Biomimetic Synthesis of Anatabine from 2,5-Dihydropyridine Produced by the Oxidative Decarboxylation of Baikiain[1]

Edward Leete* and Mark E. Mueller

Contribution from the Natural Products Laboratory,[2] School of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received March 26, 1982*

Abstract: The tobacco alkaloid anatabine (1,2,3,6-tetrahydro-2,3′-bipyridine) is formed, along with small amounts of pyridine and glutaconaldehyde, when baikiain (1,2,3,6-tetrahydropyridine-2-carboxylic acid) is oxidized with sodium hypochlorite. Baikiain, labeled with deuterium or ^{13}C at C-2, yielded anatabine labeled only at the C-2 and C-2′ positions, the location of the isotopes being determined by NMR spectroscopy. The formation of anatabine in this reaction is considered to involve the intermediacy of 2,5-dihydropyridine, a compound that has previously been proposed as an intermediate in the biosynthesis of the alkaloid from nicotinic acid in *Nicotiana* species.

Introduction

Anatabine (**8**)[3] is the most abundant of the minor alkaloids of tobacco.[4] it has been established[5a,6,7] that both rings of this alkaloid are derived from nicotinic acid (**1**). Our current views on the biosynthesis of anatabine and nicotine are shown in Scheme I. The fate of the hydrogen (indicated with a T) initially present at C-6 of nicotinic acid and labeled carbon at C-2 (indicated with a heavy dot) is indicated in this scheme to illustrate the regio- and stereospecific details that have been established for their biosynthesis. It is proposed that nicotinic acid is reduced to 3,6-dihydronicotinic acid (**2**). This reduction is stereospecific at C-6, the incoming hydrogen entering the *pro-R* position.[7] Nicotine (**9**) is formed by reaction of **2** with the *N*-methyl-Δ^1-pyrrolinium salt (**3**), via the intermediate **6**. The hydrogen originally present at C-6 of nicotinic acid is lost by a stereospecific dehydrogenation of **6**. Since **2** is a β-imino acid, it is expected to undergo facile decarboxylation to yield 2,5-dihydropyridine (**5**). Anatabine could be formed by a route, analogous to that proposed for the formation of nicotine, by reaction of C-3 of the dihydronicotinic acid with C-6 of the 2,5-dihydropyridine. An alternative route is illustrated whereby some of the 2,5-dihydropyridine isomerized to 1,2-dihydropyridine (**4**). This enamine then condenses with **5** to yield a dihydroanatabine (**7**). The final step is considered to be a stereospecific 1,4-elimination of hydrogen with a hydride acceptor (Z^+). It is the *pro-S* hydrogen that is eliminated from the C-6 position since anatabine derived from [6-^{3}H]nicotinic acid contained no tritium in its pyridine ring.[7]

The present study is concerned with a biomimetic synthesis of anatabine that is considered to involve the same reactions and intermediates proposed for the biosynthesis of the alkaloid in the tobacco plant. Despite a vast amount of work on the dihydropyridines[8,9] little is known of the chemistry of the simple compounds that lack substituents. 1,4-Dihydropyridine is the only unsubstituted dihydropyridine that has been well characterized.[10-12] Some substituted 2,5-dihydropyridines have been synthesized;[13,14] however, the methods used for their preparation are not applicable for the production of **5**. Imines have been formed by the oxidative decarboxylation of cyclic α-amino acids.[15] Thus, a plausible precursor of 2,5-dihydropyridine would be 1,2,3,6-tetrahydropyridine-2-carboxylic acid (**10**), known trivially as baikiain.[16]

Results and Discussion

Preliminary experiments[1] were carried out by adding sodium hypochlorite to a solution of L-baikiain in aqueous buffers at pH 7, 8, and 10. There was an immediate loss of optical activity, indicating that N-chlorination to **11** and subsequent decarboxylation to **5** are rapid reactions. The course of the reaction was followed spectroscopically in the UV. There was a slow development of absorptions at 255-265 nm characteristic of pyridine derivatives, and extraction of the solution with ether yielded a mixture of pyridine and anatabine, the latter being identical with an authentic specimen of (*RS*)-anatabine.[17] The maximum yield (26%) of anatabine was obtained at pH 10. At this pH (but not at pH 7) an intense absorption at 362 nm was produced as soon as the hypochlorite was added to the baikiain solution. Acidification of the solution with hydrochloric acid completely eliminated this absorption, which reappeared, with somewhat reduced intensity, on addition of sodium hydroxide. It was established[18] that the absorption at 362 nm is due to the production of some glutaconaldehyde (**13**, 5-hydroxy-2,4-pentadienal). Scheme II illustrates a plausible route for its formation from 2,5-dihydropyridine (**5**). Hydration of the imine in base yields **12**, which could undergo N-chlorination, dehydrohalogenation, and ring opening. The aqueous solution from the reaction of baikiain with hypochlorite at pH 10 showed spectral shifts in the UV and visible spectrum identical with authentic glutaconaldehyde.[20] Thus, the

