2-^{14}C]nicotinic acid yielded [2,2′-^{14}C]anatabine, while [6-^{14}C]nicotinic acid yielded [6,6′-^{14}C]anatabine. In several feeding experiments involving nicotinic acid, equal labeling was obtained in both rings of anatabine, suggesting that the immediate precursors of the two rings are identical. One could hypothesize that one half of anatabine was derived from 3,6-dihydronicotinic acid, and the other half from 2,5-dihydropyridine. However, if this were the case I would have expected some disparity in the labeling of the two rings in some of the feeding experiments.

This biosynthesis of anatabine was a surprise since it is quite different from the previously known route to anabasine (**5**). The piperidine ring of this alkaloid is derived

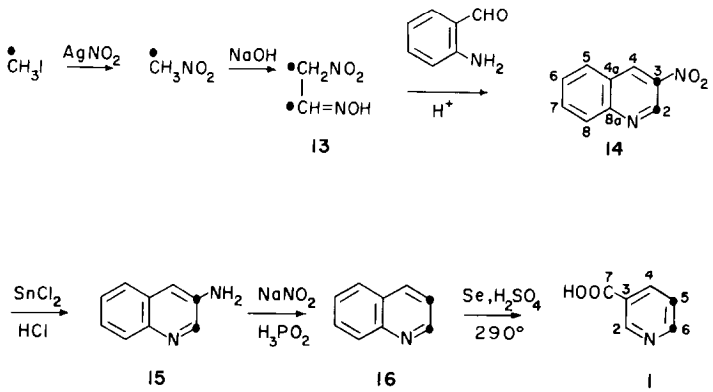

FIG. 4. Synthesis of [5,6-^{14}C,$^{13}C_2$]nicotinic acid (labeled atoms indicated with heavy dots).

from lysine (*31*), via Δ^1-piperideine (**6**) (*32*) with retention of the ε-amino group of lysine (*33*).

[5,6-^{14}C,$^{13}C_2$]Nicotinic acid was prepared by the sequence illustrated in Fig. 4. [^{14}C,^{13}C]Methyl iodide was converted to nitromethane by heating with silver nitrite (*34*). 2-Nitroacetaldehyde oxime (**13**) was obtained by the action of 50% sodium hydroxide on nitromethane (*35*). Condensation of this oxime with *o*-aminobenzaldehyde in the presence of acid yielded 3-nitroquinoline (**14**) (*36*). The 3-aminoquinoline (**15**) obtained by reduction of **14** with stannous chloride was deaminated by treatment with sodium nitrite and hypophosphorous acid affording quinoline (**16**). Attempted reduction of the diazonium fluoroborate derived from 3-aminoquinoline with sodium borohydride (*37*) failed. Oxidation of the quinoline with selenium in sulfuric acid at

TABLE 1

CHEMICAL SHIFTS (PPM FROM Me_4Si) OF NICOTINIC ACID AND ITS SYNTHETIC PRECURSORS

Carbon number	3-Nitroquinoline[a]	3-Aminoquinoline[a]	Quinoline[a]	Nicotinic acid[b]
2	144.2	143.1	150.2	150.3
3	141.6	140.2	120.9	133.5
4	132.4	114.6	135.6	138.6
4a	126.2	126.2	128.1	
5	133.6	125.8	127.6	124.8
6	129.0	126.8	126.2	151.5
7	130.0	125.3	129.1	173.8
8	130.1	128.8	129.4	
8a	150.3	142.4	148.3	

[a] In $CDCl_3$.
[b] In D_2O with NaOH.

