References and Notes

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Biosynthetic Studies of Secondary Plant Metabolites with $^{13}CO_2$. *Nicotiana* Alkaloids. 2.[1] New Synthesis of Nornicotine and Nicotine. Quantitative Carbon-13 NMR Spectroscopic Analysis of [2′,3′,*N*-CH_3-$^{13}C_3$]Nicotine[2]

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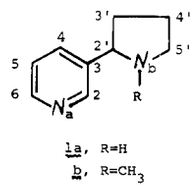
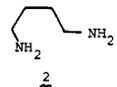
An efficient synthesis of the tobacco alkaloids, nornicotine (**1a**) and nicotine (**1b**), is achieved by Michael condensation of the α-lithiomethoxime of 3-acetylpyridine (**11**) with a ketene thioacetal monoxide (**4**) to give **12a**, thus providing all the pyrrolidine ring atoms of **1a** in masked form. Subsequent reduction with diborane and reductive cyclization in refluxing 97% formic acid are used to produce *N*-formyl-**1a** in high yield. The latter is converted to **1a** or **1b** by literature procedures in 60% overall yield from 3-acetylpyridine. The chemistry of several alternative, but inefficient, synthetic approaches to **1a** also is discussed, in particular a route from 3-acetylpyridine and mesylaziridine (**16**). The synthesis of [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b** is achieved by this route from [1,2-$^{13}C_2$]acetic acid and [^{13}C]formaldehyde via [1′,2′-$^{13}C_2$]-3-acetylpyridine. Analysis of the proton-decoupled ^{13}C NMR spectrum of the triply ^{13}C-labeled **1b** is done to certify the accuracy of the quantitative determination of the relative ^{13}C enrichment in **1b** biosynthetically labeled by $^{13}CO_2$. Thereby an earlier conclusion about the symmetry of ^{13}C labeling of carbons 2′ and 5′ of **1b** is circumstantially validated, i.e., that these four carbons are unequally ^{13}C labeled by $^{13}CO_2$ within experimental error.

We are studying the applicability of highly enriched $^{13}CO_2$ as a biosynthetic probe of secondary plant metabolites, particularly alkaloids. In our first paper concerning the tobacco alkaloids[1] the results of some initial feeding experiments using 97 atom % $^{13}CO_2$, in which we studied the biosynthesis of nicotine (**1b**), the major alkaloid of *N. tabacum* and *N. glutinosa*, were described and *tentatively* interpreted as corroborating some of Rapoport's earlier observations obtained with $^{14}CO_2$:[4] that the *N*-methylpyrrolidine ring of **1b** could become unsymmetrically labeled by incorporation of isotopically labeled CO_2. Since such conclusions are in variance with all of the other data concerning nicotine's biosynthesis,[1,5] i.e., that the *N*-methylpyrrolidine ring of **1b** is formed in vivo via putrescine (**2**) and thereby should be symmetrically labeled by isotopic carbon labeled precursors, it is very important to certify the experimental error of our technique of ^{13}C label distribution analysis (^{13}C NMR spectroscopy). This is especially important since the intramolecular ^{13}C labeling inequality of **1b** that we reported was C(2′) (62%), C(3′) (65%), C(4′) (58%), and C(5′) (49%),[1] such values perhaps being equivalent within experimental error, although Matwiyoff and Burnham had certified that the technique we used was accurate to within ±1.4% for uniformly and nonuniformly ^{13}C-labeled acetate.[6] For this reason we developed a new synthesis of nornicotine (**1a**) and **1b** designed to meet our special needs for the synthesis of [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b**. The chemistry that was encountered during the development of our most efficient synthetic route to **1a** and **1b** is reported here as well as the synthesis and quantitative ^{13}C NMR spectroscopic analysis of the triply ^{13}C-labeled **1b**. From our new results the earlier



1a, R=H
b, R=CH_3



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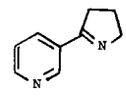

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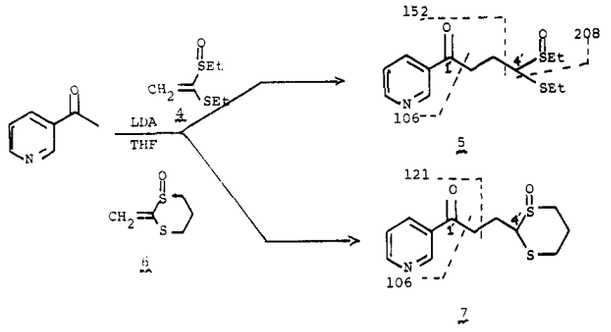
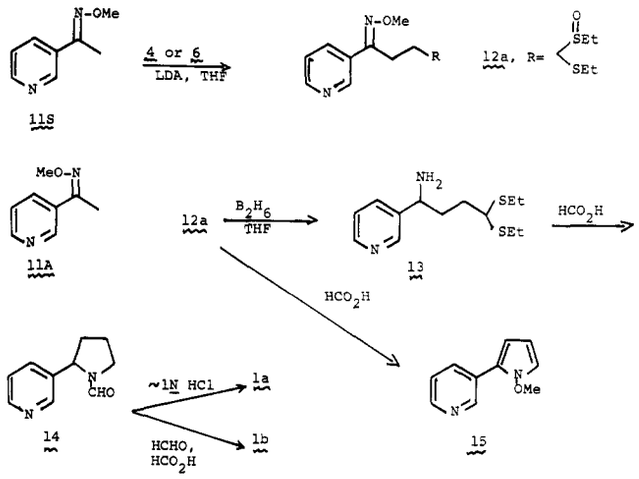
Scheme I



Scheme II

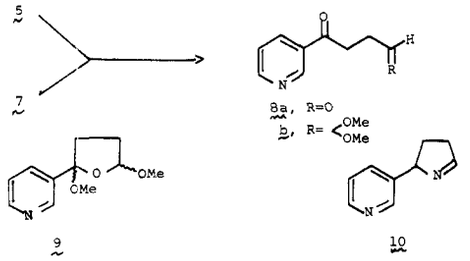


conclusion about the labeling of **1b** by $^{13}CO_2$[1] is partly validated.

Results and Discussion

I. The Synthesis of Nornicotine (1a) and Nicotine (1b). The several syntheses of **1b** reported to date[7] have two general strategies. The most frequently used synthetic route has been the one in which pyridine with a one-carbon C(3) substituent at various oxidation levels is condensed with a three-carbon equivalent. The N-CH_3 group is either contained in the three-carbon unit or introduced by reductive amination of a 1,4-dicarbonyl intermediate. The most novel syntheses of **1b** are Leete's biogenetically modeled route,[7h] and Steven's cyclopropylimine rearrangement route,[8a] which gives myosmine (**3**), a minor tobacco alkaloid easily converted into **1b** by reduction and reductive N-methylation.[7i,9] None of these strategies was felt to be applicable to our special needs for the synthesis of [2′,3′,N-CH_3-$^{13}C_3$]-**1b** either due to their low overall yields or to the limited commercial availability of a suitably ^{13}C-labeled starting material. Hence we chose to develop an alternative strategy wherein the N-methylpyrrolidine ring of **1b** is constructed from 3-acetylpyridine methoxime (**11**), Schlessinger's[10] ketene thioacetal monoxide (**4**), and formaldehyde by Eschweiler–Clarke reductive methylation. The same strategy is employed in a different tactical development in which 3-acetylpyridine and mesylaziridine (**16**) are used to synthesize **3.** In either case our initial assumption was that the desired ^{13}C labeling of **1b** could be achieved straightforwardly by synthesis of doubly ^{13}C-labeled 3-acetylpyridine from [1,2-^{13}C]acetic acid and reductive methylation using [^{13}C]formaldehyde.

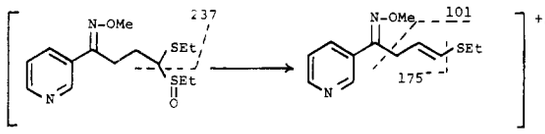
Ketene thioacetal monoxides **4** and **6** (Scheme I) were prepared by a convenient modification of Schlessinger's procedure[10a] in 69 and 57% overall yield, respectively (Experimental Section). Condensation of **4** with the lithium enolate (from lithium diisopropylamide) of 3-acetylpyridine occurred at −10 °C in THF overnight, giving **5** in 38% yield; similiarly **7** was obtained in 46% yield using **6.** The structures of **5** and **7** were consistent with the principal spectral data: characteristic ^1H NMR signals for the pyridyl and C(4′) thioacetal protons and electron impact mass spectral fragments as indicated in Scheme I. Sodium hydride also could be used as the base in THF/HMPA solvent mixtures at −78 °C, but the yield of **5** was much lower than with LDA and dialkylation of 3-acetylpyridine appeared to be a serious problem. It also was noticed that in the formation of **5** prolonged reaction times resulted in the reappearance of starting materials (TLC), indicating that the reaction was partially reversible under the conditions we used.



Hydrolysis of **5** or **7** to the 1,4-dicarbonyl compound (**8a**), followed by reductive amination, was anticipated to lead to **1a** or **1b.** Both **5** and **7** were resistant to hydrolysis with aqueous acid/CH_3CN,[10] aqueous acid/$HgCl_2$, boron trifluoride etherate/HgO,[11] or boron trifluoride etherate/$Hg(OAc)_2$ in acetic acid.[11] By treatment of **5** with $HgCl_2$ and p-toluenesulfonic acid in methanol at room temperature for 24 h the desired hydrolysis could be achieved but in very low yield: **8a** (11%) and **8b** (15%).[12] Since the major byproduct under these conditions was the tetrahydrofuran (**9**),[12] attempts were made to reductively aminate the carbonyl of **5.** We assumed that during the subsequent hydrolysis the primary amine would more effectively trap the incipient C(4′) carbonium ion than the solvent molecules to give isomyosmine (**10**), which would be reducible to **1a** by precedent.[7i,9] However, treatment of the hydrochloride salt of **5** with $NH_4Br/NaCNBH_3$[13] or its oxime with $NaCNBH_3$ at pH 3[13] did not give any of the desired products; overreduction of the pyridine ring and poor material balance in the reaction resulted instead.

