Phytochemistry, Vol. 27, No. 2, pp. 401–406, 1988.
Printed in Great Britain.

0031-9422/88 $3.00 + 0.00

THE INCORPORATION OF [2-^{13}C, ^{14}C, ^{15}N]-1-METHYL-Δ^1-PYRROLINIUM CHLORIDE INTO CUSCOHYGRINE IN *ERYTHROXYLUM COCA**†

EDWARD LEETE, SUNG HOON KIM and JATINDER RANA

Natural Products Laboratory, Department of Chemistry, University of Minnesota, Minneapolis, MN 55455, U.S.A.

(*Received* 5 *May* 1987)

Key Word Index—*Erythroxylum coca*; Erythroxylaceae; alkaloids; biosynthesis; cuscohygrine; 1-methyl-Δ^1-pyrrolinium chloride and its dimer.

Abstract—[2-^{13}C, ^{14}C, ^{15}N]-1-Methyl-Δ^1-pyrrolinium chloride was synthesized from potassium [^{13}C, ^{14}C]cyanide and [^{15}N]methylamine. The λ_{max} at 267 nm previously reported for this iminium salt, was shown to be due to an impurity. Feth, F. *et al. Phytochemistry* **24**, 1653). The structure of this impurity was established as 1-methyl-3-(*E*)-(4′-methylaminobutylidene)-Δ^1-pyrrolinium chloride formed by the acid cleavage of the dimer of the iminium salt. Administration of the labelled pyrrolinium salt to *Erythroxylum coca* plants resulted in the formation of radioactive cuscohygrine (specific inc. of ^{14}C: 0.25%). The ^{13}C NMR spectrum of this alkaloid exhibited satellites at the signals due to C-2 and C-2′ arising from coupling of these carbons with the adjacent ^{15}N. The multiplicity of signals in the ^{13}C NMR spectrum of both natural and synthetic cuscohygrine indicated that the alkaloid is a mixture of its *meso*, (2*S*,2′*R*), and optically active (2*S*,2′*S*), (2*R*,2′*R*) diastereomers.

INTRODUCTION

The 1-methyl-Δ^1-pyrrolinium salt (**5**) or its open form, 4-methylaminobutanal (**7**) has been established as a precursor of the 1-methylpyrrolidine ring of nicotine (**4**) in *Nicotiana* species. It is formed in this species from ornithine (**1**), via putrescine (**2**) and *N*-methylputrescine (**3**). Enzymes catalysing these steps have been isolated and characterized [1–7].

The iminium salt is also considered to be a precursor of other alkaloids containing a 1-methylpyrrolidine ring and non-enzymatic 'biomimetic syntheses' of hygrine (**8**) [3, 8, 9], cuscohygrine (**9**) [9, 10], ficine (**10**) [11], and brevicolline (**11**) [12] have been achieved from **5**, the carbinolamine **6**, or **7** (Fig. 1).

The present article describes the synthesis of the pyrrolinium salt **5** labelled with ^{15}N and with ^{13}C and ^{14}C at C-2. The incorporation of this compound into cuscohygrine in *Erythroxylum coca* was then monitored by ^{14}C assay and ^{13}C NMR spectroscopy.

RESULTS AND DISCUSSION

The unequivocal synthesis of [2-^{13}C, ^{14}C, ^{15}N]-1-methyl-Δ^1-pyrrolinium chloride (**5**) is illustrated in Fig. 2. Reaction of 1-bromo-3-chloropropane (**12**) with a mixture of potassium [^{13}C] and [^{14}C] cyanide afforded 4-chlorbutyronitrile (**13**). Reduction with diisobutyl aluminum hydride yielded 4-chlorobutanal which was converted to 4-chlorobutanal diethyl acetal (**14**) by reaction with ethanol in the presence of calcium chloride. [^{15}N] Benzylmethylamine (**15**) was obtained by the reductive amination of benzaldehyde with sodium cyanoborohydride in the presence of [^{15}N]methylamine. Reaction of the [^{15}N]benzylmethylamine with the chloro compound **14** in the presence of potassium carbonate yielded *N*-benzyl-*N*-methyl-4-aminobutanal diethyl acetal (**17**). Hydrogenolysis in the presence of palladium on charcoal yielded 4-methylaminobutanol diethyl acetal (**16**). Hydrolysis of this acetal with hydrochloric acid yielded the iminium chloride **5**. This last reaction was also used by Wagner and co-workers [13] to prepare an authentic specimen of this iminium salt. They reported the ^{13}C NMR spectrum of this salt and the spectrum which we determined was essentially the same, with the additional multiplicity of signals expected due to spin–spin couplings with the ^{15}N enriched nitrogen (See Experimental). We previously [3] reported that the iminium salt **5** had an absorption in the ultraviolet (λ_{max} 267, ε 2240 in 95% ethanol). Wagner [13] purified this iminium salt by HPLC and found that this absorption was due to an impurity. We have confirmed this observation and determined the structure of this impurity to be 1-methyl-Δ^1-3-(*E*)-(4′-methylaminobutylidene)-Δ^1-pyrrolinium chloride-hydrochloride (**21**) (λ_{max} 267 nm, ε 13 000). It is considered that this compound is formed by the mechanism illustrated in Fig. 2. Loss of HCl from the iminium salt **5** affords 1-methyl Δ^2-pyrroline (**18**). This enamine than condenses with the iminium salt **5** to yield **19**. Loss of HCl from this compound affords 1-methyl-3-(1′-methyl-2′-pyrrolidinyl)-Δ^2-pyrroline (**20**). This compound was first obtained [14] by the oxidation of 1-methylpyrrolidine

