

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

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SUMMARY

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

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

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,

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<sup>(1), (2), (3)</sup>.

The chemical synthesis of creatine-<sup>15</sup>N, (guanidino-1-<sup>15</sup>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<sup>(2), (4), (5)</sup> but we prefer to use ion exchange techniques for isolation of sarcosine rather than the insoluble salt methods described by Cocker and Lapworth<sup>(4)</sup>. We have also modified the creatine reaction mixture<sup>(2), (5)</sup>, 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<sup>(6)</sup>.

Preparation of sarcosine-<sup>15</sup>N from glycine-<sup>15</sup>N.

In a 250ml beaker were mixed 75ml M NaOH and 67 millimoles glycine-<sup>15</sup>N (97.3 atom % <sup>15</sup>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-<sup>15</sup>N was collected by filtration and dried in vacuo. Yield 64 millimoles (96% of theory).

Benzenesulphonyl glycine-<sup>15</sup>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-<sup>15</sup>N was collected by filtration and dried in vacuo. Yield 63 millimoles (98% of theory).

Benzenesulphonyl sarcosine-<sup>15</sup>N (63 millimoles) was hydrolysed by boiling for 5 hrs in 7M H<sub>2</sub>SO<sub>4</sub> under

reflux. After cooling, filtering and diluting to 150ml with distilled  $\text{H}_2\text{O}$  the solution was applied to a 35cm x 3cm column of cation exchange resin (Amberlite IR120  $\text{H}^+$ ). Sarcosine- $^{15}\text{N}$  was retained by the column and eluted with 0.2M  $\text{NH}_4\text{OH}$ - $^{14}\text{N}$ . The fractions containing sarcosine- $^{15}\text{N}$  were concentrated at  $60^\circ$  in a rotary vacuum evaporator to a small volume (c. 5ml) and 100ml ethanol added. Sarcosine- $^{15}\text{N}$  precipitated overnight on standing at  $4^\circ\text{C}$ ; it was collected by filtration and dried in vacuo. Yield 52 millimoles (83% of theory).

Preparation of creatine- $^{15}\text{N}$  from sarcosine- $^{15}\text{N}$ .

In 10ml  $\text{H}_2\text{O}$  were dissolved 52 millimoles sarcosine- $^{15}\text{N}$  and 52 millimoles sodium chloride; 98 millimoles of cyanamide (Sigma) dissolved in 2.5ml  $\text{H}_2\text{O}$  and 0.3ml concentrated ammonia- $^{14}\text{N}$  solution were added. The addition of ammonia catalyses the reaction and the addition of sodium chloride appears to increase the yield of creatine- $^{15}\text{N}$  by about 20%, perhaps by salting out the creatine- $^{15}\text{N}$ . The solution was left for 2 days at room temperature ( $25^\circ\text{C}$ ) and the precipitated creatine- $^{15}\text{N}$  filtered off and dried in vacuo. Yield 45 millimoles (87% of theory).

The creatine- $^{15}\text{N}$  (45 millimoles) was recrystallized by dissolving in 50ml boiling  $\text{H}_2\text{O}$ , filtering through a heated filter funnel and crystallizing for 48 hrs at  $5^\circ$ . 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<sub>4</sub>H<sub>9</sub>N<sub>3</sub>O<sub>2</sub>·H<sub>2</sub>O (assuming 1 atom is <sup>15</sup>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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