GENERAL METHODS FOR THE PREPARATION OF DEUTERIUM AND TRITIUM-LABELLED PHENETHYLAMINES AND PHENETHANOLAMINES: SYNTHESIS OF RADIOACTIVE 6-HYDROXYDOPAMINE

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SUMMARY

A convenient synthetic method for preparation of phenethylamines and phenethanolamines labeled with deuterium or tritium has been developed. A substituted benzaldedyde is condensed with nitromethane or nitroethane and the resultant nitrostyrene is reduced with sodium borotritide or borodeuteride. Subsequent reduction of the nitro-group and removal of blocking groups yields a phenethylamine or an α-methylphenethylamine containing a tritium or deuterium in the benzylic position. Condensation of the substituted benzaldehyde with nitromethane or nitroethane at low temperature yields a nitroalcohol which is oxidized to a ketone with Jones reagent. Reduction with sodium borotritide or borodeuteride and subsequent reduction of the nitro-group and removal of blocking groups yields a phenethanolamine or α-methylphenethanolamine labeled in the benzylic position. Preparation of deutero- and/or tritio-labeled 2,4,5-trihydroxyphenethylamine (6-hydroxydopamine), α-methyl-6-hydroxydopamine, α-methyl-p-tyramine, β-phenethanolamine, 2-hydroxyphenethanolamine and α-methyl-4-benzyloxyphenethanolamine is described.

Key Words: 6-Hydroxydopamine, Catecholamines, Tyramine, Phenethanoamine, Octopamine, Tritium-labeling.

A variety of phenethylamines and phenethanolamines are important pharmacological tools for the study of noradrenergic and dopaminergic function. Certain amines, for example, α-methyltyramine, displace norepinephrine from

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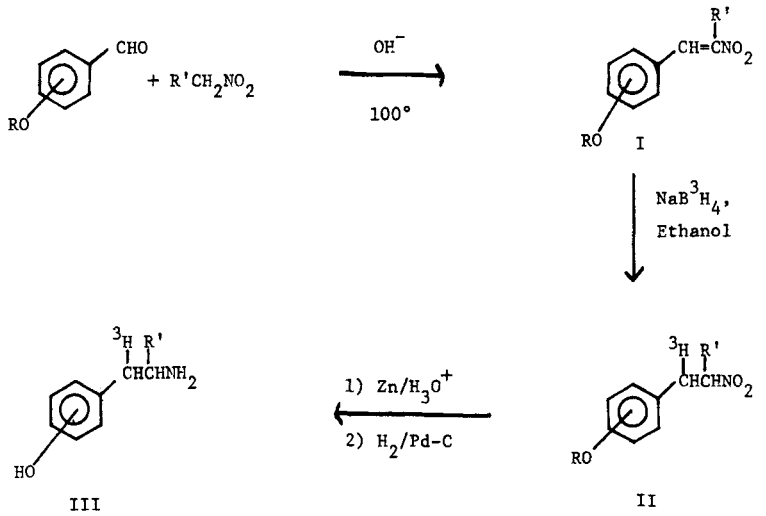
storage sites and may also be converted by the action of dopamine-β-hydroxylase to β-phenethanolamines. These amines may then serve as "false transmitters" being released by physiological stimuli to interact with adrenergic receptors (1) Other amines such as 6-hydroxydopamine bind irreversibly to tissue constituents and cause destruction of monoaminergic terminals in the nervous system (2). The present paper describes simple and convenient procedures for the preparation of tritium and deuterium-labelled phenethylamines and phenthanolamines from readily available substituted benzaldehydes. The uptake, storage, metabolism and possible binding of such amines to intracellular macromolecules may then be easily studied both in vivo and with isolated biological preparations.