*Part 38 in the series 'Chemistry of the Tropane Alkaloids and Related Compounds'.

†Contribution No. 204 from this Laboratory. Presented in part at the 15th IUPAC meeting on the Chemistry of Natural Products, The Hague, The Netherlands, 17–22 August 1986.

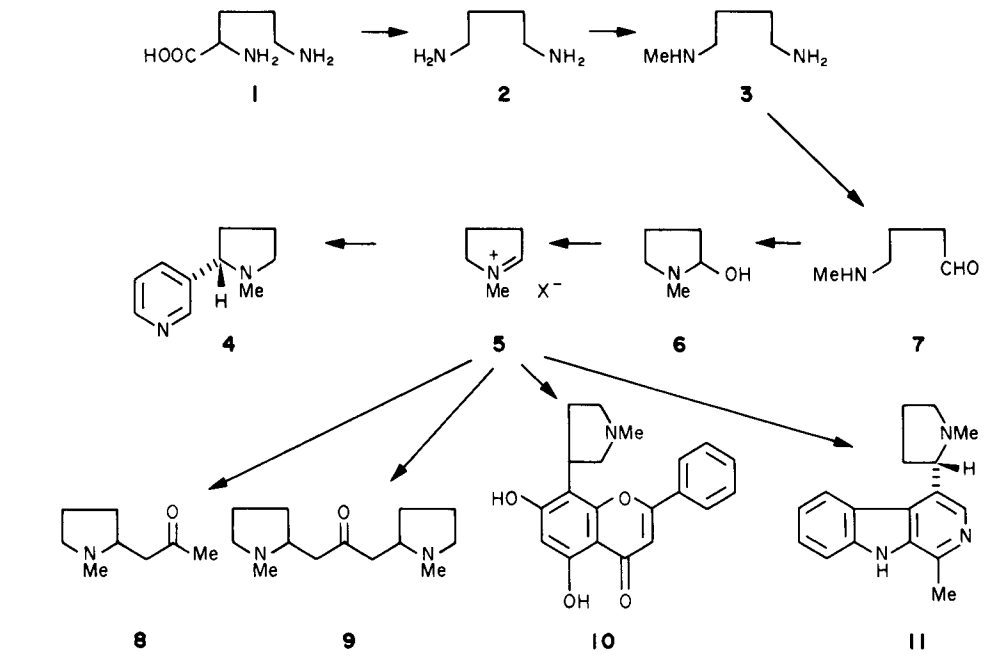

Fig. 1. Some alkaloids derived from the 1-methyl-Δ-^{1}-pyrrolinium salt.

