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Research Article

Synthesis of a deuterium-labelled standard of bufotenine (5-HO-DMT)

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Abstract: The Batcho–Leimgruber strategy was employed to synthesize 3-(2-dimethylamino-[$^2H_4$]-ethyl)-1*H*-indol-5-ol (bufotenine, 5-HO-DMT) (**8**) from commercial 3-methyl-4-nitro-phenol (**1**), benzyl bromide and *N*,*N*–dimethylformamide–dimethylacetal. Compound **4** was synthesized from compound **3** using the Batcho–Leimgruber strategy in the presence of Raney nickel and hydrazine hydrate. Compound **4** was treated with oxalyl chloride, dimethylamine and lithium aluminum [$^2H_4$]-hydride to yield [2-(5-benzyloxy-1*H*-indol-3-yl)-[$^2H_4$]-ethyl]-dimethyl-amine (**7**). The benzyl ether in compound **7** was cleaved by hydrogenolysis to give bufotenine **8**. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: bufotenine; deuterium labelled; internal standard

Introduction

The allegedly psychedelic drug, bufotenine, is an alkaloid found in frog and toad skins, mushrooms, high-order plants, and mammals, especially in the brain, plasma and urine of schizophrenics.[1,2] Bufotenine, commonly known as 5-hydroxy-DMT (5-OH-DMT), is a tryptamine related to the neurotransmitter serotonin.[3]

Unknown abused drugs are typically detected and identified by gas chromatography–mass spectrometry (GC–MS) because of the high sensitivity of this method and its ability to separate complex mixtures of organic compounds.[4–7] Standard samples used for analyzing controlled drugs in Taiwan are very difficult to obtain. Many studies have been reported for the preparation of deuterium-labelled control drugs as internal standards for use as GC–MS analysis.[8–18] This work describes synthetic routes to bufotenine-d_4, and presents related characteristic analytical data.

Results and discussion

Although bufotenine has been prepared via several synthetic routes,[19–25] [$^2H_4$]-bufotenine has been prepared using a different method.[19] This study describes alternative procedures for preparing [$^2H_4$]-bufotenine. Scheme 1 presents the preparation of 3-(2-dimethylamino-[$^2H_4$]-ethyl)-1*H*-indol-5-ol (**8**). 3-Methyl-4-nitro-phenol (**1**) was treated with benzyl bromide in the presence of potassium carbonate to give benzyl-(3-methyl-4-nitro-phenyl)-ether (**2**) in a 99% yield.[26] As in the Batcho–Leimgruber synthesis of indole,[20–25,27–32] compound **2** was condensed with *N*,*N*-dimethylformamide–dimethyl acetal to yield [2-(5-benzyloxy-2-nitro-phenyl)-vinyl]-dimethyl-amine (**3**).[27] Hydrogenolysis of compound **3** with Raney nickel and hydrazine hydrate gave 5-benzyloxy-1*H*-indole (**4**) in a 62% yield.[26] A reaction of compound **4** and oxalyl chloride[33,34] and then dimethyl amine[20] formed 2-(5-benzyloxy-1*H*-indol-3-yl)-*N*,*N*-dimethyl-2-oxo-acetamide (**6**) in a 79% yield. Reduction of compound **6** with lithium aluminum [$^2H_4$]-hydride produced [2-(5-benzyloxy-1*H*-indol-3-yl)-[$^2H_4$]-ethyl]-dimethyl-amine (**7**) in a 95% crude yield.[19] Hydrogenolysis of the benzyl ether group in compound **7** with hydrogen (1 atm.) and Pd/C (10%) gave 3-(2-dimethylamino-[$^2H_4$]-ethyl)-1*H*-indol-5-ol (**8**) in a 99% crude yield.[20] In order to purify compounds **7** and **8** by recrystallization, compound **7** was converted to its oxalic acid salt which was recrystallized from methanol

