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2,3-DEHYDROCOCAINE: NOT A DIRECT PRECURSOR OF COCAINE IN *ERYTHROXYLUM COCA*[1]

EDWARD LEETE[2]

Natural Products Laboratory,[3] Department of Chemistry, University of Minnesota, Minneapolis, Minnesota 55455

ABSTRACT.—(±)-3-[4-^{3}H]Benzoyloxy-2-[*carbonyl*-^{13}C, ^{14}C]carbomethoxy-2-tropene (2,3-dehydrococaine) was sythesized from Ba [^{13}C, ^{14}C]CO_3 and [4-^{3}H]benzoic acid. This labeled compound (^{3}H/^{14}C 0.65) was administered to *Erythroxylum coca* plants for 3 and 15 days. After these times, cocaine was isolated and was found to have a ^{3}H/^{14}C ratio quite different from the administered dehydrococaine (3.0 and 10.6 for the 3- and 15-day feeding experiments, respectively). Degradations of the cocaine indicated that tritium was all located in the 4-position of its benzoyl moiety. ^{13}C-nmr spectroscopy indicated enrichment of the carbomethoxy group of cocaine, consistent with the observed specific incorporation of the ^{14}C. From the 3-day feeding experiment unmetabolized dehydrococaine was isolated having the same specific activity and ^{3}H/^{14}C ratio as the administered compound. Also from this feeding experiment 2-carbomethoxy-3-tropinone was isolated having about ⅓ the specific activity (^{14}C) of the administered dehydrococaine and having a (−) optical rotation. These results indicate that cocaine is not formed by the direct reduction of dehydrococaine. The results are rationalized by proposing initial hydrolysis of the dehydrococaine to benzoic acid and 2-carbomethoxy-3-tropinone. The latter compound is then reduced to methyl ecgonine, which is then esterified with benzoic acid to yield cocaine. The change in the ^{3}H/^{14}C ratio is due to the fact that only the (+) enantiomer of 2-carbomethoxy-3-tropinone is converted to cocaine, and that different pool sizes of the nonlabeled intermediates exist in the plant.

It was recently (1) established that 2-carbomethoxy-3-tropinone [**1**] is converted to cocaine [**6**] in plants of *Erythroxylum coca* Lamarck (Erythroxylaceae). In particular, it was shown that the *O*-methyl group (labeled with ^{3}H) of this compound was incorporated with the same effficiency as a ^{14}C label at C-9. This result indicated that **1** is an advanced intermediate in the biosynthesis of cocaine, and Figure 1 illustrates two possible routes whereby this could occur. Route A involves a stereospecific reduction of **1** to yield methyl ecgonine [**2**] which is then converted to cocaine, possibly by reaction with benzoyl coenzyme A [**4**]. Route B involves the benzoylation of the enol of 2-carbomethoxy-3-tropinone [**3**] to yield 3-benzoyloxy-2-carbomethoxy-2-tropene [**5**], conveniently named 2,3-dehydrococaine. A stereospecific (*cis*) hydrogenation of this enol ester would then yield cocaine. Compound **5** was first described by Willstätter (2) and its structure established unequivocally by spectroscopic methods (3). Even though it has not been possible to convert **5** to cocaine by a variety of chemical reducing agents (3), it seemed worthwhile to examine it as a potential biochemical intermediate between **1** and cocaine.

Accordingly, 3-[4-^{3}H]benzoyloxy-2-[*carbonyl*-^{13}C, ^{14}C]carbomethoxy-2-tropene was prepared by the method illustrated in Figure 2. ^{13}C was introduced into this compound so that its incorporation could hopefully be monitored by ^{13}C-nmr spectroscopy. Reaction of a mixture of Ba [^{13}C] and [^{14}C]CO_3 with aqueous $AgNO_3$ gave a quantitative yield of Ag_2 [^{13}C, ^{14}C]CO_3 (4). Reaction of this carbonate with methyl iodide in

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[2]Member of the Editorial Advisory Board of the *Journal of Natural Products* (*Lloydia*) since 1984.

