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1-METHYLPYRROLIDINE-2-ACETIC ACID IS NOT A PRECURSOR OF TROPANE ALKALOIDS*†

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Key Word Index—*Erythroxylum coca*; Erythroxylaceae; *Datura innoxia*; Solanaceae; biosynthesis; alkaloid; cuscohygrine; tropane; cocaine; scopolamine; hyoscyamine.

Abstract—1-Methylpyrrolidine-2-acetic acid and related compounds were studied as precursors in the biosynthesis of the tropane alkaloids in *Erythroxylum coca* and *Datura innoxia*. (*R,S*)-[1′,2-$^{13}C_2$,2-^{14}C,^{15}N]-1-Methylpyrrolidine-2-acetic acid, (*R,S*)-[1′,2′-$^{13}C_2$,1′-^{14}C]-1-methylpyrrolidine-2-acetic acid, (*R,S*) ethyl [1′,2′-$^{13}C_2$,1′-^{14}C]-1-methylpyrrolidine-2-acetate, and (*R,S*)-[2′-^{14}C]-1-methylpyrrolidine-2-acetic acid *N*-acetylcysteamine thioester were synthesized and fed to intact plants by leaf-painting or hydroponic-feeding. Specific incorporation of these compounds into (−)-hyoscyamine, (−)-scopolamine, (−)-cocaine and the biosynthetically related cuscohygrine were very low. These results indicate that 1-methylpyrrolidine-2-acetic acid is not an efficient precursor of tropane alkaloids.

INTRODUCTION

The biosynthesis of the tropane alkaloids (−)-hyoscyamine (**10**) and (−)-scopolamine (**11**) (*Datura*, *Hyoscyamus*, *Atropa* and *Duboisia* species), and (−)-cocaine (*Erythroxylum coca*) are related [1]. The pyrrolidine alkaloid, cuscohygrine (**8**), is also found in the majority of plants that contain tropane alkaloids. It has been established that it is formed as an offshoot of the tropane biosynthetic pathway [2]. The generally accepted hypothesis for the formation of these alkaloids begins with the formation of the *N*-methyl-Δ^1-pyrrolinium ion (**2**) from the amino acid ornithine (**1**) (Scheme 1) [1]. The β-keto ester (**3**) was established as a precursor to cocaine (**9**) by feeding methyl (*R,S*)-[1,2-$^{13}C_2$,1-^{14}C]-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate to *E. coca* [3]. In the coca plant, cyclization of **3** gives carbomethoxytropinone (**5**), which is then stereospecifically reduced to methyl ecgonine (**4**). Cocaine is formed after benzoylation with benzoic acid, most likely as the CoA thioester (**6**) [4].

In *Datura innoxia*, high specific incorporations into compounds **10** and **11** were obtained after feeding ethyl (*R,S*)-[2,3-$^{13}C_2$,3-^{14}C]-4-(1-methyl-1-2-pyrrolidinyl)-3-oxobutanoate, an analogue of **3** [5]. Interestingly, hygrine [6–8], which was previously established as a precursor to these alkaloids, gave minimal incorporation. Consequently, the current hypothesis for the formation of hyoscyamine (**10**) and scopolamine (**11**) is even more similar to cocaine (**9**) biosynthesis than previously believed (Scheme 1). After formation of **3** and subsequent cyclization and decarboxylation, tropinone (**7**) is formed. Reduction of **7** gives tropine, which forms the (*S*)-tropic acid ester, hyoscyamine (**10**). Compound **10** is then hydroxylated to form 6β-hydroxyhyoscyamine, which then forms **11**. Several enzymes in this pathway have been studied [9].

Hygrine was also established as a precursor to **8** [6–8]. However, a comparison feeding of [2-^{14}C]hygrine and ethyl (*R,S*)-[2,3-$^{13}C_2$,3-^{14}C]-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate showed that, in coca, the β-keto ester was a much more efficient precursor for cuscohygrine than for hygrine [10]. The current hypothesis is that **3** condenses with *N*-methylpyrrolinium ion to form cuscohygrine.

RESULTS AND DISCUSSION

The next step in elucidating the biosynthesis of the tropane alkaloids and cuscohygrine would be to identify the intermediate between the pyrrolinium salt (**2**) and the β-ketoester (**3**). One possibility is the direct condensation of a four-carbon unit, such as acetoacetyl CoA. A previous feeding experiment [11] found that C-2 of the pyrrolinium salt becomes C-5 of (−)-cocaine, so reaction with acetoacetyl CoA would then require condensation at the methyl and not the chemically more activated methylene position, as in pelletierine biosynthesis [12]. Another possible intermediate is 1-methylpyrrolidine-2-acetic acid (**12a**) (Fig. 1), which would be formed from the

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†Dedicated to the memory of E. Leete, 1928–1992.

