The Incorporation of [1-^{13}C,^{14}C,*methylamino*-^{15}N]-*N*-Methylputrescine into Nicotine and Scopolamine Established by Means of Carbon-13 Nuclear Magnetic Resonance

Edward Leete* and James A. McDonell[1]

Contribution from the Natural Products Laboratory,[2] Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received August 11, 1980*

Abstract: [1-^{13}C,^{14}C,*methylamino*-^{15}N]-*N*-Methylputrescine was prepared from a mixture of potassium [^{14}C]cyanide and [^{13}C,^{15}N]cyanide. Administration of this compound to *Nicotiana tabacum* plants afforded radioactive nicotine (0.10% specific incorporation). Examination of its ^{13}C NMR revealed the presence of satellites about the signal for C-5′ of the pyrrolidine ring due to the presence of contiguous ^{13}C and ^{15}N atoms ($J_{^{13}C,^{15}N}$ = 4.2 Hz). Scopolamine (0.17% specific incorporation) was isolated from *Datura innoxia* plants which had been fed the same precursor. The bridgehead carbons of the tropane moiety of this alkaloid afford ^{13}C NMR signals with slightly different chemical shifts ($\Delta\delta$ = 0.09 ppm) due to the presence of the chiral tropic acid half of the molecule, and only one of these signals exhibited satellites ($J_{^{13}C,^{15}N}$ = 2.9 Hz) due to contiguous ^{13}C and ^{15}N atoms. These results substantiate previous hypotheses on the biosynthesis of these alkaloids.

Introduction

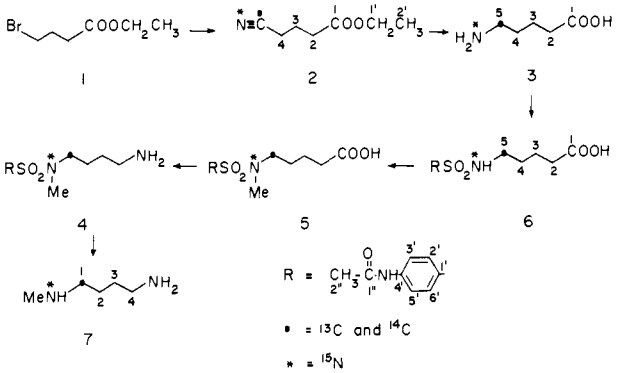
In the last few years an increasing number of biosynthetic studies has been carried out by using precursors which are labeled with contiguous ^{13}C atoms.[3] These adjacent ^{13}C atoms give rise to satellites (due to spin–spin coupling) in the ^{13}C NMR, located about central peaks which arise from natural abundance ^{13}C. Since the natural occurrence of two ^{13}C atoms is only 0.0123%, quite high dilutions of a highly enriched precursor into the ultimate natural product can be tolerated.[4] Much less work has been published utilizing precursors labeled with contiguous ^{13}C and ^{15}N atoms.[5] All the previous studies have been concerned with the biosynthesis of natural products in microbial systems where high specific incorporations are often obtained. Even though the natural abundance of contiguous ^{13}C and ^{15}N atoms is only 0.004%, the observation of satellites in the ^{13}C NMR spectra is often difficult because the coupling constants of ^{13}C to ^{15}N are usually much smaller than typical ^{13}C to ^{13}C coupling constants.[6]

The present work involving the use of [^{13}C,^{15}N]-*N*-methylputrescine was undertaken to establish the validity of the biosynthetic routes to nicotine and the tropane alkaloids.

Results

(1) Synthesis of [1-^{13}C,^{14}C,*methylamino*-^{15}N]-*N*-Methylputrescine. Previous syntheses of unlabeled *N*-methylputrescine[7] were not economical for the preparation of the desired compound containing contiguous ^{13}C and ^{15}N atoms. Numerous attempts to use a method previously described for the preparation of [1-^{14}C,*methylamino*-^{15}N]-*N*-methylputrescine[8] were unsuccessful in our hands. Scheme I illustrates a new synthesis of *N*-methylputrescine which was finally used for the preparation of the labeled compound. Reaction of ethyl 4-bromobutanoate (**1**) with a mixture of potassium [^{14}C]cyanide and potassium [^{13}C,^{15}N]-cyanide yielded ethyl [*cyano*-^{13}C,^{14}C,^{15}N]-4-cyanobutanoate (**2**) (67%). The labeled potassium cyanide contained a considerable amount of potassium hydroxide, and the yield of **2** was very poor if this was not removed prior to reaction with the bromo ester (see Experimental Section). It is of interest to note that the labeled **2** exhibited three absorptions in the IR at 2246, 2209, and 2153 cm^{-1} due to the presence of ^{12}C≡^{14}N, ^{12}C≡^{15}N, and ^{13}C≡^{15}N, respectively. These frequencies are consistent with those calculated

Scheme I. Synthesis of [1-^{13}C,^{14}C,*methylamino*-^{15}N]-*N*-Methylputrescine



(1) Bush Foundation Predoctoral Fellow, 1976–1978. This paper is dedicated to Professor Kurt Mothes, who celebrated his 80th birthday on November 3, 1980.

(2) Contribution No. 174 from this Laboratory. Contribution No. 173: Leete, E. *Can. J. Chem.* **1980**, *58*, 1806.

(3) (a) Leete, E. *Rev. Latinoamer. Quim.* **1980**, *11*, 8. (b) Leete, E. *J. Nat. Products* **1980**, *43*, 130. (c) Leete, E.; Yu, M.-L. *Phytochemistry* **1980**, *19*, 1093.

(4) Significant satellites were observed when the specific incorporation of [2,3-$^{13}C_2$]ornithine into nornicotine was only 0.025%,[3c] i.e., a dilution of 3200 of the precursor which contained 79% of the $^{13}C_2$ species.

