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BIOSYNTHESIS OF COCAINE AND CUSCOHYGRINE FROM [1-^{14}C]ACETATE AND [4-^{3}H]PHENYLALANINE IN *ERYTHROXYLON COCA**

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Key Word Index—*Erythroxylon coca*; Erythroxylaceae; biosynthesis; cocaine; cuscohygrine.

Abstract—A mixture of sodium [1-^{14}C]acetate and DL-[4-^{3}H]phenylalanine (^{3}H/^{14}C = 1.0) was administered to *Erythroxylon coca* plants by painting an aqueous solution of these compounds on the leaves. The leaves were removed after two weeks and yielded labeled cocaine (^{3}H/^{14}C = 11.4) and cuscohygrine. A systematic degradation of the cocaine indicated that 96% of the ^{3}H was located in the *para*-position of its benzoyl moiety, and the ^{14}C was located in the ecgonine residue at positions in accord with the accepted biosynthesis of this alkaloid. The cuscohygrine, containing a negligible amount of ^{3}H, was found to have 67% of its ^{14}C located on the carbonyl carbon. Cocaine, isolated from a second crop of leaves, harvested 10 weeks after the initial feeding, was still labeled at specific positions.

INTRODUCTION

Recently, it has been established that [5-^{14}C]ornithine serves as a precursor of the pyrrolidine rings of cocaine (**17**) and cuscohygrine (**8**) [1]. Figure 1 illustrates the generally accepted biosynthetic pathway for the formation of these alkaloids. Since the cocaine derived from [5-^{14}C]ornithine was labeled equally at the bridgehead carbons (C-1 and C-5), it is proposed that the ornithine (**1**) is incorporated via putrescine (**2**), a symmetrical intermediate. *N*-Methylation affords *N*-methylputrescine (**3**) which is oxidized at its primary amino group to yield 4-methylaminobutanal (**4**). Cyclization affords the *N*-methyl-Δ^{1}-pyrrolinium salt (**7**) which yields hygrine-1′-carboxylic acid (**6**) on reaction with acetoacetic acid. Hygrine (**5**) is formed from **6** by decarboxylation, and condensation with a second molecule of the pyrrolinium salt, **7**, affords cuscohygrine (**8**) [2–4]. It is considered that cocaine is formed from **6** probably with some kind of protection of the carboxyl group to inhibit decarboxylation of this β-keto acid. In the proposed biosynthetic scheme the carboxyl group is converted to its methyl ester and the pyrrolidine ring dehydrogenated to yield **9**. Cyclization then yields 2-carbomethoxytropinone (**10**). Reduction of the ketone yields methylecgonine (**12**), a minor alkaloid of *Erythroxylon coca* [5]. It has been established that the benzoic acid moiety of cocaine is derived from phenylalanine [6]. It was shown that the radioactive cocaine derived from [3′-^{14}C]phenylalanine had all its activity located in the benzoyl moiety, however, the specific location of the ^{14}C (presumably on the carbonyl group) was not determined. The benzoic acid (**16**) is plausibly formed from phenylalanine (**11**) via cinnamic acid (**13**), 3-hydroxy-3-phenylpropanic acid (**14**) and benzoyl acetic acid (**15**). Previous experiments [3] involving the feeding of [1-^{14}C]acetate to *E. coca* led to inconclusive results. The distribution of activity found in the cocaine is illustrated in Fig. 2. Less than 10% of the total activity was present in the ecgonine moiety. Having obtained a successful incorporation of [5-^{14}C]ornithine into cocaine by the leaf-painting technique [1], the role of acetic acid as a precursor of the four-carbon unit (C-2–C-4, and C-9) of cocaine has now been reinvestigated.

