vs TMS) δ 0.82-0.91 (2 H, m), 1.27 (3 H, t), 1.45 (9 H, s), 1.24-1.60 (4 H, m), 2.40 (2 H, t), 3.74 (2 H, s), 3.81 (3 H, s), 4.20 (2 H, q), 4.22-4.32 (1 H, m), 4.99 (1 H, d, D_2O exch), 6.85 (2 H, d), 7.22 (2 H, d); IR (NaCl, $CDCl_3$) 3370, 1738, 1710 cm^{-1}; mass spectrum (NH_3, CI), *m/e* 412 ($M^+ + 1$, 3.4).

(2*R*)-2-(*tert*-Butyloxycarbonyl)amino-6-[(*p*-methoxybenzyl)thio]hexanoic Acid (18). To a stirred solution of (2*R*)-ethyl 2-(*tert*-butyloxycarbonyl)amino-6-[(*p*-methoxybenzyl)thio]heptanoate (**17**) obtained above (1.54 g, 3.74 mmol, 1 equiv) in EtOH (120 mL) was added lithium hydroxide monohydrate (785 mg, 18.71 mmol, 5 equiv). After 15 h, ethanol was evaporated off and the residue was diluted with water. The aqueous solution was acidified to pH 2 with 1 N HCl and then extracted three times with EtOAc. The combined organic layer was dried over anhydrous magnesium sulfate, filtered, concentrated, and separated by radial chromatography on silica gel (eluted with 3% acetic acid in CH_2Cl_2) to afford 1.31 g (91.3%) of **18** as a light amber oil: ^1H NMR (270 MHz, DMSO-d_6, vs TMS) δ 1.37 (9 H, s), 1.20-1.69 (6 H, m), 2.34 (2 H, t, *J* = 7.03 Hz), 3.65 (2 H, s), 3.73 (3 H, s), 3.74-3.85 (1 H, m), 6.86 (2 H, d, *J* = 8.47 Hz), 6.99 (1 H, d, D_2O exch, *J* = 8.72 Hz), 7.21 (2 H, d, *J* = 8.50 Hz); IR (NaCl, $CDCl_3$) 3335, 1709, 1654 (sh) cm^{-1}; $[\alpha]^{25}_D$ -0.7° (*c* 1.2, CH_2Cl_2); exact mass calcd for $C_{19}H_{30}NO_5S$ 384.1845, found 384.1841.

(3*R*,5*R*,6*S*)-4-(*tert*-Butyloxycarbonyl)-5,6-diphenyl-3-(3′-chloropropyl)-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (3b, $R_1 = (CH_2)_3Cl$). To a stirred solution of **1b** (707 mg, 2 mmol, 1 equiv) and 1-iodo-3-chloropropane (107 μL, 10 mmol, 5 eq) in THF (20 mL) and HMPA (2 mL) was added lithium bis(trimethylsilyl)amide (3 mL, 3 mmol, 1.5 equiv, 1.0 M solution in THF) dropwise via syringe at -78 °C. After 30 min, the reaction mixture was poured into ethyl acetate. The organic layer was washed with saturated aqueous NH_4Cl (20 mL), water, and brine, dried over anhydrous magnesium sulfate, filtered, concentrated, and triturated with *n*-hexanes to give the chloride (620 mg, 72%), which could be recrystallized from ethanol (mp 168 °C): ^1H NMR (270 MHz, $CDCl_3$) δ 1.10 (6 H, s), 1.26 (3 H, s), 1.47-2.54 (4 H, m), 3.66 (2 H, m), 4.81 ($^1/_2$ H, m), 5.02 (1 H, d, *J* = 3 Hz), 5.24 ($^1/_2$ H, d, *J* = 2 Hz), 5.95 (1 H, d, *J* = 3 Hz), 6.53-6.67 (2 H, m), 6.94-7.40 (8 H, m); IR (NaCl, Nujol) 1747, 1702, 1315, 1257, 1169, 1053, 886, 703 cm^{-1}. Anal. Calcd for $C_{24}H_{28}ClNO_4$: C, 67.05; H, 6.56; N, 3.26. Found: C, 66.91; H, 6.65; N, 3.11.

(3*R*,5*R*,6*S*)-4-(*tert*-Butyloxycarbonyl)-5,6-diphenyl-3-(3′-iodopropyl)-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (3b, $R_1 = (CH_2)_3I$). To a stirred solution of **1b** (3.0 g, 8.5 mmol, 1 equiv) and 1,3-diiodopropane (5.6 mL, 42.4 mmol, 5 equiv) in THF (85 mL) and HMPA (8.5 mL) was added lithium bis(trimethylsilyl)amide (12.7 mL, 12.7 mmol, 1.5 equiv, 1.0 M solution in THF) dropwise via syringe at -78 °C. After 30 min, the reaction mixture was allowed to come to room temperature and then poured into ethyl acetate. The organic layer was washed with saturated aqueous NH_4Cl (20 mL), saturated aqueous $Na_2S_2O_3$, water, and brine, dried over anhydrous magnesium sulfate, filtered, concentrated, and triturated with *n*-hexanes to give the iodide (3.5 g, 86%), which could be recrystallized from ethanol (mp 172-173 °C): ^1H NMR (270 MHz, $CDCl_3$) δ 1.07 (6 H, s), 1.45 (3 H, s), 1.66-1.81 (2 H, m), 1.85-2.10 (2 H, m), 2.10-2.26 (1 H, m), 3.24 (2 H, t, *J* = 7 Hz), 4.82 ($^1/_2$ H, m), 5.01 (1 H, m), 5.21 ($^1/_2$ H, m), 5.92 (1 H, br s), 6.52-6.63 (2 H, m), 6.93-7.40 (8 H, m); IR (NaCl, Nujol) 1747, 1702, 1315, 1257, 1169, 1053, 886, 703 cm^{-1}. Anal. Calcd for $C_{24}H_{28}INO_4$: C, 55.29; H, 5.41; N, 2.69. Found: C, 55.33; H, 5.50; N, 2.53.

(3*R*,5*R*,6*S*)-4-Aza-5,6-diphenyl-2-oxo-1-oxabicyclo[4.3.0]nonane. To a solution of (3*R*,5*R*,6*S*)-4-(*tert*-butyloxycarbonyl)-5,6-diphenyl-3-(3′-chloropropyl)-2,3,5,6-tetrahydro-4*H*-1,4-oxazin-2-one (**3b**, $R_1 = (CH_2)_3Cl$) (36 mg, 0.08 mmol, 1 equiv) in CH_2Cl_2 (0.3 mL) was added trifluoroacetic acid (0.1 mL) at 0 °C. The mixture was stirred for 20 min at 0 °C and for 1 h at room temperature. The solvent was removed under reduced pressure, and the residue was partitioned between ethyl acetate and saturated aqueous $NaHCO_3$. The organic layer was separated, washed with H_2O, dried over anhydrous $MgSO_4$, and purified by silica gel TLC (eluted with EtOAc/hexanes, 2:5) to give the bicyclic product (22 mg, 80%) as an oil: ^1H NMR (270 MHz, $CDCl_3$) δ 1.70-1.94 (2 H, m), 2.10-2.26 (1 H, m), 2.32-2.46 (1 H, m), 2.50-2.62 (1 H, m), 3.08-3.18 (1 H, m), 4.15 (1 H, dd, *J* = 7.8 Hz and 9.4 Hz), 4.27 (1 H, d, *J* = 3.8 Hz), 5.59 (1 H, d, *J* = 3.8 Hz), 6.93-7.02 (2 H, m), 7.10-7.40 (8 H, m); IR (NaCl, neat) 1734, 1484, 1443, 1368, 1335, 1229, 1191, 1144, 1038 cm^{-1}; $[\alpha]^{25}_D$ -130.4° (*c* 2.6, CH_2Cl_2).

Acknowledgment. We are indebted to the National Science Foundation (CHE 8717017), the National Institutes of Health (GM 40988), and Hoffman-La Roche, Inc. for providing financial support. High-resolution mass spectra were obtained at the Midwest Center for Mass Spectrometry, Lincoln, NE (an NSF-funded Regional Instrumentation Facility). We are grateful to Professor Dave Evans and Dr. Joseph Dellaria for communicating their enolate alkylation results to us on a related system prior to publication.

