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Synthesis of D-(-)-[1,7-$^{13}C_2$]Shikimic Acid

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SUMMARY

The chemical synthesis of 99% enriched D(-)-[1,7-$^{13}C_2$]shikimic acid from [1,2-$^{13}C_2$]acetate in 25% overall yield is described. A two-carbon phosphonate synthon was prepared from [1,2-$^{13}C_2$]acetate and used in the shikimate synthesis of Fleet and coworkers.

(Key words: ^{13}C labeling, chemical synthesis, shikimic acid, optically active)

INTRODUCTION

Shikimic acid is an important intermediate in the biosynthesis of the aromatic amino acids, but also gives rise to a variety of structurally diverse secondary metabolites in plants and microorganisms (1,2). For studies on the origin of the p-hydroxybenzoic acid precursor of the plant pigment, shikonin (3), we needed shikimic acid labeled specifically and intramolecularly with carbon-13 in positions 1 and 7. This arrangement of isotopic labels allows the distinction of biosynthetic pathways in which shikimic acid is incorporated into a product directly, with all seven carbon atoms, from ones in which it is incorporated via the aromatic amino acids, i.e., with loss of the carboxyl group. We therefore developed and describe here an efficient synthesis of D-(-)-[1,7-$^{13}C_2$]shikimic acid.

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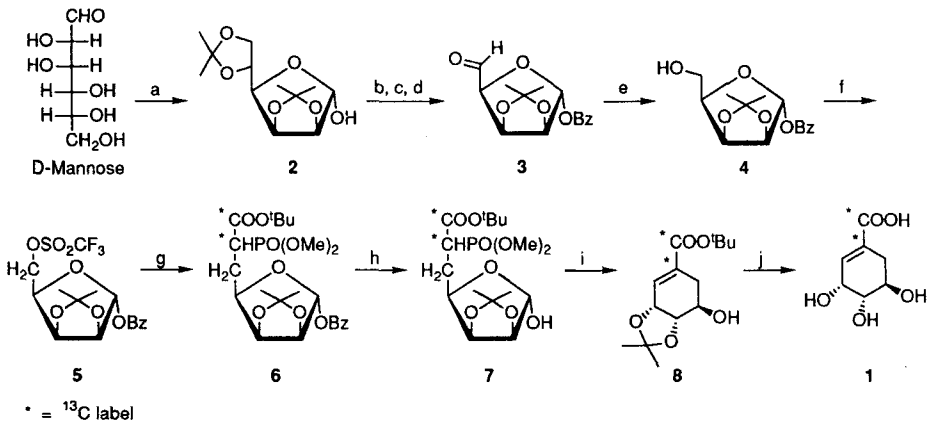
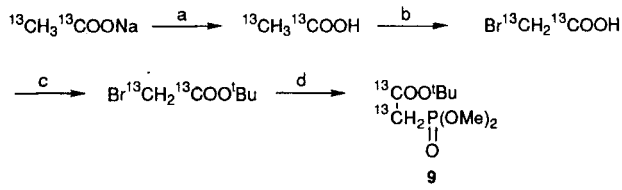
RESULTS AND DISCUSSION

Fleet *et al.* (4) have reported a synthesis of optically pure (-)-shikimic acid (**1**), the natural enantiomer, from D-mannose in 39% yield (Scheme 1). That procedure attracted our attention because it not only gave an excellent yield but also should easily permit isotopic labeling at various positions of shikimic acid. We adopted their procedure to synthesize several specifically labeled shikimic acids in optically pure form by replacing a starting material or a reagent by a labeled one. The only modification of the original procedure was the debenzylation step (**67**). We removed the benzyl protecting group of **6** by treatment with $HCOONH_4$ and Pd/C in methanol instead of using Pd/C and hydrogen gas as a matter of practical convenience.



Scheme 1 The synthesis of D-(-)-[1,7-$^{13}C_2$]shikimic acid by the route of Fleet *et al.*
a) acetone, H^+ b) BzCl, NaH c) HCl, aq. MeOH d) $NaIO_4$ e) $NaBH_4$
f) $(CF_3SO_2)_2O$, py. g) **9**, NaH h) $HCOONH_4$, Pd/C i) NaH
j) 60% aq. CF_3COOH

In the Fleet synthesis, carbon atoms 1 and 7 of shikimate arise from carbons 1 and 2 of the phosphonoacetate reagent used in step g (Scheme 1). Thus, to introduce the desired label we prepared the labeled reagent **9** from sodium [1,2-$^{13}C_2$]acetate in 65% yield as shown in Scheme 2. Condensation with a slight excess of triflate **5** followed by debenzylation, ring closure by Wittig reaction and deprotection then gave D-(-)-[1,7-$^{13}C_2$]shikimic acid in 38% yield (24.7% overall based on sodium acetate).