RESULTS AND DISCUSSION

It was previously discovered [1] that the absolute incorporation of activity from [5-^{14}C]ornithine into cocaine and cuscohygrine is apparently dependent on the season, a much higher incorporation being obtained when feedings were carried out in the spring or summer rather than the autumn. This seasonal variation was also observed in the present work involving the administration of [1-^{14}C]acetate. A very poor incorporation was obtained in October, but the feeding carried out in March afforded a much higher incorporation (by a factor of 28) into cocaine. (See Table 1 which also records the previous feedings of [5-^{14}C]ornithine.) In this latter experiment (No. 5) DL-[4-^{3}H]phenylalanine was fed along with the [1-^{14}C]acetate so that the efficiency of the formation of benzoic acid (from phenylalanine) could be compared with incorporation of acetic acid into the tropane skeleton. The ratio ^{3}H/^{14}C in the administered mixture was 1.0, however, the cocaine isolated two weeks later had a ^{3}H/^{14}C ratio of 11.4. It was subsequently shown that almost all the tritium was located in the benzoyl moiety and the ^{14}C was in the tropane nucleus. At this time it is not possible to explain the more efficient incorporation of phenylalanine into the benzoic acid moiety of cocaine. As expected, the cuscohygrine contained a negligible amount of tritium.

The cocaine from experiment 5 was degraded by means

*Part 31 in the series "Chemistry of the Tropane Alkaloids". For Part 30 see, Leete, E. *Rev. Latinoam. Quim.* (in press).

†Contribution No. 185 from this laboratory. This paper is dedicated to Professor Holger Erdtman who recently celebrated his 80th birthday.

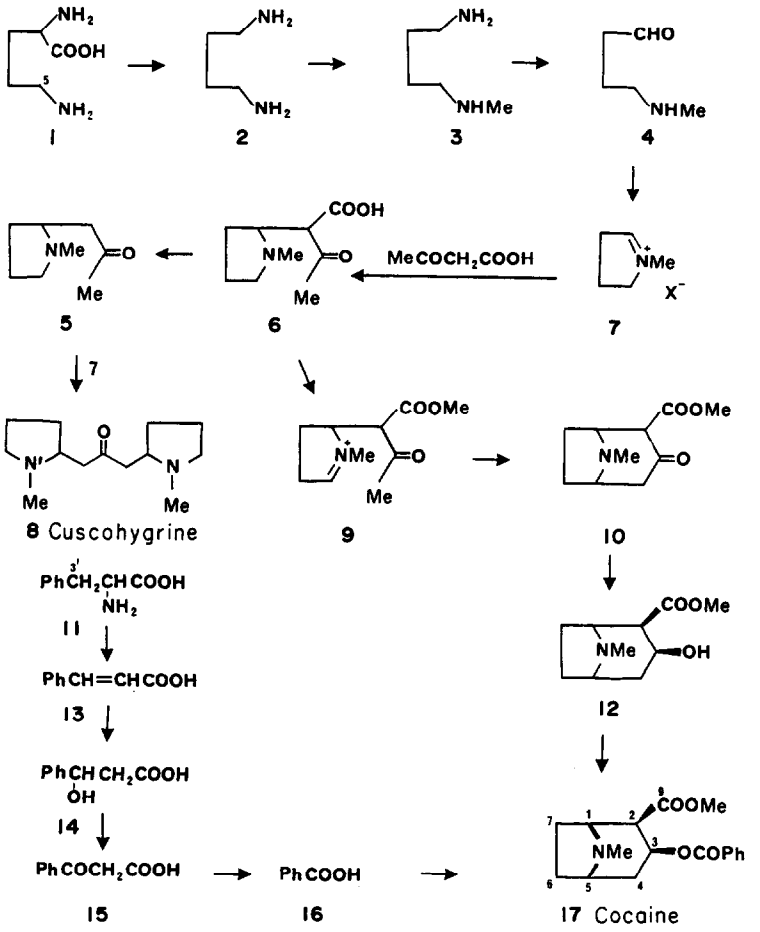

Fig. 1. Biosynthesis of cuscohygrine and cocaine.

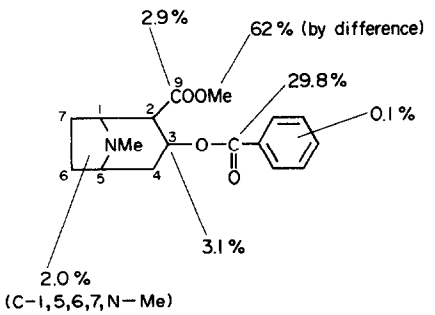

Fig. 2. Distribution of activity in cocaine labelled from [1-^{14}C]acetate [3].