Late Intermediates in the Biosynthesis of Cocaine: 4-(1-Methyl-2-pyrrolidinyl)-3-oxobutanoate and Methyl Ecgonine

Edward Leete,* Jeffrey A. Bjorklund, Maria M. Couladis, and Sung Hoon Kim

Contribution No. 223 from the Natural Products Laboratory, Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455. *Received May 24, 1991*

Abstract: Methyl (*RS*)-[1,2-$^{13}C_2$,1-^{14}C]-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate was synthesized from a mixture of sodium [1,2-$^{13}C_2$]- and [1-^{14}C]acetate. This β-keto ester was administered to intact *Erythroxylum coca* plants, resulting in the formation of labeled cocaine and methyl ecgonine. The presence of contiguous ^{13}C atoms in these alkaloids at C-2 and C-9 was established by ^{13}C NMR spectroscopy, and the presence of ^{14}C at C-9 was established by a chemical degradation. These results are consistent with our new hypothesis for the biosynthesis of cocaine, which involves the intermediacy of 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate (rather than 2-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate) in the formation of the tropane moiety of cocaine. Support for this biogenetic scheme was also obtained by a biomimetic synthesis of 2-carbomethoxy-3-tropinone by the oxidation of methyl 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate with mercuric acetate. The formation of labeled cocaine and methyl ecgonine in leaf cuttings of *Erythroxylum coca* was observed after incubation with [9-^{14}C]-2-carbomethoxy-3-tropinone. The degree of incorporation of this precursor into cocaine was significantly increased by the concomitant administration of the *N*-acetylcysteamine thioester of benzoic acid, with a corresponding reduction in the degree of incorporation into methyl ecgonine.

Our current hypothesis for the biosynthesis of cocaine is illustrated in Scheme I. This pathway has been recently reviewed[1] and is based entirely on feeding experiments with putative labeled precursors, which were administered (by leaf painting) to intact plants of *Erythroxylum coca*. No work has been published on the enzymology of any of these hypothetical steps in the coca plant. In this scheme, the compounds which have so far been established as precursors of cocaine are enclosed in boxes. DL-[5-^{14}C]Ornithine (**1**) afforded labeled cocaine (**17**) in which the ^{14}C was equally distributed between its C-1 and C-5 positions.[2] This result led

(1) Leete, E. *Planta Med.* **1990**, *56*, 339-352.

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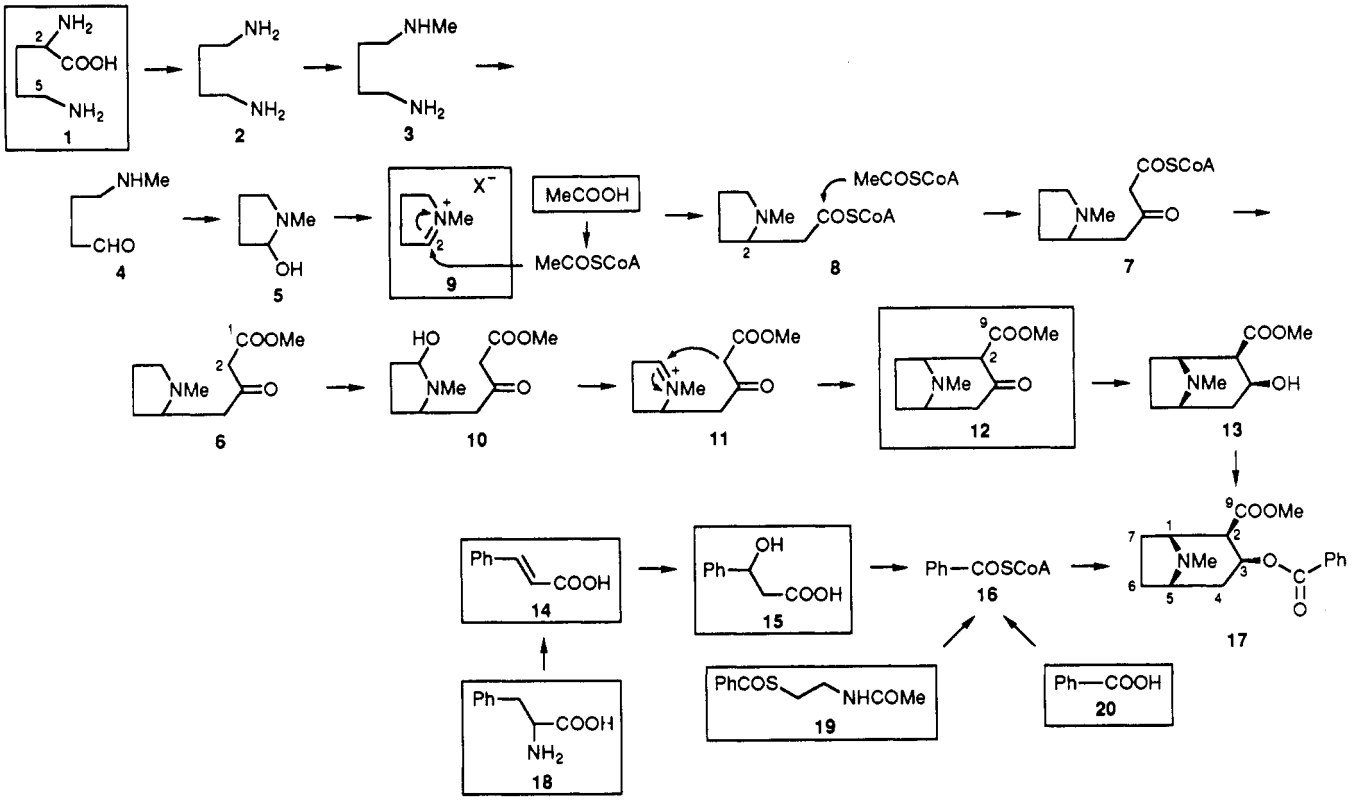
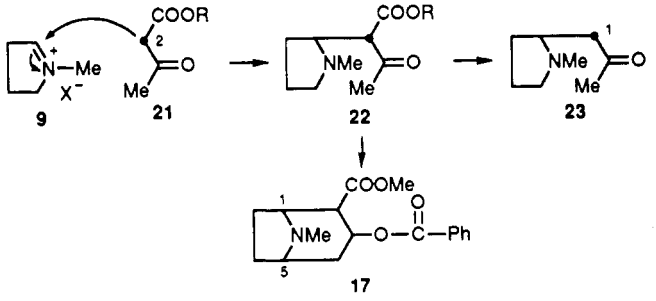
Scheme I. Hypothetical Biosynthesis of Cocaine



Scheme II. Old Hypothesis for the Biosynthesis of Cocaine, Involving the Condensation of the 1-Methyl-Δ^1-pyrrolinium Salt with C-2 of Acetoacetate and the Biomimetic Synthesis of Hygrine (**23**) by this Route

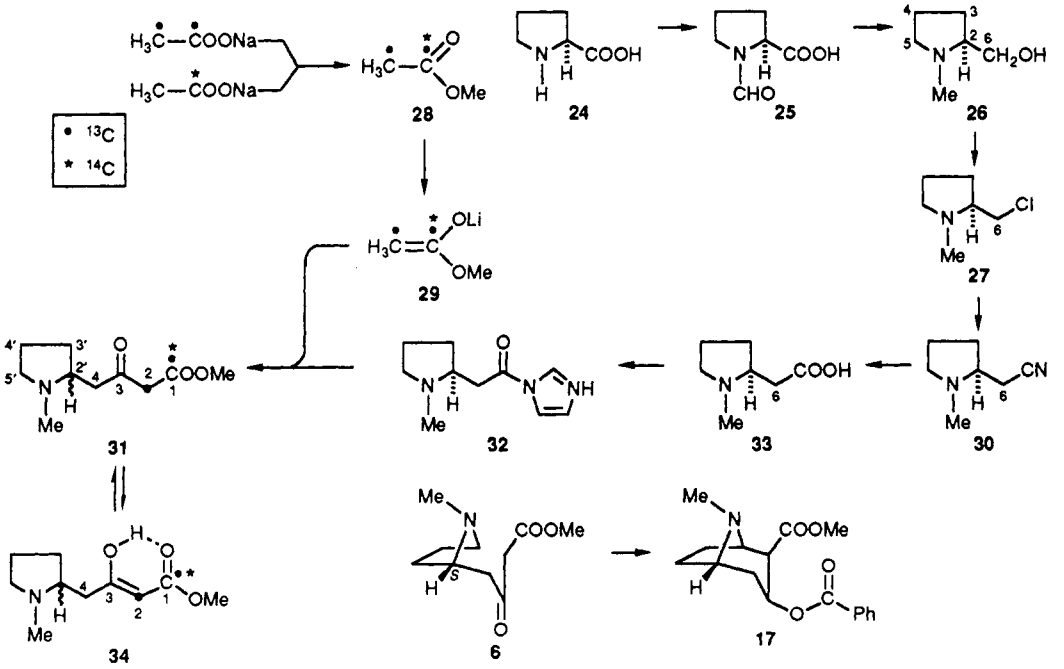


us to propose the intermediacy of putrescine (**2**), formed by the decarboxylation of ornithine. It is then proposed that the putrescine is methylated to yield *N*-methylputrescine (**3**), which is then oxidized to 4-(methylamino)butanal (**4**). This amino aldehyde cyclizes to yield 2-hydroxyl-1-methylpyrrolidine (**5**). In acid media this carbinolamine exists as the iminium salt, 1-methyl-Δ^1-pyrrolinium salt (**9**). This compound, labeled with ^{15}N and at C-2 with ^{13}C and ^{14}C, was shown to be a precursor of cocaine, which was labeled with ^{15}N and with ^{13}C and ^{14}C at its C-5 position.[3] This result was unexpected since the generally accepted route to cocaine involved a condensation between the iminium salt **9** and acetoacetate **21** as illustrated in Scheme II. Such a condensation would have ultimately yielded cocaine labeled with ^{13}C and ^{14}C at C-1 not C-5. Indeed, the chemical condensation of the iminium salt **9** with ethyl [2-^{13}C]acetoacetate (**21**, R = Et) yielded hygrine (**23**), which was enriched with ^{13}C only at its C-1′ position. This result was established by ^{13}C NMR spectroscopy. The intermediate β-keto ester **22** undergoes hydrolysis and decarboxylation under the workup conditions.