290°C yielded [5,6-^{14}C,$^{13}C_2$]nicotinic acid. These synthetic precursors of nicotinic acid, highly enriched with ^{13}C at specific positions, afforded interesting ^{13}C NMR spectra. Vicinal (three bond), geminal (two bond), as well as single bond couplings were observed in these enriched materials. The chemical shifts of 3-nitroquinoline, 3-aminoquinoline, quinoline, and nicotinic acid are recorded in Table 1. The ^{13}C NMR spectra of quinoline has been previously reported (*38*). The chemical shifts of the other compounds were assigned by comparison with model compounds and by the use of known substituent parameters of benzene derivatives (*39*). Unambiguous assignments of certain carbons was made possible by the presence of the excess ^{13}C at certain positions. For example, in 3-nitroquinoline C-7 and C-8 differed by only 0.1 ppm. However, in 3-nitro-[2,3-$^{13}C_2$]quinoline the resonance at 130.1 was split into a doublet ($J \sim 2$ Hz) because of vicinal coupling with C-2 and is therefore assigned to C-8. In 3-nitro-[2,3-$^{13}C_2$]quinoline the difference in the chemical shifts of C-2 and C-3 (75.6 Hz) was close to the coupling constant between C-2 and C-3 (62.4 Hz). The resultant spectrum of this enriched material is thus an AB quartet (Fig. 5), superimposed on the singlet resonances resulting from the presence of 3-nitro-[2-^{13}C]- and 3-nitro-[3-^{13}C]-quinoline in the synthetic preparation. A similar spectrum was obtained for 3-amino-[2,3-$^{13}C_2$]quinoline. The difference in the chemical shifts of the enriched carbons in [5,6-$^{13}C_2$]nicotinic acid and [2,3-^{13}C]quinoline was much larger than the

coupling constant of the contiguous ^{13}C atoms, and normal satellites were observed. However, even in these compounds the satellites are not completely symmetrically located around the singlet peaks due to singly labeled species. The inner peaks of the coupled carbons are closer to the central singlet peak than the outer satellite peaks. This effect has been previously observed (*40*). The calculated (*41*) distances of the inner satellite to the central singlet peak, recorded in Table 2, are in good agreement with the observed distances. Figure 6 illustrates the ^{13}C NMR spectrum of [5,6-$^{13}C_2$]nicotinic acid. Coupling of C-4 to C-5 is observed along with a geminal coupling of C-4 to C-6 (2.8 Hz). The signal at C-3 was split 12.5 Hz and is ascribed to a vicinal coupling with C-6. A $^3J_{2,5}$ coupling of 13.9 Hz was observed in pyridine (*42*). The signal at C-2 could not be observed since it is located under the upfield satellite of C-6.

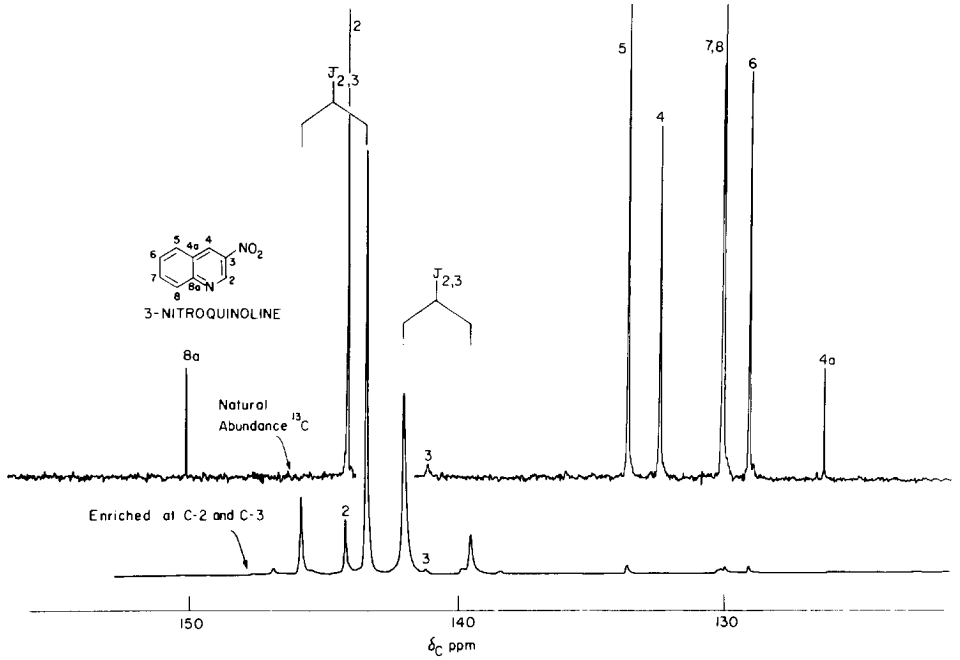

FIG. 5. Proton noise-decoupled FT ^{13}C NMR spectrum of 3-nitroquinoline. Upper spectrum: natural abundance ^{13}C (300 mg in 3 ml of $CDCl_3$ in a 12-mm tube, 2K transients, 0.8-sec acquisition time, 1.25 Hz/data point). Lower spectrum: 79% [2,3-$^{13}C_2$]-, 10% [2-^{13}C]-, and 10% [3-^{13}C]-3-nitroquinoline (47 mg in 0.5 ml of $CDCl_3$ in a 5 mm tube; 19K transients; 4-sec acquisition time; 0.25 Hz/data point).