(5) (a) Gould, S. J.; Chang, C. C.; Darling, D. S.; Roberts, J. D.; Squillacote, M. *J. Am. Chem. Soc.* **1980**, *102*, 1707. (b) Haber, A.; Johnson, R. D.; Rinehart, K. L. *Ibid.* **1977**, *99*, 3541. (c) Burton, G.; Nordlov, H.; Hosozawa, S.; Matsumoto, H.; Jordan, P. M.; Faterness, P. E.; Pryde, L. M.; Scott, A. I. *Ibid.* **1979**, *101*, 3114.

(6) (a) Berger, S.; Roberts, J. D. *J. Am. Chem. Soc.* **1974**, *96*, 6757. (b) Kainsho, M. *Ibid.* **1979**, *101*, 1031. (c) DiBlasi, R.; Kopple, K. D. *J. Chem. Soc., Chem. Commun.* **1975**, 33. (d) Fritz, H.; Clerin, D.; Fleury, J. P. *Org. Magn. Reson.* **1976**, *8*, 269. (e) Berger, S.; Kaletsch, H. *Ibid.* **1976**, *8*, 438. (f) Severge, A.; Juttner, F.; Breitmaier, E.; Jung, G. *Biochim. Biophys. Acta* **1976**, *437*, 289. (g) Schulman, J. M.; Venanzi, T. *J. Am. Chem. Soc.* **1976**, *98*, 4701, 6739.

(7) (a) Dudley, H. W.; Thorpe, W. V. *Biochem. J.* **1925**, *19*, 854. (b) Keil, W. *Hoppe-Seyler's Z. Physiol. Chem.* **1931**, *6*, 81.

(8) Maier, W.; Schütte, H. R. *Z. Chem.* **1967**, *7*, 155. In this method it was claimed that *N*-(4-aminobutyl)phthalimide was obtained by the catalytic reduction of *N*-(3-cyanopropyl)phthalimide. This amine was then tosylated, N-methylated, and hydrolyzed to yield *N*-methylputrescine. Our experiments indicated that the *N*-(4-aminobutyl)phthalimide is a very labile compound undergoing rapid conversion to a phthalamic acid derivative by a neighboring group participation of the primary amino group in the hydrolysis of the phthalimido protecting group. This facile hydrolysis of *N*-(aminoalkyl)-phthalimides has been observed.[9] Earlier attempts to prepare *N*-(4-aminobutyl)phthalimide by reduction of the corresponding cyanide also failed.[10] Hydrogenation of *N*-(3-cyanopropyl)phthalimide in $(CF_3CO)_2O$ yielded *N*-(4-(trifluoroacetamido)butyl)phthalimide. However, attempted methylation of this amide with methyl iodide in acetone in the presence of KOH followed by hydrolysis of the product with HCl afforded only putrescine.

(9) Peck, R. M. *J. Org. Chem.* **1962**, *27*, 2677.

(10) Keil, W. *Ber. Dtsch. Chem. Ges.* **1926**, *59*, 2816.

0002-7863/81/1503-658$01.00/0

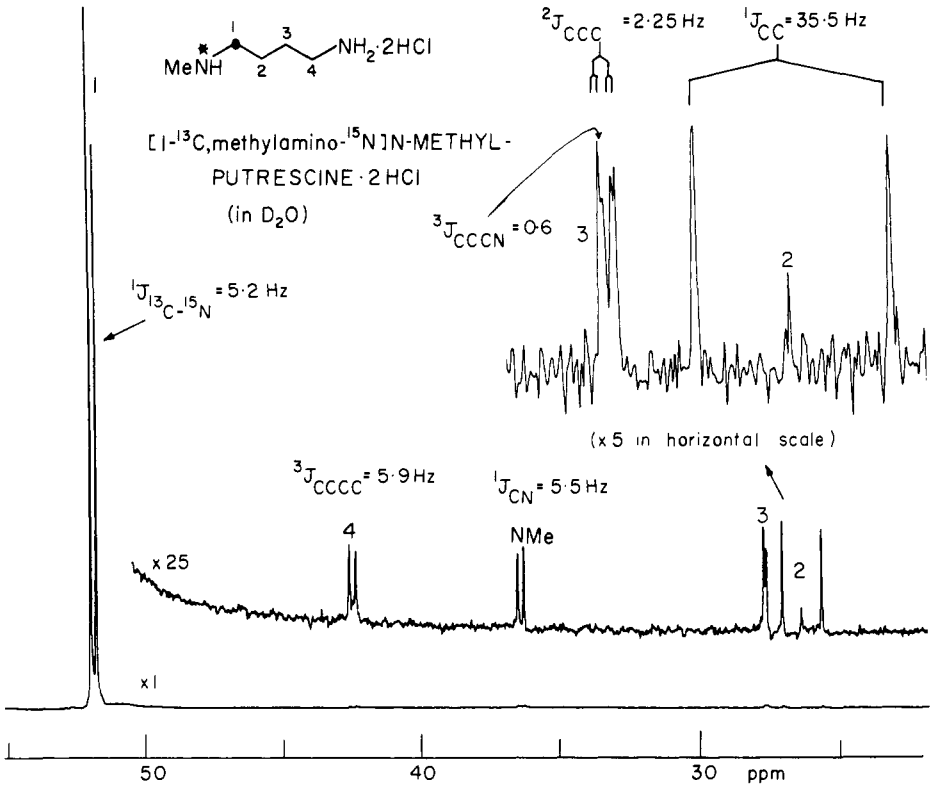


Figure 1. Proton-noise-decoupled ^{13}C NMR spectrum of [1-^{13}C,*methylamino*-^{15}N]-*N*-methylputrescine dihydrochloride in D_2O.