The unexpected mode of incorporation of the iminium salt **9** into cocaine caused us to propose a new hypothesis for the formation of the bicyclic tropane moiety of cocaine. It was proposed[3,4] that the iminium salt reacts with acetyl coenzyme A (or perhaps malonyl coenzyme A with accompanying decarboxylation) to yield the coenzyme A ester of 1-methylpyrrolidine-2-acetic acid (**8**). Circumstantial evidence favoring the intermediacy of this compound was the isolation of radioactive 1-methylpyrrolidine-2-acetic acid from coca plants which had been fed [2-^{14}C]-**9**. Degradation established that the 1-methylpyrrolidine-2-acetic acid had all of its radioactivity located at its C-2 position.[4] Reaction of the ester **8** with a second molecule of acetyl coenzyme A then affords the coenzyme A thioester of 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoic acid (**7**). The administration of sodium [1-^{14}C]acetate to coca yielded labeled cocaine, which had a preponderance of its activity located at its C-3 (48%) and C-9 (38%) positions.[5] The thioester function of **7** eventually becomes the carbomethoxy group of cocaine; hence in Scheme I, an ester interchange with methanol is proposed to yield the corresponding methyl ester (**6**). This ester interchange could well occur at a later step in the biosynthetic process, and its depiction at this point is purely arbitrary. The pyrrolidine ring of **6** is then oxidized to yield the carbinolamine **10**, which will afford the iminium salt **11** by elimination of hydroxide. A Mannich reaction then yields 2-carbomethoxy-3-tropinone (**12**). This compound labeled with ^{13}C and ^{14}C at C-9 and with tritium on its *O*-methyl group was incorporated into cocaine with complete retention of tritium relative to ^{14}C.[6] A stereospecific reduction of this β-keto ester then affords methyl ecgonine (**13**), which is a fairly abundant alkaloid in *Erythroxylum coca*. The alkaloids, isolated from fresh leaves of coca plants grown in a greenhouse, had the following average composition: cocaine (47%), cuscohygrine (24%), methyl ecgonine (17%), *trans*-cinnamoylcocaine (6.7%), and *cis*-cinnamoylcocaine (4.1%) (determined by capillary GC). Cocaine is then presumably obtained by reaction of methyl ecgonine with benzoyl coenzyme A (**16**).

(2) Leete, E. *J. Am. Chem. Soc.* **1982**, *104*, 1403–1408.
(3) Leete, E.; Kim, S. H. *J. Am. Chem. Soc.* **1988**, *110*, 2976–2978. The actual compound fed to *E. coca* was the diethyl acetal of [2-^{13}C,^{14}C,^{15}N]-4-(methylamino)butanal. However, it is considered that this acetal undergoes hydrolysis to the free aldehyde under the acidic condition (pH 4.5) prevailing in the coca leaves.
(4) Leete, E. *Heterocycles* **1989**, *28*, 481–487.
(5) Leete, E. *Phytochemistry* **1983**, *22*, 699–704.
(6) Leete, E. *J. Am. Chem. Soc.* **1983**, *105*, 6727.

Scheme III. Synthesis of Methyl (*RS*)-4-(1-Methyl-2-pyrrolidinyl)-3-oxobutanoate



Thus, the administration of labeled benzoic acid (**20**)[7] or its *N*-acetylcysteamine thioester (**19**)[7] afforded cocaine labeled in its benzoyl moiety. More remote precursors of the benzoyl moiety are phenylalanine (**18**),[5,8] cinnamic acid (**14**),[9] and 3-hydroxy-3-phenylpropanoic acid (**15**).[9]

We have now examined the β-keto ester **6** as a precursor of methyl ecgonine and cocaine. It was synthesized by the route illustrated in Scheme III. Since it was predicted that the *S* isomer of the β-keto ester **6** would be the stereoisomer incorporated into cocaine (**17**), as illustrated in the bottom part of Scheme III, the synthesis was started with L-proline (**24**), which would be expected to yield this desired isomer. Unfortunately, racemization occurred during the final step in this synthesis, and it was the *RS* compound which was ultimately fed to the coca plants.

L-Proline was converted to its *N*-formyl derivative (**25**) by treatment with formic acid in the presence of acetic anhydride. Reduction with lithium aluminum hydride yielded (*S*)-2-(hydroxymethyl)-1-methylpyrrolidine (**26**).[11] The hydrochloride salt of 2-(chloromethyl)-1-methylpyrrolidine (**27**) was obtained on treatment of this alcohol with thionyl chloride in chloroform. The nitrile **30** was obtained on treatment with sodium cyanide in 80% ethanol. This nitrile also readily undergoes racemization, especially during purification on silica gel. Hydrolysis by refluxing with concentrated HCl afforded 1-methylpyrrolidine-2-acetic acid (**33**). A mixture of sodium [1,2-$^{13}C_2$]acetate and sodium [1-^{14}C]acetate was refluxed with trimethyl phosphate to afford methyl [1,2-$^{13}C_2$,1-^{14}C]acetate (**28**), which was converted to its lithium enolate (**29**), which was added to the imidazole derivative of 1-methylpyrrolidine-2-acetic acid (**32**). The product, methyl (*RS*)-[1,2-$^{13}C_2$,1-^{14}C]-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate (**31**), exhibited the expected doublets at C-1 and C-2 in its ^{13}C NMR spectrum, with a coupling constant of 58.7 Hz. ^{13}C NMR chemical shifts of the pyrrolidine carbons were assigned by comparison with those of hygrine. In a $CDCl_3$ solution, the β-keto ester **31** existed to the extent of about 5% in the enol form **34**. In this tautomer the coupling between C-1 and C-2 was 74.4 Hz. A similar proportion of its enol form was observable in the ^{13}C NMR spectrum of ethyl acetoacetate.[12]

The β-keto ester **31** dissolved in water, along with a little Tween 80, was painted on the leaves of *Erythroxylum coca* plants which were growing in a greenhouse. The feeding was spread over several

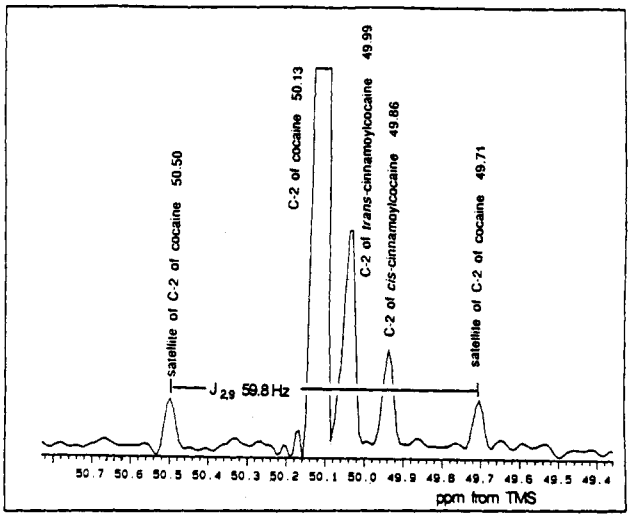
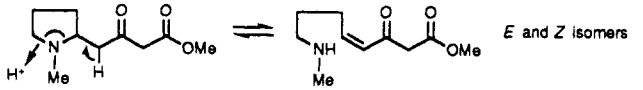


Figure 1. Cocaine (26 mg) in $CDCl_3$ (0.4 mL) run with the following instrument (Varian VXR 300) parameters: frequency, 75.4 MHz; spectral width, 20,000 Hz; acquisition time, 0.4 s; relaxation delay, 1.0 s; pulse width, 3.5 μs, no. of acquisitions, 45,000; total acquisition time, 17.5 h. The satellites are not completely symmetrical: the central signal of C-2 of cocaine is presumably unsymmetrically arranged between the satellites due to an isotope shift (1.1 Hz or 0.014 ppm). Specific incorporation (deduced from area of satellite peaks/area of C-2 peak − 0.01) = 0.08%.