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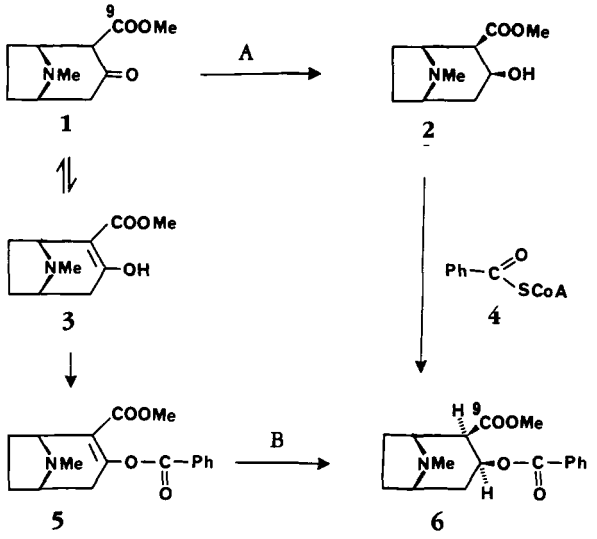

FIGURE 1. Two hypothetical biosynthetic routes to cocaine

Et_2O yielded dimethyl [*carbonyl*-^{13}C, ^{14}C]carbonate [**8**]. Reaction of this ester with 3-tropinone [**7**] in cyclohexane in the presence of sodium hydride yielded [9-^{13}C, ^{14}C]-2-carbomethoxy-3-tropinone [**1**] (5,6). [4-^{3}H]-Benzoic acid [**10**] was obtained by the oxidation of [4-$_3$H]phenylalanine [**11**] (7) with $KMnO_4$. [4-^{3}H]Benzoyl chloride [**9**] obtained by reaction with thionyl chloride was reacted with **1** as previously described (3) in the presence of pyridine to yield **5**. The ^{13}C-nmr spectrum of the labeled 2,3-dehydrococaine [**5**] showed the expected enhancement of the signal at 164.9 ppm (C-9), and the natural abundance signal at 121.6 ppm (C-2) was split into a doublet ^{1}J=75.5 Hz due to the contiguous highly enriched carbon. This labeled material, which was racemic, had a ^{3}H/^{14}C ratio of 0.65 and was administered [by leaf painting (8)] to *E.*

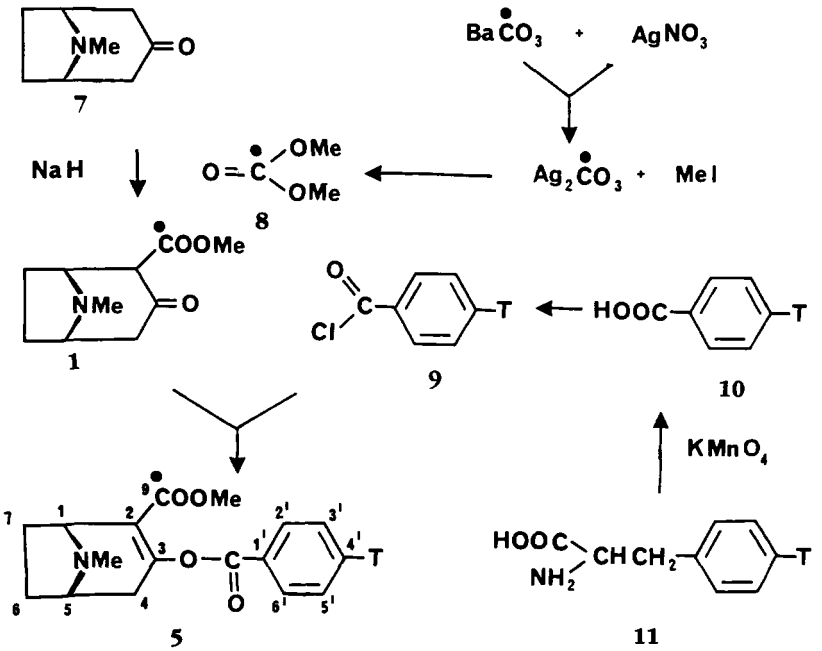

FIGURE 2. Synthesis of labeled 2,3-dehydrococaine

coca plants (3 to 4 years old) as a solution in dilute HOAc containing a little Tween 80. Two feeding experiments were carried out for 3 and 15 days. The cocaine was then isolated from the leaves of the plants as previously described (8), except that a chromatographic system was used that gave a clean separation of cocaine and dehydrococaine (see Experimental section).