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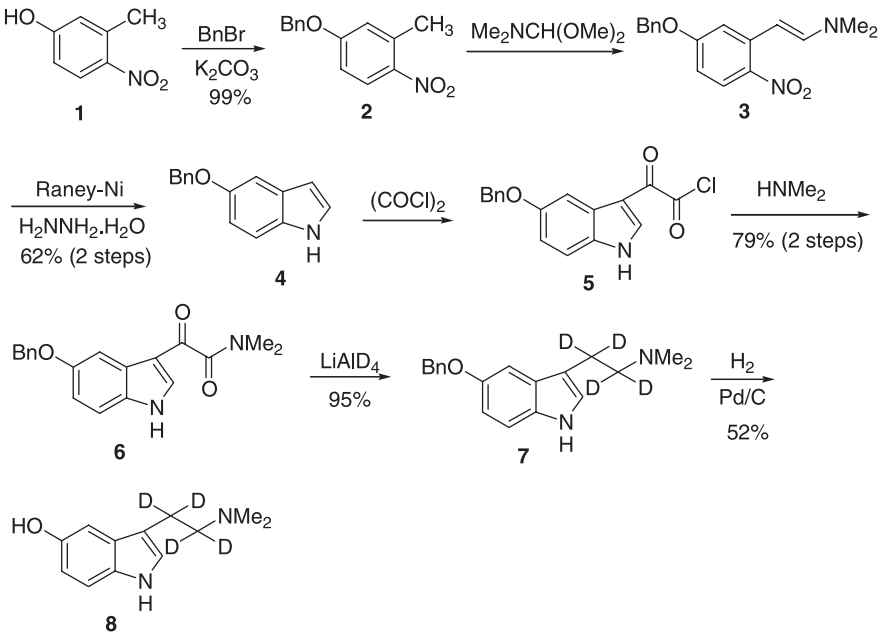

Scheme 1

to give the oxalic acid salt of compound **7** in an 86% yield. Hydrogenolysis of the benzyl ether group in the oxalic acid salt of compound **7** gave the oxalic salt of compound **8** which was recrystallized from methanol/diethyl ether to give the pure oxalic acid salt of compound **8** in a 61% yield. The overall yield for the seven steps is 24%.

Experimental

General

^{1}H NMR spectra were obtained at 300 or 400 MHz (indicated in each case), and ^{13}C NMR spectra were obtained at 100.6 MHz using a Bruker NMR spectrometer. Chemical shifts (δ) are reported in ppm relative to $CHCl_3$ (7.26 and 77.0 ppm). Mass spectra (MS) were obtained using a Micromass Platform II mass spectrometer at 70 eV. High-resolution MS (HRMS) were obtained using a Finnigan/Thermo Quest MAT 95XL mass spectrometer. Infrared spectra were recorded using an ATI Mattson spectrometer. All reactions were performed in anhydrous solvents. Tetrahydrofuran and diethyl ether were distilled from sodium-benzophenone under argon. Benzene and *N,N*-dimethylformamide were distilled from calcium hydride. All air-sensitive reactions were performed in dry glassware under nitrogen using a standard glovebox. Flash column chromatography was performed using MN silica gel 60 (70–230 mesh) which was purchased from Macherey–Nagel.

All reactions were initially optimized using unlabelled compounds.

Synthesis of benzyl-(3-methyl-4-nitro-phenyl)-ether (2).[26] A mixture of benzyl bromide (1.53 g, 10.0 mmol), 3-methyl-4-nitrophenol (**1**) (2.22 g, 13.0 mmol) and potassium carbonate (2.07 g, 15.0 mmol) in dry acetone (20 mL) was refluxed for 5 h until compound **1** was consumed. Filtration and concentration yielded a residue, which was purified by flash column chromatography using silica gel as the stationary phase and ethyl acetate–hexane (1:19) as the mobile phase to generate compound **2** (2.40 g, 9.9 mmol). Yield: 99%. m.p.: 69°C (lit.[35] 70–71°C). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.08 (d, J = 9.9 Hz, 1H), 7.42–7.35 (m, 5H), 6.89–6.85 (m, 2H), 5.13 (s, 2H), 2.63 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 162.2, 142.4, 137.1, 135.7, 128.8, 128.5, 127.6, 127.5, 118.4, 112.6, 70.5, 21.7. IR (KBr, thin film): 2929, 1588, 1518, 1334, 1252, 1076, 993, 837, 761, 702, 655 cm^{-1}. MS-EI (m/z): 243 (M^+, 6), 92 (9), 91 (100), 77 (1), 65 (8), 51 (1).

Synthesis of [2-(5-benzyloxy-2-nitro-phenyl)-vinyl]-dimethyl-amine (3).[27] A solution of benzyl-(3-methyl-4-nitro-phenyl)-ether (**2**) (2.20 g, 9.1 mmol) in *N,N*-dimethylformamide–dimethyl acetal (3.6 mL, 27.2 mmol) and *N,N*-dimethylformamide (9.0 mL) was heated at 130°C using a reflux condenser for 38 h. The condenser was replaced with a distillation apparatus to remove the remaining *N,N*-dimethylformamide and *N,N*-dimethylformamide–dimethyl acetal. The dark brown

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residue was used in the subsequent reaction without further purification.