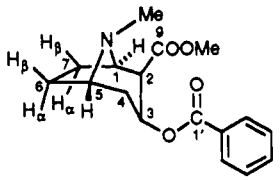
(7) Leete, E.; Bjorklund, J. A.; Kim, S. H. *Phytochemistry* **1988**, *27*, 2553–2556.
(8) Gross, D.; Schütte, H. R. *Arch. Pharm. (Weinheim, Ger.)* **1963**, *296*, 1–15.
(9) Leete, E.; Bjorklund, J. A. Unpublished work.
(10) The racemization is considered to occur by the following mechanism, involving opening of the pyrrolidine ring.



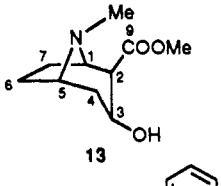
(11) Chavdarian, C. G.; Sanders, E. B. *Org. Prep. Proc. Int.* **1981**, *13*, 389–393. (*S*)-2-(Hydroxymethyl)-1-methylpyrrolidine is now commercially available from Aldrich Chemical Co., although it is somewhat expensive.

(12) Johnson, L. F.; Jankowski, W. C. *Carbon-13 NMR Spectra*; John Wiley and Sons: New York, 1972; Spectrum no. 181. The ^{13}C signals (in $CDCl_3$) of ethyl acetoacetate are at the following positions: 200.6 (C-3), 167.2 (C-1), 50.0 (C-2), and 29.9 (C-4) ppm from TMS. In the enol form, C-2 is at 90.0 ppm, essentially the same position as C-2 of the enol form of the β-keto ester **31**.

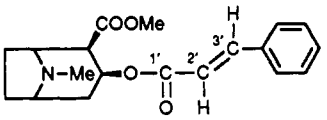
Table I. ^{13}C NMR Chemical Shifts (Relative to Tetramethylsilane) of the *Erythroxylum coca* Alkaloids, Dissolved in $CDCl_3$



17



13



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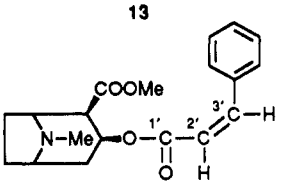
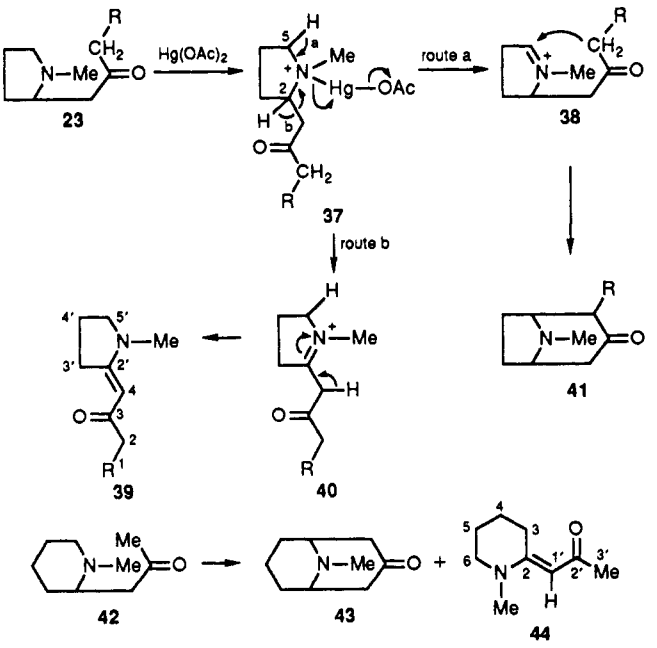


36

carbon no.	cocaine (17)	methyl ecgonine (13)	*trans*-cinnamoyl-cocaine (35)	*cis*-cinnamoyl-cocaine (36)
1	64.78	64.18	64.70	64.68
2	50.13	51.59	49.99	49.86
3	66.84	64.12	66.50	66.40
4	35.47	40.15	35.39	35.21
5	61.50	61.45	61.46	61.40
6	25.19	25.48	25.10	25.00
7	25.36	24.74	25.31	25.19
9	170.66	174.11	170.69	170.61
NMe	41.10	41.04	41.05	41.01
OMe	51.35	51.56	51.33	51.17
1′(CO)	166.08		166.58	165.49
2′			118.13	119.66
3′			144.81	143.58
		Aromatic Carbons		
ipso	130.20		134.21	134.62
ortho	129.62		128.01	127.77
meta	128.22		128.66	128.82
para	132.84		130.15	129.89

days, and the leaves were removed after 2 weeks. Two separate feedings were carried out, in December and March. Since it had been previously established that the level of incorporation of radioactivity into cocaine from [9-^{14}C]-2-carbomethoxy-3-tropinone was increased by feeding the *N*-acetylcysteamine thioester of benzoic acid, an equivalent amount of this compound was also administered in the March feeding. The cocaine and methyl ecgonine obtained from the March feeding had a higher specific activity than the alkaloids isolated from the December feeding. Even though the specific incorporation (based on the incorporation of ^{14}C) into cocaine was low (0.1%), satellites were readily observable at the signal for C-2 of cocaine (see Figure 1), with a coupling constant of 59.8 Hz. The two peaks upfield of the C-2 signal for cocaine are due to the presence of small amounts of *trans*-cinnamoylcocaine (**35**) and *cis*-cinnamoylcocaine (**36**), which could not be completely removed by crystallization from the small amount of cocaine (30 mg) available. Since C-9 of cocaine is a carbonyl group, its signal in the ^{13}C NMR spectrum is of much lower intensity, and no satellites could be detected at the C-9 signal. No satellites were detected at the signal assigned to C-4 (35.47 ppm) either. This is an important observation since it obviates the possibility that the β-keto ester **31** had undergone a retroactive Claisen condensation to yield [1,2-$^{13}C_2$]acetate, which would have afforded cocaine labeled with contiguous ^{13}C atoms at C-4–C-3 and C-2–C-9. This possibility was also eliminated by a chemical degradation. Essentially all of the ^{14}C was located on the carboxyl group of cycloheptanecarboxylic acid, which was obtained, as previously described,[2] from methyl ecgonine. The ^{13}C NMR spectrum of the methyl ecgonine obtained from these feeding experiments also exhibited satellites at its C-2 signal, ($^1J_{29}$ = 57.3 Hz). This result is not completely unequivocal since the chemical shift of the C-2 of methyl ecgonine is almost identical with that of its *O*-methyl group. However, there is no reason for

Scheme IV. Biomimetic Synthesis



the *O*-methyl group to exhibit satellites with a coupling constant of this magnitude.

The ^{13}C NMR spectrum of cocaine has been previously reported,[13] and our data, recorded in Table I, are in agreement with this earlier work. In addition, we have been able to unequivocally assign the signals for C-6 and C-7, which was not previously done. It was possible to make a definitive assignment of the hydrogens in the ^1H NMR spectrum of cocaine by carrying out a two-dimensional homonuclear correlation (COSY) spectrum. The multiplet at 2.18 ppm arises from 6-Hα and 7-Hα. However, only the downfield half of this multiplet couples with the C-1 hydrogen (3.55 ppm), and thus this portion is assigned to 7-Hα. The upfield portion of the signal at 2.18 ppm couples with the C-5 hydrogen (3.00 ppm). With this information in hand, it was then possible to carry out a two-dimensional heteronuclear correlation spectrum of ^1H–^{13}C (HETCOR) in which it was shown that the ^{13}C signal at 25.19 coupled with the 6-Hα and was thus assigned to C-6 and the ^{13}C signal at 25.36 coupled with the 7-Hα was assigned to C-7. Similar correlations were carried out on methyl ecgonine, *cis*-cinnamoylcocaine, and *trans*-cinnamoylcocaine, and their ^{13}C NMR spectra are recorded in Table I.

We have recently[14] achieved the oxidation of hygrine (**23**, R = H) to tropinone (**41**, R = H) with mercuric acetate. The proposed intermediate in this cyclization is the quaternary ammonium compound (**37**, R = H). Proton loss from C-5 (route a) and elimination of mercury and acetate affords the iminium salt (**38**, R = H), which yields tropinone by a Mannich reaction (Scheme IV). Using the methyl 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate (**23**, R = COOMe in Scheme IV) as a substrate in this reaction gave a small yield of 2-carbomethoxy-3-tropinone (**41**, R = COOMe). The low yield is partially attributed to the instability of this compound in the reaction conditions. Since the yield of 2-carbomethoxy-3-tropinone was so low, the reaction was repeated with methyl [1-^{14}C]-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate. At the end of the reaction, nonradioactive 2-carbomethoxy-3-tropinone was added as a carrier. Reisolation of this compound and purification by sublimation and crystallization yielded material which had a constant specific activity that was equivalent to a 1.2% yield of **41** (R = COOMe).