[5,6-^{14}C,$^{13}C_2$]Nicotinic acid was fed to both *N. tabacum* and *N. glauca* by the wick method. The alkaloids were isolated as previously described (*30*), and the specific incorporation of the nicotinic acid into these alkaloids was determined initially by radioactive assay. These results are recorded in Table 3. In the feeding experiment involving *N. glauca*, the nornicotine (**8**) had a higher specific activity than nicotine. This was surprising because nornicotine is formed from nicotine by demethylation (*43*). We rationalize this result by postulating a fairly rapid metabolism of nicotine in this species affording nornicotine which then accumulates. In shorter feeding times the nicotine had a higher specific activity than the nornicotine, a result consistent with this hypothesis.

TABLE 2

COUPLING CONSTANTS FOR [5,6-$^{13}C_2$]NICOTINIC ACID, ITS SYNTHETIC PRECURSORS AND THE ALKALOIDS DERIVED FROM IT

Compound	Coupled carbons[a]	V_{AB}[b] (Hz)	J_{AB} (Hz)	Distance between central peaks and satellites[c] (Hz)	Calculated distance between central peak and inner satellite[d]
[5,6-$^{13}C_2$]Nicotinic acid	5 6	667	55.0 55.0	+25.6 −29.4 +28.0 −27.0	26.8
3-Nitro-[2,3-$^{13}C_2$]-quinoline	2 3	75.6	62.4 62.5	+42.0 −20.4 +19.4 −43.1	20.0
3-Amino-[2,3-$^{13}C_2$]-quinoline	2 3	72.5	56.3 56.3	+37.5 −18.8 +17.5 −38.8	18.5
[2,3-$^{13}C_2$]Quinoline	2 3	738	51.1 51.1	+26.2 −24.9 +24.2 −26.9	24.7
Anabasine	5 6	636	55.2 55.0	+25.6 −29.6 +27.9 −27.1	26.4
Anatabine	5 6	354	59.0 59.2	+26.2 −32.8 +31.4 −27.8	27.1
	5′ 6′	1944	42.0 42.0	+21.0 −21.0 +21.0 −21.0	20.8
Nicotine	5 6	629	54.8 —	+25.7 −29.1 —[e] −26.4	26.2
Nornicotine	5 6	647	56.2 55.9	+27.3 −28.9 +28.5 −27.4	26.7

[a] Other observed couplings were as follows: nicotinic acid, $^1J_{4,5} = 54$, $^2J_{4,6} = 2.8$, $^2J_{3,5} = 2.4$, $^3J_{3,6} = 12.5$; 3-nitroquinoline, $^1J_{3,4} = 60$, $^2J_{2,4} = 9$, $^3J_{2,8} = 3$, $^3J_{2,4a} = 7.5$, $^3J_{3,8a} = 10$; 3-aminoquinoline, $^1J_{3,4} = 58$; quinoline, $^1J_{3,4} = 58$ Hz.

[b] V_{AB} = difference in chemical shift between the coupled carbons.

[c] Positive downfield, negative upfield from central peak.

[d] Calculated using the formula: $\{V_{AB} + J_{AB} - [(V_{AB})^2 + (J_{AB})^2]^{1/2}\}/2$ (*41*).

[e] Not observable due to overlap with the C-2 signal.

The ^{13}C NMR spectra of anabasine, anatabine, nicotine, and nornicotine are recorded in Table 4. The spectrum of nicotine has been previously determined (*44*), and we agree with the reported assignments. The assignments of C-2 and C-6 were tentative, and in the present work they were unambiguously assigned by low power single frequency spin decoupling of the protons at C-2 and C-6 at 23 KG, where the proton chemical shifts differ by 6 Hz (0.06 ppm). Any doubt regarding the assignments of C-2 and C-6 was removed when nicotine enriched with ^{13}C at C-5 and C-6 was examined (see Fig. 7). Hutchinson et al. (*18*) found that the resonance due to C-6 was downfield of C-2 in the N_b-monoethane sulfonate of nicotine (in D_2O). He has also observed that the chemical shifts of nicotine and its salts are quite pH-dependent (*45*). The chemical shifts of the carbons located in the piperidine ring of anabasine and the pyrrolidine ring of nornicotine have been determined by Wenkert and co-workers (*46*), and our results are in good agreement with their reported values. The chemical shifts of C-2 and C-6 in nornicotine were assigned as previously described for nicotine, by single frequency spin decoupling of the protons at C-2 and C-6, having ^1H NMR ($CDCl_3$) δ 8.56 (doublet)