by Hookes law.[11] Hydrogenation of **2** in aqueous ethanol containing sodium hydroxide yielded, after ion-exchange chromatography, 5-aminopentanoic acid (**3**) (68%). The *N*-(2,4-dinitrophenyl) derivative of **3** was prepared for determination of its isotope content by mass spectrometry. The spectrum was consistent with an enrichment of 91% ^{13}C and >99% ^{15}N. Reaction of **3** with *N*-acetylsulfanilyl chloride yielded the sulfonamide **6** (89%). This sulfonamide was obtained in much higher yield than the tosylate. Methylation of **6** in aqueous sodium hydroxide with dimethyl sulfate afforded **5** (82%) which was subjected to a Schmidt reaction with hydrazoic acid in chloroform to yield the amine **4**. This sulfonamide was hydrolyzed with 48% hydrobromic acid in the presence of phenol[12] to yield [1-^{13}C,^{14}C,*methylamino*-^{15}N]-*N*-methylputrescine isolated as its dihydrochloride (8.7% from **5**). In this synthesis ^{14}C was introduced so that the specific incorporation of this compound into the ultimate alkaloids could readily be determined by radioactive assay. The ^{13}C NMR spectrum of this *N*-methylputrescine dihydrochloride enriched at C-1 with 91% ^{13}C and on the adjacent nitrogen with >99% ^{15}N is illustrated in Figure 1 and exhibits several interesting couplings. The chemical shifts were assigned by off-resonance decoupling and comparison with the spectrum of putrescine. In the off-resonance decoupled spectrum the signal at 36.4 ppm was a quartet, and it thus arises from the *N*-methyl group. Putrescine dihydrochloride (in D_2O) has two signals at 42.8 and 27.7 ppm (from Me_4Si) arising from the carbons adjacent to the nitrogens and those two bonds away, respectively. The signal at 42.5 ppm in *N*-methylputrescine is thus assigned to C-4. Carbons 2 and 3 can be differentiated by examination of the spectrum of the enriched material. The signal centered at 26.2 ppm is a triplet and is assigned to C-2. The triplet arises by a one bond coupling to the 91% enriched C-1 (J = 35.5 Hz, typical for sp^3–sp^3 coupling[13]). The satellites are not completely symmetrical about the central peak. The inner satellite is 17.1 Hz from the central C-2 peak in excellent agreement with the calculated distance (17.2 Hz).[14] No geminal coupling of C-2 to the enriched ^{15}N was observed. On the other hand the signal for C-3 at 27.5 ppm is a doublet of doublets. The larger coupling of 2.25 Hz is assigned to geminal coupling of C-3 to C-1. The smaller splitting of about 0.6 Hz is assigned to a vicinal coupling of C-3 to the ^{15}N. Other couplings of interest are the vicinal coupling of C-4 to C-1 (3.9 Hz) and the one bond couplings of C-1 and the *N*-methyl group to the ^{15}N (5.2 and 5.5 Hz, respectively).

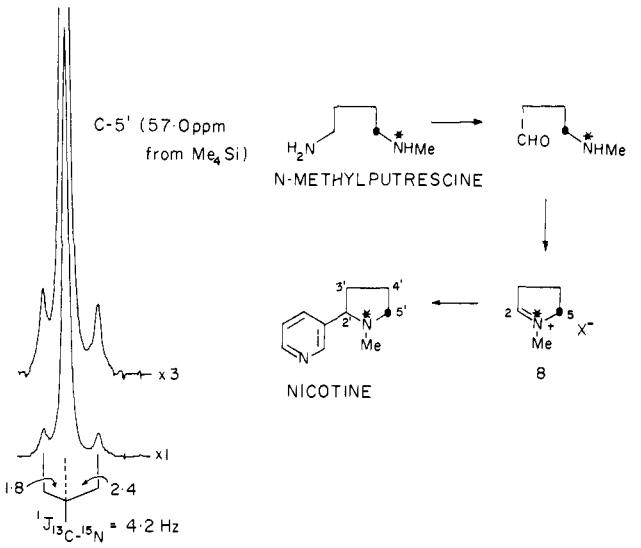


Figure 2. ^{13}C NMR signal of C-5′ of nicotine derived from [1-^{13}C,-*methylamino*-^{15}N]-*N*-methylputrescine.

(2) The Pattern of Labeling in Nicotine and the Tropane Alkaloids Derived from [1-^{13}C,^{14}C,*methylamino*-^{15}N]-*N*-Methylputrescine. The pattern of labeling expected in nicotine derived from the [1-^{13}C,*methylamino*-^{15}N]-*N*-methylputrescine is illustrated in Figure 2. The immediate precursor of the pyrrolidine

(11) Colthup, N. B.; Daly, L. H.; Wiberly, S. E. "Introduction to Infrared and Raman Spectroscopy"; Academic Press: New York, 1964, p 169.
(12) Snyder, H. R.; Heckert, R. E. *J. Am. Chem. Soc.* **1952**, *74*, 2006.
(13) Stothers, J. B. "Carbon-13 NMR Spectroscopy"; Academic Press: New York, 1972; p 327.
(14) Calculated from the formula $0.5(V_{AB} + J_{AB} - (V_{AB}^2 + J_{AB}^2)^{1/2})$ where V_{AB} = difference in chemical shift between the coupled carbons and J_{AB} = coupling constant.