The cocaine isolated from the 3- and 15-day feeding experiment had $^{3}H/^{14}C$ ratios of 3.0 and 10.6 respectively, quite different from that of the administered dehydrococaine. The specific labeling of the benzoyl group with tritium was established as previously described (7) by chemical degradation. The benzoic acid obtained by hydrolysis of the cocaine was subjected to a Schmidt reaction affording aniline. The was acetylated to yield acetanilide, which was brominated to give *p*-bromoacetanilide, almost devoid of tritium, thus establishing that essentially all the tritium was located in the *para* position of the benzoic acid. The location of the ^{13}C (at the same position as ^{14}C) was established by ^{13}C-nmr spectroscopy. Figure 3 illustrates the carbonyl region of the ^{13}C-nmr spectra of natural and the labeled cocaine from the 15-day feeding experiment. The specific incorporation of ^{14}C into this labeled cocaine was 0.03%. The expected enhancement of the signal at 175.8 ppm due to C-9 in the ^{13}C-nmr spectrum of the enriched cocaine was 0.30×91% (27%) inasmuch as the ^{13}C enrichment at C-9 in the dehydrococaine was 91%. Figure 3 indicates that the labeled cocaine had exactly the expected enhancement at C-9. The peak heights of the two carbonyl groups are normalized; the peak height of the benzoyl carbonyl group is 80% that of the C-9 carbonyl group in natural cocaine.

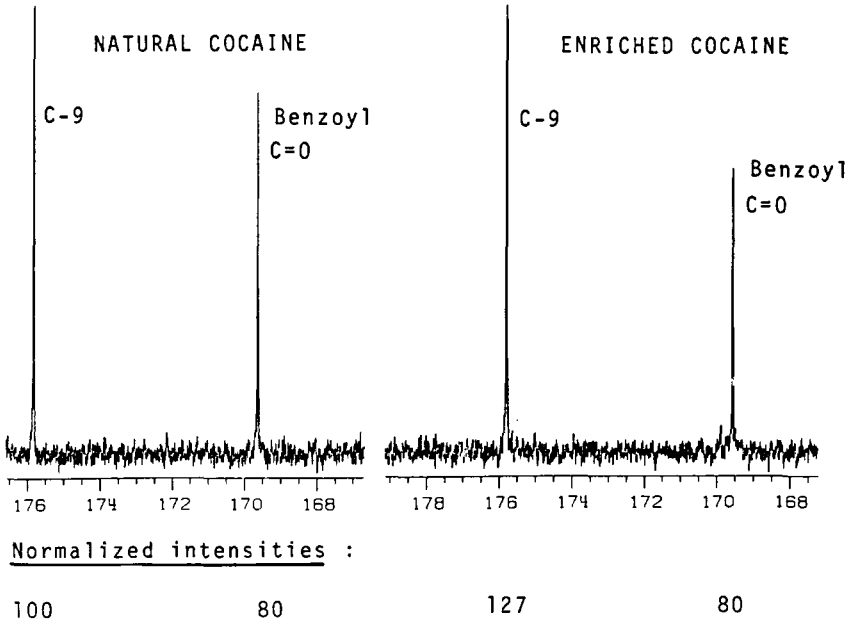

FIGURE 3. ^{13}C-nmr spectra of natural and enriched (from the 15-day feeding) cocaine HCl (carbonyl region)

Degradations and ^{13}C-nmr spectroscopy of the cocaine isolated from the 3-day feeding experiment also indicated specific labeling with ^{3}H and ^{13}C. In this experiment, in contrast to the 15-day experiment, the crude alkaloid fraction revealed on tlc the presence of dehydrococaine. This was isolated and found to have the same $^{3}H/^{14}C$ ratio (0.65) as the administered compound. Also its specific activity was the same as material fed to the plant. If dehydrococaine were indeed an intermediate in the biosynthesis of

cocaine, one would have expected some dilution with reduction in specific activity of the reisolated material. It was also possible to isolate 2-carbomethoxy-3-tropinone from this 3-day feeding experiment. This material, as expected, contained no ^{3}H, however its ^{14}C specific activity (3.88×10^{7} dpm/mmol) was less than that of the administered dehydrococaine (1.08×10^{8} dpm/mmol). It was found to be optically active $[\alpha]^{25}D$ $-6.7°$. Findlay (5) found that 2-carbomethoxy-3-tropinone obtained by the oxidation of methyl ecgonine derived from the natural (−)-cocaine was dextrorotary ($[\alpha]^{20}D$ $+18.3°$).

