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Synthesis of ^{14}C-labelled aminoguanidine

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SUMMARY

[^{14}C]Aminoguanidine was synthesized by the reaction of hydrazine sulphate with barium [^{14}C]cyanamide in a one-step synthesis, and conveniently isolated by crystallization as the bicarbonate salt. The yield was 32%.

KEY WORDS aminoguanidine, dicarbonyl compounds, advanced glycation endproducts, diabetes, nitric oxide.

INTRODUCTION

Aminoguanidine is currently under investigation for prospective therapeutic use (1). It inhibits the formation of advanced glycation endproducts (AGE), which are compounds formed from the degradation of fructosamines produced in the early stages of the non-enzymatic glycation of proteins (2). This is thought to be achieved by scavenging reactive α,β-dicarbonyl compounds, such as 3-deoxyglucosone and 2-glucosulose, formed from the degradation of fructosamines under physiological

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conditions, thereby preventing the further irreversible modification of protein and associated formation of characteristic fluorescent pigments (3,4). It has been suggested that the formation of AGE is an etiological factor in the development of chronic clinical complications of diabetes mellitus (retinopathy, neuropathy, nephropathy) (5). Aminoguanidine prevents the development of albuminuria, decreased sciatic nerve conductiyon velocity and protein crosslinking in diabetic rats (6,7,8) and is now under clinical evaluation for the preventive therapy of diabetic complications.

Aminoguanidine is also a competitive inhibitor of nitric oxide synthase (9). Inhibition of nitric oxide production may in part mediate the beneficial effects of aminoguanidine in reversing microvascular complications in diabetes mellitus (10).

During studies of the pharmacological effects of aminoguanidine, there has been a requirement for a radiolabelled derivative. We describe in this note the convenient one-step synthesis and isolation of [^{14}C]aminoguanidine bicarbonate from commercially available barium [^{14}C]cyanamide.

MATERIALS AND METHODS

Materials

Barium [^{14}C]cyanamide $BaCN_2$, 1 mCi, was purchased from Sigma Chem. Co. Ltd. (Poole, Dorset, U.K.) with a specific activity 7.9 mCi/mmol. Hydrazine sulphate, methylglyoxal methylacetal and methylglyoxal bisguanylhydrazone were purchased from Sigma, and potassium bicarbonate from Fisons Scientific Supplies (Loughborough, Leicestershire, U.K.). Methylglyoxal was prepared by acid hydrolysis of methylglyoxal methylacetal and purified by fractional vacuum distillation as described (11).

Methods

Preparation of [^{14}C]aminoguanidine bicarbonate

Aminoguanidine was synthesized by the reaction of cyanamide with hydrazine (12). The reaction conditions were optimized with unlabelled reagents prior to radiosynthesis.

Barium [^{14}C]cyanamide (22.6 mg, 0.127 mmol, 1 mCi) was dissolved in 0.716 M sodium cyanamide solution (1.22 ml, 0.873 mmol) to give a cyanamide solution of specific activity 1 mCi per mmol. Hydrazine sulphate (0.130g, 1 mmol) was added and the solution stirred at room temperature for 24 h. The resulting suspension was centrifuged (11,000g, 15 min, room temperature), the supernatant removed and added to 0.8 ml of saturated potassium bicarbonate. This solution was stirred for 24 h during which time the solid product crystallized. The solid product was collected by centrifugation (11,000g, 15 min, room temperature) and washed with saturated potassium bicarbonate solution (1.5 ml), distilled water (1 ml) and dried under vacuum.

The solid product was derivatized by dissolving 0.27 mg of solid in 0.1 ml of 10 mM methylglyoxal in 1 M hydrochloric acid and allowing the reaction to proceed for 1 h at room temperature. The product mixture was analyzed by thin layer chromatography on silica 60 F_{254} plates (BDH, Poole Dorset) with a mobile phase of ethanol:acetic acid:water, 10:5:5 (v/v/v), and compared with a standard methylglyoxal bisguanylhydrazone solution.

RESULTS

Synthesis and isolation of [^{14}C]aminoguanidine bicarbonate

When 1 mmol of [^{14}C]labelled barium/sodium cyanamide was reacted with hydrazine sulphate in aqueous solution, [^{14}C]-labelled aminoguanidine was formed along with a white precipitate of barium sulphate. This was sedimented by centrifugation and the supernatant containing [^{14}C]aminoguanidine and sodium sulphate

removed and retained. [^{14}C]Aminoguanidine was conveniently isolated by addition of saturated potassium bicarbonate solution which induced crystallization of the bicarbonate salt. The solid product was sedimented by centrifugation, washed and dried under vacuum.

Aminoguanidine bicarbonate was characterized by determination of the melting point 167-170°C (dec.) - literature value 170-172°C (dec.), and by derivatization with methylglyoxal to form methylglyoxal bisguanylhydrazone which had a R_f value of 0.81. Under acidic conditions, methylglyoxal reacts with aminoguanidine to form a 1:2 adduct, methylglyoxal bisguanylhydrazone (13).

DISCUSSION

Hydrazine reacts with cyanamide to form aminoguanidine,

$$CN_2^{2-} + 2H^+ + H_2N\text{-}NH_2 \rightarrow H_2N\text{-}NH\text{-}C(=NH)\text{-}NH_2$$

The method previously described (12) employed calcium cyanamide, and an excess of cyanamide over hydrazine sulphate. Pilot syntheses showed that the yield was optimum when a 1:1 molar ratio of cyanamide and hydrazine sulphate was used. Adaptation of this method to use commercially available barium [^{14}C]cyanamide produced a precipitate of barium sulphate in the reaction mixture which was readily removed.

[^{14}C]Aminoguanidine is required for pharmacological studies in the development of aminoguanidine for therapeutic use. [^{14}C]Aminoguanidine, in protein-binding assays, will also enable the determination of α,ß-dicarbonyl compounds bound to proteins, such implicated as intermediate in the formation of N_ϵ-carboxylmethyl-lysine residues during the degradation of protein fructosamines (14); other low molecular mass α,ß-dicarbonyl compounds may be determined by high performance liquid chromatography following derivatization with 1,2-diaminobenzene

derivatives (15). The method described for the synthesis and isolation of [^{14}C]aminoguanidine bicarbonate is a very simple one and involves little technical difficulty.

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