ring of nicotine is the *N*-methyl-Δ^1-pyrrolinium salt (**8**) which is incorporated into nicotine without any equilibration of the C-2 and C-5 positions.[15] It is considered that this compound is formed by the oxidation of the primary amino group of *N*-methylputrescine. Indeed, an enzyme has been isolated from *Nicotiana tabacum* roots which catalyzes this transformation.[16] In the present work the administration of the labeled *N*-methylputrescine to intact *Nicotiana tabacum* plants by the wick method yielded radioactive nicotine (0.104% specific incorporation) and nornicotine (0.054% specific incorporation). Examination of the ^{13}C NMR spectrum of the nicotine run with a sweep width of 2800 Hz revealed no satellites on any of the carbon signals. This disappointing result was attributed to the fact that the ^{13}C signals at the base line have a spectral width of about 5 Hz, the same magnitude as the expected $^{13}C-^{15}N$ coupling. When a much smaller spectral window was chosen (507 Hz), satellites were observed on either side of the signal for C-5′ (Figure 2). It should be noted that the satellites are not symmetrically arranged about the central peak. This is attributed to an isotope effect; i.e., the central singlet peak is due to ^{13}C adjacent to ^{14}N, whereas the satellites arise from ^{13}C adjacent to ^{15}N. The shift is 0.3 Hz upfield for the $^{13}C-^{15}N$ signal.[17] The specific incorporation of the *N*-methylputrescine into nicotine was calculated by measurement of the intensity of the central C-5′ peak and the upfield satellite, the latter being chosen since the downfield satellite is overlapping significantly with the central peak. These measurements indicated that at C-5′ of nicotine there was 0.0830% of ^{13}C contiguous with ^{15}N. When the natural occurrence of $^{13}C-^{15}N$ is corrected for, this figure indicates that the specific incorporation of the *N*-methylputrescine, containing 90% of the doubly labeled ^{13}C, ^{15}N species, was 0.10%, in excellent agreement with the incorporation of ^{14}C determined by radioactivity measurement. No satellites were detected at the C-2′ signal. This observation indicates that the proposed intermediate is incorporated into nicotine without any equilibration of the 2- and 5-positions, in agreement with earlier results.[15] This result also indicates that the *N*-methylputrescine does not undergo any demethylation to yield putrescine in the course of the feeding experiment. If this had occurred, the symmetry of this compound would have resulted in distribution of the ^{13}C label between C-2′ and C-5′ of nicotine. This result differs from that found by Schütte et al.[18] On administering [*methyl*-^{14}C,*methylamino*-^{15}N]-*N*-methylputrescine to the shoots of *N. rustica*, they obtained nicotine in which the specific incorporation of the ^{15}N (0.28%) was more than twice that of the ^{14}C (0.13%). They proposed that the *N*-methylputrescine underwent partial demethylation on the way from the shoot to the root which is the principal site of nicotine synthesis. Later feeding experiments by the same group[19] with [1-^{14}C,*methylamino*-^{15}N]-*N*-methylputrescine yielded nicotine which had most (>90%) of its ^{14}C located at C-5′ in accord with the present work.

N-Methylputrescine has also been shown to be a precursor of the tropane alkaloids,[20] although the labeled hyoscyamine and scopolamine obtained after feeding [*methyl*-^{14}C,*methylamino*-^{15}N]-*N*-methylputrescine to a root culture of *Datura metel* were not degraded to establish the location of the ^{14}C. The incorporation of *N*-methylputrescine into the pyrrolidine ring of the tropane nucleus is consistent with extensive tracer work on the origin of this class of alkaloids carried out during the past 25 years.[21] A significant result was the discovery that [2-^{14}C]ornithine labeled only C-1′ of the tropane nucleus.[22,23] The biosynthetic route is

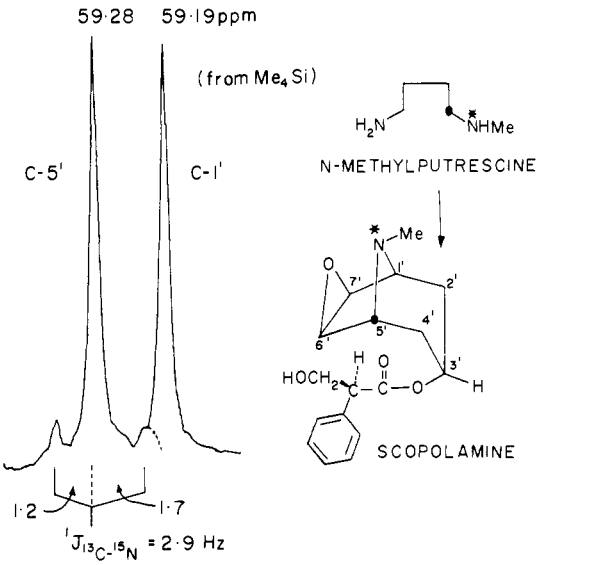


Figure 3. ^{13}C NMR Signals of C-1′ and C-5′ of scopolamine derived from [1-^{13}C,*methylamino*-^{15}N]-*N*-methylputrescine.

considered to proceed via δ-*N*-methylornithine,[24,25] *N*-methylputrescine, *N*-methyl-Δ^1-pyrrolinium salt,[26] hygrine,[27-29] tropinone, and tropine. Thus our [1-^{13}C,*methylamino*-^{15}N]-*N*-methylputrescine would be expected to label C-5′ of the tropane nucleus with ^{13}C. Previously[22,23] it required an extensive elaborate degradative scheme to prove that [2-^{14}C]ornithine yielded hyoscyamine labeled at the C-1′ position. Although the tropane nucleus has a plane of symmetry, C-7′, C-1′, and C-3′ are magnetically nonequivalent to the diastereotopic positions C-6′, C-5′ and C-4′, respectively, because of the presence of the chiral tropic acid moiety. This effect was first observed in the ^{13}C NMR spectra of hyoscyamine and scopolamine by Simeral and Maciel.[30] We independently[31] observed the chemical shift differences (up to 0.5 ppm) for the diastereotopic carbons of scopolamine and hyoscyamine. Administration of our labeled *N*-methylputrescine to *Datura innoxia* plants by the wick method yielded radioactive scopolamine (0.17% specific incorporation) and hyoscyamine (0.15% specific incorporation). Hyoscyamine readily racemizes to atropine under normal conditions of isolation. In such racemic material, there will still be two signals for the bridgehead carbons C-1′ and C-5′; however, each signal will be the average of interactions of C-1′ and C-5′ with the two enantiomorphic forms of tropic acid. Fortunately, scopolamine is quite optically stable. Examination of its ^{13}C NMR spectra revealed the presence of a satellite on the downfield signal (59.28 ppm) (Figure 3). The other satellite is partially obscured by the signal for the other bridgehead carbon; however, a $^{13}C-^{15}N$ coupling constant of about 2.9 Hz is apparent. Again, an isotope shift of about 0.25 Hz is observed. The incorporation calculated from the intensity of the downfield satellite to the central peak (0.15%) is in good agreement with that obtained from the radioactive assay. Our present results do not, of course, prove that it is the C-5′ (rather than C-1′) position of scopolamine which is labeled with ^{13}C and we are unwilling to rationalize the assignment of C-5′ to the downfield signal based on examination of models in which the positions of