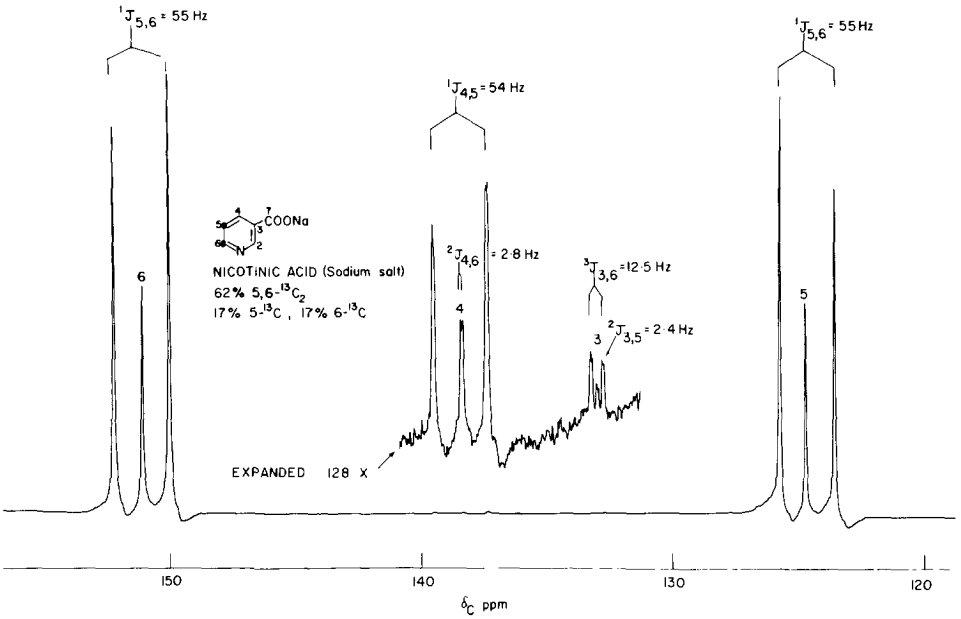

FIG. 6. Proton noise-decoupled FT ^{13}C NMR spectrum of enriched nicotinic acid: 62% [5,6-$^{13}C_2$], 17% [5-^{13}C], 17% [6-^{13}C] (40 mg in 0.3 ml of D_2O + NaOH in a 5-mm tube, containing dioxane as an internal standard; 2K transients, 2-sec acquisition time; 0.5 Hz/data point).

TABLE 3

INCORPORATION OF [5,6-^{14}C, $^{13}C_2$]NICOTINIC ACID INTO THE TOBACCO ALKALOIDS

	Species fed	
	Nicotiana tabacum	*Nicotiana glauca*
[5,6-^{14}C, $^{13}C_2$]Nicotinic acid		
Weight (mg)	17.85	29.15
^{14}C Activity (dpm/mmol)	3.48×10^8	6.52×10^8
^{13}C Distribution	79% $^{13}C_2$, 20% $^{13}C_1$	55% $^{13}C_2$, 38% $^{13}C_1$
Time of feeding	6 days (April)	7 days (June)
Fresh weight of plants (g)	1400	1800
Anabasine		
Weight (mg)	1.3	530
Specific incorporation (^{14}C)	0.27	1.64
Specific incorporation (^{13}C)		1.83
Anatabine		
Weight (mg)	13.7	8.1
Specific incorporation (^{14}C)	0.41	4.5
Specific incorporation (^{13}C)		2.8 (C-5,6)
		2.5 (C-5′,6′)
Nicotine		
Weight (mg)	391	15
Specific incorporation (^{14}C)	0.067	0.3
Specific incorporation (^{13}C)	0.07	
Nornicotine		
Weight (mg)	9.4	30
Specific incorporation (^{14}C)	0.06	0.92
Specific incorporation (^{13}C)		0.93

TABLE 4

CHEMICAL SHIFTS (PPM FROM Me_4Si) OF THE TOBACCO ALKALOIDS

Carbon number	Anabasine[a]	Anatabine[b]	Nicotine[a]	Nornicotine[a]
2	148.9	147.6	149.5	148.6
3	140.9	137.4	138.8	140.5
4	134.3	142.5	134.9	134.0
5	123.6	129.9	123.6	123.2
6	148.8	144.1	148.5	148.0
2′	59.9	54.7[d][c]	68.9	60.0
3′	34.9	29.5[t]	35.2	34.5
4′	24.3	126.3[d]	22.6	25.6
5′	25.7	121.6[d]	57.0	47.0
6′	47.7	44.4[t]		
N-Me			40.3	

[a] In $CDCl_3$.