(1) A preliminary account of part of this work has appeared: Leete, E. *J. Chem. Soc., Chem. Commun.* **1978**, 1055.
(2) Contribution No. 183 from this laboratory. Part 47 in the series "Tobacco Alkaloids and Related Compounds"; Part 46: Leete, E. *J. Nat. Prod.* **1982**, *45*, 197.
(3) Anatabine is currently indexed in Chemical Abstracts under the entry: 1,2,3,6-tetrahydro-2,3′-bipyridine. If this nomenclature is followed the pyridine ring should be numbered with prime numbers. However, it has been standard practice to number the tetrahydropyridine ring with prime numbers. To avoid confusion and to facilitate reference to the other pyridine alkaloids of tobacco, this method of numbering, indicated on structure **8**, has been used in this article.
(4) In fresh *Nicotiana tabacum*, the species most commonly used for the production of cigarette tobacco, the alkaloid mixture consisted of 93% nicotine, 3.9% anatabine, 2.4% nornicotine, and 0.5% anabasine.[5a] Similar percentages were reported for the alkaloid composition of processed tobacco.[5b]
(5) (a) Leete, E.; Slattery, S. A. *J. Am. Chem. Soc.* **1976**, *98*, 6326. (b) Elmenhorst, H. *Bull. Inf. Coresta, Special Issue*, **1978**, *abstr. 513*, 120.
(6) Leete, E. *Bioorg. Chem.* **1977**, *6*, 273.
(7) Leete, E. *J. Chem. Soc., Chem. Commun.* **1978**, 610.
(8) (a) Eisner, U.; Kuthan, J. *Chem. Rev.* **1972**, *72*, 1. (b) Stout, D. M.; Meyers, A. I. *Chem. Rev.* **1982**, *82*, 223.
(9) Lyle, R. E. "Heterocyclic Compounds, Pyridine and Its Derivatives"; Abramovitch, R. A., Ed.; Wiley; New York, 1974, Vol. 14, Part I, p. 137.
(10) Cook, N. C.; Lyons, J. E. *J. Am. Chem. Soc.* **1965**, *87*, 3283.
(11) Fowler, F. W. *J. Org. Chem.* **1972**, *37*, 1321.
(12) Fowler, F. W. *J. Am. Chem. Soc.* **1972**, *94*, 5926.
(13) (a) Giam, C. S.; Knaus, E. E.; Pasutto, F. M. *J. Org. Chem.* **1974**, *39*, 3565. (b) Francis, R. F.; Davis, W.; Wisener, J. T. *J. Org. Chem.* **1978**, *43*, 3227. (c) Finch, N.; Gemenden, C. W. *J. Org. Chem.* **1975**, *40*, 569. (d) Knaus, E. E.; Ondrus, T. G.; Giam, C. S. *J. Heterocycl. Chem.* **1976**, *13*, 789.
(14) Marsais, F.; Granger, P.; Quéguiner, *J. Org. Chem.* **1981**, *46*, 4494.
(15) Bragg, P. D.; Hough, L. *J. Chem. Soc.* **1958**, 4050.
(16) King, F. E.; King, T. J.; Warwick, A. J. *J. Chem. Soc.* **1950**, 3590.
(17) Quan, P. M.; Karns, T. K. B.; Quin, L. D. *J. Org. Chem.* **1965**, *30*, 2769.
(18) We are indebted to James L. Kilgore, a member of our research group, who serendipitously discovered in the literature[19] that the enolate anion of glutaconaldehyde (**14**) has an intense absorption at 362 nm (log ϵ 4.77).
(19) Becher, J. *Acta Chem. Scand.* **1972**, *26*, 3627.
(20) Baumgarten, P. *Chem. Ber.* **1924**, *57*, 1622.

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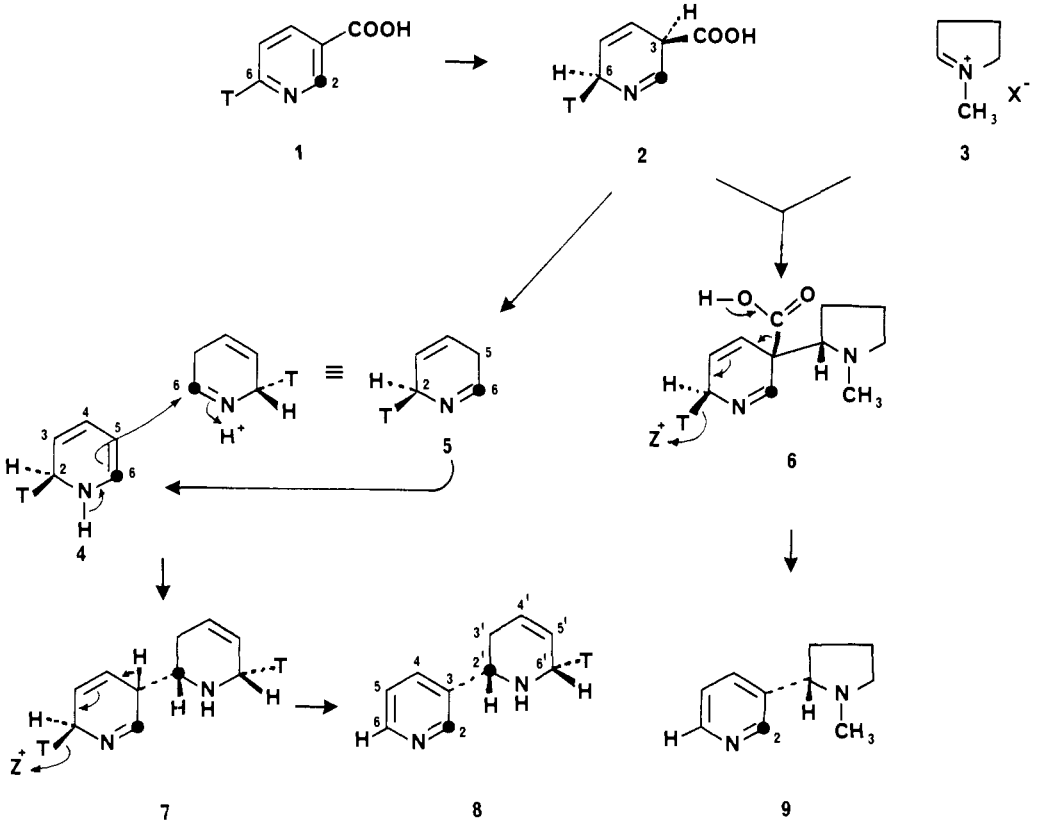
Scheme I. Hypothetical Biosynthesis of Anatabine and Nicotine from Nicotinic Acid



addition of carbon dioxide to the alkaline reaction mixture yielded free glutaconaldehyde.[21] Aniline in the presence of hydrochloric acid yielded the red dianilide salt (**15**) having an absorption at 485 nm.[22] The yield of glutaconaldehyde from baikiain at pH 10, estimated from the UV absorption of its enolate anion, was 5–10%. It was isolated and characterized as its *O*-benzoyl derivative (**16**).[20] Control experiments established that glutaconaldehyde was not obtained from pyridine on treatment with hypochlorite at pH 10.