The general methods for the synthesis of deuterium or tritium-labelled phenethylamines and phenethanolamines are illustrated in Scheme Iab with NaB^3H_4 as reducing agent. The required intermediate nitrostyrenes (I) and phenylnitroethanols (IV) are readily available by condensation of substituted benzaldehydes with nitromethane (R' = H) or nitroethane (R' = CH_3). Many examples of such condensations are described in the literature (3-12). The nitroethanol (IV) can be oxidized in good yield to a nitroacetophenone (V) with Jones reagent; i.e., CrO_3 in aqueous sulfuric acid. Reduction of either I or V with deutero- or tritio-$NaBH_4$ then provides in near quantitative yield a stable, easily isolated, organic soluble product, II or VI, in which one heavy hydrogen atom has been incorporated. This product (II or VI) may now be converted to the desired amine by reduction of the nitro-group with either zinc-acetic acid, $LiAlH_4$ or for best results with Red-Al (Aldrich Chem. Co.), followed by removal of benzyl groups by reduction with hydrogen using palladium on charcoal as catalyst. The methods are simple and widely applicable and utilize a relatively inexpensive and stable source of isotopic hydrogen, NaB^2H_4 or NaB^3H_4. The methods have been applied to the synthesis of isotopically labeled 6-hydroxydopamine, α-methyl-6-hydroxydopamine, α-methylyl-p-tyramine, phenethanolamine 2-hydroxyphenethanolamine (o-octopamine) and α-methyl-4-benzyloxyphenethanolamine (precursor for α-methyl-p-octopamine).

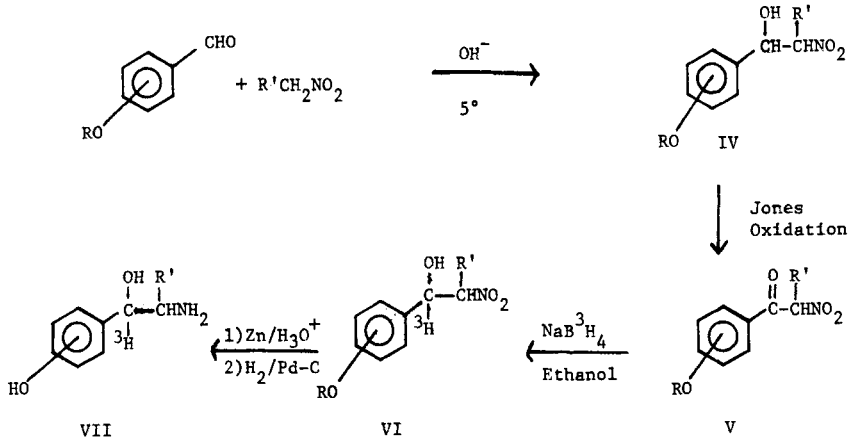
6-Hydroxydopamine. Condensation of 2,4,5-tribenzyloxybenzaldehyde with nitromethane and elimination of water yielded 2,4,5-tribenzyloxy-β-nitrostyrene. Reduction with NaB^2H_4 in ethanol led in excellent yield to the phenylnitroethane

Scheme I: R = Benzyl, R' = H or CH_3.

a) Phenethylamines



b) Phenethanolamines



with nearly quantitative incorporation of one deuterium as determined by electron impact or chemical ionization mass spectra. Nuclear magnetic resonance (n.m.r.) spectral analysis indicated a selective incorporation of the isotope on the benzylic carbon. The other incorporated hydrogen atom must be derived from the solvent, ethanol. Incorporation of two isotopic hydrogens would, thus, occur if $CH_3CH_2O^2H$ or CH_3O^2H were used as solvent. The phenylnitroethanol was reduced with Red-Al to yield 2,4,5-tribenzyloxyphenethylamine without loss of deuterium as shown by mass spectral analysis. Catalytic removal of the benzyl groups afforded monodeutero-6-hydroxydopamine identical in chromatographic properties to commercial sample of 6-hydroxydopamine (Aldrich Chemical Co.). The same procedure, but with NaB^3H_4 afforded radioactive 6-hydroxydopamine in good yield.

α-Methyl-6-hydroxydopamine. A similar reaction sequence using nitroethane in the initial condensation afforded monodeutero-α-methyl-6-hydroxydopamine in good yield and with quantitative incorporation of the isotopic hydrogen on the benzylic carbon. With NaB^3H_4 radioactive α-methyl-6-hydroxydopamine was obtained in good yield.

