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The Synthesis of (2R)- and (2S)-[2-^{3}H]-Propionic Acid

Ronald J. Parry* and Rajesh Turakhia

Department of Chemistry, Rice University, Houston, Texas 77251

Summary

A practical synthesis of (2R)- and (2S)-[2-^{3}H]-propionic acid is described. The key steps in the synthesis are the reduction of [*formyl*-^{3}H]-3-methoxy-4-mesyloxybenzaldehyde with R- or S-Alpine Borane to (7S)- and (7R)-[7-^{3}H]-3-methoxy-4-mesyloxybenzyl alcohol and the use of the vanillyl moiety as a masked carboxyl group.

Key Words: propionic acid, tritium, stereospecific labeling, Alpine Borane

Introduction

In conjunction with biosynthetic studies on the antibiotic furanomycin,[1] (2R)- and (2S)-[2-^{3}H]-propionic acid were required. A survey of the literature revealed that no convenient method for the preparation of these labeled compounds has been devised. An enzymatic synthesis[2] reported in 1975 utilizes the commercially unavailable enzyme γ-cystathionase, while the chemical syntheses reported for the preparation of the corresponding stereospecifically deuterated propionates[3-6] all suffer from extremely low yields as well as the inconvenience of handling a series of volatile intermediates. We have therefore developed a more practical synthesis of stereospecifically tritiated propionic acid.

Results and Discussion

The synthesis (Scheme I) began with 3-methoxy-4-mesyloxy-benzaldehyde[7] (**1**) which was reduced with [^{3}H]-potassium borohydride (100 mCi) in methanol to yield [7-^{3}H]-3-methoxy-4-mesyloxybenzyl alcohol (**2**) in 95% yield with a radiochemical yield of 57%. Oxidation of the labeled benzyl alcohol with chromium trioxide in pyridine[7] then produced [*formyl*-^{3}H]-3-methoxy-4-mesyloxybenzaldehyde (**3**) in *ca.* 95% yield. Reduction of the labeled benzaldehyde with R- or S-Alpine Borane[8] proceeded smoothly to give (7S)- and (7R)-[7-^{3}H]-3-methoxy-4-mesyloxybenzyl

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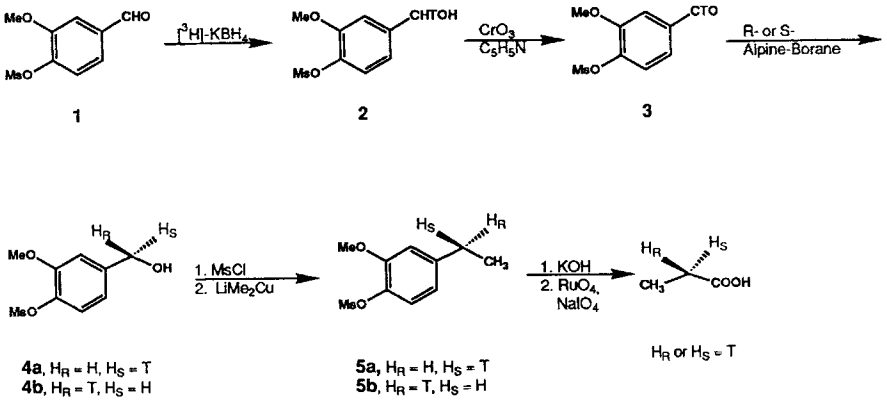
alcohol (**4a,b**) (52% yield). The stereospecifically tritiated alcohols were each treated with mesyl chloride and triethylamine to form the corresponding dimesylates which were isolated in 85% yield after recrystallization. The next stage in the synthesis involved the coupling of each of the stereospecifically tritiated dimesylates with lithium dimethylcuprate. This coupling reaction was anticipated to proceed with inversion of configuration[9] provided there was no S_N1 character in the transition state. On the basis of literature precedent,[7] it appeared that the presence of the electron withdrawing mesyl group would suppress this undesirable reaction path. In the event, each coupling reaction proceeded in about 56% yield to generate the stereospecifically labeled ethylbenzene derivatives **5a** and **5b**. Subsequent studies (*vide infra*) demonstrated that the displacement had indeed occurred with inversion of configuration. The final stages of the synthesis involved base-catalyzed removal of the mesyl deactivating group (91%) followed by ruthenium tetroxide oxidation[10] of the aromatic ring to unmask the propionate carboxyl group. The propionic acid was isolated by steam-distillation and then converted to its sodium salt. The overall chemical yield was *ca.* 10%, while the overall radiochemical yield was *ca.* 5%.