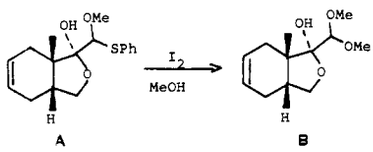
In view of these disappointing results a different approach to **1a** and **1b** was investigated. Since the low yields in the formation of **5** were felt to be due in part to the condensation's reversibility, we considered the use of α-lithio-3-acetylpyridine methoxime (**11**) as the nucleophilic partner. Spenser and Leong[14] had reported that the methoxime of dibenzyl ketone was deprotonated and regiospecifically deuterated on the syn α carbon. Similar observations have been described about ketoxime dianions,[15,16] which are stabler than methoxime monoanions,[15] although Kofron and Yeh reported that dilithioacetone oxime did not give 1,4 adducts with acrolein, only aldol condensation products in low yield.[16] We reasoned that the α-monoanion of **11** would be a more reactive nucleophile than α-lithio-3-acetylpyridine and, because of the lower acidity of **11** than 3-acetylpyridine, would disfavor the reversibility of the 1,4 addition to **4.** Methoxime **11** was shown to be composed of two geometrical isomers in a ratio of 83:17 by the relative integrals of the O-CH_3 and pyridyl ring protons. Since the methyl carbon syn to the hydroxyl of 2-butanone ketoxime resonates at ~6–6.8 ppm higher field than the anti methyl carbon,[17] and since in geraniol the (*Z*)-4-CH_3 resonates at δ_C 16.0 relative to the (*E*)-4-CH_3 (δ_C 23.5) in nerol,[18] the more abundant compound in the **11** mixture must be the syn isomer (**11S**), δ_C 11.9, and the less abundant the anti isomer (**11A**), δ_C 20.8 (Scheme II). Michael addition of α-lithio-**11** to **4** proceeded readily in THF at −78 °C giving **12a**

(86–95%). Compound **12a** was only the C(1′) *E* isomer based on the chemical shift of its *O*-CH_3 protons at δ 3.99, since the methoxime prepared from **5** was a 74:26 mixture of the syn (δ_H 4.00) and anti (δ_H 3.85) isomers.[19] It had a mass spectral fragmentation pattern similar to **5.**



In accord with the known reduction of methoximes to primary amines[20] and sulfoxides to thioethers[21] using diborane, **12a** gave the amino thioacetal (**13**) in quantitative yield[22] by reduction with excess diborane in THF at 25–65 °C. When this reduction was carried out at 25 °C, **13** (40%) plus a second substance (21%) were obtained, the latter proving to be the *O*-methylhydroxylamine derivative of **13** based on its proton resonances at δ 3.40 (OCH_3), 3.76 (H-1′), and 3.98 (H-4′).

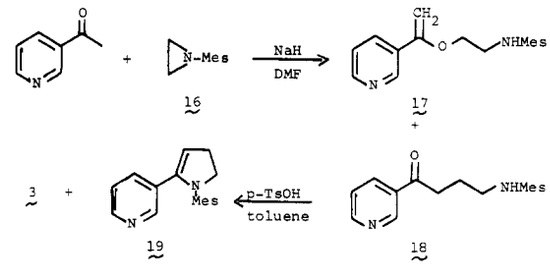
As with **5** the hydrolysis of **13** to a 4-amino aldehyde (or **10**) proved to be troublesome. Not surprisingly treatment of **13** in CH_3CN/H_2O with $HgCl_2$ resulted in the immediate precipitation of a mercuric complex, which did not give any of the desired products after 13 h at room temperature, nor after reduction of the crude reaction mixture with $NaBH_4$. The use of $CdCO_3$[23] or $CuO/CuCl_2$[24] gave only unreacted **13.** Hydrolysis through alkylation[25] or oxidation at sulfur[26] was not attempted because of the presence of primary amine and pyridine functionality and the sensitivity of the expected products to oxidation. Since Trost and Miller reported that the transacetalization A to B proceeded smoothly using I_2 in



refluxing methanol,[27] it was anticipated that **13** could be converted to **10** under similar conditions. Indeed, treatment of **13** with 2 equiv of I_2 in CH_3CN/H_2O at 25 °C for 1 h followed by appropriate workup gave **10** in 41–56% yield plus $(EtS)_2$, although **10** seemed to be quite sensitive to oxidation conditions since excess I_2 or silver oxide caused the formation of polar byproducts and markedly lowered the yield of **10.** The structure of **10** was fully consistent with its principal spectral characteristics: δ_H at 7.24 (q, H-5′) and 5.12 (t, H-2′); molecular ion at *m/e* 146. Reduction of **10** using $NaBH_4$ gave **1a** (66%), which also was obtained directly from **13** without isolation of **10** in 26% yield. The best overall yields of **1a** from **13** (30–40%) were obtained by using AcOH as the reaction solvent, in which evidence was obtained (^1H NMR) for the formation of an intermediate 4-amino diacetoxyacetal, although this substance was too unstable to isolate and characterize fully.

At this stage the capriciousness of the latter hydrolysis technique resulted in the loss of ~40% of our precious ^{13}C-labeled **13** (vide infra), causing us to consider yet another method for conversion of **13** to **1a,** which turned out to work very well. We reasoned that if formic acid was acidic enough to cause a small amount of **10** to form from hydrolysis and intramolecular cyclization of **13,** its well known propensity for imine reduction [28] would drive the reaction in the desired direction. Thus it was gratifying to observe the formation of **14** in 75% overall yield from **12a** when crude **13** was refluxed for 3 h in 97% formic acid.[29] *N*-Formyl-**1a** (**14**) had a doubled formyl proton resonance at δ 8.37 (0.4 H) and 8.10 (0.6 H) indicative of restricted rotation about its amide bond and an expected rapid loss of HCO from the molecular ion in its mass spectrum, giving a fragment ion at *m/e* 147. When **12a** was refluxed 1 h in 97% formic acid, the *N*-methoxypyrrole (**15**)

Scheme III



was formed in 54% yield[12] along with several other more polar compounds. Apparently, the resistance of the methoxime to reduction by HCO_2H in this case directs the reaction's course toward aromatized products, rather than toward *N*-OMe-**3** or -**1a.** Conversion of **14** to **1a** by refluxing in 3 N HCl[28] or to **1b** by Eschweiler–Clark reductive methylation[7i,28] was achieved in 91–93% yield. Furthermore, **1b** could be obtained in 66% overall yield from **12a** without isolation of any intermediates. The resulting overall yield of nicotine or nornicotine was 60% from 3-acetylpyridine, one of the best yielding syntheses of these alkaloids reported to date.[30]

During the initial stages of our work a synthesis of **1a** alternative to that shown in Scheme II was investigated briefly (Scheme III). Although the yield of **3** was too low to warrant further development, the observations are worth mentioning.

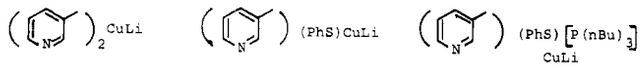
Alkylation of the sodium enolate of 3-acetylpyridine with mesylaziridine (**16**)[31] in DMF at 0 °C gave O-alkylated (**17**) and C-alkylated (**18**) products in a 1:1 ratio in only moderate yield (38%). The use of lithium (LDA in THF) or potassium (KO-*t*-Bu in *t*-BuOH) enolates was less successful than the former and did not markedly change the O to C alkylation ratio. One can conjecture that the presence of O-alkylation here in contrast to its lack using **4** or **6** as the electrophile can be rationalized according to the hard–soft acid–base principles.[32] Since **4** is predicted to be a softer electrophile than **16,** attack by the softer carbanion center of 3-acetylpyridine's enolate would be favored over attack by the harder oxyanion center, whereas with **16,** O-alkylation predominates. Solvent polarity differences also are an important factor here. When **18** was treated subsequently with 1 equiv of *p*-toluenesulfonic acid in refluxing toluene for 3 h with azeotropic water removal, two major products were formed: a mesylate, **19,** the expected product, in 26% yield, which was characterized by its ^1H NMR resonances at δ 2.81 (mesylate) and 5.73 (H-3′) and rapid loss of methanesulfinic acid upon mass spectral fragmentation; and myosmine (**3**) in 15% yield, identified spectrally and by reduction with $NaBH_4$ to **1a** in 66% yield. The appearance of **3** in this reaction was rather surprising, since mesylates usually are rather resistant to acid-catalyzed hydrolysis.

II. The Synthesis of [2′,3′,*N*-CH_3-$^{13}C_3$]Nicotine. A synthesis of [1′2′-$^{13}C_2$]-3-acetylpyridine was required for the synthesis of [2′,3′-$^{13}C_2$]nornicotine. The normal method used to synthesize 3-acetylpyridine, Claisen condensation of ethyl nicotinate and ethyl acetate followed by acid-catalyzed ester hydrolysis and decarboxylation,[33] was felt to be inexpedient and expensive since the ethyl acetate is used in large excess. One attempt was made to react 1 equiv of dilithioacetate with methyl nicotinate (THF at −78 to −30 °C), but the initial results were not encouraging so this route was abandoned. Consequently, we investigated the use of several of the literature methods for ketone synthesis,[34] which were based on the reaction of 3-lithiopyridine with a two-carbon electrophile. Such condensations had to be carried out at <−30 °C due to the instability of 3-lithiopyridine at higher temperatures.[35] However, when acetronitrile or lithium acetate were reacted with 3-lithiopyridine the heterogenous reactions did not proceed until −30 to −10 °C and in both cases the yields of

3-acetylpyridine were low (1–27%) and very poorly reproducible. The use of the more reactive acetylimidazole,[36] dimethylacetamide, or 2-pyridyl thioacetate[37] as electrophile in THF at ~ −50 °C was also unsuccessful, although a small amount of 3-acetylpyridine was formed with the first two reactants. We attribute these failures to the high basicity and low nucleophilicity of 3-lithiopyridine, which favored deprotonation of the electrophile rather than its nucleophilic arylation.

The ketone synthesis of Mukaiyama et al.,[37] in which Grignard reagents are acylated by 2-pyridyl thioacetate, was attempted using the Grignard reagent prpeared from a threefold excess of highly reactive magnesium[38] and 3-bromopyridine at 25 °C. No reaction occurred at 0–25 °C, which may have been due to the excess magnesium needed to prepare the Grignard reagent in good yield.

