EFFICIENT SYNTHESES OF DEUTERIUM LABELED EPININES

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SUMMARY

Syntheses of N-methyl-2-(3,4-dihydroxyphenyl)ethylamine hydrochlorides (epinines) labeled with deuterium in the ring, in the N-methyl group and both in the ring and N-methyl group are described. The N-trideuteromethyl group was generated by the $LiAlD_4$ reduction of the corresponding carbamate while the ring deuterated epinines were produced by the DCl catalyzed exchange in D_2O of the corresponding ring unlabeled epinines.

Key Words: Epinine-N-CD_3, epinine-2,5,6-D_3, epinine-2,5,6-D_3-NCD_3, deuterium labeling, lithium aluminum deuteride.

INTRODUCTION

Epinine, the N-methylated derivative of dopamine, has been shown to occur in a number of mammalian tissues (1-5). Although norepinephrine is universally accepted as the biosynthetic precursor of epinephrine, it has been suggested

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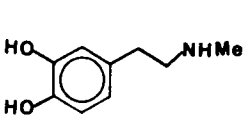
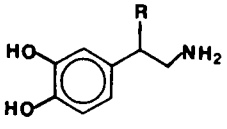
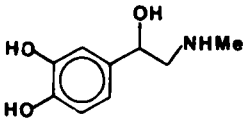
that epinephrine could also be formed by the β-hydroxylation of epinine which in turn is formed by the N-methylation of dopamine (1-4). Later studies, however, suggest that the formation of epinephrine from epinine may at best represent only a minor pathway for epinephrine biosynthesis (5,6). Thus the biochemical significance of occurrence of epinine in mammalian tissues is not clear. In order to study the physiological role played by epinine, we have been interested in establishing the metabolic fate and tissue levels of epinine. One of the analytical techniques we plan to utilize for this purpose is mass fragmentography which has been used for detecting very small (< picomole) amounts of epinine (5) and related catecholamines, e.g., dopamine and norepinephrine (7). For quantitative assay by mass fragmentography the ideal internal standard is the same compound labeled with a stable isotope (7). Thus we needed access to a number of deuterium labeled epinines. In this report we describe the synthesis of epinines labeled with deuterium in the ring, in the N-methyl group and both in the ring and N-methyl group.



epinine



dopamine (R=H)



epinephrine

norepinephrine (R=OH)

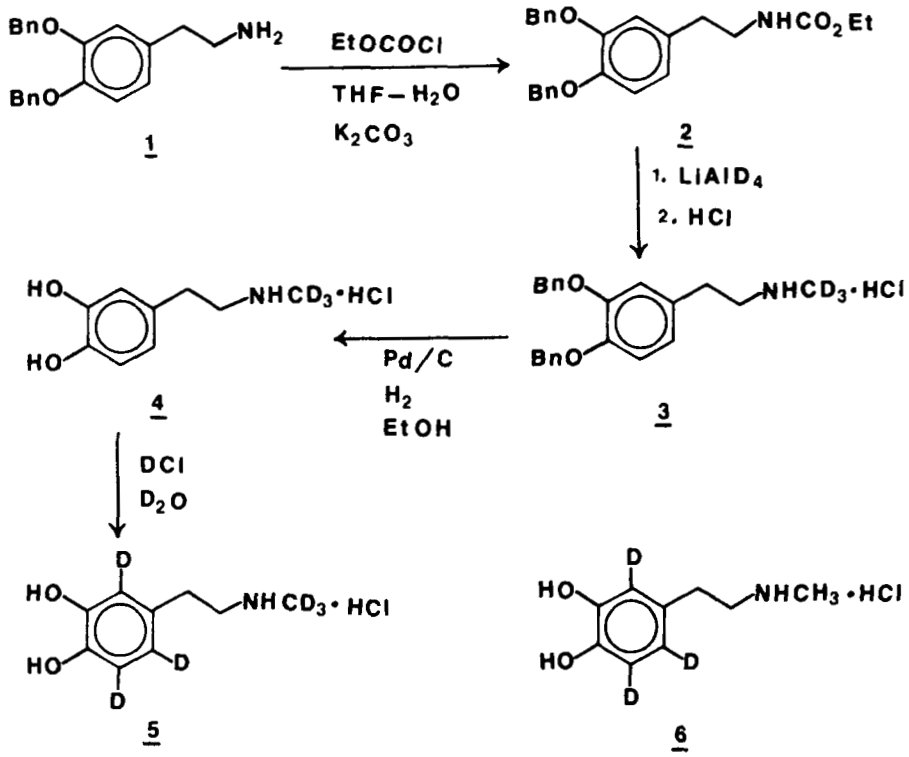
RESULTS AND DISCUSSION

For the synthesis of epinine-N-CD_3 (4), we started with 3,4-bis(benzyloxy)benzaldehyde which was first converted to the