In the oxidation of hygrine to tropinone, the major product formed was (*E*)-1′,2-dehydrohygrine (**39**, R = H) with a different

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Table II. Incorporation of [9-^{14}C]-2-Carbomethoxy-3-tropinone into Cocaine and Methyl Ecgonine in Isolated Leaves of *Erythroxylum coca*

experiment no.	experimental conditions	time of incubation (h)	absolute incorporation (%) cocaine	methyl ecgonine
1	new leaves	24	0.2	1.4
2	middle-aged leaves	24	0.04	0.1
3	old leaves	24	0.02	0.05
4	new leaves	6	0.23	1.38
5	new leaves	24	0.26	5.12
6	new leaves	70	0.27	13.6
7	new leaves + the *N*-acetylcysteamine thioester of benzoic acid	24	2.1	0.42
8	new leaves + the *N*-acetylcysteamine thioester of benzoic acid	70	9.7	0.32

numbering system, which is considered to arise by loss of a proton from C-2 of **37** (route b) to yield **40**, followed by a proton shift as illustrated. Similarly, the major product produced from the β-keto ester (**23**, R = COOMe) was the dehydro derivative (**39**, R = COOMe), the structure being established spectroscopically. During the reaction there was apparently considerable conversion of the β-keto ester (**23**, R = COOMe) to hygrine, which was oxidized to 1′,2-dehydrohygrine and tropinone.

A similar oxidation of *N*-methylpelletierine (**42**) afforded ψ-pelletierine (**43**) and (*E*)-1′,2-dehydro-*N*-methylpelletierine (**44**). The structure of **44** was established spectroscopically and by an independent synthesis from 1-methylpiperidine-2-thione and bromoacetone by Eschenmoser's synthesis of vinylogous amides.[15] This biomimetic conversion is in accord with the established biochemical conversion of *N*-methylpelletierine to ψ-pelletierine in *Punica granatum*.[16]

In all of our successful feeding experiments a solution of the labeled putative precursor in water or aqueous ethanol was painted on the leaves of the coca plant. It has thus been our general feeling that the leaves of the plant are the main site of cocaine biosynthesis, at least for the later steps in the biosynthetic pathway. This has now been confirmed by carrying out feeding experiments involving leaves which were cut into small pieces and suspended in a solution of [9-^{14}C]-2-carbomethoxy-3-tropinone (**12**)[17] dissolved in a phosphate buffer (pH 7) along with sucrose. The activity found in cocaine and methyl ecgonine is recorded in Table II. It should be noted that there was a significantly greater degree of incorporation into these alkaloids in the young new leaves compared with the older leaves. In the conditions prevailing in the greenhouse, where the plants are grown, the "old leaves" were 4–6 months old. The "new leaves" were a bright light-green color, and appeared 3–4 weeks after the plant had been completely defoliated by hand picking. On one occasion an inadvertent loss of heat in our greenhouse during a Minnesota winter resulted in the plants being exposed to a temperature of 30 °F. The plants did not die, but all of the leaves fell off. The subsequent regrowth of the plants was excellent with luxuriant new leaves. The greenhouse was normally maintained at 78 °F with diurnal variation of temperature of ±10 °F. Since a higher level of incorporation was obtained with the new leaves, these were used in all subsequent experiments. The second set of experiments, 4, 5, and 6 where the cut leaves were incubated for varying lengths of time, showed a fairly constant level of incorporation into cocaine, but the incorporation into methyl ecgonine increased with time. This suggested to us that perhaps the amount of benzoyl coenzyme A in the leaf was limiting the production of cocaine. Thus, in the final two experiments (7 and 8) the leaves were fed the *N*-acetylcysteamine thioester of benzoic acid along with the labeled 2-carbomethoxy-3-tropinone. We did, indeed, find a dramatic increase in the incorporation of activity into cocaine (9.7%). We have previously shown that this thioester of benzoic acid is an excellent precursor of the benzoyl moiety of cocaine.[7]

Conclusions

We have presented strong evidence that the terminal steps in the biosynthesis of cocaine occur in the leaves of *Erythroxylum coca*. Our new results also favor the formation of the bicyclic tropane moiety of cocaine from 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate. Our experiments were carried out with the methyl ester of this β-keto acid. However, the cyclization of the 3-oxobutanoate could well involve the corresponding thioester with coenzyme A. Our positive result could have involved an ester interchange with coenzyme A prior to cyclization. We will attempt to answer this question by carrying out feeding experiments with the multiply labeled ester containing isotopes in the *O*-methyl group and the rest of the molecule. The time is now ripe for a study of the biosynthesis of cocaine at the enzyme level.

Experimental Section

General Methods. Melting points are corrected. Radioactive materials were assayed by liquid scintillation counting using dioxane–EtOH as a solvent with the usual scintillators.[18] GC was carried out in a Hewlett-Packard gas chromatograph Model 5890A on a 25-m glass capillary column (0.31-mm i.d.) coated with cross-linked dimethyl silicone (0.52 μm thick) using the following instrument parameters: He 1 mL/min, injection temperature 250 °C (an injection temperature of 150 °C was used for some compounds, such as 2-carbomethoxy-3-tropinone which is partially decomposed to tropinone when an injection of 250 °C is used), initial oven temperature 50 °C, equilibration time 4 min, rate of temperature increase 30 °C/min, final temperature 250 °C. The final run time was held at 250 °C for 19.34 min, making a total run time of 30 min. GC retention times are recorded as GC rt in minutes. Mass spectra were determined by Dr. Edmund Larka at the University of Minnesota on an AEI MS-30 mass spectrometer (EI) or a Finnegan 4000 mass spectrometer (CI) with methane as the carrier gas. NMR spectra were determined on Brüker/IBM NR 300 or Varian VXR 300 or 500 spectrometers. ^{13}C NMR spectra were determined at 75.4 MHz and ^{1}H NMR spectra at 300 or 500 MHz. All spectra were recorded as ppm from tetramethylsilane. Infrared spectra were determined on a Beckman Acculab I. Ultraviolet spectra were determined on a Cary 17D spectrophotometer. Optical rotations were determined at ambient temperature (22 ± 2 °C) on a Perkin-Elmer Model 241 polarimeter. Elemental analyses were carried out by MHW Laboratories, Phoenix, AZ. Preparative TLC was carried out on Merck silica gel PF_{254}. An analytical high-performance liquid chromatography (HPLC) was performed on a Gilson dual pump gradient chromatograph, connected to a Model 811 Dynamic mixer, using an Alltech Associates 10 × 250 mm RSIL Silica 10 μm column. Peaks were detected with a Gilson Model 116 variable wavelength UV detector set at 254 nm.

Ethyl [2-^{13}-C]Acetoacetate (21) and [1′-^{13}C]Hygrine (23). Ethyl [2-^{13}C]acetoacetate was synthesized by a modification of the synthesis of diethyl [2-^{13}C]malonate.[19] *n*-Butyllithium (9.2 mL of a 2.5 M solution in hexane, 23 mmol) was added slowly to a magnetically stirred solution of hexamethyldisilazane (4.52 g, 30.9 mmol) in dry THF (20 mL) under a N_2 atmosphere maintained below −65 °C. The reaction mixture was allowed to warm to 20 °C over 15 min and then cooled to −74°C. Ethyl [2-^{13}C]acetate (1.0 g 11.2 mmol, 99% ^{13}C, Aldrich Chemical Co.) dissolved in THF (1 mL) was added to the reaction mixture during 5 min. After the solution was stirred for 20 min, freshly distilled acetyl chloride (0.84 mL, 11.2 mmol) was slowly added over 5 min, keeping the reaction mixture below −55 °C. After 2 h the reaction mixture was worked up as previously described,[19] affording ethyl [2-^{13}C]acetoacetate which was purified by distillation (bp 70–80 °C, 18 mm) (1.14 g, 8.7 mmol, 78% yield). The highly enriched carbon at C-2 had δ_c at 50.13 ppm (in $CDCl_3$). Its ^{1}H NMR had a $^{1}J_{^{13}C-^{1}H}$ of 130.2 Hz for the H at C-2. Ethyl [2-^{13}C]acetoacetate (0.615 g, 5.0 mmol) was added to a solution of 1-methyl-Δ^1-pyrrolinium chloride[20] (0.52 g, 5.0 mmol) in 50% aqueous EtOH (2.5 mL) and the mixture stirred at room temperature for 5 days. Concentrated HCl (0.5 mL) was then added and the mixture refluxed for 5 min. The cooled solution was made basic with Na_2CO_3 and extracted with Et_2O (20 × 2 mL). The dried (Na_2SO_4) extract was

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evaporated to afford [1′-^{13}C]hygrine (0.4 g) which was purified by chromatography on silica gel, eluting with CH_2Cl_2 containing 10% MeOH and 1% concentrated NH_4OH: ^{13}C NMR ($CDCl_3$) δ 207.9 (C-2′), 61.7 (C-2, d, $^1J_{1',2}$ = 38.2 Hz), 56.7 (C-5), 48.2 (C-1′, highly enriched, which showed natural abundance satellites, $^1J_{1',2}$ = 37.4 Hz), 40.4 (NMe), 31.2 (C-3′), 30.8 (C-3, d, $^2J_{1'-3'}$ = 1.9 Hz), 22.0 (C-4). There was no enrichment of the C-3′ signal.