α-Methyltyramine. 4-Benzyloxy-α-methylnitrostyrene was prepared by condensation of 4-benzyloxybenzaldehyde and nitroethane and was converted with NaB^3H_4 to a monotritio-product which on reduction of the nitro group and the removal of the benzyl group afforded monotritio-α-methyl-p-tyramine in good yield. In a similar sequence with NaB^2H_4 incorporation of one deuterium into the benzylic position was nearly quantitative as judged from mass and n.m.r. spectral analysis.

Phenethanolamine. The key steps in the present method for synthesis of labelled phenethanolamines are oxidation and then reduction at the β-carbon of the side-chain. The intermediate, β-phenyl-β-hydroxynitroethane, was prepared by condensation of benzaldehyde and nitromethane at low temperatures. Oxidation with Jones reagent proceeded smoothly to yield a keto-intermediate which was reduced back to the alcohol with NaB^2H_4. The alcohol contained one atom of deuterium as shown by mass spectrometry. Reduction to the desired phenethanolamine was with $LiAlH_4$ or zinc dust in acetic acid.

2-Hydroxyphenethanolamine. Preparation of this compound followed the procedure described above for phenethanolamine. Incorporation of one deuterium was

nearly quantitative. The benzyl group was removed by catalytic hydrogenation to afford monodeutero-2-hydroxyphenethanolamine (o-octopamine) identical in properties to a reference sample.

α-Methyl-4-benzyloxyphenethanolamine. Preparation of this compound followed the procedure described above for phenethanolamine. Incorporation of deuterium was nearly quantitative. Removal of the benzyl groups by catalytic hydrogenation would afford labelled α-methyl-p-octopamine.

EXPERIMENTAL

Chemical ionization mass spectra were recorded on a Finnegan 1015 mass spectrometer with ammonia gas or isobutane. N.m.r. spectra were obtained with a Varian HA 100 instrument. In instances where both tritium and deuterium-labelled amines were prepared, details are presented only for synthesis of tritium-labelled amines. Procedures for the synthesis of deuterium-labelled amines with NaB^2H_4 were in these cases essentially the same, but on a larger scale.

β-[^{3}H]-2,4,5-Trihydroxyphenethylamine (6-hydroxydopamine). To a solution of 70 mg (0.15 mmoles) of 2,4,5-tribenzyloxynitrostyrene (4) in 5 ml absolute ethanol was added at room temperature 6.8 mg (0.18 mmole) of NaB^3H_4 (sp. activity 45.6 mCi/mmole). After two hours of stirring, 5 mg of nonradioactive $NaBH_4$ was added and the mixture stirred overnight. The ethanol was evaporated in vacuo and the product, [^{3}H]-2,4,5-tribenzyloxyphenylnitroethane was extracted with ether in nearly quantitative yield. This crude product (50 mg, 0.106 mmole) was dissolved in 5 ml of benzene and reduced with 3 ml Red-Al solution (70% in benzene) at room temperature overnight. After cooling, water was added, the mixture filtered and the benzene evaporated in vacuo. The product, [^{3}H]-2,4,5-tribenzyloxyphenethylamine, was then extracted from the aqueous mixture into ether. The yield was about 50 percent. This crude product (10 mg, 0.02 mmole) was dissolved in 0.5 ml of ethanol and reduced with 50 psi hydrogen gas and 5 mg of 10% palladium on charcoal for 12 hrs. The catalyst was removed by centrifugation and the ethanol evaporated *in vacuo*. Final purification of [^{3}H]-6-hydroxydopamine was by chromatography on Whatman 1 paper with butanol-2N HCl (1:1) and elution with methanol. The final specific activity was 41.0 mCi/mmole.