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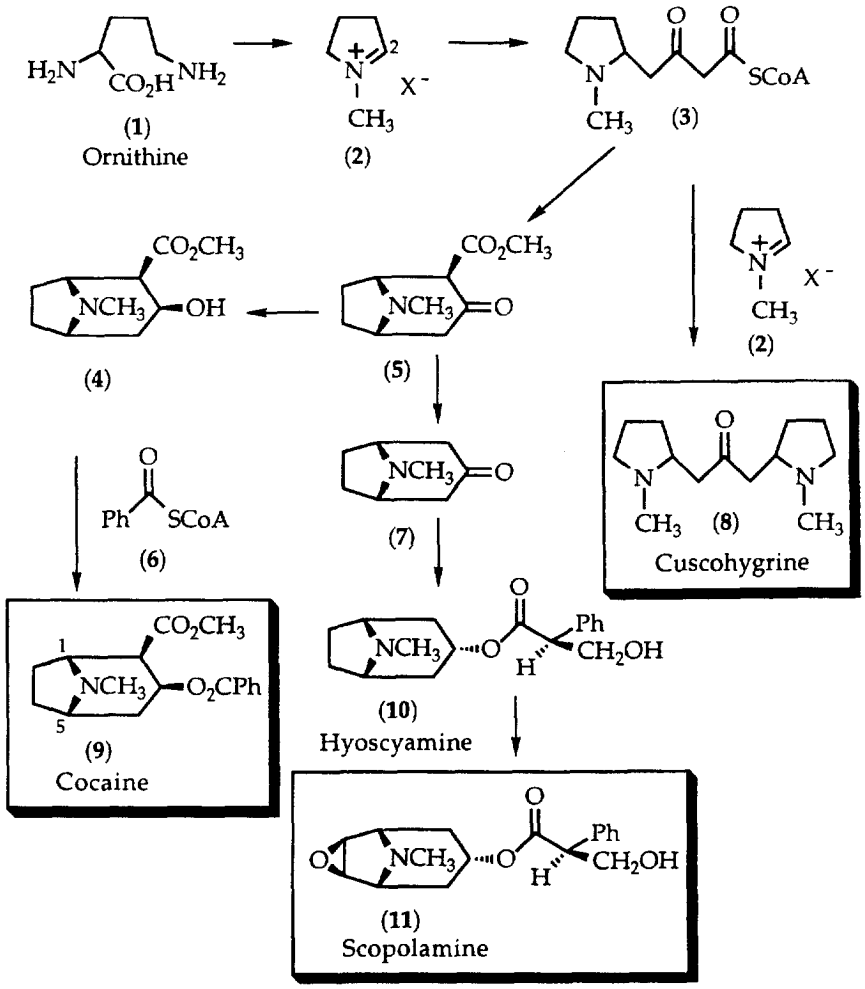
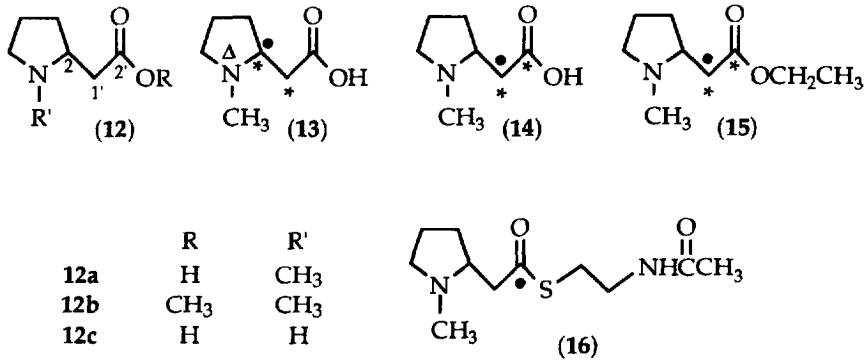


Scheme 1. Biosynthetic pathway to cocaine, hyoscyamine, scopolamine and cuscohygrine.

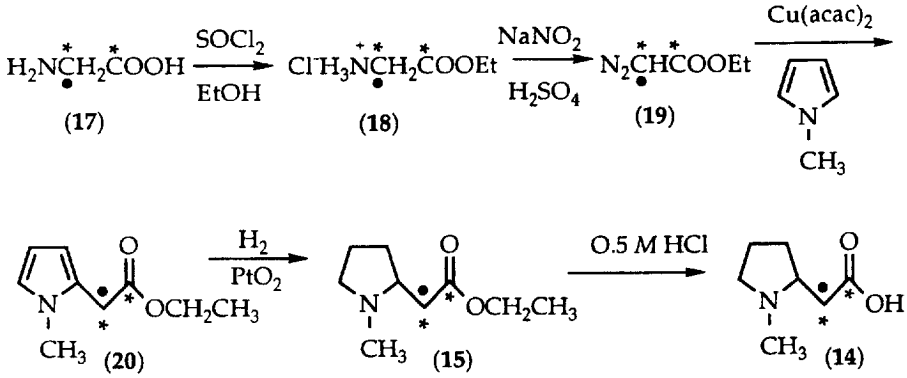


	R	R'
12a	H	CH_3
12b	CH_3	CH_3
12c	H	H

Fig. 1. *N*-Methylpyrrolidine-2-acetic acid and analogues for feeding experiments to *Erythoxylum coca* and *Datura innoxia*: * = ^{13}C; ● = ^{14}C; Δ = ^{15}N.

initial condensation of a two-carbon unit (such as acetate) with **2**. In the plant, this reaction could proceed as two consecutive acetyl coenzyme A units (or other activated compounds) adding to the salt (**2**), first forming the coenzyme A thioester of the acid (**12**) and then the β-ketothioester (**3**), an established precursor to (−)-cocaine, (−)-hyoscyamine, (−)-scopolamine and cuscohygrine.