Scheme 2. Synthesis of t-butyl dimethyl phosphono-[1,2-$^{13}C_2$]acetate
a) H_3PO_4 b) PBr_3, Br_2, $(CF_3)CO)_2O$ c) t-BuOH, DCC, 4-pyrrolidinopyridine
d) $P(OMe)_3$

As the above example demonstrates, the synthesis of Fleet *et al.* is a very versatile route for the introduction of specific isotopic labels into shikimic acid. By the same route we have also synthesized D-(-)-[2-^{13}C]shikimic acid (5) (from D-[1-^{13}C]mannose), D-(-)-[2-^{2}H]shikimic acid (6) (by oxidation of intermediate **2**, Scheme 1, and reduction of the lactone with $NaBD_4$), D-(-)-[6-$^{2}H_1$]shikimic acid, 6R:6S isomer 7:3 (6) (by reduction of intermediate **3**, Scheme 1, with $NaBD_4$) and D-(-)-[4-^{2}H]shikimic acid (6) (by preparing [3-^{2}H]-**7** by an alternate route from D-arabinose). Position 3 of shikimic acid, likewise, could readily be labeled with deuterium or ^{13}C starting from 2-labeled D-mannose which in turn can be easily prepared from the corresponding 1-labeled D-glucose by the Barker/Serianni method (7). Only position 5 of shikimic acid is not readily accessible to labeling by this chemistry. The route gives reproducibly good yields, making it very suitable for isotopic syntheses.

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EXPERIMENTAL

$^{13}CH_3{}^{13}COOH$ from $^{13}CH_3{}^{13}COONa$ (8) A 100 mL round-bottom flask, fitted with a magnetic stirring bar, was charged with 17.3 g (0.123 mol) of P_2O_5 and capped with a rubber septum. Water (6.7 mL, 0.37 mol) was slowly added from a syringe to the cooled flask (ice bath) at a rate such that no appreciable buildup of pressure occurred. After the addition was complete, the flask was heated at 70° C for 2h. The contents of the flask were allowed to cool and $^{13}CH3{}^{13}COONa$ (3.93 g, 46.8 mmol, 99% ^{13}C) was added. After the flask had been fitted with a short-path distillation apparatus, it was carefully heated with a Bunsen burner. Distillation was complete in less than 5 min. to give crude acetic acid (4.2 g) which contained about 10% of water.

$Br^{13}CH_2{}^{13}COOH$ from $^{13}CH_3{}^{13}COOH$ (8) Trifluoroacetic anhydride (30.0 g, 0.14 mol) was carefully added to the cooled (ice bath) $^{13}CH_3{}^{13}COOH$ obtained above. After the addition was complete, PBr_3 (0.27 g, 1.0 mmol) was added to the reaction mixture and the reaction flask was heated to 60° C. When trifluoroacetic anhydride started to reflux, bromine (4.0 mL) was slowly added to the reaction flask at a rate such that a pale yellow color was just maintained in the flask. After the addition was complete the reaction mixture was heated at 70° C for 2h. It was then cooled to room temperature and water (2.4 mL) was slowly added. Water was then removed by distillation (bath temp. 110° C) and the remaining liquid was dissolved in dry ether (100 mL) and dried over Na_2SO_4. After filtration it was distilled at atmospheric pressure and then at 10 torr (bath temp. 130° C) for 10 min. to remove volatile substances. The remaining liquid (5.5 g) solidified upon cooling in a water bath. It was used directly for the next reaction.

$Br^{13}CH_2{}^{13}COO^{13}Bu$ from $Br^{13}CH^{13}COOH$ (9) To a stirred solution of $Br^{13}CH_2$-$^{13}COOH$ in dry CH_2Cl_2 (180 mL) was added dicyclohexylcarbodiimide (14.2 g, 69 mmol), butanol (5.1 g, 69 mmol), and 4-pyrrolidinopyridine (0.93 g, 6.3 mmol). After stirring overnight the mixture was filtered to remove dicyclohexylurea and washed successively with water (3 x 200 mL), 5 % acetic acid (3 x 200 mL), and water (3 x 200 mL). The dark brown solution was gravity filtered through ca. 50 mL of silica gel. Removal of solvent under reduced pressure afforded a brown oil (6.1 g), which was used directly for the next reaction.
$Br^{13}C_2{}^{13}COOtBu$: GC/MS (m/z, rel intensity) 183 (M^+-CH_3, 6), 181 (M^+-CH_3, 6), 125(M^+-^{t}BuO, 14), 123 (M^+-^{t}BuO, 14), 96 (M^+-COO^tBu, 7), 94 (M^+-COO^tBu, 7), 59 (53), 57 (100).

