# ENZYMATIC SYNTHESIS AND CHROMATOGRAPHIC PURIFICATION OF L-3-[$^{11}$C]-LACTIC ACID VIA D,L-3-[$^{11}$C]-ALANINE

G. Kloster and P. Laufer

Institut für Chemie 1 (Nuklearchemie)

der Kernforschungsanlage Jülich GmbH, D-5170 Jülich, FRG

## SUMMARY

D,L-3-[$^{11}$C]-Alanine was prepared by methylation with $^{11}CH_3I$ of tert.butyl 2-isocyanoacetate and subsequent hydrolysis. Enzymatic reaction using glutamate pyruvate transaminase and lactate dehydrogenase in a coupled reaction yields L-3-[$^{11}$C]-lactic acid. The radiochemical yield is 45% for D,L-alanine and 26.5% for L-lactic acid at a specific activity of 0.45 mCi/μmole. Synthesis time including purification by hplc is 23 min for D,L-3-[$^{11}$C]-alanine and 45 min for L-3-[$^{11}$C]-lactic acid, respectively.

Key words: Carbon-11, Alanine, Lactic Acid, Enzymatic synthesis

## INTRODUCTION

L-lactic acid is a normal intermediate of carbohydrate and amino acid metabolism. During oxidative metabolism, it is oxidized to $CO_2$ and $H_2O$, whereas during oxygen deprivation, it is generated from glycolysis and accumulates in tissues and blood. Normal myocardium is known to take up free fatty acids to generate 2/3 of its energy needs; the other 1/3 is generated from carbohydrate derivatives, including L-lactate. In addition to our investigations of labelled fatty acids (1,2), in particular

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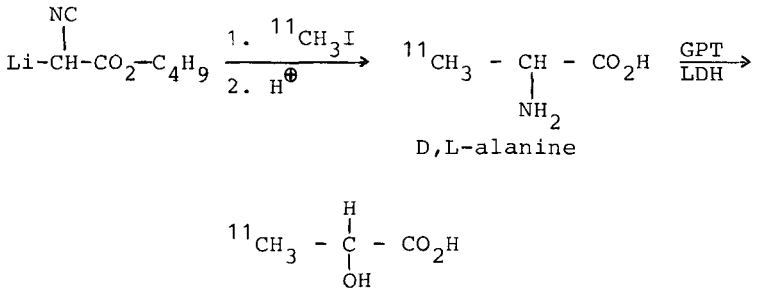
$\omega$-$^{123}$I-heptadecanoic acid, as tools for probing regional myocardial metabolism (3), we decided to synthesize L-lactic acid, which should enable us to probe regional myocardial metabolism using the path of carbohydrate derivatives. D,L-$^{11}$C-lactic acid was one of the first $^{11}$C-labelled compounds, and was synthesized by Cramer and Kistiakowsky in 1941 (4). Later, D,L-1-$^{11}$C-lactic acid was prepared via H$^{11}$CN by Winstead et al. (5). L-1-$^{11}$C-lactic acid was enzymatically prepared from $^{11}CO_2$ by Cohen et al. (6). Those syntheses, however, have some drawbacks: in two of them (4,5) D,L-lactic acid is obtained as product, which means that 50% of the activity to be injected is in the D-form which cannot be utilized by man. The third one (6), though producing pure L-lactic acid, was reported to have a rather low radiochemical yield of only 3 to 5%. We therefore decided to follow a different route, introducing the label into the 3-position via $^{11}CH_3I$ (Scheme 1).




SCHEME 1

## EXPERIMENTAL

Tertiary butyl 2-isocyanoacetate and its anion was prepared according to Schöllkopf et al. (7).

Practically carrier-free $^{11}CH_3I$ was prepared according to Marazano et al. (8).

A typical preparation of L-3-$^{11}$C-lactate runs as follows:

t = 0 min — After transferring practically carrier-free $^{11}CH_3I$ (22.4 mCi) into a solution of 50 mg of the lithium salt of tert.butyl 2-isocyanoacetate in 500 µl THF at -78 °C, the mixture is stirred for 3 min at -78 °C and another 3 min at room temperature.

t = 6 min — 2 ml of 5 N HCl are added to the solution, which is decolourized immediately. The resulting mixture is heated at 80 °C for 2 min. The solution is evaporated to complete dryness using a rotary evaporator.

