SYNTHESIS OF [3-^{11}C]PHENYLPYRUVIC ACID AND ITS USE IN AN ENZYMATIC TRANSAMINATION TO [3-^{11}C]PHENYLALANINE

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SUMMARY

The synthesis of [3-^{11}C]phenylpyruvic acid, starting with $^{11}CO_2$, is reported. This α-keto acid was prepared via a condensation reaction, using [^{11}C]benzaldehyde (prepared as described elsewhere), and 2-phenyl-5-oxazolone, using diazabicyclooctane (DABCO) as a base. The condensation product, [α-^{11}C]-4-benzylidene-2-phenyl-5-oxazolone, was converted by basic hydrolysis to give [3-^{11}C]phenylpyruvic acid in 40 % radiochemical yield, starting with $^{11}CO_2$, within 40 min. The potential of [3-^{11}C]phenylpyruvic acid in enzymatic transamination, catalysed by glutamic/oxaloacetic acid transaminase (GOT) (EC 2.6.1.1) immobilized on CNBr-activated Sepharose was also studied.

Key Words: ^{11}C-phenylpyruvic acid, ^{11}C-phenylalanine, [3-^{11}C]amino acid, ^{11}C-α-keto acid.

INTRODUCTION

Amino acids labelled with short-lived radionuclides, such as ^{11}C, are of great medical interest because of their role

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in human protein synthesis[1] and, in some cases, as precursors of neurotransmitters.[2] Human metabolism may be studied in vivo by use of positron emission tomography (PET). We have been particularly interested in the possibility of synthesizing amino acids labelled in various positions, in pure optical form.[3,4]

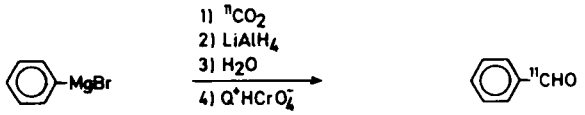
Employing enzymatic reactions, L-[4-^{11}C]aspartic acid was prepared from $^{11}CO_2$ by use of phosphoenolpyruvate carboxylase and glutamic/oxaloacetic acid transaminase immobilized on Sepharose supports, as reported by Barrio et al.[5]

Both ^{13}N-labelled aliphatic[6] and aromatic amino acids[7] have been prepared in pure optical form by use of enzymatic transamination, but in these cases ^{13}N-labelled L-glutamate and unlabelled α-keto acid were used. ^{13}N-Labelled phenylalanine was thus prepared in 10 % yield by the same transamination reaction as used in the investigation presented in this paper.

In this work, the synthesis of [3-^{11}C]phenylpyruvic acid from the corresponding unsaturated oxazolone and the use of the α-keto acid in a transamination reaction, yielding [3-^{11}C]phenylalanine, are reported.

SYNTHETIC PATHWAYS

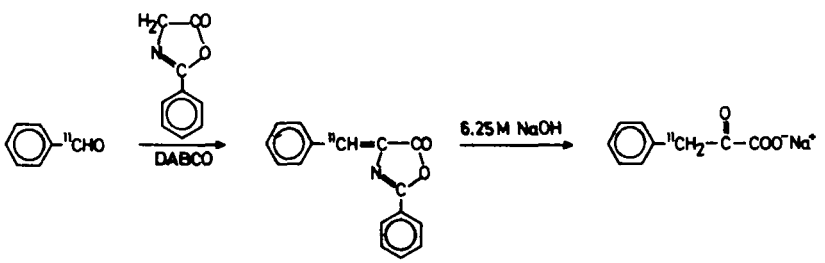
[^{11}C]Benzaldehyde was prepared from the corresponding [^{11}C]acid salt, according to Scheme 1, as reported elsewhere.[8]



Scheme 1

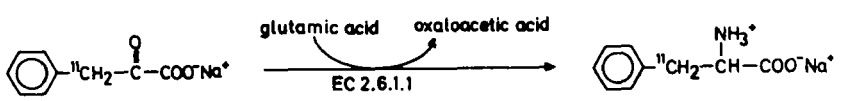
The [^{11}C]acid salt was reduced directly with lithium aluminium hydride (LAH) to give the corresponding alcohol. [^{11}C]Benzaldehyde was obtained by using tetrabutylammonium hydrogen chromate with 3 M sulfuric acid/ethyl acetate as solvent in an ion-pair oxidation.

The solution containing the labelled aldehyde was used, after being washed with water and diluted sodium hydroxide, in a condensation reaction with 2-phenyl-5-oxazolone in the presence of a tertiary amine diazabicyclooctane (DABCO), according to Scheme 2. The resultant [α-^{11}C]-4-benzylidene-2-phenyl-5-oxazolone was converted to [3-^{11}C]phenylpyruvic acid with 6.25 M sodium hydroxide.



Scheme 2

The [3-^{11}C]phenylpyruvic acid was converted to [3-^{11}C]-phenylalanine by enzymatic transamination catalysed by glutamic/oxaloacetic acid transaminase (GOT) (EC 2.6.1.1) immobilized on Sepharose supports, according to Scheme 3.



Scheme 3

RESULTS

[^{11}C]Benzaldehyde was prepared routinely in a radiochemical yield > 95 %, starting with $^{11}CO_2$. The [α-^{11}C]-4-benzyl-

idene-2-phenyl-5-oxazolone was prepared in 80 % radiochemical yield within 4 min by a condensation reaction, and converted to [3-^{11}C]phenylpyruvic acid in 70 % radiochemical yield within another 7 min. The overall synthesis time was 40 min and the radiochemical yield was of the order of 40 %, starting with $^{11}CO_2$, including 10-12 min for transportation of the reaction vessel to the chemistry laboratory and 5-10 min for the washing procedure.