Sequential addition of acetate was demonstrated in feeding experiments with *Hyoscyamus albus* root cultures



Scheme 2. Synthesis of ethyl [1′2′-$^{13}C_2$,1′-^{14}C]-1-methylpyrrolidine-2-acetate and [1′,2′-$^{13}C_2$,1′-^{14}C]-1-methylpyrrolidine-2-acetic acid.

[13] and with *Datura stramonium* [14]. Dual-labelled acetate ($C^2H_3{}^{13}COONa$) gave ^2H incorporation at the C-2 and C-4 positions in the tropine moiety of several alkaloids in *H. albus*, but in unequal amounts. In *D. stramonium*, the labelling pattern in isolated 6β-hydroxytropine following [1,2,3,4-$^{13}C_4$]acetoacetate feeding was identical with that from [1,2-$^{13}C_2$]acetate, indicating that the acetoacetate was cleaved to acetate before being incorporated. Additionally, **12a** was isolated labelled with ^{14}C from *E. coca* which had been fed a precursor to the [2-^{14}C]pyrrolinium salt [15]. Degradation of this compound showed that all the radioactivity was located at the C-2 position.

1-Methylpyrrolidine-2-acetic acid (**12a**) and analogues have also been found naturally in several types of plants [16–18] (Fig. 1). This gives circumstantial evidence that this compound may be a precursor common to several natural products. The methyl ester (**12b**) (methyl 1-methylpyrrolidine-2-acetate) was isolated from *Solanum sturtianum*, a small shrub in Western Australia, which contains steroidal alkaloids [16]. The nor-methyl free acid (**12c**) (pyrrolidine-2-acetic acid) was found in cured leaves of *Nicotiana tabacum* [17]. This same compound (**12c**) was also found in the alkaloid fraction of the flowers of *Tussilago farfara* and several *Arnica* species [18]. Identifying this β-amino acid gave evidence that **12** was a biosynthetic intermediate of the pyrrolizidium alkaloids found in these species.

Labelled 1-methylpyrrolidine-2-acetic acid and the corresponding ethyl ester (Fig. 1) were synthesized and fed to *E. coca* or to *D. innoxia*. [1′,2-$^{13}C_2$,2-^{14}C,^{15}N]-1-Methylpyrrolidine-2-acetic acid (**13**) was synthesized from diethyl [2-^{13}C]malonate and [2-^{13}C,2-^{14}C,^{15}N]-1-methyl-Δ^1-pyrrolinium chloride [19]. Scheme 2 outlines the synthesis of ethyl (*R,S*)-[1′,2′-$^{13}C_2$,1′-^{14}C]-1-methylpyrrolidine-2-acetate (**15**). [1,2-$^{13}C_2$,2-^{14}C]-Glycine (**17**) on reaction with a mixture of thionyl chloride and ethanol gave ethyl [1,2-$^{13}C_2$,2-^{14}C]glycinate hydrochloride (**18**) [20]. Diazotization with sodium nitrite afforded ethyl [1,2-$^{13}C_2$,2-^{14}C]diazoacetate (**19**) [21]. The reaction of *N*-methylpyrrole with **19** in the presence of cupric acetylacetonate gave ethyl [1,2-$^{13}C_2$,2-^{14}C]-2-(1-methyl-2-pyrryl) acetate (**20**) [22]. Hydrogenation of the pyrrylacetate (**20**) in the presence of Adams catalyst afforded ethyl (*R,S*)-[1′,2′-$^{13}C_2$,1′-^{14}C]-1-methylpyrrolidine-2-acetate (**15**). The free acid (**14**) was obtained from the hydrolysis of the ethyl ester (**15**) with 0.5 M HCl.

Table 1 outlines the results of feeding 1-methylpyrrolidine-2-acetic acid and related compounds to *E. coca* and *D. innoxia*. Feeding the free acid (**13**) to *E. coca* initially gave 0.17% specific incorporation into (−)-cocaine. However, when the alkaloid was hydrolysed to methyl ecgonine to determine the position of the label, the specific incorporation dropped to 0.006%. This indicates that the radioactivity was either mainly from an impurity or present in the phenyl ring, which is incompatible with the labelling pattern of the fed compound. The ethyl ester (**15**) was fed to *E. coca* as well, giving nominal incorporation of less than 0.004%.