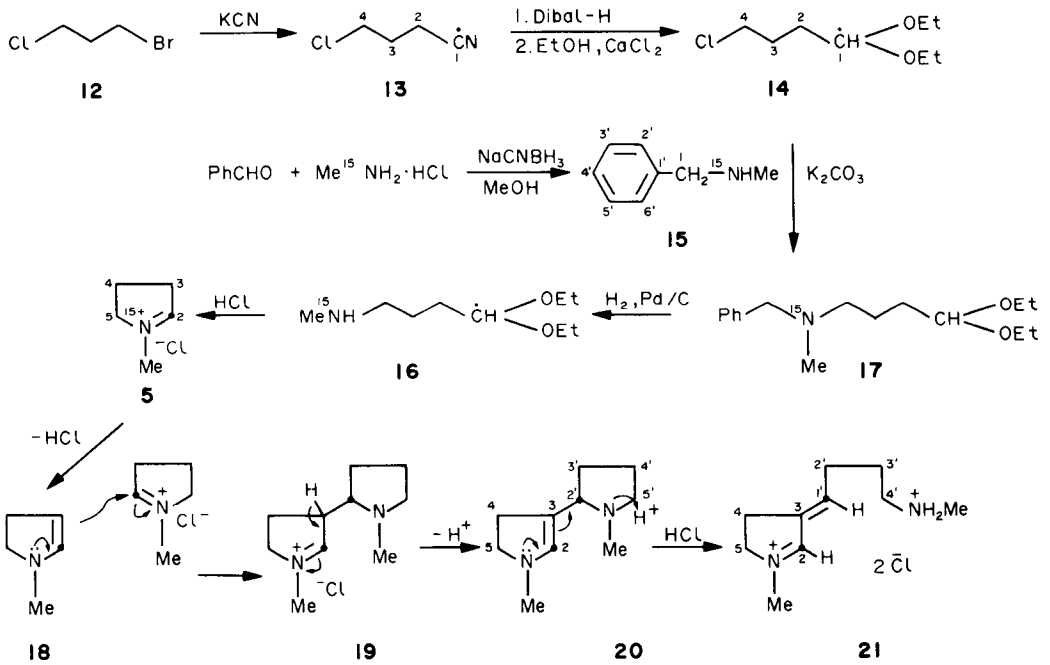

Fig. 2. Synthesis of labelled 1-methyl-Δ^1-pyrrolinium chloride and the formation of its protonated dimer.

(also [15]). This dimer is the vinylog of a *gem*-diamino compound and the depicted ring opening of the pyrrolidine ring with HCl is analogous to the acid cleavage of a 1,1-diamine to an amine and an iminium salt illustrated below:

$$R-\overset{\curvearrowright}{NH}-CH_2-\overset{\curvearrowright}{\overset{+}{N}}H_2-R' \rightarrow R-\overset{+}{N}H=CH_2 + H_2N-R'$$

The ^{13}C NMR spectrum of the dimer (**20**) and its ring opened derivative **21** were consistent with the proposed structures. Especially revealing was the spectrum of **21** derived from [2-^{13}C]-1-methyl-Δ^1-pyrrolinium chloride. Carbons 2 and 1′ were the enriched positions and appeared as enhanced signals, the signal at 150.2 ppm (C-1′) appearing as a doublet (J = 4.9 Hz) due to a two bond coupling between these enriched positions. In the ^{13}C NMR spectrum of unenriched **21** only 10 signals were

observed indicating that only one geometric isomer was formed on ring opening of the pyrrolidine ring of the dimer. The (*E*)-configuration was assigned to the butylidene side chain on the basis of its ^{1}H NMR spectrum. An NOE experiment indicated that the proton at C-2 (δ8.45, *s*, 12% enhancement) is *cis* to the proton at C-1′ (δ6.76, *t*, 11% enhancement). Compound **21** could be isolated as its chloride-hydrochloride salt and was purified by chromatography on Dowex 50 × 8. The strong absorption at 267 nm is assigned to the α,β-unsaturated iminium ion. This chromophore is present in the enamine derived from progesterone and pyrrolidine. This compound had λ_{max} 277 nm (ε = 20,475) in MeOH–HCl solution [16]. On making a solution of **21** in water basic, the absorption at 267 nm disappeared with regeneration of the dimer **20**.

