SYNTHESIS OF 2-(BROMOMETHYL-^{13}C)-FUMARIC ACID (BROMOMESACONIC ACID)

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SUMMARY

The fumarase inhibitor, bromomescaconic acid, has been synthesized with ^{13}C labeling in the bromomethyl group. Di-t-butyl acetylenedicarboxylate was converted with Li $(^{13}CH_3)_2Cu$ to di-t-butyl (methyl-^{13}C)citraconate. Photochemical isomerization of this to di-t-butyl (methyl-^{13}C)mesaconate, followed by deblocking with trifluoroacetic acid gave the title compound.

Key Words: Bromomesaconic Acid, fumarase

INTRODUCTION

Bromomesaconic acid (1a) was reported (1,2) to be a very rapidly acting, irreversible inhibitor of fumarase. Subsequently, (1a) was also shown to be an effective inhibitor of succinic dehydrogenase (3). The inactivation of fumarase by bromomesaconic acid was shown to involve the alkylation of an essential glutamic acid residue at the active site (4). For use in a ^{13}C nmr study of the mechanism of fumarase inhibition by (1a), particularly to attempt to distinguish between possible S_N2 or S_N2' pathways (Scheme I) for the alkylation of glutamate residues by (1a), 2-(bromomethyl-^{13}C)-fumaric acid (^{13}C-bromomesaconic acid), (1b), has been synthesized.

DISCUSSION

^{13}C-Bromomesaconic acid (1b) was synthesized by the route shown below (Scheme II). Acetylenedicarboxylic acid (2a) was converted with isobutylene and

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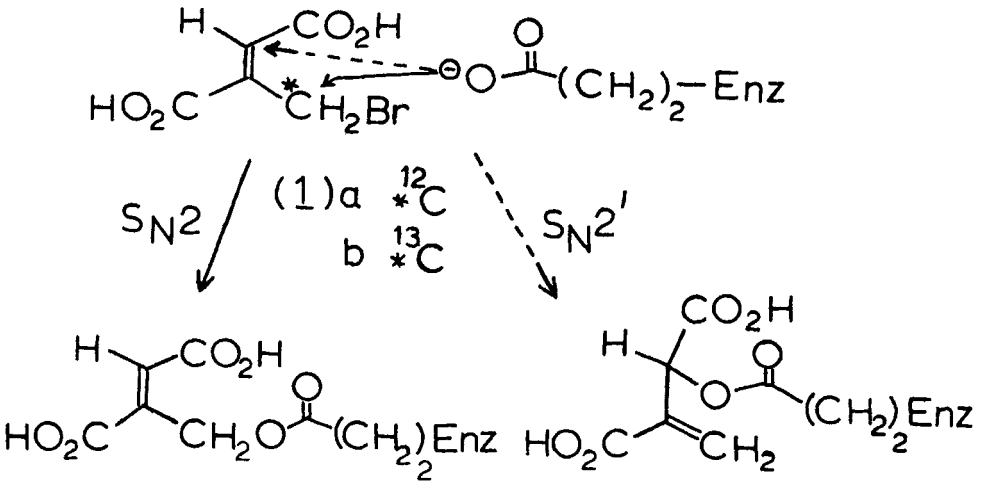
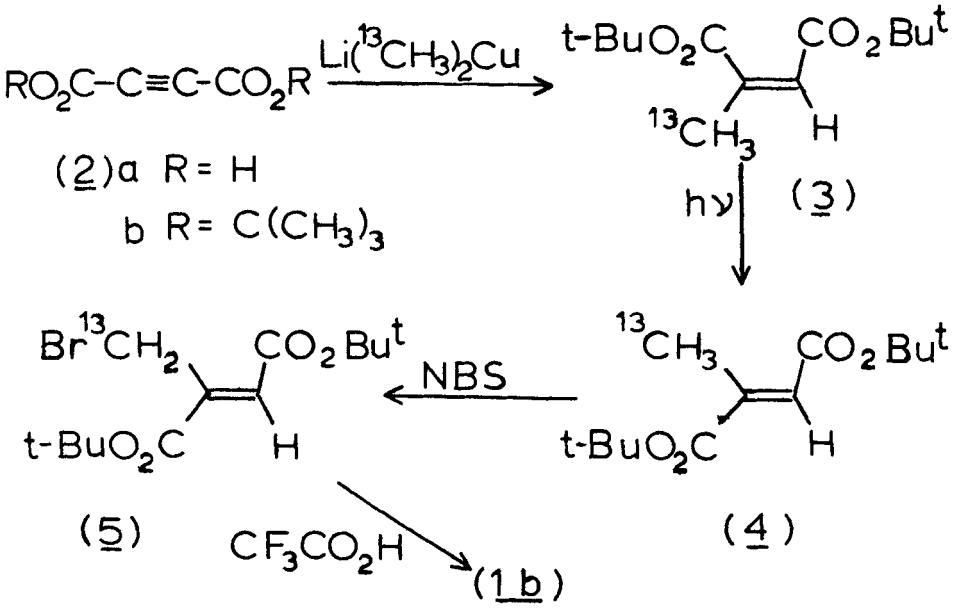