The use of the mixed cuprate(I) reagents, **20–22,** reported to yield ketones in good yield by reaction with acid chlorides,[39] initially appeared to be encouraging, since 3-benzoylpyridine was prepared from **22** (only; none of this ketone was formed



20 **21** **22**

using **20** or **21**) and benzoyl chloride in 67% yield.[12] However, the same reaction carried out with acetyl chloride failed to yield any 3-acetylpyridine, nor was any of this ketone obtained using **20** or **21.** Since lithium diorganocuprates are not as basic as organolithiums,[39b] the lack of 3-acetylpyridine formation probably is not due to deprotonation of acetyl chloride, but may be due to the difference in reduction potential of radical anion intermediates, *if* these reactions proceed by a two-stage mechanism as proposed by House for conjugate additions of lithium organocuprates.[39b,41]

Since all our attempted ketone syntheses via acetic acid derivatives had been fruitless, we next considered the synthesis of 3-ethylpyridine (**23**), since we had discovered that this could be oxidized to 3-acetylpyridine in 70–83% yield in buffered $KMnO_4$. We attempted to reproduce the synthesis of Giam[41] in which the dihydropyridine reagent prepared from $LiAlH_4$ in excess pyridine was reported to be alkylatable at C(3) by ethyl iodide in 89% yield (gas chromatographic analysis). In our hands this method worked with benzyl chloride as reported, giving 3-benzylpyridine in 24% isolated yield calculated from benzyl chloride, but 3-ethylpyridine was obtained in only 17% yield (GLC) despite several variations in experimental conditions, the major product being quaternized pyridine.[12] At this point we recalled that during the preparation of 3-lithiopyridine from *n*-butyllithium and 3-bromopyridine at −78 °C, a small amount of 3-*n*-butylpyridine usually was formed from alkylation of the *n*-butyl bromide produced in the initial transmetalation. Accordingly, we found that **23** was produced in 77% yield by reaction of ethyl iodide with 3-lithiopyridine at −95 °C. After we completed this work a paper by Parham and Piccirilli appeared in which was described the alkylation of 2- and 3-bromopyridine with *n*-butyl bromide under conditions identical with ours.[42]

Thus, the synthesis of [2′,3′,*N*-CH_3-$^{13}C_3$]nicotine was completed in good overall yield using the chemistry described above as shown in Scheme IV.

III. Quantitative ^{13}C NMR Spectroscopic Analysis of [2′,3′-*N*-CH_3-$^{13}C_3$]Nicotine. The procedures described in our earlier paper[1] were used to prepare samples of the N_b-monoethanesulfonate of [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b** for ^{13}C NMR analysis. It was found that care had to be taken to prepare such samples immediately after elution from the chromatographic adsorbent to avoid line-broadening effects in the carbon spectra. Since all paramagnetic ions extractable with dithiazone in CCl_4 were customarily removed, this line-broadening problem may have been due to oxidation of **1b** in which unpaired electron-containing species were generated in situ; however, as sample degassing by argon did not always alleviate this problem, our hypothesis is conjectural.

Table I. Relative Percentage ^{13}C Enrichment of [2′,3′,*N*-CH_3-$^{13}C_3$]Nicotine

method	intramolecular[a] C(2′)	intramolecular[a] C(3′)	intermolecular[a] C(2′)	intermolecular[a] C(3′)	intermolecular[a] *N*-CH_3
mass spectral[b]	85.6 (89.6)	93.0 (90.8)	5.8	6.2	4.5
^1H NMR	86.1	93.0			
^{13}C NMR					
A[c]	90.9	92.8	6.6	7.5	5.7
B[d]	89.6	92.2	7.0	7.2	*e*
C[f,h]	91.8	94.0	*e*	*e*	*e*
D[g,h]	91.5	93.8	*e*	*e*	*e*

[a] The precision of all peak area measurements was ≤3.5%. [b] Calculated from the relative isotopic mass spectral ion abundances as described in the Experimental Section. The values in parentheses were calculated from an undiluted sample of [2′,3′,-*N*-CH_3-$^{13}C_3$]nicotine, the *N*-CH_3 of which contained 56–62 mol % excess ^{13}C. [c] Calculated by the methods described in ref 1 from ^{13}C NMR spectrum of the N_b-monoethanesulfonate. [d] Calculated by the method described in the Experimental Section from ^{13}C NMR spectrum of N_b-monoethanesulfonate. [e] Not determined. [f] Calculated from relative peak areas of ^{13}C NMR spectrum of bis(trifluoroacetate). [g] Calculated from relative peak areas of ^{13}C NMR spectrum of free base (pH 8.2). [h] Sample of ~90% [2′,3′,-*N*-CH_3-$^{13}C_3$]nicotine in which the contribution of natural ^{13}C abundance **1b** to I_S was *not* subtracted when calculating the intramolecular ^{13}C enrichment.[1]

The ^{13}C enrichment of one sample of [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b** containing ~7 mol % excess ^{13}C was determined by mass spectrometry using the *N*-methylpyrrolidinium fragment ion at *m/e* 84 and the fragment ion at *m/e* 133 [M^+· − C(3′) and C(4′)][45] to measure the ^{13}C enrichment, by ^1H NMR spectroscopy in which the $^1J_{CH}$ and $^2J_{CCH}$ satellite peak areas of a sample of [1′,2′-$^{13}C_2$]-3-acetylpyridine containing ~90 mol % excess ^{13}C were measured, and by ^{13}C NMR spectroscopy using the two methods described in our earlier paper, the *inter*molecular ^{13}C enrichment (total signal area enhancement) and the *intra*molecular ^{13}C enrichment (singlet-to-satellite area ratios corrected for the presence of natural ^{13}C abundance nicotine).[1] The results are shown in Table I(A), in which also is shown (B) the relative ^{13}C enrichment of the triply labeled **1b** calculated by a variation of the original methods (Experimental Section) and of a sample of triply labeled **1b** bis(trifluoroacetate) (C) and free base (D) containing 60–90 mol % excess ^{13}C at the labeled carbons.

The accuracy of our ^{13}C NMR technique for measuring the relative ^{13}C enrichment of **1b** (shown in Table II) is dependent, of course, on what values one chooses as the standard on which the comparison is based. Use of the mass spectral values as the standard is necessary when considering the relative *inter*molecular ^{13}C enrichment, i.e., the total amount of all ^{13}C-labeled molecules present in the sample; here there is

Scheme IV

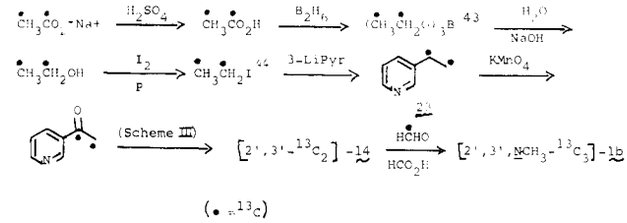


Table II. Relative Error (%) in the Determination of ^{13}C Enrichment of [2′,3′,*N*-CH_3-$^{13}C_3$]Nicotine

	% error of relative ^{13}C enrichment[a]				
	intramolecular		intermolecular		
standard	C(2′)	C(3′)	C(2′)	C(3′)	*N*-CH_3
mass spectrum	−5.8[b,c]	+0.3[c]	−12.1[c]	−17.3[c]	−21.0[c]
	(−1.4)[d]	(−2.2)[d]			
	−4.5[e] (0)	+1.0 (−1.4)	−17.0	−13.9	*f*
^1H NMR	−5.3[b]	+0.2			
	−3.9[e]	+0.9			
^{13}C NMR[g]	+1.0[b]	−3.0			
	−2.4[e]	−2.3			

[a] Refers to data of Table I: A (+) sign indicates the amount the value is greater than the reference value; a (−) sign, the amount the value is less than the reference value. [b] Relative to the values of Table I (A). [c] Based on the mass spectrally determined ^{13}C enrichment of a sample of [2′,3′,*N*-Me-$^{13}C_3$]-**1b** containing ~7 mol % excess ^{13}C at the labeled positions. [d] As in *c* but using a sample of **1b** containing 60–90 mol % excess ^{13}C at the labeled positions. [e] Relative to the values of Table I (B). [f] Not determined. [g] Based on the values of Table I (C) as the reference standard.

Table III. T_1 Values for Nicotine N_b-Monoethanesulfonate[a,b]

carbon	T_1,[c] s	T_1,[d] s
C(2)	1.64 ± 0.26	1.54 ± 0.20
C(3)	~6	~6
C(4)	1.41 ± 0.08	1.39 ± 0.08
C(5)	1.39 ± 0.08	1.43 ± 0.10
C(6)	1.12 ± 0.05	1.21 ± 0.07
C(2′)	1.73 ± 0.29	1.97 ± 0.23
C(3′)	0.90 ± 0.14	0.97 ± 0.12
C(4′)	1.23 ± 0.14	1.25 ± 0.11
C(5′)	1.04 ± 0.14	1.16 ± 0.10
N-CH_3	1.11 ± 0.14	1.19 ± 0.10
CH_2[e]	2.74 ± 0.42	2.78 ± 0.34
CH_3[e]	3.40 ± 0.33	3.38 ± 0.37

[a] Determined by the inversion–recovery method using a 1 M solution in D_2O at 37 °C, pH 5.5. [b] These values are significant only as an indication of relative carbon relaxation rates under the same experimental conditions used to obtain relative ^{13}C enrichments of synthetically and biosynthetically ^{13}C-labeled **1b**. [c] Calculated from peak area. [d] Calculated from peak intensity. [e] Carbons of sulfonate anion.

a large apparent error of ±12–21%. This must be due to the inaccuracy in measuring the rather small mass spectral isotopic peak intensities, particularly of the *m/e* 133 fragment ions, of the diluted sample of ^{13}C-labeled **1b**, since good agreement between ^{13}C and ^{14}C derived relative intermolecular isotopic labeling of **1b** was obtained (see Table IV). The best accuracy is obtained when the relative *intra*molecular ^{13}C enrichment is considered, as expected, based on the report of Matwiyoff and Burnham.[6] Again, the largest apparent error was encountered when the mass spectral data from the diluted sample of ^{13}C-labeled **1b** was used as the standard. We note particularly that the values shown in Table I (A–D) are in excellent agreement with the stated relative ^{13}C content of the commercial sample of [1,2-$^{13}C_2$]acetate used in our synthesis: C(1), 91.6 atom %; and C(2), 92.8 atom % ^{13}C.

