SYNTHESIS OF DEUTERIUM LABELLED COCAINE AND PSEUDOCOCAINE

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SUMMARY

Cocaine and pseudococaine were mass-labelled with deuterium at various positions on the tropane ring. The synthetic procedures followed were adaptations of those previously published for the unlabelled compounds. The isotopic purity was greater than 95% for 2-[^{2}H]-, 4,4-[$^2H_2$]-, and 1,5,6,6,7,7-[$^2H_6$]-cocaine and 3-[^{2}H]-, 4,4-[$^2H_2$]-, and 1,5,6,6,7,7-[$^2H_6$]-pseudococaine, while that of 3-[^{2}H]-cocaine exceeded 90%.

Key words: Cocaine, pseudococaine, deuterium labelling, GC-MS, NMR

INTRODUCTION

As part of an ongoing research program investigating the chemistry of the cocaines, we required cocaine(**2g**) and pseudococaine(**3e**) specifically mass-labelled at selective positions on the tropane ring. Only the preparation of 4-[^{3}H] -cocaine and 2-[^{2}H]-pseudococaine have been reported in the literature(1,2). Ring labelled cocaines and their esters are of recent interest in biochemical, pharmacological, and analytical studies(3). Incorporation into the tropane ring provides a label at metabolically non-labile positions which can be useful in

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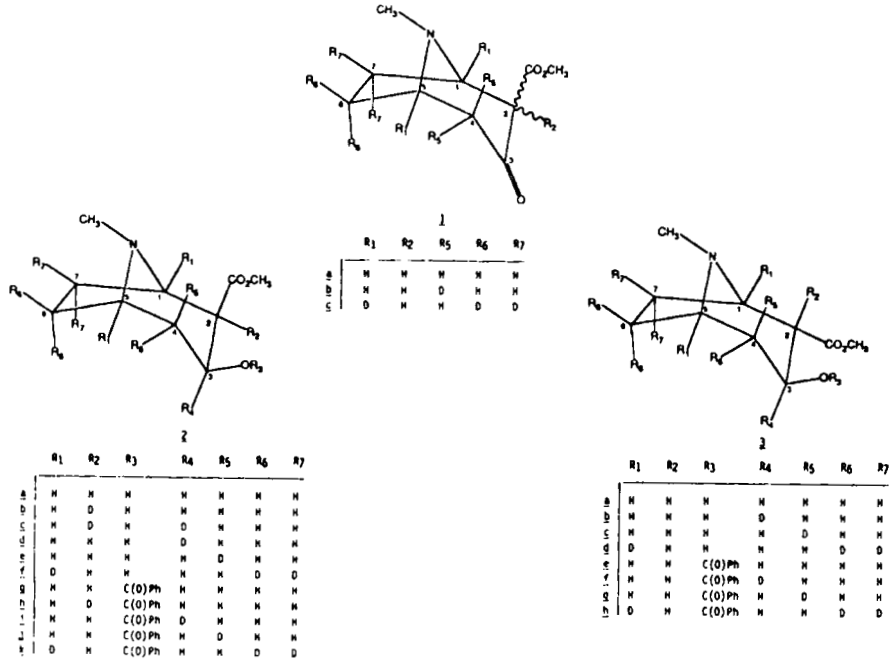
metabolic and pharmacokinetic studies. Specifically labelled positions on the tropane ring are essential in determining the electron impact fragmentation mechanisms of the cocaines in mass spectrometry. Thus we have prepared deuterium labelled isotopomers of cocaine(**2h-k**), pseudococaine(**3f-h**), ecgonine methyl ester(**2b-f**), and pseudoecgonine methyl ester(**3b-d**) with specific labels, as shown below.



MASS SPECTROMETRY

All intermediates and final products were analyzed by a Finnigan Model 5100 quadrupole gas chromatograph mass spectrometer(GC-MS) operated under electron impact ionization conditions at 70 eV and in full scan operation. Isotopic purities were determined by a Hewlett Packard Model 5971A Mass Selective Detector(EI,70 eV) by monitering the molecular ion region of M-1 to M+2 by selective ion monitoring(SIM). The isotopic purity of all compounds

exceeded 95% with the exception of 3-[^{2}H]-cocaine(**2i**) (ca. 90%).

