SYNTHESIS AND *N*-METHYLATION OF β-^{14}C-*p*-CHLOROAMPHETAMINE HYDROCHLORIDE

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SUMMARY

β-^{14}C-p-Chloroamphetamine hydrochloride was prepared by condensation of carbonyl-^{14}C-p-chlorobenzaldehyde with nitroethane followed by lithium aluminum hydride (LAH) reduction of the resulting chlorophenylnitropropene. After purification the overall yield was about 25%. N-Methyl- and N,N-dimethyl-β-^{14}C-p-chloroamphetamine were prepared, respectively, by LAH reduction of N-carbobenzoxy-β-^{14}C-p-chloroamphetamine and by reductive (Eschweiler-Clarke) methylation of β-^{14}C-p-chloroamphetamine.

INTRODUCTION

The long-lasting effects of administration of single doses of *p*-chloroamphetamine (1a) in depression of brain serotonin (5-HT) levels and tryptophane hydroxylase activity in rats have been extensively documented[1-5] but are not well understood. These effects persist for up to four months while the level of unchanged drug decreases exponentially to <1% after four days.[6] Further, recent

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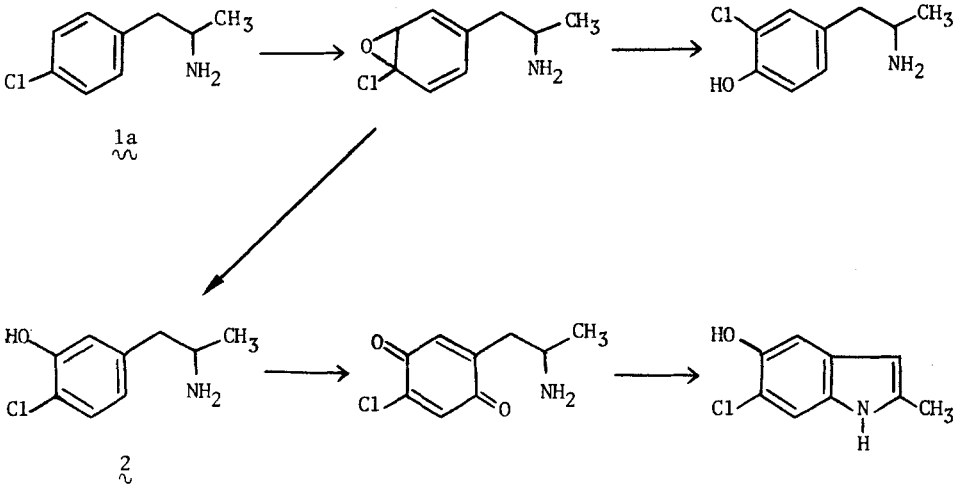
evidence has been presented for a selective cytopathological effect on serotonergic cell bodies produced by administration of 1a.[7] It is generally believed that a neurotoxic metabolite of 1a is responsible for these effects, and it has been suggested that serotonergic cells may have a unique ability to convert 1a to such a metabolite.[7] The identity of the toxic metabolite has provoked some speculation and, in view of the known neurotoxicity of 5,6-dihydroxytryptamine,[8] a 5,6-dihydroxyindole was proposed.[4] Oxidative cyclization of catecholamines to indoles has been observed chemically[9] and enzymatically,[10] and while we agree this is quite possible, the pathway outlined in Scheme I is more credible

