MODIFIED AND IMPROVED METHOD FOR SYNTHESIS OF p-HALOGENATED-N-ISOPROPYLAMPHETAMINE LEADING TO SYNTHESIS OF "NO CARRIER ADDED" I-131 IODOAMPHETAMINE

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SUMMARY

I-123 labeled N-isopropyl-p-iodoamphetamine (IMP) has been shown to be an important and useful radiopharmaceutical for measurement of cerebral blood flow (1-4). Since substituted amphetamines have a strong pharmacological activity, the synthesis of "No Carrier" Added" (NCA) labeled IMP would be desirable (5). In this paper we would like to report a modified and improved method for the synthesis of para-halogenated-N-isopropylamphetamine and the preparation of NCA I-131 IMP. This procedure involves a labeling reaction using I-131 sodium iodide and para-bromo-N-isopropyl amphetamine (BrMP) followed by HPLC separation of I-131 IMP and BrMP using reverse phase chromatography.

KEY WORDS: Brain Imaging, Labeled Amphetamine, I-131 Iodoamphetamine, Bromoamphetamine

INTRODUCTION

Regional parameters of brain physiology have been estimated by using a number of positron labeled tracers such as N-13 ammonia (6) and (F-18)2-Fluoro-2-Deoxy-D-Glucose (7)....etc, in conjunction with transaxial emission computed tomography. The exciting results obtained with this technique have stimulated efforts to develop single-photon radiopharmaceuticals such as I-123 IMP (1), I-123 HIPDM (8),....etc. These are considerably less expensive and therefore could have widespread clinical utility limited only by the availability of the imaging instrumentation. Regional Brain perfusion studies in humans using I-123 IMP is under extensive investigation in a growing number of centers. Synthesis of IMP and subsequent radiolabeling has been reported (9). However, the

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published method sufters from low synthetic yield (overall yield of 5.5% from phenylacetic acid) of the unlabeled IMP and low specific activity of the labeled product (2-4mCi/mg). The latter point is very important since amphetamine derivatives have strong pharmacological activities. We have modified the synthetic method to improve the overall yield of the nonradiolabelled product to 76% (from commercially available bromophenylacetic acid). We have also used BrMP as the precursor for radiolabeling. Upon separation of BrMP from radioiodinated IMP, NCA radioiodine labeled IMP is isolated (Scheme I). Although we have used only I-131 as labeling nuclide in this work, this procedure can be easily modified for I-123.

METHODS AND RESULTS

^{1}H n.m.r. spectra were recorded on a Varian EM360A. Mass spectra were obtained from the Marine Biomedical Institute at UTMB. Elemental analysis were obtained from Galbraith Laboratory, Knoxville, TN. All chemicals were purchased from Aldrich Chemical Company and used without further purification, except sodium acetate which was dried in an oven at 200°C and atmospheric pressure for 24 hours.

p-IODOPHENYLACETIC ACID

P-Iodophenylacetic acid was prepared by the method described previously (10). Sodium nitrate (19.0g, 0.28 mol) was added portionwise to a solution of phenylacetic acid (34.0g, 0.25 mol) in glacial acetic acid 200 ml and concentrated sulfuric acid 20 ml and iodine (31.7g, 0.125 mol) with stirring. The vigorous stirring continued until the iodine color disappeared. When the reaction was complete, the hot mixture was poured onto crushed ice and the product was filtered and recrystalized from petroleum ether (b.p.100-120): mp = 133°C [lit.(10) mp = 135°C] (yield 55%) ^{1}H n.m.r. ($CDCl_3$) δ 3.73(2H, s), 7.13(2H,d, J=8Hz), 7.8(2H,d, J=8Hz).

p-IODOPHENYLPROPANONE

p-Iodophenylacetic acid (23g,0.09 mol) was refluxed in acetic anhydride 75 ml (0.8 mol) and dried sodium acetate (7.4g, 0.09 mol) for 48 hours. The resulting mixture was allowed to cool to room temperature before 200ml of water was added. The mixture was neutralized with concentrated sodium hydroxide (30% w/w) and extracted with chloroform 3x100 ml. The combined organic phase was dried with magnesium sulfate and evaporated to dryness which resulted in a reddish colored oil. This oil was refluxed in concentrated hydrochloric acid (80 ml) for one hour. After neutralization with a solution of sodium hydroxide (30% W/W) the mixture was extracted with 3x100 ml ether. After treatment with activated carbon the combined organic phase was dried with magnesium sulfate and evaporated to give the desired product $bp_{0.6}$ = 105-107°C [Lit. (9) $bp_{0.1}$ = 100-101°C] (20g, 85%). ^1H n.m.r. ($CDCl_3$) δ 2.13 (3H, s), 3.65 (2H, s), 7.0 (2H, d, J-8Hz), 7.7 (2H, d, J=8Hz).

p-BROMOPHENYLPROPANONE

The same reaction as above was employed using p-bromophenylacetic acid to give p-bromophenylpropanone $bp_{0.6}$ = 93-95°C,(16.5g, 86%) ^1H n.m.r. ($CDCl_3$) δ 2.13(3H,s), 3.66 (2H, s), 7.08 (2H, d, J=8Hz), 7.5 (2H, d, J= 8Hz).

