1-METHYLPYRROLIDINE-2-ACETIC ACID, A PLAUSIBLE INTERMEDIATE IN THE BIOSYNTHESIS OF COCAINE*

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Abstract — Radioactive 1-methylpyrrolidine-2-acetic acid (0.5% absolute incorporation) was isolated from Erythroxylum coca plants which had been fed [2-^{14}C]-1-methyl-Δ^1-pyrrolinium chloride. A systematic degradation of the 1-methylpyrrolidine-2-acetic acid established that all the radioactivity was located at its C-2 position. Since the cocaine isolated from this feeding experiment was labeled at C-5, this result is consistent with the hypothesis that 1-methylpyrrolidine-2-acetic acid, or a suitably activated ester, is an intermediate in the biosynthesis of cocaine.

In 1982[1] it was established that [5-^{14}C]-ornithine is incorporated into the tropane moiety of cocaine (15). Equal activity was found at the bridgehead carbons C-1 and C-5. It was thus proposed that the ornithine (1) is incorporated via a symmetrical intermediate, namely putrescine (2). Subsequent steps were considered to be methylation to N-methylputrescine (3), then oxidation to 4-methylaminobutanal (7). In acid solution this amino-aldehyde exists as the 1-methyl-Δ^1-pyrrolinium salt (5). We recently[2] made the surprising discovery that this iminium salt, when labeled at C-2 with ^{14}C and ^{13}C, is incorporated into cocaine which is labeled exclusively at its C-5 position (as in 15b). This result is inconsistent with the generally accepted hypothesis that the tropane nucleus is formed by reaction of the iminium salt with a four carbon unit (e.g., acetoacetyl coenzyme A (4)) to yield an intermediate such as (8) which then undergoes further oxidation to (11) and subsequent ring closure and trans-

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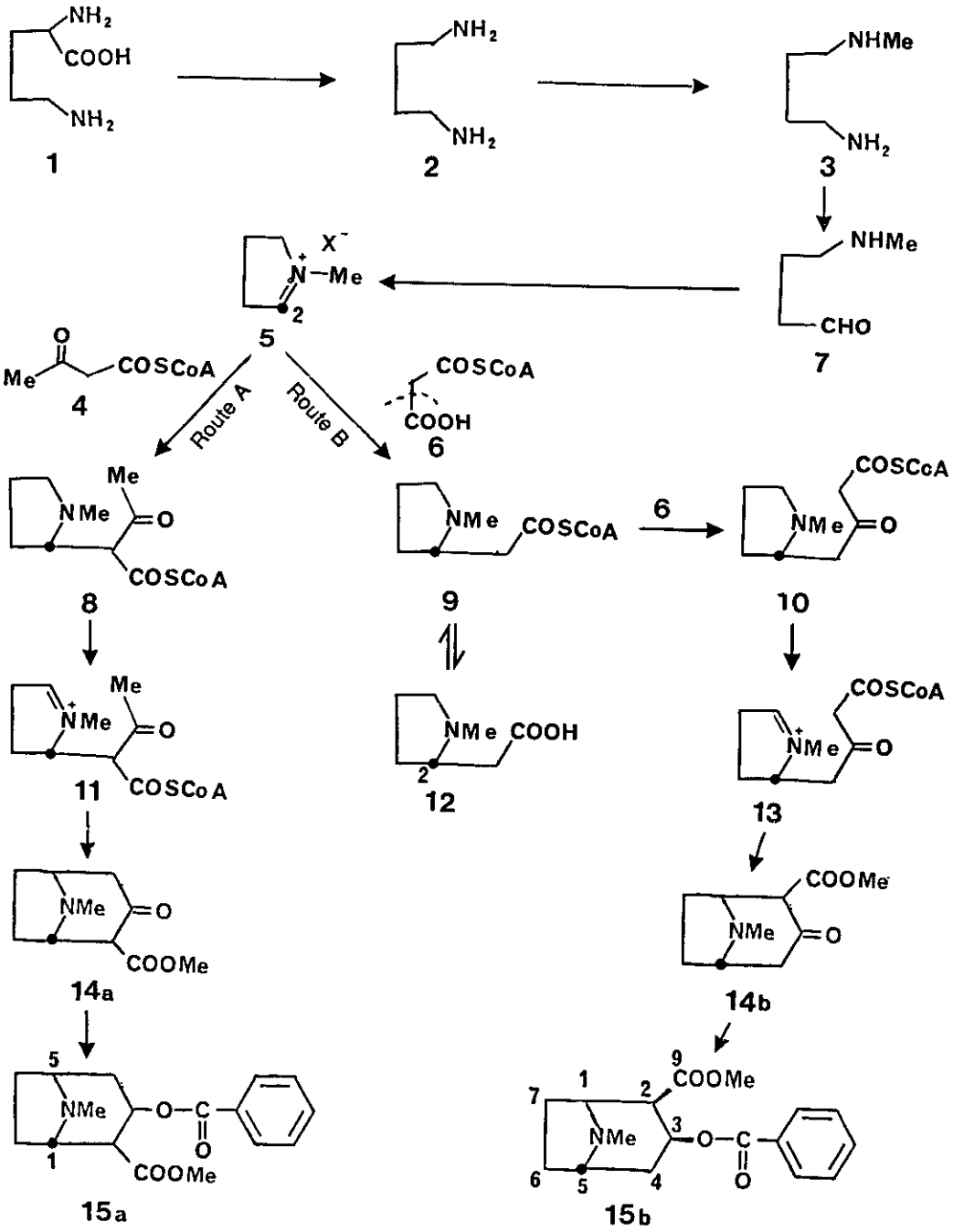
*This paper is dedicated to Professor D.H.R. Barton who celebrated his 70th birthday on September 8, 1988. I have long admired his pioneering and elegant research. From my perspective one of his most exciting discoveries was the elucidation of the late stages in the biosynthesis of the opium, Erythrina and Amaryllidaceae alkaloids, which involve oxidative coupling of phenolic intermediates.