The reactions leading to the formation of anatabine were further investigated by labeling the baikiain with deuterium and ^{13}C at C-2. Several syntheses of baikiain have been reported,[16,23,24] and we have used a modification of Burgstahler's method,[24] illustrated in Scheme III, for the introduction of the isotopic labels. The sodium salt of diethyl acetamidomalonate (**18**) (labeled with ^{13}C at C-2 for the production of [2-^{13}C]baikiain) was condensed with a large excess of 1,4-dichloro-(*Z*)-2-butene (**17**) in ethanol to yield ethyl 2-acetamido-2-carbethoxy-6-chloro-(*Z*)-4-hexenoate (**19**). This compound was cyclized to diethyl 1-acetyl-1,2,3,6-tetrahydropyridine-2,2-dicarboxylate (**20**) with lithium bis(trimethylsilyl)amide. Hydrolysis of this ester with hydrochloric acid yielded DL-baikiain (38% from **18**). When the ultimate hydrolysis was carried out with DCl in D_2O, baikiain labeled with deuterium (>95%) at C-2 was obtained. The [2-^{2}H]baikiain and [2-^{13}C]-baikiain exhibited the expected ^{13}C and ^1H NMR spectra (see Experimental Section) consistent with the presence of these isotopes at the C-2 position.

The mass spectrum of the anatabine derived from the [2-^{2}H]baikiain indicated that it contained two deuterium atoms. Its ^{13}C NMR spectra (Figure 1) indicated that the deuterium was located at the C-2 and C-2′ positions, since these signals were absent in the proton noise-decoupled spectra. Initial observation of the spectra was disconcerting since the signal for C-4 was

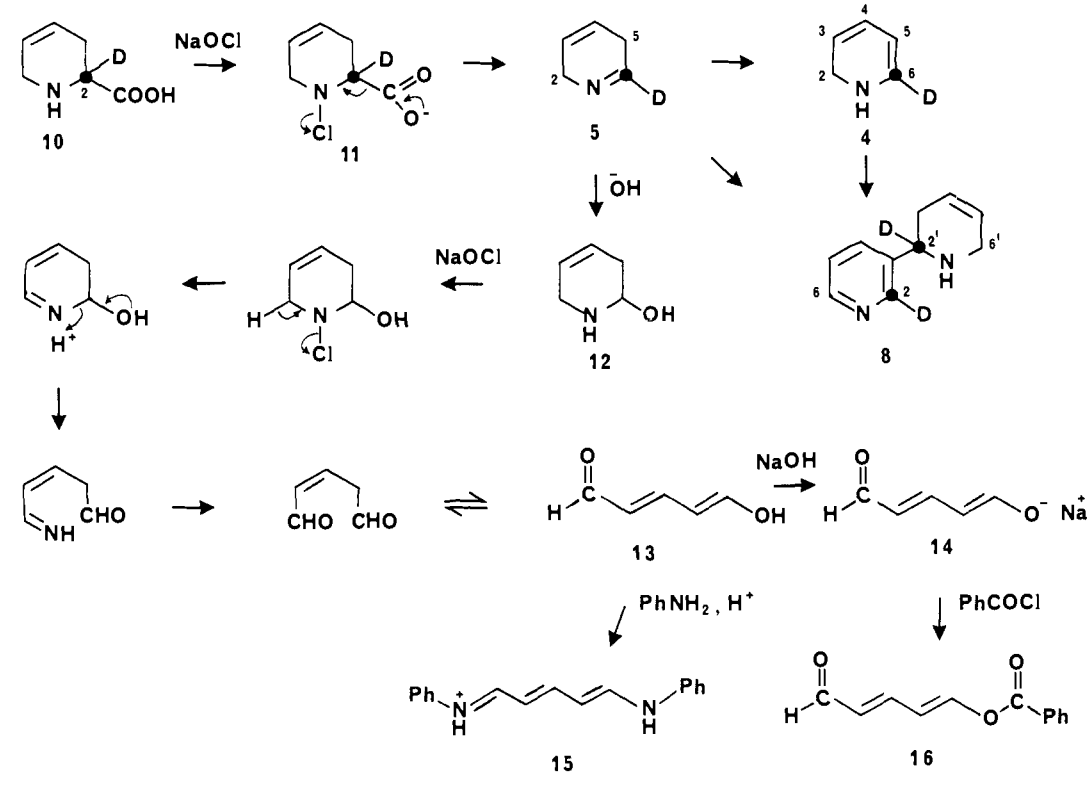
Table I. ^{13}C NMR of Anatabine and Nicotine (ppm from Me_4Si)