[1′,2′-$^{13}C_2$,1′-^{14}C]-1-Methylpyrrolidine-2-acetic acid (**14**) was fed to *D. innoxia* plants growing in a hydroponic solution. The incorporation into **10** and **11** was 0.03 and 0.04%, respectively. When the ethyl ester (**15**) was fed, there was little incorporation into the tropane alkaloids. Also, an unnatural peak was found by GC in the crude alkaloid extract, which corresponded to the unmetabolized labelled compound.

Due to the low incorporations of labelled **12** or its ethyl ester (**15**), it was felt that feeding an analogue of acetyl-CoA could enhance uptake by the plant. One technique which is common in biosynthetic studies is to feed a carboxylic acid precursor as the *N*-acetyl cysteamine (NAC) thioester [23]. This type of thioester is believed to be similar enough to a coenzyme A-bound substrate and, therefore, is incorporated more readily into natural products. The enhanced incorporation of a thioester has been demonstrated in our laboratory in the final step of cocaine biosynthesis [14].

(*R,S*)-[2′-^{14}C]-1-Methylpyrrolidine-2-acetic acid *N*-acetylcysteamine thioester (**16**) was synthesized as shown in Scheme 3. (*S*)-1-Methyl-2-(chloromethyl)pyrrolidine (**21**) was treated with labelled sodium [^{14}C]cyanide mixed with non-radioactive sodium cyanide using

Table 1. Precursors fed to *Erythroxylum coca* and *Datura innoxia*

Precursor (wt, specific acitivity)	Species	Feeding details	Specific incorporation*			
			Cocaine	Cuscohygrine	Hyoscyamine	Scopolamine
[1′,2-$^{13}C_2$,2-^{14}C,^{15}N]-1-Methylpyrrolidine-2-acetic acid (**13**) (33 mg, 1.2×10^8 dpm mmol^{-1})	*E. coca*	Leaf painting 28 days	< 0.006%	not isolated	—	—
Ethyl [1′,2′-$^{13}C_2$,1′-^{14}C]-1-methylpyrrolidine-2-acetate (**15**) (101 mg, 5.2×10^7 dpm mmol^{-1})	*E. coca*	Leaf painting 14 days	< 0.004%	not isolated	—	—
[2′-^{14}C]-1-Methylpyrrolidine-2-acetic acid *N*-acetylcysteamine thioester (**16**) (28 mg, 1.1×10^8 dpm mmol^{-1})	*E. coca*	Leaf painting 28 days	0.012%	0.007%	—	—
[2′-^{14}C]-1-Methylpyrrolidine-2-acetic acid *N*-acetylcysteamine thioester (**16**) (73 mg, 1.0×10^8 dpm mmol^{-1})	*E. coca*	Leaf painting† 14 days	0.011%	0.004%	—	—
[1′,2′-$^{13}C_2$,1′-^{14}C]-1-Methylpyrrolidine-2-acetic acid (**14**) (106 mg, 5.5×10^7 dpm mmol^{-1})	*D. innoxia*	Hydroponic 14 days	—	not isolated	0.03%	0.04%
Ethyl [1′,2′-$^{13}C_2$,1′-^{14}C]-1-methylpyrrolidine-2-acetate (**15**) (102 mg, 6.2×10^7 dpm mmol^{-1})	*D. innoxia*	Hydroponic 14 days	—	not isolated	0.16%‡	0.36%‡

*Specific incorporation: specific activity of isolated alkaloid (dpm mmol^{-1}) divided by specific activity of administered precursor × 100.

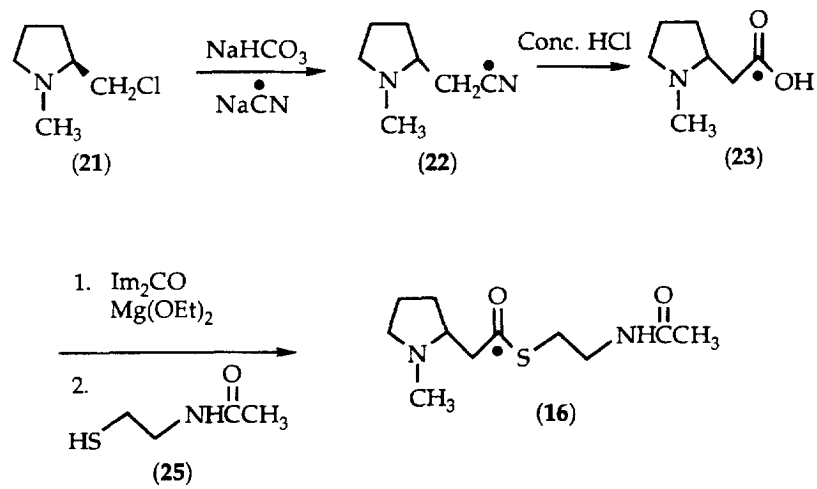
†Aqueous Tween 80 solution painted on leaves each day for duration of feeding.

‡Specific incorporation stated most likely due to impurity since ^{13}C NMR spectra for both alkaloids did not show presence of $^{13}C_2$ labelled compound.