Scheme 1: *Synthesis of (2R)- and (2S)-[2-^{3}H]-Propionate*



The optical purity and configuration of the labeled propionic acid obtained from this synthesis was evaluated by carrying out the synthesis of one of the stereoisomers in deuterated form. Mesylation of methyl vanillate followed by reduction with lithium aluminum deuteride yielded (7-$^2H_2$)-3-methoxy-4-mesyloxybenzyl alcohol. The deuterated alcohol was oxidized in the usual way to the corresponding deuterated aldehyde and the latter was reduced with R-Alpine Borane. The resulting monodeuterated alcohol was converted into sodium propionate in the manner previously outlined. The sodium propionate was treated with dry HCl gas to form free propionic acid and the latter was reacted

with (S)-(+)-methyl mandelate in the presence of DCC and DMAP to yield the corresponding ester.[11] The ^{1}H NMR spectrum of the mandelate ester revealed[11] that the deuterated propionic acid had the expected configuration (S) and an optical purity of *ca.* 90%. Since the commercially available R-Alpine Borane has an optical purity of about 91%,[12] it follows that both the reduction and displacement steps exhibit a very high degree of stereoselectivity.

In summary, a new synthesis of stereospecifically tritiated propionic acid has been developed. This synthesis has a number of advantages over previously published methods. It proceeds in acceptable overall yield, it utilizes a series of non-volatile intermediates that are easily handled and purified, and the tritium is introduced from labeled borohydride so it is possible to obtain propionic acid with a high specific radioacitivity.

Experimental Section

General Methods. ^{1}H NMR spectra were recorded on either a Jeol FX-90Q (90 MHz) or an IBM AF300 (300 MHz) spectrometer using $CDCl_3$ as solvent unless otherwise noted. Chemical shifts are given in parts per million downfield from tetramethylsilane. All melting points were taken on a Fischer-Johns melting point apparatus and are uncorrected. Mass spectra were run on Finnegan 3300 and CEC 111021-110B mass spectrometers. Analytical thin layer chromatography was performed with precoated Merck silica gel type 60, F-254 glass plates (0.25 mm layers). Column chromatography was performed using Baker silica gel (60-200 mesh or 200-400 mesh). Chemical reagents were purchased from the Aldrich Chemical Company while the tritiated potassium borohydride was purchased from Amersham/Searle Corporation.

3-Methoxy-4-mesyloxybenzaldehyde (1). This compound was synthesized from 3-methoxy-4-hydroxybenzaldehyde (vanillin) using the procedure of Battersby *et al.*[7] and was obtained in 68% yield after recrystallization from 95% ethanol; m.p. 92-94° (lit.,[7] 93-94°).