It is thus concluded that the administered dehydrococaine, which was racemic, is hydrolyzed in the plant to afford benzoic acid and (±)-2-carbomethoxy-3-tropinone. The (+)-enantiomer of this compound is then reduced to methyl ecgonine which is then benzoylated to afford cocaine. If there was no dilution of [^{3}H]benzoic acid and [^{14}C]-2-carbomethoxy-3-tropinone, the resultant cocaine would have a $^{3}H/^{14}C$ ratio of 1.30. Because the observed ratios in the two feeding experiments were higher than this, it is presumed that pool size non-labeled 2-carbomethoxy-3-tropinone was higher than that of benzoic acid.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points are corrected. Radioactivity measurements were carried out in a Tractor Analytic Mark III liquid scintillation counter. Samples (assayed in duplicate) were dissolved in H_2O or EtOH (1 ml), which was then diluted with liquid scintillation solution (10 ml). The liquid scintillation solution was made by dissolving naphthalene (50 g), 2,5-diphenyloxazole (3.5 g), and 1,4-(5-phenyloxazol-2-yl)benzene (0.25 g) in spectroscopic grade dioxane (500 ml). The backgrounds in the ^{14}C and ^{3}H channels were 8 and 14 dpm, respectively. In unquenched samples the efficiency of counting in the ^{14}C and ^{3}H channels was 60 and 45%, respectively, and the spill-over of ^{14}C into the ^{3}H channel was 10%. Gc 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 microns thick) internal diameter 0.31 mm, using the following instrument parameters: He flow rate 1 ml/min, injection temp 280°, initial oven temp 50°, equilibration time 4 min, rate of temp increase 30°/min, oven maximum 250°. Under these conditions the following retention times (min) were obtained: 2,3-dehydrococaine 18.39, cocaine 18.72, *cis*-cinnamoylcocaine 22.75, *trans*-cinnamoylcocaine 27.30. The two latter compounds, minor alkaloids in *E. coca* (9), are readily assayed by this method. ^{13}C-nmr spectra are reported in ppm from TMS. Spectra were obtained by Dr. Stephen B. Philson in a Nicolet 300 spectrometer operating at 75.5 MHz. Mass spectra were determined by Dr. Edward Larka on an AEI-30 (Kratos) spectrometer.

DIMETHYL [*carbonyl*-^{13}C, ^{14}C]CARBONATE [**8**].—Ba [^{13}C]CO_3 (91.7% ^{13}C) (20 g) and Ba [^{14}C]CO_3 (nominal activity 5 mCi, 53 mCi/mmol, 0.02 g) were added to a solution of $AgNO_3$ (34.4 g) in distilled H_2O (60 ml) in a 250 ml conical flask protected from light with Aluminum foil. The mixture was sonicated in ice H_2O for 30 min and then shaken at 25° for 30 min. The mixture was then filtered; the yellow residue was washed with H_2O, EtOH, and Et_2O. The residue was dried in vacuo at 25° for 18 h. This dried Ag_2CO_3 (28 g) was suspended in Et_2O (100 ml), and methyl iodide (20 ml) was added to this mixture in a conical flask closed with a cork. The mixture was kept at 22° for 5 days with occasional shaking. The filtered reaction mixture was then distilled with a short Vigreaux fractioning column affording dimethyl [*carbonyl*-^{13}C, ^{14}C]carbonate, bp 89-90° (7.6 g, 82%) ir (neat) 1710 cm^{-1} ($^{13}C=O$) compared with 1750 cm^{-1} for $^{12}C=O$ in unenriched dimethyl carbonate, ^{13}C nmr ($CDCl_3$) 156.6 (C=O), 54.8 ppm (CH_3).