	anatabine		nicotine	
solvent	D_2O/HCl	$CDCl_3$	D_2O/HCl[26a]	$CDCl_3$[6]
C no.				
2	142.5	148.9	142.8	149.5
3	137.4	140.3	133.8	138.8
4	147.6	134.8	148.2	134.9
5	129.9	126.6	129.3	123.6
6	144.1	148.9	143.7	148.5
2′	54.7	55.3	69.4	68.9
3′	29.5	33.7	31.6	35.2
4′	126.3	125.4	22.5	22.6
5′	121.6	123.9	57.5	57.0
6′	44.4	46.0		
NMe			39.7	40.3

apparently missing, but it was then realized that the signals for C-2 and C-4 of anatabine hydrochloride in D_2O had been previously misassigned.[6] The assignments had been made by comparison with nicotine and anabasine whose spectra were run in $CDCl_3$. It has since been established that the chemical shifts of nicotine and the other tobacco alkaloids are very sensitive to changes in the pH of the solvent.[25,26] Table I records the chemical shifts of anatabine and nicotine in chloroform and as their hydrochloride salts in D_2O. The assignments of C-2 and C-4 of anatabine were further substantiated by the preparation of anatabine specifically labeled at C-2 and C-4 with deuterium, starting with [2-^{2}H]- and [4-^{2}H]nicotinic acid, respectively.[27,28] These labeled nicotinic acids were converted to pyridine-3-carboxaldehyde

(21) Becher, J.; Christensen, M. C. *Tetrahedron* **1979**, *35*, 1523. See also: Becher, J. *Synthesis* **1980**, 589.
(22) Brooker, L. G. S.; White, F. L.; Keyes, G. H.; Smyth, C. P.; Oesper, P. F. *J. Am. Chem. Soc.* **1941**, *63*, 3192.
(23) Dobson, N. A.; Raphael, R. A. *J. Chem. Soc.* **1958**, 3642.
(24) Burgstahler, A. W.; Aiman, C. E. *J. Org. Chem.* **1960**, *25*, 489.
(25) Pitner, T. P.; Seeman, J. I.; Whidby, J. F. *J. Heterocycl. Chem.* **1978**, *15*, 585.
(26) Slaven, R. W. 35th Tobacco Chemists' Res. Conf., Winston-Salem, NC, Oct 6–9, 1981; Abstr. no. 27.
(27) Zoltewicz, J. A.; Smith, C. L.; Meyer, J. D. *Tetrahedron* **1968**, *24*, 2269.
(28) Dawson, R. F.; Christman, R. K.; D'Adamo, A.; Solt, M. L.; Wolf, A. P. *J. Am. Chem. Soc.* **1960**, *82*, 2628.

Scheme II. Biomimetic Formation of Anatabine from Baikiain



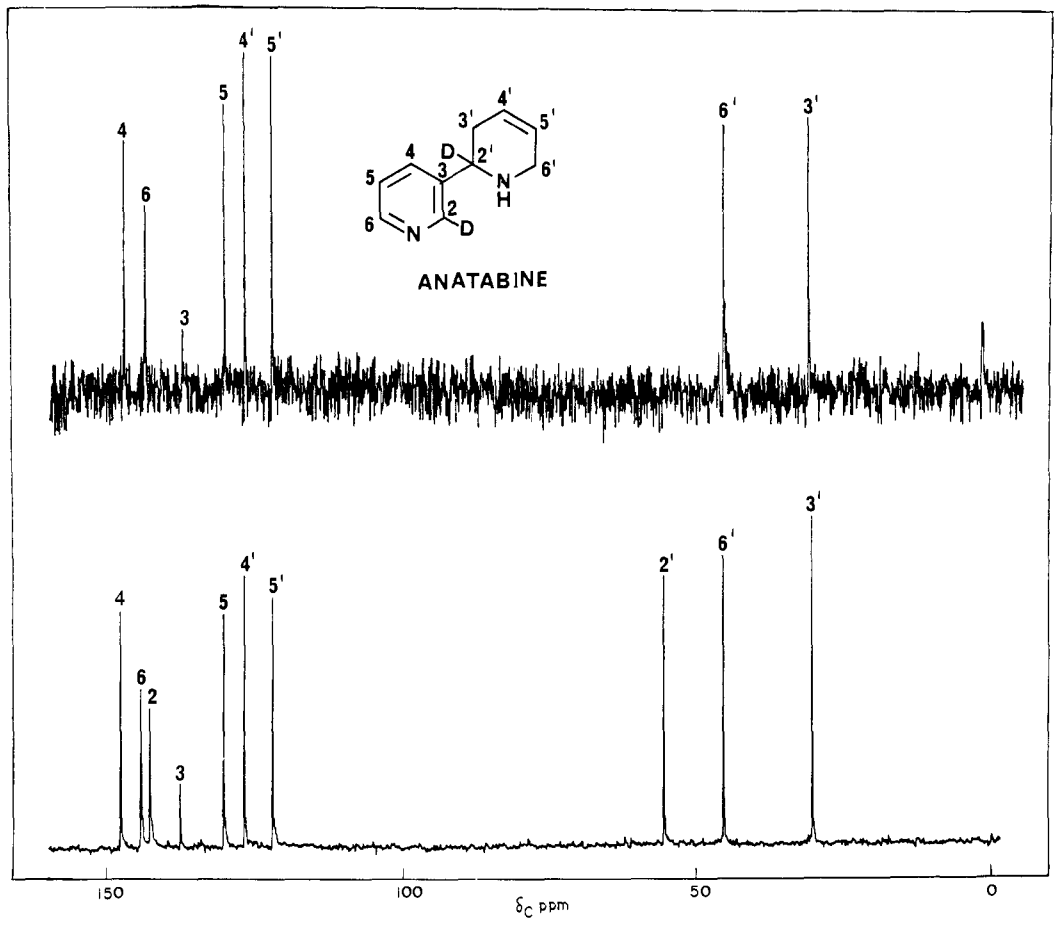


Figure 1. Proton noise-decoupled ^{13}C NMR spectra of anatabine hydrochloride (lower spectrum) and [2,2′-$^2H_2$]anatabine hydrochloride derived from [2-^2H]baikiain (upper spectrum).

and then to anatabine by previously described methods.[17,29] These deuterated anatabines showed the expected degenerate signals in their ^{13}C NMR spectra at C-2 and C-4.