EXPERIMENTAL PROCEDURES

All reagents and solvents were products of Aldrich Chemical except for 2,3,3,4,4,5-[$^2H_6$]-2,5-diethoxytetrahydrofuran (Cambridge Isotope Labs) and were used without further purification. Reactions were performed in oven-dried glassware and protected from moisture with dry nitrogen. Total syntheses of labelled cocaines were performed as described in the literature(4), substituting appropriate deuterium labelled reagents and solvents where necessary. The Mannich route was employed for incorporation of deuterium at carbons 1,4,5,6,and 7 in the intermediate ketones(**1b** and **1c**). Deuterium incorporation at C-2 and C-3 was accomplished via dissolving metal reduction of the ketone, and through hydrogen-deuterium exchange/epimerization of the alcohols (**2a**,**2c**, and **2e**)(5). Benzoylations were performed with a 50% molar excess of benzoyl chloride in dry pyridine(6). The reported yields have not been optimized. Melting points were obtained on a Mel-Temp capillary tube apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian Model EM-360 spectrometer using TMS as internal standard.

(-)-2-[^{2}H]-Ecgonine methyl ester HCl (2b)

A mixture of nonlabelled (-)-ecgonine methyl ester (**2a**) (50 mg, 0.251 mmoles) and $NaOCH_3$ (6 mg, 0.111 mmoles) in 1.0 ml MeOD was capped for 72 hrs. at 25° C. The reaction mixture was then quenched with 250 µl of D_2O and diluted with 2 ml of 10% sodium bicarbonate in H_2O. The mixture was promptly extracted three times with 15 ml $CHCl_3$. The combined extracts were dried over $MgSO_4$ and evaporated to an oil, converted into its hydrochloride salt, and recrystallized from MeOH and $CHCl_3$ to yield 24.5

mg(41%) of 2b as white crystals, mp. 210° C (lit[4] 213.5-214.5°C nonlabelled).

(-)-2-[^{2}H]-Cocaine (2h)

Benzoylation of 2b (22 mg, 0.093 mmoles) with 50% excess benzoyl chloride in pyridine afforded 15 mg(53%) of 2h as an oil. Mass spectra: 304(5%, $M^{+\cdot}$), 82(100%, N-methylpyrrolidinium), 183(54%), 83(40%), 105(30%), 77(30%), 95(28%), 123(8%), 199(7%), 152(4%). ^{1}H-NMR($CDCl_3$): 1.7 to 2.15(m,5H,H-6,H-7,$H\text{-}4_{eq}$), 2.4(m,1H,$H\text{-}4_{ax}$), 2.2 (s,3H,NCH_3), 3.3(m,1H,H-5), 3.6(m,1H,H-1), 3.7(s,3H,OCH_3), 5.15(dd,$J_{3,4eq}$=5.0 Hz,$J_{3,4ax}$=11.0 Hz,1H,H-3).

(±)-2,3-[$^2H_2$]-Ecgonine methyl ester HCl (2c)

To a solution of 2-carbomethoxy-3-tropinone (1a) (400 mg, 1.9 mmoles) (prepared from reported procedure[4]) in 5 ml of 10% D_2SO_4 at 0-5°C was added 62 g of 1.5% NaHg over 1.5 hr. A pH between 3-4 was maintained with periodic addition of 30% D_2SO_4. Extraction and recrystallization afforded 156 mg(35%) of 2c as white crystals, mp 195-196° C (lit[7] 194.5°C nonlabelled).

(±)-3-[^{2}H]-Pseudoecgonine methyl ester (3b)

A mixture of 2c base (15 mg, 0.074 mmoles) and $NaOCH_3$ (20 mg, 0.370 mmoles) in MeOH was refluxed 3.5 hrs to afford 3b and was recrystallized from ether to yield 2 mg(17%) of white crystals, mp. 125-126° C (lit[7] 128.5-130.5°C nonlabelled).

(±)-3-[^{2}H]-Pseudococaine (3f)

Benzoylation of 3b (5 mg, 0.025 mmoles) afforded 4.6 mg(61%) of 3f as an oil. Mass spectra: 304(9%, $M^{+\cdot}$), 82(100%, N-methylpyrrolidinium), 183(52%), 77(26%), 105(22%), 96(20%), 95(14%), 153(10%), 123(7%), 199(6%). ^{1}H-NMR($CDCl_3$): 1.7 to 2.2(m,6H,$H\text{-}4_{ax}$,$H\text{-}4_{eq}$,H-6,H-7), 2.4(s,3H,NCH_3), 3.15(m,1H,H-2), 3.3(m,1H,H-5), 3.5(m,1H,H-1), 3.7(s,3H,OCH_3).

(±)-3-[^{2}H]-Ecgonine methyl ester HCl (2d)

A mixture of 2c base (40 mg, 0.200 mmoles) and $NaOCH_3$ (5 mg, 92

umoles) in MeOH was capped and let stand 60 days at 7°C. The reaction mixture was quenched with H_2O and worked up in the usual manner to afford 7 mg(15%) of **2d** as white crystals, mp. 194.5-195.5°C (lit[7] 194.5 °C nonlabelled).