[b] The spectrum was that of anatabine diperchlorate in D_2O with 2,2-dimethyl-2-silapentane sulfonic acid, Na salt (DSS), as an internal reference. The chemical shifts reported in the table are expressed relative to Me_4Si by applying a correction of −1.1 ppm (the chemical shift of the methyl groups in DSS).

[c] Multiplicity of the off resonance decoupled spectra.

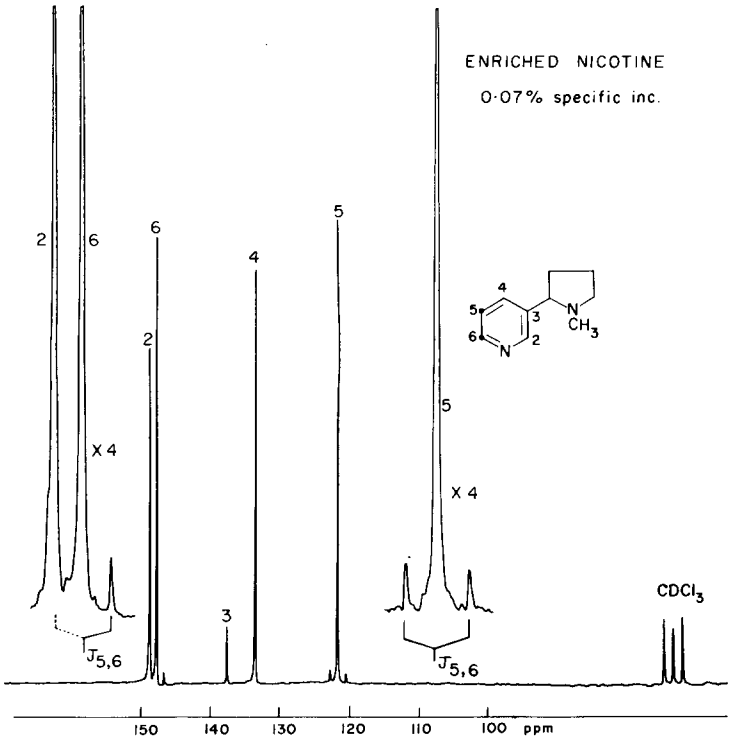

FIG. 7. Proton noise-decoupled FT ^{13}C NMR spectrum of enriched nicotine, aromatic region (317 mg in 3 ml of $CDCl_3$ in a 12-mm tube, accumulated for 16 hr: 64K transients; 0.8-sec acquisition time; 1.25 Hz/data point). The satellites at C-5 and C-6 are also shown expanded fourfold in both the horizontal and vertical scales.

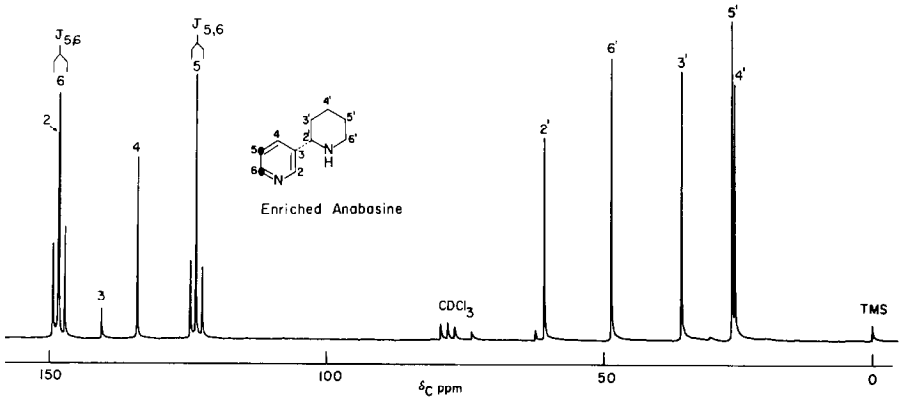

FIG. 8. Proton noise-decoupled FT ^{13}C NMR spectrum of enriched anabasine (470 mg in 2.5 ml of $CDCl_3$ in a 12-mm tube; 5K transients; 0.8-sec acquisition time; 1.25 Hz/data point).

and 8.41 (doublet of doublets), respectively. In anabasine the chemical shifts of C-2 and C-6 were so close that they could only be assigned by reference to the alkaloid which was enriched at C-5 and C-6 with ^{13}C (Fig. 8).