Previously it has been established that the pyrrolidine rings of cuscohygrine are derived from ornithine [17, 18]. Hygrine (**8**) was also found to be a direct precursor of cuscohygrine [18, 19], the former alkaloid being hypothetically derived from the iminium salt **5** and acetoacetate [20]. It was assumed that cuscohygrine is formed by reaction of hygrine with a second molecule of the iminium salt. In the present work it was thus expected that [2-^{13}C, ^{14}C, ^{15}N]-1-methyl-Δ^1-pyrrolinium chloride would afford cuscohygrine enrichd with ^{13}C at the C-2 and C-2′ positions (Fig. 3) Cuscohygrine isolated from *Erythroxylum coca* plants which had been fed (by leaf painting) this labelled precursor was significantly labelled with ^{14}C (0.25% specific incorporation) and had 0° optical rotation. Examination of its ^{13}C NMR spectrum revealed the presence of satellites (J = 4.2–4.3 Hz) at the signals due to C-2 and C-2′ (Fig. 4). The asymmetry of these satellites is due to an isotope shift (~ 1.1 Hz) of ^{13}C adjacent to ^{15}N compared with ^{13}C adjacent to ^{14}N. Both the cuscohygrine isolated from the *coca* plant and synthetic alkaloid [8] exhibited double peaks for the C-2,2′, C-6,6′, and the two *N*-methyl groups. The multiplicity of these peaks is considered to indicate that cuscohygrine, both natural and synthetic, is an equilibrium mixture of the *meso* form (2*S*, 2′*R*) (**9b**) and a racemic mixture of the two optically active forms (2*R*, 2′*R*) (**9a**) and (2*S*, 2′*S*) (**9c**). This equilibrium is considered to proceed via the ring opened intermediate (**22**). In support of this mechanism the hydrogens present at C-6 and C-6′ of cuscohygrine are rapidly exchanged with solvent in acidic D_2O. *meso*-Cuscohygrine (**9b**) is expected to give rise to seven different signals in its ^{13}C NMR spectrum, i.e. C-2 will have the same chemical shift as C-2′. Likewise the optically active forms of cuscohygrine (**9a, 9c**) will both have seven signals. However, since **9b** and (**9a, 9c**) contain different chiral environments, the chemical shifts of C-2 in **9b** is different from the chemical shift of C-2 in (**9a, 9c**). The signals of equivalent carbons which appeared as doublets in the ^{13}C NMR spectrum of cuscohygrine in $CDCl_3$ were of almost equal intensity. This indicates that the alkaloid in this solvent is an equilibrium mixture of 50% *meso* and 25% each of the two optically active forms. The failure of previous workers [21, 22] to resolve either natural or synthetic cuscohygrine with a variety of optically active acids is probably due to this rapid equilibrium of the forms **9a–c**.

EXPERIMENTAL

General methods. Radioactive materials were assayed by liquid scintillation counting using dioxane–EtOH as the solvent with the usual scintillators [23]. NMR spectra were determined in a Nicolet 300 spectrometer operating at 300 and 75.5 MHz respectively for ^{1}H and ^{13}C, with the assistance of Dr S. B. Philson, All recorded spectra are ppm from Me_4Si. Mass spectra were determined by Dr E. Larka and his assistants at the University of

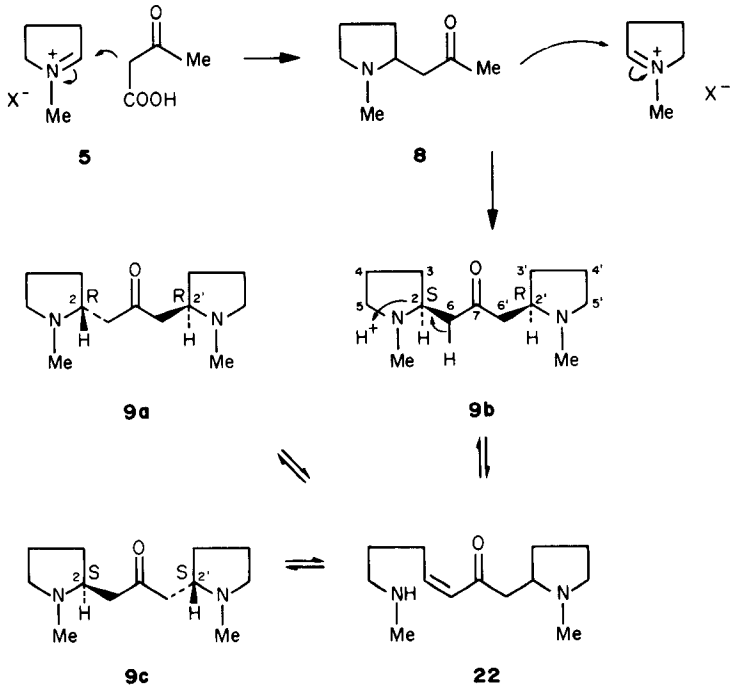

Fig. 3. Proposed biosynthesis of cuscohygrine from the 1-methyl-Δ^1-pyrrolinium salt.

