A CONVENIENT SYNTHESIS OF N^G-MONO[^{14}C-METHYL]-L-ARGININE

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SUMMARY

N^G-Mono[^{14}C-methyl]-L-arginine was prepared in a two-step synthesis. N,S-dimethylthiopseudouronium iodide [methyl-^{14}C] was prepared in excellent yield and afforded the labelled amino acid on coupling with L-ornithine.

KEY WORDS: [^{14}C]Methylthiourea, N,S-Dimethylthiopseudouronium Iodide [Methyl-^{14}C], Selective Amination

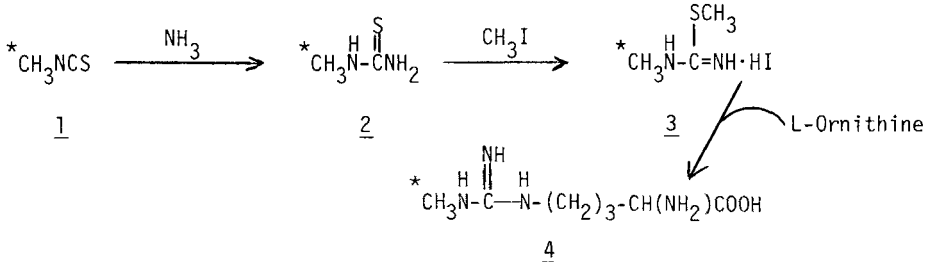
INTRODUCTION

N-Monomethyl-L-arginine arises from the enzymatic methylation of protein and subsequent degradation of the methylated product.[1]

Our interest in the metabolic fate of N^G-monomethyl-L-arginine *in vivo* after injection into rats or *in vitro* after incubation with rat kidney homogenate led us to prepare N^G-mono[^{14}C-methyl]-L-arginine (4).

DISCUSSION

Among the reported methods for preparing N^G-monomethyl-L-arginine,[2] the use of thiopseudouronium salts[3] leads to higher yields of methylated product. N,S-Dimethylthiopseudouronium iodide (methyl-^{14}C] (3) was prepared in 93% yield by methylation of [^{14}C]methylthiourea (2) with methyl iodide. The reaction of the thiopseudouronium salt (3) with L-ornithine afforded (4) with 7% radioactivity in an overall yield of 23%.



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EXPERIMENTAL

Methylamine hydrochloride-^{14}C (1 mCi/0.025 mmol) was purchased from Amersham Corp. All solvents were reagent grade unless otherwise specified and were used without purification. Radioactivity was determined using a Packard Tri-Carb liquid scintillation spectrophotometer with an efficiency of approximately 85%. All melting points are uncorrected.

[^{14}C]-Methylisothiocyanate (1):

In a 250-ml three-neck flask, surrounded by an ice bath and fitted with a reflux condenser and a 250-ml dropping funnel, are placed 5.5 ml of carbon disulfide (6.8 g, 90 mmol), a solution of methylamine hydrochloride (6.1 g, 90 mmol) in 8 ml of water and a solution of ^{14}C-methylamine hydrochloride (0.0017 g, 0.025 mmol; 2 x 10^9 cpm) in 2 ml of water. To the stirred mixture at 0-5° a solution of sodium hydroxide (7.2 g, 180 mmol) in 16 ml of water was added over a period of 30 minutes. The stirred bright orange reaction mixture was warmed to 70-80° on a water bath for 2 hrs and then cooled to 40°. Ethyl chloroformate (8.5 ml. 9.8 g, 90 mmol) was added over 1 hr and stirring was continued for an additional 30 minutes. The ^{14}C-methylisothiocyanate which separated was dried over anhydrous sodium sulfate, filtered and distilled at atmospheric pressure through a short Vigreux column; the fraction which boiled at 115-121° was collected (4.5 g, 66% yield). The product solidified on cooling, mp 36-37°; IR (neat) 1420, 2120 and 2200 cm^{-1}. Recovery = 1.32 x 10^9cpm.