β-[^{2}H]-α-Methyl-2,4,5-trihydroxyphenethylamine (α-methyl-6-hydroxydopamine). Condensation of 2,4,5-tribenzyloxybenzaldehyde to form the nitrostyrene was as described (4), except that nitroethane was substituted for nitromethane. The product, 2,4,5-tribenzyloxy-α-methyl-α-nitrostyrene (mp 122-123°, ethanol), was obtained in 70 percent yield. This nitrostyrene (1 gr) was dissolved in 2 ml of tetrahydrofuran and 1 ml of ethanol, followed by addition of 150 mg of NaB^2H_4 and stirring at room temperature for 2 hr. The solution was neutralized with 1 N HCl and the solvent evaporated in vacuo. The product was purified by column chromatography on silica gel GF_{254} with CH_2Cl_2: petroleum ether, bp. 30-60° (3:7). The yield was 60 percent and the product, a tribenzyloxyphenylnitroproane, contained 1 deuterium in the benzylic position by mass and n.m.r. spectral analysis. This nitro compound (600 mg) was dissolved in 5 ml dry benzene, followed by addition of 4 ml Red-Al solution (70% in benzene) and stirring at room temperature overnight. After addition of water the suspension was filtered and the benzene filtrate was dried over Na_2SO_4. After removal of benzene in vacuo, the product was dissolved in ether and precipitated by addition of ether saturated with HCl. Recrystallization was from ethanol-ether to yield β-[^{2}H]-α-methyl-2,4,5-tribenzyloxyphenethylamine in 60 percent yield and containing 1 deuterium atom by mass spectral analysis. This amine (150 mg) was dissolved in 0.5 ml absolute ethanol and reduced with 45 psi of hydrogen gas and 10 mg 10% palladium on charcoal overnight. The catalyst was removed by filtration and the product precipitated by addition of ether. Recrystallization was from ethanol-ether afforded β-[^{2}H]-α-methyl-6-hydroxydopamine (Rf 0.26, Whatman 1; butanol-2N HCl, 1:1) in 50 percent yield.

β-[^{3}H]-α-Methyl-4-hydroxyphenethylamine (α-methyl-p-tyramine). 4-Benzyloxybenzaldehyde was condensed with nitromethane using the procedure described for such condensations by Lee and Dickson (4). The product, 4-benzyloxy-α-methyl-α-nitrostyrene was reduced with NaB^3H_4 was essentially as described above in the synthesis of 6-hydroxydopamine and the product tritio-4-benzyloxyphenyl-nitropropane was obtained in nearly quantitative yield. This crude product was reduced with zinc dust in acetic acid/ethanol to afford [^{3}H]-α-methyl-4-benzyloxyphenylethylamine in 35 percent yield. Reduction with hydrogen gas of this crude product was as described above. Final purification was by silica gel

column chromatography with CH_2Cl_2-methanol (95:5) to afford β-[^{3}H]-α-methyl-p-tyramine (sp. activity 13.9 mCi/mmole). The material was identical in chromatographic properties to authentic α-methyl-p-tyramine.

β-[^{3}H]-Phenethanolamine. 1-Phenyl-1-hydroxy-2-nitroethane was prepared by condensation of benzaldehyde and nitromethane according to a standard procedure described by Heacock and Hutzinger (12). This compound (1 g) was dissolved in 10 ml acetone and Jones reagent (70 g of CrO_3 in 500 ml water to which 61 ml H_2SO_4 was added, cf ref. 13) was added slowly at room temperature until a slight excess was present. The mixture was then stirred for slightly over 1 hr followed by addition of an excess of methanol and concentration *in vacuo*. The dark green oily residue was dissolved in water and the product was extracted into ether. After removal of the ether, the residue was crystallized from methanol to yield benzoylnitromethane (mp. 110°) in 70 percent yield. To a solution of 20 mg (0.12 mmole) of benzoylnitromethane in 2 ml absolute ethanol was added 1 mg of NaB^3H_4 (sp. activity 45 mCi/mmole) and 1 mg of $NaBH_4$ followed by stirring for 10 min at room temperature. The precipitate was removed by filtration and crude product, 1-[^{3}H]-1-phenyl-1-hydroxy-2-nitroethane, was obtained by evaporation of the ethanol *in vacuo*. This crude product (10 mg, 0.06 mmole) was dissolved in dry tetrahydrofuran and reduced with 50 mg $LiAlH_4$ at reflux for 15 hr. After cooling water was added and the mixture filtered on celite. The solvent was evaporated and the oily residue was dissolved in ether, dried over Na_2SO_4 and evaporated *in vacuo*. The product was purified by silica gel chromatography eluted with CH_2Cl_2: petroleum ether bp. 30-60° (10:1). The final product (2.1 mg, overall yield 27%, sp. activity 3.07 mCi/mmole) was identical in chromatographic properties to authentic β-phenethanolamine.