(*S*)-1-Methyl-2(hydroxymethyl)pyrrolidine (26). L-Proline (20 g, 174 mmol) was dissolved in 85% formic acid (370 mL) and cooled to 0 °C. Acetic anhydride (120 mL) was added and the mixture stirred at room temperature for 2 h. Ice cold water (140 mL) was then added and the solvent removed under reduced pressure. The residual pale yellow oil was dissolved in THF (90 mL) and added slowly to a slurry of $LiAlH_4$ (33 g) in refluxing THF (500 mL). After refluxing for 48 h, water (44 mL) and 10% NaOH (10 mL) were added. After refluxing for 10 min, the mixture was filtered. The filtrate was evaporated, dissolved in CH_2Cl_2 (30 mL), dried ($MgSO_4$), and evaporated. The residue was distilled (bp 80–82 °C, 22 mm) to afford (*S*)-1-methyl-2-(hydroxymethyl)pyrrolidine (15.0 g, 75% yield): $[\alpha]_D$ −50.3° (*c* 5.03 in MeOH), lit.[11] $[\alpha]_D$ −49.5° (*c* 5.0 in MeOH); ^{13}C NMR ($CDCl_3$) δ 66.5 (C-6), 63.1 (C-2), 57.4 (C-5), 40.8 (NMe), 27.2 (C-3), 22.8 (C-4); ^{1}H NMR ($CDCl_3$) δ 4.12 (bs, 1 H, OH), 3.63 (dd, 1 H, C-6, J_{vic} = 5.8 Hz, J_{gem} = 13.1 Hz), 3.48 (dd, 1 H, C-6, J_{vic} = 5.4 Hz, J_{gem} = 12.0 Hz), 3.07 (m, 1 H, C-2), 2.37 (s, NMe), 2.25 (bm, 2 H, C-3), 1.8 (bm, 4 H, C-4 and C-5); MS-CI (CH_4) *m/z* (relative intensity) 116 (M^+ + 1, 40), 98 ($C_6H_{11}N^+$ + 1, 42), 84 (100).

(*S*)-1-Methyl-2-(chloromethyl)pyrrolidine Hydrochloride (27). Freshly distilled thionyl chloride (3.4 mL) dissolved in EtOH-free $CHCl_3$ (15 mL) was added slowly to an ice cold solution of (*S*)-1-methyl-2-(hydroxymethyl)pyrrolidine (4.26 g 37 mmol) in EtOH-free $CHCl_3$ (30 mL) stirred in a N_2 atmosphere. After the addition the reaction mixture was stirred at room temperature for 2 h and then refluxed for 30 min. The solvent was then removed under reduced pressure and the residue dissolved in a small amount of absolute EtOH. Et_2O was added and (*S*)-1-methyl-2-(chloromethyl)pyrrolidine hydrochloride separated (5.78 g, 34.2 mmol, 93% yield: mp 155–156.5 °C; $[\alpha]_D$ −30.2° (*c* 2.4 in MeOH); ^{13}C NMR (D_2O) δ 71.9 (C-2), 60.0 (C-5), 44.7 (C-6), 43.2 (NMe), 30.0 (C-3), 24.5 (C-4); ^{1}H NMR (D_2O) δ 3.91 (dd, 1 H, C-6, J_{vic} = 6.0 Hz, J_{gem} = 12.3 Hz), 3.79 (dd, 1 H, C-6, J_{vic} = 6.2 Hz, J_{gem} = 12.0 Hz), 3.70 (m, 1 H, C-3), 3.56 (m, 1 H, C-3), 3.18 (m, 1 H, C-2), 2.84 (s, NMe), 1.8–2.2 (bm, 4 H, C-4 and C-5).

(*S*)-1-Methyl-2-(cyanomethyl)pyrrolidine (30). (*S*)-1-Methyl-2-(chloromethyl)pyrrolidine hydrochloride (1.0 g, 5.9 mmol) was dissolved in 80% aqueous EtOH (15 mL) and cooled in an ice bath. $NaHCO_3$ (556 mg, 6.6 mmol) dissolved in water (7 mL) was slowly added and the mixture stirred for 15 min. Sodium cyanide (430 mg, 8.8 mmol) in 80% EtOH (15 mL) was added and the mixture refluxed for 30 min. The EtOH was then removed under reduced pressure and the residue extracted with Et_2O (4 × 25 mL). The dried ($MgSO_4$) extract was evaporated and the residual oil distilled (bp 45–46 °C, 0.5 mm) to afford (*S*)-1-methyl-2-(cyanomethyl)pyrrolidine (549 mg, 4.3 mmol, 75% yield): GC rt 8.47; $[\alpha]_D$ −36.6° (*c* 3.0 in MeOH); ^{13}C NMR ($CDCl_3$) δ 117.8 (CN), 61.0 (C-2), 56.5 (C-5), 39.9 (NMe), 30.3 (C-6), 21.9 (C-3), 21.7 (C-4); ^{1}H NMR ($CDCl_3$) δ 2.99 (t, 1 H, C-2), 2.36 (bm, 2 H, C-6), 2.36 (3 H, NMe), 2.18 (m, 1 H, C-3), 1.98 (m, 1 H, C-3), 1.5–1.8 (bm, 4 H, C-4 and C-5).

(*S*)-1-Methylpyrrolidine-2-acetic Acid (33). (*S*)-1-Methyl-2-(cyanomethyl)pyrrolidine (3.69 g, 29.7 mmol) was added to concentrated HCl (20 mL) and the mixture refluxed for 2 h. The solution was then evaporated to dryness and the residue dissolved in water (3 mL). The solution was chromatographed on a Bio-Rad ion exchange resin AG 50 W × 8, H^+ form, 100–200 mesh. The column was washed with water and the product eluted with a 3% NH_4OH solution. The fractions which contained the product were combined and evaporated to dryness. The residue was dissolved in CH_2Cl_2, decolorized with charcoal, and crystallized from benzene to yield colorless plates (3.57 g, 84% yield): mp 120–122 °C; $[\alpha_D]$ −31.3° (*c* 1.0 in EtOH); GC rt 9.99; ^{13}C NMR ($CDCl_3$) δ 174.5 (COOH), 64.6 (C-2), 55.2 (C-5), 39.0 (NMe), 36.3 (C-6), 30.1, (C-3), 21.7 (C-4); ^{1}H NMR ($CDCl_3$) δ 3.62 (m, 1 H, C-2), 3.29 (m, 1 H, C-6), 2.71 (s, NMe), 2.5-2.8 (bm, 2 H, C-3), 2.3 (m, 1 H, C-6), 1.75–2.1 (bm, 4 H, C-4 and C-5).

Methyl [1,2-$^{13}C_2$,1-^{14}C]Acetate (28). Sodium [1-^{14}C]acetate (nominal activity 0.5 mCi, specific activity 49.9 mCi/mmol, Sigma Chemical Co.) in H_2O (0.5 mL) was added to a solution of sodium [1,2-$^{13}C_2$]acetate (1.2 g, 99% ^{13}C at C-1, 99.4% ^{13}C at C-2, Aldrich) in 25 mL of 80% aqueous EtOH. The solution was evaporated to dryness and then dried at 100 °C in vacuo, yielding sodium [1,2-$^{13}C_2$,1-^{14}C]acetate (1.2 g) with a specific activity of 7.3 × 10^7 dpm/mmol. This labeled sodium acetate (413 mg, 4.9 mmol) was added to trimethyl phosphate (1.5 mL) in a 10-mL flask containing a plug of glass wool. The mixture was refluxed (oil bath temperature 190–200 °C) under N_2 for 2 h. The top of the reflux condenser was then connected to a vacuum source (0.2 mmHg), drawing the vapors first through a cold trap at 0 °C and a second trap cooled in liquid nitrogen. The reaction flask was heated at 90–130 °C for 1 h. The trap cooled in liquid N_2 contained methyl [1,2-$^{13}C_2$,1-^{14}C]acetate, which was dissolved in dry THF (3 mL) and used as such in the following reaction.

