

SYNTHESIS OF 1, 2, 6, 7-<sup>14</sup>C-CHELIDONIC ACID

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Received on June 13, 1975.

## SUMMARY

*A semimicro synthesis was devised to prepare pure 1,2,6,7-<sup>14</sup>C-chelidonic acid of high specific activity from 1,2-<sup>14</sup>C-oxalic acid.*

INTRODUCTION

Specifically labelled chelidonic acid, required to continue investigations on the biosynthesis and metabolism of chelidonic acid (1), is not available commercially. The most appropriate starting material available for the synthesis of radioactive chelidonic acid is 1,2-<sup>14</sup>C-oxalic acid. Oxalic acid can be converted to diethyloxalate (2), which by reaction with acetone yields chelidonic acid (3) (Fig. 1).

DISCUSSION AND RESULTS

The procedures involved in the macro conversion of oxalic acid to chelidonic acid (2,3), were modified to yield a useful semimicro synthesis. The modifications described allow the synthesis of small amounts of specifically labelled chelidonic acid of sufficient activity to be useful in biosynthetic experiments. In this case 1 g of oxalic acid containing 1 mC of carbon-14 yielded 475 mg of pure 1,2,6,7-<sup>14</sup>C-chelidonic acid (chemical and radiochemical yield 46.6%) with a specific activity of  $4.0 \times 10^8$  counts per minute per millimole (1  $\mu$ C per mg). A sample of this chelidonic acid was degraded as described previously (1) (Fig. 1), to yield inactive hexa-

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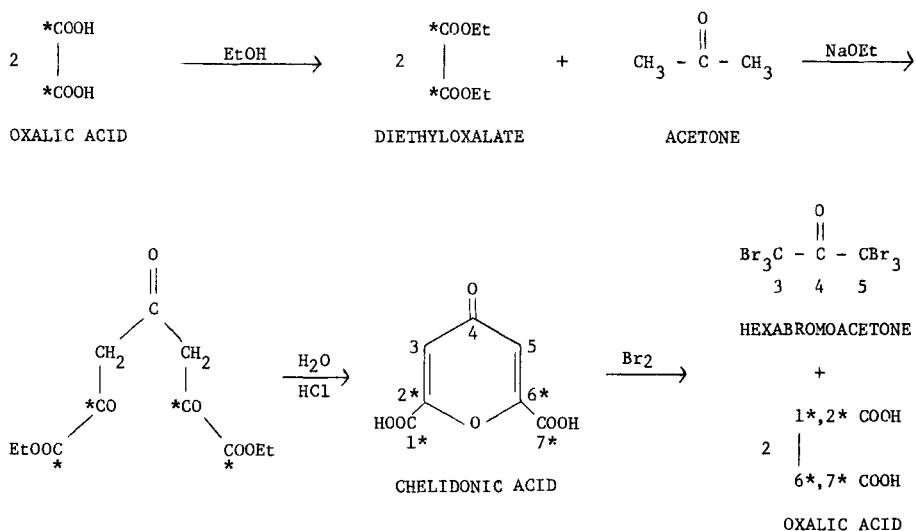


Fig. 1. Synthesis and Degradation of Chelidonic acid.

bromoacetone, and active oxalic acid. The results demonstrated that the carbon-14 had entered the chelidonic acid with expected specificity at the 1,2,6, and 7 positions.

The procedure described is capable of operation with the same yield using one-fifth of the quantities used in this synthesis. Hence by use of undiluted radioactive oxalic acid, chelidonic acid could be synthesized with specific activities up to  $2.0 \times 10^{10}$  counts per minute per millimole (50  $\mu\text{C}$  per mg).

#### EXPERIMENTAL

Radioactivity was measured by means of a Nuclear Chicago Low Background Counter.

#### Synthesis of 1,2-<sup>14</sup>C-Diethyloxalate

1,2-<sup>14</sup>C-Oxalic acid (5.5 mg; 1 mC; purchased from ICN), and pure anhydrous oxalic acid (994.5 mg) were placed in a flask equipped for distillation, and protected with  $\text{CaCl}_2$ . A mixture of absolute ethanol : carbon tetrachloride (13 : 7; 150 ml) was added, and the solution distilled slowly for 9 hr, adding solvent mixture as required to keep the volume above 75 ml.

The solvent was removed by evaporation at 15° and 20 mm to yield diethyloxalate (1.23 g; 76%) which was used without further purification.

Synthesis of 1,2,6,7-<sup>14</sup>C-Chelidonic acid

Sodium (416 mg) was added to absolute ethanol (5.5 ml; prepared by distillation from sodium (4)) in a test tube protected by CaCl<sub>2</sub>, and dissolved by heating to 80-90°. The synthesized radioactive diethyloxalate was mixed with acetone (0.7 ml; dried over CaCl<sub>2</sub> and distilled) in a stoppered tube.

Using a prewarmed Pasteur pipet sodium ethoxide (2 ml) was transferred to a fresh tube protected by CaCl<sub>2</sub>, and as soon as precipitation began diethyloxalate-acetone (0.3 ml) was introduced below the surface by means of a Pasteur pipet. An exothermic reaction ensued and the solution turned yellow and then brown. After a few minutes, when a precipitate began to form, the rest of the sodium ethoxide and diethyloxalate-acetone were added simultaneously by means of Pasteur pipets. The tube was heated at 75° for 45 min during which time the first solid dissolved and a new one formed. The mixture was cooled to 20°, added to conc. HCl (5 ml) and ice (8 g), and well mixed. The solid material was separated, heated at 75° for 18 hr in conc. HCl (4 ml), and cooled to yield crude chelidonic acid separated by filtration.

Purification of Chelidonic acid

Bio-Rad AG1-X10 (100-200 mesh; 5 g) in water (15 ml) in a 1 cm column was washed with HCl (20%; 100 ml) and with water until the pH of the eluate was 7. The chelidonic acid was dissolved in water and added to the column. Elution with 0.1 M HCl was carried out until the chelidonic acid first appeared in the eluate (recognized by formation of a yellow color with 10% KOH) which was discarded. Elution was continued with 0.5 M HCl until the eluate showed no further traces of chelidonic acid (1000-1200 ml), whereupon evaporation yielded purified chelidonic acid. This procedure was repeated twice with fresh columns of Bio-Rad AG1-X10. The solid isolated from the third column was dissolved in water (20 ml) to which conc. HCl (1 ml) was added. Evaporation to 10 ml, standing, and filtration yielded

pure 1,2,6,7-<sup>14</sup>C-chelidonic acid (475 mg; 46.4% based on oxalic acid; m. pt. 287-290<sup>o</sup>) identical in all respects except for the radioactivity to authentic chelidonic acid.

#### REFERENCES

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