Scheme I



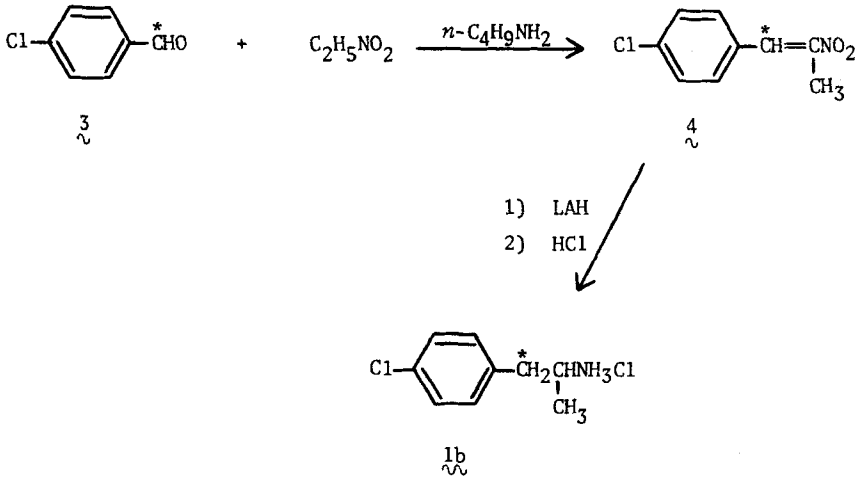
than that proposed.[4] Of the two possible chlorophenols arising from 1a via the arene oxide,[11] only 4-chloro-3-hydroxyamphetamine (2) is capable of cyclization to an indole. However, the observation that 2 is significantly less effective than 1a in lowering brain 5-HT levels[12] does not appear consistent with the indole hypothesis. Thus the need for studies of the metabolism of 1a in brain and for the positive identification of metabolites and the implication of one or more of these as serotenergic neurotoxins is clearly apparent. To this end we sought a convenient synthesis of ring-^{14}C or β-^{14}C labeled 1a of a minimum

specific activity of 5 mCi/mmol from a commercially available precursor.

RESULTS AND DISCUSSION

Preliminary experiments with unlabeled materials showed that synthetic routes utilizing ring-^{14}C-*p*-chlorobenzoic acid would not be feasible. However, a two-step procedure described earlier for the preparation of certain other substituted amphetamines[13] afforded 25-35% overall yields of *p*-chloroamphetamine hydrochloride (1b) from *p*-chlorobenzaldehyde (3) (Scheme II). In larger scale

Scheme II



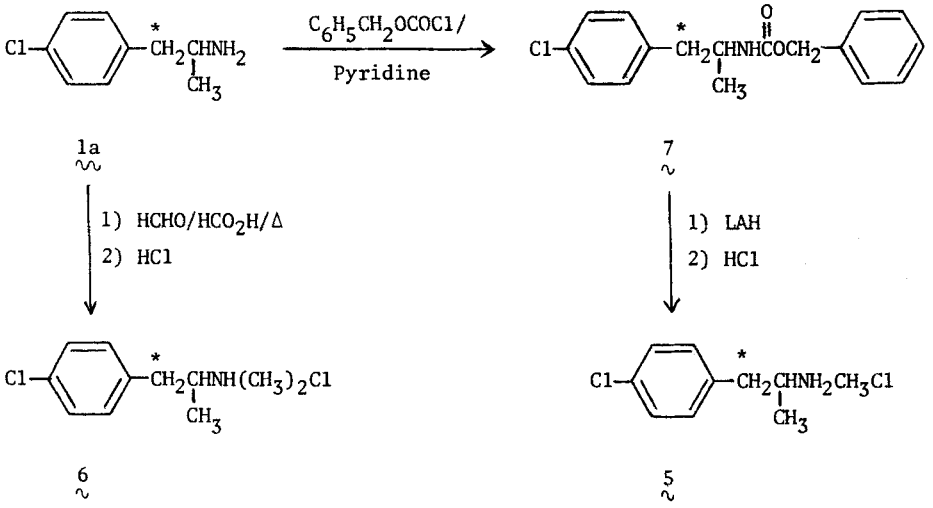
3 4 1b

runs with unlabeled material both the intermediate nitropropene 4 and the final product 1b were purified by recrystallization. For synthesis of β-^{14}C-1b from carbonyl-^{14}C-3, the small quantity of material of high specific activity necessitated LAH reduction without purification of the nitropropene and purification of the resulting hydrochloride by successive extractions and countercurrent distribution. The identity and chemical purity of the β-^{14}C-1b were confirmed by gas chromatography of the *N*-trichloroacetyl derivative,[6] and radiochemical purity was demonstrated by co-crystallization with authentic material.