This result with [2-^2H]baikiain indicates that the dimerization

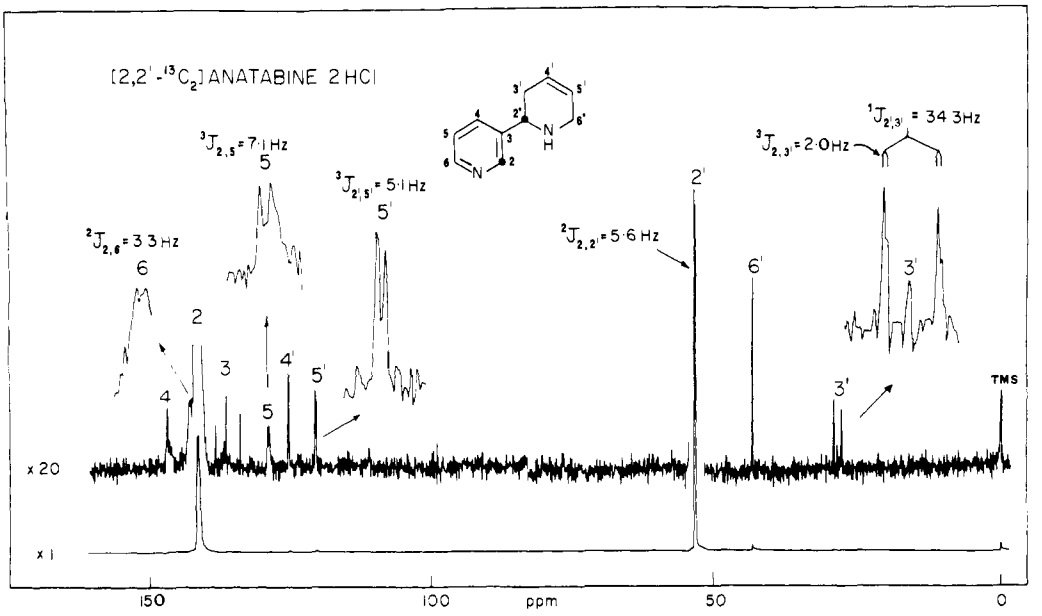
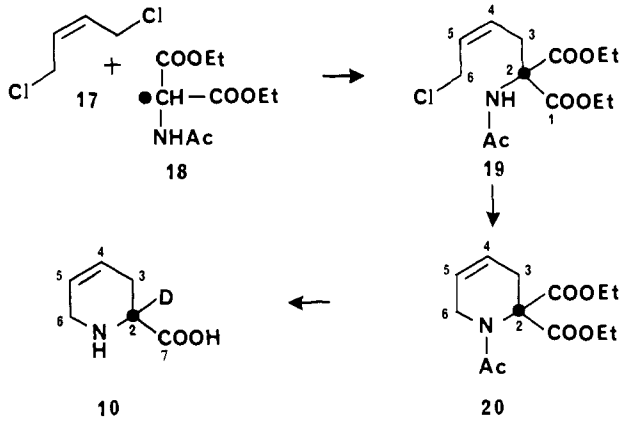


Figure 2. Proton noise-decoupled ^{13}C NMR spectrum of [2,2'-$^{13}C_2$]anatabine hydrochloride derived from [2-^{13}C]baikiain. Expansion of the horizontal scale to illustrate the couplings is ×7.

Scheme III. Synthesis of [2-^{2}H]- and [2-^{13}C]Baikiain



of 2,5-dihydropyridine is apparently regiospecific. However, the reaction was further investigated with [2-^{13}C]baikiain since a small amount of scrambling of deuterium between C-2 and C-6 of the pyridine ring of anatabine would be undetectable by the method of analysis used.

The ^{13}C NMR spectrum of the anatabine obtained from [2-^{13}C]baikiain is illustrated in Figure 2. It is clear that only the C-2 and C-2′ positions are enriched with ^{13}C, no enhancement of C-6 or C-6′ being apparent. This spectrum also illustrates the various short- and long-range couplings that can be observed in this enriched material which are in accord with the literature.[30,31] Pyridine was also isolated from the reaction of [2-^{13}C]baikiain with hypochlorite. The ^{13}C NMR spectrum of its hydrochloride in D_2O exhibited only a single peak at 142.6 ppm, having a chemical shift identical with C-2,6 of unenriched pyridine hydrochloride.[32]

Since the regiospecificity observed in the formation of anatabine from the labeled baikiain in vitro is the same as that observed in the formation of the alkaloid from nicotinic acid in vivo (Scheme I), strong support has been provided for the intermediacy of the previously unknown 2,5-dihydropyridine in both reactions. If, indeed, some of the 2,5-dihydropyridine isomerizes to 1,2-dihydropyridine prior to self-condensation, the present results indicate that the 1,2-dihydropyridine reacts entirely at C-5 with no reaction at C-3. This finding is in accord with studies on the electrophilic attack of dienamines,[33] which takes place regioselectively at the position β to the nitrogen.