The accuracy of our quantitative ^{13}C NMR spectroscopic analysis of **1b** also is dependent on the relative spin–lattice relaxation rate of the compound's carbon atoms. When close correspondence between the number of carbons and signal intensity is desired, ^{13}C NMR spectra usually are determined with a long delay time (five or more times a carbon's T_1 value) between successive pulses in the Fourier transform mode, or

Table IV. Relative Percentage Isotopic Labeling of [^{13}C,^{14}C]-1b after a 240 h Total Metabolism Period[a]

carbon	relative ^{14}C specific radioactivity,[b] %	relative intermolecular ^{13}C enrichment, %
2′	6.7	6.1[c]
5′	6.9	6.8
2 to 6 plus 2′	66	68
2 to 6	59	62

[a] Exposed to $^{13}CO_2$ for 14 h then grown in the normal atmosphere for 226 h. [b] Taken from Table I of ref 5a. [c] Represents percentage of total intermolecular ^{13}C enrichment calculated by subtracting 1.0 from each carbon's value in column 6, Table I, ref 1, and figuring the percentage this value is of the total (13.2).

by the addition of a paramagnetic relaxation reagent.[46] This has been observed to be unnecessary by Matwiyoff and coworkers when the relative intensities of the singlet and satellite resonances of ^{13}C-enriched compounds are being measured under proton noise-decoupling conditions, *if* the carbon in question bears a directly attached proton.[47] Since in our earlier paper the ^{13}C NMR spectra of ^{13}C-labeled **1b** had been determined with an 0.8-s pulse repetition rate, the data recorded in Table I were obtained under similar spectrometer parameters, even though the T_1 values for nicotine's protonated carbons have a range of 0.90–1.97 s when determined on its N_b-monoethanesulfonate salt (Table III). Since the T_1's of the *N*-methylpyrrolidine ring's carbons do not differ greatly under our experimental conditions, it is unlikely that the relaxation of any of these carbons is dominated by processes like spin rotation, which could lead to considerable inaccuracy in the quantitative spectral analysis. Thus such considerations are neglected in analyzing the accuracy of our quantitative ^{13}C NMR analysis of **1b**.

After considering the above data, it is important to reevaluate the accuracy of our spectral techniques for determining the *inter*molecular ^{13}C enrichment of **1b**. Since this value was defined[1] to represent the total contribution that all ^{13}C-labeled species make to the ^{13}C NMR signal intensity of a given carbon, it can be compared directly to the relative labeling of **1b** by carbon-14,[1] whose quantitative analysis can be carried out with high accuracy. Professor Edward Leete has chemically degraded one of our biosynthetically ^{13}C,^{14}C-labeled samples of **1b**[1] by a new method developed in his laboratory. His findings[5a] are summarized in Table IV, from which it can be seen that for carbon 5′ and the carbon sets, C(2) to C(6) plus C(2′), and C(2) to C(6), the accuracy of the ^{13}C NMR technique for quantifying the *relative* intermolecular ^{13}C enrichment of **1b** is good, i.e., within a ±5.1% error range; for carbon 2′ alone, however, the error is larger (−9.0%). Since a ^{14}C reference label was not included during the synthesis of [2′,3′,*N*-Me-$^{13}C_3$]-**1b**, a similar certification of accuracy for the synthetically ^{13}C-labeled **1b** cannot be made. It is our belief, however, that the accuracy of this technique is at least ±10%, better than that shown in Table II, where the accuracy of the mass spectral analysis is too poor to permit much significance to be placed on this part of our results.

Finally, the proton-decoupled ^{13}C NMR spectrum of [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b** (Figure 1) is clear proof of an assumption we made in our earlier paper: that the mirror image relationship of the satellite resonances for C(2′)/C(3′) and C(4′)/C(5′) was the result of $^1J_{CC}$ coupling in these pairs of carbons, which form AB spectral subsets.[48] This relationship of satellite intensities is well known in proton NMR spectroscopy, but it has particular value in the present study for easy identification of some ^{13}C labeling relationships in the complex mixture of multiply ^{13}C-labeled molecules when recourse to ^{13}C–^{13}C

homonuclear decoupling techniques is not available. Consequently, it now is certain that the second-order $^1J_{CC}$ relationships are evidence for the biosynthetic origin of these two-carbon sets from biochemical C_2 units. This conclusion is consistent with the expected flow of carbon-13 via acetic acid through the tricarboxylic acid cycle and the labeling of the N-methylpyrrolidine ring of **1b** by [^{14}C]acetate reported several years ago by Zielke and Byerrum.[49]

Conclusions

The development of an efficient synthesis of **1a** and **1b**, which was used to prepare a sample of (±)-[2′,3′,N-Me-$^{13}C_3$]nicotine, has permitted us to certify that the accuracy of the ^{13}C NMR techniques used to determine the relative ^{13}C enrichment of nicotine, biosynthetically labeled by $^{13}CO_2$, is sufficient to validate part of the data presented in our initial paper.[1] That is, that the difference in the relative *intra*molecular ^{13}C labeling of the C(2′)/C(3′) and C(4′)/C(5′) two-carbon units of **1b** (8–21%) is larger than the experimental error, determined in this study to be ≤±6%. On the other hand, the apparent differences in the *inter*molecular ^{13}C enrichment of **1b** reported earlier are less than the experimental error determined in this study, and are unlikely to be significant.

It is tempting to propose a biochemical explanation of our results at this time, which others find puzzling.[5b] However, *since only one biosynthetically ^{13}C-labeled sample of* **1b** *was measured,*[1] *we feel it is too early to determine what significance this has to nicotine biosynthesis.* Yet we believe that it is quite possible to encounter equal intermolecular ^{13}C labeling via biochemical passage of carbon-13 through a symmetrical intermediate, e.g., **2**, but to encounter unequal intramolecular ^{13}C labeling of a carbon subset of the same intermediate, since the latter's intramolecular ^{13}C labeling can be "symmetrized" only by a bond breakage and statistically random recombination of the two coupled carbon atoms. There simply is not any basis at present to assume that as a result of the metabolic processes which join the two carbons (from $^{13}CO_2$ in our case) that ultimately form C(4′) and C(5′) of **1b**, these carbons will be equally intramolecularly ^{13}C labeled. Hopefully in future work we are planning the results obtained will permit us to shed some light on this question.

Experimental Section

General. Reagents and organic chemicals were of commercial quality. The 90% sodium[1,2-$^{13}C_2$]acetate was purchased from Merck and Co. and the 90% [^{13}C]formaldehyde from Stohler Isotopes. Solvents were redistilled and THF was distilled from $LiAlH_4$ immediately before its use. Oxygen-free nitrogen was obtained through use of a deoxygenation catalyst (BASF R-33). Thick-layer (PLC), thin layer (TLC), and column chromatography were done using the appropriate Brinkmann silica gel. Infrared spectra were determined on a Perkin-Elmer 237 grating spectrophotometer. Mass spectra were run on an AEI MS-9 interfaced to a Nova 2 computer or a Finnegan quadrupole 1015 GC/mass spectrometer interfaced to a Finnegan M6000 computer. Nuclear magnetic resonance spectra were determined at 90 MHz (^{1}H) on a Bruker HX-90E or Varian EM 390 spectrometer, at 22.63 MHz (^{13}C) on a Bruker HX-90E using the parameters described under each figure, or those reported earlier.[1]

2,2-Bis(ethylthio)ethanal. To 1,1-bis(ethylthio)methane (10.88 g, 0.08 mol) in dry tetrahydrofuran (160 mL) magnetically stirred at −30 °C under a nitrogen atmosphere was added *n*-butyllithium (35.2 mL, 2.5 M, 0.088 mol) as a hexane solution. After 1 h additional stirring, the resulting dithioalkyllithium was transferred into previously cooled (−10 °C) dry dimethylformamide (24 mL) by a cannula using a slight positive nitrogen pressure. The reaction mixture was kept at −10 °C overnight, then poured into ice water (150 mL). The basic aqueous solution was extracted with Skellysolve A (3 × 150 mL), followed by acidification with 3 N hydrochloric acid to pH 6, and extracted with diethyl ether (3 × 150 mL). The combined ether extracts were dried over anhydrous magnesium sulfate and the solvent was removed in vacuo. The product was purified by distillation to give 11.86 g (91%) of a colorless liquid: bp 67 °C (0.3 Torr); IR (neat) ν 2708, 1715 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 9.20 (d, J = 4.2 Hz, 1 H), 4.25 (d, J = 4.2 Hz, 1 H), 2.63 (q, J = 7.4 Hz, 4 H), and 1.27 (t, J = 7.4 Hz, 6 H). Anal. ($C_5H_{12}S_2O$) C, H, S.

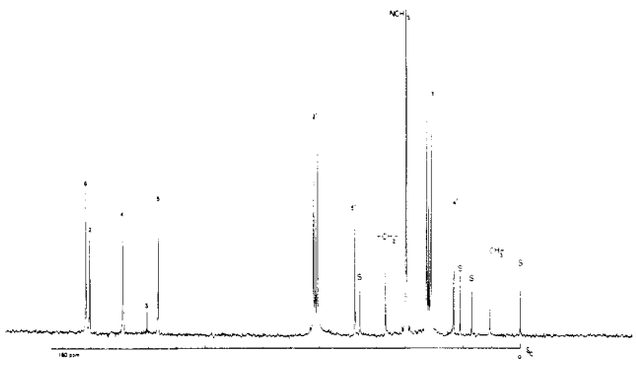


Figure 1. Proton-decoupled ^{13}C NMR spectrum of the monoethanesfulfonate salt of [2′,3′,N-CH_3-$^{13}C_3$]nicotine (**1b**), ~0.3 M, in D_2O. The resonances marked *S* are of the DSS internal standard and signal assignments are taken from ref 1 as recently confirmed by T. P. Pitner, J. I. Seeman, and J. F. Whidby, *J. Heterocycl. Chem.*, in press.

2-Formyl-1,3-dithiane. Prepared from 1,3-dithiane as above to give a colorless liquid in 81% yield: bp 93 °C (0.8 Torr) [lit.[50] 83–85 °C (0.45 Torr)]; IR (neat) ν 2700, 1715, 910 cm^{-1}.