For use in *in vivo* demethylation studies, *N*-methyl- and *N*,*N*-dimethyl-β-^{14}C-*p*-chloroamphetamine hydrochlorides (5 and 6) were also prepared, by LAH

reduction of *N*-carbobenzoxy-β-^{14}C-*p*-chloroamphetamine (7) and by reductive (Eschweiler-Clarke) methylation of β-^{14}C-*p*-chloroamphetamine (1a), respectively (Scheme III). The specific activity of these samples was low enough to permit purification by recrystallization.

Scheme III



EXPERIMENTAL

A Beckman LS-100 scintillation counter was used for radioactivity determinations. Amine hydrochlorides were weighed into vials, dissolved in water (1 ml) and mixed with toluene-based scintillation fluid (4 g of PPO and 50 mg of POPOP/1.)(10 ml) and Beckman Biosolve 3 (3 ml).

β-^{14}C-*p*-Chloroamphetamine Hydrochloride (1b). A. A mixture of 3 (85 mg, 0.605 mmol, 8.27 mCi/mmol*), nitroethane (73 mg, 0.97 mmol), *n*-butylamine

*This was the specific activity stated by the supplier (New England Nuclear, Boston, Mass. 02218). We had inexplicable difficulty counting the material. Aliquots gave extremely small and unreproducible values. Specific activities of 1b and 3b, which gave normal reproducible counts, indicate that the specific activity of 2b when used by us was 6-7 mCi/mmol.

(7μl) and absolute ethanol (30 drops) was heated under reflux for 15 hr. After cooling the mixture was evaporated to complete dryness, and the residue was added in portions to a stirred ice-cooled slurry of LAH (0.7 g) in ether. Small portions of ether were then used to transfer the remaining crude 4 to the LAH reduction mixture. The mixture was stirred 2 hr at room temperature and then cooled with ice while water was added slowly. The suspension was filtered, the filter cake was washed repeatedly with ether and the filtrate was dried over potassium hydroxide. Crude hydrochloride 1b, obtained by treatment of the ether solution with hydrogen chloride, was a purple semi-solid (77 mg, 2.9 mCi). It was dissolved in 0.5 *N* hydrochloric acid, and the solution was extracted with *n*-butyl chloride leaving 2.1 mCi of basic material. A small amount (2%) of a weakly basic impurity was removed by adjustment of pH to 6 and extraction with cyclopentane. Countercurrent distribution (4 transfers) of an aliquot of the remaining basic material between cyclopentane (20 ml) and 0.2 *M* pH 8 borate-phosphate buffer (6.8 ml) showed about 15% of a more polar impurity in the aqueous phase. Therefore 1b was purified by a 6-transfer countercurrent distribution using the above system. The material obtained from the cyclopentane fractions was greater than 98% radiochemically pure as shown by countercurrent distribution of an aliquot (Figure 1), and the radiochemical yield was 0.9 mCi.

A 5.4 μCi aliquot of pure 1b was mixed with 540 mg of unlabeled material and recrystallized three successive times from acetone-methanol. After each recrystallization, the crystals had 20,000 ± 200 dpm/mg. Finally, a portion of pure 1b was converted to its *N*-trichloroacetyl derivative and subjected to gas chromatographic analysis as described earlier.[6] The gas chromatogram showed only one peak with a retention time identical to that of the *N*-trichloroacetyl derivative of an authentic sample of 1a.[6]

B. Crude 4 (118 mg, 3.2 mCi), prepared as described above, was diluted with unlabeled material[15] (1.382 g). It was reduced with LAH as described above yielding 1.038 g (81%) of 1a. This material was used to prepare 5 and 6 as outlined below.