Experimental Section[34]

Ethyl 2-Acetamido-2-carbethoxy-6-chloro-(*Z*)-4-hexenoate (19). Diethyl acetamidomalonate (21.75 g, 0.10 mol) in ethanol (250 mL) was added to a solution of sodium (2.52 g, 0.11 g atom) in ethanol (200 mL), and this solution was added slowly during 1.5 h to 1,4-dichloro-(*Z*)-2-butene (57 g, 0.45 mol) dissolved in ethanol (250 mL) at 25 °C in a N_2 atomosphere. After stirring for an additional 13 h, the pale orange reaction mixture was filtered and the filtrate evaporated in vacuo below 20 °C to yield a residue which was dissolved in ether (250 mL) and washed with water and $NaHCO_3$ solution. The dried (Na_2SO_4) ether extract was evaporated to yield a residue which was dissolved in a little ethanol. Addition of petroleum ether (bp 30–40 °C) afforded colorless needles of ethyl 2-acetamido-2-carbethoxy-6-chloro-(*Z*)-4-hexenoate (21.5 g, 70%): mp 85.5–86.5 °C; ^1H NMR ($CDCl_3$) δ 6.80 (s, 1 H, NH), 5.55 (m, 2 H, H-4 and H-5), 4.25 (q, 4 H, OCH_2CH_3), 4.04 (d, 2 H, CH_2Cl), 3.17 (d, 2 H, H-3), 2.03 (s, 3 H, $COCH_3$), 1.26 (t, 6 H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$) δ 169.5 (NHCO), 167.5 (*C*OOEt), 130.3 (5), 126.6 (4), 66.0 (2), 62.7 (OCH_2CH_3), 39.1 (6), 30.5 (3), 22.9 ($COCH_3$), 14.0 (OCH_2CH_3); IR (KBr) 3260 (NH), 1760 (ester CO), 1650, 1540 cm^{-1} (amide I, II); mass spectrum (70 eV, relative percent), *m/z* 270 (92, M − Cl), 174 (100).

Anal. Calcd for $C_{13}H_{20}ClNO_5$: C, 51.07; H, 6.59; N, 4.58. Found: C, 51.32; H, 6.55; N, 4.72.

Diethyl 1-Acetyl-1,2,3,6-tetrahydropyridine-2,2-dicarboxylate (20). The chloro compound **19** (9.3 g, 30 mmol) dissolved in ether (500 mL) was added during 0.5 h to a solution of lithium bis(trimethylsilyl)amide (made in situ from 10 g of bis(trimethylsilyl)amine and an equivalent amount of butyllithium) in ether (500 mL) stirred under N_2 at 25 °C. After stirring for 12 h, a precipitate had formed in the reaction mixture, which was then diluted with water (200 mL). The ether layer was washed with 5% $NaHCO_3$ and dried over Na_2SO_4. Evaporation afforded a golden colored oil of **20** (8.0 g) contaminated with some of the starting material. Purification by distillation in vacuo or by chromatography was

(29) Leete, E.; Ranbom, K. C.; Riddle, R. M. *Phytochemistry* **1978**, *18*, 75.
(30) Wray, V. *Prog. Nucl. Magn. Reson. Spectrosc.* **1979**, *13*, 177.
(31) Weigert, F. J.; Roberts, J. D. *J. Am. Chem. Soc.* **1972**, *94*, 6021.
(32) Pugmire, R. J.; Grant, D. M. *J. Am. Chem. Soc.* **1968**, *90*, 697.
(33) (a) Stork, G.; Birnbaum, G. *Tetrahedron Lett.* **1961**, 313. See also: (b) Velluz, L.; Nomine, G.; Bucourt, R.; Pierdet, A.; Dufay, P. *Ibid.* **1961**, 127. (c) Opitz, G.; Merz, W. *Justus Liebigs Ann. Chem.* **1962**, *652*, 163.
(34) Melting points are uncorrected. ^1H NMR spectra were recorded on a Varian HFT-80 spectrometer. ^{13}C and deuterium NMR spectra were recorded on a Varian XL-100-15 Fourier transform spectrometer operating at 25.16 and 15.36 MHz, respectively, by Stephen B. Philson and Brad Roberts. All chemical shifts are given relative to tetramethyl silane. Mass spectra were determined by Roger Upham and his associates at the University of Minnesota on an AEI-MS-30 or Finnigan 4000 mass spectrometer. Ultraviolet spectra were determined on a Cary 17D spectrometer (purchased with NSF equipment Grant CHE 78-23857). Infrared spectra were determined on a Perkin-Elmer 727 spectrometer. Elementary analyses were performed by MHW Laboratories, Phoenix, AZ.

unsatisfactory. However, this material was converted successfully to baikiain in good yield in the subsequent step. A sample of **20** obtained by distillation (110 °C, 10^{-4} mmHg) was a colorless viscous oil having the following spectral properties: ^{1}H NMR ($CDCl_3$) δ 5.77 (br m, 2 H, H-4 and H-5), 4.12 (q, 4 H, OCH_2CH_3), 4.02 (m, 2 H, H-6) 2.86 (m, 2 H, H-3) 2.16 (s, 3 H, $COCH_3$), 1.26 (t, 6 H, OCH_3CH_3); ^{13}C NMR ($CDCl_3$) δ 172.5 ($COCH_3$), 168.1 (COOEt), 123.2 (4), 122.9 (5), 66.6 (2), 61.9 (OCH_2CH_3), 45.5 (6), 32.1 (3), 22.2 ($COCH_3$), 14.0 (OCH_2CH_3); IR (neat) 1755 (ester CO), 1645 cm^{-1} (amide CO); chemical ionization mass spectrum (NH_3 carrier gas), m/z 287 (M + 18).

