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Synthesis of deuterium-labeled hydroxybupropion

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This paper describes the synthesis of deuterium-labeled hydroxybupropion. Mass spectrometry analysis of the compound revealed over 98% deuterium enrichment.

Keywords: deuterium-labeled; bupropion; hydroxybupropion; metabolite; synthesis

Introduction

Bupropion is commonly used for the treatment of depression or smoking cessation and has been studied for a number of other uses such as bipolar disorder, attention-deficit hyperactivity disorder, and weight loss.[1–3] Bupropion is extensively metabolized in the body with less than 0.5% of product recovered intact in urine.[4] Three active metabolites have been identified: hydroxybupropion, resulting from cytochrome P450-catalyzed oxidation, and erythrohydrobupropion and threohydrobupropion, reduced diastereomers formed via non-P450-dependent pathways (Figure 1).[4] Hydroxybupropion is an active metabolite that contributes to the antidepressant effect of the parent drug. Hydroxybupropion has a longer elimination half-life than the parent drug, peak cerebrospinal fluid, and plasma concentration exceed those of bupropion by fourfold to sevenfold, and the plasma metabolite/parent area under the curve ratio is approximately 10 or greater after a single dose.[5–9]

Identification and quantification of drugs and metabolites by liquid chromatography-mass spectrometry (LC-MS) relies largely on stable isotope-labeled analogs.[10,11] A renewed interest has been recently raised to develop a robust and validated LC-MS method to determine hydroxybupropion, the major metabolite of bupropion in biological fluids. The preparation of the stable-labeled version of the title compound was requested. Although ^{2}H and ^{3}H have been prepared for pharmacological studies, the studies of its stable-labeled internal standard have not been described in detail.[12–15] In this paper, the synthetic route to [$^{2}H_6$] hydroxybupropion is described in detail.

Results and discussion

Although bupropion and hydroxybupropion have been readily prepared via several synthetic routes,[16–19] the synthesis of [$^{2}H_6$] hydroxybupropion has not been described previously. Many approaches can be followed to prepare stable-labeled version of hydroxybupropion. Based on protocols from the literature, [$^{2}H_3$] hydroxybupropion and [$^{2}H_4$] hydroxybupropion are both synthesized in three steps starting from 3-chlorobenzonitrile. Scheme 1 presents the general synthetic scheme for preparing [$^{2}H_3$] hydroxybupropion. 3-Chlorobenzonitrile (1) was alkylated with [$^{2}H_3$] ethyl magnesium bromide and then quenching with HCl aqueous solution to afford ketone (2). Bromination of compound (2) with Br_2 in carbon tetrachloride, monobromide (3) was obtained. Compound (3) was cyclized with 2-amino-2-methylpropan-1-ol to afforded [$^{2}H_3$] hydroxybupropion (4). However, MS showed compound (4) have lost about 12% deuterium label in cyclization process.

Scheme 2 presents the general synthetic scheme for preparing [$^{2}H_4$] hydroxybupropion. 3-Chlorobenzonitrile (1) was alkylated with [$^{2}H_5$] ethyl magnesium bromide and then quenched with HCl aqueous solution to afford ketone (5). Bromination of compound (5) with Br_2 in carbon tetrachloride, monobromide (6) was obtained. Compound (6) was cyclized with 2-amino-2-methylpropan-1-ol to afford [$^{2}H_4$] hydroxybupropion (7). However, MS showed compound (7) have lost about 50% deuterium label in cyclization process.

Scheme 3 presents the general synthetic scheme for preparing [$^{2}H_6$] hydroxybupropion (14). Treatment of [$^{2}H_6$] acetone with NaCN and ND_4Cl in D_2O afford α-amino alcohol (9a) and α-amino nitrile (9b) through the Strecker reaction. The by-product (9a) can be removed by evaporating under the reduced pressure (0.5 mmHg). The Strecker reaction is a key step that influenced deuterium enrichment. In order to effectively prevent significant loss or scrambling of deuterium label, H_2O and NH_4Cl were replaced by D_2O and ND_4Cl. α-Amino nitrile (9a) was hydrolyzed by DBr in D_2O to afford α-amino acid (10). Application of DBr

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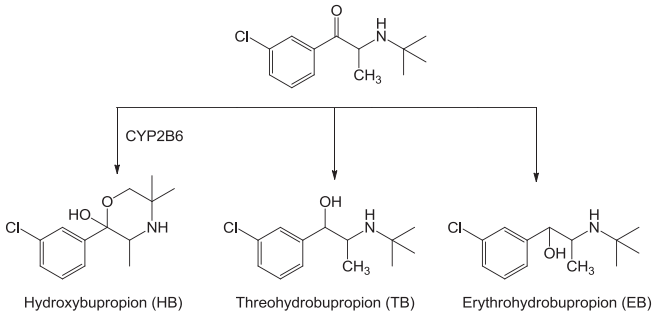