N-ISOPROPYLIODOAMPHETAMINE

p-Iodophenylpropanone (16g, 0.06 mol) was added, and stirred in isopropylamine (100 ml) at room temperature for 24 hours. The solvent was evaporated and the residue was dissolved in a solution of 50% ethanol and water (200 ml). This solution was then reduced by the addition of sodium borohydride (2.2g, 0.06 mol). The final mixture was stirred for an hour and the solvent was evaporated. The residue was dissolved in 1 N hydrochloric acid solution (100 ml) and extracted with ether 3x100 ml. The aqueous phase was brought to pH=11 by addition of concentrated sodium hydroxide (30%

W/W). The alkaline solution was extracted with ether 3x100 ml, and the combined organic phase was washed with water and dried with magnesium sulfate prior to treatment with activated carbon. The ether solution was filtered and evaporated to give the desired product which was purified by distillation: $bp_{0.6}$ = 98-99°C [Lit. (9) $bp_{0.05}$ = 91-92°C], (16g,88%), analyzed for C, H. ^1H n.m.r. ($CDCL_3$) δ 1.0 (9H,m), 2.46 - 3.05 (4H,m), 7.0 (2H,d, J=8Hz), 7.8 (2H,d, J=8Hz). MS: m/e 304 (M + 1), 217 (M-86).

N-ISOPROPYLBROMOAMPHETAMINE

The same reaction as above was employed using p-Bromophenyl-propanone to yield BrMP. $bp_{0.6}$=88-89°C (90% yield) analyzed for C, H. ^1H n.m.r. ($CDCL_3$ δ 1.0 (9H,m), 2.55 - 3.2 (4H,m), 7.1 (2H,d, J=8Hz), 7.6 (2H,d, J=8Hz). MS: m/e, 256-258 (M+1), 169-171 (M-86)

Liquid Chromatographic Separation of IAMP and BrAMP

Samples of IMP and BrMP dissolved in 60% ethanol: phosphate buffer 0.05M pH = 7.0 (mobile phase) and applied to an HPLC

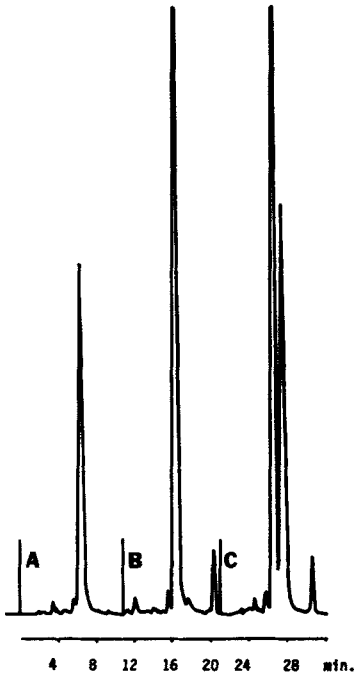


Figure 1: HPLC results on known samples of A) IMP, B) BrMP, and C) mixture of BrMP and IMP

equipped with Nova pack C-18 column (Waters Associates, Milford, MA). The results are shown in Figure 1 which shows a clear separation of IMP (retention time = 6.5 min) and BrMP (retention time = 5.8 min). In this experiment we used a flow rate of 0.3 ml/min and a chart rate of 2.5mm/min.

Synthesis of I-131 Labeled IMP

a) A solution of IMP (1.0mg) in concentrated acetic acid (25 µl),4N solution of copper (II) nitrate (10µl), and 0.5ml solution of I-131 sodium iodide was placed in a sealed tube and heated at 120°C - 150°C for 2 hours. After cooling to room temperature the solution was made alkaline by addition of 1N sodium hydroxide and then extracted with ether (2.0ml). The organic phase was separated and washed with water 2xlml and the ether evaporated to give the radio-labeled product (20-35% yield). HPLC analysis of the product showed that more than 97% of radioactivity was associated with IMP, about 3% of radioactivity eluted with the solvent front which was assigned to be free iodine (retention time = 1.6 min). b) The same reaction carried out on BrMP yielded the same result. When the product was applied to the HPLC column using 55% ethanol: phosphate buffer 0.05M pH = 0.7 as eluate, the retention times of IMP and BrMP increased to 8.2 and 6.4 minutes respectively; I-131 IMP and non-radioactive BrMP were separated (Figure 2). Reinjection of the purified product showed very little BrMP impurity. We have estimated the specific activity of I-131 IMP to be 750 mCi/mg. The mass of I-131 IMP was estimated with a calibrated HPLC equipped with a U.V. detector (Model 441, Waters Associates, Milford, MA) operating at 214 nm. This labeled product was contaminated with almost the same mass amount of BrMP which reduced the effective specific activity to 375mCi/mg as shown in Figure 2. When both of the above reactions were repeated using 10mg of either IMP of BrMP, the radiolabeling yield increased to more than 50%, with specific activity of 2-3 mCi/mg.

