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Synthesis of *N*-Methyl-Trideuterium-Labelled *m*-Hydroxybenzoylecgonine As An Internal Standard for GC/MS Analysis

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SUMMARY

m-Hydroxybenzoylecgonine is an important metabolite of cocaine. A synthesis of [8-C^2H_3]-*m*-hydroxybenzoylecgonine (**7**) for use as an internal standard in GC/MS analysis of *m*-hydroxybenzoylecgonine, is described. *N*-Demethylation of cocaine (**1**) gave norcocaine (**2**) which was converted to [8-C^2H_3]cocaine (**3**) upon treatment with CD_3I. Hydrolysis of **3** followed by esterification gave [8-C^2H_3]ecgonine methyl ester (**5**). [8-C^2H_3]-*m*-Hydroxycocaine (**6**) was obtained from **5** by first acylation and then selective hydrolysis of the protected *m*-hydroxy moiety under acidic conditions. The title compound, **7**, was obtained by selective hydrolysis of **6** in refluxing water.

Key Words: [8-C^2H_3]-*m*-Hydroxybezoylecgonine, [8-C^2H_3]-*m*-Hydroxycocaine Hydrochloride, Norcocaine, Deuterium Labelling.

INTRODUCTION

Cocaine is rapidly transformed through enzymatic or chemical processes to as many as 11 metabolites which all have been found in human urine (1). Among them, benzoylecgonine (BE) and ecgonine methyl ester (EME) are the two major metabolites commonly used for detection of cocaine administration (2-4). However, because the hydrolysis of cocaine occurs in biological fluids when left unfrozen at a pH value greater than 7 (5-7), the presence of these two compounds in urine does not necessarily confirm cocaine ingestion because the specimen could have been simply contaminated. Although *m*-hydroxybenzoylecgonine (*m*-OH-BE) is a minor metabolite of cocaine in urine (1), ElSohly *et al.* recently reported (8) that *m*-OH-BE, an alternative "true" metabolite, could be used as a valuable marker of cocaine ingestion in addition to BE and EME in a GC/MS confirmatory urine test. In addition, *m*-OH-BE was also found to be an important metabolite in the meconium of newborn infants (Elsohly, *et al.*, unpublished). As part of our ongoing studies targeted toward the identification of major metabolites in urine and

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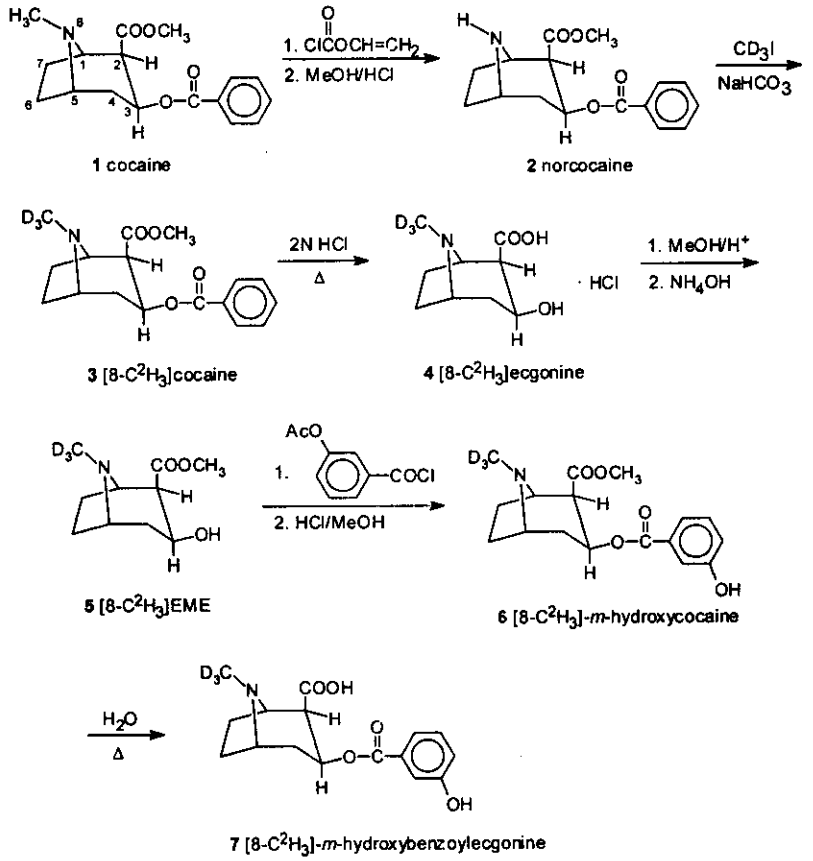
meconium, we developed a facile synthesis of *N*-methyl-trideuterium-labelled *m*-hydroxybenzoylecgonine (**7**) as an internal standard for GC/MS analysis (**8**).