DL-[**2-^{2}H**]**Baikiain (10).** Compound **20** (2.7 g, 10 mmol) was refluxed for 6 h in D_2O (30 mL) containing 6 N DCl (3 mL) in a N_2 atmosphere. The solvent and the DCl nominally contained >99.8% D. The brown crystalline residue obtained on evaporation was redissolved in water (H_2O), decolorized with charcoal, and again evaporated to dryness. The residue was crystallized from a mixture of methanol and acetone, affording baikiain hydrochloride as colorless plates, mp 258–263 °C, (lit.[16] mp 264 °C for the natural L isomer). This material was dissolved in water and chromatographed on a column of Amberlite IR-120 ion exchange resin (H^+ form), washing with water until the eluant was free of Cl^- ion. The baikiain was eluted from the column with 2 N ammonium hydroxide. The residue obtained on evaporation of this eluant was crystallized from a mixture of methanol and acetone to yield DL-[2-^{2}H]baikiain as colorless prisms (0.7 g, 55%): mp 255–261 °C dec (lit.[16] mp 273–274 °C); ^{1}H NMR (D_2O) δ 6.0–5.55 (m, 2 H, H-4 and H-5), 3.60 (m, 2 H, H-6), no triplet at δ 3.55 which is present in undeuterated baikiain, assigned to H at C-2, 2.75–2.05 (m, 2 H, H-3); ^{13}C NMR (D_2O) δ 174.1 (7), 126.2 (4), 121.2 (5), 55.8 (2, a degenerate triplet due to ^{2}H at this position), 43.4 (6), 26.6 (3); mass spectrum (70 eV, relative percent), m/z 128 (6.2, M), 83 (100, M − COOH).

Diethyl [2-^{13}C]Acetamidomalonate (18).[35] Sodium nitrite (11.8 g, 170 mmol) in water (18 mL) was added during 1 h to a stirred solution of diethyl [2-^{13}C]malonate[36] (4.40 g, 27.3 mmol) in acetic acid (11 mL) cooled in an ice bath in a N_2 atomosphere. The solution was stirred for 24 h, allowing the temperature to rise to 25 °C. The reaction mixture was extracted with CH_2Cl_2 which was dried (Na_2SO_4) and evaporated to afford a yellow oil. This oil was dissolved in CH_2Cl_2 (150 mL) and stirred with Celite (5 g) and anhydrous sodium acetate (6 g) for 2 h. Evaporation of the filtered mixture afforded the solid tris(diethyl-[2-^{13}C]oximomalonate)–sodium acetate complex (5.1 g), which was dissolved in a mixture of acetic acid (20 mL) and acetic anhydride (27 mL). Zinc dust (7.5 g) was added slowly to this solution, which was stirred at 95 °C. The mixture was refluxed for 45 min and then filtered, the residue being washed with hot acetic acid (50 mL). The residue obtained on evaporation of the combined filtrates was crystallized from benzene, affording colorless plates of diethyl [2-^{13}C]acetamidomalonate (4.26 g, 19.5 mmol, 71%): mp 96–97 °C, (lit.[35] mp 97–98 °C); ^{1}H NMR ($CDCl_3$) δ 6.52 (m, 1 H, NH), 5.13 (d of d, 1 H, CH, $^3J_{H,H}$ = 7.1 Hz, $^1J_{^{13}C,H}$ = 146 Hz), 4.23 (q, 4 H, OCH_2CH_3), 2.08 (s, 3 H, $COCH_3$), 1.32 (t, 6 H, OCH_2CH_3); ^{13}C NMR (CD_3COCD_3) δ 169.8 (CONH), 166.7 (COOEt, $^1J_{1,2}$ = 61.8 Hz), 61.9 (OCH_2CH_3), 56.7 (COCHCO, $^1J_{1,2}$ = 61.8 Hz), 21.7 ($COCH_3$), 13.7 (OCH_2CH_3); mass spectrum (70 eV, relative percent); m/z 219 (2.8, M + 1), 103 (100).

DL-[**2-^{13}C**]**Baikiain (10).** Diethyl [2-^{13}C]acetamidomalonate was converted to [2-^{13}C]baikiain with the same experimental conditions used for the synthesis of [2-^{2}H]baikiain. The following coupling constants were observed in the ^{13}C NMR of the intermediates: ethyl [2-^{13}C]-2-acetamido-2-carbethoxy-6-chloro-(*Z*)-4-hexenoate (**19**) ($CDCl_3$), δ 167.5 (COOEt, $^1J_{1,2}$ = 60.0 Hz), 30.4 (3, $^1J_{2,3}$ = 36.1 Hz); diethyl [2-^{13}C]-1-acetyl-1,2,3,6-tetrahydropyridine-2,2-dicarboxylate (**20**), ($CDCl_3$) δ 168.1 (COOEt) $^1J_{2,7}$ = 63.1 Hz. [2-^{13}C]Baikiain had the following spectral characteristics, different from unenriched material: ^{1}H NMR (Na^+ salt in D_2O) δ 3.55 (d, 1 H, H-2, $^1J_{^{13}C,H}$ = 140 Hz); ^{13}C NMR (Na^+ salt in D_2O) δ 174.1 (7, $^1J_{2,7}$ = 59.1), 121.2 (5, $^3J_{2,5}$ = 3.5 Hz), 55.8 (2, enriched carbon, greatly enhanced signal), 26.6 (3, $^1J_{2,3}$ = 33.4 Hz); mass spectrum (70 eV, relative percent); m/z 129 (0.9), 128 (1.8), 83 (34.5), the spectrum indicated a ^{13}C enrichment of 91%.