[9-^{13}C, ^{14}C]-2-CARBOMETHOXY-3-TROPINONE [**1**].—Sodium hydride (75 mmol, 3.0 g of a 60% suspension in mineral oil) was washed (by decantation) with dry cyclohexane. The dimethyl [*carbonyl*-^{13}C, ^{14}C]carbonate (3.5 g, 38.5 mmol) was added to a suspension of this NaH in cyclohexane (40 ml) in a N_2 atmosphere, the mixture being stirred with a magnetic stirrer. 3-Tropinone (5.5 g, 39 mmol) and MeOH (0.15 ml) were added, and the mixture was refluxed in an oil bath for 4 h. The mixture was then stirred at 22° for 18 h. H_2O and ice (50 g) were then added to the gray semi-solid mass, and the brown aqueous layer was separated. The cyclohexane was then washed with more H_2O. NH_4Cl (10 g) was added to the combined aqueous layers which then extracted with $CHCl_3$ (5×100 ml). The residue obtained on evaporation of the dried (Na_2SO_4) extract was dissolved in Me_2CO (50 ml) and H_2O (5 ml) added. On cooling to −20° crystals (3.9 g) separated. Sublimation at 110° (10^{-4} mm Hg) afforded 2-[*carbonyl*-^{13}C, ^{14}C]carbomethoxy-3-tropinone as a white solid (3.5 g, 17.7 mmol 46%) mp 101°, ^{14}C activity: 1.08×10^{8} dpm/mmol.

[4-^{3}H]BENZOIC ACID [**10**].—*RS*-[4-^{3}H]Phenylalanine (165 mg, 1 mmol) (1.1×10^9 dpm/mmol), NaOH (100 mg), and $KMnO_4$ (600 mg) were dissolved in H_2O (50 ml), and the mixture was refluxed for 18 h. The filtered reaction mixture was acidified with HCl and extracted with Et_2O in a continuous extractor. The residue obtained on evaporation of this extract was sublimed (90°, 10^{-3} mm Hg) affording [4-^{3}H]benzoic acid (110 mg, 90%). 1.11×10^9 dpm/mmol.

(*RS*)-3-[4-^{3}H]BENZOYLOXY-2-[*carbonyl*-^{13}C, ^{14}C]CARBOMETHOXY-2-TROPENE (2,3-DEHYDROCOCAINE) [**5**].—A mixture of [4-^{3}H]benzoic acid (0.9 g, 7.4 mmol, 7.0×10^7 dpm/mmol) and thionyl chloride (1 ml) was refluxed for 1 h. The solution was then evaporated at 25°, and the residual benzoyl chloride was dissolved in pyridine (2.5 ml) and added to a solution of the sublimed 2-[*carbonyl*-^{13}C, ^{14}C]carbomethoxy-3-tropinone (0.935 g, 4.7 mmol, 1.08×10^8 dpm/mmol) in pyridine (2.5 ml); the mixture and stirred at 25° for 24 h. Ice H_2O (~20 g) was then added and the mixture adjusted to pH 5.8 with HCl. This solution was extracted with Et_2O, which was then discarded. The residual aqueous solution was evaporated to dryness in vacuo. The residue was made basic with 10% aqueous $NaHCO_3$ and extracted with $CHCl_3$. The residue obtained on evaporation of the dried (Na_2SO_4) extract was subjected to radial chromatography on the Chromatotron®. Silica gel GF (4 nm thick) was used as the absorbant, developing initially with a mixture of $CHCl_3$-EtOAc-conc. NH_3 (50:50:1). Later 10% MeOH was added to this solvent mixture. A good separation of **5** and unreacted 2-carbomethoxy-3-tropinone (slower moving) was achieved. The fractions containing **5** were evaporated , and the residue was crystallized from hexane affording beautiful rhombic needles (550 mg, 1.82 mmol, ^{14}C: 1.08×10^8 dpm/mmol, ^{3}H/^{14}C 0.65) mp 76-77° [lit (2,3) mp 75-76°] ms *m/z* (rel. int.) 302 (M^+) (14), 105 (PhCO) (100). The ratio of the 302/301 peaks indicated a ^{13}C enrichment of 91%. ^{13}C nmr ($CDCl_3$) 164.9 (C-9, enriched), 164.3 (PhCO), 153.8 (C-3) 133.4 (C-4′), 130.1 (C-2′,6′), 129.4 (C-1′), 128.6 (C-3′,5′), 121.1, 120.1 (C-2, $^1J_{2,9}$=75.5 Hz), 59.3 (C-1), 57.3 (C-5), 51.3 (OMe), 35.5 (NMe), 35.0 (C-6), 33.7 (C-7), 30.3 ppm (C-4). Assignments were made by off-resonance decoupling, DEPT pulse sequences, and by comparison with model compounds: cocaine (6) and 2-tropene (10). Gc indicated that the labeled dehydrococaine was >98.6% pure. Tlc on silica gel Pf 254 developing with a mixture of $CHCl_3$-EtOAc-conc. NH_3 (50:50:1) afforded the following Rf values: cocaine (0.80) 2,3-dehydrococaine (0.53, 2-carbomethoxy-3-tropinone (0.10), these compounds being detected by uv or by exposure to I_2 vapor.