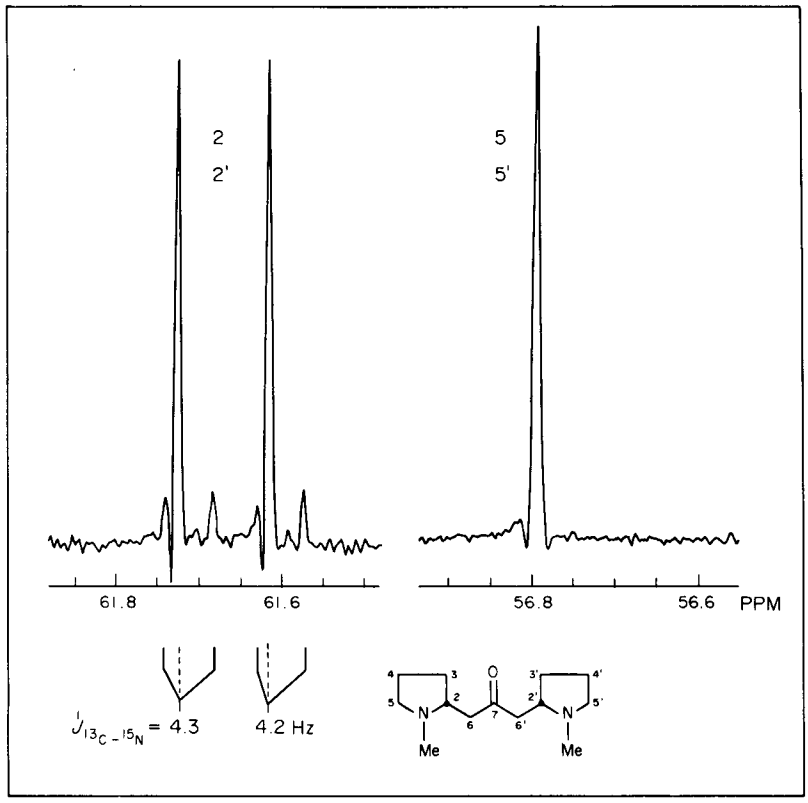

Fig. 4. ^{13}C NMR ($CDCl_3$) of cuscohygrine, (C-2) and C-5 signals), isolated from E. coca fed [2-^{13}C, ^{14}C, ^{15}N]-1-methyl-Δ^1-pyrrolinium chloride.

Minnesota. Gas chromatography was carried out in a Hewlett–Packard model 5890A gas chromatogram on a 25 m glass capillary column coated with cross-linked methyl silicone (0.52μ m thick) i.d. 0.31 mm, using the following instrument parameters: He flow rate 1 ml/min, injection temp. 250°, initial oven temp. 50°, equilibration time 4 min, rate of temp. increase 30°/min, final oven temp. 250°. R_t in min.

[1-^{13}C, ^{14}C]-*4-chlorobutanal diethyl acetal* (**14**). A solution of K ^{13}CN (1.0 g, 15 mmol, 99% ^{13}C) (MDS Isotopes) and K ^{14}CN (nominal act. 1 mCi, 1.5 mg) (Amersham-Searle) in H_2O (1 ml) was added a solution a 1-bromo-3-chloropropane (7.8 g, 49.5 mmol) and KI (0.17 g, 1.03 mmol) in EtOH (13 ml) and the mixture refluxed for 7 hr. The cooled reaction mixture was evapd *in vacuo*, the residue mixed with H_2O and extracted with $CHCl_3$ (5 × 10 ml). The residue obtained on evapn of the dried ($CaCl_2$) extract was chromatographed on a SiO_2 gel column, developing with hexane–$CHCl_3$ affording crude 4-chlorobutyronitrile (1.15 g, 73% based on K^{13}CN). GC indicated the presence of 94% 4-chlorobutyronitrile (R_t 7.04) and 4% 4-bromobutyronitrile (R_t 7.98). DIBAL-H (12.2 ml of a 1.0 M solution in CH_2Cl_2, 12.2 mmol) was added dropwise during 10 min to a solution of the crude 4-chlorobutyronitrile (1.15 g, 11 mmol) in CH_2Cl_2 (10 ml) at −10°. After stirring for 7 hr at room temp., the mixture was added to a mixture of ice (50 g), Et_2O (50 ml) and 10% H_2SO_4 (10 ml). The organic layer was separated and the aqueous layer extracted with more Et_2O (4 × 20 ml). The Et_2O layer was washed with 10% H_2SO_4, H_2O, and dried ($MgSO_4$). Evaporation yielded [1-^{13}C]-4-chlorobutanal (GC R_t 6.12) which was dissolved in EtOH (25 ml) and stirred at 20° over solid $CaCl_2$ for 5 days.

The residue obtained on evapn of of the EtOH soln was added to 5% $NaHCO_3$ (25 ml) and extracted with $CHCl_3$ (3 × 25 ml). Evaporation of the dried (Na_2SO_4) extract yielded an oil which was distilled (88–90°, 14 mm Hg) affording [1-^{13}C]-4-chlorobutanol diethyl acetal as a colourless oil (840 mg, 1.55 × 10^8 dpm/mmol, 30% yield from K^{13}CN). GC R_t (9.53). ^{13}C NMR ($CDCl_3$): δ102.2 (enriched, C-1), 61.0 (O-$\underline{C}H_2$-Me), 44.7 (C-4), 30.8 (*d*, C-2, $^1J_{1,2}$ = 35.2 Hz), 27.8 (C-3), 15.1 (O-CH_2-$\underline{Me}$).

