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The Synthesis of Deuterium-Labelled Cocaine, Cocaethylene and Metabolites

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SUMMARY

We describe the syntheses of benzoylecgonine (1,1,1-$^2H_3$)methyl ester [($^2H_3$)cocaine], ($^2H_5$)benzoylecgonine, ($^2H_5$)benzoylecgonine methyl ester [($^2H_5$)cocaine], benzoylecgonine (2,2,2-$^2H_3$)ethyl ester [($^2H_3$)cocaethylene], ($^2H_5$)benzoylecgonine ethyl ester [($^2H_5$)cocaethylene], ($^2H_5$)benzoylecgonine (2,2,2-$^2H_3$)ethyl ester [($^2H_8$)cocaethylene], ecgonine (1,1,1-$^2H_3$)methyl ester, ecgonine (2,2,2-$^2H_3$)ethyl ester, ecgonine (1,1,2,2,2-$^2H_5$)ethyl ester and anhydroecgonine (1,1,1-$^2H_3$)methyl ester. ($^2H_5$)Cocaine and ($^2H_3$)cocaethylene have been administered to human subjects to study the interactions of cocaine and ethanol. The other eight compounds were utilized as analytical standards or internal standards for GC-MS quantitation of cocaine and its metabolites in biological fluids.

Key Words: Cocaine, cocaethylene, metabolites, deuterium labelling

INTRODUCTION

Recreational use of cocaine without the concomitant use of alcohol is rare.[1-4] The concurrent use of cocaine and ethanol is associated with greater risk of sudden death than cocaine alone.[5] Ethanol alters the biotransformation of cocaine (**1 a**), resulting in the transesterification to a metabolite, cocaethylene (benzoylecgonine ethyl ester, ethyl cocaine, **1 f**, Fig. 1).[3,6] Cocaethylene was found in substantial amounts in biological fluids and tissues of people who died after combined use of

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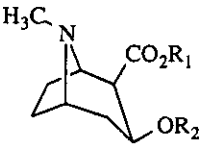
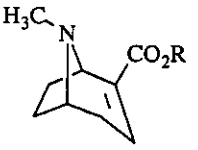
cocaine and ethanol,[7] and in urine of patients ingesting large amounts of cocaine and ethanol together.[8] In animal, human, and *in vitro* studies, cocaethylene has pharmacologic effects similar to cocaine,[9-12] and cocaethylene formation may augment cocaine's reinforcing and toxic effects. No studies yet fully characterize the pharmacokinetics and metabolism of this interaction.





1 a, $R^1 = CH_3$, $R^2 = C_6H_5CO$
b, $R^1 = CH_3$, $R^2 = C_6D_5CO$
c, $R^1 = CD_3$, $R^2 = C_6H_5CO$
d, $R^1 = H$, $R^2 = C_6H_5CO$
e, $R^1 = H$, $R^2 = C_6D_5CO$
f, $R^1 = CH_2CH_3$, $R^2 = C_6H_5CO$
g, $R^1 = CH_2CD_3$, $R^2 = C_6H_5CO$
h, $R^1 = CH_2CH_3$, $R^2 = C_6D_5CO$
i, $R^1 = CH_2CD_3$, $R^2 = C_6D_5CO$
j, $R^1 = H$, $R^2 = H$
k, $R^1 = CH_3$, $R^2 = H$
l, $R^1 = CD_3$, $R^2 = H$
m, $R^1 = CH_2CH_3$, $R^2 = H$
n, $R^1 = CH_2CD_3$, $R^2 = H$
o, $R^1 = CD_2CD_3$, $R^2 = H$
2 a, R = H
b, $R = CH_3$
c, $R = CD_3$