of the reactions illustrated in Fig. 3. Hydrolysis of cocaine with concentrated hydrochloric acid yielded ecgonidine (**19**) and benzoic acid (**20**). A Schmidt reaction on the latter afforded carbon dioxide (C-7′) and aniline (**21**) which was acetylated to yield acetanilide (**28**). Essentially all the tritium was lost from this compound on bromination yielding *p*-bromoacetanilide (**34**) [7], indicating that the initial benzoic acid was labeled solely at its *para*-position. The ecgonidine was converted to the methiodide of its methyl ester (**25**) which was subjected to a Hofmann degradation yielding dimethylamine (**27**), collected as its *N*-benzoyl derivative (**33**), and cycloheptatriene carboxylic acid (**26**) [8]. Catalytic reduction afforded cycloheptane carboxylic acid (**32**), also assayed as its anilide. A Schmidt reaction on **32** yielded carbon dioxide (C-9) and cycloheptylamine (**31**) which was converted to trimethylcycloheptyl ammonium iodide (**35**). The radiochemical purity of this fragment was authenticated by a Hofmann reaction yielding cycloheptene (**36**) which was oxidized to pimelic acid (**37**) as previously described [1]. Another portion of ecgonidine was oxidized with chromium trioxide in sulfuric acid affording *N*-methylsuccinimide (**24**). Reaction of this compound with phenylmagnesium bromide yielded 1-methyl-2,5-diphenylpyrrole (**30**) [9]. Oxidation of this pyrrole with permanganate yielded benzoic acid representing the activity at C-1 and C-5. Activity on the *O*-methyl group of cocaine was determined by an SN_2 cleavage of the ester [10] with the sodium salt of *p*-acetamidothiophenol in hexamethylphosphoramide, affording methyl-(*p*-acetamidophenyl)sulfide (**23**). Mild hydrolysis of cocaine with sodium hydroxide yielded ecgonine (**18**) which was oxidized with chromic acid in dilute acetic acid yielding tropinone (**22**) [11]. Reaction with phenyl lithium yielded 3-phenyltropine (**29**) [12] which on oxidation with permanganate afforded benzoic

Table 1. Effect of season on the incorporation of labeled precursors into cocaine and cuscohygrine

Expt No.	Precursor	Date feeding started [duration (weeks)]	100 × absolute incorporation* (%)	
			Cocaine	Cuscohygrine
1	DL-[5-^{14}C]Ornithine	9 April (4)	2.9	0.86
2	DL-[5-^{14}C]Ornithine	3 July (4)	3.9	0.43
3	DL-[5-^{14}C]Ornithine	20 November (5)	0.21	0.086
4	Sodium [1-^{14}C]acetate	21 October (2)	0.11	0.013
5	Sodium [1-^{14}C]acetate	4 March (2)	3.1	0.45

*Absolute incorporation = $\frac{\text{total activity (dpm) found in the isolated alkaloid}}{\text{total activity (dpm) administered to leaves}}$.

acid, representing the activity at C-3. The activities of these degradation products of cocaine are recorded in Table 2. The distribution of ^{3}H and ^{14}C in the cocaine is illustrated in Fig. 4. It is seen that the bulk of the ^{14}C activity is located at C-3 and C-9, in accord with the formation of [1, 3-^{14}C]acetoacetate from [1-^{14}C]acetate, and subsequent incorporation as previously described. The somewhat higher activity at C-3 may be significant, and can be rationalized on the basis of C-4 and C-3 being the 'end' acetate unit (as acetyl coenzyme A) whilst C-2 and C-9 are introduced later (as malonyl coenzyme A) [13]. A significant amount of activity was detected at the bridgehead carbons. This result is consistent with the entry of [1-^{14}C]acetate into the Krebs cycle with subsequent labeling of α-ketoglutaric acid at the C-1 and C-5 positions. Glutamic acid and ornithine derived from this keto acid will also be labeled at the C-1 and C-5 positions. Since it has been previously established that [5-^{14}C]ornithine labels C-1 and C-5 of cocaine equally, the [1-^{14}C]acetate will also label these positions equally. The lower activity found at the bridgehead carbons is consistent with acetate being metabolically more remote (more biochemical steps) from these positions than from the four-carbon unit derived from acetoacetate. A similar distribution of activity was found in tropine derived from [1-^{14}C]acetate [14, 15] (81% at C-3, 19% at C-5). Although almost all of the tritium was located in the *para*-position of the benzoic acid moiety a significant amount (2.5%) resided in the tropane skeleton. Most of this is apparently at C-3 since tropinone, and subsequent degradation products, were devoid of tritium. Tritium presumably enters this position when the proposed intermediate **10** undergoes reduction, tritium having entered the general metabolic pool as water by the catabolism of the [4-^{3}H]phenylalanine.