+Contribution No. 208 from this Laboratory. Part 41 in the series "Chemistry of the Tropane Alkaloids and Related Compounds". Presented in part at the 28th Annual meeting of the American Society of Pharmacognosy, Kingston, RI, July 19-22, 1987.

esterification to the tropane derivative (14a). This is depicted as Route A in Scheme 1. 2-Carbomethoxy-3-tropinone (14) is indeed a precursor of cocaine[3], and final steps to cocaine are presumably stereospecific reduction of the ketone to the equitorial alcohol (methylecgonine) and benzoylation[4,5].



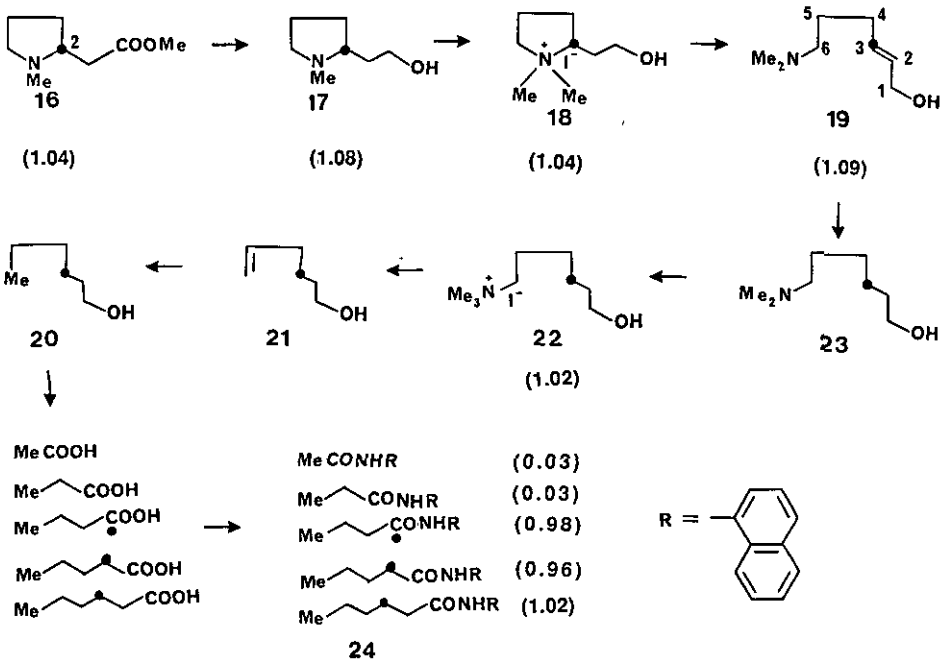
Scheme 1. Biosynthetic Routes to Cocaine

We thus proposed a new hypothesis (Route B) for the formation of the tropane nucleus of cocaine. In this pathway the iminium salt (5) serves as a starter unit for a polyketide, a two-carbon unit being added (perhaps by activation of acetyl coenzyme A by conversion to malonyl coenzyme A (6)) first to yield the thioester of 1-methylpyrrolidine-2-acetic acid (9). The addition of a second two-carbon unit then affords 4-(1-methyl-2-pyrrolidinyl)acetoacetate (10). 2-Carbomethoxy-3-tropinone labeled at C-5 (as in 14b) will then be formed via the iminium salt (13).