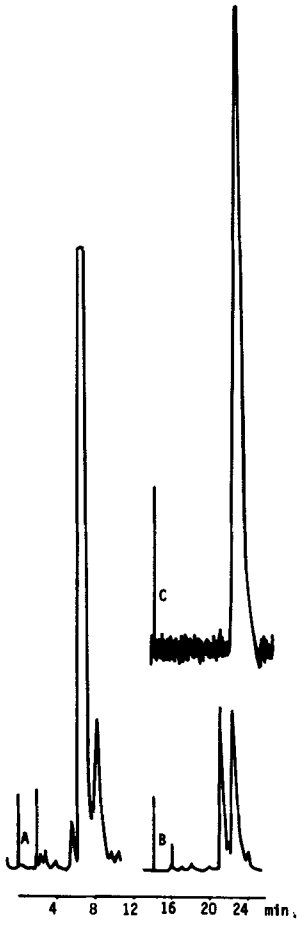


Figure 2: HPLC results on A. Crude I-131 IMP and BrMP. B. Reinjection of purified sample of I-131 IMP which is contaminated with BrMP. C. Radioactivity trace of the purified sample of I-131 IMP.

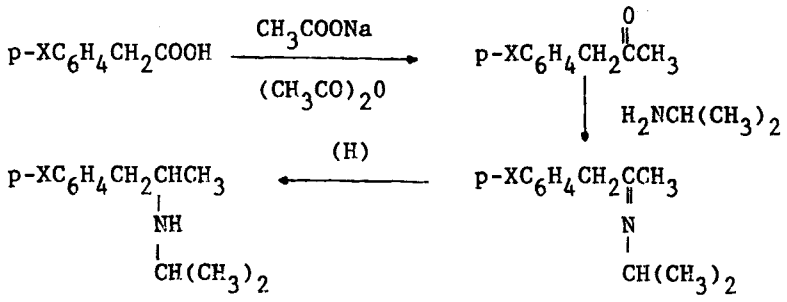
Animal Biodistribution

Five female Sprague-Dawley rats weighing 200-300g were anesthetized with sodium pentobarbital and were injected in a tail vein with 0.10-0.15mCi of the high specific activity I-131 labeled IMP in a volume of 0.2-0.5ml. The rats were sacrificed by cordiectomy one hour post injection. Each organ to be assayed was weighed wet and counted in standardized geometry with a sodium iodide scintillation counter. The results of this study agreed very closely with that of previously published data (3).

DISCUSSION

Our modified method for synthesis of p-halogenated-N-isopropyl amphetamine is summarized in scheme I. This shows 1) acetylation/decarboxylation of p-bromophenylacetic acid. This reaction has been reported to yield 43% p-bromophenylpropanone, but

SCHEME I



X = Br, I

by using thoroughly dried sodium acetate this reaction yielded over 85% product; 2) condensation of p-bromophenylpropanone with isopropylamine, followed by reduction of the Schiff base by sodium borohydride, gave racemic N-isopropyl-p-bromoamphetamine in almost quantitative yield. Both non-radioactive BrMP and IMP were prepared by this method in good yields and were identified by different spectroscopic techniques. Figure 1 shows the HPLC chromatograms on these products. These chromatograms were produced by using a reverse phase column and a solution of 60% ethanol: 0.05 M phosphate buffer pH = 7.0 as mobile phase. A flow rate of 0.3 ml/min and chart rate of 2.5 mm/min were used. These chromatograms show that BrMP and IMP can be separated. However, when the same experiment was repeated using 55% ethanol: 0.05 M phosphate buffer pH = 7.0, as mobile phase, a slightly better separation and slightly longer retention time was observed. Therefore, this solvent was chosen for the separation of BrMP and I-131 IMP.

Nevertheless, as a result of tailing we found some BrMP contamination in the final product. Of course this product could be purified further by re-chromatography, however this would involve one more evaporation step of the eluting solvant which would decrease the radiolabeling yield considerably. We have tried other solvent systems and larger columns, however we could not improve the result. We have estimated the specific activity of I-131 IMP to be 750 mCi/mg. This means that a patient dose of about 5 mCi would contain about 6.7 μg of IMP and about 6.7 μg of BrMP, which is still an improvement over existing method which produces 5 mCi of I-131 IMP contaminated with few mg of non-radioactive IMP. We have performed many experiments with different amounts of I-131 sodium iodide activity from 4 to 80 mCi. Since the radiolabeling yields were producible, we have only quoted the percentage of the radiolabeled product in the experimental section. It was obvious that by using more IMP and BrMP the radiolabeling yield would be increased. While we were able to demonstrate this, we were unable to separate I-131 IMP from large amounts of BrMP even with the use of larger columns. It was surprising to find that the specific activity of I-131 IMP was as low as 750 mCi/mg since the theoretical value for carrier free I-131 IMP is 54 Ci/mg. When we communicated with the manufacturer (Mallinckrodt), we were advised that their I-131 sodium iodide is almost carrier free and they added, "The specific activity is 1.6×10^7 mCi/mmol." However, the bottles in which the product is delivered are prewashed with a concentrated solution of non-radioactive sodium ilodide to prevent surface asorption of I-131 sodium iodide.

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