t = 15 min — The residue is dissolved in 1 ml of 50 mM phosphate buffer pH 7.5 and heated at 37 °C. 5 mg of NADH, 5 mg of α-ketoglutarate and 550 U of lactate dehydrogenase are added to this solution. The enzymatic reaction is started by addition of 8 U of glutamate pyruvate transaminase. After a 6 min reaction time, the reaction is stopped by heating the mixture at 100 °C for 30 sec. The denatured enzymes are separated by centrifugation.

t = 28 min — The clear supernatant is evaporated to dryness; $^{11}$C-lactic acid is extracted from the residue with 2x1 ml ethanol. This treatment dissolves almost all $^{11}$C-lactic acid, while leaving most of the NADH and α-ketoglutarate undissolved.

t = 37 min — The solution is injected onto a hplc-column (LiChroSorb KAT/$H^+$ 10 µ 25x1 cm) via a sample valve. At a flow rate of 9.9 ml/min using ethanol as eluent, L-3-$^{11}$C-lactic acid elutes with a retention time of 2 min. The L-3-$^{11}$C-lactic acid peak is collected and evaporated to dryness.

A radiochemical yield of 5.9 mCi ≙ 26.5% (decay corrected) was obtained with a specific activity of about 0.45 mCi/μmole.

This reaction sequence can be used to prepare D,L-3-$^{11}$C-alanine as well. In this case, the residue left over after the t = 15 min step is dissolved in 1 ml of ethanol. The solution is injected onto a hplc-column (Latek $SiO_2$ 10 μ 21.5x1 cm) via a sample valve. At a flow rate of 5 ml/min, a mixture of 4 parts of ethanol with 1 part of 0.4% aqueous $Na_2HPO_4$-solution is used as eluent. Under these conditions D,L-3-$^{11}$C-alanine is eluted with a retention time of 4 min. In a typical preparation, 4.0 mCi of D,L-3-$^{11}$C-alanine are obtained from 8.8 mCi of $^{11}CH_3I$ in 23 min; the radiochemical yield is 45% at the same specific activity as stated above for L-3-$^{11}$C-lactic acid.

Addition of carrier methyl iodide to the reaction mixture does not change the radiochemical yield. During preparation of $^{11}CH_3I$, about 2 μmole of nonradioactive $CH_3I$ are also generated, probably from atmospheric $CO_2$; therefore, alanine and lactic acid are not carrier-free.

The hplc conditions (see Table 1) were selected to yield a salt-free residue of L-3-$^{11}$C-lactic acid. In order to characterize the L-3-$^{11}$C-lactic acid unequivocally, we chromatographed the $^{11}$C-compound eluted from the preparative column at k' = 0.7 on a second column (LiChroSorb RP18 10 μ, 25x1 cm). In both cases, the radioactivity is eluted at the same position as authentic L-lactic acid.

Table 1. HPLC-Data

| column/eluent | k' alanine | k' lactate |
|---|---|---|
| Latek $SiO_2$ 10 µ/0.4% $Na_2HPO_4$: EtOH 4:1 (5 ml/min) | 1.35 | - |
| LiChroSorb RP18 10 µ 0.01 N HCl (5 ml/min) | 1.2 | 1.8 |
| LiChroSorb KAT/$H^{\oplus}$ 10 µ EtOH (9.9 ml/min) | ∞ | 0.7 |

$k' = (v-v_o)/v_o$

## CONCLUSION

We report here a synthesis of L-3-[$^{11}$C]-lactic acid that proceeds with 26.5% radiochemical yield in 45 min. Compared to the synthesis of Winstead et al. (5) it has the advantage of producing only the natural L-stereoisomer instead of the racemate in shorter time and comparable yield. In comparison to the synthesis of Cohen et al. (6), who produced L-1-[$^{11}$C]-lactic acid from $^{11}CO_2$, it has the advantage of using only commercially available enzymes and achieving a higher yield.

The yield of L-3-[$^{11}$C]-lactic acid in our synthesis may still be enhanced if one succeeds in producing partially resolved alanine, which contains an excess of L-alanine, the precursor of L-lactic acid. This synthesis has been reported by Långström and Stridsberg (9) during the final stages of our work. They obtained a 2.9 fold excess of L-3-[$^{11}$C]-alanine over D-3-[$^{11}$C]-alanine using asymmetric induction. Using their synthesis of enriched L-alanine and our procedure to synthesize L-lactic acid should yield L-3-[$^{11}$C]-lactic

acid in radiochemical yields up to 40%.

Studies on the pharmacokinetics of L-3-[$^{11}$C]-alanine in rabbits are being performed presently. It remains to be seen whether it is possible to measure regional myocardial metabolism in rabbits and, finally, in man using L-3-[$^{11}$C]-lactic acid as a tracer.

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