Fig. 1. Analogs of cocaine and anhydroecgonine

For studies of the metabolic disposition of cocaine and alcohol, cocaine and cocaethylene were required in sufficient purity and quantity for administration of pharmacologically relevant doses to human subjects. A potentially confounding factor in studies of abused drugs in a laboratory setting is the possibility of illicit drug use by research subjects prior to experimental sessions. Therefore, to accurately determine the pharmacokinetics of cocaine and quantitative aspects of its metabolism in drug abusers, we have employed stable isotope methodology. Using analytical techniques that employ mass spectrometry, the labelled drug and metabolites derived from the labelled drug can be distinguished from the illicit drug and its metabolites. Stable isotope methods have frequently been used for determination of metabolic pathways and pharmacokinetics in both acute and chronic dosing conditions. In order to study the metabolism of cocaine to cocaethylene and to its major metabolite benzoylecgonine (**1 d**), the phenyl moeity was chosen for the introduction of five deuterium atoms. To determine the pharmacokinetic parameters of cocaethylene in

the presence of deuterium-labelled cocaine and ethanol, cocaethylene was synthesized with three deuterium atoms located on the ethyl group (**1 g**, Fig. 1). Therefore, following simultaneous administration of ($^2H_5$)cocaine (**1 b**), ($^2H_3$)cocaethylene (**1 g**) and ethanol, ($^2H_5$)cocaethylene (**1 h**) derived from ($^2H_5$)cocaine (**1 b**) and ethanol can be distinguished from administered ($^2H_3$)cocaethylene (**1 g**) and from unlabelled cocaethylene (**1 f**) derived from illicit drug use.

There appear to be only three reports in the literature that describe syntheses of deuterium-labelled cocaine and cocaine metabolites. Bosin et al.[13] prepared two deuterium-labelled analogs of cocaine: one labelled with deuterium in the 4-position of the phenyl moiety and a second labelled in the 3- and 5- positions. Jindal et al.[14, 15] described the syntheses of cocaine and several cocaine metabolites labelled on the *N*-methyl group as well as on the methyl ester group.[15] Preparation of cocaine randomly labelled with tritium using two methods has also been reported.[16]

In this paper, methods for the preparation of ($^2H_5$)cocaine (**1 b**) hydrochloride, labelled on the phenyl moiety, and ($^2H_3$)cocaethylene (**1 g**) fumarate, labelled on the ethyl group, in sufficient purity and quantity for carrying out large-scale pharmacologic and metabolic studies in humans, are described. Syntheses of various other deuterium-labelled analogs of cocaine and cocaethylene, their metabolites, and the cocaine pyrolysis product anhydroecgonine methyl ester (**2 b**),[17,18] labelled on the ester moieties, are also described.

RESULTS AND DISCUSSION

The key intermediates for the synthesis of the labelled compounds were benzoylecgonine (**1 d**), ecgonine (**1 j**), anhydroecgonine (**2 a**), and ecgonine methyl ester (**1 k**). These intermediates were all synthesized from cocaine (**1 a**). Benzoylecgonine (**1 d**) was obtained by hydrolysis of cocaine base under neutral conditions.[13] Heating cocaine under reflux with dilute HCl provided ecgonine (**1 j**), and heating under reflux with concentrated HCl yielded anhydroecgonine (**2 a**).[19] Ecgonine methyl ester (**1 k**) was prepared by esterification of ecgonine (**1 j**) with methanol and HCl.

($^2H_5$)Cocaine (**1 b**) was prepared by the condensation of ecgonine methyl ester (**1 k**) with ($^2H_5$)benzoyl chloride according to the method described by Bosin et al.[13] for the preparation of the 4-chlorophenyl- and 3,5-dichlorophenyl-substituted

analogs. This condensation was performed in benzene and required two moles of ($^2H_5$)benzoyl chloride. A drawback of the method is precipitation of the hydrochloride salts of both the product and starting material during the course of the reaction, limiting the yield to approximately 50%. An alternative method, requiring only a slight (10%) excess of the expensive ($^2H_5$)benzoyl chloride and carried out in chloroform stabilized with amylene hydrocarbons, and with triethylamine as an acid scavenger, maintains the reaction medium as a homogeneous solution and results in a yield approaching 95%. The new procedure is outlined in Fig. 2. The product was purified as the hydrochloride salt.

