SYNTHESIS OF 6-QUINOXALIN-2,3-$^{14}C_2$-AMINE AND DERIVATIVES VIA ETHANEDIAL-1,2-$^{14}C_2$

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SUMMARY

High specific activity ethanedial-1,2-$^{14}C_2$ has been prepared in 74% radiochemical yield by oxidation of paraldehyde-$^{14}C_6$ with selenious acid. The crude ethanedial-$^{14}C_2$ was directly condensed with 1,2,4-benzenetriamine dihydrochloride in aqueous sodium carbonate giving 6-quinoxalin-2,3-$^{14}C_2$-amine in 56% yield. The quinoxaline was brominated and then converted to its isothiocyanate by reaction with thiophosgene. The isothiocyanate was directly converted to 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline-2,3-$^{14}C_2$ by reaction with ethylenediamine in refluxing 1:1 methanol toluene. The overall radiochemical yield was 11% from paraldehyde-$^{14}C_6$.

Key Words: ethanedial, glyoxal, paraldehyde, 6-quinoxalin-2,3-amine, quinoxaline

INTRODUCTION

5-Bromo-6-(2-imidazolin-2-ylamino)quinoxaline is a potent and selective α_2 adrenoceptor agonist (1). It has been used extensively in studies of α-adrenergic receptor binding (2-4) and vasoconstriction (5-7). Although tritium labeled 5-bromo-6-(2-imidazolin-2-ylamino)quinoxaline is commercially available, we required the carbon-14 labeled material with the label in the quinoxaline ring system.

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There are few examples of syntheses of carbon-14 labeled quinoxaline ring systems. Lont and van der Plas (8) synthesized 2-quinoxalinamine-2-^{14}C via condensation of carbon-14 labeled hydrogen cyanide and formaldehyde with 1,2-phenylenediamine followed by base hydrolysis and peroxide oxidation. Saari and Lumma (9) synthesized 2,6-dichloroquinoxaline-2,3-$^{14}C_2$ from labeled glyoxylic acid and 4-chloro-1,2-phenylenediamine. The quinoxalinone thus produced was converted to the desired compound by treatment with phosphorus oxychloride. Neither of these two routes could be adapted to our target compound, however.

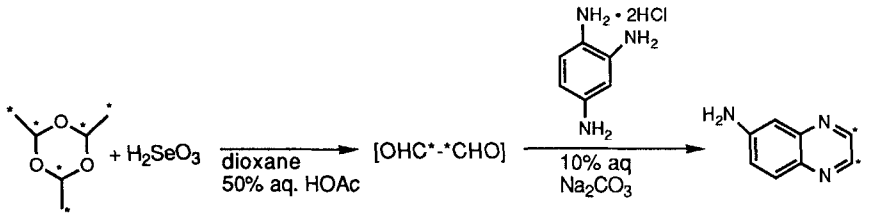
The classical synthesis of quinoxalines involves the condensation of 1,2-phenylenediamines with ethanedial (glyoxal), an α-ketoaldehyde or an α-diketone (10). We reasoned that carbon-14 labeled ethanedial could be prepared from commercially available paraldehyde-$^{14}C_6$. Thus, condensation of 1,2,4-benzenetriamine with carbon-14 labeled ethanedial would provide our key intermediate, 6-quinoxalinamine. It is this route that we chose for our synthesis.

RESULTS AND DISCUSSION

Synthesis of Ethanedial-$^{14}C_2$. Only one synthesis of carbon-14 labeled ethanedial has been reported. Nystrom and Mann (11) degraded 1,3-butadiene-2,3-$^{14}C_2$ with ozone and isolated the ethanedial-$^{14}C_2$ as its 2,4-dinitrophenylosazone. Since, a more practical method was required, we chose to modify a synthesis of ethanedial bisulfite. Ronzio and Waugh (12) oxidized excess paraldehyde (acetaldehyde trimer) with selenious acid. The ethanedial was not directly isolated but rather was treated with sodium bisulfite and the solid ethanedial bisulfite collected by filtration. The authors report a 72-74% yield of ethanedial bisulfite based on selenious acid, but only 20% based on paraldehyde. This stoichiometry is not acceptable for the synthesis of labeled ethanedial since maximum conversion of paraldehyde, and not selenious acid, is required. We found that