[^{15}N]*Benzylmethylamine* (**15**). [^{15}N]Methylamine hydrochloride (1.0 g, 14.8 mmol, 99%^{15}N) (MDS Isotopes) and benzaldehyde (800 mg, 7.54 mmol) were dissolved in dry MeOH (15 ml) and stirred in a N_2 atoms. in the presence of 3Å molecular sieves (2 g). Sodium cyanoborohydride (1.5 g, 24 mmol) was added in small protions. The reaction was kept acidic by the slow addition of HOAc (2–3 ml). After 3 hr stirring at 20° the reaction mixture was filtered and the filtrate evapd. The residue was made basic with 10% NaOH and extracted with $CHCl_3$ (5 × 20 ml). The residue (1.15 g) obtained on evaporation of the dried ($MgSO_4$) extract was subjected to chromatography on a SiO_2 gel column. Elution with $CHCl_3$–MeOH (17:3) afforded benzyl alcohol, (GC R_t 8.63), followed by dibenzylmethylamine (GC R_t 13.98). Elution with $CHCl_3$, MeOH, conc NH_4OH (85:14:1) then afforded [^{15}N]-benzylmethylamine (500 mg, 4.55 mmol, 31% yield from [^{15}N]methylamine), R_t 8.87. ^{13}C NMR ($CDCl_3$): δ140.2 (C-1'), 128.4 (C-2', 6'), 128.2 (C-3', 5'), 127.0 (C-4'), 56.1 (d, C-1, $^1J_{1,^{15}N}$ = 4.5 Hz), 36.0 (*d*, NMe, $^1J_{Me^{15}N}$ = 3.8 Hz).

[1-^{13}C, ^{14}C, ^{15}N]-N-*Benzyl*-N-*methyl*-4-*aminobutanal diethyl acetal*. (**17**). [1-^{13}C, ^{14}C]-4-Chlorobutanal diethyl acetal (800 mg, 4.42 mmol) dissolved in dry 1-BuOH (3 ml) was added during 10 min to a stirred mixture of [^{15}N]benzylmethylamine (550 mg, 4.5 mmol), and anhydrous K_2CO_3 (523 mg, 4.51 mmol)

and KI (75 mg, 0.45 mmol) in 1-BuOH (15 ml). The mixture was then refluxed for 48 hr, cooled and diluted with Et_2O (15 ml). The filtered reaction mixture was evaporated to yield a viscous oil which was subjected to chromatography on a SiO_2 gel column, eluting with $CHCl_3$–MeOH–NH_4OH (90:9:1) affording [1-^{13}C, ^{14}C, ^{15}N]-*N*-benzyl-*N*-methyl-4-aminobutanal diethyl acetal as a pale yellow oil (888 mg, 62%) R_t 13.53 (97% pure). ^{13}C NMR ($CDCl_3$) (on unlabelled material) δ139.3 (C-1'), 129.0 (C-2', 6'), 128.1 (C-3', 5'), 126.8 (C-4'), 102.8 (C-1, enhanced in labelled material), 62.3 (–$\underline{C}H_2$Ph), 60.9 (O–$\underline{C}H_2$–Me), 57.1 (C-4), 42.1 (NMe), 31.4 (C-2), 22.6 (C-3), 15.4 (O–CH_2–$\underline{Me}$).

[1-^{13}C, ^{14}C, ^{15}N]-4-*Methylaminobutanal diethyl acetal* (**16**). A solution of **17** (400 mg, 1.5 mmol) in absolute EtOH (15 ml) was hydrogenated in the presence of 10% Pd/C (20 mg) at 22° and atm. pres. for 8 hr. The reaction mixture was filtered through celite and evapd to yield a pale yellow oil which was subjected to chromatography on SiO_2 gel eluting with a mixture of $CHCl_3$, MeOH, conc NH_4OH (90:9:1 changing to 75:24:1) affording [1-^{13}C, ^{14}C, ^{15}N]-4-methylaminobutanal diethyl acetal (165 mg, 62% 1.56×10^8 dpm/mmol) as a colourless oil, R_t 9.87 (100% pure). ^{13}C NMR ($CDCl_3$): δ102.8 (C-1, signal enhanced), 60.8 (*s*, O–$\underline{C}H_2$Me), 51.7 (*dd*, $^1J_{4,^{15}N}$ = 4.5 Hz, $^3J_{1,4}$ = 4.1 Hz, C-4), 36.2 (*d*, $^1J_{Me,^{15}N}$ = 4.4 Hz, NMe) 31.2 (*d*, $^1J_{1,2}$ = 45.5 Hz, C-2), 25.0 (*d*, $^2J_{3,^{15}N}$ = 2.1 Hz, C-3), 15.4 (*s*, O–$CH_2\underline{Me}$).