RESULTS AND DISCUSSION

As outlined in scheme 1, the synthesis of **7** started from cocaine (**1**), which was *N*-demethylated to give *N*-norcocaine (**2**). Thus, cocaine was treated with vinyl chloroformate (9-10) in 1,2-dichloroethane to give a vinylurethane, which was not isolated, but converted to norcocaine by selective hydrolysis in methanol solution containing hydrochloric acid. Pure norcccaine was obtained in 82% yield after column chromatography. Other methods for preparing norcocaine have been reported (11-13) where the yields ranged from 56% to 71%. Upon treatment of **2** with CD_3I in the presence of $NaHCO_3$, [8-C^2H_3]cocaine (**3**) was obtained in 89% yield. Jindal and Vestergaard (14)



Scheme 1

reported the synthesis of **3** in an unspecified yield. Compound **3** was converted to [8-C^2H_3]ecgonine methyl ester ([8-C^2H_3]EME) (**5**) in good yield (81%) by first hydrolysis in 2N HCl followed by methylation in MeOH with conc. sulfuric acid.

[8-C^2H_3]EME (**5**) reacted with *m*-acetoxybenzoyl chloride in THF containing triethylamine gave [8-C^2H_3]-*m*-acetoxycocaine. Without purification, this intermediate was subjected to the exclusive hydrolysis of the acetyl moiety in methanol solution containing 6N HCl at room temperature to give [8-C^2H_3]-*m*-hydroxycocaine (**6**) in 85% yield. In a reported procedure for the preparation of non-labelled *m*-hydroxybenzoylecgonine, Steele *et al* (15) used *m*-acetoxybenzoic anhydride to couple with EME followed by hydrolysis under basic condition which gave the product in low yield. Selective hydrolysis of methoxycarbonyl ester function in refluxing water gave [8-C^2H_3]-*m*-hydroxybenzoylecgonine (**7**) in 60% yield. The isotopic purity of **7** was determined to be greater than 98% by mass spectrometry (figure 1).

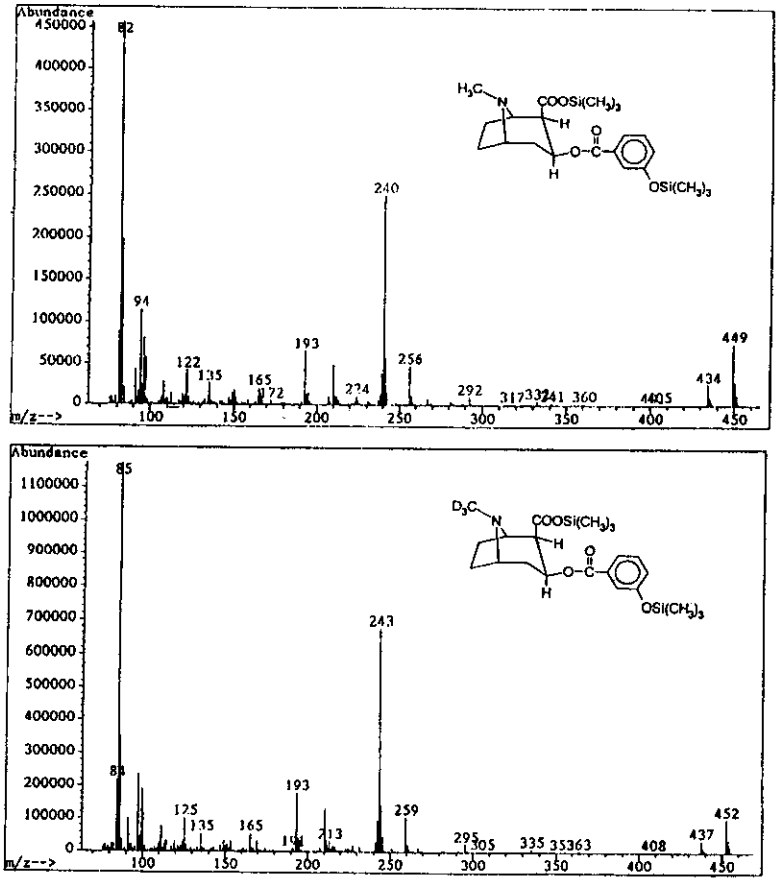


Figure 1. Mass spectrum of (*N*-methyl-d_3)-*m*-hydroxybenzoylecgonine (bottom) compared with mass spectrum of *m*-hydroxybenzoylecgonine (top), as di-TMS derivatives.

[8-C^2H_3]-*p*-Hydroxybenzoylecgonine, as well as non-labelled hydroxylated metabolites of cocaine can also be prepared in a similar manner starting from the appropriate starting materials.