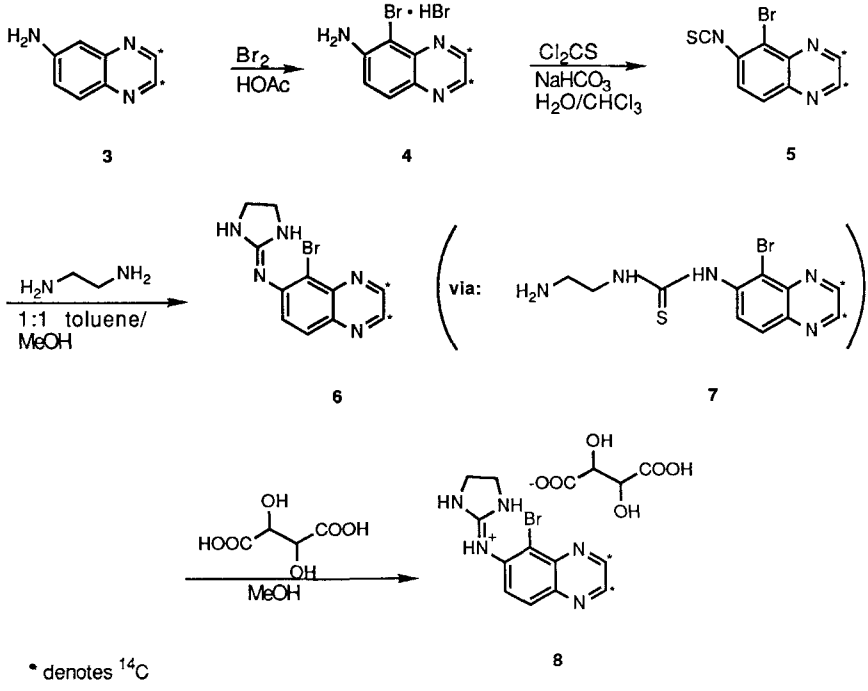
this oxidation was very successful on our small (mmol) scale when an equivalent, or an excess, of selenious acid was used. Thus, in the synthesis of carbon-14 labeled ethanedial (Scheme I), 42.8 mCi of paraldehyde-$^{14}C_6$ (324 mCi/mmol) was oxidized to 31.9 mCi of ethanedial-$^{14}C_2$ (74.5% radiochemical yield based on paraldehyde-$^{14}C_6$). Because of operational simplicity, we chose to directly use

Scheme I



1 2 3

Scheme II



* denotes ^{14}C

the crude ethanedial-$^{14}C_2$ in our 6-quinoxalinamine synthesis, rather than convert this product to the sodium bisulfite adduct. Also, the small amount of labeled material produced, and its reactivity, precluded any analysis of the ethandial-$^{14}C_2$.

Synthesis of 6-Quinoxalin-2,3-$^{14}C_2$-amine. Ethanedial bisulfite has previously been condensed with both 1,2-phenylenediamine to form quinoxaline (13) and with 1,2,4-benzenetriamine to form 6-quinoxalinamine (14-15). Jones and McLaughlin (13) in their synthesis of quinoxaline indicated that bisulfite was necessary for the reaction to proceed in 85-90% yield; the yield fell to 30% without bisulfite present. We found that, on a 0.3 mmol scale, the direct condensation of 1,2,4-benzenetriamine dihydrochloride with either freshly prepared ethanedial or ethanedial bisulfite proceeded in nearly the same yield (60-70%). Thus, ethanedial-$^{14}C_2$ was directly cyclized with 1,2,4-benzenetriamine in aqueous sodium carbonate (Scheme I). This gave the desired 6-quinoxalin-2,3-$^{14}C_2$-amine in 56% radiochemical yield.

Synthesis of 5-Bromo-6-(2-imidazolin-2-ylamino)-quinoxaline-2,3-$^{14}C_2$. 6-Quinoxalin-2,3-$^{14}C_2$-amine (3) was converted to the title compound as shown in Scheme II by modifying the procedure of Danielewicz (16). The amine 3 was brominated with bromine in glacial acetic acid. The bromoquinoxalinamine 4 was then converted to its isothiocyanate 5 by treatment with thiophosgene. This isothiocyanate was obtained from these two steps in 50% overall radiochemical yield after chromatographic purification. Isothiocyanate 5 was directly converted to the imidazolinylamino-quinoxaline 6 in 52% radiochemical yield by a one-pot reaction with ethylenediamine in 1:1 refluxing toluene/methanol. This modification is an improvement over the published procedure which required two steps: condensation of the isothiocyanate with

ethylenediamine in toluene, and then cyclization of the 5-bromo-6-(N'-2-[aminoethyl]thioureido)quinoxaline (7) intermediate in refluxing methanol. Unlike many isothiocyanates which react with alcohols to give thiocarbamates, isothiocyanate 5 appeared to be stable in in 1:1 toluene/methanol. Thus, the two reaction sequence, which did not require isolation of the thioureido intermediate, could be satisfactorily conducted in one simple operation. Yields were typically 85% for this procedure, though the yield in carbon-14 synthesis was somewhat lower as noted above.