[2-^{13}C, ^{14}C, ^{15}N]-1-*Methyl*-Δ^1-*pyrrolinium chloride* (**5**). The acetal **16** (160 mg, 0.90 mmol) was heated at 60° in 2M HCl (1.5 ml) for 20 min in a N_2 atmosphere. The lyopholized solution was redissolved in 2M HCl and passed through a small column of Dowex 50 × 8 (H^+). Elution with 2N HCL afforded [2-^{13}C, ^{14}C, ^{15}N]-1-methyl-Δ^1-pyrrolinium chloride (90 mg, 82%, 1.50×10^8 dpm/mmol). ^{13}C NMR (D_2O): δ184.4 (*d*, $^1J_2$, ^{15}N = 18.2 Hz, enhanced signal. This signal appears as a triplet in the [^{14}N]-**5**, J = 12.2 Hz due to a quadrupolar coupling of C-2 to $^{14}N^+$ [13], C-2), 63.5 (*d*, $^1J_{5,^{15}N}$ = 5.05 Hz, C-5), 43.3 (*d*, $^1J_{Me,^{15}N}$ = 5.3 Hz, 1-Me), 38.7 (*d*, $^1J_{2,3}$ = 37.3 Hz, C-3), 22.5 (*s*, C-4).

1-*Methyl* 1-3-(1'-*methyl*-2'-*pyrrolidinyl*)-Δ^2-*pyrrolidine* (**20**). This was prepared by the mercuric acetate oxidation of 1-methylpyrrolidine as previously described [14]. ^{13}C NMR ($CDCl_3$): δ139.6 (C-2), 117.6 (C-3), 65.2 (C-2'), 56.6 (C-5), 56.1 (C-5'), 41.0 (1-Me) 40.2 (1'-Me), 29.9 (C-4), 28.9 (C-3'), 21.9 (C-4'). On dissolving this material in D_2O and adding HCl there was an immediate change in the NMR spectra due to the formation of 3-(*E*)-(4'-methylaminobutylidene)-1-methyl-Δ^1-pyrrolinium chloride-hydrochloride (**21**) ^{13}C NMR (D_2O, H^+): δ174.3 (C-2), 150.2 (C-1'), 142.1 (C-3), 62.1 (C-5), 51.2 (C-4'), 42.7 (1-Me), 35.6 (N'-Me), 30.1 (C-2'), 27.1 (C-4), 26.7 (C-3'). A sample of **21** was prepared from [2-^{13}C]-**5** (made from $K^{13}CN$) by incubating an aqueous solution at pH 9, then acdifying with HCl and subjected to chromatography on Dowex 50 × 8. The signals enhanced due to the presence of excess ^{13}C were at 174.3 (*br s*, C-2) and 150.1 (*d*, C-1', $^2J_{1',2}$ = 4.9 Hz). ^{1}H NMR (D_2O, H^+) δ1.79 (*p*, 3'), 2.28 (*q*, 2'), 2.58 (*s*, 4' NMe), 2.87 (*m*, 4), 2.93 (*m*, 4'), 3.49 (*s*, 1-Me), 4.11 (*t*, 5), 6.76 (*t*, 1'), 8.44 (*s*, 2). An NOE experiment produced enhancements (12 and 11% respectively) of the signals due to the hydrogens at C-2 and C-1', respectively indicating that they were *cis*. UV (95% EtOH, H^+) λ_{max} 267 nm (ε = 13,000). EIMS *m/z* (rel. int.) 167 (1.5) (M^+), 137 (10.1), 82 (61.3).