The natural abundance ^{13}C NMR spectrum of anatabine diperchlorate in D_2O is illustrated in Fig. 9. Assignment of the pyridine carbons was straightforward. However, the previously reported chemical shifts of Δ^3-piperideines were not too helpful for distinguishing C-4′ and C-5′. The models considered, with the assigned chemical shifts (*46*, *47*), were 1-methyl-Δ^3-piperideine (**17**), 1,6-dimethyl-Δ^3-piperideine (**18**), and tropidine (**19**), and they are illustrated in Fig. 10. The trend in these compounds seems to be that the δ-carbon is upfield of the carbon which is β to the nitrogen. However, examination of the ^{13}C NMR spectra of the anatabine obtained from *N. glauca* which had been fed the enriched nicotinic acid clearly indicated that C-4′ was downfield of C-5′, since the signal at 121.6 ppm exhibited satellites because of coupling with C-6′. We also noted that the introduction of a double bond in the piperidine ring results in a

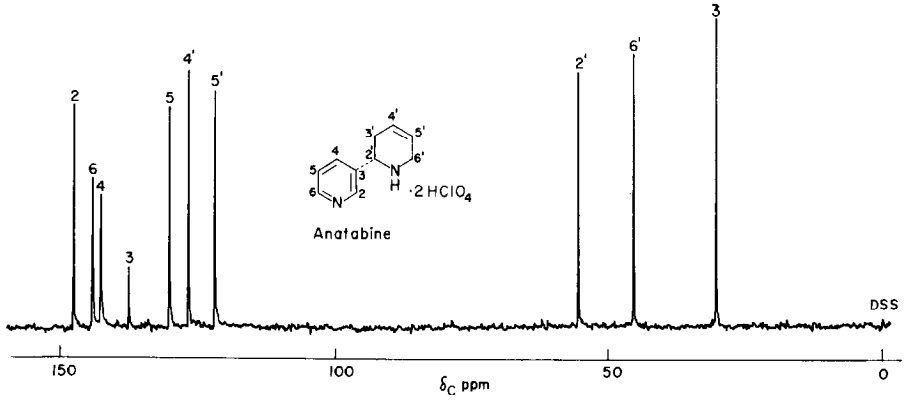

FIG. 9. Proton noise-decoupled FT ^{13}C NMR spectrum of natural abundance anatabine diperchlorate (100 mg in 0.5 ml of D_2O in a 5-mm tube; 10K transients; 0.73-sec acquisition time; 1.47 Hz/data point).

shielding of the homoallylic carbon at C-2′, causing an upfield shift (59.9 ppm in anabasine to 54.7 ppm in anatabine), this effect being first observed by Wenkert (*47*).

The ^{13}C NMR spectra of the enriched alkaloids clearly indicated the presence of contiguous ^{13}C atoms. In the feeding experiment involving *N. tabacum* the specific incorporation of the [5,6-^{14}C,$^{13}C_2$]nicotinic acid into nicotine was low (0.067%, a dilution of 1500). However, satellites were readily detected at C-5 and C-6 in the ^{13}C NMR spectrum of this nicotine (Fig. 7). Alkaloids with much higher specific incorporation were obtained from the feeding experiment with *N. glauca*. The spectra of the enriched anabasine and anatabine are illustrated. Specific incorporations of ^{13}C could be determined by measurement of the intensity of the satellite peaks relative to the central singlet peaks, the calculation taking into account the contribution of singly