Figure 1. The metabolite routes of bupropion.

and D_2O can greatly decrease the loss of deuterium. In addition, methanol was employed to treat the reaction residue instead of methanol with ammonia, which avoids aminolysis, and greatly decreased the loss of deuterium. α-Amino acid (10) was treated with $SOCl_2$ in methanol to give α-amino ester (11), which was reduced by $LiAlH_4$ in THF to afford β-amino alcohol (12). Compound (12) was reacted with 2-bromo-1-(3-chlorophenyl) propan-1-one by cyclization reaction to give the [$^2H_6$] hydroxybupropion (13).

The HPLC results showed that [$^2H_6$] hydroxybupropion (13) was obtained with over 98% chemical purity. MS analysis of [$^2H_6$] hydroxybupropion (13) revealed over 98% deuterium enrichment. The synthesis provided important internal standards for the clinical studies of bupropion.

Experimental

General

All reagents were obtained from Sigma-Aldrich and CDN Isotope. Mass spectra were recorded using a Quattro micro API mass spectrometer. ^1H NMR spectra were recorded on a Bruker 300-MHz instrument (Bruker Corporation, Germany). Chemical purities were determined by an Agilent 1200 HPLC with a XDB-C18 column, 5 μm, 4.6 × 150 mm (Agilent, USA).

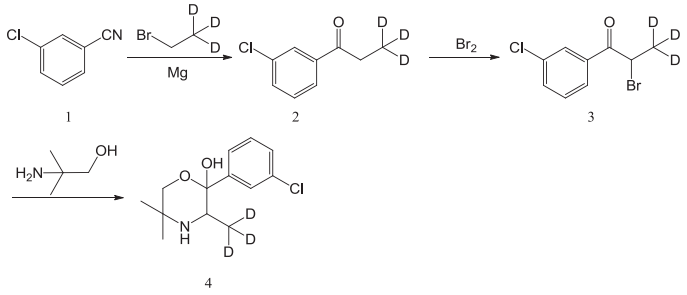
Synthesis of [$^2H_6$]2-amino-2-methylpropanoic acid(10)

To a stirred solution of ND_4Cl (15.0 g, 0.261 mol) in D_2O (40 mL) was added [$^2H_6$] acetone (8) (13.5 g, 0.21 mol) in diethyl ether (40 mL), while the temperature was maintained at 5–8 °C. Then the solution of NaCN (11.0 g, 0.224 mol) in D_2O (24.5 mL) was added to the aforementioned solution, while the temperature was maintained at 3–6 °C. After addition, stirring was continued at 5–10 °C for 1.5 h. The organic phase was separated, and the aqueous phase was extracted with diethyl ether (50 mL × 8). The organic layers were combined, dried over Na_2SO_4, and concentrated under reduced pressure to give a blue oil residue (18.7 g). ^1H NMR showed the residues contain 80% compound (9b) and 20% compound (9a). The aforementioned residue was dissolved in D_2O (40 mL) containing DBr (40 g, 0.49 mol) to give a light brown solution under ice–water bath. The solution was heated at 50–60 °C, evaporated to dryness to afford (9b) as a light yellow semi-solid, and dried under the reduced pressure (0.5 mmHg). Thin layer chromatography (TLC) analysis showed that more than 90% by-product was removed.

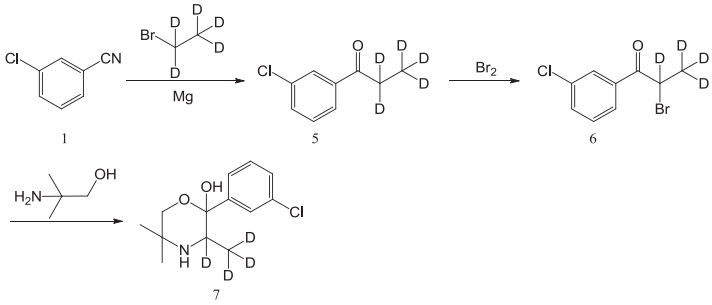
Compound (9b) was dissolved in D_2O (40 mL) containing DBr (40 g, 0.49 mol), and the mixture was stirred at 125 °C for 8 h. The mixture was evaporated to dryness, co-evaporated with 30% H_2O_2 (45 mL) three times, and deeply dried in vacuum to give a white semi-solid. The solid was dissolved in methanol, followed by filtering inorganic salts. The filtrate was adjusted to pH 6 by adding pyridine and stirred for 8 h, followed by filtering the white solid. The filter cake was rinsed with methanol and dried under vacuum to give (10) as a white solid (12.0 g, 52.3%).