Synthesis of 5-benzyloxy-1*H*-indole (4).[26] To a solution of crude compound **3** in tetrahydrofuran (9.05 mL), methanol (9.05 mL) and Raney nickel (ca. 2 g) was added hydrazine hydrate (0.9 mL, 18.1 mmol) slowly, and the reaction mixture was stirred at room temperature. After stirring for 30 min, the Raney nickel was removed by filtration through a bed of celite. Concentration left a residue, which was purified by flash column chromatography using silica gel as the stationary phase and ethyl acetate–hexane (1:9) as the mobile phase to produce compound **4** (1.25 g, 5.6 mmol). Yield: 62%. m.p.: 114–116°C (lit.[36] 109–111°C). ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.05 (s, br, 1H), 7.53–7.17 (m, 8H), 7.99 (d, J= 2.4 Hz, 1H), 6.50 (s, 1H), 5.14 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 153.5, 137.8, 131.2, 128.6, 128.3, 127.8, 127.6, 125.0, 113.1, 111.8, 104.0, 102.5, 70.9. IR (thin film): 3313, 3029, 2867, 1580, 1481, 1456, 1223, 1149, 996, 728, 698 cm^{-1}. MS-EI (*m/z*): 223 (M^+, 75), 132 (73), 104 (32), 91 (100), 77 (12), 65 (12), 51 (8).

Synthesis of 2-(5-benzyloxy-1*H*-indol-3-yl)-*N,N*-dimethyl-2-oxo-acetamide (6). 5-Benzyloxy-1*H*-indole (2.90 g, 13.0 mmol) was added in portions to a cool and well-stirred solution of oxalyl chloride (2.28 mL, 26.1 mmol) in anhydrous diethyl ether (35 mL). The mixture was stirred for an additional 30 min. Red solids that formed were triturated with diethyl ether to give compound **5**.[33,34] Compound **5** in a small amount of anhydrous diethyl ether was added to a solution of dimethylamine hydrochloride (45.80 g, 0.56 mmol) and sodium hydroxide (22.40 g, 0.56 mmol) in water (54 mL).[20] Stirring was continued for a further 30 min, and the resulting white solid was collected and washed with diethyl ether to give 2-(5-benzyloxy-1*H*-indol-3-yl)-*N,N*-dimethyl-2-oxo-acetamide (**6**) (3.31 g, 10.3 mmol). Yield: 79%. m.p.: 178–179°C (lit.[19] 183–184°C).^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.93 (s, 1H), 7.96 (d, J= 2.2 Hz, 1H), 7.87 (d, J= 3.0 Hz, 1H), 7.51–7.29 (m, 6H), 7.02 (dd, J = 8.7, 2.2 Hz, 1H), 5.11 (s, 2H), 3.08 (d, J= 7.3 Hz, 6H) . ^{13}C NMR (75 MHz, $CDCl_3$, δ): 186.0, 168.3, 155.9, 137.2, 136.0, 131.8, 128.6, 127.9, 127.7, 126.1, 115.1, 114.0, 113.1, 104.7, 70.6, 37.6, 34.4. IR (thin film): 3187, 1641, 1605, 1475, 1434, 1268, 1081, 789, 743, 643 cm^{-1}. MS-EI (*m/z*): 322 (M^+, 56), 250 (100), 194 (3), 159 (31), 131 (18), 103 (5), 91 (44), 72 (10), 65 (4). HRMS-EI (*m/z*): [M^+] calcd for $C_{19}H_{18}N_2O_3$, 322.1317; found 322.1321.