β-[^{3}H]-β-2-hydroxyphenethanolamine (o-octopamine). The synthetic procedure was essentially as described above for β-phenethanolamine with 1-[2'-benzyloxyphenyl]-1-hydroxy-2-nitroethane as starting material. Oxidation with Jones reagent, reduction with NaB^3H_4 and then $LiAlH_4$ afforded β-[^{3}H]-β-2-benzyloxyphenethanolamine in 20 percent yield. This material was dissolved in 5 ml ethanol and reduced overnight with 45 psi of hydrogen gas and 2 mg 10% palladium on charcoal catalyst. The catalyst was centrifuged and the alcohol evaporated

to yield 2-hydroxyphenethanolamine (40% yield, sp. activity 3 mCi/mmole) which was identical in chromatographic properties to authentic amine.

α-Methyl-β-[^{2}H]-β-4-benzyloxyphenethanolamine. The nitroalcohol was prepared by condensation of 4-benzyloxybenzaldehyde with nitroethane (cf. ref. 4). Oxidation of 300 mg of nitroalcohol in 20 ml acetone with Jones reagent at 10-15° was essentially as described above for β-phenethanolamine. The product, a nitropropiophenone was obtained in 87% yield (mp. 97-8°, recryst. ethanol). This nitropropiophenone (220 mg) was dissolved in a mixture of 5 ml tetrahydrofuran and 7 ml ethanol, and 20 mg NaB^2H_4 was added at 0°. Stirring was continued for 10 min, and an additional 10 mg NaB^2H_4 was added. After 20 min of further stirring, the solution was adjusted to pH 7.0 with dilute HCl and evaporated *in vacuo*. The product was extracted into CH_2Cl_2 and chromatographed on silica gel with CH_2Cl_2 to yield 140 mg of the deuterium labelled nitroalcohol (63%, mp 111-112°), which was reduced overnight with Red-Al (see above) to yield α-methyl-β-[^{2}H]-β-4-benzyloxyphenethanolamine.

REFERENCES

1. Kopin, I. J., - Annual Rev. Pharmacol. 8: 377 (1968).
2. Kostrzewa, R. M. and Jacobowitz, D. M. - Pharmacol. Rev. 26: 199 (1974).
3. Daly, J. W., Benigni, J., Minnis, R., Kanaoka, Y., and Witkop, B. - Biochemistry 4: 2513 (1965).
4. Lee, F. G. H., and Dickson, D. E. - J. Med. Chem. 14: 266 (1971).
5. Wehrli, P. A., Pigott, F., and Chu, V. - Canadian J. Chemistry 50: 3075 (1972).
6. Merchant, J. R. and Khan, M. S. - J. Indian Chem. Soc. 39: 227 (1962).
7. Ramirez, F. A. and Burger, A. - J. Am. Chem. Soc. 72: 2781 (1950).
8. Howe, R., Young, E.H.P. and Ainley, A. D. - J. Med. Chemistry 12: 998 (1969).
9. Axelrod, J., Senoh, S. and Witkop, B. - J. Biol. Chem. 233: 697 (1958).
10. Benington, F., Morin, R. D., and Clark Jr., L. C. - J. Org. Chem. 20: 1292 (1955).
11. Heacock, R. A. and Hutzinger, O. - Canad. J. Chem. 41, 543 (1963).
12. Meinwald, J., Crandall, J. and Hymans, W. E. - Org. Syn. 45: 77 (1965).