1-Acetyl-2,2-bis(ethylthio)ethanol. To 2,2-bis(ethylthio)ethanal (11.85 g, 0.072 mol) in ethanol (110 mL) was added sodium borohydride (1.5 g, 0.039 mol) in several portions while magnetically stirring at 25 °C. The reaction mixture was stirred overnight and acidified with 3 N hydrochloric acid to pH 5–6, and the ethanol was mostly removed by evaporation in vacuo. The resulting material was extracted with diethyl ether (3 × 200 mL) and the combined ether extracts were dried over anhydrous magnesium sulfate. The crude alcohol, obtained as a colorless liquid upon removal of the solvent in vacuo, was acetylated at 25 °C using acetic anhydride (1.5 mL) and pyridine (5 mL). Excess acetic anhydride was destroyed by an addition of water (1 mL) to the reaction mixture and acidification with 3 N hydrochloric acid to pH 1. The resulting solution was extracted with diethyl ether (3 × 200 mL), the combined ether extracts were washed with saturated aqueous sodium bicarbonate and brine, and the ether solution was dried over anhydrous magnesium sulfate. Removal of ether in vacuo followed by distillation gave a colorless liquid (13.51 g, 91%): bp 87 °C (0.3 Torr); IR (neat) ν 1750, 1230 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.5–3.8 (m, 3 H), 2.70 (q, J = 7.8 Hz, 4 H), 2.10 (s, 3 H), and 1.27 (t, J = 7.8 Hz, 6 H). Anal. ($C_8H_{16}S_2O_2$) C, H, S.

2-Acetoxymethyl-1,3-dithiane. Prepared from 2-formyl-1,3-dithiane as above to give the intermediate alcohol as a colorless crystalline solid in 95% yield: mp 35.0–35.8 °C (from dichloromethane/Skellysolve A (1:3)); IR (KBr) ν 3400, 1069, 908 cm^{-1}. Anal. ($C_5H_{10}OS_2$) C, H, S. Its acetate was prepared as above to give a colorless liquid in 94% yield: bp 110–111 °C (1.5–2 Torr); IR (neat) ν 1745, 1230 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 4.5–3.9 (m, 3 H), 3.2–2.5 (m, 4 H), 2.08 (s, 3 H), and 2.3–1.9 (m, 2H). Anal. ($C_7H_{12}O_2S_2$) C, H, S.

Ketene Thioacetal Monoxide 4. To 1-acetyl-2,2-bis(ethylthio)-ethanol (13.4 g, 0.0644 mol) dissolved in methanol (90 mL) and water (28 mL) was added sodium metaperiodate (13.8 g, 0.0644 mol) in 3-g portions over 15-min intervals at 0 °C with vigorous stirring. Stirring was continued at 22 °C for 24 h, whereupon the white solid precipitate was separated by filtration and the filter cake was washed with diethyl ether (100 mL). The volume of the combined ether/aqueous methanol filtrate was reduced to ~100 mL. The filter cake was triturated with dichloromethane (200 mL) and this organic filtrate was combined with the former. The resulting two-phase solution was extracted with dichloromethane (3 × 150 mL) and the resulting combined dichloromethane extracts were washed with 10% aqueous sodium bisulfite (50 mL) and then saturated aqueous sodium carbonate. The solution was dried over anhydrous magnesium sulfate and evaporated in vacuo to give the monosulfoxide acetate as a colorless liquid (14.0 g).

To a vigorously stirred solution of the monosulfoxide acetate (14.0 g, 0.063 mol) dissolved in benzene (63 mL) was added powdered potassium hydroxide (7.1 g, 0.126 mol) and anhydrous potassium carbonate (17.4 g, 0.126 mol). The reaction mixture was stirred at 22 °C for 3 h, whereupon dichloromethane (100 mL) and Celite (5–10 g) were added and the resulting mixture was then filtered under vacuum through anhydrous magnesium sulfate. Evaporation of the solvent gave a residual oil, which on distillation afforded pure **4** (8.8 g, 84%): bp 62 °C (0.38 Torr); IR (neat) ν 3095, 1750, 1060 cm^{-1}; ^{1}H NMR

($CDCl_3$) δ 6.30 (d, J = 1 Hz, 1 H), 5.96 (d, J = 1 Hz, 1 H), 2.87 (m, 4 H), and 1.20 (m, 6 H). Anal. ($C_6H_{12}OS_2$) C, H, S.

Ketene Thioacetal Monoxide 6. Prepared from 2-acetoxymethyl-1,3-dithiane as above, giving **6** as a colorless solid in 82% yield (two steps): mp 44.7–45.1 °C; bp 98 °C (0.3 Torr); IR (neat) ν 3090, 1747, 1055, 920 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.14 (s, 1 H), 6.28 (s, 1 H), 3.6–3.2 (m, 1 H), and 3.0–2.2 (m, 5 H). Anal. ($C_5H_8OS_2$) C, H, S.

δ-Keto Sulfoxide 5. 3-Acetylpyridine (234 mg, 1.93 mmol) was added dropwise to lithium diisopropylamide (LDA, 2.25 mmol) in THF (5 mL) at −78 °C under an N_2 atmosphere. The resulting solution was warmed to −5 °C for 30 min and cooled to −78 °C, and **4** (298 μL, 2.0 mmol) was added dropwise with magnetic stirring. The reaction was stirred at −10 °C for 19 h then worked up by addition of water (1 mL) followed by acidification with 2 N HCl (10 mL). The acidified aqueous solution was extracted with EtOAc (2 × 20 mL), and then an acidic (2 N HCl, 10 mL) backwash of the EtOAc layer was combined with the first acidic aqueous layer, which was basified with concentrated NH_4OH and saturated with solid NaCl. Extraction of the resulting aqueous solution with EtOAc (3 × 50 mL), drying of the combined organic extracts (Na_2SO_4), and solvent removal in vacuo gave a crude viscous gum. This residue was purified by PLC in $CHCl_3$/MeOH/NH_4OH (180:20:1) and the major UV absorbing product was eluted with CH_2Cl_2/MeOH (4:1) to give **5** after solvent removal in vacuo as a viscous oil (215 mg, 37.7%): IR (neat) ν 1700, 1600, 1460, 1430, 1390, 1280, 1060, and 1040 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.22 (d, J = 2.0 Hz, 1 H), 8.82 (dd, J = 1.6, 4.8 Hz, 1 H), 8.29 (dt, J = 1.7, 8.0 Hz, 1 H), 7.46 (dd, J = 4.8, 8.0 Hz, 1 H), 4.03–3.63 (m, 1 H), 3.60–1.80 (m, 8 H), and 1.33 (pseudo q, J = 7.6 Hz, 6 H); MS m/e (rel intensity) 207 (M^+ · − $HOSC_2H_5$, 62) [$C_{11}H_{13}NOS$ calcd 207.0698, found 207.0708], 152 (4), 146 (51), 118 (30), 117 (12), 106 (100), 101 (20), 92 (4), 91 (5), 78 (75).

δ-Keto Sulfoxide 7. 3-Acetylpyridine (244 mg, 2 mmol) was slowly added to LDA (2 mmol) in THF (4 mL) at −78 °C with magnetic stirring under an N_2 atmosphere. After 30 min, **6** (296 mg, 2 mmol) was added dropwise. The reaction was stirred at − 78 °C for 2 h, at −10 °C for 4.5 h, then at 25 °C for 15 h. Workup as for **5** gave **7** (246 mg, 45.5%) as a colorless oil: IR ($CHCl_3$) ν 1690, 1590, 1483, 1421, 1368, 1315, 1235, 1030, 790, 700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 9.35 (br s, 1 H), 8.96 (dd, J = 1.9, 4.8 Hz, 1 H), 8.44 (dt, J = 1.9, 8.3 Hz, 1 H), 7.62 (dd, J = 4.8, 8.3 Hz, 1 H), 3.98 (dd, J = 5.2, 7.6 Hz, 1 H), 3.50 (br t, J = 6 Hz, 2 H), and 3.3–1.8 (m, 8 H); MS m/e (rel intensity) 269 (M^+, 17) [$C_{12}H_{15}NO_2S_2$ calcd 269.0538, found 269.0541], 146 (72), 121 (14), 118 (19), 106 (95), 91 (34), 87 (44), 79 (41), 78 (100).

3-Acetylpyridine Methoxime 11. Powdered NaOH (3.20 g, 80 mmol) was added to a magnetically stirred solution of 3-acetylpyridine (2.2 g, 20 mmol) and methoxyamine hydrochloride (2.5 g, 30 mmol) in 95% ethanol (14 mL)–water (3 mL). After a 5-min reflux of the reaction the mixture was saturated with solid NH_4Cl and NaCl, and the products were extracted with EtOAc (5 × 60 mL). The combined organic extracts were dried ($MgSO_4$) and distilled to give **11** (2.98 g, 99%): bp 114 °C (25 mmHg); IR (neat) ν 1615, 1590, 1568, 1055, 900, and 711 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.84 (d, J = 1.5 Hz, 0.87 H), 8.73 (d, J = 1.5 Hz, 0.13 H), 8.55 (dd, J = 1.5, 5.1 Hz, 1 H), 7.93 (dt, J = 1.5, 7.8 Hz, 0.87 H), 7.82 (dt, J = 1.5, 7.8 Hz, 0.13 H), 7.24 (dd, J = 5.1, 7.8 Hz, 1 H), 3.98 (s, 2.67 H), 3.84 (s, 0.33 H), and 2.19 (s, 3 H); ^{13}C NMR δ ($CDCl_3$) (rel signal area) 11.87 (6.5), 20.82 (1.8), 62.03 (10.6), 61.66 (2.0), 123.20 (17.9), 132.97 (16.4), 135.50 (15.4), 147.53 (17.1), 149.31 (2.3), 150.01 (24.9), and 151.85 (1.6); MS m/e (rel intensity) 150, M^+·, 100) [$C_8H_{10}H_2O$ calcd 150.0793, found 150.0786], 119 (20), 112 (47), 104 (20), 78 (97).

