# THE SYNTHESIS OF DEUTERATED HYDROXYPHENYLETHANOLAMINES AND THEIR METABOLITES

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## SUMMARY

The o-, m- and p-NC[$^2H_3$] synephrines were prepared via a three step reaction from the corresponding oxazolidones. Ring deuterated m-synephrine, m-octopamine and m-hydroxymandelic acid were prepared by [$^1H_1$] - [$^2H_1$] exchange in dilute [$^2H_1$]Cl medium. Reduction of o- and m-mandelic acid with lithium aluminum deuteride afforded the corresponding hydroxyphenylglycols. Exclusive side chain deuteration of 2-dibenzyl-1-(4-hydroxyphenyl)ethanone and 2-methylamino-1-(3,4-dihydroxyphenyl)ethanone were accomplished in both acidic and basic media. The former compound was deuterated on both the side chain and aromatic ring using 45% [$^2H_1$]Br.

Key words: Hydroxyphenylethanolamines-[$^2H_1$], synephrine-[$^2H_3$], octopamine-[$^2H_3$] or -[$^2H_4$], adrenalone-[$^2H_2$], hydroxymandelic acid [$^2H_3$], hydroxyphenylglycol [$^2H_2$] and [$^2H_3$].

## INTRODUCTION

Evidence is accumulating that catecholamines[1] and various non-catecholic phenylethylamines[2,3] (eg. phenylethylamine, phenylethanolamine, tyramine and octopamine) are implicated in certain disease states. Elucidation of the normal and pathological mechanisms by which these amines act requires the identification and quantitative determination of very small quantities of them (and their metabolites) in biological media by methods which are specific, sensitive, reproducible and rapid. In recent years several techniques (eg. radioimmunoassay[4], enzymology[5], spectrophotometry[6], chromatography[7] and gas chromatography mass spectrometry with selected ion monitoring (GC-MS-SIM)[8]) have been developed for these purposes and the latter method has become firmly established in such investigations.

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In order to assay such compounds reliably by GC-MS-SIM it is important that suitable isotopically labeled (eg. deuterated) compounds be available for use as internal standards. Requirements for such an internal standard are:

1. It should be an analog or, preferably, an isotopomer of the compound under investigation.
2. Two (preferably 3 or 4) atoms of deuterium per molecule are desired. Thus natural isotopes in the undeuterated substance will not make a significant contribution to the intensity of that fragment of the internal standard which is being measured by GC-MS-SIM. With a larger number of deuterium atoms, a significant shift in the GC retention time of the deuterated standard relative to that of the undeuterated compound will be noted.
3. Ideally, the internal standard should not contain any of the undeuterated isotopomer; effectively 0-2% of the latter is tolerable for quantitative measurements by GC-MS-SIM.
4. It is important to know the position of the deuterium atoms in the molecule. Frequently it is not possible to use $M^+$ for GC-MS-SIM determinations and the deuterium atoms must reside in those characteristic ions measureable by GC-MS-SIM.
5. The deuterium atoms must not be exchangeable for hydrogen atoms under the conditions used for processing biological samples for GC-MS-SIM.
6. Synthesis of such compounds should preferably be simple and afford crystalline products.

In our investigations of the occurrence, function and metabolism of endogenous amines it has become necessary to prepare deuterated analogs of the isomeric octopamines, synephrines, hydroxymandelic acids and hydroxyphenylglycols. In this paper we describe the synthesis and mass spectra of a variety of such compounds labeled in the aromatic ring and/or the side chain.

## MATERIALS AND METHODS

### Instrumentation

$^1H$ nmr spectra were recorded at room temperature on a Varian EM-360L and a Jeol FX-100 spectrometer. A flip angle of 90° was used and the free induction decay (FID) was accumulated in 16K data points per 1200 Hz spectral width with

repetition times of 8-10 sec. The corresponding parameters for $^{13}C$ nmr spectra were: flip angle 45°, FID in 8K data points per 6002 Hz spectral width. Chemical shifts are expressed in ppm relative to the signal of the appropriate internal standard.