ADMINISTRATION OF 2,3-DEHYDROCOCAINE TO *ERYTHROXYLUM COCA* AND ISOLATION OF COCAINE.—The following are the experimental details of the feeding lasting 3 days. The labeled 2,3-dehydrococaine (60.9 mg, ^{14}C activity 1.08×10^8 dpm/mmol, total ^{14}C: 2.18×10^7 dpm, ^{3}H/^{14}C 0.65) was dissolved in H_2O (8 ml) containing Tween 80 (0.1 ml) and HOAc (0.03 ml), and the solution painted on the leaves of two *E. coca* plants (3 to 4 years old) growing in soil in a greenhouse. After 3 days, the leaves (fresh wt. 55 g) were removed and chopped up with $CHCl_3$ (1 liter) and 10% Na_2CO_3 (100 ml) in a Waring Blender. After filtration the $CHCl_3$ layer was evaporated and the residue dissolved in Et_2O, which was then extracted with 0.5 N HCl (3×100 ml). This acid extract was made basic with Na_2CO_3 and extracted with $CHCl_3$ (4×200 ml). Evaporation of this dried (Na_2SO_4) extract yielded the crude alkaloids (^{14}C: 1.23×10^7 dpm, 56% of ^{14} C activity fed. Preparative tlc was carried out using the $CHCl_3$-EtOAc-NH_3 (50:50:1) solvent system. Radioactive assay of the plate indicated the following distribution of the ^{14}C activity with the ^{3}H/^{14}C ratio of each zone indicated in parenthesis: cocaine 4.3% (3.0), dehydrococaine 84% (0.65), 2-carbomethoxy-3-tropinone 12% (no ^{3}H). These zones were extracted with MeOH affording cocaine, crystallized to constant activity as it HCl salt (52 mg, ^{14}C: 9.84×10^5 dpm/mmol, specific inc. 0.91% ^{3}H/^{14}C 3.0), dehydrococaine, crystallized from C_6H_6/hexane (12 mg, ^{14}C: 1.07×10^8 dpm/mmol, ^{3}H/^{14}C 0.65), and 2-carbomethoxy-3-tropinone (5.0 mg, purified by sublimation, ^{14}C: 3.88×10^7 dpm/mmol, $[\alpha]^{25}D$ −6.7°, $[\alpha]^{25}_{365}$ −22.2°, MeOH).

The plants which were allowed to grow for 15 days after feeding the same amount of the labeled dehydrococaine afforded from the leaves (fresh wt. 110g) cocaine HCl (88 mg, ^{14}C: 3.27×10^5 dpm/mmol, specific inc. 0.30%, ^{3}H/^{14}C 10.6). The crude alkaloids from this experiment did not contain detectable amounts of dehydrococaine. The crude cocaine before crystallization of its HCl salt consisted of (by gc analysis) 89% cocaine, 3% *cis*-cinnamoylcocaine, and 8% *trans*-cinnamoylcocaine.

DEGRADATION AND NMR SPECTROSCOPY ON THE LABELED COCAINE.—The ^{13}C nmr of the natural and enriched (from the 15-day feeding experiment) cocaine HCl (50 mg in 0.4 ml of D_2O in a 5 mm tube) were obtained using the following parameters: a spectral window of 847 Hz spanning the carbonyl region (167-179 ppm), 3.18 sec acquisition time, 5.0 sec delay time between 45° pulses, number of acquisitions 328.

Hydrolysis of the labeled cocaine HCl (^{14}C: $3.27=10^5$ dpm/mmol, ^{3}H: $3.46\times10_6$ dpm/mmol) with HCl (8) yielded ecgonidine HCl (^{14}C: 3.16×10^5 dpm/mmol, ^{3}H: 6.4×10^3 dpm/mmol) and benzoic acid (^{14}C: negligible, ^{3}H: 3.36×10^6 dpm/mmol). The benzoic acid was degraded (7) to yield acetanilide (^{3}H: 3.37×10^6 dpm/mmol) and *p*-bromoacetanilide (^{3}H: 1.65×10^4 dpm/mmol).

Degradation of the cocaine HCl isolated from the 3-day feeding experiment indicated similar specific labeling of its benzoyl moiety with tritium.

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