Chavdarian's methodology [24], yielding the nitrile (**22**). [2′-^{14}C]-1-Methyl-2-(cyanomethyl)pyrrolidine (**22**) was hydrolysed with concentrated hydrochloric acid to give **23**. The acid (**23**) was activated with carbonyldiimidazole and treated with freshly prepared *N*-acetylcysteamine (**24**) (synthesized from the diacetyl compound [23]) and a catalytic amount of magnesium ethoxide [25], giving compound **16**.

Compound **16** was fed by the leaf-painting method to *E. coca* for 14 days. However, low specific incorporations were found (0.007% for cuscohygrine and 0.012% for cocaine). The thioester (**16**) was again fed to *E. coca*, using a modification of the feeding method [4]. Following the initial leaf-painting, 2–4% aqueous solution of Tween 80 was painted on to the leaves daily for the duration of the feeding period. This second feeding experiment gave similar results for **9** (0.011% specific incorporation) and even lower specific incorporation into **8** (0.004%).

The results obtained from the experiments described above indicate that 1-methylpyrrolidine-2-acetic acid (**13**



Scheme 3. Synthesis of [2′-^{14}C]-1-methylpyrrolidine-2-acetic acid *N*-acetylcysteamine thioester.

and **14**), the ethyl ester (**15**) and even the NAC thioester (**16**) were not efficient precursors to the tropane alkaloids or cuscohygrine in *E. coca* and *D. innoxia*. In comparison with **2** and **3** in these species, the incorporation of 1-methylpyrrolidine-2-acetic acid and related compounds into **8–11** was disappointingly low.

Currently, the intermediate(s) between **2** and **3** remain(s) unknown. There is indirect evidence [13–15] for the presence of 1-methylpyrrolidine-2-acetic acid as an intermediate in the tropane pathway, however, our feeding studies did not give much experimental support. Clearly, further work into the identification of the intermediate(s) (by isolation from *E. coca* or *Datura*) and the biosynthetic process between metabolites **2** and **3** merits further investigation.

EXPERIMENTAL

General. Mps: corr. Radioactive materials were assayed by liquid scintillation counting using either Ecoscint A (National Diagnostics) or a dioxane–EtOH-based soln (for picrates). NMR spectra were determined at 300 and 75.5 MHz for ^1H and ^{13}C, respectively. All recorded spectra are ppm from TMS. MS were determined by Dr E. Larka at the University of Minnesota. Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. GC was carried out on a 25 m glass capillary coated with a cross-linked Me silicone (0.52 μm thick), i.d. 0.31 mm, NPD and FID detectors, using the following instrument parameters: He flow rate 1 ml min^{-1}, injector temp. 250°, initial oven temp. 50°, equilibration time 4 min, rate of temp. increase 30° min^{-1}.

[1′,2-$^{13}C_2$,2-^{14}C,^{15}N]-1-*Methylpyrrolidine-2-acetic acid* (**13**). [2-^{13}C,2-^{14}C,^{15}N]-1-Methyl-Δ^1-pyrrolinium chloride [17] (0.173 g, 1.43 mmol, 2.2×10^8 dpm mmol^{-1}) was treated with diethyl [2-^{13}C]malonate (0.50 g, 3.10 mmol) in 50% EtOH (3 ml) at 30–40°. After 3 days, 3 M HCl (10 ml) was added and the soln refluxed for 3 hr. After cooling, the reaction mixt. was concd and purified on a Dowex 50 × 8 (H^+ form) column. Elution with H_2O, followed by 3% NH_4OH, afforded **13**. After concn, the product was dried by azeotropic distillation with CH_2Cl_2 and then vacuum dist. at 0.01 mm Hg to give pure **13** (0.132 g, 0.903 mmol, 2.34×10^8 dpm mmol^{-1}, 63% yield). ^{13}C NMR ($CDCl_3$): δ63.52, 63.47, 63.05, 63.00, 34.02, 33.54. $^1J_{C\text{-}C} = 36$ Hz, $^1J_{C\text{-}^{15}N} = 3.7$ Hz.

Ethyl [1,2-$^{13}C_2$,2-14C]*glycinate hydrochloride* (**18**). [1,2-$^{13}C_2$]Glycine (1 g, 13 mmol, Isotec) and [2-^{14}C]-glycine (7.8 mg, 0.5 mCi, from E. Leete) were dissolved in the minimum vol of H_2O–EtOH (1:1) and combined. The solvent was evapd and the resulting solid (**17**) was dried overnight at 100° and 5 mm Hg in an Abderhalden drying pistol containing KOH. EtOH (5 ml) was cooled to −10° under N_2 and then $SOCl_2$ (1 ml, 13.9 mmol) was added dropwise. The soln was stirred for 10 min and then **17** (0.998 g 12.96 mmol) was added and the soln stirred at −10° for 10 min. After warming to room temp., the mixt. was stirred for 5 hr and then refluxed for 20 min. On cooling, crystals (**18**) formed which were vacuum-filtered, washed with dry Et_2O and dried overnight (56° and 5 mm Hg in an Abderhalden drying pistol containing KOH). Compound **18** (1.7517 g, 6.2×10^7 dpm mmol^{-1}) was obtained in 95.5% yield. ^{13}C NMR (CD_3OD): δ168.5 (*d*, C-1, $^1J_{1\text{-}2} = 62$ Hz), 41.0 (*dt*, $^1J_{2\text{-}1} = 62$ Hz, $^1J^{13}C\text{–}^{14}N = 3$ Hz).

