PREPARATION OF ACETIC-1-^{13}C AND MALONIC-1-^{13}C ACIDS FOR BIOSYNTHETIC STUDIES

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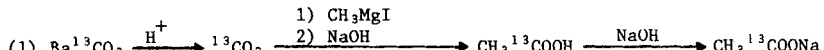
INTRODUCTION

Malonic acid is an important precursor of fungal polyketides produced through condensation of malonyl CoA-derived acetate units (1). Until recently the role of precursors in biosynthetic processes was determined experimentally by extensive degradation of end products produced through ^{14}C isotope labelling. Development of ^{13}C nuclear magnetic resonance techniques has now made possible determination of the labelling pattern without need for chemical degradation, using non-radioactive ^{13}C-labelled precursors (2). Several examples utilizing ^{13}C-labelled acetate have appeared recently (3-8).

Since malonic-^{13}C acid is not yet commercially available, we have adapted synthetic techniques described for preparation of unlabelled and ^{14}C-labelled malonic acid to the preparation of gram quantities of malonic-1-^{13}C acid, using barium carbonate-^{13}C as precursor. The syntheses and isotopic purity determinations of malonic-1-^{13}C acid and an isolated intermediate, sodium acetate-1-^{13}C, are included in this report.

RESULTS AND DISCUSSION

The synthetic scheme is summarized in the following reaction sequences:



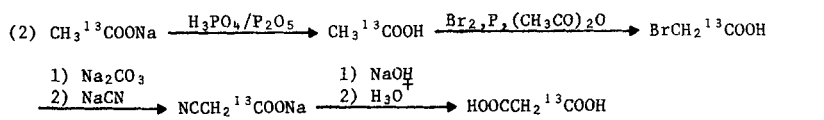


Table I. Yields and isotopic enrichments in the synthesis of acetic and malonic acids

starting material		Product		
wt (g)	% enrichment	wt (g)	% yield	% enrichment
barium carbonate		sodium acetate		
5	---	1.51	72.7	---
6.74*	85-90	2.50	89.2	87.1
sodium acetate		malonic acid**		
2	---	1.22	48.0	---
3	---	2.44	64.2	---
2.46*	87.1	1.32	42.3	82.4

*Denotes ^{13}C-labelled precursors.
**All values are uncorrected for acetic anhydride used in bromination step (equivalent to ~ 4% of acetate used.)

Synthetic methods were tested using both unlabelled and ^{13}C-labelled barium carbonate. Illustrative results (Table 1) confirm the satisfactory scale-up of the method of Murray (9) for preparation of sodium acetate. Average yields through the second sequence, to malonic acid, were somewhat lower than expected but reasonably consistent. The methods of Ropp (10) and Kögl (11) were modified by substitution of commercial polyphosphoric acid in the protonation of sodium acetate, and addition of a calcium chloride drying tube in the bromination step. Both modifications suppressed the troublesome formation of glycolic acid, by minimizing the amount of moisture available for hydrolysis of bromoacetic acid.

Overall, malonic-1-^{13}C acid was produced in 37.8% yield from barium carbonate-^{13}C, with essentially quantitative incorporation of label. Results using unlabelled precursors indicate overall yields in excess of 50% were attainable. Furthermore, suitable modifications of these procedures may prove useful for preparation of 2-^{13}C-labelled acetic and malonic acids.

EXPERIMENTAL

Sodium Acetate-1-^{13}C. The procedure of Murray (9) was modified slightly, to accomodate approximately 10-fold larger quantities of reactants. A vacuum

manifold similar to that described for preparation of benzoic-^{14}C acid (12) was employed for carbon dioxide evolution and Grignard reagent carbonation, except that a third manifold fitting was added to allow for intermediate condensation of carbon dioxide prior to the carbonation reaction. Typically, the carbon dioxide from 6.74 g (0.0341 mole) of barium carbonate-^{13}C (Mallinckrodt Chemical Works, St. Louis, Mo.) and 6.0 ml of conc. sulfuric acid was condensed in a 150 ml gas-tight vacuum flask cooled in liquid nitrogen. The evolution assembly was then isolated from the system, and the vacuum flask allowed to warm slightly (to -80°C) to release carbon dioxide at a constant rate to a stirred solution of methylmagnesium iodide (0.102 mole) in 150 ml of ethyl ether. The work-up of the sodium acetate was that of Murray (9) with the exception that the salt was rendered anhydrous by heating for 2 hrs at 130-140°C.