EXPERIMENTAL

Melting points were measured with a Fisher-Johns melting point apparatus and were uncorrected. ^{1}H-NMR spectra were taken on a Bruker AC-300 spectrometer. GC/MS analyses were performed on a HP5890 gas chromatograph with a HP5970 mass selective detector in the E.I. mode (70 eV), equipped with a 25 m × 0.2 mm × 0.33 *um* DB-5MS column. TMS derivatization was carried out by heating the analytes with excess bis(trimethylsilyl)trifluoroacetamide (BSTFA) in sealed GC vials. Reagents were purchased from Aldrich Chemical Company and used without further treatment. Cocaine HCl was provided by the National Institute on Drug Abuse and was converted to free base of cocaine in the usual manner. Small quantities of norcocaine, [8-C^2H_3]cocaine, [8-C^2H_3]ecgonine HCl, and [8-C^2H_3]EME HCl were obtained from Sigma for comparision purpose. Elemental analysis were carried out by Galbraith Lab., Inc.

Norcocaine (2): A solution of cocaine (8.9 g, 0.029 mmole) and vinyl chloroformate (17 mL, 21.3 g, 0.2 mole) in 80 mL of 1,2-dichloroethane were heated under reflux under N_2 for 12 hrs. A second portion of vinyl chloroformate (3 mL) was added and the mixture was continued to reflux for 4 hrs. The solvent and excess reagent were removed under reduced pressure to give a yellow oil. This oil was dissolved in 80 mL of methanol and 5 mL of conc. HCl was added. The mixture was heated under reflux for 16 hrs. Methanol was evaporated under reduced pressure. The residue was partitioned between 100 mL of water and 30 mL of ethyl acetate. The aqueous phase was basified with 30% NH_4OH and extracted with chloroform (3 × 100 mL). The chloroform layers were combined and dried over $MgSO_4$. Solvent was evaporated *in vacuo* to give an oil (8.2 g), which was purified by flash column chromatography (silica gel) eluting with hexane:acetone:TEA (65:35:5) as a colorless oil (6.93 g, 82%). The HCl salt had mp 112-114 °C [lit. (12) 111-113 °C]; *m/z*: 289 (M^+, 17%); 168 (100%). GC/MS showed one single peak and was identical to an authentic sample.

[8-C^2H_3]Cocaine (3): To a suspension of norcocaine (6.90 g, 0.024 mole) and $NaHCO_3$ (2.02 g, 0.024 mole) in 60 mL of benzene, was added dropwise with stirring a solution of CD_3I (1.66 mL, 3.86 g, 0.027 mole, 99.5 atom % D) in 20 mL of benzene during 2 hrs. After stirring overnight, a second portion of CD_3I (0.84 mL) in 10 mL of benzene was added dropwise during 1 hr. The mixture was continued to stir at rt for 6 hrs. The solid was filtered and washed succesively with cold benzene and water to afford the

first crop of product (3.02 g, 41%) as a white solid after drying *in vacuo*, mp 97-97.5 °C [lit. (16) 98 °C for unlabelled]; *m/z*: 306 (M^+, 35%), 275 (12%), 185 (88%), 105 (31%) and 85 (100%). The filtrate and the washings were combined and acidified with conc. HCl to pH 2. The aqueous layer was separated and washed one more time with benzene (20 mL). The product precipitated upon addition of 30% NH_4OH solution to the aqueous phase until pH 9. The precipitate was filtered and washed with cold water to give the second crop of product as a white solid (3.50 g, 48%) after drying *in vacuo*, mp 97-97.5 °C. The two crops of products (89% total yield) were pure and identical to an authentic sample by GC/MS.

[8-C^2H_3]Ecgonine HCl (4) and [8-C^2H_3]ecgonine methyl ester (5): A solution of [8-C^2H_3]cocaine (5.5 g, 0.018 mole) in 60 mL of 2N HCl was refluxed for 16 hrs. The solution was cooled to rt and partitioned with ether (2 × 15 mL) to remove the benzoic acid. The aqueous layer was evaporated to dryness under reduced pressure and the resulting solid was triturated with acetone to give the product as a white solid (3.35 g, 84%), mp 245-246 °C [lit. (17) 246 °C for unlabelled]. To a solution of this solid (3.35 g, 0.015 mole) in 80 mL of methanol, carefully was added 2 mL of conc. H_2SO_4. The resulting mixture was refluxed for 16 hrs. After cooling to rt, solvent was evaporated to about 20 mL under reduced pressure at below 40 °C. The residue was added slowly to a mixture of crushed-ice (150 g) and 10 mL of 30% NH_4OH. The product was partitioned into chloroform (5 × 50 mL) and the combined organic layers were dried over $MgSO_4$. Solvent was removed *in vacuo* to afford the crude [8-C^2H_3]ecgonine methyl ester (2.92 g, 97%) as a colorless oil. The HCl salt had mp 210-211 °C [lit. (18) 212.5-213 °C for unlabelled]; *m/z* (as TMS derivative): 274 (M^+, 10%), 243 (9%), 185 (14%), 99 (64%), 85 (100%). This material was identical to an authentic sample by GC/MS and was used for the next reaction without further purification.