($^2H_3$)Cocaine (**1 c**) and ($^2H_3$)cocaethylene (**1 g**) were prepared by the reaction of (1,1,1-$^2H_3$)methan(^{2}H)ol and (2,2,2-$^2H_3$)ethan(^{2}H)ol, respectively, with the acid chloride of benzoylecgonine. These compounds were converted to the perchlorate salts for use as analytical standards. ($^2H_3$)Cocaethylene (**1 g**) was also converted to the pharmacologically acceptable fumarate salt and purified by recrystallization for administration to human subjects. ($^2H_5$)Cocaethylene (**1 h**) and ($^2H_8$)cocaethylene (**1 i**) were synthesized by the reaction of ethanol and (2,2,2-$^2H_3$)ethan(^{2}H)ol, respectively, with the acid chloride of ($^2H_5$)benzoylecgonine (**1 e**). The perchlorate salts

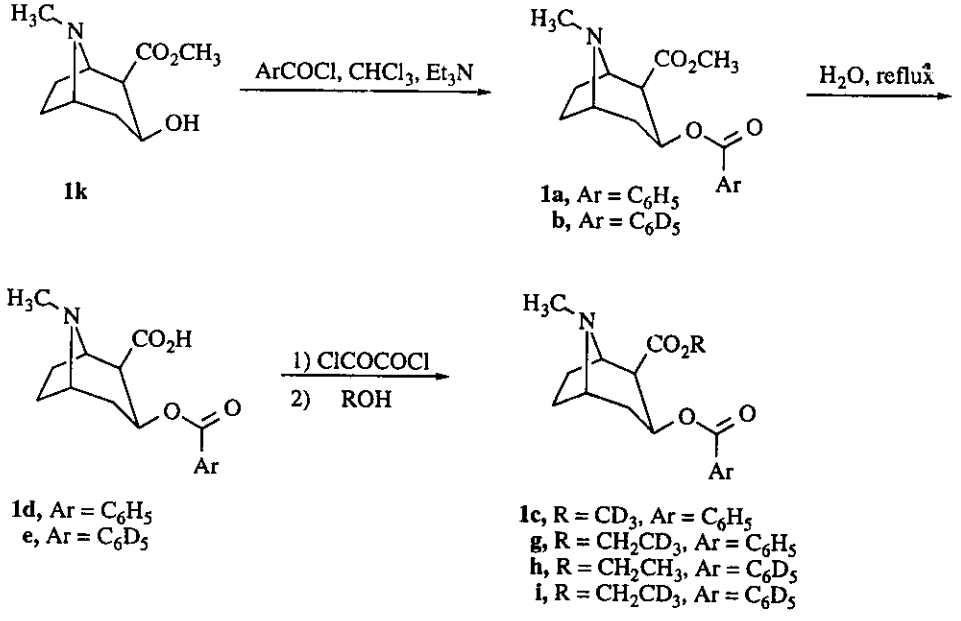


Fig. 2. Synthetic procedure for isotopically substituted cocaine, cocaethylene and benzoylecgonine

were prepared and utilized as analytical standards. The syntheses of these four compounds are also illustrated in Fig. 2.

Ecgonine (1,1,1-$^2H_3$)methyl ester (**1 l**), (2,2,2-$^2H_3$)ethyl ester (**1 n**) and (1,1,2,2,2-$^2H_5$)ethyl ester (**1 o**) were prepared by the reaction of ecgonine (**1 j**) with (1,1,1-$^2H_3$) methan(^{2}H)ol, (2,2,2-$^2H_3$)ethan(^{2}H)ol and (1,1,2,2,2-$^2H_5$)ethan(^{2}H)ol, respectively, saturated with anhydrous hydrogen chloride. These compounds were also converted to the perchlorate salts and used as analytical standards.