(15) Leete, E. *J. Am. Chem. Soc.* **1967**, *89*, 7081.
(16) Mizusaki, S.; Tanabe, Y.; Noguchi, M.; Tamaki, E. *Phytochemistry* **1972**, *11*, 2757.
(17) Isotope effects on NMR signals have been recently summarized: Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1980**, *102*, 4609.
(18) Schütte, H. R.; Maier, W.; Mothes, K. *Acta Biochim. Pol.* **1966**, *13*, 401.
(19) Schütte, H. R.; Maier, W.; Stephan, U. *Z. Naturforsch., B: Anorg. Chem., Org. Chem., Biochem., Biophys., Biol.* **1968**, *23*, 1426.
(20) Liebisch, H. W.; Maier, W.; Schütte, H. R. *Tetrahedron Lett.* **1966**, 4079.
(21) This topic has been reviewed: Leete, E. *Planta Med.* **1979**, *36*, 97.
(22) Leete, E. *J. Am. Chem. Soc.* **1962**, *84*, 55.
(23) Leete, E. *Tetrahedron Lett.* **1964**, 1619.
(24) Ahmad, A.; Leete, E. *Phytochemistry* **1970**, *9*, 2345.
(25) Baralle, F. E.; Gros, E. G. *Chem. Commun.* **1969**, 721.
(26) This compound was detected in *Datura* plants which had been fed labeled ornithine: Mizusaki, S.; Kisaki, T.; Tamaki, E. *Plant Physiol.* **1967**, *43*, 93.
(27) Liebisch, H. W.; Peisker, K.; Radwan, S.; Schütte, H. R. *Z. Pflanzenphysiol.* **1972**, *67*, 1.
(28) O'Donovan, D. G.; Keogh, M. F. *J. Chem. Soc. C* 1969, 223.
(29) McGaw, B. A.; Woolley, J. G. *Phytochemistry* **1978**, *17*, 257.
(30) Simeral, L.; Maciel, G. E. *Org. Magn. Reson.* **1974**, *6*, 226.
(31) Leete, E.; Kowanko, N.; Newmark, R. A. *J. Am. Chem. Soc.* **1975**, *97*, 6826.

the phenyl and the hydroxymethyl group of tropic acid are oriented relative to C-1′ and C-5′. All our present results prove is that *N*-methylputrescine is incorporated unsymmetrically into the tropane nucleus of scopolamine in accord with previous ideas on the biosynthesis of this class of alkaloid.

Experimental Section

General Remarks. Elemental analyses were performed by Clark, Means and Perkins Microanalytical Laboratory, Urbana, Ill, or by the Scandinavian Microanalytical Laboratory, Herley, Denmark. Melting points and boiling points are uncorrected. ^{1}H NMR spectra were recorded on a Varian HFT-80 spectrometer. Carbon-13 NMR spectra were determined on a Varian XL-100 operating at 25.1 MHz on a Varian CFT-20 spectrometer. All spectra are recorded as parts per million from Me_4Si. Infrared spectra were recorded on a Beckman Model 33 instrument. Mass spectra were determined by Dr. Roger Upham at the University of Minnesota by using an AEI-MS30 double-beam spectrometer. Radioactivity measurements were carried out in a Nuclear Chicago Mark II liquid scintillation system by using the usual scintillators[32] in dioxane. Evaporations were carried out in vacuo in a rotary evaporator at 50 °C.

Ethyl [*cyano*-^{13}C,^{14}C,^{15}N]-4-Cyanobutanoate (2). Ethyl 4-bromobutanoate[33] (3.30 mL, 25 mmol) was slowly added by means of a syringe to a magnetically stirred solution of a mixture of potassium [^{13}C,^{15}N]-cyanide (1.95 g, 30 mmol, 91% ^{13}C, 99.7% ^{15}N, Stable Isotope Resource) and potassium [^{14}C]cyanide (<1.0 mg, nominal activity 0.5 mCi, Amersham Searle) in dry dimethyl sulfoxide (40 mL)[34] in an N_2 atmosphere at 20 °C. After 30 min the solution was stirred at 60 °C for 20 h. The cooled reaction mixture was then diluted with chloroform (30 mL) and water (20 mL). The separated aqueous solution was extracted with more chloroform (2 × 30 mL). The combined organic extract was washed with water (5 × 20 mL) and saturated sodium chloride (30 mL). Evaporation of the dried (Na_2SO_4) exctract yielded a pale brown oil which was distilled (100 °C (5-mm Hg)) to afford ethyl 4-cyanobutanoate as a colorless oil (2.84 g, 66% based on the potassium cyanide): IR (neat) 2246 (^{12}C≡^{14}N), 2209 (^{12}C≡^{15}N), 2153 cm^{-1} (^{13}C≡^{15}N) with relative intensities of 0.4:10:100, respectively; mass spectrum, *m/e* (relative intensity) 143 (2.2), 96 (100, –OEt), identical with unlabeled material except for M + 2 shifts due to the presence of ^{13}C and ^{15}N; ^{1}H NMR (acetone-d_6) δ 1.22 (3 H, t, 2′), 1.97 (2 H, m, 3), 2.47 (4 H, m, 2 and 4), 4.10 (2 H, q, 1′); ^{13}C NMR ($CDCl_3$) δ 14.2 (2′), 16.4 (4, $^1J_{4,CN}$ = 56.6 Hz, $^2J_{4,^{15}N}$ = 2.8 Hz), 21.0 (3, $^2J_{3,CN}$ = 1.7 Hz), 32.6 (2, $^3J_{2,CN}$ = 4.1 Hz), 60.8 (1′), 119.3 (CN, $^1J_{C,^{15}N}$ = 16.6 Hz), 172.3 (1).