Synthesis of [2-(5-benzyloxy-1*H*-indol-3-yl)-[$^2H_4$]-ethyl]-dimethyl-amine (7).[19] To a well-stirred suspension of lithium aluminum [$^2H_4$]-hydride (0.56 g, 13.3 mmol) in anhydrous tetrahydrofuran (15 mL) was added, in small portions, a suspension of 2-(5-benzyloxy-1*H*-indol-3-yl)-*N,N*-dimethyl-2-oxo-acetamide (**6**) (1.0 g, 3.33 mmol) in tetrahydrofuran (40 mL) and then the mixture was refluxed for 3.5 h. Following cooling in an external ice bath, the reaction complex and excess hydride were decomposed by cautious addition of 2 M aqueous NaOH. The inorganic solid was removed by filtration and the filter cake was washed using additional diethyl ether. The filtrate and washes were combined and dried over anhydrous magnesium sulfate, and the solvents were removed *in vacuo*, yielding [2-(5-benzyloxy-1*H*-indol-3-yl)-[$^2H_4$]-ethyl]-dimethyl-amine (**7**) (0.94 g, 3.2 mmol) in a 95% crude yield. A solution of oxalic acid (0.35 g, 3.86 mmol) in anhydrous diethyl ether (30 mL) was added to a solution of compound **7** (0.82 g, 2.75 mmol) in anhydrous diethyl ether (20 mL) to form a white precipitate. White precipitate that formed was filtered and washed with diethyl ether. The white solid was dissolved in hot methanol (60 mL); when this saturated solution was cooled in an ice bath it formed a white powder of the oxalic acid salt of compound **7** (0.92 g, 2.37 mmol) with 86% yield. m.p.: 180–181°C. The spectra data of compound **7** are as follows. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 7.94 (s, 1H), 7.50–7.32 (m, 5H), 7.22 (s, 1H), 7.13 (d, J= 2.3 Hz, 1H), 6.99 (d, J= 2.4 Hz, 1H), 6.94 (m, 1H), 5.12 (s, 2H), 2.33 (s, 6H). ^{13}C NMR (75 MHz, $CDCl_3$, δ): 153.1, 138.1, 132.3, 128.8, 128.5, 128.1, 128.0, 123.3, 113.2, 112.7, 112.4, 102.7, 71.3, 59.5 (m), 45.2, 23.1 (m). IR (KBr, thin film): 3037, 2823, 2200, 2065, 1586, 1451, 1219, 1051, 937, 789, 732 cm^{-1}. MS-EI (*m/z*): 298 (M^+, 41), 238 (5), 160 (5), 147 (8), 133 (2), 119 (5), 91 (24), 65 (3), 60 (100). HRMS-EI (*m/z*): [M^+] calcd for $C_{19}H_{18}D_2N_2O$, 298.1979; found 298.1981.

Synthesis of 3-(2-dimethylamino-[$^2H_4$]-ethyl)-1*H*-indol-5-ol (8). To a solution of [2-(5-benzyloxy-1*H*-indol-3-yl)-[$^2H_4$]-ethyl]-dimethyl-amine (**7**) (0.92 g, 3.13 mmol) in anhydrous methanol (10 mL), Pd/C (10%, 92 mg) at room temperature was added and the reaction mixture was stirred under a hydrogen atmosphere (1 atm.) for 20.5 h. The catalyst was removed by filtration through a bed of celite. Concentration left a dark brown solid **8** (0.64 g, 3.1 mmol) in a 99% crude yield. A mixture of oxalic acid salt of compound **7** (0.90 g, 2.32 mmol) and Pd/C (10%, 0.09 g) in anhydrous methanol (225 mL) was stirred under a hydrogen atmosphere (1 atm.) for 24 h. The catalyst was removed by filtration through a bed of celite. Concentration left a purple solid. The purple solid was recrystallized from methanol/diethyl ether to give a purple crystalline of the oxalic acid salt

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of compound **8** (0.42 g, 1.41 mmol) in a 61% yield. m.p.: 103–104°C. The spectra data of compound **8** are as follows. ^{1}H NMR (400 MHz, CD_3OD, δ): 7.81 (s, 1H), 7.07 (d, J= 8.6 Hz, 1H), 6.90 (s, 1H), 6.82 (d, J= 2.3 Hz, 1H), 6.58 (dd, J = 8.6, 2.3 Hz, 1H), 2.27 (s, 6H). ^{13}C NMR (100.6 MHz, CD_3OD, δ): 149.1, 131.1, 127.3, 121.9, 110.7, 110.6, 110.3, 101.4, 60.6 (m), 43.2, 22.2 (m). IR (KBr, thin film): 3406, 3280, 2956, 2867, 2200, 2057, 1624, 1583, 1466, 1204, 800 cm^{-1}. MS-EI (m/z): 208 (M^+, 39), 164 (5), 148 (19), 133 (2), 119 (5), 93 (3), 83 (4), 79 (2), 60 (100). HRMS-EI (m/z): [M^+] calcd for $C_{12}H_{12}D_4N_2O$, 208.1510; found 208.1507.

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