The labeled cuscohygrine was degraded by reaction with phenyl lithium to yield 1,3-bis-(1′-methyl-2′-pyrrolidyl)-2-phenyl-2-propanol (**38**). It has been shown that the cuscohygrine isolated from *E. coca* is a mixture of *meso*- and (*RS*)-forms [16]. There are thus four theoretically possible stereoisomers of **38** (2′*S*,2*R*,2″*R*; 2′*S*,2*S*,2″*R*; 2′*S*,2″*S*; 2′*R*,2″*R*), the first two being derived from the *meso*-form, and the other two from the optically active forms. In the present work material, apparently homogeneous (by TLC), mp 61–62° was obtained. Previous workers have reported mps of 118–120° [2] and 63–64° [17] for this compound formed from cuscohygrine isolated from *Scopolia lurida* and *Atropa belladonna*, respectively. Oxidiation of **38** with permanganate yielded benzoic acid representing the activity at C-2 of cuscohygrine. The activity found at this position was 67%, and it is presumed that the rest of the activity is located at the α-positions of the pyrrolidine rings of this alkaloid. The distribution of activity between C-2 and these positions is the same as that found in cocaine, comparing the activity at C-3 (48%) with that at the bridgehead carbons (9%), in accord with a common biosynthetic origin for the two alkaloids. Somewhat surprisingly the cuscohygrine isolated from *A. belladonna* which had been fed [1-^{14}C]acetate was found to have 98% of its activity at C-2 [17].

In the present study the cocaine and cuscohygrine were isolated from the leaves of *E. coca* which were removed from the plant two weeks after the initial feeding. New leaves appeared on the plants a few days after this harvest and they were removed 10 weeks after the initial feeding. The cocaine and cuscohygrine obtained from the second harvest had much lower specific activities (see Experimental). Even though the activity of the cocaine was quite low, it was possible to carry out a partial degradation, and the alkaloid was still labeled at specific positions: 98% of the tritium being at the *para*-position of the benzoic acid moiety and 74% of the ^{14}C was located in the ecgonine residue.

EXPERIMENTAL

General. Mps are corr. MS were determined by Thomas L. Guggenheim on an AE1-30 spectrometer. ^{13}C NMR were determined on a Nicolet 300 spectrometer in $CDCl_3$ with TMS as an int. standard by Dr. S. Philson. Radioactive materials were assayed in a Nuclear Chicago Mark II liquid scintillation counter using dioxane–EtOH as a solvent with the usual scintillators [18]. The *E. coca* plants were cultivated in a greenhouse, the original seeds having been obtained from Dr. Fernandez Gabieses, University of San Marcos, Lima, Peru, in 1968.

Administration of [1-^{14}C]*acetate and* DL-[4-^{3}H]*phenylalanine to* E. coca *and isolation of the alkaloids.* In the initial feeding (expt 4, Table 1) sodium [1-^{14}C]acetate (Amersham) (41 mg, 2.0 × 10^{9} dpm) dissolved in H_2O (10 ml) containing Tween 80 (30 mg) was administered to eight *E. coca* plants (2–6 years old) by painting on the leaves (during three days). Two weeks after the initial feeding the leaves (230 g) were removed and extracted as previously described [1] yielding cocaine (366 mg, 1.79 × 10^{4}