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Anhydroecgonine methyl ester (AEME, **2 b**) is a pyrolysis product of smoked cocaine (**1 a**) and is found in the urine of cocaine smokers.[17,18] We report the synthesis of anhydroecgonine (1,1,1-$^2H_3$)methyl ester [($^2H_3$)AEME, **2 c**], required as an internal standard for the quantitation of AEME (**2 b**) in biological fluids using GC-MS. This compound was prepared by the reaction of the acid chloride of anhydro-ecgonine (**2 a**) with (1,1,1-$^2H_3$)methan(^{2}H)ol and converted to the perchlorate salt.

EXPERIMENTAL

Materials. ($^2H_5$)Benzoic acid, 99 + atom % D, (2,2,2-$^2H_3$)ethan(^{2}H)ol, 99 atom % D, (1,1,2,2,2-$^2H_5$)ethan(^{2}H)ol, anhydrous, 99 atom % D and (1,1,1-$^2H_3$)methan(^{2}H)ol, 99.8 atom % D, were purchased from Aldrich (Milwaukee, WI, USA). Cocaine free base was obtained from Sigma (St. Louis, MO, USA). Cocaethylene fumarate was obtained from the National Institute on Drug Abuse, Drug Supply Program (Rockville, MD, USA). All other reagents and chemicals employed were either ACS or HPLC grade.

Ecgonine (1 j) hydrochloride. Cocaine (**1 a**) (5.06g, 14.9 mmol) was converted to ecgonine hydrochloride (3.29g, 14.9 mmol, 100%) by the method of Bell and Archer.[20]

Ecgonine methyl ester (1 k). Ecgonine (**1 j**) hydrochloride (33.0 g, 149 mmol) was dissolved in 1800 mL of methanol saturated with HCl gas, protected from atmospheric moisture and refluxed for 24 h. After cooling to room temperature, the

methanol was removed under reduced pressure and the residue dissolved in 500 mL of distilled water. This solution was saturated with potassium carbonate and rapidly extracted three times with 500 mL of diethyl ether. The combined extracts were washed with 500 mL of saturated sodium chloride, dried over sodium sulfate, filtered and evaporated under reduced pressure to yield 22.8 g (114 mmol, 77%) of a pale yellow oil.

($^2H_5$)Benzoyl chloride. ($^2H_5$)Benzoic acid (34.3 g, 270 mmol) was dissolved in 40 mL (65.2 g, 548 mmol) of thionyl chloride. After the addition of 260 mg (4.45 mmol) of sodium chloride, the mixture was protected from atmospheric moisture and refluxed overnight. After cooling to room temperature, the excess thionyl chloride was evaporated and the residue distilled at 81-82 °C/15 mm Hg to yield 36.1 g (248 mmol, 92%) of the colorless acid chloride.

($^2H_5$)Cocaine (1 b) hydrochloride. Ecgonine methyl ester (5.05 g, 25.3 mmol) was dissolved in 60 mL of amylenes-stabilized, anhydrous chloroform, treated with 7.80 mL (5.66 g, 56.1 mmol) of triethylamine and 3.24 mL (4.06 g, 27.9 mmol) of ($^2H_5$)benzoyl chloride dissolved in 15 mL of chloroform, protected from atmospheric moisture and refluxed for 24 h. The reaction mixture was cooled to room temperature, the solvent evaporated and the residue partitioned between 100 mL of ice-cold 0.5 M HCl and 100 mL of ether. The layers were separated and the aqueous phase was washed with ether (100 mL x 2) and diluted with 350 mL of 11% ammonium carbonate. Extraction of the precipitated ($^2H_5$)cocaine free base with ether (300 mL x 2) was followed by washing the combined extracts with saturated NaCl, drying over sodium sulfate, decolorizing with Norit, filtering and evaporating to yield 7.41 g (24.0 mmol, 94.9%) of ($^2H_5$)cocaine free base as viscous pale yellow oil which slowly crystallized. The material was converted to the hydrochloride salt and recrystallized from 2-propanol to give white, crystalline ($^2H_5$)cocaine hydrochloride, melting at 186-187 °C. Microanalysis: calculated for $C_{17}H_{17}D_5NO_4Cl$: C, 59.21; H + D, 6.43; N, 4.06; O, 18.56; Cl, 10.28. Found: C, 58.92; H + D, 6.64; N, 3.96; O, 18.28; Cl, 10.06. Label incorporation: D_1, 0.0%; D_2, 0.2%; D_3, 0.2%; D_4, 1.4%; D_5 98.2%.