Synthesis of [$^2H_6$] methyl 2-amino-2-methylpropanoate hydrochloride (11)

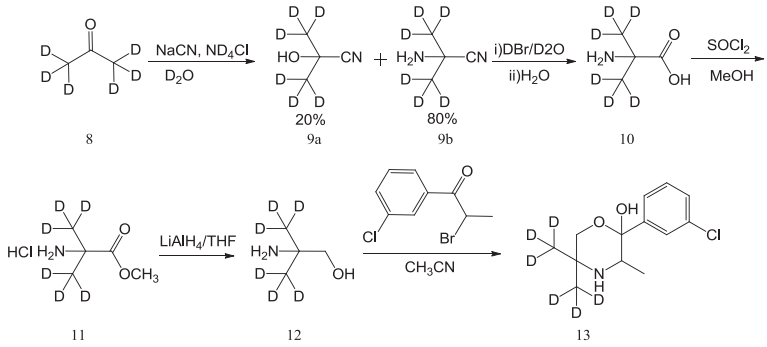
To a suspension of compound (10) (2 g, 18.3 mmol) in methanol (15 mL), was added $SOCl_2$ (5.4 mL) dropwise, and the mixture was stirred at 50 °C



Scheme 1. Synthesis of [$^2H_3$] hydroxybupropion.



Scheme 2. Synthesis of [$^2H_4$] hydroxybupropion.



Scheme 3. Synthesis of [$^2H_6$] hydroxybupropion.

for 4 h. The reaction mixture was concentrated under reduced pressure to give a white solid. The solid was redissolved in CH_2Cl_2 (60 mL), followed by filtering the insoluble matter. The filter cake was rinsed thoroughly with CH_2Cl_2 (20 mL), and the combined organic layer was concentrated under reduced pressure to give (11) as a white solid (2.9 g, 99.2%).

^{1}H NMR ($CDCl_3$, 300 Hz): δ 8.93 (s, 3H), 3.82 (s, 3H).

Synthesis of [$^2H_6$] 2-amino-2-methylpropan-1-ol (12)

To a solution of compound (11) (2.9 g, 18.1 mmol) in freshly distilled THF (60 mL), was added $LiAlH_4$ in two batches by cooling with an ice–water bath as necessary. After addition, the suspension was stirred at room temperature for 1.5 h. TLC showed the reaction was finished. The reaction solution was cooled in an ice–water bath and quenched by adding diethyl ether (80 mL), 30% H_2O_2 (1.5 mL), 15% NaOH (3.0 mL), and H_2O (7.5 mL). The mixture was stirred for a further 1 h at room temperature, then, filtered through celite. The filtrate was dried with anhydrous Na_2SO_4 and concentrated under reduced pressure to give a light brown oil. The oil was redisolved in diethyl ether (35 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford (12) as a colorless oil (1.2 g, 69.6%).

^{1}H NMR ($CDCl_3$, 300 Hz): δ 7.77(s, 2H), 3.63(s, 2H).

Synthesis of [$^2H_6$] hydroxybupropion (13)

To a stirred solution of compound (12) (1.2 g, 12.61 mmol) in dry CH_3CN (13.5 mL), was added 2-bromo-1-(3-chlorophenyl)propan-1-one (1.1 g, 4.44 mmol). The reaction solution was heated at 95 °C and refluxed for 6 h. TLC showed little starting material remained. The solution was co-evaporated with ethanol (150 mL × 3) to afford a yellow solid. The solid was purified by column chromatography on silica gel column, eluted with CH_2Cl_2/saturated methanol ammonia (10:0.3) to afford (6) as a silver gray solid (0.54 g, 46.5%).

^{1}H NMR ($CDCl_3$, 300 Hz): δ 7.61(d, 1H, J = 1.5 Hz), 7.49(d, 1H, J = 7.5 Hz), 7.30 (d, 2H, J = 4.0 Hz), 3.94(d, 1H, J = 9.0 Hz), 3.80(s, 1H), 3.40(d, 1H, J = 6.0 Hz), 3.25 (m, 1H), 1.28 (b, 1H), 0.91(d, 3H, J = 6.0 Hz). MS-EI, (m/z): 262.1 (MH^+, 100), 263.1 (18), 264.1 (30), 265.1(5). HPLC (XDB-C18, CH_3OH/20 mmol/L NaH_2PO_4 + 0.05% TEA = 57/43, 1.0 mL/min): t_R 11.02 min (98.5%). Isotopic enrichment determined by MS was over 98%.

Conflict of interest

The authors did not report any conflict of interest.

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