Methyl (*RS*)-[1,2-$^{13}C_2$,1-^{14}C]-4-(1-Methyl-2-pyrrolidinyl)-3-oxobutanoate (31). *n*-Butyllithium (4.3 mL of a 2.34 M solution in hexane, 10.1 mmol) was added to a solution of hexamethyldisilazane (2.2 mL, 10.5 mmol) in dry THF (10 mL) cooled to −70 °C in a N_2 atmosphere. The solution was allowed to warm to −10 °C and then cooled down to −78 °C. The methyl [1,2-$^{13}C_2$,1-^{14}C]acetate prepared in the previous section was then added slowly, and the mixture was stirred for 30 min at −78 °C. (*S*)-1-Methylpyrrolidine-2-acetic acid (750 mg, 5.24 mmol) was added to a solution of 1,1′-carbonyldiimidazole (938 mg, 5.78 mmol) in dry THF (6 mL) and stirred at room temperature for 2 h. This solution containing the imidazole derivative of 1-methylpyrrolidine-2-acetic acid (**32**) was then added in one portion to the solution of the lithium enolate of methyl acetate cooled to −70 °C. After the solution was stirred at this temperature for 7 h, the flask was warmed to 0 °C, and 2 N HCl (30 mL) and Et_2O (50 mL) were added. The aqueous acid solution was washed with more Et_2O, made basic with K_2CO_3, and extracted with $CHCl_3$ (3 × 20 mL). The dried (Na_2SO_4) extract was evaporated to yield an oil, which was chromatographed on silica gel 60 (230–400 mesh) eluting with a mixture of $CHCl_3$ and MeOH (1:1) to afford, after evaporation, methyl (*RS*)-[1,2-$^{13}C_2$,1-^{14}C]-3-oxobutanoate (591 mg, 2.94 mmol, 60% yield): specific activity 7.44 × 10^7 dpm/mmol; ^{13}C NMR ($CDCl_3$) δ 201.8 (d, C-3, $^1J_{2-3}$ = 36.2 Hz), 167.5 (highly enriched, d, C-1, $^1J_{1-2}$ = 58.7 Hz), 61.4 (C-2′), 56.7 (C-5′), 49.7 (highly enriched, d, C-2, J_{1-2} = 58.6 Hz), 47.7 (C-4), 46.9 (OMe), 40.4 (NMe), 31.1 (C-3′), 22.1 (C-4′). The highly enriched carbons of the enol form of this β-keto ester (**34**) were observable: ^{13}C NMR δ 172.5 (d, C-1, $^1J_{1-2}$ = 73.9 Hz), 90.1 (d, C-2, $^1J_{1-2}$ = 74.9 Hz); ^{1}H NMR ($CDCl_3$) δ 3.75 (d, 3 H, OMe, $^3J_{H-^{13}C}$ = 5.4 Hz), 3.45 (dd, 2 H, C-2, J_{gem} = 11.7 Hz, $^1J_{H-^{13}C}$ = 120.0 Hz), 3.05 (m, 1 H, C-2′), 2.88 (m, 1 H, C-4), 2.58 (m, 2 H, C-5′), 2.32 (s, NMe), 2.20 (dd, 1 H, C-4), 2.10 (m, 1 H, C-3′), 1.75 (m, 2 H, C-4′), 1.44 (m, 1 H, C-3′); MS-CI (CH_4) *m/z* (relative intensity) 203 (0.1), 202 (0.5), 201 (0.4), 200 (1.34), 170 (3.0), 141 (0.8), 84 (100). An unlabeled sample of the β-keto ester afforded a picrate, prisms from EtOH, mp 120–121 °C. Anal. Calcd for $C_{16}H_{20}N_4O_{10}$: C, 44.87; H, 4.71; N, 13.08. Found: C, 44.97; H, 4.69; N, 13.03.

Oxidation of Methyl 4-(1-Methyl-2-pyrrolidinyl)-3-oxobutanoate (31) with Mercuric Acetate. Methyl 4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate (100 mg, 0.5 mmol) was added to a solution of $NaOAc·3H_2O$ (2.8 g) and acetic acid (0.5 g) in water (40 mL). This solution was added to a solution of mercuric acetate (2.39 g, 7.5 mmol) in 40 mL of the same sodium acetate–acetic acid buffer solution. The solution (pH 4.7) was refluxed for 1 h and then kept at room temperature for 2 days. Sodium cyanoborohydride (2 g) was then added to the reaction mixture, and the resultant black mixture was filtered through Celite. The pale yellow filtrate was made basic with K_2CO_3 and extracted with $CHCl_3$ (3 × 50 mL). The reaction products obtained on evaporation of the dried (Na_2SO_4) extract were analyzed by GC, and it was found to be a mixture of hygrine (**23**, R = H) (12%), tropinone (**41**, R = H) (3.2%), 1′,2-dehydrohygrine (**39**, R = H) (43%), methyl 4-(1-methyl-2-pyrrolidylidene)-3-oxobutanoate (**39**, R = COOMe) (16%), and 2-carbomethoxy-3-tropinone (**41**, R = COOMe) (0.45%). The methyl 4-(1-methyl-2-pyrrolidylidene)-3-oxobutanoate was obtained as a colorless oil: GC rt 13.81; ^{13}C NMR ($CDCl_3$) δ 186.6 (C-3), 169.9 (C-1), 167.6 (C-2′), 89.7 (C-4), 54.6 (C-5′), 49.9 (C-2), 49.6 (OMe), 33.5 (NMe), 33.2 (C-3′), 20.6 (C-4′), (the carbons in the vinylogous amide portion of the molecule are consistent with those of model compounds[21]); high-resolution MS-EI *m/z* 197.1033 ($C_{10}H_{15}NO_3$); UV (in MeOH) λ_{max} 307.5 nm (ϵ 1.6 × 10^4); IR (neat) 2960, 2930, 2875, 2860, 1725, 1600, 1580, 1460, 1380, 1270, 1115, 1065 cm^{-1}. We have previously established[14] that the double bond in 1′,2-dehydrohygrine has the *E* geometry, and compound **39** (R = COOMe) is assumed to have the same stereochemistry. In view of the very small yield of 2-carbomethoxy-3-tropinone, the reaction was repeated using methyl [1-^{14}C]-4-(1-methyl-2-pyrrolidinyl)-3-oxobutanoate as a substrate in this reaction. The reaction mixture was diluted with nonradioactive 2-carbomethoxy-3-tropinone. Reisolation of this keto ester, purification by sublimation, and crystallization from aqueous acetone yielded material with a constant specific activity, equivalent to a yield in the reaction of 1.2%.

(*E*)-1′,2-Dehydro-*N*-methylpelletierine (44). *N*-Methyl-2-piperidone (10 g, Aldrich) was dissolved in THF (500 mL). Finely powdered $NaHCO_3$ (15 g) and phosphorus pentasulfide (24 g) were added, and the

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mixture was refluxed for 18 h. The reaction mixture was filtered, and the residue obtained on evaporation of the filtrate was distilled (bp 120 °C, 0.01 mm) to yield 1-methylpiperidine-2-thione (4.95 g), GC rt 10.58. This thione (2.5 g) was dissolved in acetonitrile (20 mL) and bromoacetone (2.2 g) was added. After standing at room temperature for 18 h, triphenylphosphine (5.0 g) and triethylamine (6 mL) were added, and the solution became warm, turned blue, and crystals separated. After shaking for 4 h at room temperature, the reaction mixture was then evaporated and the residue dissolved in $CHCl_3$. This solution was extracted with 2 N HCl (3 × 60 mL). This solution was made basic with Na_2CO_3 and extracted with $CHCl_3$. The dried (Na_2SO_4) extract was evaporated and distilled (140 °C, 0.001 mm) to afford a colorless oil (1.56 g). This material was dissolved in EtOH (20 mL), and an EtOH solution of picric acid (2.30 g) was added. The resultant picrate, which separated as bright yellow prisms (2.91 g), was crystallized from EtOH to yield the picrate of (*E*)-1′,2-dehydro-*N*-methylpelletierine, mp 157–8 °C. Anal. Calcd for $C_{15}H_{18}N_4O_8$: C, 47.12; H, 4.75; N, 14.65. Found: C, 47.28; H, 4.83; N, 14.44. The free base (GC rt 11.20) regenerated from the picrate was obtained as a colorless oil with the following spectral characteristics: ^{13}C NMR ($CDCl_3$) δ 193.8 (C-2′), 162.9 (C-2), 93.2 (C-1′), 51.7 (C-6), 40.0 (NMe), 31.4 (C-3), 27.7 (C-3′), 23.2 (C-5), 19.4 (C-4); ^1H NMR ($CDCl_3$) (A 2D NOESY experiment unequivocally established the assignment of the hydrogens at C-4 and C-5. The *E* configuration of the double bond was also established by the NOE interaction between the C-1′ hydrogen (4.95 ppm) and *N*-methyl hydrogens (2.81 ppm)) δ 4.95 (s, 1 H, C-1′), 3.22 (t, 2 H, C-6), 3.09 (t, 2 H, C-3), 2.81 (s, NMe), 2.00 (s, 3 H, C-3′), 1.72 (p, 2 H, C-5), 1.57 (p, 2 H, C-4); IR (neat) 2943, 2885, 1632 (C=O), 1540 (C=C) cm^{-1}; UV (95% EtOH) λ_{max} 307 nm (ε 2.7 × 10^4); MS-EI *m/z* (relative intensity) 153 (M^+, 34), 138 (M − C-3′ Me, 100), 110 (22).