[7-^{3}H]-3-Methoxy-4-mesyloxybenzyl Alcohol (2). To a stirred solution of 3-methoxy-4-mesyloxybenzaldehyde (1.1 g, 4.8 mM) in 60 mL of anhydrous methanol, sodium borohydride (12 mg, 0.31 mM) was added. Stirring was continued for 1.5 h after which time [^{3}H]-potassium borohydride (0.44 mg, 100 mCi) was added. The resulting solution was stirred for an additional 4 h and sodium borohydride (67 mg, 1.8 mM) was then added. The reduction was allowed to proceed overnight. It was then quenched with cold 2N HCl. The methanol was removed *in vacuo*, saturated

brine (40 mL) added to the residue, and the tritiated alcohol extracted with dichloromethane. Removal of the solvent left a colorless oil weighing 1.01 g (95%) with a total activity of 57 mCi. The ^{1}H NMR spectrum (d_6-DMSO) was in accordance with the expected structure and was similar to that previously reported:[7] δ 3.41 (s, 3H, CH_3SO_2), 3.72 (s, 3H, CH_3O), 4.50 (d, J = 4 Hz, 2H, CH_2OH), 5.24-5.39 (t, J = 4 Hz, 1H, OH), 6.92-7.31 (m, 3H, ArH).

[*formyl*-^{3}H]-3-Methoxy-4-mesyloxybenzaldehyde (3). This labeled aldehyde was prepared from the preceding labeled alcohol using a published procedure.[7] The crude aldehyde (1.03 g) was obtained in about 95% yield and had a total activity of 52.1 mCi (radiochemical yield of 91%).

(7S)-[7-^{3}H]-3-Methoxy-4-mesyloxybenzyl Alcohol (4a). The crude benzaldehyde **3** (0.98 g, 4.3 mM) was dissolved in dry THF (20 mL) and a 0.5 M solution of R-Alpine Borane (14.4 mL, 7.2 mM) was added with stirring. Stirring of the reaction mixture was continued for 4 h at room temperature, at the end of which time the reaction was quenched by the addition of acetaldehyde (1.0 mL). The reaction mixture was stirred for an additional 15 min and the solvents were then removed *in vacuo*. α-Pinene was removed from the residue by warming at 40° for 2 h under high vacuum. The resulting greenish oil was dissolved in ether (25 mL), the solution cooled to 0°, and ethanolamine added (0.5 mL). The precipitate was removed by filtration and the filtrate taken to dryness *in vacuo*. Flash chromatography of the residue (silica gel, ether) yielded pure (7S)-[7-^{3}H]-3-methoxy-4-mesyloxybenzyl alcohol (0.51 g, 52% yield).

(7S)-[7-^{3}H]-3-Methoxy-4-mesyloxybenzyl Methane Sulphonate. The labeled methane sulphonate (0.59 g) was prepared from 0.51 g of the (7S)-[7-^{3}H]-alcohol **4a** in 85% yield using a previously reported procedure.[7] The recrystallized methane sulfonate had a m.p. of 112-115° (lit.,[7] 114-115°) and a total activity of 13.5 mCi (52% radiochemical yield from **3**). The ^{1}H NMR spectrum was similar to that reported[7] previously: δ 2.92 (s, 3H, CH_3SO_2), 3.12 (s, 3H, CH_3SO_2), 3.84 (s, 3H, CH_3O), 5.12 (s, 2H, CH_2O), 6.88-7.29 (m, 3H, ArH).

(7S)-[7-^{3}H]-3-Methoxy-4-mesyloxyethylbenzene (5a). Purified copper(I) iodide[13] (0.87 g, 4.56 mM) was suspended in anhydrous ether (5 mL) and the mixture cooled to -10°. A solution of methyllithium in ether (1.2 M, 6.3 mL, 7.6 mM) was added while stirring the reaction mixture at -10° under nitrogen. The resulting colorless solution was held at -10° while a solution of