In this article we present evidence which favors this new biosynthetic scheme for cocaine. We reasoned that the Erythroxylun coca plant which had been fed [2-^{14}C]-1-methyl-Δ^1-pyrrolinium chloride (5) would contain some labeled 1-methylpyrrolidine-2-acetic acid (12) formed by hydrolysis of its thioester (9). Accordingly a trapping experiment was carried out to detect this amino acid in the coca plant. The coca leaves which had been fed [2-^{14}C]-(5) were harvested after two weeks and worked up in the usual way[1] by chopping up on a Waring blendor with a mixture of chloroform and aqueous Na_2CO_3 solution. After filtering the mixture non-radioactive (RS)-1-methylpyrrolidine-2-acetic acid was added to the aqueous extract. The amino acids in this extract were isolated by ion exchange chromatography on Dowex 50. The crude mixture of amino acids was esterified with methanolic HCl. Methyl 1-methylpyrrolidine-2-acetate (16) was separated from this mixture by preparative TLC, distilled and converted to its picrate. After several crystallizations, this picrate had a constant specific activity, equivalent to a 0.5% conversion of the labeled iminium salt into the amino acid (12). The degradation illustrated in Scheme 2 was devised to determine activity at all the carbons of the 1-methylpyrrolidine-2-acetic, especially at C-2, the expected location of the carbon-14. Reduction of the methyl ester (16) with lithium aluminum hydride yielded the alcohol 17 which was converted to its methiodide (18). A Hofmann degradation on this methiodide carried out at 125 °C yielded 6-dimethylamino-2(E)-hexen-1-ol (19) as the sole product. The (E)-geometry of this allylic alcohol was established by ^{13}C-nmr spectroscopy, by comparison with model (E) and (Z)-allylic alcohols[6]. Hydrogenation of this allylic alcohol in the presence of platinum yielded 6-dimethylamino-1-hexanol (23) which was converted to its methiodide 22 and subjected to another Hofmann degradation yielding 5-hexen-1-ol (21). Hydrogenation afforded 1-hexanol (20) which was subjected to a modified Kuhn-Roth oxidation. The C_2 to C_6 carboxylic acids from this oxidation were converted to their 1-naphthylamides (24)[7] and separated by TLC. The specific activities (dpm/mM) of these degradation products are recorded in Scheme 2. The absence of change in the specific activity of these degradation products up to 1-hexanol indicates that the methyl ester 16 was radiochemically pure. The lack of significant activity in the amides of acetic and propanoic acid, and essentially the same specific activity of the

C_4, C_5, and C_6 amides indicates that all the activity of the 1-methylpyrrolidine-2-acetic acid was located at its C-2 position, a result consistent with Route B to cocaine.

Methyl (S)-1-methylpyrrolidine-2-acetate (16) has been found in the plant Solanum sturtianium (Solanaceae)[8], which provides circumstantial evidence in favor of the intermediacy of 1-methylpyrrolidine-2-acetic acid, or a suitably activated derivative, in the biosynthesis of cocaine. We are currently examining this amino acid as a precursor of cocaine and other tropane alkaloids.



Scheme 2. Degradation of Methyl 1-Methylpyrrolidine-2-acetate (numbers in parenthesis are specific activities dpm/mM x 10^{-5})

EXPERIMENTAL

General Methods. Radioactive materials were assayed by liquid scintillation counting using dioxane-EtOH as the solvent with the usual scintillators[9]. ^{13}C-Nmr spectra were determined at 75.5 MHz by Dr. S.B. Philson. All recorded spectra are ppm from TMS. 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 (0.52 μm thick), internal diameter 0.31 mm, with the following instrument parameters (unless otherwise stated): He flow rate 1 ml/min, injection temp 250 °C, initial oven temp 50 °C, equilibration time 4 min, rate of temp increase 30 °C/min, final temp 250 °C. Retention times (min) are reported: GC R_t. Elemental analyses were carried out by MHW Laboratories, Phoenix, Arizona.

(RS)-1-Methylpyrrolidine-2-acetic acid (12). 1-Methylpyrrole-2-acetate (Chemical Dynamics Corp.), GC R_t 9.91, (15 g) dissolved in acetic acid (100 ml) was hydrogenated in the presence of Adam's catalyst (0.5 g) at 3 atmospheres pressure and 25 °C for 72 h. The filtered solution was evaporated in vacuo and the viscous residue was made basic with 10% Na_2CO_3, and then extracted with CH_2Cl_2. The residue obtained on evaporation of the dried (Na_2SO_4) extract was distilled (120 °C, 10^{-2} mm) to afford methyl 1-methylpyrrolidine acetate (16) (12 g) as a colorless oil, GC R_t 9.35, picrate from EtOH, yellow plates, mp 135 °C (lit.[8] mp 135-136 °C). The ester afforded a methiodide, plates from EtOAc, mp 172-173 °C. Anal. Calcd for $C_9H_{18}INO_2$: C, 36.13; H, 6.06; N, 4.68. Found: C, 35.97; H, 6.14; N, 4.61%.