N-[^{14}C-Methyl]thiourea (2):

In a 100-ml three-neck flask equipped with a reflux condenser and a dropping funnel, concentrated ammonium hydroxide (7 ml, 1.7 g, 100 mmol) was placed. The freshly prepared (1) (4.5 g, 65 mmol) was added with stirring over a period of 1 hr. The condenser was then removed and the solution was heated on a boiling water bath for 30 minutes. The solution was boiled with charcoal, filtered and chilled in an ice bath. The colorless compact crystals of (2) which separated were filtered and dried; yield 2.7 g (75%), mp 117-119° (lit[5] 119-120.5°); IR (KBr) 1950 and 3200 cm^{-1}. Recovery ≒ 1 x 10^9 cpm.

N,S-Dimethylthiopseudouronium Iodide [Methyl-^{14}C] (3):

To a slurry of (2) (2.5 g, 25 mmol) in 10 ml of acetone, methyl iodide (3.55 g, 25 mmol) was added. The reaction mixture was refluxed for 10 minutes and 5 ml of absolute ethanol was added to dissolve the desired product. The solution was filtered hot and hexane was added to the filtrate to the cloud point. On cooling, 5.4 g (90% yield) of (3) was obtained, mp 135-136° (lit[2] (135-136°). Recovery = 0.9 x 10^9 cpm.

Coupling of L-Ornithine and (3):

L-Ornithine hydrochloride (4.57 g, 27.2 mmol) and copper acetate (2.72 g, 13.6 mmol) were dissolved in 52 ml of 25% ammonium hydroxide solution in a 100-ml roundbottom flask. To the stirred mixture compound (3) (6.39 g, 27.2 mmol) was added and stirring was continued for 24 hrs at room temperature. The yellowish copper mercaptide was separated by filtration and the precipitate was washed with 94 ml of aqueous ammonium hydroxide (1:2). The filtrate was concentrated to approximately 70 ml under reduced pressure at about 40° (arginine derivatives are not stable in base at high temperatures), and the concentrate was charged on a Dowex 50 H^+ column (3 cm x 13 cm; 200-400 mesh) which had been washed with 300 ml of water. Elution was conducted with 300 ml of 3 N ammonium hydroxide. The eluate was concentrated to about 80 ml under reduced pressure at about 40°, and flavianic acid (27.2 mmol) in 54 ml of water was added to the concentrate. The mixture was chilled in ice. The yellow crystals were filtered and washed with cold water. The methylated amino acid (4) was recrystallized from water (52% yield); mp 252-253° (lit[2] 252-253°). Final recovery = 1.38 x 10^8 cpm.

Purity of N^G-Monomethyl-L-arginine

The purity of the N^G-mono[methyl-^{14}C]-L-arginine was determined with an automatic amino acid analyzer.[1] Briefly stated, the column (Aminex A-5 resin; 0.9 cm x 30 cm) was eluted with 0.42 N (Na^+) sodium citrate buffer, pH 6.48 at 26°C at a flow rate of 30 ml/hr. Under this condition, ε-N-methylated lysines are clearly separated from N^G-monomethyl-L-arginine. Analysis indicated that the final product is completely pure.

Specific Activity of N^G-Mono[methyl-^{14}C]-L-arginine: 40,500 cpm/μmole of N^G-monomethyl-L-arginine.

REFERENCES

1. Paik, W.K. and Kim, S. Advances in Enzymology, Meister, A. (ed.) John Wiley & Sons, N.Y., Vol. 42, 227-286 (1975).
2. Reporter, M. Arch. Biochem. Biophys., 158:577 (1973).
3. Simons, C. and Young, H.T., Brit. Pat. 672,684; Chem. Abstr., 47:P5436C (1953).
4. Radioactivity was determined with respect to the starting [^{14}C]methylamine hydrochloride.
5. Moore, M.L. and Crossley, F.S. Org. Syn. Coll. Vol. 3, 617 (1955).