(7S)-[7-^{3}H]-3-methoxy-4-mesyloxybenzyl methane sulfonate (0.59 g, 1.89 mM, 13.5 mCi) in a mixture of dry toluene (5 mL) and dry methylene chloride (15 mL) was added slowly with stirring. After 15 min, the reaction mixture was quenched with excess, saturated ammonium chloride solution and filtered to remove insoluble salts. Extraction of the filtrate with methylene chloride and removal of the solvent from the dried extract *in vacuo* yielded a colorless oil that was purified by flash chromatography(silica gel, 3:1 hexanes: ethyl acetate). The purified product (0.25 g, 56%; 7.5 mCi, 55%) exhibited spectral data consistent with the assigned structure: ^{1}H NMR: δ 1.18-1.32 (t, J = 7 Hz, 3H, $\mathbf{CH_3}CH_2$), 2.56-2.79 (q, J = 7 Hz, 2H, $CH_3\mathbf{CH_2}$), 3.21 (s, 3H, CH_3SO_2), 3.92 (s, 3H, CH_3O), 6.75-7.24m (m, 3H, ArH); MS: *m/e* 230. 0627 (230.0613 calc. for $C_{10}H_{14}O_4S$).

(7S)-[7-^{3}H]-3-Methoxy-4-hydroxyethylbenzene. A mixture of mesylate **5a** (0.25 g, 1.09 mM), 2N KOH (25 mL), and methanol (25 mL) was stirred overnight at room temperature. The methanol was then removed *in vacuo* and the pH of the aqueous solution adjusted to 2 with conc. HCl. The product was isolated by repeated extraction with dichloromethane. The combined organic extracts were washed with brine, dried, and the solvent removed *in vacuo* to yield 0.15 g of a colorless oil (91%). The product exhibited spectral data consistent with the assigned structure: ^{1}H NMR: δ 1.06-1.15 (t, J = 7 Hz, 3H, $\mathbf{CH_3}CH_2$), 2.44-2.66 (q, J = 7 Hz, 2H, $CH_3\mathbf{CH_2}$), 3.94 (s, 3H, CH_3O), 6.60-6.90 (m, 3H, ArH); MS: *m/e* 152.0813 (152.0837 calcd. for $C_9H_{12}O_2$).

Oxidation of (7S)-[7-^{3}H]-3-Methoxy-4-hydroxyethylbenzene to (2S)-[2-^{3}H]-Propionic Acid. To a solution of (7S)-[7-^{3}H]-3-methoxy-4-hydroxyethylbenzene (0.153 g, 1.0 mM) in a mixture of carbon tetrachloride (5 mL), acetonitrile (5 mL), and water (6.2 mL) sodium periodate (3.84 g, 18 mM) and ruthenium trichloride (5 mg, 0.022 mM) were added. The resulting mixture was stirred overnight. The solution was then filtered, and the pH of the filtrate adjusted to 9 with dilute NaOH. The solution was refiltered and the alkaline filtrate was lyophilized. The residue was dissolved in water (100 mL) and the pH of the solution adjusted to 2 with 2N sulfuric acid. The resulting solution was steam-distilled and distillate collected until no significant quantity of radioactivity distilled. The pH of the distillate was adjusted to 8 with dilute sodium hydroxide and the alkaline distillate lyophilized to yield sodium propionate as a white solid (80 mg, 48% yield, sp. act. 5.07 mCi/mM), ^{1}H NMR (D_2O/p-dioxane): δ 0.76-0.96 (t, J = 8 Hz, 3H, $\mathbf{CH_3}CH_2$), 1.84-2.05 (q, J = 8 Hz, 3H, $CH_3\mathbf{CH_2}$).

Synthesis of (2R)-[2-^{3}H]-Propionic Acid. (2R)-[2-^{3}H]-Propionic acid was synthesized from [*formyl*-^{3}H]-3-methoxy-4-mesyloxybenzaldehyde (**3**) via the same reaction sequence used to prepare the (2S) compound except that the reduction of **3** was carried out with S-Alpine Borane. Since S-Alpine Borane is prepared commercially from (S)-(-)-α-pinene of 87% optical purity, the optical purity of the resulting propionic acid should be close to 87%.