$(MeO)_2P(O)^{13}CH_2{}^{13}COO^tBu$ (9) from $Br^{13}CH_2{}^{13}COO^tBu$ A mixture of t-butyl bromoacetate (6.1 g) and trimethylphosphite (6.1 g) was refluxed (oil bath temp. 130° C) for 4h. The resulting mixture was cooled to room temperature and purified by column chromatography (ether-pentane 1:1) to give **9** (6.9 g, 65% from $^{13}CH_3{}^{13}COONa$): R_f 0.51 (hexane-EtOAc 1:4); ^{1}H-NMR ($CDCl_3$, 300 MHz) ∂1.43 (s, 9H), 2.85 (ddd, 2H, J=129 Hz, 21.4 Hz, 7.2 Hz), 3.75 (d,

6H, J=9 Hz); ^{13}C-NMR ($CDCl_3$, 75.5 MHz) ∂ 34.62 (dd, J=134 Hz, 58 Hz), 164.65 (dd, J=56 Hz, 6 Hz); GC-MS (m/z, rel. intensity) 211 (M^+-CH_3, 0.1), 171 (21), 153 (100), 125 (23), 109 (28), 95 (17), 79 (13), 57 (43).

t-Butyl (benzyl 5,6-dideoxy-6-dimethylphosphono-2,3-O-isopropylidene-a-D--[6,7-$^{13}C_2$]lyxoheptofuranosid)uronate ([6,7-$^{13}C_2$]-6). Prepared in 57% yield from **5** (1.6 equiv.) and **9** (1 equiv.) according to the procedure of Fleet *et al.* (4). ^{1}H-NMR ($CDCl_3$, 300 MHz) ∂ 1.43 (s, 3H), 1.44 (s, 9H), 1.46 (s, 3H), 2.22-2.30 (m, 2H), 2.75-3.60 (m, 1H), 3.74-3.81 (m, 6H), 4.6-4.10 (m, 1H), 4.41 (dd, 1H, J=11.6 Hz, 1.2 Hz), 4.59-4.65 (m, 3H), 4.99 (d, 1H, J=2.8 Hz), 7.25-7.31 (m, 5H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz) ∂ 42.97 and 43.77 (dd, J=131 Hz, 57 Hz), 167.76 and 167.95 (dd, J=57 Hz, 6 Hz) (mixture of diastereomers); GC-MS (m/z, rel. intensity) 372 (1.3), 325 (0.8), 280 (2), 232 (11), 223 (14), 222 (7), 210 (10), 205 (15), 193 (22), 168 (20), 166 (10), 165 (12), 164 (24), 125 (16), 122 (14), 109 (10), 95 (10), 91 (100).

t-Butyl (5,6-dideoxy-6-dimethylphosphono-2,3-O-isopropylidene-a-D-[6,7-^{13}C 2]lyxo-heptofuranosid)uronate ([6,7-$^{13}C_2$]-7). To a stirred solution of [6,7-$^{13}C_2$]-**6** (8.50 g) in MeOH (350 mL) was added 10% Pd/C (6.10 g) in small portions, and then $HCOONH_4$ (5.25 g). The mixture was slowly heated to 75° C and stirred for 1h at that temperature. After cooling to room temperature the reaction mixture was filtered to remove the catalyst, and the methanolic solution was concentrated under reduced pressure. The resulting oil was dissolved in benzene (30 mL) and concentrated again. This procedure was repeated once more to obtain a thick syrup (6.58 g). This material was used directly for the next reaction.

t-Butyl (3R,4S,5R)-3,4,5-trihydroxy-3,4-O-isopropylidene-[1,7-$^{13}C_2$]cyclohex-1-enecarboxylate ([1,7-$^{13}C_2$]-8). Prepared from [6,7-$^{13}C_2$]-7 as described (4) (68% from [6,7-$^{13}C_2$]-**6**). ^{1}H-NMR ($CDCl_3$, 300 MHz) ∂ 1.38 (s, 3H), 1.44 (s, 3H), 1.47 (s, 9H), 2.07-2.22 (m, 1H), 2.70-2.83 (m, 1H), 3.78-3.87 (m, 1H), 4.03 (dd, 1H, J=7.8 Hz, 6.4 Hz), 4.71 (m, 1H), 6.80-6.84 (m, 1H); ^{13}C-NMR ($CDCl_3$, 75.5 MHz) ∂ 132.77 (d, J=73 Hz), 165.16 (d, J=73 Hz); GC-MS (m/z, rel. intensity) 257 (M^+-CH_3, 38), 199 (M^+-^{t}BuO, 11), 141 (67), 96 (23), 59 (18), 57 (100).

[1,7-$^{13}C_2$]Shikimic acid (99% ^{13}C by ^{13}C-NMR). Prepared in 98% yield from [1,7-$^{13}C_2$]-**8** as described (4). ^1H-NMR (CD_3OD, 300 MHz) ∂ 2.12-2.24 (m, 1H), 2.64-2.75 (m, 1H), 3.66 (dd, 1H, J=7.4 Hz, 4.2 Hz), 3.94-4.03 (m, 1H), 4.33-4.40 (m, 1H), 6.76-6.82 (m, 1H); ^{13}C-NMR (CD_3OD, 75.5 MHz) ∂ 130.80 (d, J=70 Hz), 170.04 (d, J=70 Hz).

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