(±)-3-[^{2}H]-Cocaine (2i)

Benzoylation of **2d** (1.2 mg, 0.005 mmoles) afforded 1.1 mg(61%) of **2i** as an oil. Mass spectra: 304(8%, $M^{+\cdot}$), 82(100%, N-methylpyrrolidinium), 183(57%), 77(41%), 96(38%), 95(35%), 105(34%), 123(8%), 199(7%), 153(5%). ^{1}H-NMR($CDCl_3$): 1.7 to 2.0(m,5H,H-6,H-7,H-4$_{eq}$), 2.5(dd,$J_{4ax,5}$=3.0 Hz,$J_{4ax,4eq}$=10.5 Hz,1H,H-4$_{ax}$), 2.2(s,3H,NCH_3), 3.0(m,1H,H-2), 3.3(m,1H,H-5), 3.6(m,1H,H-1), 3.7(s,3H,OCH_3).

(±)-4,4-[$^2H_2$]-2-Carbomethoxy-3-tropinone (1b)

The title compound was prepared following the reported procedure(4) for the nonlabelled ketone. D_2SO_4, MeOD, and D_2O were substituted for H_2SO_4, MeOH, and H_2O, respectively, to yield 1.3 g (39%) of **1b** as a white crystals, mp. 89-93°C (lit[9] 93-96°C nonlabelled dihydrate).

(±)-4,4-[$^2H_2$]-Ecgonine methyl ester HCl (2e)

Sodium-mercury amalgam reduction of **1b** (350 mg, 1.6 mmoles) provided 64 mg(15%) of **2e** as white crystals, mp. 192-193°C (lit[7] 194.5°C nonlabelled).

(±)-4,4-[$^2H_2$]-Cocaine (2j)

Benzoylation of **2e** (25 mg, 0.105 mmoles) afforded 15 mg(47%) of **2j** as a white solid, mp. 71-73°C (lit[8] 79-80 °C nonlabelled). Mass spectra: 305(8%,$M^{+\cdot}$), 82(100%, N-methylpyrrolidinium), 184(56%), 83(50%), 105(32%), 77(28%), 95(25%), 200(7%), 122(5%), 153(4%). ^{1}H-NMR($CDCl_3$): 1.7 to 2.0(m,4H,H-6,H-7), 2.2(s,3H,NCH_3), 3.0(dd,$J_{2,3}$=6.0 Hz,$J_{2,1}$=3.5 Hz,1H,H-2), 3.3(m,1H,H-5), 3.6(m,1H,H-1), 3.7(s,3H,OCH_3), 5.3(d,$J_{3,2}$=6.0 Hz, 1H,H-3).

(±)-4,4-[$^2H_2$]-Pseudoecgonine methyl ester (3c)

A mixture of **2e** (9 mg, 0.045 mmoles) and $NaOCH_3$ (3 mg, 0.055 mmoles) in 600 µl MeOH was heated at 80°C for 27 hrs and then quenched with 50 µl H_2O. The reaction was worked up in the usual manner to afford 4.3 mg(48%) of **3c** as white crystals, mp. 121-122°C (lit[7] 128.5-130.5°C nonlabelled).

(±)-4,4-[$^2H_2$]-Pseudococaine (3g)

Benzoylation of **3c** (4.1 mg, 0.020 mmoles) afforded 4.8 mg(77%) of **3g** as a white solid, mp. 74-75°C (lit[8] 81.5°C nonlabelled). Mass spectra: 305(9%, $M^{+\cdot}$), 82(100%, N-methylpyrrolidinium), 83(67%), 184(58%), 77(40%), 105(34%), 96(18%), 153(12%), 122(7%), 200(7%). ^{1}H-NMR($CDCl_3$): 1.7 to 1.9(m,4H,H-6,H-7), 2.4(s,3H,NCH_3), 3.15(m,1H,H-2), 3.3(m,1H,H-5), 3.5(m,1H,H-1), 3.7(s,3H,OCH_3), 5.5(d,$J_{3,2}$=10.5 Hz,1H,H-3).

(±)-1,5,6,6,7,7-[$^2H_6$]-2-Carbomethoxy-3-tropinone (1c)

The title compound was prepared following the reported procedure(4). Substituting 2,3,3,4,4,5-[$^2H_6$]-2,5-diethoxytetrahydrofuran for the unlabeled furan afforded 1.06 g (50%) of **1c** as white crystals, mp.101-102.5°C (lit[9] 93-96°C nonlabelled dihydrate).

(±)-1,5,6,6,7,7-[$^2H_6$]-Ecgonine methyl ester HCl (2f)

Sodium-mercury amalgam reduction of **1c** (870 mg, 3.9 mmoles) provided 70 mg(7%) of **2f** as white crystals, mp. 178-179.5°C (lit[7] 194.5°C nonlabelled).