Benzoylecgonine (1 d). Cocaine (1 a) was converted to benzoylecgonine (1 d) by a published method.[13]

($^2H_3$)Cocaethylene (1 g) base. To 0.50 g (1.39 mmol) of benzoylecgonine (1 d) tetrahydrate in a culture tube was added dropwise 2.5 mL of oxalyl chloride. The

mixture was allowed to stand 15 minutes, and the excess oxalyl chloride was evaporated. The tube was cooled in an ice bath, and (2,2,2-$^2H_3$)ethan(^2H)ol was added slowly, dropwise (exothermic). The tube was capped and let stand overnight at room temperature. Diethyl ether, 25 mL, was added portionwise, which led to crystallization of ($^2H_3$)cocaethylene hydrochloride. This was collected by filtration and air dried to give 0.53 g of white solid. The salt was dissolved in 2 mL of distilled water containing 2 drops of concentrated sulfuric acid. The solution was washed with 4 mL of diethyl ether, and basified by addition of concentrated aqueous ammonia. The mixture was extracted twice with 4 mL portions of diethyl ether and the combined extracts were dried over potassium carbonate and evaporated with a current of nitrogen to give 0.44 g (1.38 mmol) of ($^2H_3$)cocaethylene base as a white solid.

Purification of ($^2H_3$)cocaethylene base by column chromatography. The above base (0.99 g) was dissolved in 10 mL of a mixture of ethyl acetate-methanol-concentrated ammonia (85:5:1, v/v/v), and then applied to a 1.5 cm diameter column packed with 10 g of silica gel that had been washed with 25 mL of the above solvent mixture. The same solvent mixture was used to elute the product; 5 mL aliquots were collected and monitored by TLC. Those fractions (5-9) that contained the product were pooled and evaporated to give 0.9 g of a white solid. This was recrystallized from 5 mL of heptane-diethyl ether (9:1, v/v) to give 0.82 g (83% recovery) of white needles.

($^2H_3$)Cocaethylene (1 g) fumarate. ($^2H_3$)Cocaethylene base (0.82 g, 2.6 mmol) was dissolved in 10 mL of 2-propanol, to which was added 0.45 g (3.8 mmol) of fumaric acid. The mixture was heated on a hot plate until the fumaric acid dissolved. The 2-propanol was evaporated to give a white solid. To this was added 10 mL of ether, and the solid lumps were broken up using a glass rod. The product was filtered from solution and dried to give 1.11 g of white powder. The salt (0.99 g, 2.0 mmol) was recrystallized from 25 mL of 2-propanol. The product was collected by filtration, washed with 5 mL cold 2-propanol, then 5 mL cold 2-propanol-diethyl ether (1:1, v/v), and finally 5 mL of diethyl ether. The product was first air dried and then under high vacuum at 0.1 mm Hg for 1 h to give 0.60 g (1.2 mmol) of white crystalline powder, mp 144-145 °C. The microanalytical results were: Calculated for $C_{24}H_{26}D_3NO_{10}$: C, 58.30; H + D, 5.91; N, 2.83; O, 32.35. Found: C, 58.01; H + D, 5.95; N, 2.77; O, 32.55. TLC was carried out on silica gel using the same solvent

system (described above) for column chromatography (described above). No impurities were detected using UV or alkaline potassium permanganate for visualization. The mass spectrum was in accord with the structure: *m/z* 320 (23%), molecular ion. Label incorporation: D_1, 0.0%; D_2, 0.3%; D_3, 99.7%.