Methyl 3-Methoxy-4-mesyloxybenzoate. Methyl 3-methoxy-4-hydroxybenzoate (methyl vanillate) (3.50 g, 19.2 mM) was dissolved in dry pyridine (40 mL), the solution chilled in an ice-bath, and distilled mesyl chloride (2.97 mL, 38.4 mM) added dropwise with stirring. The resulting solution was stirred for 6 h at 0° and then poured into excess cold 2N H_2SO_4. The acidic mixture was stirred for 30 min and the precipitated solid filtered off, washed with cold water, and dried *in vacuo* to yield 3.8 g of colorless crystals (76%) which appeared pure by tlc and ^{1}H NMR: δ 3.20 (s, 3H, CH_3SO_2), 3.91 (s, 3H, CH_3O), 3.95 (s, 3H, CH_3O), 7.26-7.40 (m, 1H, ArH), 7.60-7.76 (m, 2H, ArH). An analytical sample was obtained by recrystallization from 95% ethanol, mp 98-99.5°. (Found: C, 46.34; H, 4.43. $C_{10}H_{12}O_6S$ requires C: 46.11, H: 4.65).

(7-$^2H_2$)-3-Methoxy-4-mesyloxybenzyl Alcohol. Methyl 3-methoxy-4-mesyloxybenzoate (1.17g 4.5 mM) was dissolved in anhydrous ether (100 mL) and lithium aluminum deuteride (0.14 g, 3.36 mM) added slowly with stirring under nitrogen. The mixture was stirred for 12 h at room temperature at the end of which time tlc showed that a considerable quantity of unreduced ester remained. Additional $LiAlD_4$ (0.070 g, 1.67 mM) was added and the reaction stirred for another 3 h. The reaction mixture was quenched by addition of water (0.20 mL), 15% aq. NaOH (0.20 mL) and finally more water (0.63 mL). The mixture was filtered and the filtrate taken to dryness *in vacuo*. The residual oil was purified by flash chromatography (silica gel, ether) to yield 0.82 g of a pale yellow oil (78%) whose ^{1}H NMR spectrum (d_6-DMSO) was identical to that of the tritiated alcohol **2** except for the complete absence of the signals due to the benzylic methylene group.

(2S)-(2-$^2H_1$)-Propionic Acid. (2S)-(2-$^2H_1$)-Propionic acid was synthesized from (7-$^2H_2$)-3-methoxy-4-mesyloxybenzyl alcohol using the same reaction sequence that was employed to synthesize the corresponding form of tritiated propionic acid. The overall yield from methyl vanillate was about 5%.

Derivatization of (2S)-(2-$^2H_1$)-Propionic Acid with (S)-(+)-Methyl Mandelate. Dry (2S)-(2-$^2H_1$)-sodium propionate (81 mg, 0.84 mM) was placed in a small three-necked flask. The flask was evacuated to less than 1 mm and then filled with dry HCl gas to a pressure of about 160 mm. Over a 30 min time span, the flask was twice warmed gently with a small flame. The flask was then cooled to -78° and evacuated with an oil pump to remove residual HCl. The flask was next warmed to room temperature and dry methylene chloride (7 mL) added to the residue. The methylene chloride solution of propionic acid was transferred to a clean flask, leaving behind NaCl and any unreacted sodium propionate. After cooling the methylene chloride solution to 0°, (S)-(+)-methyl mandelate (138 mg, 0.84 mM), 4-dimethylaminopyridine (10 mg, 0.084 mM), and dicyclohexylcarbodiimide (172 mg, 0.84 mM) were added. The resulting reaction mixture was stirred at 5° for 3 h. The mixture was then filtered and the filtrate evaporated to dryness *in vacuo*. Flash chromatography of the residue (silica gel, 7:3 hexane/ethyl acetate) yielded the mandelate ester as a colorless oil (40 mg, 21%). The 300 mHz ^{1}H NMR spectrum (d_6-benzene) of the deuterated mandelate ester exhibited a multiplet centered at δ 2.40 (H_S) and a multiplet centered at 2.52 (H_R) in a ratio 1:9.

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12. The R-Alpine Borane is prepared commercially from (R)-(+)-a-pinene of 91% optical purity.