(±)-1,5,6,6,7,7-[$^2H_6$]-Cocaine (2k)

Benzoylation of **2f** (1.5 mg, 0.006 mmoles) afforded 1.2 mg(63%) of **2k** as an oil. Mass spectra: 309(7%, $M^{+\cdot}$), 87(100%, N-methyl-2,3,3,4,4-[$^2H_5$]-pyrrolidinium), 188(69%), 77(44%), 105(41%), 89(40%), 96(40%), 204(8%), 128(5%), 278(4%). ^{1}H-NMR($CDCl_3$): 1.85(dd,$J_{4eq,4ax}$=10.3 Hz,1H,H-4eq), 2.2(s,3H,NCH_3), 2.45(t,$J_{4ax,3}$=10.3 Hz,1H,H-4ax), 3.0(d,$J_{2,3}$=5.2 Hz,1H,H-2),

3.75(s,3H,OCH_3), 5.25(dt,$J_{3,4ax}$=10.3 Hz,$J_{3,2}$=5.2 Hz,$J_{3,4eq}$=5.2 Hz,1H,H-3).

(±)-1,5,6,6,7,7-[$^2H_6$]-Pseudoecgonine methyl ester (3d)

A total of 6.4 mg of white crystals was obtained as an ether insoluble by-product from the preparation of **2f**, mp. 128.5-129.5°C (lit[7] 128.5-130.5°C for nonlabelled).

(±)-1,5,6,6,7,7-[$^2H_6$]-Pseudococaine (3h)

Benzoylation of **3d** (1.5 mg, 0.007 mmoles) afforded 1.0 mg(53%) of **3h** as a white solid, mp. 76-77.5°C (lit[8] 81.5°C nonlabelled). Mass spectra: 309(7%, $M^{+\cdot}$), 87(100%, N-methyl-2,3,3,4,4-[$^2H_5$]-pyrrolidinium), 188(59%), 89(35%), 77(34%), 105(28%), 86(25%), 154(10%), 204(8%), 278(3%). ^{1}H-NMR($CDCl_3$): 1.9(t,$J_{4ax,3}$=10.5 Hz, 1H,H-4ax), 2.05(dd,$J_{4eq,3}$=6.5 Hz,$J_{4eq,4ax}$=13.0 Hz,1H,H-4eq), 2.4(s,3H,NCH_3), 3.2(d,1H,H-2), 3.6(s,3H,OCH_3), 5.5(dt,$J_{3,2}$=10.5 Hz,$J_{3,4eq}$=6.5 Hz,$J_{3,4ax}$=10.5 Hz,1H,H-3).

RESULTS AND DISCUSSION

This paper reports the development of synthetic routes for the incorporation of up to six deuterium atoms in the tropane ring. The reported procedures use total synthesis alleviating the problem of unlabelled cocaines remaining as an impurity from retro-synthesis. To help establish the electron impact ionization fragmentation mechanisms of these important compounds through high resolution mass spectrometry, site specific mono and dideuterococaines are required. Mannich condensation allowed incorporation at carbons 1,4,5,6, and 7. Amalgam reduction of the unlabelled ketone(**1a**) in deuterated medium led to incorporation at C-2 and C-3 of the alcohol(**2c**). Hydrogen-deuterium exchange and epimerization at C-2 afforded the appropriately labelled alcohols(**2b**, **2d**, **3b**, and **3c**). The

cocaines were incorporated with isotopic purities exceeding 95% except for **2i** (ca. 90%). With the exception of **2i**, no evidence of isotopic exchange or scrambling was observed (i.e. from extraction, crystallization, or benzoylation). The synthesis of **2i** proved to be the most difficult, due to back-exchange of deuterium at C-2 and the facile epimerization of **2c** to **3b**.
For the investigation of metabolic pathways, a very distinct and unique mass ion is desirable. Although the N-methyl, carbomethoxy, and benzoyl functional groups are easily labelled via chemical syntheses, these positions are metabolically labile(10) and consequently compounds labelled at such positions are not suitable for biological studies. As a result of the present work the availability of hexadeuterococaines **2k** and **3h** with the label in the pyrrolidine ring portion of the molecule, provides compounds suitable for metabolic studies. Furthermore these isotopomers would also be suitable for use as internal standards for GC-MS assays. A mass increase of 6 amu in each of the three ions usually monitored by SIM (m/z's 182, 272,303 to 188, 278, 309) are without interferences. Since the new deuterated analogs of cocaine were fully characterized and no incorporation of deuterium atoms was observed in undesignated positions, the synthetic routes outlined should also allow cocaine and its analogs to be selectively deuterated in other combinations.

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