($^2H_3$)Cocaethylene (1 g) perchlorate. ($^2H_3$)Cocaethylene (1 g) base (0.1 g, 0.31 mmol) was dissolved in 1 mL of 2-propanol. To this was added 52 μL of 60% perchloric acid. The solution turned cloudy and, on scratching with a glass rod, a white solid precipitated. After cooling in an ice bath, the product was collected by filtration and washed with 0.5 mL of cold 2-propanol, 1 mL of 2-propanol/diethyl ether (1:1, v/v) and then 1 mL of ether. After air drying there was obtained 0.12 g (0.28 mmol, 87%) of white needles, mp 169-170 °C. Microanalysis: Calculated for $C_{18}H_{21}D_3NO_8$: C, 51.57; H + D, 5.75; N, 3.33. Found: C, 51.63; H + D, 5.86; N, 3.48.

Benzoylecgonine (1,1,1-$^2H_3$)methyl ester [1 c, ($^2H_3$)Cocaine] perchlorate. ($^2H_3$)Cocaine (1 c) base was prepared from benzoylecgonine (1 d) and (1,1,1-$^2H_3$)-methan(^{2}H)ol as described above for the synthesis of ($^2H_3$)coca-ethylene (1 g), and converted to the perchlorate salt, mp 169-170 °C. The mass spectrum of the free base was in accord with the structure: *m/z* 306 (24%) molecular ion. Label incorporation: D_1, 0.0%; D_2, 0.3%; D_3, 99.7%.

($^2H_5$)Benzoylecgonine (1 e). ($^2H_5$)Cocaine (1 b) hydrochloride (0.76 g, 2.2 mmol) was converted to the free base and then hydrolyzed as described for the synthesis of benzoylecgonine (1 d). The product was recrystallized from 2 mL boiling water to give 0.32 g (0.87 mmol, 40%, as the tetrahydrate) of fluffy white crystalline solid, mp 88-90 °C.

($^2H_5$)Cocaethylene (1 h) perchlorate. ($^2H_5$)Cocaethylene (1 h) base was prepared from ($^2H_5$)benzoylecgonine (1 e) and ethanol as described for the synthesis of ($^2H_3$)cocaethylene (1 g), and converted to the perchlorate salt as described above, mp 165-166 °C. The mass spectrum of the free base was in accord with the structure: *m/z* 322 (21%), molecular ion. Label incorporation: D_0, 0.1%; D_1, 0.0%; D_2, 0.0%, D_3, 0.3%; D_4, 1.5%; D_5, 98.1%.

($^2H_8$)Cocaethylene (1 i) perchlorate. This was prepared from ($^2H_5$)benzoyl-ecgonine (1 e) and (2,2,2-$^2H_3$)ethan(^{2}H)ol as described above for the D_3 (1 g) and D_5 (1 h) analogs, mp 168-169 °C. The mass spectrum of the free base was in accord

with the structure: *m/z* 325 (16%), molecular ion. Label incorporation: D_2, 0.0%; D_3, 0.1%; D_4, 0.0%; D_5, 0.3%; D_6, 0.3%; D_7, 3.0%; D_8, 96.3%.

Ecgonine (2,2,2-$^2H_3$)ethyl ester (1 n). A suspension of 0.22 g (1 mmol) of ecgonine (1 j) hydrochloride in 2 mL (2,2,2-$^2H_3$)ethan(^{2}H)ol contained in a glass culture tube was saturated with HCl gas, and the mixture was heated at 85° C for 4 h. The ($^2H_3$)ethanol was evaporated to give a viscous liquid. To this was added 1 mL of water and 0.5 mL of 50% potassium carbonate, and the solution was extracted twice with 3 mL portions of methylene chloride. Solvent was evaporated from the combined extracts and the product was dissolved in 1 mL of 2-propanol. To this was added a solution of 0.1 mL of 60% perchloric acid in 1 mL 2-propanol. Stirring and scratching with a glass rod caused the salt to crystallize. The product was collected by filtration, washed with 0.5 mL of 2-propanol, 2 mL of 2-propanol/ether (1:1, v/v), and then 2 mL ether. After air drying there was obtained 0.17 g (52% yield), mp 166-167° C. Ecgonine ethyl ester (1 m) perchlorate prepared in a similar fashion had mp 167-168° C. Microanalysis: calculated for $C_{11}H_{20}NClO_7$: C, 42.11: H, 6.43; N, 4.47. Found: C, 42.28; H, 6.59; N, 4.30. The mass spectrum of the acetylated, labelled free base was in accord with the structure: *m/z* 258 (17%), molecular ion. Label incorporation: D_0, 0.3%; D_1, 0.0%; D_2, 1.3%; D_3, 98.4%.