phenylethylamine 1 using literature procedure (8). The amine 1 was then reacted with EtOCOCl in a mixture of water and THF in the presence of K_2CO_3 to give carbamate 2 in 97% yield. Reduction of carbamate 2 with $LiAlD_4$ gave O-benzylated epinine-NCD_3 3 with complete deuterium incorporation as determined by NMR. The protio homolog of 3 was also prepared by reduction of 2 with $LiAlH_4$ under identical conditions for comparison (see Experimental section). Catalytic debenzylation of 3 furnished epinine-NCD_3 (4) in excellent overall yields. No loss of deuterium label occurred during the conversion of 3 to 4 as judged by NMR.

For the ring deuteration of epinine-NCD_3 (4) and epinine, a number of methods were considered, both acid and base catalyzed (9). The most suitable method found was that described by Kirby and Ogunkoya (10) for a number of catechol derivatives using DCl in D_2O. Thus the exchange of the ring hydrogens was carried out by refluxing 4 or epinine in 4N DCl in D_2O, generated *in situ* by reacting $SOCl_2$ with D_2O. It was found that a significantly smaller proportion of the reagent system DCl/D_2O was needed for achieving comparable deuterium incorporation if anhydrous 4 or epinine were used with their labile hydrogens (as in OH and NH) already exchanged with deuterium. The optimum time of reflux for maximum deuterium incorporation was found to be 6h as determined by NMR. Interestingly, the extent of deuterium incorporation was 84% in each case and even the relative amounts of undeuterated, monodeuterated, dideuterated and trideuterated homologs present in the product mixture representing 5 and 6, respectively, were the same as determined

by mass spectrometry. For 5 these relative amounts were 4.6, 4.3, 26.8 and 64.3%, respectively, and the corresponding values for 6 were 4.1, 4.4, 27.3 and 64.2%, respectively.



EXPERIMENTAL

General Methods: ^1H NMR spectra were recorded on Varian T-60 and FT-80A spectrometers, with chemical shifts reported in ppm downfield from Me_4Si. For compounds whose spectra were recorded in D_2O, the chemical shifts were measured with respect to p-dioxane (δ Me_4Si 3.56) as the internal standard. Electron impact mass spectra were recorded on a Varian MAT CH-5 mass spectrometer with RDS Data System for computer analysis of spectra. The $LiAlD_4$ contained 99 atom % D and was

purchased from Merck Sharp & Dohme (Canada Limited) and epinine hydrochloride was from Sigma Chemical Company (St. Louis, MO).

N-Trideuteromethyl-2-[3,4-bis(benzyloxy)phenyl]ethylamine Hydrochloride (3): To a stirred solution of 2-[3,4-bis(benzyloxy)phenyl]ethylamine (1) (2g, 6 mmol), prepared from 3,4-bis(benzyloxy)benzaldehyde following the procedure described by Cava and Buck (8) in 20 mL of THF was added a solution of K_2CO_3 (828 mg, 6 mmol) in 10 mL of H_2O. Ethyl chloroformate (760 mg, 7 mmol) was then added dropwise keeping the temperature at 25° C. TLC (silica gel, CH_2Cl_2) indicated completion of reaction after 1.5 h. The mixture was then concentrated *in vacuo* to 10 mL and then extracted with CH_2Cl_2. The CH_2Cl_2 extract was washed, in order with 2N HCl, $NaHCO_3$ solution, dried (Na_2SO_4) and then evaporated *in vacuo* to dryness to give 2.36 g (97%) of the carbamate 2 as a gum, homogeneous by TLC (silica gel, CH_2Cl_2): ^1H NMR ($CDCl_3$) δ 1.17 (t, J=7Hz, 3, CH_3), 2.61 (t, J=7Hz, 2, $C\underline{H}_2CH_2N$), 3.30 (q, 2, $CH_2C\underline{H}_2N$), 4.07 (1, J=7Hz, 2, $C\underline{H}_2CH_3$), 5.04 (s, 4, $OC\underline{H}_2Ph$), 6.52-6.87 (m, 3, Ar), 7.13-7.41 (m, 10, Ph). To a stirred suspension of $LiAlD_4$ (504 mg, 12 mmol) in 50 mL of anhydrous THF under an N_2 atmosphere was added a solution of carbamate 2 (1.62 g, 4 mmol) in 10 mL of THF and the mixture was refluxed for 6 h. After cooling to 0-5° C, the mixture was diluted with 100 mL of Et_2O and the excess $LiAlD_4$ was decomposed by carefully adding water with cooling. More water was added gradually until the inorganics precipitated as a gel. The organic layer was collected by decantation and the gel was washed several times with Et_2O. The combined organic layers