Ethyl [1,2-$^{13}C_2$,2-^{14}C] *diazoacetate* (**19**). NaOAc (9 mg, 0.11 mmol) and **18** (1.75 g, 12.4 mmol) were dissolved in de-ionized H_2O (4 ml) and cooled below 20°. $NaNO_2$ (1.31 g, 19 mmol) in de-ionized H_2O (4 ml) was added followed by Et_2O (10 ml). Aq. H_2SO_4 (10%, 0.35 ml) was added and the two-phase system stirred for 15 min. The two layers were sepd and the organic phase shaken with 10% aq. Na_2CO_3 (25 ml) and then dried ($MgSO_4$). The aq. phase was extracted (3 ×) with Et_2O (10 ml) and 10% aq. H_2SO_4 (0.35 ml). The combined dried Et_2O extracts were concd, giving a yellow liquid (**19**) (1.24 g, 86.3% yield, 5.45×10^7 dpm mmol^{-1}). ^{13}C NMR ($CDCl_3$): δ166.8 (*d*, C-1, $^1J_{1\text{-}2} = 96.5$ Hz), 60.5 (OCH_2), 46.1 (*d*, C-2, $^1J_{2\text{-}1} = 96.5$ Hz), 14.4 ($OCH_2\underline{C}H_3$).

Ethyl [1,2-$^{13}C_2$,2-^{14}C]-2-(1-*methyl-2-pyrryl)acetate* (**20**). Into a two-necked flask, equipped with a magnetic stirrer, dropping funnel and reflux condenser, was added *N*-methylpyrrole (3.52 g, 43.5 mmol) and cupric acetylacetonate (85 mg, 0.32 mmol). A mixt. of *N*-methylpyrrole (1.73 g, 21.4 mmol) and **19** (1.2377 g, 10.7 mmol) was placed in the dropping funnel and a few drops were added to the flask. The reaction mixt. was heated to 70° under N_2. After the mixt. turned dark red, the rest of the soln was added dropwise over 10 min and the resulting mixt. heated at 70° for 1 hr. After cooling to room temp., dry Et_2O (20 ml) was added and the soln filtered through a cotton wool plug. The crude product was obtained after concn and purified by silica gel chromatography using a hexane–EtOAc gradient. The product was obtained as a light yellow oil (**20**) (0.640 g, 35.5% yield, 6.36×10^7 dpm mmol^{-1}). ^{13}C NMR ($CDCl_3$); δ170.4 (*d*, C-1, $^1J_{1\text{-}2} = 59$ Hz), 124.8 (*dd*, C-2′, $^1J_{2'\text{-}2} = 52.5$ Hz), 122.3 (*d*, C-5′, $^3J_{5'\text{-}3} = 3.7$ Hz), 108.4 (C-3′), 106.8 (C-4′), 60.8 (OCH_2), 33.9 (NCH_3), 32.5 (*d*, C-2, $^1J_{2\text{-}1} = 59$ Hz), 14.3 (OCH_2CH_3).

Ethyl (*R,S*)-[1′,2′-$^{13}C_2$,1′-^{14}C]-1-*methylpyrrolidine-2-acetate* (**15**). Compound **20** (0.64 g, 3.79 mmol) was dissolved in HOAc (30 ml) and placed in a Parr bottle. Adams catalyst (PtO_2, 60 mg) was added and the mixt. hydrogenated at 40 psi for 4 hr. The catalyst was removed by filtration and the filtrate diluted with de-ionized H_2O (25 ml). After cooling to 0°, the soln was made basic with satd K_2CO_3 and extracted with CH_2Cl_2 (4 × 25 ml). The combined organic extracts were dried (Na_2SO_4) and the solvent evapd. The crude product was purified by silica gel chromatography, eluting with a $CHCl_3$–MeOH–NH_4OH gradient. Compound **15** was obtained as a light brown oil (50.26 mg, 79.6% yield, 5.49×10^7 dpm mmol^{-1}). ^{13}C NMR ($CDCl_3$): δ172.2 (enriched *d*, C-1, $^1J_{1\text{-}2} = 57.3$ Hz), 62.4 (*d* C-2′, $^1J_{2'\text{-}2} = 38.99$ Hz), 60.2 (*d*, OCH_2, $^2J_{C\text{-}O\text{-}C} = 2.4$ Hz), 56.8 (*d*, C-5′, $^3J_{5'\text{-}2} = 3.7$ Hz), 40.3 (NCH_3), 39.3

(enriched *d*, C-2, $^1J_{2\text{-}1} = 57.3$ Hz, $^1J_{2\text{-}2'} = 39$ Hz), 31.1 (C-3'), 21.9 (*d*, C-4', $^3J_{4'\text{-}2} = 3.7$ Hz), 14.2 (OCH_2CH_3). EIMS *m/z* (rel. int.) 173 (0.61), 149 (1.19), 144 (0.74), 111 (0.65), 99 (0.79), 98 (1.42), 97 (2.59), 95 (1.19), 84 (100). Elemental analysis (unlabelled sample): Found: 63.05% C, 9.95% H, 8.3% N. $C_9H_{17}NO_2$ requires 63.13% C, 10.01% H, 8.18% N.