Malonic-1-^{13}C acid. Typically, an excess of polyphosphoric acid (10 ml) and 2.46 g (0.0300 mole) of sodium acetate-1-^{13}C, contained in a 25 ml pear-shaped flask, was heated to 170-180°C with an oil bath. The acetic acid was slowly distilled under partial pressure into a 10 ml two-neck pear-shaped flask cooled in dry ice-acetone. Following distillation, 0.05 ml of acetic anhydride (0.00106 mole acetic acid) and 0.01 g of red phosphorus were added to the distillate; the flask was fitted with a reflux condenser and calcium chloride drying tube, and was heated to 125-135°C with an oil bath. Then 2.5 ml (0.0488 mole) of bromine was carefully added through the condenser and the mixture refluxed for 3 hrs. The temperature was reduced to 50°C and dry carbon dioxide bubbled through to remove excess bromine. Crystalline bromoacetic acid, formed upon cooling the solution to room temperature, was then dissolved in 30 ml of water, transfered to a 100 ml round-bottom flask and adjusted to pH 8 with solid sodium carbonate. To the contents of the flask, stirred with a magnetic stirring bar and cooled in an ice bath, was added slowly a solution of 3 g (0.0612 mole) of sodium cyanide in 20 ml of water. The reaction was completed by heating for 1 hr on a steam bath. The flask was cooled slightly, and 3 g (0.075 mole) of sodium hydroxide was added. It was again heated on a steam bath, for 2 hrs; then steam was bubbled through the solution to remove the last traces of ammonia

by-product (13). After the volume of the resulting solution was reduced to ca. 50 ml by distillation of water, it was cooled in an ice bath and ca. 10 ml of conc. hydrochloric acid was added. The solution was extracted with ether (3 x 50 ml), and the extracts combined, dried over anhydrous sodium sulfate, and filtered. The ether was removed under reduced pressure to yield malonic-1-^{13}C acid. It was then sublimed at 90-100°C and less than 1 mm Hg, m.p. 130-133°. Although it was of sufficient purity for most applications, the acid could be further purified by recrystallization (14).

Analysis of product isotope enrichment. For analysis of sodium acetate -1-^{13}C, 0.05 g of dry sodium acetate and 1 ml of 2N sulfuric acid was added to a 10 ml screw-cap test tube. The solution was extracted by shaking with ether (3 x 1 ml); the combined ether layers were dried over anhydrous sodium sulfate, filtered, and concentrated under reduced pressure to ca. 0.15 ml. A portion of the resulting solution (0.05 ml) was introduced through a metal inlet maintained at 55° to a CEC Model 104 mass spectrometer (ionization voltage, 70 electron volts; filament current, 10 μamps; filament temperature, 250°C). A mass spectrum of ether was obtained under the same conditions, so that its contribution to the peaks at m/e 60 (parent peak of acetic-1-^{12}C acid), m/e 45 (P-CH_3 fragment of acetic-1-^{12}C acid) and m/e 46 (P-CH_3 fragment of acetic-1-^{13}C acid) could be subtracted. No peak appeared at m/e 61 in the ether spectrum. An 87.1% enrichment of ^{13}C was then calculated from the ratio of the m/e 61 to the sum of the m/e 60 and m/e 61 peaks. Close agreement was achieved with a similar analysis of the m/e 45 and m/e 46 peaks.

Malonic-1-^{13}C acid was converted to its dimethyl ester with diazomethane before analysis of isotopic enrichment in order to enhance the P and P+1 peaks. The sample was run on the same instrument and under the same conditions as the acetic-1-^{13}C acid. A spectrum of unlabelled dimethyl malonate was run under the same conditions in order to correct the P+1 peak (m/e 133) for other isotopic contributions. The ratio of the P+1 to the sum of the P and P+1 peaks was then used to determine an enrichment of 82.4% at one of the carboxyl positions. A

similar comparison of the m/e 101 (P-OCH_3) and m/e 102 peaks, in addition to ^{14}C spiking experiments confirmed the enrichment level.

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