Methoxime Sulfoxide 12a. Methoxime **11** (2.25 g, 15 mmol) was added to LDA (16.5 mmol) in THF (30 mL) at −78 °C under an N_2 atmosphere with magnetic stirring. After 20 min **4** (2.23 mL, 15 mmol) was added to the yellow solution and stirring was continued for 3 h at −78 °C. The reaction was quenched at −78 °C by the addition of H_2O and solid NH_4Cl. After warming and saturation with NaCl the reaction mixture was extracted with EtOAc (4 × 100 mL), the combined organic extracts were dried (Na_2SO_4), and the solvent was removed in vacuo. The resulting residue was purified by column chromatography in ether to give **12a** (4.1 g, 86%) as a colorless oil: IR (neat) ν 1650, 1585, 1565, 1450, 1410, 1050, and 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.90 (d, J = 2.1 Hz, 1 H), 8.58 (dd, J = 2.1, 5.1 Hz, 1 H), 7.97 (dt, J = 8.4, 2.1 Hz, 1 H), 7.28 (dd, J = 5.1, 8.4 Hz, 1 H), 3.99 (s, 3 H), 3.72–3.45 (m, 1 H), 3.22–2.23 (m, 8 H), 1.35 (t, J = 7.5 Hz, 3 H), and 1.33 (t, J = 7.5 Hz, 3 H); MS m/e (rel intensity) 237 (34), 206 (4), 175 (38), 162 (3), 161 (3), 160 (3), 145 (14), 144 (12), 131 (5), 118 (8), 117 (6), 106 (16), 101 (100). Anal. ($C_{14}H_{22}N_2O_2S_2$) C, H, N, S.

4-Amino Thioacetal 13. Methoxime sulfoxide **12a** (1.74 g, 5.54 mmol) was cooled to ice bath temperatures with magnetic stirring and diborane (1 M solution in THF, 38.8 mL) was added. The reaction was stirred 10 min, allowed to warm to room temperature and kept there for 12–16 h, and then refluxed for 3.5 h. After cooling to room temperature excess B_2H_6 was destroyed by the addition of MeOH and the reaction was stirred for 12–16 h. After solvent removal in vacuo the residue was treated with 10% NaOH (38 mL) and the resulting solution was refluxed for 1 h. After cooling to room temperature the reaction mixture was neutralized with solid NH_4Cl, saturated with KCl, and extracted with EtOAc (6 × 50 mL). The combined organic extracts were dried (Na_2SO_4) and evaporated in vacuo to give **13** as a colorless liquid (1.50 g, 100%), pure by ^1H NMR analysis: IR (neat) ν 3370, 3280, 1595, 1580, 1450, 1425, 1265, 1170, 1025, 915, and 717 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.62–8.43 (m, 2 H), 7.72 (dt, J = 7.8, 1.5 Hz, 1 H), 7.24 (dd, J = 4.8, 7.8 Hz, 1 H), 3.98 (t, J = 6.0 Hz, 1 H), 3.76 (t, J = 6.6 Hz, 1 H), 2.60 (q, J = 7.2 Hz, 1 H), 2.57 (q, J = 7.2 Hz, 1 H), 2.15–1.60 (m, 4 H), and 1.21 (t, J = 7.2 Hz, 6 H); MS m/e (rel intensity) 270 (M^+, 23) [$C_{13}H_{22}N_2S_2$ calcd 270.1225, found 270.1229], 241 (35), 224 (55), 210 (16), 208 (33), 192 (74), 147 (31), 146 (11), 131 (12), 130 (74), 121 (16), 120 (100), 107 (56), 105 (11).

Isomyosmine (10). Iodine (88 mg, 0.35 mmol) was added to magnetically stirred **13** (92 mg, 0.34 mmol) dissolved in CH_3CN/H_2O (3:1) at room temperature. After 1 h, AgOAc (56 mg, 0.34 mmol) was added and the reaction again was treated with the same amount of iodine then AgOAc, followed by filtration to remove precipitated solids. The filtrate was decolorized by addition of 10% aqueous $Na_2S_2O_3$, basified with saturated aqueous $NaHCO_3$, and continuously extracted with CH_2Cl_2 for 48 h. After drying the CH_2Cl_2 extract (Na_2SO_4) and evaporation in vacuo, the resulting oil was purified by PLC as for **5** to give **10**[51] (20.5 mg, 41%) as a colorless oil: ^1H NMR ($CDCl_3$) δ 8.60–8.43 (m, 2 H), 7.83 (br s, 1 H), 7.55 (dt, J = 1.5, 7.8 Hz, 1 H), 7.24 (dd, J = 4.5, 7.8 Hz, 1 H), 5.12 (t, J = 8.1 Hz, 1 H), and 2.90–2.17 (m, 4 H); MS m/e (rel intensity) 146 (M^+, 3), 145 (8), 118 (100), 105 (7), 104 (5), 91 (35).

Reduction of **10** (51.5 mg, 0.35 mmol) with $NaBH_4$ according to a literature method[7i] gave **1a** (34 mg, 66%) identified by TLC comparison with an authentic reference standard.

Hydrolysis of **13** (360 mg, 1.33 mmol) in glacial AcOH (13 mL) using iodine (1.65 g, 6.65 mmol) for 1 h followed by reduction of excess I_2 with aqueous 10% $Na_2S_2O_3$, addition of NaOAc (6.65 mmol), evaporation of the solvent, pH adjustment to 7–8 with aqueous $NaHCO_3$, addition of solid NaOH (110 mg) in MeOH (13 mL), and reduction using $NaBH_4$ (48 mg) gave **1a** (0.46 mmol, 35%) after the workup described above. The yield of **1a** (30–40%) varied seemingly dependent on the purity of **13.**

N_b-Formylnornicotine (14). The crude 4-amino thioacetal **13** (105 mg, 0.39 mmol) was refluxed in 97% HCOOH (1.5 mL) for 3 h. The reaction mixture was concentrated in vacuo at <30 °C and treated with a few drops of saturated aqueous $NaHCO_3$ followed by solid Na_2SO_4. The resulting semisolid mass was extracted with EtOAc (5 × 50 mL) until no more UV-absorbing material was detectable. The combined EtOAc extracts were concentrated in vacuo and purified by PLC as for **5** to give **14**[52] (53 mg, 75%) as a pale yellow oil: IR (neat) ν 2875, 1665, 1580, 1425, 1380, 1025, and 718 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.63–8.40 (m, 2 H), 8.37 (s, 0.4 H), 8.10 (s, 0.6 H), 7.53 (m, 1 H), 7.25 (m, 1 H), 5.18–4.85 (m, 1 H), 3.76–3.55 (m, 2 H), 2.56–2.25 (m, 1 H), and 2.15–1.83 (m, 1 H); MS m/e (rel intensity) 176 (M^+, 100) [$C_{10}H_{12}N_2O$ calcd 176.0950, found 176.0950], 147 (57), 120 (21), 119 (36), 118 (17).

Nornicotine (1a). N-Formylnornicotine (19 mg, 0.11 mmol) was refluxed with 3 N HCl (7.2 mL) for 2 h. The reaction was basified with saturated aqueous $NaHCO_3$ and continuously extracted with CH_2Cl_2 for 48 h. Final purification as described for **5** gave the dihydrochloride salt of **1a** (22 mg, 0.1 mmol, 92%) identified by TLC and NMR of its free base vs. authentic **1a.**

Nicotine (1b) N-Formylnornicotine (21 mg, 0.12 mmol) in 97% HCOOH (0.5 mL) and 37% formalin (0.5 mL) was heated in a sealed tube at 100 °C for 18 h. The reaction mixture was evaporated in vacuo at <30 °C, 2 drops of H_2O and solid $NaHCO_3$ were added, and the resulting solid mass was extracted with Et_2O (5 × 2 mL). The combined ether extracts were concentrated in vacuo at 0–10 °C and purified by PLC as with **5** to give the dihydrochloride salt of **1b** (26 mg, 93%), identified by TLC and NMR of its free base vs. authentic **1b.**

This alkaloid (45 mg, dihydrochloride) also was obtained directly from **13** (75 mg) in 68% overall yield without isolation of intermediate products; the overall yield of one other preparation was 81%.

Mesylates 17 and 18. 3-Acetylpyridine (230 μL, 2.1 mmol) was added to a magnetically stirred suspension of Skellysolve A washed NaH (2.1 mmol) in DMF (5 mL) under an N_2 atmosphere and stirred for 2 h at 0 °C. Mesylaziridine[31] **16** (242 mg, 2 mmol) in DMF (2 mL) was added slowly and the reaction mixture was stirred for 5 h at 0 °C. The reaction was quenched by addition of NH_4Cl (1 g) in H_2O (10 mL)

followed by saturation of the solution with NaCl and extraction with EtOAc (5 × 40 mL). The combined organic extracts were dried ($MgSO_4$), the solvents were removed in vacuo, and the resulting residue was purified by PLC as for **5**. Compound **17** (100 mg, 21%) was obtained as a colorless oil: ^1H NMR ($CDCl_3$) δ 8.83 (br s, 1 H), 8.50 (d, *J* = 4.5 Hz, 1 H), 7.87 (dt, *J* = 1.5, 7.8 Hz, 1 H), 7.24 (dd, *J* = 4.5, 7.8 Hz, 1 H), 4.74 (d, *J* = 3.0 Hz, 1 H), 4.34 (d, *J* = 3.0 Hz, 1 H), 4.02 (t, *J* = 4.8 Hz, 2 H), 3.56 (t, *J* = 4.8 Hz, 2 H), and 2.97 (s, 3 H).

Compound **18** (91 mg, 19%) also was obtained as a colorless oil: ^1H NMR (Me_2SO-d_6) δ 9.10 (d, *J* = 1.5 Hz, 1 H), 8.78 (dd, *J* = 1.5, 4.8 Hz, 1 H), 8.28 (dt, *J* = 1.5, 7.8 Hz, 1 H), 7.56 (dd, *J* = 4.8, 7.8 Hz, 1 H), 3.26–2.94 (m, 4 H), 2.88 (s, 3 H), and 1.82 (q, *J* = 6.9 Hz, 2 H); MS *m/e* (rel intensity) 242 (M^+) [$C_{10}H_{14}N_2O_3S$ calcd 242.0775, found 242.0750] 163 (71), 146 (15), 134 (9), 122 (35), 121 (27), 106 (100), 79 (40), 78 (79).