were washed with brine, dried (K_2CO_3) and then evaporated *in vacuo* to dryness to give the free base of 3 as a syrup (1.3 g, 96%). ^1H NMR indicated complete deuterium incorporation: ($CDCl_3$) δ1.41 (br, s, 1, NH), 2.69 (br, s, 4, CH_2CH_2), 5.06 (s, 2, OCH_2), 5.10 (s, 2, OCH_2), 6.58-6.90 (m, 3, Ar), 7.15-7.43 (m, 10, Ph). Addition of Et_2O saturated with anhydrous HCl to a solution of the free base in Et_2O gave 3 as a white solid which was recrystallized from EtOH-Et_2O: mp 180-181° C.

N-Methyl-2-[3,4-bis(benzyloxy)phenyl]ethylamine Hydrochloride, the protio homolog of 3, was prepared in comparable yields by reducing carbamate 2 with $LiAlH_4$ under identical conditions: ^1H NMR of free base, ($CDCl_3$) δ 1.42 (br, s, 1 NH), 2.33 (s, 3, NMe), 2.68 (br, s, 4, CH_2CH_2), 5.05 (s, 2, OCH_2), 5.09 (s, 2, OCH_2), 6.57-6.90 (m, 3, Ar), 7.15-7.42 (m, 10, Ph); hydrochloride salt: mp 180-181° C. Anal. Calcd. for $C_{23}H_{25}NO_2$ HCl: C, 71.96; H, 6.83; N, 3.65. Found: C, 71.86; H, 6.90; N, 3.49.

N-Trideuteromethyl-2-(3,4-dihydroxyphenyl)ethylamine Hydrochloride (4). A mixture of 3 (774 mg, 2 mmol) and 5% Pd/C (500 mg) in 120 mL of 95% EtOH was shaken in a Parr Apparatus under 40 psi of H_2 for 6 h at 25° C. The mixture was then filtered and the filtrate was evaporated *in vacuo* to dryness. The resulting crude product on recrystallization from EtOH-Et_2O gave 398 mg (95%) of 4 as a colorless solid: mp 176-177° C (epinine HCl, from Sigma, mp 175-177° C); ^1H NMR (D_2O) δ 2.60-3.18 (m, 4, CH_2CH_2), 4.58 (HDO), 6.47-6.78 (m, 3, Ar).

N-Trideuteromethyl-2-(3,4-dihydroxy-2,5,6-trideutero-phenyl)ethylamine Hydrochloride (5). The reagent 4N DCl in D_2O was prepared by reacting 0.6 mL $SOCl_2$ with 3.5 mL of D_2O at 25° C under an N_2 atmosphere. The solution was then freed of SO_2 by bubbling N_2 for 15 min (10). The labile hydrogens (as in OH and NH) of 4 were first exchanged with deuterium by evaporating a solution of 4 (207 mg, 1 mmol) in 4 mL of D_2O to dryness to give anhydrous powder. This powder was then added to the 4N DCl in D_2O and then refluxed for 6 h under an N_2 atmosphere. The mixture was cooled to 25° C, diluted with EtOH and then evaporated in vacuo to dryness. The crude residue was recrystallized from EtOH-Et_2O to give 191 mg (93%) of 5 as a colorless solid: mp 174-176° C; ^1H NMR (D_2O) δ 2.57-3.17 (m, CH_2CH_2), 4.58 (HDO). m/e (relative intensity %) 123 (5), 124 (8.7), 125 (32.3), 126 (85.3), 127 (56.8). Epinine HCl: m/e (relative intensity %): 122 (2.9), 123 (74.0), 124 (71.5), 125 (1.3).

N-Methyl-2-(3,4-dihydroxy-2,5,6-trideuterophenyl)ethyl-amine Hydrochloride (6), was prepared from epinine hydrochloride as above: mp 174-176° C; ^1H NMR (D_2O) δ 2.55 (s, 3, NMe) 2.58-3.17 (m, 4, CH_2CH_2), 4.57 (HDO); m/e (relative intensity %): 123 (5.1), 124 (10.1), 125 (39.1), 126 (100), 127 (73.5).

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