Since a water soluble form of the quinoxaline 6 was required for biological testing, the free base was converted to its tartrate salt by reaction with L-tartaric acid in methanol.

EXPERIMENTAL

General. Paraldehyde-$^{14}C_6$ was purchased from American Radiolabeled Chemicals, Inc (St. Louis, MO). All reagents were of analytical reagent grade or better. Thin layer chromatography was performed on 5 cm x 20 cm Silica Gel Gf plates (250 μm, Merck). Radiochromatograms were analyzed on a Berthold LB 2832L Linear Analyzer. HPLC was monitored for radioactivity using a Ramona Radioactivity Flow Detector using TruCount Scintillant at 5.0 mL/min in a 0.75 mL flow cell.

Ethanedial-1,2-$^{14}C_2$ (2). Selenious acid (251 mg, 1.94 mmol, K&K), dioxane (0.45 mL), and 50% aqueous acetic acid (33 μL) were placed in a 1 mL Reacti-Vial. 2,4,6-Tri(methyl-^{14}C)-1,3,5-trioxane-2,4,6-$^{14}C_3$ (42.8 mCi at 324 mCi/mmol, paraldehyde, 1) was added to the mixture and the reaction was heated to 70°C in the closed Reacti-Vial. A black precipitate of selenium formed within a few minutes. The reaction mixture was stirred for 18 hours and then filtered through a cotton plug into an 8 mL round bottom flask. The selenium metal was rinsed with 2 mL of water. The filtrate and rinsings were combined and concentrated by

distillation. The 0.85 mL of distillate contained 1.8 mCi. The concentrated solution was treated with 25% (w/w) lead acetate in water which immediately gave a white precipitate of lead selenite. The mixture was stirred 5 minutes. A drop of the mixture was filtered through a cotton plug and a drop of 25% (w/w) lead acetate solution was added; no precipitate formed, indicating that the conversion of selenium to lead selenite was complete. The entire reaction mixture was filtered through Celite; the Celite was rinsed with 1 mL of water. Hydrogen sulfide gas was bubbled through the clear filtrate for 7 minutes. After a few minutes a black precipitate of lead sulfide formed. After standing for 15 minutes, the mixture was filtered through Celite and the Celite was rinsed with 1 mL of water. The filtrate contained 31.9 mCi (74% radiochemical yield) of crude ethanedial-1,2-$^{14}C_2$ in 4.2 mL of water. This product was used directly in the next step without further purification or characterization.

6-Quinoxalin-2,3-$^{14}C_2$-amine (3). 1,2,4-Benzenetriamine dihydrochloride (247 mg, 1.26 mmol), prepared as described by Sherif (17), was dissolved in 5 mL of 10% aqueous sodium carbonate. This solution was added to the 31.9 mCi of ethanedial-1,2-$^{14}C_2$ obtained in step 1. The reaction mixture was stirred for 19 hours at 90°C in a closed, 20 mL screw-capped test tube, then cooled to room temperature and extracted with ten 4 mL portions of ether. The combined ether extracts were dried over magnesium sulfate, filtered, and concentrated *in vacuo* to give 18.0 mCi of 6-quinoxalin-2,3-$^{14}C_2$-amine (3) as a golden brown oil. The radiochemical purity of 3 was 93.0% (TLC on Silica Gel GF, ether, R_f = 0.1)

5-Bromo-6-quinoxalin-2,3-$^{14}C_2$-amine hydrobromide (4). 6-Quinoxalin-2,3-$^{14}C_2$-amine (3, 18.0 mCi) from step 2 was dissolved in 1 mL of glacial acetic acid. To this solution at 10°C was added

28 μL of bromine (0.54 mmol) in 58 μL of glacial acetic acid. The reaction mixture was stirred for 1 hour under an argon atmosphere and then was diluted with ether. The red precipitate was collected by filtration, washed with ether, and air dried. This procedure gave 14.8 mCi (82% radiochemical yield, 80 mg) of 5-bromo-6-quinoxalin-2,3-$^{14}C_2$-amine hydrobromide (4) as a brick red solid. Radiochemical purity by TLC was 57.1% (Silica Gel GF, ether, R_f = 0.26).