So far, [3-^{11}C]phenylalanine has been obtained in 15-20 % radiochemical yield from [3-^{11}C]phenylpyruvic acid within 10 min. Increasing the reaction time did not produce a significant increase in yield. Technical handling takes approximately 30 min, but it should be possible to improve upon this time.

The glutamic/oxaloacetic acid transaminase was immobilized on CNBr-activated Sepharose and the column, when stored at 4 ^{o}C in 2 M KCl/30 mM sodium phosphate pH 7.5, gave reproducible results for two weeks. Such parameters as concentrations of substrates, pH, temperature, metal ions and solubility of substrates have not yet been optimized and work is in progress.

EXPERIMENTAL

General. The ^{11}C was produced at the Tandem Van de Graaff accelerator at the University of Uppsala by means of the $^{14}N(p,\alpha)^{11}C$ reaction on a nitrogen gas target. The [^{11}C]-carbon dioxide was trapped in a reaction vessel containing the phenylmagnesium bromide solution. When trapping was completed, the reaction vessel containing the Grignard solution was transported to the chemistry laboratory.

Analytical LC was performed on a Hewlett-Packard 1084 B equipped with a 250 x 4.6 mm Spherisorb C-18 10 µm column

and a variable wavelength detector in series with a β-flow detector.[9] Aqueous 0.1 M ammonium formate, pH 3.50, (A), and methanol (B) were used as solvents.

The substrate, 2-phenyl-5-oxazolone was prepared according to the method of Crawford.[10]

Porcine heart glutamic/oxaloacetic acid transaminase (GOT) (EC 2.6.1.1) was obtained from Sigma Chemical Co. Cyanogen bromide-activated Sepharose was obtained from Pharmacia, and ion-exchange resin (Bio-Rad AG1148) from Bio-Rad Laboratories.

[^{11}C]Benzaldehyde[8] (Scheme 1). [^{11}C]Carbon dioxide was trapped in a reaction vessel containing 2.5 cm^3 of 0.20 M phenylmagnesium bromide in ether at 0 °C. When trapping was completed, the solution of the magnesium salt of the labelled acid was transported to the chemistry laboratory. The salt of the [^{11}C]benzoic acid was reduced by means of 0.4 cm^3 1.0 M lithium aluminium hydride (LAH) in ether. After 1 min the LAH complex was hydrolysed and the ethereal solution of [^{11}C]benzyl alcohol was oxidized (1 min) with a two-phase mixture containing 2.0 cm^3 ethyl acetate, 1.0 cm^3 3 M sulfuric acid, 0.25 g (0.74 mmol) tetrabutylammonium hydrogen sulfate and 0.50 g (1.7 mmol) sodium dichromate. The organic solution was separated and carefully washed successively with water and 0.25 M sodium hydroxide before use in the following step.

LC analysis was performed using the C-18 column and solvents A and B in the following program: flow 3.0 cm^3/min, column temperature 60 °C, UV 257 nm, LC gradient time 0-4.5, A/B from 65/35-60/40; time 4.5-5.0, A/B from 60/40-20/80; time 5.0-9.0, A/B 20/80 isocratic. The retention times for benzoic acid, benzyl alcohol and benzaldehyde were about 2.4, 2.1 and 4.1 min, respectively.

[3-^{11}C]Phenylpyruvic acid (Scheme 2). To the solution containing the [^{11}C]benzaldehyde, 0.25 g (1.6 mmol) 2-phenyl--5-oxazolone in 2.0 cm^3 absolute ethanol and 0.50 g (4.5 mmol) DABCO in 1.0 cm^3 absolute ethanol (both heated to homogeneous solution at 80 °C) were added and the mixture was kept at 140 °C for 4 min while being flushed with nitrogen to remove most of the solvent. To the residue, 2.0 cm^3 6.25 m sodium hydroxide was added and the mixture was kept at 210 °C for 7 min. After cooling the crude mixture, 2.0 cm^3 6.0 M hydrochloric acid was added and the product was analysed by LC in the following program: flow 3.0 cm^3, column temperature 60 °C, UV 257 nm, LC gradient time 0-4.5, A/B 80/20 isocratic; time 4.5-5.0, A/B from 80/20-20/80; time 5.0-9.0, A/B 20/80 isocratic.

[3-^{11}C]Phenylalanine (Scheme 3). Enzyme immobilization: glutamic/oxaloacetic acid transaminase (GOT) (EC 2.6.1.1), 1000 units, was immobilized on 1000 mg of CNBr-activated Sepharose, using a previously described procedure.[6] The columns were stored at 4 °C in 2 M KCl/30 mM sodium phosphate, pH 7.5. In the cold runs, a degradation of the immobilized enzyme column was noted. The column was stable and reusable for approximately two weeks.

The mixture containing the [3-^{11}C]phenylpyruvic acid was eluted through an ion-exchange column (Bio-Rad AG1148) (4 cm^3 ion-exchange resin in a 10 cm^3 syringe). The resin was washed with water (2 x 2.0 cm^3). The eluate was collected in a vessel and evaporated. To the residue, 2.5 cm^3 30 mM sodium phosphate, pH 7.5, and 20.0 mg (0.14 mmol) L-glutamic acid were added. The mixture was heated to a homogeneous solution, cooled (37 °C), and the pH was adjusted (7.5). The solution was then allowed to pass through the

glutamic/oxaloacetic acid transaminase column. The solution was reinjected subsequently by means of a pump for 10 min. The conversion of [3-^{11}C]phenylpyruvic acid to [3-^{11}C]-phenylalanine was followed by LC, using the program described above.

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