[8-C^2H_3]-*m*-Hydroxycocaine HCl (6): A mixture of *m*-acetoxybenzoic acid (2.70 g, 0.015 mole) and thionyl chloride (10 mL) was heated under reflux with stirring for 0.5 hr. The excess thionyl chloride was removed under reduced pressure. The residue was dissolved in 5 mL of dry THF. To this solution under argon and in an ice bath, was added successively triethylamine (1.5 g, 0.015 mole) and [8-C^2H_3]EME (1.06 g, 0.0053 mole) in dry THF (8 mL). The resulting mixture was refluxed for 3 hrs. The reaction was quenched with water. THF was removed under reduced pressure and the remaining aqueous mixture was extracted with ethyl acetate (3 × 50 mL). The organic layers were combined and washed with 0.5 N NaOH (2 × 30 mL), water and brine, and dried over $MgSO_4$. Solvent was removed under reduced pressure to give an oil which was dissolved in 62 mL of methanol followed by the slow addition of 6N HCl (34 mL) in an ice-bath.

The resulting mixture was allowed to stir at rt for 24 hrs. Conc. NH_4OH (30%, 25 mL) was added dropwise with stirring at 0-5 °C. Methanol was evaporated under reduced pressure. The residue was dissolved in 6N HCl (30 mL) and partitioned with ether (2 × 30 mL). The aqueous phase was basified with conc. NH_4OH and extracted with chloroform (5 × 30 mL). The extract was washed with brine several times and dried over $MgSO_4$. Solvent was removed *in vacuo* to afford crude [8-C^2H_3]-*m*-hydroxycocaine (**6**) as an amorphous foam (1.442 g, 85%). *m/z* (as TMS derivative): 394 ($M^{+\cdot}$, 17%), 363 (5%), 185 (100%), 121 (17%), 85 (91%). A portion (0.567 g, 0.0018 mole) of this material was converted to its HCl salt by mixing with 1 mL of acetone containing conc. HCl (0.15 mL, 0.0018 mole). It slowly solidified after cooling in an ice-bath. The solid was filtered and washed with acetone to give [8-C^2H_3]-*m*-hydroxycocaine HCl as a white solid (0.502 g, 80%), mp 181-183 °C. ^{1}H-NMR (DMSO-d_6) δ: 10.20 (1H, s) and 8.96 (1H, s) for OH and N^+H, 7.30 (3H, m, ArH), 7.11 (1H, m, ArH), 5.45 (1H, m, 3-H), 4.25 (1H, bd, J = 6.1 Hz) and 3.95 (1H, s) for 1-H and 5-H, 3.65 (3H, s, $COOCH_3$), 3.51 (1H, dd, J = 2.0 Hz, J = 7.1 Hz, 2-H), 2.05-2.39 (6H, m, 3 × CH_2). *Anal.* Calcd for $C_{17}H_{18}D_3NO_5 \cdot HCl \cdot 2H_2O$: C, 51.71; H, 6.73; N, 3.55. Found: C, 51.63; H, 6.73; N, 3.68.

[8-C^2H_3]-*m*-Hydroxybenzoylecgonine (7): [8-C^2H_3]-*m*-Hydroxycocaine (amorphous foam, 0.875 g, 0.0027 mole) was suspended in water (10 mL) and was heated under reflux for 3 hrs. Solvent was evaporated to dryness under reduced pressure. The resulting solid was triturated with acetone and filtered to give [8-C^2H_3]-*m*-hydroxybenzoylecgonine (**7**) as a white solid (0.500 g, 60%), mp 244-245 °C [lit. (15) 236-237 °C and (19) 243-244 °C for unlabelled]. ^{1}H-NMR (DMSO-d_6) δ: 9.94 (1H, s, COOH), 7.29 (3H, m, ArH), 7.02 (1H, m, ArH), 5.22 (1H, m, 3-H), 3.72 (1H, s) and 3.66 (1H, s) for 1-H and 5-H, 2.82 (1H, dd, J = 6.4 Hz, J = 2.7 Hz, 2-H), 2.04-2.21 (5H, m,) and 1.80-1.94 (2H, m) for 3 × CH_2 and OH. *m/z* (as di-TMS derivative): 452 ($M^{+\cdot}$, 9%), 437 (3%), 259 (9%), 243 (58%), 85 (100%). *Anal.* Calcd for $C_{16}H_{16}D_3NO_5$: C, 62.32; H, 6.33; N, 4.54. Found: C, 62.40; H, 6.35; N, 4.47.

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