Oxidation of *N*-Methylpelletierine (42) to ψ-Pelletierine (43) and (*E*)-1′,2-Dehydro-*N*-methylpelletierine (44). *N*-Methylpelletierine[22] (310 mg, 2 mmol) dissolved in acetic acid (10 mL) was added to a solution of mercuric acetate (10 g) in hot water (500 mL) and the mixture refluxed for 2 days. Sodium cyanoborohydride (2 g) was added to the cooled reaction mixture, and the resultant black mixture was filtered after 5 min through Celite. The pale yellow filtrate was made basic with K_2CO_3 and extracted with $CHCl_3$. The dried (Na_2SO_4) extract contained (by GC) the following compounds: *N*-methylpelletierine (**42**) (GC rt 9.75) (40%), ψ-pelletierine (**43**) (GC rt 10.54) (8%), and (*E*)-1′,2-dehydro-*N*-methylpelletierine (**44**) (GC rt 11.20) (24%). These compounds were separated by preparative TLC on silica gel, developing with a mixture of $CHCl_3$, EtOH, and concentrated NH_4OH (90:10:1). Compound **44** (R_f 0.8) was readily detected by its strong absorption in the UV. Compounds **43** and **42** were detected by spraying a thin strip on the side of the plate with I_2 in benzene and had R_f values of 0.62 and 0.48, respectively. The zones were extracted with MeOH and evaporated, and the residues were distilled or sublimed, affording ψ-pelletierine (mp 54 °C) identical (IR, NMR, picrate) with an authentic specimen[23] and (*E*)-1′,2-dehydro-*N*-methylpelletierine, identical with the material synthesized in the previous section.

Administration of Methyl (*RS*)-[1,2-$^{13}C_2$,1-^{14}C]-4-(1-Methyl-2-pyrrolidinyl)-3-oxobutanoate (31) to *Erythroxylum coca* and Isolation of the Alkaloids. The following procedure describes the feeding carried out in March, 1991, when the β-keto ester **31** was administered along with the *N*-acetylcysteamine thioester of benzoic acid (**19**). The β-keto ester **31** (76 mg, 0.378 mmol, total activity 2.79 × 10^7 dpm) was dissolved in water (50 mL) which contained Tween 80 (0.75 mL). A solution of the *N*-acetylcysteamine thioester of benzoic acid[7] (83 mg, 0.372 mmol) in EtOH (2 mL) was added to this aqueous solution which was then painted on the leaves (upper and lower surfaces) of four coca plants (6–8 years old) growing in a greenhouse. The feeding was spread over 4 days, and the plants were allowed to grow for a total time of 2 weeks after the initial feeding. The leaves were then all removed (fresh wt 96 g) and the alkaloids extracted as previously described,[2] with cocaine and methyl ecgonine being separated by preparative TLC on silica gel, developing with a mixture of ethyl acetate, methanol, water, and concentrated NH_4OH (85:13:1:0.5). In this system, cocaine and the cinnamoylcocaines have an R_f value of 0.6 (detected by UV) and methyl ecgonine (detected by I_2) an R_f of 0.3. Cocaine and methyl ecgonine were purified as their hydrochloride salts. The cocaine was largely separated from the cinnamoylcocaines by crystallization of its free base from a mixture of CH_2Cl_2 (a minimal amount) and heptane. The yields and activities of the isolated alkaloids were as follows: cocaine hydrochloride (77 mg, 7.32 × 10^4 dpm/mmol, 0.1% specific incorporation) and methyl ecgonine hydrochloride (36 mg, 1.52 × 10^5 dpm/mmol, 0.20% specific incorporation). The alkalids were converted to their free bases for the ^{13}C NMR spectra determinations in $CDCl_3$. The C-2 signal in the ^{13}C NMR spectrum of the methyl ecgonine (determined under similar conditions as those recorded in Figure 1 for cocaine) exhibited satellites, $^1J_{2-9}$ = 57.3 Hz. Again the satellites were arranged slightly unsymmetrically, 28.0 Hz downfield and 29.3 Hz upfield, from the central C-2 peak. No satellites were observable at the signal at 40.15 ppm (C-4). In the December feeding, the β-keto ester **31** (97 mg, 7.44 × 10^7 dpm/mmol) dissolved in water (40 mL) containing Tween 80 (0.8 mL) was fed for a total time of 2 weeks. The fresh leaves (83 g) yielded cocaine hydrochloride (151 mg) having an activity of 1.6 × 10^4 dpm/mmol (0.02% specific incorporation) and methyl ecgonine hydrochloride (68 mg) with an activity of 7.0 × 10^4 dpm/mmol (0.1% specific incorporation). Our previous experience on the biosynthesis of cocaine has indicated that the best incorporation of potential precursors are obtained when the feedings are carried out between May and August.

Chemical Degradation of Methyl Ecgonine. The degradation previously described for cocaine[2] was carried out on the methyl ecgonine (7.0 × 10^4 dpm/mmol) obtained from the above feeding of methyl (*RS*)-[1,2-$^{13}C_2$,1-^{14}C]-3-oxobutanoate carried out in December, 1990. Refluxing the methyl ecgonine with concentrated HCl yielded ecgonidine hydrochloride (6.8 × 10^4 dpm/mmol). This compound was converted to its methiodide, which was subjected to a Hofmann degradation yielding dimethylamine (collected as its hydrochloride) (<0.1 × 10^4 dpm/mmol) and cycloheptatrienecarboxylic acid. Hydrogenation of this acid afforded cycloheptanecarboxylic acid (6.9 × 10^4 dpm/mmol), which on subjection to a Schmidt reaction yielded carbon dioxide (collected as $BaCO_3$, 6.5 × 10^4 dpm/mmol, representing the activity at C-9 of methyl ecgonine) and cycloheptylamine (converted to (dimethylamino)cycloheptane methiodide for assay) (0.3 × 10^4 dpm/mmol).

Feeding of (*RS*)-[9-^{14}C]-2-Carbomethoxy-3-tropinone (12) to Leaf Fragments of *Erythroxylum coca*. The incubations were carried out in an aqueous solution (100 mL) containing K_2HPO_4 (1.14 g), which was adjusted to pH 7 by the addition of a solution of KH_2PO_4 (680 mg) in water (100 mL). Sucrose (1.71 g) was added to each 100 mL of this solution along with 0.1 mL of Tween 80. For each experiment the weight of fresh leaves was 300–320 mg. They were cut up with a razor blade into pieces about 3-mm square and placed in 3 mL of the phosphate buffer. In each experiment, 0.1 mL of a 0.1 M solution of (*RS*)-[9-^{14}C]-2-carbomethoxy-3-tropinone[17] (1.2 × 10^8 dpm/mmol, 1.97 mg) was added. At the end of each experiment 2 N HCl (3 mL) was added to the incubation mixture, and the whole was ground up with sand in a mortar. The solids were then removed by centrifugation. At this stage methyl ecgonine hydrochloride (3 mg) was added to the supernatant solution to facilitate its detection by UV spectroscopy in the subsequent HPLC. The supernatant solution was made basic with Na_2CO_3 and extracted with $CHCl_3$ (3 × 7 mL). The dried (Na_2SO_4) extract was evaporated, and the residue was dissolved in $CHCl_3$ (0.2 mL) and filtered through a centrifuge filter. An additional 0.2 mL of $CHCl_3$ was used to wash the evaporation flask and centrifuge filter. The $CHCl_3$ solution was then analyzed on the HPLC system described in the General Methods section on a silica column with a solvent flow rate of 2 mL/min. The alkaloids were detected as they came off the column by a UV detector set at 254 nm. The gradient system was $CHCl_3$ (containing 1% NH_3) with increasing amounts of MeOH. At zero time there was 0% MeOH, which was increased to 2% MeOH during 2 min and held constant for 15 min. The MeOH content was then increased to 20% over the next 60 min. Under these gradient conditions the elution times for cocaine, methyl ecgonine, and 2-carbomethoxy-3-tropinone were 18–20 min, 28–32 min, and 36–40 min, respectively. By carrying out dilutions it was established that the activity in these fractions corresponded to these compounds. The results are recorded in Table II. In experiments 7 and 8 the *N*-acetylcysteamine thioester of benzoic acid (2 mg) was added to the incubation mixture.

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