Myosmine (3) and N_b-Mesylmyosmine (19). The C-alkylated mesylate **18** (82 mg, 0.34 mmol) was refluxed in toluene (10 mL) containing *p*-toluenesulfonic acid hydrate (65 mg, 0.34 mmol) for 3 h in an apparatus set up to permit the condensing toluene to pass through Linde 4A molecular sieves. The reaction was basified to pH 10 with 10% aqueous NaOH, solid NaCl was added to saturation, and the mixture was extracted with EtOAc (5 × 50 mL). The combined organic extracts were dried (Na_2SO_4), the solvents were removed in vacuo and the resulting residue was purified by PLC as for **5**. Two major products were isolated as colorless oils.

N_b-Mesylmyosmine (**19**; 20 mg, 26%): ^1H NMR ($CDCl_3$) δ 8.73 (br s, 1 H), 8.57 (d, *J* = 4.8 Hz, 1 H), 7.58 (dt, *J* = 1.5, 7.8 Hz, 1 H), 7.21 (dd, *J* = 4.8, 7.8 Hz, 1 H), 5.73 (t, *J* = 7.8, 1 H), 4.11 (t, *J* = 8.1 Hz, 2 H), 2.81 (s, 3 H), and 2.70 (dt, *J* = 2.8, 8.1 Hz, 2 H); MS *m/e* (rel intensity) 224 (M^+, 41), 161 (4), 145 (100), 144 (37), 118 (45), 117 (74), 105 (25), 92 (22), 91 (22), 90 (12), 89 (16), 79 (10), 78 (15).

Myosmine (**3**;[7i] 8 mg, 15%): ^1H NMR ($CDCl_3$) δ 9.00 (d, *J* = 1.5 Hz, 1 H), 8.65 (dd, *J* = 1.5, 4.8 Hz, 1 H), 8.21 (dt, *J* = 1.5, 8.1 Hz, 1 H), 7.36 (dd, *J* = 4.8, 8.1 Hz, 1 H), 4.12 (m, 2 H), 2.98 (m, 2 H), and 2.11 (m, 2 H); MS *m/e* (rel intensity) 146 (M^+, 50), 145 (45), 118 (100), 105 (21), 91 (16), 78 (34).

Reduction of **3** with $NaBH_4$ as for **10** gave the dihydrochloride salt of **1a** (8 mg, 66%), identified as before.

[1′,2′-$^{13}C_2$]-3-Acetylpyridine. A typical experiment for the unlabeled synthesis is presented followed by the yields obtained in the ^{13}C-labeled synthesis.

A magnetically stirred suspension of NaOAc (1.23 g, 15 mmol) in THF (5 mL) contained in a heavy-walled tube fitted with a pressure stopcock was treated with concentrated H_2SO_4 (450 μL). The mixture was cooled to 0 °C, diborane in THF (1 M, 18 mL) was added, and the reaction mixture was stirred for 1 h at 0 °C then overnight at room temperature. The THF was removed in vacuo under anhydrous conditions and NaOH (0.8 g) in H_2O (4 mL) was added to decompose the boroxine. The resulting mixture was frozen in liquid N_2 and evacuated on a high vacuum line to 5 × 10^{-3} Torr. The mixture of water and ethanol thus was vacuum transferred into a heavy-walled tube, the upper one-half of which was jacketed with a cold-water condenser, containing I_2 (6.8 g) and red phosphorus (0.67 g), and the resulting mixture was heated in a steam bath for 2 h. The reaction tube was frozen in liquid N_2 and the contents was vacuum transferred into a tube containing solid Na_2CO_3 (5 g). After CO_2 evolution had ceased, the ethyl iodide was purified by vacuum transfer through solid NaOH into P_2O_5 (10 g) and the resulting mixture was warmed, frozen, and then vacuum transferred through Linde 4A molecular sieves to obtain a colorless liquid (1.52 g, 65%).

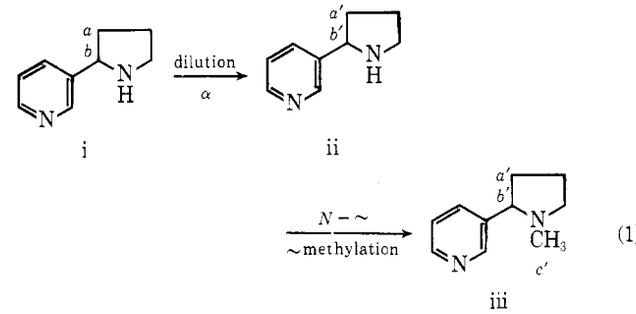
3-Bromopyridine (250 μL, 2.5 mmol) was slowly added to magnetically stirred *n*-butyllithium (2.0 M, 1.25 mL) in THF (10 mL) at −95 °C (liquid N_2–toluene mixture). After 30 min ethyl iodide (82 μL, 1 mmol) was added dropwise to the brown solution of 3-lithiopyridine and stirring was continued for 3 h at −95 °C. The cold reaction was quenched by addition of H_2O, acidified with 6 N HCl (10 mL), and extracted with Et_2O (2 × 25 mL) to remove *n*-butyl bromide. The acidic aqueous layer was basified with solid $NaHCO_3$, saturated with KCl, and extracted with Et_2O (5 × 25 mL). The combined ether extracts were dried (Na_2SO_4) and evaporated in vacuo at <0 °C, and the residue was purified by PLC as for **5**. The hydrochloride salt of 3-ethylpyridine (110 mg, 77%) thus was obtained as a white solid; it was identified by TLC and NMR comparison (free base) with an authentic reference standard.

3-Ethylypridine (86 mg, 0.8 mmol) was mixed with MgO (160 mg, 4 mmol), H_2O (4 mL), $KMnO_4$ (632 mg, 4 mmol), and concentrated HNO_3 (0.54 mL, 8.2 mmol), and the mixture was stirred 5 h at room temperature. Aqueous 10% $Na_2S_2O_3$ was added to reduce the excess $KMnO_4$, the precipitated MnO_2 was filtered off and washed with 2% aqueous HCl (3 × 3 mL), and the combined filtrates were basified ($NaHCO_3$), saturated with NaCl, and extracted with EtOAc (4 × 20 mL). After addition of 2% aqueous HCl (10 mL), the combined organic layers were evaporated in vacuo to give pure 3-acetylpyridine (89 mg, 71%) as its hydrochloride salt. When pyridine (fivefold excess relative to 3-ethylpyridine) also was added to the oxidation reaction mixture, the yield of 3-acetylpyridine increased to 83% after PLC purification.

The following yields were obtained during the ^{13}C-labeled preparation starting with 90% sodium [1,3-$^{13}C_2$]acetate (1 g, 12 mmol): [1,2-$^{13}C_2$]ethyl iodide (0.9 g, 48%); [1′,2′-$^{13}C_2$]-3-acetylpyridine methoxime (201 mg, 23%). The lower yield of ethyl iodide obtained here was the result of inadventitious bumping during the THF removal after boroxine formation.

[2′,3′,*N*-CH_3-$^{13}C_3$]Nicotine. The [1′2′-$^{13}C_2$]-3-acetylpyridine methoxime was converted into **12a** (307 mg, 73%), which was divided into three portions. The first portion (135 mg) was lost when the conversion of **13** into **1a** using I_2/AcOH and $NaBH_4$ failed. The second portion (18 mg) was diluted (~4.8-fold) with unlabeled **12a** (68 mg) and gave [1′,2′-$^{13}C_2$]-**13** (75 mg, 100%). This was converted into the dihydrochloride salt of [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b** (45 mg, 69%) using ~15 atom % [^{13}C]formaldehyde. The third portion (154 mg) was converted without dilution into ~90% [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b** dihydrochloride (7 mg, 6%), the reason for the low overall yield being unaccountable.

Mass Spectral Analysis of [2′,3′,*N*-CH_3-$^{13}C_3$]-1b. The following method was used to calculate the intermolecular and intramolecular ^{13}C labeling shown in Table I of the text. Let eq 1 represent the operations carried out in the preparation of the diluted sample of ^{13}C-labeled **1a** where α is a dilution coefficient and *a*, *b*, *a*′, *b*′, and *c*′ are ^{13}C labeling probabilities such that $a = \alpha a'$ and $b = \alpha b'$ by definition. The diluted, triply ^{13}C-labeled **1b** is represented by iii.



Since the fragment ion at *m/e* 84 contains *a*′, *b*′, and *c*′, the intensity of the *m/e* 85 fragment ion contains contributions which can be expressed by $[a(1-b)(1-c')/\alpha] + [b(1-a)(1-c')/\alpha] + c'[1 - \{[a(1-b)/\alpha] + [b(1-a)/\alpha] + [ab/\alpha]\}]$, of the *m/e* 86 fragment ion by $[ab(1-c')/\alpha] + [a(1-b)c'/\alpha] + [b(1-a)c'/\alpha]$, and of the *m/e* 87 fragment ion by abc'/α. Thus by simplification of the contributions, the intensity of the *m/e* 85 fragment ion can be expressed as $a' + b' + c' - 2a'b'\alpha - 2a'c' - 2b'c' + a'b'c'\alpha$, of the *m/e* 86 fragment ion as $a'b'\alpha + a'c' + b'c' - 3a'b'c'\alpha$, and of the *m/e* 87 fragment ion as $a'b'c'\alpha$. Since the mass spectrum of the diluted sample of [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b** did not exhibit an *m/e* 87 fragment ion of significant intensity, a further approximation of these ion intensity expressions can be made: the *m/e* 85 fragment ion's intensity can now be approximated by $a' + b' + c' - 2a'b'\alpha$], and the *m/e* 86 fragment ion's by $a'b'\alpha$. The measured mass spectral ion intensities were

	unlabeled **1b**		^{13}C-labeled **1b**		
m/e	85	86	85	86	87
intensity	5.5 ± 0.18	0.09 ± 0.02	12.14 ± 0.38	6.48 ± 0.19	0.18 ± 0.03

from which the corrected relative intensity of the *m/e* 85 fragment ion (5.83%) and the *m/e* 86 fragment ion (5.35%), relative to the intensity of the *m/e* 84 fragment ion, were calculated.

