

THE USE OF  $^{13}\text{C}$  IN THE SYNTHESIS OF DOUBLE LABELLED PRECURSORS.

Morteza Ansarin

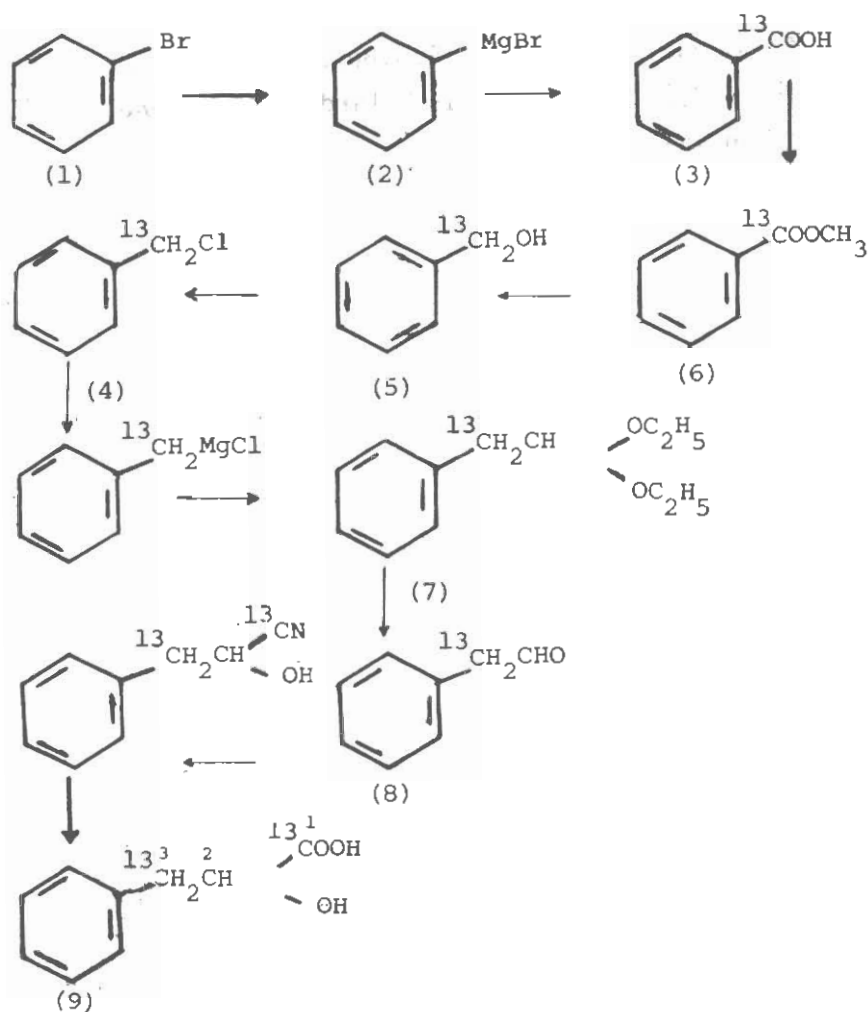
Department of Pharmacognosy, School of Pharmacy, Medical Sciences  
University, Tabriz, Iran. Iran.

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## ABSTRACT

Discovery of  $^{13}\text{C}$  NMR and its application in the biosynthetic pathways prompted the synthesis of several  $^{13}\text{C}$  labelled precursors. To investigate the metabolism of phenyllactic acid in the Solanacea family, the double labelled acid was synthesised from barium carbonate ( $^{13}\text{C}$ ) and sodium cyanide ( $^{13}\text{C}$ ) (Scheme 1)



## INTRODUCTION

The discovery of the alkaloid littorine which is the phenyllactic acid ester of tropine in the *Datura sanguinea* and *Anthocercis littorea*, [1], prompted an investigation into the role of this acid in the biosynthesis of tropic acid. Tropic acid is the acid moiety of the medicinal alkaloids hyoscine and Hyoscyamine (Atropine). The skeleton of tropic acid is formed from the amino acid phenylalanine [2]. Relationship between these two groups of alkaloids, lead to the investigation of the role of phenyllactic acid in the biosynthesis of tropic acid in *Datura Stramonium* (containing hyoscine and atropine). Double labelled Phenyllactic acid was not available commercially. For the reasons mentioned phenyllactic acid (1,3- $^{13}\text{C}$ ) was prepared from barium carbonate ( $^{13}\text{C}$ ) and sodium cyanide ( $^{13}\text{C}$ ) (Scheme 1). An independent estimation of the isotope enrichment of the phenyllactic acid was made by an examination of its  $^{13}\text{C}$  NMR, and Mass spectrums. Spectroscopy was used in investigating the biosynthetic pathway.

## EXPERIMENTAL

Melting points were determined in capillaries (Buchi mp apparatus, 510), pH measurements were made using a Pye Unicam pH meter model 292.  $^{13}\text{C}$  NMR spectra were determined via Bruker

spectrospin, 62,901 MHz  $^{13}\text{C}$  NMR. Mass spectra and Mass measurements were determined by the VGI Micromass, 16F-70<sup>EV</sup>. Infrared spectra were obtained using the KBr disc method on a Perkin Elmer 257. All  $^{13}\text{C}$  materials were purchased from Prochem Ltd, BOC, London.