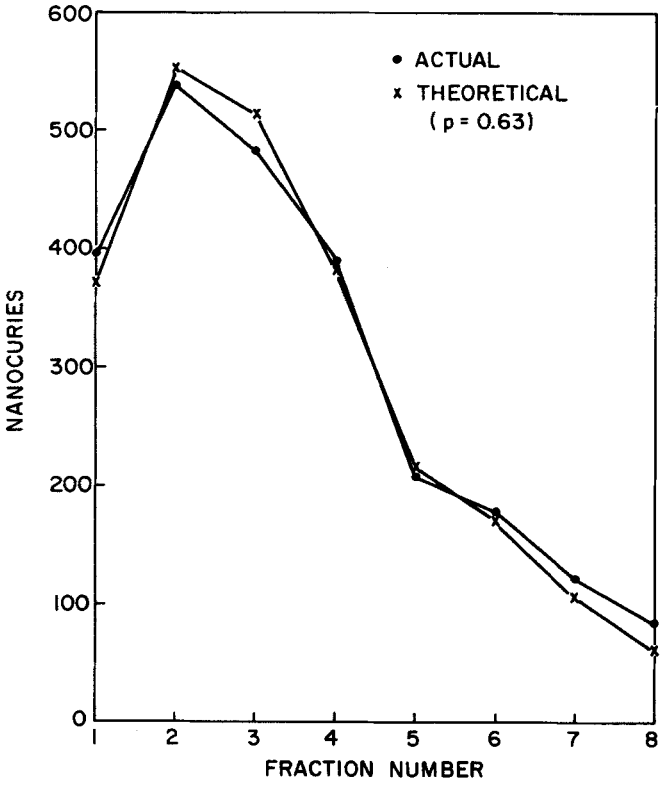


Fig. 1. Countercurrent distribution of 2.6 μCi of 1b in cyclopentane (13.5 ml) and pH 8.5 borate-phosphate buffer (6.5 ml). Fractions 1-4 refer to the organic phases in order; fractions 5-8 refer to the aqueous phases in reverse order. The theoretical distribution was calculated according to Bush and Densen.[14]

N-Methyl-β-^{14}C-*p*-chloroamphetamine Hydrochloride (5). To a stirred, ice-cooled solution of β-^{14}C-*p*-chloroamphetamine (1a) (668 mg, 3.94 mmol) in pyridine (2 ml) was added dropwise during 30 min carbobenzoxy chloride (1.5 g, 8.8 mmol). The ice bath was removed and stirring was continued for 1.5 hr before water was added. The resulting mixture was extracted with ether, and the ether solution was washed with 2 *N* hydrochloric acid until the aqueous phase remained acidic, then with sodium bicarbonate solution and two portions of water. The ether solution was dried ($MgSO_4$), evaporated to 15 ml, and then stirred and cooled while excess LAH was added in small portions. The mixture was stirred 15 hr at room temperature and then cooled while water was added. The suspension was filtered and the filtrate was extracted with 1 *N* hydro-

chloric acid (5 ml). The aqueous layer was evaporated to dryness and the residue was recrystallized from acetone and dried at 60° (0.1 mm) to give 5 (382 mg, 44%). Co-crystallization of this material (5.0 mg) with authentic *N*-methyl-*p*-chloroamphetamine hydrochloride[16] (495 mg) three successive times gave samples of unchanged specific activity, 21,400 ± 300 dpm/mg.

N,*N*-Dimethyl-β-^{14}C-*p*-chloroamphetamine Hydrochloride (6). A mixture of 1a (370 mg, 2.18 mmol), 90% formic acid (0.8 g, 16 mmol) and 36% aqueous formaldehyde (0.7 ml) was heated 14 hr at 90-95°. The mixture was cooled, mixed with 6 *N* hydrochloric acid (1 ml) and evaporated to dryness. The dark brown residue was dissolved in water (5 ml) and extracted twice with methylene chloride. Evaporation of the aqueous solution and recrystallization of the residue from acetone yielded, after drying at 60° (0.1 mm), 241 g (47%) of 6. Co-crystallization of this material (6.0 mg) with authentic *N*,*N*-dimethyl-*p*-chloroamphetamine hydrochloride[16] (494 mg) three successive times gave samples of unchanged specific activity, 26,000 ± 500 dpm/mg.

ACKNOWLEDGMENT

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