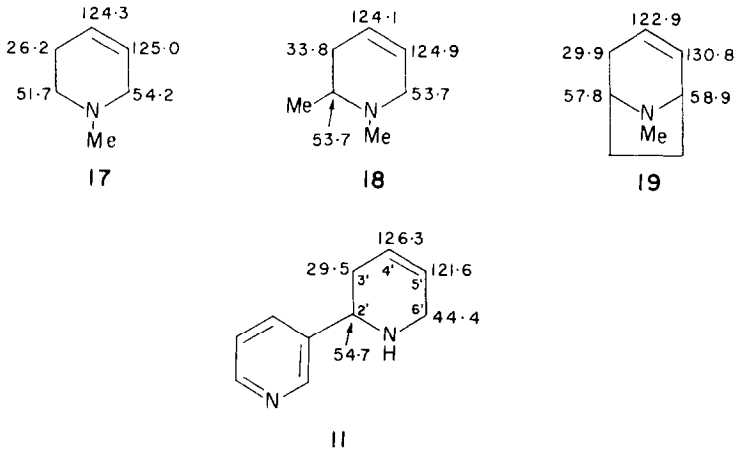

FIG. 10. Chemical shifts of some Δ^3-piperideines.

labeled species to the central peak (*12*). The values reported in Table 3 are the average values obtained from measurements of the two separate contiguous carbons. Good agreement between the incorporation determined by this method and by ^{14}C assay was obtained. The specific incorporation of tracer into the two rings of anatabine, determined by the ^{13}C method, was not exactly the same. However, the difference from equality is probably within the experimental error of this method, and the present results do not render our previous conclusion regarding the biosynthesis of anatabine invalid. The observed coupling constants found for C-5 and C-6 in the pyridine ring of these alkaloids (55–59 Hz) are in agreement with previously reported values for pyridine (*42*). A coupling constant of 42 Hz between C-5′ and C-6′ of anatabine is also typical (*48*) for an sp^3–sp^2 bond. As previously mentioned (*40*) the satellites are not completely symmetrically located about the central singlet peaks. Good agreement between the calculated and observed inner distances was obtained. In an AB spin system, the heights of the inner satellites are higher than the outer satellites. This is readily observed in the spectrum of the enriched anatabine diperchlorate (Fig. 11). Only fair agreement between the calculated (*41*) and observed heights was found. Thus in anatabine where $J_{5,6}$ is 59.1 Hz and V_{AB} is 354 Hz, the calculated ratio of the height of the inner satellite to the outer one is 1.39. The observed values were 1.11 (for C-5) and 1.33 (for C-6).

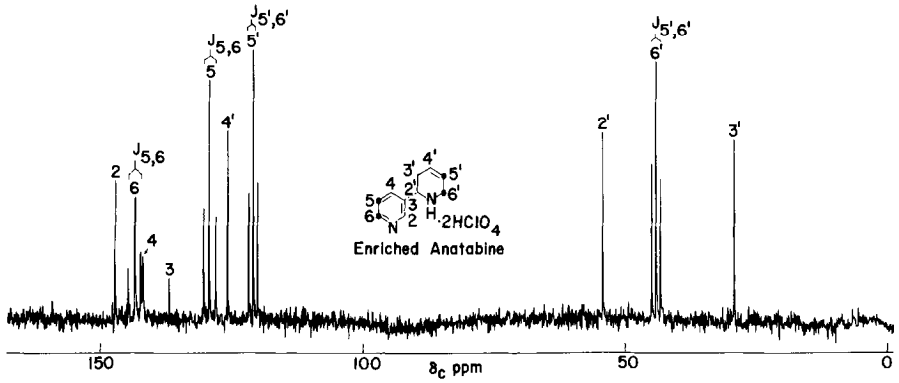

FIG. 11. Proton noise-decoupled FT ^{13}C NMR spectrum of enriched anatabine diperchlorate (18.3 mg in 0.3 ml of D_2O in a 5-mm tube; 86K transients; 1.0-sec acquisition time; 1.0 Hz/data point).

The utility of precursors labeled with contiguous ^{13}C atoms has thus been clearly demonstrated, even at low levels of incorporation. It is predicted that much information will be obtained in the future by the use of complex precursors which are labeled in this way.

EXPERIMENTAL

Radioactive materials were assayed in a Nuclear Chicago Mark II liquid scintillation counter, using as a solvent dioxane–ethanol with the usual scintillators (*49*). Mass spectra were determined by Dr. Roger Upham at the University of Minnesota on an AEI-MS-30 instrument. Carbon-13 NMR spectra were recorded on a Varian XL-100-15 spectrometer operating at 25.2 MHz, equipped with a VFT-100 Fourier transform accessory.