[5-^{13}C,^{14}C,^{15}N]-5-Aminopentanoic Acid (3). The labeled cyano ester **2** (2.86 g, 19.9 mmol) dissolved in ethanol (5 mL) was added to a suspension of prehydrogenated W2 Raney nickel (2.0 g) in a mixture of ethanol (30 mL) and 10% aqueous sodium hydroxide (15 mL). Hydrogenation was carried out at 20 °C for 18 h at 3 atm of pressure when 85% of the theoretical uptake of H_2 had occurred. The reaction mixture was then filtered, and the catalyst washed with ethanol and 10% sodium hydroxide. The combined filtrates were evaporated until all the ethanol was removed. The residual aqueous solution was adjusted to pH 4 with concentrated hydrochloric acid, filtered, and applied to a 20 × 2-cm column of Bio-Rad AG 50W-8 (H^+ form). The column was washed with water until the eluate was free of chloride. The amino acid was then eluted with 1 N ammonium hydroxide (150 mL). Evaporation of this eluate and trituration of the residue with ether afforded 5-aminopentanoic acid (1.80 g 76%), mp 135–145 °C (lit.[35] 157–158 °C). This material was used without further purification in the next step. *N*-(2,4-Dinitrophenyl)-[5-^{13}C,^{14}C,^{15}N]-5-aminopentanoic acid was prepared from this labeled material (50 mg) by shaking with 2,4-dinitrofluorobenzene (0.2 mL), potassium bicarbonate (0.2 g) in water (1 mL), and ethanol (1 mL). The reaction mixture was extracted with ether and then acidified with concentrated hydrochloric acid when the 2,4-dinitrophenyl derivative separated. Recrystallization from aqueous acetone afforded colorless plates, mp 167–168 °C (lit.[36] 167.5–169.5 °C). Comparison of the molecular ion peak intensities in the mass spectrum of this material (16 eV) (*m/e* (relative intensity) 285 (30.9), 284 (3.6), 283 (<0.2)) with unenriched material (285 (2), 284 (5), 283 (28)) indicated that the enrichments of ^{13}C and ^{15}N were the same as in the initial potassium [^{13}C,^{15}N]cyanide: ^{1}H NMR (acetone-d_6) δ 2.82, 4.52 (2 H, br d, 5, $^1J_{^{13}CH}$ = 136 Hz); ^{13}C NMR (Me_2SO-d_6) δ 21.8 (3), 27.6 (4, $^1J_{4,5}$ = 35.7 Hz), 33.3 (2, $^3J_{2,5}$ = 3.4 Hz), 42.5 (5, $^1J_{5,^{15}N}$ = 9.7 Hz), 115.4 (6′), 123.8 (5′), 130.1 (3′), 134.8 (4′), 148.3 (1′, $^1J_{1',^{15}N}$ = 20.0 Hz), 174.6 (1). The aromatic carbons were assigned by comparison with *o*- and *p*-nitroaniline;[37] C-2′ was not detected in the spectrum.

Anal. Calcd for $C_{11}H_{13}N_3O_6$ (enriched material): C, 46.64; H, 4.61; N, 15.07. Found: C, 46.24; H, 4.59; N, 14.29.

5-(*N*-Acetylsulfanil[^{15}N]amido)[5-^{13}C,^{14}C]pentanoic Acid (6). *N*-Acetylsulfanilyl chloride (5.86 g, 25 mmol, mp 147–149 °C, crystallized from benzene) was added slowly to a vigorously stirred solution of the crude 5-aminopentanoic acid (1.60 g, 13.4 mmol) in a mixture of 0.8 N sodium hydroxide (34 mL, 27 mmol) and ether (50 mL). The mixture was stirred for 20 h, adding sodium hydroxide periodically to keep to pH close to neutrality. At the end of the reaction the ether was evaporated and the solution filtered and adjusted to pH 5 with acetic acid, when a white solid separated. This material was crystallized from aqueous ethanol to yield colorless plates of the sulfonamide **6** (3.39 g, 89%): mp 160–161 °C (lit.[36] 159.5–160.5 °C); IR (KBr) 3400 (amide NH), 3280 (sulfonamide NH), 1700 (amide I), 1660, 1600, 1535 (amide II), 1320, 1160 (SO_2), 1070 (^{12}C–^{15}N stretch), 1040 cm^{-1} (^{13}C–^{15}N stretch); ^{1}H NMR (acetone-d_6) δ 1.56 (4 H, m, 3 and 4), 2.24 (2 H, t, 2), 2.88 (1 H, br s, acetyl NH), 6.36 (1 H, d of t, ^{15}NH, $^1J_{H,^{15}N}$ = 84.0 Hz, $^2J_{H,^{13}C}$ = 5.8 Hz), 7.75 (4 H, m, Ar H), 9.5 (1 H, br s, CO_2H); ^{13}C NMR (acetone-d_6) δ 21.6 (3), 24.1 (2″), 28.4 (4, $^1J_{4,5}$ = 36.4 Hz), 33.1 (2, $^3J_{2,5}$ = 4.1 Hz), 42.2 (5, $^1J_{5,^{15}N}$ = 5.4 Hz), 118.7 (3′,5′), 127.7 (2′,6′), 134.4 (1′, with a 2.8-Hz splitting = $^2J_{1',^{15}N}$ or $^3J_{1',5}$), 142.8 (4′), 169.1 (1″), 174.4 (1).[38]