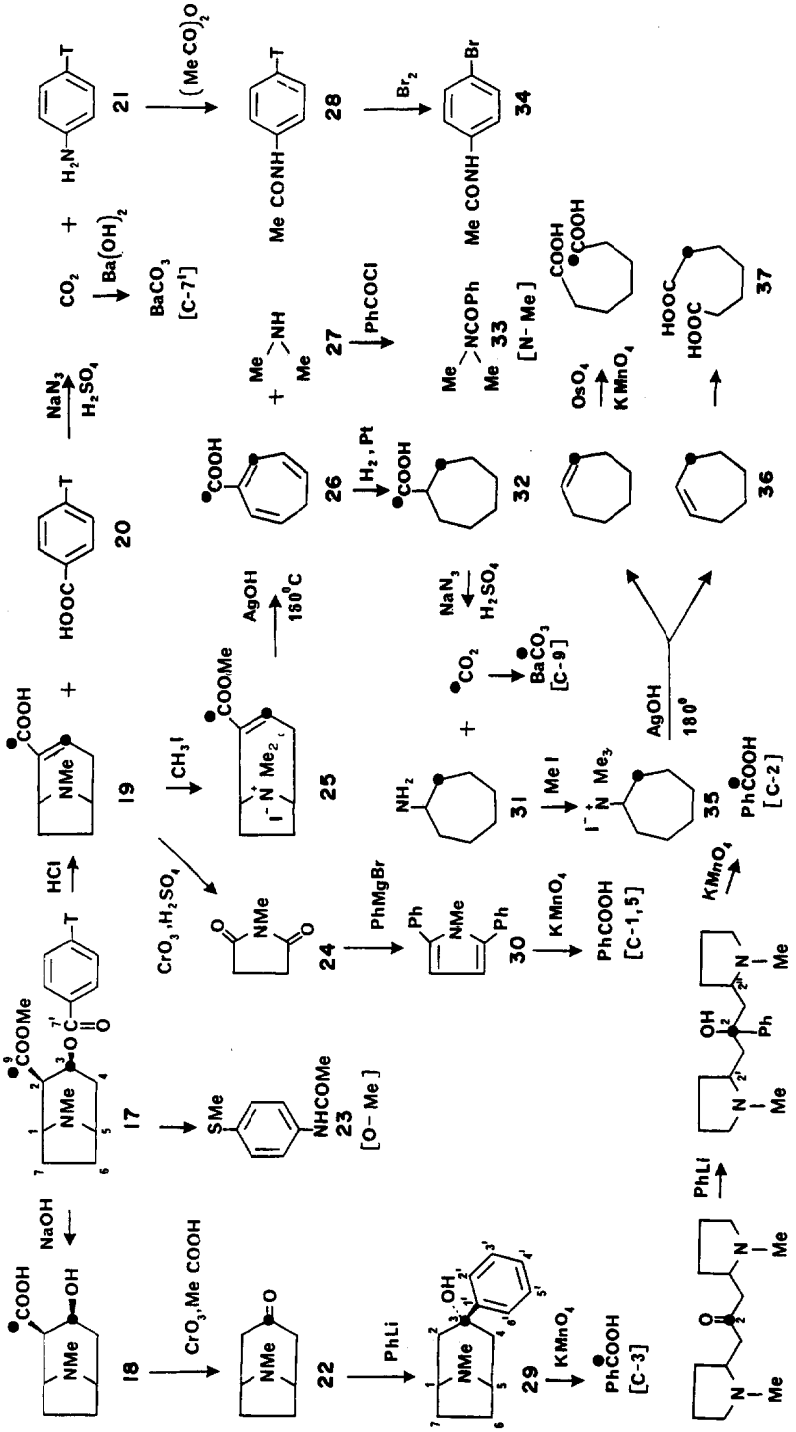

Fig. 3. Degradation of cocaine and cuscohygrine to determine the location of ^{14}C and ^{3}H.

Table 2. Activities of the degradation products of cocaine and cuscohygrine derived from [1-^{14}C]acetate and [4-^{3}H]phenylalanine

