THE SYNTHESIS OF TRITIATED METHOXAMINE WITH HIGH SPECIFIC ACTIVITY

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SUMMARY

Tritiated methoxamine with a specific activity of 28.5 Ci/mmol was prepared by iodination of methoxamine followed by catalytic tritiation.

Key Words: Methoxamine, Tritiation

INTRODUCTION

Methoxamine[1] [α-(1-aminoethyl)-2,5-dimethoxybenzenmethanol] is an adrenergic agonist which is selective for the $alpha_1$ receptor.[2,3] While there are available a number of radiolabelled ligands such as WB-4101 and prazosin which are selective antagonists at the $alpha_1$ receptor, no radiolabelled agonists are available. Since we wished to do binding studies which required an agonist ligand of high specific activity, we have synthesized tritiated methoxamine. This was carried out by iodination of methoxamine (1) with iodine in the presence of silver trifluoroacetate[4] followed by catalytic tritiation in DMF. Aprotic DMF was necessary in order to minimize exchange and achieve maximum specific activity. The tritiated material was purified by HPLC and stored in aqueous solution at 4°C. Under these conditions, extensive exchange[5] occurred, resulting in a drop of specific activity from 28.5 Ci/mmol to 7 Ci/mmol (Table 1). The material was repurified and stored at a concentration of 0.2 mCi/ml in 70% ethanol containing 1% ascorbic acid as a radical scavenger.[5] These conditions greatly improve storage stability (Table 2).

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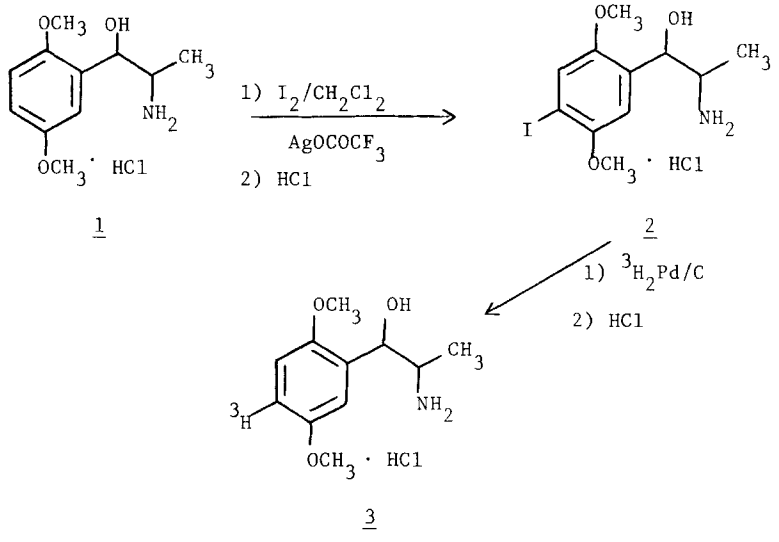

Table 1. Stability of 3 in Water[a,b,c]

Elapsed time(days)	Radiopurity(%)
0	94
7	90
14	84

a-storage temperature 4°C, b-concentration 37 mCi/ml, c-specific activity 28.5 Ci/mmol.

Table 2. Stability of 3 in 70% Ethanol[a,b,c,d]

Elapsed time(days)	Radiopurity(%)
0	98.4-99.3
13	98.7-99.4

a-storage temperature 4°C, b-concentration 0.2 mCi/ml, c-1% ascorbic acid, d-specific activity 7.0 Ci/mmol.

EXPERIMENTAL

Mass spectra were run on a Hitachi-Perkin Elmer RMU 6E Spectrometer. Elemental analyses were done on a Perkin Elmer 240 CHN Analyzer. Radioactivity measurements were obtained on a Searle Mark III (model 6880) scintillation counter and radiochromatographs were done on a Berthold TLC-Scanner (model

LB 2760). Tritiation was run at New England Nuclear.