Phenyllactic acid (1,3- $^{13}\text{C}$ ) was prepared according to standard procedures [3] from barium carbonate ( $^{13}\text{C}$ ) 90% and sodium cyanide ( $^{13}\text{C}$ ) 91%. Benzoic acid (1- $^{13}\text{C}$ ) [3] was prepared from phenylmagnesium bromide (2) and barium carbonate ( $^{13}\text{C}$ ) which was subsequently methylated to methyl benzoate (1- $^{13}\text{C}$ ) (6) by the action of methanol and sulphuric acid [4], (Scheme 1). Reduction of the methylbenzoate with aluminium hydride gave benzyl alcohol (1- $^{13}\text{C}$ ) (5), which was then converted to benzyl chloride (4). Phenylacetaldehyde-diethylactal (2- $^{13}\text{C}$ ) (7) was prepared through the reaction of benzyl magnesium chloride with ethyl orthoformate, which was later converted to phenylacetaldehyde (2- $^{13}\text{C}$ ) (8) with 2N sulphuric acid. Finally phenyllactic acid (1,3- $^{13}\text{C}$ ) (9) was prepared from phenylacetaldehyde (2- $^{13}\text{C}$ ) (8) and sodium cyanide- ( $^{13}\text{C}$ ) sodium metabisulphite. The phenyllactic acid (1,3- $^{13}\text{C}$ ) (9) crystallised from carbon tetrachloride as long needles (82% based on NaCN). The product was identical in all respects with an authentic sample of phenyllactic acid, (mp 94°-IR).

An independent estimation of the

isotope enrichment of the phenyllactic acid(1,3- $^{13}\text{C}$ ) was obtained by an examination of its  $^{13}\text{C}$  NMR spectrum. The highly enriched carbon(1) and (3) are observed as singlets, C(1) 175.5 ppm, C(3) 40.99 ppm. (Figs.1 and 2)

## RESULTS AND DISCUSSION

The phenyllactic acid(1,3- $^{13}\text{C}$ ) crystallised as long needles, (1.8 g, 82% based on  $\text{Na}^{13}\text{CN}$ ). The product was identical in all respects with an authentic sample of phenyllactic acid, (IR, mp  $94^\circ$ ). The  $^{13}\text{C}$  NMR spectrum of the  $^{13}\text{C}$  labelled phenyllactic acid in (acetone D), tube 1mm, frequency 25.150 MHz, using TMS as reference is illustrated in Figs.1 and 2, (Fig.1, uncoupled, Fig.2, proton noise decoupled). The highly enriched carbon(1) and (3) are observed as singlets, C(1) 175.5 ppm, C(3) 40.99 ppm. The Mass spectrum of the acid(1,3- $^{13}\text{C}$ ) had a parent peak at m/e 168, corresponding to 81.75% the doubly labelled species and a base peak at m/e 92, corresponding to 84%  $^{13}\text{C}$  enrichment in the benzyl position of the molecule.

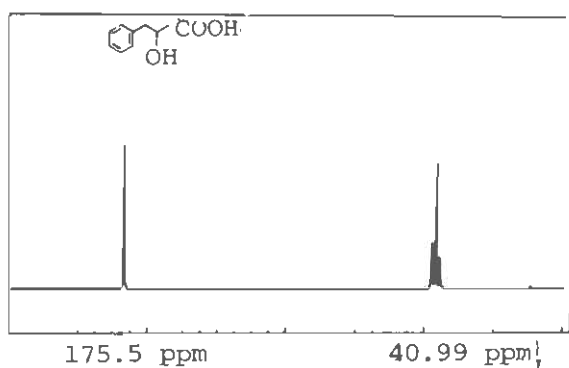


Fig 1.  $^{13}\text{C}$  NMR spectrum of phenyllactic acid(1,3- $^{13}\text{C}$ ).

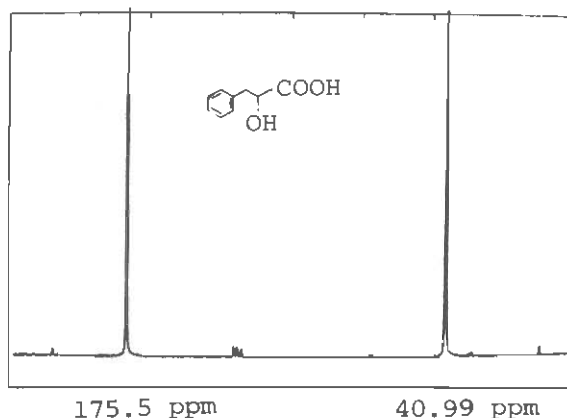


Fig 2.  $^{13}\text{C}$  NMR of phenyllactic acid(1,3- $^{13}\text{C}$ )(Proton noise decoupled spectrum) .

## REFERENCES

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