5-(*N*-Acetylsulfanil[^{15}N]-*N*-methylamido)[5-^{13}C,^{14}C]pentanoic Acid (5). Dimethyl sulfate (4.0 mL, 42 mmol) was added slowly over 30 min to a stirred solution of the sulfonamide **6** (3.33 g, 10.5 mmol) in 0.6 N sodium hydroxide (36 mL, 21.6 mmol) at 0 °C. After 16 h 10% sodium hydroxide was slowly added during 4 h until the solution became clear and alkaline. Acetic acid was then added until the pH was 5. The precipitated solid was filtered off and crystallized from aqueous ethanol (charcoal) to yield colorless needles of the sulfonamide **5** (2.83 g, 82%): mp 134–136 °C; IR (KBr) similar to **6**, except that the sulfonamide NH had disappeared and the SO_2 absorptions were shifted 40 cm^{-1} to a higher frequency; ^{1}H NMR (acetone-d_6) δ 1.65 (4 H, m, 3 and 4), 2.38 (2 H, t, 2), 2.69 (3 H, d, NMe, $^2J_{H,^{15}N}$ = 3.1 Hz), 3.88 (2 H, t, 5 $^2J_{H,^{15}N}$ = 6.3 Hz), 4.70 (1 H, br s, acetyl NH), 7.79 (4 H, q, ArH), 9.63 (1 H, s, CO_2H); ^{13}C NMR (acetone-d_6) δ 21.2 (3), 23.9 (2″), 27.5 (4, $^1J_{4,5}$ = 37.2 Hz), 32.3 (2, $^3J_{2,5}$ = 4.0 Hz), 33.7 (NMe, $^1J_{NMe,^{15}N}$ = 5.9 Hz), 49.1 (5, $^1J_{5,^{15}N}$ = 5.9 Hz), 118.5 (3′,5′), 128.1 (2′,6′), 131.5 (1′), 143.0 (4′), 168.8 (1″), 173.8 (1).

Anal. Calcd for $C_{14}H_{20}N_2O_5S$ (unlabeled material): C, 51.19; H, 6.15; N, 8.53. Found: C, 50.89; H, 5.97; N, 8.54.

[1-^{13}C,^{14}C,*methylamino*-^{15}N]-*N*-Methylputrescine (7). A solution of hydrazoic acid in chloroform (18 mL, 2.13 M, 38 mmol) was added slowly to a solution of the *N*-methylsulfonamide **5** (2.83 g 8.57 mmol) in a mixture of chloroform (25 mL), acetonitrile (1 mL), and concentrated sufuric acid (2 mL)[39] at 20 °C. The mixture was then refluxed for 4 h. The cooled reaction mixture was neutralized with sodium hydroxide, extracted with chloroform, and dried (Na_2SO_4). Evaporation of this extract afforded a solid which still had an absorption in the carbonyl region of the IR (1720 cm^{-1}). This material was thus recycled through the reaction with hydrazoic acid. The final product, *N*-acetylsulfanil[^{15}N]-*N*-methyl-([1-^{13}C,^{14}C]-4-aminobutyl)amide (**4**) was obtained as an oil which failed to crystallize and was used in the next step without further purification. This oil was refluxed with a mixture of 48% hydrobromic acid (25 mL) and phenol (2.0 g) for 17 h. After being cooled, the solution was extracted with ether (4 × 20 mL) and the aqueous solution then made basic with sodium hydroxide. This solution was extracted with chloroform (5 × 20 mL) which was dried (Na_2SO_4) and evaporated to yield a brown oil. This material was dissolved in 2-propanol which was saturated with HCl gas. The precipitated solid was crystallized from 95% ethanol to yield *N*-methylputrescine dihydrochloride as a colorless fluffy solid (144 mg, 8.7%, mp 179–181 °C (lit.[7a]

(32) Friedman, A. R.; Leete, E. *J. Am. Chem. Soc.* **1963**, *85*, 2141.
(33) Wohlgemuth, H. *Ann. Chim. (Paris)* **1914**, [9] *2*, 298.
(34) The potassium [^{13}C,^{15}N]cyanide was freed from potassium hydroxide by the following method. Anhydrous ammonia (10 mL) was condensed into a flask (25 mL) which contained the cyanide. After being stirred for several minutes, the mixture was allowed to stand and the ammonia solution of potassium cyanide decanted into a 50-mL flask, leaving behind potassium hydroxide. Several washings and decantations were required to transfer all the cyanide to the second flask. The ammonia was evaporated in a current of dry N_2 leaving a residue of potassium cyanide which was dissolved in dry dimethyl sulfoxide for subsequent reaction.
(35) Wallach, O. *Justus Liebigs Ann. Chem.* **1900**, *312*, 171.
(36) Burmistrov, S. I.; Plit, V. A. *Tr. Dnepropetr. Khim.-Tekhnol. Inst.* **1959**, [I] *12*, 153; *Chem. Abstr.* **1962**, *57*, 16550*a*.
(37) Ref 13, p 197.
(38) The aromatic carbons of this compound and **5** were assigned by comparison with benzene sulfonyl derivatives: Horyna, J.; Lycka, A.; Snobl, D. *Collect. Czech. Chem. Commun.* **1980**, *45*, 1575.
(39) This procedure was based on analogous Schmidt reactions: (a) Adamson, D. W. *J. Chem. Soc.* **1939**, 1564; (b) Rothchild, S.; Fields, M. *J. Org. Chem.* **1951**, *16*, 1080.

179 °C)). Its specific activity (^{14}C) was 5.19×10^7 dpm/mmol, somewhat higher than that calculated (4.4×10^7 dpm/mmol), indicating that the commercial potassium [^{14}C]cyanide contained about 18% more activity than expected. Its ^{13}C NMR is illustrated in Figure 1. The sample was dissolved in D_2O with sodium (trimethylsilyl)propionate as internal standard. The recorded chemical shifts are corrected (+1.1 ppm) so that they are relative to Me_4Si. The spectrum was obtained with a spectral width of 1500 Hz, 2-s acquistion time (0.5 Hz/data point): δ 26.2 (2, $^1J_{2,1}$ = 35.5 Hz), 27.5 (3, $^2J_{3,1}$ = 2.3 Hz, $^3J_{3,^{15}N}$ = 0.6 Hz), 36.4 (NMe, $^1J_{NMe,^{15}N}$ = 5.5 Hz), 42.5 (4, $^3J_{4,1}$ = 5.9 Hz), 51.9 (1, $^1J_{1,^{15}N}$ = 5.2 Hz). It afforded a dipicrate (mp 229–231 °C dec (lit.[7a] mp 229–230.5 °C dec)) identical with authentic *N*-methylputrescine dipicrate.