4-Iodomethoxamine (2). Into 150 ml of CH_2Cl_2 was suspended 1.48g (6 mmol) of methoxamine hydrochloride and 2.65g (12 mmol) of silver trifluoroacetate. The mixture was stirred under argon at room temperature while a solution of 2.0g (8 mmol) of iodine in 40 ml of CH_2Cl_2 was added dropwise until a purple color persisted. The precipitated solids were removed by filtration and the filtrate evaporated. The residue was dissolved in 100 ml of 3N HCl and extracted with 100 ml of Et_2O. The aqueous portion was layered with fresh Et_2O, adjusted to pH 10.5 with 10% NaOH and extracted with three 100-ml portions of Et_2O. The combined extracts were dried over sodium sulfate and treated with excess ethereal HCl. The resulting yellow precipitate was removed by filtration, washed with ether and dried to give 1.2g of yellow powder. Recrystallization twice from MeOH-Et_2O gave 835mg (37%) of pale yellow crystals mp 232-234°. Anal. Calcd. for $C_{11}H_{16}INO_3 \cdot$ HCl: C, 35.6; H, 4.59; N, 3.75. Found: C, 35.49; H, 4.55; N, 3.73 M^+/e = 337. The compound was 98% pure by HPLC analyses. A 100mg sample was purified by preparative HPLC on an Altex LiChrosorb Si 100 column, 10μ, 10mm I.D. The mobile phase was acetonitrile/methanol/water/ammonium hydroxide (165:30:4:1) at a flow rate of 4 ml/min. Final purity was greater than 99%.

^{3}H Methoxamine (3). A sample of 15mg of 10% Pd/C was suspended in 1.0 ml of DMF and 20 Ci of tritium gas was added. The mixture was stirred for 30 min. at room temperature. This step was repeated with 20 Ci of fresh tritium gas. A solution of 37.4mg (0.1 mmol) of iodomethoxamine in 2ml of DMF containing 20mg of Et_3N was added. After 75 min. 2.1ml of tritium had been taken up. Stirring was continued for 30 minutes and labile tritium was removed *in vacuo* using methanol as solvent. The catalyst was removed by filtration and the product taken to dryness and again taken up in 10ml of methanol. The methanol was again evaporated to dryness and the residue dissolved in 2ml of mobile phase and purified by HPLC under the same conditions used to purify 4-iodomethoxamine. The solution of crude ^{3}H methoxamine was put on the column (1ml load) and the major peak collected. The retention time for methoxamine was approximately 14 minutes. The combined product fractions were evaporated under vacuum to dryness. The dry residue (free of any ammonia) was treated

with 1ml of 2N isopropanolic hydrogen chloride and again concentrated to dryness under vacuum. The residue (hydrochloride salt) was made up to a volume of 15ml with sterile water. The radiochromatographic purity of the ^{3}H methoxamine hydrochloride solution was 94% by TLC and 91% by HPLC-LSC. The specific activity of the solution was 36.8mCi/ml for a total activity of 552mCi. The solution had a concentration of methoxamine of 0.32mg/ml and from this, the molar specific activity was calculated to be 28.5 Ci/mmol. This material gradually decomposed upon storage as shown in Table 1. It was repurified by preparative HPLC on an Altex LiChrosorb RP-18, 10μ column, 10mm I.D. with a mobile phase consisting of 0.1M NaH_2PO_4 (pH 4.5)/methanol/acetic acid (70:30:10) at a flow rate of 4ml/min. The product peak was collected in approximately 5ml of mobile phase and made up to 50ml with 70% ethanol containing 1% ascorbic acid. The radiochromatographic purity of the material was 98.4-99.3% and had a specific activity of 0.2mCi/ml. The overall radiochemical yield was 38% and the chemical concentration was 0.0069mg/ml by HPLC analysis. The molar specific activity was calculated to be 7Ci/mmol.

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