[*5,6-*^{14}C,$^{13}C_2$]*Nicotinic Acid*

Several syntheses starting with [^{13}C]methyl iodide and varying amounts of [^{14}C]methyl iodide were carried out. The following is a typical preparation.

2-Nitro-[*1,2-*^{14}C,$^{13}C_2$]*acetaldehyde oxime* **13**

[^{13}C]Methyl iodide (90% ^{13}C) (Merck, Sharp, and Dohme, Canada Ltd.) (2.0 g) and [^{14}C]methyl iodide (nominal activity 1 mCi) (0.28 g) were added to a mixture of silver nitrite (4.2 g) and sand (4 g) and heated for 2 hr on a steam bath with a dry ice condenser. Ether (1 ml) was then added to the cooled reaction mixture, and the whole was distilled (100°C, 0.1 mm) into a U-tube cooled in dry ice. The contents of this tube were washed out with ether into a solution of sodium hydroxide (0.5 g) in water (1 ml) stirred at 50°C. The ether was allowed to evaporate, and the solution was stirred for 30 min. The pale brown reaction mixture was cooled, acidified with 2 *N* sulfuric acid, and extracted with ether. The dried ($MgSO_4$) extract was evaporated to afford 2-nitroacetaldehyde oxime (538 mg, 64%).

3-Nitro-[2,3-^{14}C,$^{13}C_2$]quinoline **14**

2-Nitroacetaldehyde oxime (538 mg) and *o*-aminobenzaldehyde (*50*) (1.2 g) in ethanol (3 ml) were stirred at room temperature with concentrated hydrochloric acid (0.4 ml) overnight. Crude 3-nitroquinoline (555 mg) separated. Additional material was obtained from the mother liquor by evaporating, dissolving the residue in benzene containing 5% methanol, and subjecting to column chromatography on alumina (activity II). Elution with the same solvent yielded 3-nitroquinoline. The combined products were crystallized from ethanol affording 3-nitroquinoline (605 mg, 68%), mp 130–131°C. Analysis of this material by mass spectrometry indicated the presence of 62% $^{13}C_2$, 34% $^{13}C_1$, and 4% $^{13}C_0$ (five scans).

[2,3-^{14}C,$^{13}C_2$]Quinoline **16**

3-Nitroquinoline (352 mg) was dissolved in concentrated hydrochloric acid (16 ml) and stannous chloride dihydrate (2.1 g) was added. After stirring for 3 hr at room temperature the mixture was cooled and made basic with sodium hydroxide. Extraction with ether, drying ($MgSO_4$), and evaporation yielded 3-aminoquinoline as pale yellow needles (290 mg, 99%). This 3-aminoquinoline (284 mg) was dissolved in a mixture of water (6 ml) and 30% hypophosphorous acid (4 ml), cooled to 0°C and stirred in a nitrogen atmosphere for 2 hr. Sodium nitrite (180 mg) in water (0.3 ml) was then added, and the mixture was allowed to warm up to room temperature and then stirred for 20 hr. The brown solution was made basic with sodium hydroxide, extracted with ether, dried ($MgSO_4$), and the residue obtained on evaporation was distilled (130°C, 10^{-2} mm) affording quinoline as a colorless oil (142 mg, 56%).

[5,6-^{14}C,$^{13}C_2$]Nicotinic Acid **1**

The labeled quinoline (140 mg) was dissolved in concentrated sulfuric acid (6 ml) and heated with selenium (200 mg) at 290°C in a metal bath for 20 hr. The cooled reaction mixture was added to ice, adjusted to pH 3 by the addition of potassium hydroxide, and extracted with ether overnight. Evaporation of the ether yielded crude nicotinic acid (139 mg) which was sublimed (160°C, 10^{-3} mm) and crystallized from ethanol yielding [5,6-^{14}C,$^{13}C_2$]nicotinic acid (114 mg, 85%). Analysis by mass spectrometry (five scans) indicated 62% $^{13}C_2$, 34% $^{13}C_1$, and 4% $^{13}C_0$. Specific activity (^{14}C): 2.75×10^8 dpm/mmol.

ACKNOWLEDGMENTS

This investigation was supported by a research Grant No. GM-13246 from the U.S. Public Health Service. I am greatly indebted to Richard A. Newmark, 3M Company, St. Paul, and Robert M. Riddle, University of Minnesota, for determination of the ^{13}C NMR spectra.

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