Using a similar development the intensity of the *m/e* 134 fragment ion, which contains isotopic contributions from C(2′) and *N*-CH_3 of **1b**, can be expressed as $b'(1-c') + c'(1-b')$, which simplified to $b' + c' - 2b'c'$, and of the *m/e* 135 fragment ion, $b'c'$. Since again the intensity of the *m/e* 135 fragment ion was insignificant in the mass spectrum of the diluted sample of [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b**, the *m/e* 134 fragment ion's intensity is approximated by $b' + c'$. The measured mass spectral ion intensities were

	unlabeled **1b**		^{13}C-labeled **1b**	
m/e	134	135	134	135
intensity	11.68 ± 0.78	0.46 ± 0.22	23.14 ± 0.69	0.74

from which the corrected relative intensity of the m/e 134 fragment ion (10.28%) was calculated. Thus, $a' + b' + c' - 2a'b'\alpha = 5.83 \times 10^{-2}$, $a'b'\alpha = 5.35 \times 10^{-2}$, and $b' + c' = 10.28 \times 10^{-2}$, from which it calculates that $a' = 7.14 \times 10^{-2}$.

From the original definitions of a and b, and from the ^{13}C labeling probabilities shown in Table I(C) for C(2′) and C(3′), it calculates that $a = 93.1$, $b = 85.6$, $a' = 6.25$, $b' = 5.75$, and $c' = 4.53$, each value $\times 10^{-2}$, when α is calculated from the ^1H NMR derived values for C(2′) and C(3′) relative ^{13}C enrichment shown in Table I.

^{13}C NMR Analysis of [2′,3′,*N*-CH_3-$^{13}C_3$]-1b. (a) The values shown in Table I(A) were calculated according to footnote c of Table I and eq 4 of ref 1 from the following relative peak area weights (milligrams) of the ^{13}C NMR resonances:

unlabeled monoethanesulfonate of **1b**

$-CH_2-$	C(3′)	C(2′)	*N*-CH_3
13.40 ± 0.27 (1)	22.78 ± 0.73 (1.70)	23.78 ± 0.56 (1.78)	21.37 ± 0.06 (1.59)

^{13}C-labeled monoethanesulfonate of **1b**

	C(3′)		C(2′)		
$-CH_2-$	I_S	I_D	I_S	I_D	*N*-CH_3
5.72 ± 0.20	15.78 ± 0.58	60.31 ± 1.19	14.56 ± 0.15	56.66 ± 1.44	56.55 ± 0.74

For example, for the intramolecular ^{13}C labeling proabability of C(2′)

$$I_{S3'} = 15.78 - (5.72 \times 1.70) = 6.06$$

$$P_{2'} = \frac{60.31}{6.06 + 60.31} = 0.9087$$

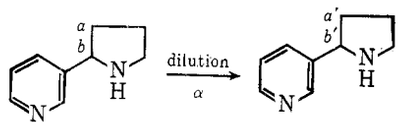
and for the intermolecular ^{13}C labeling of C(2′)

$$I_{S2'} = 14.59 - (5.72 \times 1.78) = 4.41$$

$$\text{total signal intensity} = 56.66 + 4.41 = 61.07$$

$$\%\ ^{13}C \text{ enrichment} = \frac{61.07 \times 1.1\%}{(5.72 \times 1.78)} = 6.60\%.$$

(b) The values shown in Table I(B) were calculated according to the following method. Let eq 2 represent the operations carried out



(2)

in preparing the diluted sample of [2′,3′-$^{13}C_2$]-**1a**, where a, b, a', and b' are the appropriate ^{13}C labeling probabilities of C(3′) and C(2′) of **1b** (or **1a**). Also let c represent the ^{13}C labeling probability of natural abundance **1b**; f_a and f_b represent intensity factors of C(3′) and C(2′) of **1b**. It follows that the ^{13}C NMR singlet's intensity (I_S) is represented by

$$I_S{}^a = a(1 - b)f_a$$

$$I_S{}^{a'} = (I_s{}^a/\alpha) + [(\alpha - 1)/\alpha]\, I_N{}^a$$

where $I_N{}^a$ is the singlet intensity of C(3′) due to natural ^{13}C abundance **1b**, that the ^{13}C NMR doublet's intensity is represented by

$$I_D{}^a = abf_a$$

$$I_D{}^{a'} = abf_a/\alpha$$

and that

$$I_N{}^a = f_a c$$

By appropriate substitution and simplification,

$$I_S{}^{a'} = \frac{a(1 - b)f_a}{\alpha} = \left(\frac{\alpha - 1}{\alpha}\right) f_a c$$

$$= f_a\left[a' - a'b'\alpha + \left(\frac{\alpha - 1}{\alpha}\right) c\right]$$

but since $I_D{}^{a'} = (\alpha a')(\alpha b')f_a/\alpha = a'b'\alpha f_a$,

$$I_S{}^{a'} = f_a a' - I_D{}^{a'} + [(\alpha - 1)/\alpha] f_a c$$

from which division by $I_N{}^a$ gives

$$\frac{I_S{}^{a'}}{I_N{}^a} = \frac{a'}{c} - \frac{I_D{}^{a'}}{I_N{}^a} + \frac{\alpha - 1}{\alpha}$$

which rearranges to

$$\frac{a'}{c} = \left(\frac{I_S{}^{a'}}{I_N{}^a} + \frac{I_D{}^{a'}}{I_N{}^a} - 1\right) + \frac{1}{\alpha} \tag{3}$$

Similarly, it can be derived that

$$\frac{b'}{c} = \left(\frac{I_S{}^{b'}}{I_N{}^b} + \frac{I_D{}^{b'}}{I_N{}^b} - 1\right) + \frac{1}{\alpha} \tag{4}$$

Since

$$\frac{I_D{}^{a'}}{I_N{}^a} = \frac{a'b'\alpha f_a}{f_a c} = \frac{a'b'\alpha}{c}$$

it follows that

$$a'b' = \frac{c}{\alpha}\left(\frac{I_D{}^{a'}}{I_N{}^a}\right) \tag{5}$$

or, similarly,

$$a'b' = \frac{c}{\alpha}\left(\frac{I_D{}^{b'}}{I_N{}^b}\right) \tag{6}$$

Let

$$F_a = \left(\frac{I_S{}^{a'}}{I_N{}^a} + \frac{I_D{}^{a'}}{I_N{}^a} - 1\right)$$

and

$$F_b = \left(\frac{I_S{}^{b'}}{I_N{}^b} + \frac{I_D{}^{b'}}{I_N{}^b} - 1\right)$$

Equations 3 and 4 now can be rewritten as

$$a' = cF_a + c/\alpha \tag{7}$$

and

$$b' = cF_b + c/\alpha \tag{8}$$

From substitution of eq 7 and 8 into eq 5 and 6

$$c^2\left(F_a + \frac{1}{\alpha}\right)\left(F_b + \frac{1}{\alpha}\right) = \frac{c}{\alpha}\left(\frac{I_D{}^{a'}}{I_N{}^a}\right) = \frac{c}{\alpha}\left(\frac{I_D{}^b}{I_N{}^b}\right)$$

which rearranges to give

$$c\left(\frac{1}{\alpha}\right)^2 + \left[c(F_a + F_b) - \left(\frac{I_D{}^a}{I_N{}^a}\right)\right]\frac{1}{\alpha} + cF_aF_b = 0$$

then solving for $1/\alpha$ gives

$$\frac{1}{\alpha} = \left\{\frac{I_D{}^{a'}}{I_N{}^a} - c(F_a + F_b) \pm \sqrt{\left[c(F_a + F_b) - \frac{I_D{}^{a'}}{I_N{}^a}\right]^2 - 4c^2F_aF_b}\right\} \Big/ 2c \tag{9}$$

From the appropriate ^{13}C NMR area weights the following values can be calculated:

$$I_D{}^{a'}/I_N{}^a = 6.20$$

$$I_D{}^{b'}/I_N{}^b = 5.57$$

$$F_a = 6.83$$

$$F_b = 6.00$$

If the average of these $I_D{}^{a'}/I_N{}^a$ and $I_D{}^{b'}/I_N{}^b$ values (5.88) is used to solve eq 9, $\alpha = 12.72$, from eq 7, $a' = 0.0725$, and from eq 8, $b' = 0.0704$. Since $a = \alpha a'$ and $b = \alpha b'$ by definition, $a = 0.922$ and $b = 0.896$.

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Registry No.—**1a**, 5746-86-1; **1a** 2HCl, 67209-89-6; [2′,3′-$^{13}C_2$]-**1a**, 67209-83-0; **1b** 2HCl, 67209-84-1; [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b**, 67209-85-2; [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b**, 67209-86-3; **1b** monoethanesulfonate salt, 67209-87-4; [2′,3′,*N*-CH_3-$^{13}C_3$]-**1b** monoethanesulfonate salt, 67209-88-5; **3**, 532-12-7; **4**, 67209-90-9; **5**, 67209-91-0; **6**, 67209-92-1; **7**, 67209-93-2; **10**, 67209-94-3; **11A** 67209-95-4; **11S**, 67209-96-5; **12A**, 67209-97-6; [1′,2′-$^{13}C_2$]-**12A**, 67209-98-7; [1′,2′-$^{13}C_2$]-**13**, 67209-99-8;

13, 67210-00-8; **14,** 3000-81-5; **16,** 930-41-6; **17,** 67210-01-9; **18,** 67210-02-0; **19,** 67210-03-2; 2,2-bis(ethylthio)ethanal, 42919-45-9; 1,1-bis(ethylthio)methane, 4396-19-4; 2-formyl-1,3-dithiane, 34906-12-2; 1,3-dithiane, 505-23-7; 1-acetyl-2,2-bis(ethylthio)ethanol, 67210-04-2; 2,2-bis(ethylthio)ethanol, 67210-05-3; 2-acetoxymethyl-1,3-dithiane, 67210-06-4; 1,3-dithiane-2-methanol, 37721-88-3; 1-acetyl-2,2-bis(ethylthio)ethanol sulfoxide, 67210-07-5; 3-acetylpyridine, 350-03-8; methoxyamine hydrochloride, 593-56-6; [1′,2′-$^{13}C_2$]-3-acetylpyridine, 67210-08-6; 3-bromopyridine, 626-55-1; 3-lithiopyridine, 60573-68-4; 3-ethylpyridine hydrochloride, 67210-09-7; 3-acetylpyridine hydrochloride, 67210-10-0; [1′,2′-$^{13}C_2$]-3-acetylpyridine methoxine, 67210-11-1; carbon-13 dioxide, 1111-72-4.

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