The ester 16 (1.57 g, 10 mM) was refluxed with $Ba(OH)_2$ (1.71 g, 10 mM) in water (100 ml) for 3 h. Carbon dioxide was passed into the solution. After removal of the $BaCO_3$ the filtrate was evaporated to dryness and the residue was extracted with benzene affording (RS)-1-methylpyrrolidine-2-acetic acid (1.21 g) as colorless hygroscopic plates, mp 123-124 °C (lit.[10] mp 124 °C).

Feeding of [2-^{14}C]-1-methyl-Δ^1-pyrrolinium chloride to Erythroxylum coca, followed by isolation of 1-methylpyrrolidine-2-acetic acid. [2-^{14}C]-1-Methyl-Δ^1-pyrrolinium chloride[11] (1.72×10^8 dpm, 1.17 mM) was administered to three E. coca plants (3-4 years old) by painting an aqueous solution (containing 5% Tween 80) on the leaves of the plants by means of an artist paint brush. After 2 weeks the leaves (fresh wt. 152 g) were removed and chopped up in a Waring blendor with $CHCl_3$ (3 l) and 10% Na_2CO_3 (300 ml). The plant debris was removed by filtration and the organic and aqueous layers were separated. Cocaine, purified as its hydrochloride (251 mg, 5.84×10^4 dpm/mM ≡ 0.025% absolute incorporation) was isolated from the $CHCl_3$ extract as previously described[1]. The aqueous Na_2CO_3 solution had a total activity of 2.33×10^7 dpm (13.5% of the activity fed). (RS)-1-Methylpyrrolidine-2-acetic acid (461 mg) was added to an aliquot (100 ml) of this aqueous extract (9.7×10^6 dpm) which was then acidified with HCl and filtered through celite. The filtrate was applied to a column of Dowex 50 x 8 (H^+ form) (100 g). The column was washed with water (500 ml) and the amino acids eluted with aqueous NH_3 (20 ml conc NH_3 + 80 ml of H_2O). The fractions containing amino acids were evaporated to dryness, and the residue was dissolved in MeOH which was then saturated with HCl gas and refluxed for 18 h. The residue obtained on evaporation was made basic with Na_2CO_3 and extracted with $CHCl_3$. The residue obtained on evaporation of the dried (Na_2SO_4) extract was subjected to preparative TLC on silica gel PF-254, developing with a mixture of $CHCl_3$, MeOH, conc NH_3 (90 : 10 : 0.5). The zone, R_f 0.3, corresponding to methyl 1-methylpyrrolidine-2-acetate (detected by spraying the edge of the plate with I_2 in benzene) was extracted in a Soxhlet with MeOH. GC on this extract indicated that it contained only the

ester 16 (GC R_t 9.35). The filtered extract was evaporated in the presence of picric acid (500 mg). The residue was crystallized several times from EtOH to afford methyl 1-methylpyrrolidine-2-acetate picrate (201 mg) with a constant specific activity of 1.04×10^5 dpm/mM. This activity corresponds to an absolute incorporation of the [2-^{14}C]-1-methyl-Δ^1-pyrrolinium chloride into 1-methylpyrrolidine-2-acetic acid of 0.5%.