Administration of [2-^{13}C, ^{14}C, ^{15}N]-1-methyl-Δ^1-pyrrolinium chloride *to Erythroxylum coca and isolation of the cuscohygrine.* The iminium chloride (99% ^{13}C, 1.50×10^8 dpm/mmol, 99% ^{15}N, 38 mg) was dissolved in H_2O (10 ml) together with 0.1 g of Tween 80. This soln was painted on the leaves of two 4-year *Erythroxylum coca* plants growing in a greenhouse (28 February 1986). The painting was extended over several days and the plants then allowed to grow for a total of 3 weeks after the initial feeding. The leaves of the plants were then picked (fr. wt 75 g) and extracted as previously described [24] affording cocaine and cuscohygrine isolated as its picrate (36 mg). The level of incorporation of ^{13}C into the cocaine was not high enough to establish unequivocally the direct incorporation of **5** into this alkaloid. The cuscohygrine dipicrate was crystallized to constant activity from aq. EtOH, ultimately affording material with an activity of 7.51 $\times 10^5$ dpm/mmol (0.25% specific inc. 100% specific inc. would be represented by an activity of 3.0×10^8 dpm/mmol, since 2 mol of the precursor are incorporated into the alkaloid). The free base was obtained from the dipicrate by dissolving the latter in 2N HCL, extracting with Et_2O to remove picric acid, then lyopholizing the solution of cuscohygrine hydrochloride. This was then made basic with K_2CO_3 and extracted with $CHCl_3$. GC R_t 12.88. Changing the rate parameter on the GC from 30°/min to 5°/min (all other parameters the same) gave a R_t of 34.06 min but only one peak was observed indicating that the meso and racemic forms are not separable under these conditions. ^{13}C NMR ($CDCl_3$): δ209.3 (C-7), 61.74, 61.63 (C-2, 2'), 56.79 (C-5, 5'), 48.52, 48.45 (C-6, 6'), 40.48, 40.17 (NMe), 31.32 (C-3, 3'), 21.19 (C-4, 4'). Essentially the same ^{13}C NMR spectrum was observed for cuscohygrine isolated from the *coca* plant and synthetic [8] material. The spectrum illustrated in Fig. 4 was obtained on 12 mg of cuscohygrine in 0.4 ml of $CDCl_3$ with following instrument parameters: A spectral window of 4900 Hz, acquisition time 3.3 sec, delay time 0.1 sec, 70° pulses, 16 500 acquisitions, size 64 K, resolution enhancement by Lorentz–Gauss transformation.

Acknowledgement—This work was supported by a research grant GM-13246-30 from the National Institutes of Health, U.S. Public Health Service.

REFERENCES

1. Kisaki, T., Mizusaki, S. and Tamaki, E. (1967) *Arch. Biochem. Biophys.* **18**, 179.
2. Mizusaki, S., Kisaki, T. and Tanaki, T. (1968) *Plant Physiol.* **43**, 93.
3. Leete, E. (1967) *J. Am. Chem. Soc.* **89**, 7081.
4. Mizusaki, S., Tanabe, Y., Noguchi, M. and Tamaki, E. (1973) *Plant Cell Physiol.* **14**, 103.
5. Yoshida, D. (1973) *Bull. Hatamo Tobacco Exp. Stn* **73**, 239.
6. Feth, F., Arfmann, H. -A., Wray, V. and Wagner, K. G. (1985) *Phytochemistry* **24**, 921.
7. Bush, L. P. and Saunders, J. L. (1977) *Proc. Am. Chem. Soc. Symp.*: Recent Advances in the Chemical Composition of Tobacco and Tobacco Smoke, p. 389. New Orleans.
8. Galinovsky, F., Wagner, A. and Weiser, R. (1951) *Monatsch* **82**, 551.
9. El-Olemy, M. M., Schwarting, A. E. and Kelleher, W. J. (1966) *Lloydia* **29**, 58.
10. Anet, E. F. L. G., Hughes, G. K. and Richie, E. (1949) *Aust. J. Sci. Res.* **2A**, 616.
11. Leete, E. (1982) *J. Nat. Prod.* **45**, 605.
12. Leete, E. (1979) *J. Chem. Soc., Chem. Commun.* 821.
13. Feth, F., Wray, V. and Wagner, K. G. (1985) *Phytochemistry* **24**, 1653.
14. Leonard, N. J. and Cook, A. G. (1959) *J. Am. Chem. Soc.* **81**, 5627.
15. Swan, G. A. and Wilcock, J. D. (1974) *J. Chem. Soc. Perkin Trans. I* 885.
16. Johnson, J. L., Herr, J. C., Babcock, A. E., Fonken, A. E.,

Stafford, J. E. and Heyl, F. W. (1956) *J. Am. Chem. Soc.* **78**, 430.
17. Leete, E. (1982) *J. Am. Chem. Soc.* **104**, 1043.
18. O'Donovan, D. G. and Keogh, M. F. (1969) *J. Chem. Soc.* (*C*) 223.
19. McGaw, B. A. and Woolley, J. G. (1978) *Phytochemistry* **17**, 257.
20. McGaw, B. A. and Woolley, J. G. (1979) *Tetrahedron Letters* 3135.
21. Hess, K. and Anseln, F. (1921) *Ber.* **54**, 2310.
22. Galinovsky, F. and Zuber, H. (1943) *Ber.* **76**, 942.
23. Friedman, A. R. and Leete, E. (1963) *J. Am. Chem. Soc.* **85**, 2141.
24. Leete, E. (1982) *J. Am. Chem. Soc.* **104**, 1403.