SYNTHESIS OF [α-^{14}C]-BENZYLGUANIDINE

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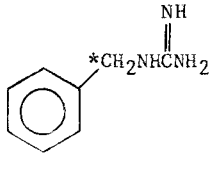
SUMMARY

[α-^{14}C]-benzylguanidine (1) having a specific activity of 14 mCi/mmol was synthesized in 55% yield (isolated) by reacting [α-^{14}C]-benzylamine hydrochloride and cyanamide in 1-butanol.

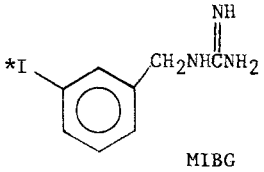
Key Words: ^{14}C-Benzylguanidine; ^{14}C-Benzylamine; Cyanamide.

INTRODUCTION

Many derivatives of benzylguanidine are potent antihypertensive agents (1,2) due to their ability to directly block the release of norepinephrine from sympathetic nerves (3). Thus, they are referred to as neuron-blocking agents. A radioiodinated derivative of benzylguanidine, m-iodobenzylguanidine (MIBG,2), has been shown to localize in adrenergic tissue (4-5). When labeled with ^{131}I or ^{123}I, MIBG in humans provides scintiphotos of catecholamine containing tumors (6), hyperfunctioning adrenal medullae (7), and organs with rich sympathetic innervation such as the heart (8).



BENZYLGUANIDINE
1



MIBG
2

In order to assess those aspects of MIBG biodistribution that are attributable to the iodine atom, we required ^{14}C-labeled benzylguanidine (1). This article decribes various attempts to synthesize this compound.

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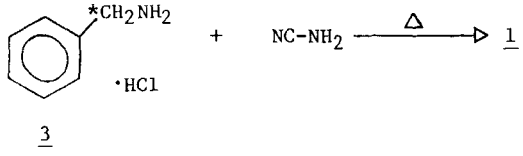
RESULTS AND DISCUSSION

Radiopharmaceutical constraints require that we obtain product of reasonably high specific activity (>10 mCi/mmol) to minimize potential pharmacological effects during *in vivo* biodistribution studies. In addition we wanted a preparation that would not require HPLC purification. *A priori*, 1 should be readily obtainable from either of the two classical syntheses (1,9-11) of aralkylguanidines:

1) Reaction of [α-^{14}C]-benzylamine with 2-methyl-2-thiopseudourea sulfate.

2) Reaction of [α-^{14}C]-benzylamine hydrochloride (3) with molten cyanamide as shown in Scheme 1.



3

Scheme 1

Our initial attempt to synthesize 1 was to react 3, an equivalent of $KHCO_3$ and 2-methyl-2-thiopseudourea sulfate in refluxing water. After 10 hours, radio-TLC showed less than 2% yield of the desired 1. Further heating did not improve the yield of 1 but led to the formation of several radioactive impurities. We next turned our attention to 1-guanyl-3,5-dimethylpyrazole nitrate (4) as the guanylation agent (11). Reaction of 3, $KHCO_3$ and 4 in refluxing water for 16 hours did not lead to any detectable amount of 1. We then focused on the cyanamide method wherein 3 and excess cyanamide were heated at 114°C for 4 hours. Compound 1 was formed in about 5% yield under these conditions as shown by radio-TLC. Further heating at 130°C for 12 hours led to an increase in the yield of 1 (ca. 25%) but an impurity (ca. 20%) that was less polar than benzylamine itself appeared in the radio-TLC.

These discouraging findings prompted a search for modifications or alternative approaches. Of special interest was the report by Miller (12) in which

p-nitrobenzylguanidine was made by reaction of p-nitrobenzylamine hydrochloride with cyanamide in refluxing 1-butanol. This synthesis proceeds in low yield when conducted under the usual molten cyanamide conditions.[13]

Thus, when 3 and excess cyanamide were allowed to react in refluxing 1-butanol as solvent at a pH of 5.5 for 5 hours, 1 was formed in 80-90% yield as judged by radio-TLC (14). Addition of more cyanamide or prolonged heating did not take the reaction to completion. Purification of the final product was achieved by extraction of the small amount of unreacted amine with ether from the aqueous solution of the reaction mixture at pH 10.2. The strongly basic benzylguanidine ($pK_a \simeq 13$) remained in the aqueous solution at this pH. Radio-HPLC showed > 98% purity after ether extraction.

Attempts are presently underway to optimize the ^{14}C-labeling of both benzylguanidine and MIBG at the guanidino carbon.

EXPERIMENTAL SECTION

Thin layer chromatography was carried out on Whatman K6F silica gel plates. Plates (20 x 2.5 cm) were developed with two solvent systems: a) ethanol/concentrated NH_4OH (3:1 v/v): benzylguanidine·HCl, R_f=0.10; benzylamine·HCl, R_f=0.74. b) 1-butanol/acetic acid/water (2:2:1 v/v/v): benzylguanidine·HCl R_f=0.47; benzylamine·HCl, R_f=0.71. The radio-TLC plates were analyzed on a Packard Model 720 radiochromatogram scanner immediately after development and drying. [α-^{14}C]-benzylamine·HCl was obtained from ICN, Irvine, Ca. as an aqueous solution of specific concentration 0.10 mCi/mL and specific activity 14 mCi/mmol. Radioactive assays were performed on a Packard 3330 liquid scintillation counter. Counting efficiency was 85-88%. Radiochemical purity of 1 and 3 were confirmed by HPLC analysis. A Waters Model 272 liquid chromatograph equipped with a Radiomatic Flo-one radioactive flow detector (200 μL solid scintillator cell) was used, employing simultaneous ultraviolet (254 nm) and radioactivity detection. A Waters-Bondapak C-18 column (4.6 x 250 mm) was used for the analysis with the following solvent system: 0.2M $NH_4H_2PO_4$/THF

(9/1) at a flow rate of 1 mL/min. The commercial ^{14}C-benzylamine·HCl was found to be 99.3% pure by radio-HPLC with a t_R value of 4.2 min.

[α-^{14}C]-benzylguanidine (1). An aqueous solution of [α-^{14}C]-benzylamine·HCl (2 ml, 2.05 mg, 200 μCi) was placed in a 5 ml Wheaton 'V'-vial and the pH of the solution was raised to 5.5 by careful addition of 0.1 N NaOH. A spin-vane was added and water was evaporated by a gentle stream of argon at 40-45°C (oil bath) over 2 hours. To the dry residue was added a solution of 1-butanol (100 μl) containing cyanamide (Sigma grade, 2 mg) and the mixture was heated and stirred at reflux temperature in an oil bath (130°C) for 5 hours. The reaction mixture was cooled, the solvent removed by a stream of argon at 45°C and the residue dissolved in warm water (0.5 ml). The warm aqueous solution was passed through a short column (1.5 x 0.6 cm) of anion exchange resin (Cellex D, OH^- form) to remove tars and washed further with three 0.5 mL portions of warm water. The pH of the eluate was adjusted to 10.2 by the addition of 0.1 N NaOH. Thorough extraction with ether (7 x 5 ml) completely removed the unreacted amine, as shown by radio-TLC and radio-HPLC. The pH of the aqueous solution was adjusted to 5.5 by the addition of 0.1N HCl and the activity was assayed to be 110 μCi - a radiochemical yield of 55% (15). Radiochemical purity of 1 was > 98% as determined by radio-HPLC; t_R=6.6 min.

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14. Although we did not carry out an extensive investigation as to the effect of pH on the yield of benzylguanidine, we did observe a dramatic decrease in yield (<50%) at a pH range of 2-4

15. Average of three runs