A CONVENIENT SYNTHESIS OF CREATINE-^{15}N FROM GLYCINE-^{15}N VIA SARCOSINE-^{15}N

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SUMMARY

The preparation of creatine-^{15}N monohydrate from glycine-^{15}N with the isolation of sarcosine-^{15}N as an intermediate is described. Glycine-^{15}N (97.3 atom% ^{15}N) is converted to benzenesulphonyl glycine-^{15}N, which is methylated to give benzenesulphonyl sarcosine-^{15}N. The latter is hydrolysed to sarcosine-^{15}N, which is isolated by ion exchange chromatography. Sarcosine-^{15}N is converted to creatine-^{15}N monohydrate by reaction with cyanamide in aqueous solution in the presence of sodium chloride and catalytic amounts of ammonium hydroxide-^{14}N. The creatine-^{15}N monohydrate precipitated from the above reaction mix is recrystallized from boiling water. The yield of sarcosine-^{15}N from glycine-^{15}N is 78% of theory and that of recrystallized creatine-^{15}N monohydrate from glycine-^{15}N is 59% of theory.

Key Words: Creatine-^{15}N, sarcosine-^{15}N

INTRODUCTION

The nitrogenous base creatine, a derivative of glycine, plays a fundamental role in the energy storage process of vertebrates. It is present as phosphocreatine,

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synthesised from ATP by the enzyme creatine kinase, and is accumulated in muscle to a much larger extent than the more toxic ATP, to provide a readily available energy source. Its metabolic synthesis and fate was investigated extensively by Bloch, Schoenheimer and Rittenberg[1], [2], [3].

The chemical synthesis of creatine-^{15}N, (guanidino-1--[^{15}N]-1-methyl-ethanoic acid), was of interest for use in future metabolic studies. The synthesis we have used is based on those previously published[2], [4], [5] but we prefer to use ion exchange techniques for isolation of sarcosine rather than the insoluble salt methods described by Cocker and Lapworth[4]. We have also modified the creatine reaction mixture[2], [5], by the addition of sodium chloride, which we have found consistently to increase the yield of creatine obtained.

EXPERIMENTAL

Analytical techniques. Gas chromatographic analysis was performed with a Pye 104 gas chromatograph with FID, using a 1.5 m x 2mm ID column containing 5% OV1 on 100-120 mesh Gas-Chrom Q. Creatine monohydrate was chromatographed as the trimethyl silyl derivative.

A VG Micromass 70-70F mass spectrometer interfaced via a glass jet separator to a Varian gas chromatograph was used to confirm isotopic composition.

The assay for creatinine was performed by the method of Edwards and Whyte[6].

Preparation of sarcosine-^{15}N from glycine-^{15}N.

In a 250ml beaker were mixed 75ml M NaOH and 67 millimoles glycine-^{15}N (97.3 atom % ^{15}N) and the solution was stirred vigorously. To this stirred solution was added 86 millimoles benzenesulphonyl chloride. During the subsequent 1 hr reaction time an additional 30ml of 3M NaOH was added in portions to keep the mixture alkaline. After the reaction was complete the solution was filtered through a glass fibre filter. The filtrate was acidified by addition of 15ml cold concentrated HCl and allowed to stand for 3 hr at 4°C. The precipitated benzenesulphonyl glycine-^{15}N was collected by filtration and dried in vacuo. Yield 64 millimoles (96% of theory).

Benzenesulphonyl glycine-^{15}N (64 millimoles) was dissolved in 65ml 3M NaOH and the solution filtered. The filtrate was stirred in a 250ml beaker and 130 millimoles of dimethyl sulphate added in 6 equal portions during a period of 1 hr. When the solution was clear (c. 30 min after final addition of dimethyl sulphate) 10ml cold concentrated HCl were added and the solution allowed to stand for 3 hrs at 4°C. The precipitated benzenesulphonyl sarcosine-^{15}N was collected by filtration and dried in vacuo. Yield 63 millimoles (98% of theory).

Benzenesulphonyl sarcosine-^{15}N (63 millimoles) was hydrolysed by boiling for 5 hrs in 7M H_2SO_4 under

reflux. After cooling, filtering and diluting to 150ml with distilled H_2O the solution was applied to a 35cm x 3cm column of cation exchange resin (Amberlite IR120 H^+). Sarcosine-^{15}N was retained by the column and eluted with 0.2M NH_4OH-^{14}N. The fractions containing sarcosine-^{15}N were concentrated at 60° in a rotary vacuum evaporator to a small volume (c. 5ml) and 100ml ethanol added. Sarcosine-^{15}N precipitated overnight on standing at 4°C; it was collected by filtration and dried in vacuo. Yield 52 millimoles (83% of theory).

Preparation of creatine-^{15}N from sarcosine-^{15}N. In 10ml H_2O were dissolved 52 millimoles sarcosine-^{15}N and 52 millimoles sodium chloride; 98 millimoles of cyanamide (Sigma) dissolved in 2.5ml H_2O and 0.3ml concentrated ammonia-^{14}N solution were added. The addition of ammonia catalyses the reaction and the addition of sodium chloride appears to increase the yield of creatine-^{15}N by about 20%, perhaps by salting out the creatine-^{15}N. The solution was left for 2 days at room temperature (25°C) and the precipitated creatine-^{15}N filtered off and dried in vacuo. Yield 45 millimoles (87% of theory).

The creatine-^{15}N (45 millimoles) was recrystallized by dissolving in 50ml boiling H_2O, filtering through a heated filter funnel and crystallizing for 48 hrs at 5°. Yield 40 millimoles (88% of theory).

Found C, 32.16; H, 7.20; N, 28.78; (N/C ratio

1/1.117). Calc. for $C_4H_9N_3O_2 \cdot H_2O$ (assuming 1 atom is ^{15}N): C, 32.01; H, 7.39; N, 28.62; (N/C ratio 1/1.118). Estimation of H & O was complicated by the double dehydration of creatine monohydrate to creatinine in the early stages of analysis. Mass spectrum m/e 314 (3), 315 (97). By gas liquid chromatography $>$ 99% pure. Assay for creatinine shows $<$ 0.01%.

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