SCHEME I



SCHEME II

conc H_2SO_4 to di-t-butyl acetylenedicarboxylate (2b)[†]. Treatment of (2b) with $Li(^{13}CH_3)_2Cu$ (9) gave di-t-butyl citraconate (methyl-^{13}C), (3) (77%). This product was isomerized photochemically (9) to yield the pure trans isomer, di-t-butyl mesaconate (methyl-^{13}C), (4)[‡]. This was brominated with N-bromo succinimide, following the published procedure (2) to give (5), (88%). Finally, treatment of (5) as previously described (2) gave 2-(bromomethyl-^{13}C) fumaric acid, (1b).

Preliminary studies on the product of the reaction of (1b) with fumarase indicated that very little ^{13}C became permanently bonded to the enzyme, although the enzymic activity was rapidly lost. Further studies on the inhibition of furmarase by (1b) are continuing.

EXPERIMENTAL

General. Infrared (IR) spectra were taken in $CHCl_3$ solutions on a Perkin-Elmer 237 spectrometer. Nuclear magnetic resonance (NMR) spectra were taken in $CDCl_3$ solutions with internal tetramethylsilane (TMS) on a Varian HA-100 spectrometer. Mass spectra were taken on a Nuclide 12-90-G mass spectrometer equipped with a Nuclide DA/CS 1.2 data acquisition system. Merck silica gel (HF 254 + 366) was used for thin layer chromatography (TLC). Tetrahydrofuran (THF) was distilled from $LiAlH_4$ before use. $^{13}CH_3I$ (90.8 atom % ^{13}C) was obtained from Prochem. Microanalyses were performed by Chemalytics, Inc., Tempe, Arizona, and Galbraith Laboratories, Knoxville, Tenn.

Di-t-butyl acetylenedicarboxylate, (2b). Isobutylene (20 g, 0.357 mole) was condensed at -78° in a 250 ml pressure bottle. With continuous stirring at -78°, a solution of acetylenedicarboxylic acid (2a) (15 g, 0.13 mole) in anhydrous ether (50 ml) was added, followed by conc. H_2SO_4 (1.5 ml). The flask was sealed, and the mixture was stirred at 0° in an ice-water bath, allowing to warm to 25° over several hours. After 18 h stirring at 25°, the solution was cooled to -78° and then poured into 20% K_2CO_3 solution (precooled to -20°). The mixture was

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[†]After the completion of this work (5), three publications on the preparation of this previously unknown ester appeared (6-8).

[‡]In contrast, similar irradiation of dimethyl citraconate gave a mixture (95:5) of dimethyl mesaconate:dimethyl citraconate (9).

extracted with ether (4 x 100 ml), and the extract was washed with 20% K_2CO_3, saturated NaCl, dried (Na_2SO_4), and evaporated to an oil. The product was purified by Kugelröhr distillation, bp 100-110° (1 mm) to give an oil, 9.83 g, which on standing at room temperature formed prisms, mp 32-34°, ν_{max} 1725, 1280, 1255, 1155 cm^{-1}; nmr ($CDCl_3$) δ 1.53(s); mass spectrum m/e (rel. intensity) 212(2), 211(13,M-CH_3), 171(32), 170(17), 155(100), 154(13), 153(100), 152(52), 126(56), 124(15), 97(35), 59(12), 57(54).

Anal. Calcd for $C_{12}H_{18}O_4$: C, 63.70; H, 8.02. Found: C, 63.77; H, 7.89.

Di-t-butyl citraconate (methyl-^{13}C), (3). A suspension of clean lithium wire (0.62 g, 0.103 g atom, cut in ca. 100 pieces) in anhydrous ether (18 ml) under N_2 at reflux was treated with a solution of $^{13}CH_3I$ (5 g, 35 mmol, 90.8 atom % ^{13}C) in anhydrous ether (18 ml), added over 30 min. Refluxing was continued for 2 h, then the solution was allowed to stand at room temperature for 18 h. The clear supernatant solution was withdrawn by syringe. The $^{13}CH_3Li$ concentration was estimated to be 1.43 M by titration, with HCl solution, of an aliquot added to water.