Anal. Calcd for $C_{17}H_{20}N_8O_{14}$ (enriched material): C, 36.49; H, 3.56; N, 20.11. Found: C, 36.16; H, 3.37; N, 19.72.

It also afforded a chloroplatinate, yellow prisms from 10% hydrochloric acid, mp 225–230 °C dec (lit.[7a] mp 230.5 °C). The *N,N'*-(2,4-dinitrophenyl) derivative of unlabeled *N*-methylputrescine was prepared by reaction with 2,4-dinitrofluorobenzene in aqueous sodium bicarbonate solution. Its dihydrochloride was obtained as yellow needles: mp 152–153 °C, mass spectrum, *m/e* (relative intensity) 435 (M + 1) (2), 268 (9), 236 (66), 210 (100), 196 (22), 180 (25), 164 (56), 118 (46), 104 (22), 91 (17).

Administration of [1-^{13}C,^{14}C,*methylamino*-^{15}N]-*N*-Methylputrescine to *Nicotiana tabacum* and Isolation of the Alkaloids. The labeled *N*-methylputrescine dihydrochloride (20 mg, 5.19×10^7 dpm/mmol, total activity = 5.86×10^6 dpm) dissolved in water (10 mL) was fed to eight 4-month-old *N. tabacum* plants growing in a greenhouse (May) by the wick mehtod. After 7 days the whole plants were harvested (fresh weight 3.9 kg). The residual activity which was not incorporated into the plants was 0.04%. The alkaloids were isolated as previously described,[40] initially macerating the fresh plants with a mixture of chloroform and concentrated ammonia. The aqueous ammonia layer had an activity of 1.21×10^6 (20.6%). The alkaloids isolated were nicotine (700 mg), purified as its diperchlorate (having a specific activity (^{14}C) equal to 5.41×10^4 dpm/mmol (0.104% specific incorporation, 4.0% absolute incorporation)), nornicotine (22 mg) purified as its dipicrate (2.82×10^4 dpm/mmol (0.054% specific incorporation, 0.07% absolute incorporation)), anabasine (1.88 mg) ($<0.1 \times 10^4$ dpm/mmol), and anatabine (13.0 mg) ($<0.1 \times 10^4$ dpm/mmol). The ^{13}C NMR spectrum of the enriched nicotine was determined on a sample (213 mg) dissolved in $CDCl_3$ (0.5 mL) in a 5-mm tube. The carbons of the pyrrolidine ring of nicotine were examined by using the following instrument parameters: 8288 scans (18 h), 135-μs (90°) pulses, 7.9-s acquisition time (0.13 Hz/data point), and a sweep width of 507 Hz. Under these conditions no trace of satellites was detectable at the signal for C-2'.

Administration of [1-^{13}C,^{14}C,*methylamino*-^{15}N]-*N*-Methylputrescine to *Datura innoxia* and Isolation of the Alkaloids. The labeled *N*-methylputrescine dihydrochloride (30 mg, 5.19×10^7 dpm/mmol, total activity = 8.80×10^6 dpm) dissolved in water was fed to 28 2-month-old *D. innoxia* plants growing in a greenhouse (May) by the wick method. After 7 days the plants were harvested (0.035% residual activity not absorbed). The roots (fresh weight 1.43 kg) and aerial parts (fresh weight 2.87 kg) were dried at 60 °C for 20 h. After finely grinding the dried material in a Wiley mill, it was extracted[41] to yield the following alkaloids which were crystallized as their picrates to constant radioactivity. From the aerial parts the following were obtained: scopolamine (30 mg), 8.80×10^4 dpm/mmol (0.17% specific incorporation, 0.10% absolute incorporation); hyoscyamine (8 mg), 7.80×10^4 dpm/mmol (0.15% specific incorporation). From the roots the following were obtained: 3α,6β-ditigloyloxytropane (9 mg), 2.63×10^4 dpm/mmol (0.051% specific incorporation); 3α,6β-ditigloyloxytropan-7β-ol (6 mg), 2.33×10^4 dpm/mmol (0.045% specific incorporation). For ^{13}C NMR the scopolamine (30 mg) was dissolved in acetone-d_6 (0.3 mL) in a 5-mm tube. The instrument parameters were essentially the same as those used for determination of the spectrum of enriched nicotine. To obtain the resolution illustrated in Figure 3, we chose a spectral window of 464 Hz to encompass the C-1' and C-5' resonances at 59.2 and 59.3 ppm from Me_4Si.

Acknowledgment. This investigation was supported by a research grant (No. GM-13246) from the National Institutes of Health, U.S. Public Health Service. We thank Dr. Robert M. Riddle of the University of Minnesota and Dr. Richard A. Newmark, 3M Company, St. Paul, for determination of the ^{13}C NMR spectra. We are indebted to the Stable Isotope Resource at the Los Alamos Scientific Laboratory (supported by Grant RR-00962 from the Division of Research Resources, National Instititutes of Health) for the potassium [^{13}C,^{15}N]cyanide. *Datura innoxia* seeds were obtained from the Zentral institut für Genetik und Kulturpflanzenforschung, Gatersleben, D.D.R.

Supplementary Material Available: ^{13}C NMR spectra of compounds **2**, **5**, **6**, and the *N*-(2,4-dinitrophenyl) derivative of **3** (4 pages). Ordering information is given on any current masthead page. These spectra will be provided with requests for reprints.

(40) Leete, E.; Slattery, S. A. *J. Am. Chem. Soc.* **1976**, *98*, 6326.

(41) Evans, W. C.; Partridge, M. W. *J. Pharm. Pharmacol.* **1952**, *4*, 769. McGaw, B. A. Ph.D. dissertation, Leichester Polytechnical Institute, Leichester, England, 1978.