A NEW APPROACH TO THE SYNTHESIS OF ^{14}C LABELLED DL-MALIC ACID

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SUMMARY

The 2-carbon units, glycine and glyoxylate are combined by a mild reaction via the copper chelate of glycine. The resulting β-hydroxy aspartic acid is converted to DL-malic acid by means of hydroxylamine O-sulfonic acid. The overall yields are in the range of 35-50% in submillimolar preparations.

Key words: Copper glycinate-^{14}C, β-hydroxy aspartic acid-^{14}C, malic acid-1-^{14}C, malic acid-3-^{14}C, malic acid-4-^{14}C, hydroxylamine O-sulfonic acid.

Malic acid is an important intermediate compound in tricarboxylic acid cycle and carbon-14 labelled malic acid has been widely used in studying its metabolism[1]. Malic acid labelled with carbon-14 has also been used to study the properties of malate synthetase in various sources and the metabolism of sedoheptulose in photosynthesis. The procedures reported so far for labelling malic acid are either lengthy involving anhydrous reaction conditions or requiring starting materials that are not easily accessible. Carbon-14 labelled malic acid has been prepared by other workers using labelled compounds such as sodium acetate-1-^{14}C, sodium acetate-2-^{14}C, DL-aspartic-3-^{14}C, L-aspartic acid-^{14}C(U) and potassium cyanide-^{14}C[2-6]. Most of these syntheses

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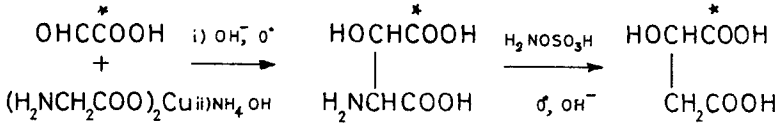
have been carried out at 10 millimoles or above of the starting labelled compounds. An enzymatic synthesis had also been reported for the synthesis of L-malic acid-1-^{14}C at micromolar levels using sodium glyoxylate-1-^{14}C[7].

We present here an alternative method which can be employed to synthesise DL-malic acid with ^{14}C or any carbon atom. This is the first chemical method to prepare DL-malic acid-1-^{14}C, using glyoxylate -1-^{14}C. This method is based on the facile condensation of copper glycinate with sodium glyoxylate in aqueous medium [8], and the conversion of the resulting β-hydroxy aspartic acid into DL-malic acid by means of hydroxylamine O-sulfonic acid (HAOS). The reaction schemes are:



This route has advantages such as easy adaptability for micromolar concentrations of reactants, dry atmosphere is not needed and there is no need for elaborate protection of functional groups before condensation of the two 2-carbon units. Employing glycine-1-^{14}C, glycine-2-^{14}C and sodium glyoxylate-1-^{14}C, the synthesis of DL-malic acid-4-^{14}C, DL-malic acid-3-^{14}C and DL-malic acid-1-^{14}C respectively have been achieved in 35-50% yields at 100 micro moles of starting labelled compounds. The lower overall yields are due to the resistance of β-hydroxy aspartic acid to react fully, a fact we have noticed in connection with another β-hydroxy amino acid namely threonine [9]. The reaction is more successful at lower concentrations of β-hydroxy amino acid (50-200 μM) since 20 fold of the reagent HAOS is needed. When the reaction is performed at millimoles of β-hydroxy aspartic acid, the addition of large amounts of HAOS is required and reaction is highly sluggish. The formation of β-hydroxy aspartic acid, the intermediate compound, proceeded in 75-80% yield, whereas glycine is quantitatively

converted to copper glycinate. The crude β-hydroxy aspartic acid was subsequently reacted wih HAOS without further purification.

The isolation and purification of malic acid from the reaction mixture have been accomplished by both ether extraction and anion exchange column chromatography and the product obtained by both methods is found to be radiochemically pure.

EXPERIMENTAL

Synthesis of DL-malic acid-3-^{14}C

Aqueous solution of glycine-2-^{14}C (20 μCi, 200 μM) was treated with copper carbonate (36 mg, 300 μM) and the solution was heated for 30 minutes at 60°C. The mixture was filtered and the filtrate was counted. Yield quantitative.

The dark blue copper glycinate-2-^{14}C was rotary evaporated to 2 ml in 50 ml round bottom flask. Sodium hydroxide (0.2 ml, 2 N, 400 μM) was added to copper glycinate, followed by addition of sodium glyoxylate (30 mg, 300 μM). The mixture was then treated with 1 ml of 25% ammonia solution and the solution was passed down a column (5 cm x 1 cm) of Dowex 50 x 8 resin in NH_4^+ form. The ammonia effluent (25 ml) was collected and an aliquot was analysed by paper chromatography in n-butanol:acetic acid:water system (2:1:1). On spraying the paper with ninhydrin, β-hydroxy aspartic acid and unreacted glycine were revealed. The radioactivity scan of paper chromatogram indicated 75-80% radioactivity in β-hydroxy aspartic acid.

The ammonia effluent obtained above (19 μCi) was thoroughly rotary evaporated to dryness and taken up in 4.5 ml of 1 N NaoH. The mixture was placed in an ice bath. Hydroxyl amino O-sulfonic acid (4 mM, 448 mg) was rapidly weighed out and added to the reaction mixture. After stirring for 4 hours, an aliquot was analysed by paper chromatography as before. The radioactivity scan indicated about 50% conversion of β-hydroxy aspartic acid into malic acid.

The reaction mixture was acidified with dilute sulphuric acid and extracted continuously with ether for 6 hours. The ether extract was counted; 10 μCi, yield 50%. An aliquot of the ether extract on analysis was found to be radiochemically pure in the solvent systems, n-butanol:acetic acid:water (4:1:5) and isopropanol:ammonia:water (8:1:1). The water phase (9 μCi) contained unreacted β-hydroxy aspartic acid and a little of malic acid.

Synthesis of DL-malic acid-4-^{14}C

In a similar manner DL-malic acid-4-^{14}C was synthesised using glycine-1-^{14}C(35 μM, 500 μCi) and sodium glyoxylate (5 mg, 50 μM) as before. The reaction mixture at the end was purified by chromatography on a column (15 cm x 1 cm) of Dowex 1 x 8 in acetate form and using 0.5 M acetic acid as the eluent. The malic acid was obtained in 80-100 ml fraction and the activity was 220 μCi, yield 44%.

Synthesis of DL-malic acid-1-^{14}C

DL-malic acid-1-^{14}C was prepared from sodium glyoxylate-1-^{14}C (100 μCi, 100 μM) and copper glycinate (15 mg, 75 μM) by the same procedure as described earlier, in a yield of 35%.

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