	^{14}C-activity		^{3}H-activity	
	Sp. act. (10^{-5} dpm/mmol)	RSA*	Sp. act. (10^{-6} dpm/mmol)	RSA*
Cocaine hydrochloride	8.26	100	9.42	100
Benzoic acid† (**20**)	0.22	2.7	9.12	97
Acetanilide (**28**)	0.16	1.9	9.18	97
p-Bromoacetanilide (**34**)	0.17	2.1	0.063	0.7
Barium carbonate (C-7′)	0.05	0.6	—	—
Ecgonidine hydrochloride (**19**)	7.94	96	0.23	2.4
Cycloheptane carboxylic acid (**32**)	7.76	94	0.22	2.3
Anilide of **32**	7.73	94	0.22	2.4
N-Benzoyldimethylamine (**33**)	0.09	1.1	< 0.02	< 0.2
Barium carbonate (C-9)	3.18	38	—	—
Trimethylcycloheptyl-ammonium iodide (**35**)	5.01	61	0.23	2.4
Pimelic acid (**37**)	5.10	62	0.24	2.5
Methyl-(*p*-acetamidophenyl)sulfide (**23**)	0.045	0.5	0.006	<0.1
Ecgonine (**18**) hydrochloride	7.92	96	0.24	2.5
Tropinone (**22**)	5.12	62	0.015	0.16
3-Phenyltropine (**29**)	5.26	64	0.014	0.15
Benzoic acid (C-3)‡	3.96	48	—	—
N-Methylsuccinimide (**24**)	0.79	9.6	< 0.02	< 0.2
1-Methyl-2,5-diphenylpyrrole (**30**)	0.76	9.2	—	—
Benzoic acid (C-1, C-5)§	0.36	4.4	—	—
Cuscohygrine (**8**) dipicrate	2.75	100	0.012	—
1,3-Bis(1′-methyl-2′-pyrrolidyl)-2-phenyl-2-propanol (**38**)	2.72	99	0.01	—
Benzoic acid (C-2)‖	1.85	67	—	—

*RSA, Relative specific activity.
†Formed by the hydrolysis of cocaine.
‡Formed by the oxidation of 3-phenyltropine.
§Formed by the oxidation of 1-methyl-2,5-diphenylpyrrole.
‖Formed by the oxidation of **38**.

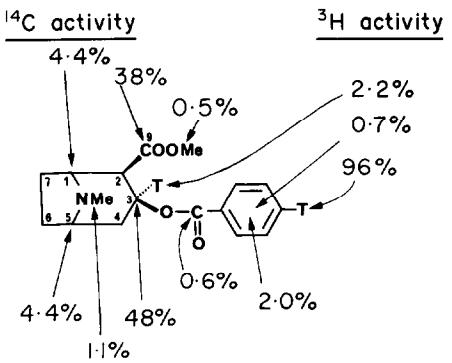

Fig. 4. Distribution of activity in cocaine labelled from [1-^{14}C]acetate and DL-[4-^{3}H]phenylalanine.

dpm/mM) and cuscohygrine (52 mg, 1.04×10^{4} dpm/mM). In expt 5 a mixture of sodium [1-^{14}C]acetate (41 mg, 3.50×10^{9} dpm) and DL-[4-^{3}H]phenylalanine [7] (38.9 mg, 3.52×10^{9} dpm) was administered as before to 11 *E. coca* plants (1–6 years old). Two weeks later the fresh leaves (205 g) afforded cocaine (359 mg, ^{14}C act. 8.26×10^{5} dpm/mM; ^{3}H act. 9.42×10^{6} dpm/mM) and cuscohygrine (128 mg, ^{14}C act. 2.72×10^{5} dpm/mM; ^{3}H act. 1.20×10^{4} dpm/mM). A second crop of leaves (140 g) obtained from these plants eight weeks later afforded cocaine (300 mg, ^{14}C act. 1.80×10^{4} dpm/mM; ^{3}H act. 2.81×10^{4} dpm/mM) and cuscohygrine (56 mg, ^{14}C act. 2.32×10^{4} dpm/mM; no ^{3}H activity).

Degradation of the labeled cocaine from expt 5. Dilutions of the cocaine and its degradation products were carried out when necessary. The sp. acts. recorded in Table 2 are calculated for undiluted material. Degradations not previously reported [1] are described.

(a) *Determination of activity on the O-methyl group.* *p*-Acetamidothiophenol (157 mg, 1 mM) was dissolved in 10% NaOH (0.4 ml) and the soln evaporated to dryness. The residue was dissolved in hexamethylphosphoramide (2.0 ml) and a soln of cocaine (120 mg, 0.4 mM) in the same solvent (0.5 ml) added. After stirring the mixture at room temp. for 18 hr, H_2O (10 ml) was added and extracted with Et_2O (3 × 30 ml). The dried (Na_2SO_4) extract was evaporated and the residue subjected to TLC on Si gel PF-254 (Merck) developed with $CHCl_3$–EtOH–NH_3 (90:10:1). The zone at R_f 0.8 (observed as a dark zone in UV) was extracted with MeOH. The residue obtained on evaporation of the MeOH was sublimed (120°, 10^{-3} mm) to yield methyl-(*p*-acetamidophenyl)sulfide (**23**) (18 mg, 25%) which afforded colourless plates from aq. EtOH, mp 130°, identical with an authentic specimen. MS (70 eV) *m/z* (rel. abundance): 181 (75), 139 (74), 124 (100).