(*R,S*)-[1',2'-$^{13}C_2$,1'-^{14}C]-1-*Methylpyrrolidine*-2-*acetic acid* (**14**). Compound **15** (0.1024 g, 0.59 mmol) in 0.5 M HCl (15 ml) was refluxed under N_2 for 2 hr. The reaction mixt. was concd and then dried by azeotropic distillation with benzene (12 hr) using a Dean–Stark trap. The tan-coloured product (**14**) was directly used as the HCl salt without further purification (5.25×10^7 dpm $mmol^{-1}$).

[2'-^{14}C]-1-*Methylpyrrolidine*-2-*acetic acid N-acetylcysteamine thioester* (**16**). Compound **21** hydrochloride (2.55 g, 15 mmol) [24] was dissolved in 80% aq. EtOH (10 ml) and cooled to 0°. $NaHCO_3$ (1.26 g, 15 mmol) in H_2O (20 ml) was slowly added and the mixt. stirred at 0° for 15 min. Na[^{14}C]CN (1 mCi, 61.2 mCi $mmol^{-1}$, ICN Biomedicals), mixed with non-radioactive NaCN (727 mg, 14.8 mmol), was dissolved in H_2O (1 ml) and added to the reaction mixt. EtOH (8 ml) was added to make an 80% aq. EtOH soln and the mixt. refluxed for 30 min. EtOH was removed under red. pres. and the remaining residue was extracted with $CHCl_3$ (4 × 25 ml). The combined organic extract was dried (Na_2SO_4) and the solvent evapd to give **22** as a brown oil. Conc. HCl (11 ml) was added and the mixt. refluxed for 2 hr. After cooling, the soln was concd to a small vol. The acid (**23**) was purified by ion-exchange chromatography [Dowex 50 × 8 (H^+ form)]. Impurities were removed with H_2O and the product (**23**) eluted with 3% aq. NH_4OH. After concn, **23** was initially dried by adding $CHCl_3$ to the oil and evapn of the solvent under red. pres. Azeotropic distillation with benzene using a Dean–Stark trap gave a tan-coloured hygroscopic solid (1.59 g, 11.1 mmol) in 74% yield starting from **21**. 1,1'-Carbonyldiimidazole (264 mg, 1.63 mmol) and $Mg(OEt)_2$ (9 mg, 0.08 mmol) were added to a stirred soln of a portion of **23** (214 mg, 1.49 mmol) dissolved in DMF (0.5 ml). After 30 min, *N*-acetylcysteamine [23] (216 mg, 1.8 mmol) was added and the soln stirred for 1.5 hr at 25° under N_2. The solvent was removed *in vacuo* (0.2 mm Hg). A few ml of 10% aq. Na_2CO_3 was added and the soln extracted with CH_2Cl_2 (3 × 20 ml). The organic phase was washed with brine, dried (Na_2SO_4) and concd. The crude product (**16**) was purified by flash CC (15 × 1.5 cm) with a $CHCl_3$–Me_2CO gradient to give a yellow solid (**16**) (202 mg, 83 mmol, 56% yield, 1.04×10^8 dpm $mmol^{-1}$). ^{13}C NMR ($CDCl_3$): δ198.3 (COS), 170.2 ($NHCOCH_3$), 62.8 (C-2), 56.6 (C-5), 48.5 (C-1'), 40.4 (NCH_3), 39.5 (CH_2NH), 30.7 (C-3), 28.5 (CH_2S), 23.1 (CH_3CO), 22.0 (C-4). CIMS (isobutane) *m/z* (rel. int.): 245 ($[M + H]^+$ 100%), 237 (25), 144 (13), 120 (14), 84 (50). FTIR (Nujol): N–H st 3290 and 2959 cm^{-1}, thioester C=O 1669 cm^{-1}, amide C=O 1652 cm^{-1}.

Feeding of precursors to E. coca *and isolation of alkaloids.* Feeding expts were carried out on intact *E. coca* Lamark plants growing in a greenhouse. Details are recorded in Table 1. Precursors were dissolved in H_2O + 3% Tween 80. If the precursor was insoluble in H_2O, up to 10% of EtOH was added.