Ecgonine (1,1,2,2,2-$^2H_5$)ethyl ester (1 o). This was prepared from ecgonine (1 j) and (1,1,2,2,2-$^2H_5$)ethan(^{2}H)ol as described above for the synthesis of ecgonine (1,1,1-$^2H_3$)ethyl ester (1 n). The perchlorate had a mp of 166-167° C. The mass spectrum of the acetylated free base was in accord with the structure: *m/z* 260 (18%), molecular ion. Label incorporation: D_3, 0.0%; D_4, 1.6%; D_5, 98.4%.

Ecgonine (1,1,1-$^2H_3$)methyl ester (1 l). This was prepared from ecgonine (1 j) and (1,1,1-$^2H_3$)methan(^{2}H)ol as described above for the synthesis of ecgonine (1,1,1-$^2H_3$)ethyl ester (1 n). The perchlorate had a mp of 185-186 °C. Ecgonine methyl ester perchlorate, prepared in the same manner, melted at 187-188 °C. Microanalysis: calculated for $C_{10}H_{18}NClO_7$: C, 40.07; H, 6.05; N, 4.67. Found: C, 40.46; H, 6.15; N, 4.30. The mass spectrum of the acetylated, labelled free base was in accord with the structure: *m/z* 244 (22%), molecular ion. Label incorporation: D_1, 0.0%; D_2, 0.8%; D_3, 99.2%.

Anhydroecgonine (1,1,1-$^2H_3$)methyl ester (2 c). Anhydroecgonine (2 a) hydrochloride[17] (0.1 g, 0.5 mmol) was suspended in 10 mL chloroform, and refluxed

with 0.2 mL of thionyl chloride for 2 hr. The solvent and excess thionyl chloride were evaporated, and 1 mL of (1,1,1-$^2H_3$)methan-(^2H)ol was added. The solution was saturated with HCl gas, and allowed to stand overnight. It was then briefly refluxed, cooled and poured into 10 mL water. The acidic solution was washed twice with 20 mL portions of methylene chloride, and then made basic with potassium carbonate. The solution was extracted with two 20 mL portions of methylene chloride, and the combined extracts were evaporated. Kugelrohr distillation (90-100 °C, 0.1 mm Hg) provided 0.05 g of the liquid free base. This was converted to the picrate salt by adding to 2 mL of a saturated solution of picric acid in ethanol. The precipitated product was collected by filtration, washed with ethanol, and air dried. Fine yellow needles were obtained, mp 211.5-212.5 °C. Microanalysis: calculated for $C_{16}H_{15}D_3N_4O_9$: C, 46.49; H + D, 4.39; N, 13.55. Found: C, 46.71; H + D, 4.28; N, 13.34. Anhydroecgonine methyl ester (**2 b**) picrate had a mp of 213.5-214.5 °C. The mass spectrum of the labelled free base was in accord with the structure: *m/z* 184 (26%), molecular ion. Label incorporation: D_1, 0.0%; D_2, 0.6%; D_3, 99.4%.

CONCLUSIONS

In summary, straightforward methods for the site-specific introduction of multiple deuterium atoms into cocaine (**1 a**), cocaethylene (**1 f**), benzoylecgonine (**1 d**), ecgonine methyl (**1 k**) and ethyl esters (**1 m**), and anhydroecgonine methyl ester (**2 b**) are described. These compounds have been used for studies of the metabolic disposition of cocaine (**1 a**) in humans.

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