(b) *Determination of activity at* C-3. Cocaine hydrochloride (513 mg) was stirred in 5% NaOH (20 ml) at 55° for 18 hr. The cooled soln was then made acidic with HCl and extracted with Et_2O to yield benzoic acid. The aq. layer was adjusted to pH 4.5 and the soln lyophilized. The residue was extracted with boiling EtOH to afford ecgonine (**18**) (220 mg) which was crystallized from a mixture of EtOH at Et_2O. Ecgonine (185 mg, 1 mM) was dissolved in HOAc (3 ml) and CrO_3 (150 mg) in H_2O (0.5 ml) added and the mixture stirred at 60° for 18 hr. The soln was cooled, made basic with NaOH and extracted with $CHCl_3$ (4 × 20 ml). The residue obtained on evaporation of the $CHCl_3$ extract (not dried) was dissolved in EtOH and picric acid (100 mg) was added. Tropinone (**22**) picrate (63 mg, 17%) mp 218–219° (dec.) separated out, identical with an authentic specimen. MS (70 eV) *m/z* (rel. abundance): 139 (20), 110 (7), 97 (11), 96 (34), 91 (16), 83 (18), 82 (100), 81 (53). The tropinone derived from this picrate was reacted with phenyl lithium as previously described [12] yielding 3-phenyltropine (**29**) mp 163–164° from EtOH–hexane. The previously recorded mps for this compound are 162–163° [12], 158–159° [19], 177–179° [2, 20]. MS (70 eV) *m/z* (rel. abundance): 217 (24), 200 (9), 159 (17), 97 (24), 96 (38), 83 (100), 82 (62); ^{13}C NMR (in $CDCl_3$): δ150.5 (C-1′ of phenyl), 127.9 (C-3′, C-5′), 126.9 (C-4′), 124.6 (C-2′, C-6′), 72.5 (C-3), 60.9 (C-1, C-5), 45.9 (C-2, C-4), 40.2 (N-Me), 25.6 (C-6, C-7). The 3-phenyltropine was oxidized with permanganate [12] to yield benzoic acid.

Degradation of cuscohygrine from expt 5. This degradation was carried out on undiluted alkaloid. Cuscohygrine dipicrate (228 mg, 0.334 mM) was dissolved in hot 2 M HCl (20 ml) and the soln extracted with Et_2O to remove picric acid. The aq. soln was made basic with NaOH and extracted with Et_2O (3 × 30 ml). The residue obtained on evaporation of the dried (Na_2SO_4) extract was redissolved in Et_2O (3 ml) and added to a soln of phenyl lithium (made from 0.14 g Li and 1.05 ml bromobenzene in 20 ml Et_2O) at 20°. After stirring for 20 hr the reaction mixture was cooled and 2 M HCl added. This acidic layer was extracted several times with Et_2O which was discarded. The aq. soln was made basic with NaOH and extracted with Et_2O. The residue obtained an evaporation of the dried (Na_2SO_4) extract was distilled (130°, 10^{-4} mm) to yield a colourless viscous oil (61 mg, 60%). This oil was dissolved in EtOH (1 ml) and H_2O (1 ml) was added when 1,3-bis(1′-methyl-2′-pyrrolidyl)-2-phenyl-2-propanol (**38**) separated as colourless needles. After drying *in vacuo* at 20° it had mp 61–62°. MS (20 eV) *m/z* (rel. abundance): 303 $[M+1]^+$ (1.6), 205 (54), 98 (53), 85 (43), 84 (100). This material (50 mg) was refluxed with $KMnO_4$ (0.4 g) in H_2O (5 ml) for 1.5 hr. The soln was decolorized wtih SO_2, acidified with HCl and extracted with Et_2O. The residue obtained on evaporation of the dried (Na_2SO_4) extract was sublimed (110°, 10^{-3} mm) affording benzoic acid (14 mg, 70%) and further purified by crystallization from hot H_2O.

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