Leaves were painted with the radioactive soln using an artist's paint brush. After 2 weeks, the leaves were picked and alkaloids isolated as previously described [26]. Alkaloids were purified by prep. TLC in $CHCl_3$–EtOH–NH_4OH (12:8:1) as eluent. Compounds were visualized with I_2 in EtOH sprayed over a small portion of the plate. Compound **8** (R_f 0.4) was recovered by placing the silica gel directly into 10% aq. Na_2CO_3. Extraction of the slurry with $CHCl_3$ gave pure **8** (GC R_t 12.1 min). Compound **8** was further purified as the dipicrate salt (mp 218–223°, lit. mp 226–227° [24]) by recrystallization from DMSO–EtOH to constant sp. activity. Cocaine (**9**) was extracted with $CHCl_3$ and MeOH from the band around R_f 0.85. Further purification of **9** was performed by additional prep. TLC (EtOAc–MeOH–NH_4OH–H_2O, 85:13:0.5:1). Cocaine (R_f 0.7) was eluted with $CHCl_3$ after visualization under UV light (254 nm), and was then recrystallized as its free base from EtOH–Et_2O or from CH_2Cl_2–heptane.

Feeding of precursors to D. innoxia *and isolation of alkaloids.* Intact *D. innoxia* plants (3–5 months old) were placed in a hydroponic system containing 2.5 $g\,l^{-1}$ Murashige and Skoog basal salt mixt. (Sigma). Air was continually bubbled through this nutrient soln to keep the roots healthy. The labelled precursor was dissolved in H_2O and equal amounts of the soln added to the hydroponic soln in which the plants were growing. Uptake of radioactivity after 48 hr was 92–95%. After a 2-week period, whole plants were worked-up following a published procedure [27]. After isolation, crude alkaloids were sepd by prep. TLC in $CHCl_3$–EtOH–NH_4OH (100:20:1). R_f values for **10** and **11** were 0.2 and 0.6, respectively. Alkaloids were visualized by UV light (254 nm) and eluted from the adsorbent with MeOH. Compounds **10** and **11** were further purified as their HCl salts by recrystallization from EtOH–Et_2O to constant sp. activity.

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REFERENCES

1. Leete, E. (1990) *Planta Med.* **56**, 339.
2. Evans, W. C., Ghani, A. and Woolley, V. A. (1972) *Phytochemistry* **11**, 2527.
3. Leete, E., Bjorklund, J. A., Couladis, M. M. and Kim, S. H. (1991) *J. Am. Chem. Soc.* **113**, 9286.
4. Leete, E., Bjorklund, J. A. and Kim, S. H. (1988) *Phytochemisty* **27**, 2553.
5. Abraham, T. W. (1992) PhD dissertation. University of Minnesota, Minneapolis.
6. McGaw, B. A. and Woolley, J. G. (1979) *Phytochemisty* **18**, 189.

7. O'Donovan, D. G. and Keogh, M. F. (1969) *J. Chem. Soc.* (C) 223.
8. McGaw, B. A. and Woolley, J. G. (1978) *Phytochemisty* **17**, 257.
9. Y. Yamada *et al.* (1990) in *Secondary Products from Plant Tissue Culture* (Charlwood, B. V. and Rhodes, M. J. C., eds), p. 225. Oxford University Press, New York.
10. Newquist, M. L. and Abraham, T. W. and Leete, E. (1993) *Phytochemistry* **33**, 1437.
11. Leete, E. and Kim, S. H. (1988) *J. Am. Chem. Soc.* **110**, 2976.
12. Hemscheidt, T. and Spenser, I. D. (1990) *J. Am. Chem. Soc*, **112**, 6360.
13. Sauerwein, M., Shimomura, K. and Wink, M. (1993) *Phytochemisty* **32**, 905.
14. Hemscheidt, T. and Spenser, I. D. (1992) *J. Am. Chem. Soc.* **114**, 5472.
15. Leete, E. (1989) *Heterocycles* **28**, 481.
16. Bremner, J. B., Cannon, J. R. and Joshi, K. R. (1973) *Aust. J. Chem.* **26**, 2559.
17. Tomita, H., Mitusaki, S. and Tamaki, E. (1964) *Agric. Biol. Chem.* **28**, 451.
18. Paßreiter, C. M. (1992) *Phytochemistry* **31**, 4135.
19. Leete, E., Kim, S. H. and Rana, J. (1988) *Phytochemistry* **27**, 401.
20. Nganie, A., Derouet, A., Fernandez and Pinazzi, C. P. (1986) *Eur. Polym. J.* **22**, 431.
21. LaForge, F. B., Gersdorff, W. A., Green, N. and Schechter, M. S. (1952) *J. Org. Chem.* **17**, 381.
22. Maryanoff, B. E. (1979) *J. Org. Chem.* **44**, 4410.
23. Schwab, J. M. and Klassen, J. B. (1984) *J. Am. Chem. Soc.* **106**, 7217.
24. Chavdarian, C. G., Sanders, E. B. and Bassfield, R. L. (1982) *J. Org. Chem.* **47**, 1069.
25. Ohta, S. and Okamoto, M. (1981) *Tetrahedron Letters* **22**, 3245.
26. Leete, E. (1982) *J. Am. Chem. Soc.* **104**, 1403.
27. Leete, E. (1972) *Phytochemsity* **11**, 1713.