To a suspension of CuI (2.93 g, 15.4 mmol) in anhydrous ether (22 ml) under N_2 at -20° was added dropwise over 30 min the above $^{13}CH_3Li$ solution (21.7 ml, 31 mmol), yielding a clear, light brown solution. The solution was cooled to -78°, then dry THF (20 ml) was added, followed by addition over 5 min of a solution of (2) (2.9 g, 15.4 mmol) in dry THF (10 ml). The resultant red solution was stirred at -78° for 2 h, followed by addition of cold water (160 ml). The mixture was filtered through a glass fibre filter and the filtrate extracted with ether. The extract was washed (5% HCl, water, saturated NaCl), dried (Na_2SO_4), and evaporated to an oil (2.93 g). The product was purified by Kugelröhr distillation giving (3), 2.39 g (77%), bp 95-100° (0.6 mm); ν_{max} ($CHCl_3$) 1720, 1650, 1160 cm^{-1}; nmr δ 1.50 (9H,s), 1.52 (9H,s), 1.94 (ca. 2.7H, dd, J_{H-H} = 2 Hz$J_{^{13}C-H}$ = 130 Hz), with a small $^{12}CH_3$ signal at 1.94 (ca. 0.3H, d, J = 2 Hz), 5.60 (1H, qu, d, J_1 (H-H) = 2 Hz, J_2 (^{13}C-H) = 7 Hz).

An unlabeled sample of (3), prepared in a similar manner (using CH_3I) had δ 1.50 (9H, s), 1.52 (9H, s), 1.94 (3H, d, J = 2 Hz), 5.60 (1H, qu, J = 2 Hz).

An analytical sample was purified by preparative glc (20% carbowax 20 M, 160°) and then distilled in a Kugelrohr tube at 90° (0.2 mm).

Anal. Calcd for $C_{13}H_{22}O_4$: C, 64.44; H, 9.15. Found: C, 64.25; H, 8.98.

Di-t-butyl mesaconate (methyl-^{13}C), (4).

The above product (3) (500 mg) in $CHCl_3$ (10 ml) plus bromine (5-10 mg) in a pyrex flask was irradiated with a 270 Watt sun lamp at a distance of 12 in. for 80 minutes. TLC (5% EtOAc-hexane) showed <1% of the starting material remaining. The solvent was evaporated to yield the product (3) as an oil (500 mg), ν_{max} 2975, 2870, 1700, 1640, 1360 cm^{-1}; nmr 1.46 (18H, s, t-butyl), 2.13 (2.7H, dd, J_1 (H-H) = 2 Hz, J_2 (^{13}C-H) = 130 Hz) with a small $^{12}CH_3$ signal at δ 2.13 (0.3 H, br s, $W_{1/2}$ = 3 Hz), 6.46 (1H, qu, d, J_1 (H-H) = 2 Hz, J_2 (^{13}C-H) = 7 Hz, vinyl H).

Di-t-butyl bromomesaconate (bromomethyl-^{13}C), (5). To a solution of (4) (160 mg, 0.66 mmole) in CCl_4 (4 ml) was added N-bromosuccinimide (170 mg) and dibenzoyl peroxide (40 mg), and the mixture was refluxed under N_2 for 4 h. After cooling, the mixture was filtered, and the filtrate was passed through a short column of silica gel (100-200 mesh - Fisher) in a Pasteur pipet, and eluted with a little additional CCl_4. After evaporation of the CCl_4 under reduced pressure, the oily residue was triturated with petroleum ether (bp 35-60°). The resultant solution was filtered and the filtrate evaporated to give crude (5), 187 mg (88%), which showed only traces of impurities (mainly dibenzoyl peroxide) on TLC (5% EtOAc-hexane). The product had (nmr) δ 1.51 (18H, s, t-butyl), 4.61 (1.8H, d, J (^{13}C-H) = 158 Hz, -$^{13}CH_2Br$) with a small signal for -$^{12}CH_2Br$ at δ 4.61 (0.2H, s), 6.53 (0.9H, qu, d, J (^{13}C-H) = 9 Hz, vinyl H) plus 6.53 (0.1 H, s).

2-Bromomethyl-^{13}C)-Fumaric Acid (Bromomesaconic acid (bromomethyl-^{13}C)), (1b). To a solution of (4) (480 mg, 1.48 mmole) in benzene (5 ml) was added trifluoroacetic acid (1.2 ml), and the mixture was refluxed with stirring for 5 h. The solvent and excess trifluoroacetic acid were evaporated under reduced pressure to give a residue which was triturated with benzene to yield crystalline (1b), 168 mg. Recrystallization from ether-petroleum ether (bp 35-60°) gave pure (1b), prisms, mp 176-178° (lit(1), 180-181°), nmr (D_2O) δ 4.64 (1.8H, d, J (^{13}C-H) =

158 Hz, $-^{13}CH_2Br$) plus 4.64 (0.2H, s, $-^{12}CH_2Br$), 6.97 (0.9H, d, J (^{13}C-H) = 9 Hz, vinyl H) plus 6.97 (0.1 H, s, vinyl H).

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