Degradation of methyl-1-methylpyrrolidine-2-acetate (16). Dilutions were carried out when necessary in the course of this degradation. The picrate of methyl 1-methylpyrrolidine-2-acetate (600 mg) was dissolved in 2 N HCl (10 ml) and the solution was extracted with Et_2O to remove picric acid. The solution was then made basic with Na_2CO_3, and extracted several times with Et_2O. The dried (Na_2SO_4) extract (80 ml) was refluxed with $LiAlH_4$ (0.5 g) for 18 h. 10% NaOH (1 ml) was then added, and after refluxing for 1 h, the mixture was filtered. The residue obtained on evaporation of the Et_2O was distilled (105 °C, 10^{-4} mm) to afford (RS)-2-(1-methyl-2-pyrrolidinyl)ethanol (17)[12] (187 mg) as a colorless oil, GC R_t 9.26. This alcohol (180 mg) was dissolved in EtOAc (5 ml) and methyl iodide (1 ml) was added. After standing 3 h a crystalline methiodide (19) separated. Its hygroscopic nature precluded an elemental analysis. This crystalline methiodide and the residue obtained on evaporation of the EtOAc mother liquor were dissolved in H_2O (10 ml) and shaken at 20 °C with AgOH (from 0.6 g of $AgNO_3$ + 10% NaOH). The filtered mixture was lyophilized and the residue was heated at 125 °C/10^{-4} mm. The volatile product from this reaction, 6-dimethylamino-2-(E)-hexen-1-ol (19) was obtained as a colorless oil, GC R_t 9.65, ^{13}C nmr ($CDCl_3$) 131.2 (2), 130.3 (3), 62.9 (1), 59.2 (6), 45.2 (N-Me), 30.0 (5), 26.9 (4). This amine afforded a methiodide, needles from EtOH-EtOAc, mp 125-126 °C. Anal. Calcd for $C_9H_{20}INO$: C, 37.90; H, 7.07; N, 4.91. Found: C, 37.85; H, 7.08; N, 4.96%. This methiodide is not part of the degradation scheme. The alcohol 19 dissolved in EtOAc (10 ml) was hydrogenated in the presence of Adam's catalyst (0.1 g) for 4 h at 2 atmospheres pressure, to afford 6-dimethylamino-1-hexanol (23). GC R_t 9.65, i.e., the same as the alkene 19. However, by changing the rate of temp increase in the GC to 5 °C/min, R_t of 19.55 and 19.68 were obtained for 19 and 23 respectively. Addition of methyl iodide (0.5 ml) to the filtered hydrogenation mixture afforded 6-dimethylamino-1-hexanol methiodide (22), (176 mg) fine colorless needles, mp 121-122 °C. Anal. Calcd for $C_9H_{22}INO$: C, 37.64; H, 7.72; N, 4.88. Found: C, 37.87; H, 7.74; N, 4.97%. The methiodide 22 (287 mg) was dissolved in H_2O (5 ml) and shaken with AgOH (from 0.5 g $AgNO_3$). The filtered solution was evaporated and the residue was distilled (130 °C, 10^{-4} mm) affording 5-hexen-1-ol (21) (pungent odor) GC R_t 6.66, which was condensed in a U-tube cooled in dry ice/acetone. The contents of the U-tube were washed out with Et_2O and the mixture was hydrogenated in the presence of Adam's catalyst (0.1 g) for 4 h at 2 atmospheres pressure. The filtered Et_2O solution was carefully evaporated at 10 °C affording 1-hexanol

(20) (85 mg) identical (ir) with an authentic specimen, GC R_t 6.79.

The 1-hexanol (145 mg) was dissolved in 2 N H_2SO_4 (20 ml) to which chromium trioxide (2.0 g) and conc H_2SO_4 (1 ml) were added. The mixture was heated on a steam bath for 10 h. The solution was then distilled, H_2O being added to maintain the volume in the distillation flask, until 150 ml had been distilled. The distillate was titrated with N/10 NaOH (phenophthalein end point, 7.5 ml required). The solution was evaporated to dryness, redissolved in H_2O (3 ml) to which was added 1-aminonaphthalene hydrochloride (180 mg) and 1-ethyl-3-[3-dimethyl-amino)propyl]carbodiimide hydrochloride (0.5 g). After 2 h the mixture was extracted with CH_2Cl_2 which was washed with 2 N HCl, and then with 10% Na_2CO_3. The dried (Na_2SO_4) extract was evaporated to yield a mixture of carboxylic acid-1-naphthylamides (175 mg) having the following GC R_t (% composition by wt): acetic 14.05 (62.1%), propanoic 14.79 (8.4%), butanoic 15.62 (4.8%), pentanoic 16.86 (6.1%), hexanoic 18.43 (18.6%). These amides were separated by TLC on silica gel PF-254, by developing 3 times with $CHCl_3$, MeOH, conc NH_3 (95 : 5 : 0.5). The zones (detected by uv) were removed and extracted with MeOH. The residues obtained on evaporation of the MeOH extracts were sublimed (~ 120 °C, 10^{-4} mm) to afford the amides. Acetyl-1-naphthylamide and hexanoyl-1-naphthylamide had the lowest and highest R_f values respectively. The C_5 and C_6 amides were extracted together and subjected to a second TLC on silica gel PF-254, a good separation being achieved with $CHCl_3$-MeOH (98 : 2). The specific activities of these degradation products of **16** are recorded in Scheme 2, taking into account the various dilutions which were performed in the course of this degradation. Samples were counted in duplicate with a SD of 3%.

ACKNOWLEDGEMENTS

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

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Received, 13th September, 1988