5-Bromo-6-isothiocyanatoquinoxaline-2,3-$^{14}C_2$ (5). The 14.8 mCi (80 mg, 0.26 mmol) of 5-bromo-6-quinoxalin-2,3-$^{14}C_2$-amine hydrobromide (4) was suspended in 1.0 mL of water. Thiophosgene (50 μL, 0.65 mmol, Aldrich), chloroform (1.40 mL), and sodium bicarbonate (48 mg, 0.57 mmol) were added to the suspension. The mixture was stirred for 14 hours at room temperature under an argon atmosphere. The reaction mixture was extracted with chloroform (4 x 3 mL), and the combined extracts were dried over magnesium sulfate, filtered, and concentrated to a dark oil *in vacuo*. The 11.9 mCi of crude product was purified by column chromatography (Baker Flash Silica Gel; eluted with chloroform). 5-Bromo-6-isothiocyanatoquinoxaline-2,3-$^{14}C_2$ (5, 9.0 mCi, 61% radiochemical yield, 28.0 mg) was obtained as a cream-colored solid having a radiochemical purity of 97.3% (TLC, Silica Gel GF, ether, R_f = 0.36).

5-Bromo-6-(2-imidazolin-2-ylamino)-quinoxaline-2,3-$^{14}C_2$ (6). 5-Bromo-6-isothiocyanatoquinoxaline-2,3-$^{14}C_2$ from step 4 (9.0 mCi, 28.0 mg, 0.105 mmol) was dissolved in 1.6 mL of 1:1 (v/v) toluene/methanol. Ethylenediamine (70 μL, 1.05 mmol, Aldrich) in 0.20 mL of 1:1 (v/v) toluene/methanol was added to the solution and the reaction mixture was heated for 18 hours at 75°C under an argon atmosphere. The solution was cooled to room temperature and filtered to remove 8.7 mg of a white powder. The yellow filtrate

was concentrated by ca. 90% in vacuo to a yellow oil. This oil was treated with methanol and the resulting bright yellow crystals were collected by filtration. The filtrate was taken to dryness in vacuo. The residue was taken up in methanol and the resulting yellow crystals were collected by filtration. The combined crops of yellow crystals were dried at 0.07 mm at room temperature for 4 hours. This procedure gave 4.7 mCi (52% radiochemical yield, 16.0 mg) of 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline-2,3-$^{14}C_2$ (6).

5-Bromo-6-(2-imidazolin-2-ylamino)-quinoxaline-2,3-$^{14}C_2$ tartrate (8). The 4.7 mCi (16.0 mg, 54.8 μmol) of 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline-2,3-$^{14}C_2$ (6) obtained above was suspended in 0.406 mL methanol. L-tartaric acid (11.6 mg, 77.3 μmol, Aldrich) in 0.209 mL methanol was added to the suspension, resulting in a color change from yellow to white. The suspension was allowed to stand at room temperature for 18 hours. The cream-colored precipitate was collected by filtration and washed with cold methanol. The crystals were dried at 0.01mm at room temperature for 18 hours. This procedure gave 2.26 mCi (48% radiochemical yield, 11.7 mg) of 5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline-2,3-$^{14}C_2$ tartrate (8) at a radiochemical purity of 98.2% (HPLC using a Beckman octyl column, 5 μm, 4.6 mm x 25 cm, eluted at 1.0 mL/min with 66:24:10 water/0.25N triethylammonium phosphate at pH 2.5/methanol, retention time = 10.1 min) and a specific activity of 101.5 mCi/mmol (by mass spectroscopy isotope abundance). Quinoxaline tartrate 8: ^{1}H-NMR ($CDCl_3$) δ3.75 (s, 4, $HNCH_2CH_2NH$), 7.88 (d, 1, J=9.0 Hz, C_8H), 8.13 (d, 1, J=9.0 Hz, C_7H), 8.92-8.93 (m, 2, C_2H + C_3H); Mass spectrum by chemical ionization (CH_4): 295(7), 294($M+H^+$, 60), 293(15), 292($M+H^+$, 61), 291(10), 241(4), 213(6), 212(4), 151(tartaric acid+H^+, 35), 145(6), 133(11), 123(10), 105(100), 87(10); tartrate analysis by HPLC (two Whatman partisil 5-ODS-3 columns in series, eluted at 1 mL/min with 80:20:0.1 water/0.25N aqueous

triethylammonium phosphate at pH 2.5/trifluoroacetic acid, UV detection at 215 nm): 37.7% (theory = 34.0%).

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