Reaction of Baikiain with Sodium Hypochlorite To Yield Anatabine and Pyridine. The following procedure is the one actually used for the synthesis of [2,2′-$^{13}C_2$]anatabine from [2-^{13}C]baikiain and is typical for other experiments, carried out at different pHs and with unlabeled baikiain. DL-[2-^{13}C]Baikiain hydrochloride (91% ^{13}C, 275 mg, 1.67 mmol) was dissolved in 80 mL of pH 10 borate buffer (43.9 mL of 0.1 N NaOH + 50 mL 0.1 M H_3BO_3 diluted to 100 mL) and 5% aqueous NaOCl (2.5 mL, 1.67 mmol) added rapidly at 25 °C. After 2 h, the pale yellow solution was extracted with ether in a liquid–liquid extractor for 18 h. This extract was evaporated at 0 °C, on a rotary evaporator, passing the evolved vapor through a trap containing 2 N HCl to collect pyridine. The residue obtained on evaporation of the ether was subjected to TLC on silica gel PF-254 (Merck), developing with a 90:10:1 mixture of chloroform–ethanol–concentrated ammonia. The upper zone (R_f 0.8) was extracted with methanol, which was then evaporated in the presence of HCl. This residue was combined with material from the 2 N HCl trap used in the initial evaporation of the ether. This residue was made basic with NaOH, extracted with ether, then evaporated, and distilled into a U-tube cooled in dry ice–acetone. The pyridine (2.03 mg, 1.5%), estimated by UV spectroscopy, was converted to its hydrochloride for determination of its ^{13}C NMR spectrum in D_2O (a single peak at 142.6 ppm (C-2) was observed, the remaining resonances being undetectable above background). The main zone (R_f 0.4) from the TLC plate was extracted with methanol and evaporated in the presence of HCl, and the residue was made basic with NaOH, extracted with ether, evaporated, and distilled into a U-tube cooled in dry ice–acetone. The contents of the U-tube were acidified with HCl and evaporated to yield anatabine dihydrochloride as a pale brown semisolid (38 mg, 19%). The ^{13}C NMR spectrum of this material in D_2O (0.4 mL) is illustrated in Figure 2, which also records the various coupling constants. The spectrum was obtained with a spectral window of 5500 Hz, 34K transients, 0.73-s acquisition time, 1.47 Hz/data point.

Preliminary reactions were carried out with L-baikiain at pH 7 and 8 (NaOH + 0.1 M KH_2PO_4 buffer) and 10 (borate buffer). The yield of anatabine (estimated by UV spectroscopy on material isolated as previously described) at pH 7, 8, and 10 was 10%, 18%, and 26%, respectively. [2,2′-$^2H_2$]Anatabine obtained from [2-^{2}H]baikiain had the following spectral characteristics: ^{1}H NMR (dihydrochloride in D_2O) δ 8.92–8.6. (overlapping d of d, 2 H, H-4 and H-6), 8.17 (d of d, 1 H, H-5), 5.90 (m, 2 H, H-4′ and H-5′), 3.85 (m, 2 H, H-6′), 2.73 (m, 2 H, H-3); ^{2}H NMR (2HCl in H_2O) δ 9.13 (D-2), 7.24 ($CDCl_3$, coaxial reference), 4.83 (D-2′). It afforded a dipicrate, mp 200–201 °C, identical (IR, mixed melting point) with an authentic specimen of (*RS*)-anatabine dipicrate; mass spectrum (70 eV, relative percent), m/z 162 (100), 161 (27), 147 (12), 133 (12), 120 (10), 109 (28), 108 (43), 107 (16), 106 (19), 83 (37), 54 (68). For unenriched anatabine dipicrate the following data were obtained: m/z (rel percent) 160 (100), 159 (47), 145 (19), 131 (46), 118 (19), 107 (21), 106 (26), 105 (17), 82 (34), 54 (31).

Isolation of 5-Benzoyloxy-2,4-pentadienal (16) from the Reaction of Baikiain with Hypochlorite. DL-Baikiain hydrochloride (163.5 mg, 1 mmol) dissolved in pH 10 borate buffer (50 mL) was treated with 5% sodium hypochlorite (3 mL, 2 mmol). Examination of an aliquot in the UV 2 min after the addition of the hypochlorite revealed a strong absorption at 362 nm. After 5 min the solution was made strongly basic by the addition of 10% NaOH (20 mL) and shaken with benzoyl chloride (1 mL) for 1 h. The solution was then extracted with ether. Evaporation of the dried ($MgSO_4$) extract afforded a pale yellow residue which was sublimed (110 °C, 10^{-4} mmHg) to afford pale yellow needles of **16** (3.5 mg, 1.7%): mp 118–119 °C (lit.[20] mp 116–118 °C); λ_{max} (95% EtOH) 283 nm (log ϵ 4.48), identical with authentic 5-benzoyloxy-2,4-pentadienal made from pyridine.[20]

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Registry No. DL-**10**, 83232-03-5; DL-**10**·HCl, 83232-04-6; DL-[2-^{13}C]-**10**, 83232-05-7; **16**, 1068-90-2; **18**, 68882-34-8; (*Z*)-**19**, 83232-02-4; **20**, 83248-22-0; [2-^{13}C]diethyl 2-oxomalonate oxime, 83248-23-1; DL-[2,2′-$^{13}C_2$]anatabine, 83232-06-8; DL-[2,2′-^{2}H]anatabine dihydrochloride, 83248-24-2; DL-[2,2′-^{2}H]anatabine dipicrate, 83248-26-4; (*Z*)-1,4-dichloro-2-butene, 1476-11-5; anatabine, 2743-90-0; baikiain, 7200-16-0.

(35) Based on the synthesis of Shaw and Nolan (Shaw, K. N. F.; Nolan, C. *J. Org. Chem.* **1957**, *22*, 1668).

(36) Mueller, M. E.; Leete, E. *J. Org. Chem.* **1981**, *46*, 3151.