Mass spectra of solid products were obtained on an AEI MS-30 and a Hewlett Packard Model 5985 mass spectrometer; source, 200°, 70 eV. A Hewlett Packard Model 5992A gas chromatograph-mass spectrometer with selected ion monitoring capability was employed for the GC-MS-SIM analyses. A silanized glass column (1.8 m x 2 mm i.d.) packed with 5% OV-101 on Chromosorb GHP 100/200 mesh (Supelco) was operated isothermally at 190° for amine derivatives and 180° for derivatives of acids and glycols. Helium was used as the carrier gas and the column effluent was diverted from the ion source for the first 1.5 min.

Melting points were recorded on an electrothermal apparatus and are uncorrected.

Materials

Reagent grade chemicals were obtained as follows: m-octopamine.HCl, Interchim, Montlucon, France; m-synephrine and p-octopamine.HCl, Regis Chemical Co., Morton Grove, Ill.; m- and p-hydroxymandelic acid, Sigma Chemical Co., St. Louis, Mo.; pentafluoropropionic anhydride (PFPA) and N,O-bis(trimethylsilyl)trifluoracetamide (BSTFA), Pierce Chemical Co., Rockford, Ill.; $\alpha,\alpha$-$[^2H_2]$-$\beta$-$[^2H_1]$-o- and p-octopamine.HCl, $[^2H_2]O$, $[^2H_1]Cl$ (38% w/w in $[^2H_2]O$), $[^2H_1]Br$ (45% w/w in $[^2H_2]O$), and $C[^2H_3]COO[^2H_1]$, $(C[^2H_3])_2SO_4$, Merck Chemical Co., St. Louis, Mo.; $LiAl[^2H_4]$ (98% atom $[^2H]$) and $NaB[^2H_4]$ (98% atom $[^2H]$), Aldrich Chem. Co., Milwaukee, Wis.; $NaO[^2H_1]$ (40% w/w in $[^2H_2]O$), Stohler Isotope Chemicals, Waltham, Mass.; phenol-$[^2H_6]$, KOR, Inc., Cambridge, Mass.

o-Octopamine was synthesized by the method of Kappe and Armstrong[9]; o-synephrine benzoate was prepared in the manner described by us earlier[10].

Sample Derivatization

The methyl bis (O-pentafluoropropionyl) ester (PFP-Me) derivatives of the hydroxymandelic acids were prepared[11] by allowing a solution of the acid in methanolic HCl (100 µl) stand at room temperature for ca. 5 min in a capped vial. Excess reagent was removed with a stream of nitrogen, PFPA (100 µl) added to the residue and the mixture heated at 60° for 30 min. Excess reagent was removed with nitrogen, dry ethyl

acetate (100 µl) was added and aliquots (1-2 µl) of this solution were used for direct injection into the gcms.

The tris-pentafluoropropionyl (PFP) derivatives of amines and glycols were similarly prepared by heating the appropriate substrate with PFPA (100 µl).

Experimental

All the crystalline compounds afforded infrared spectra and elemental analyses consistent with the proposed structures. Lit. mp's of deuterated compounds refer to those of the corresponding undeuterated isotopomers.

2-Dibenzylamino-1-(4-hydroxyphenyl)ethanone (1a); hydrochloride

Reaction of 2-chloro-1-(4-hydroxyphenyl)ethanone[12] (17.5 g, 0.1 mole) with dibenzylamine (45.2 ml, 0.2 mole) gave the hydrochloride salt (17.1 g, 46.5%, mp 232-3° d; lit.[12] mp 239-241° d) as pale yellow prisms from ethanol.

$^1$H nmr: $\delta^{DMSO}$ (TMS): 4.54 (s, 4H, -C<u>H</u>$_2$-C$_6$H$_5$ x 2), 4.65 (s, 2H, -C<u>H</u>$_2$-C=O),6.92 (d, 2H, J=8.8 Hz), 7.34-7.40 (m, 6H), 7.64-7.73 (m, 6H, J=8.8 Hz). $^{13}$C nmr: $\delta^{DMSO}$ (DMSO at 39.5 ppm): 189.3 (C=O), 163.5 ($C_4$-aryl), 115.4 ($C_3$,$C_5$-aryl), 57.8(-<u>C</u>H$_2$-C$_6$H$_5$), 54.9 (-<u>C</u>H$_2$-C=O); other unassigned aryl signals occurred at 131.2, 130.4, 129.9, 129.2, 128.4, 124.7.

The corresponding free base was precipitated by the addition of aqueous sodium bicarbonate solution (200 ml, 0.54 M) to a warm solution of the hydrochloride (2.8 g, 7.6 mmole) in 95% ethanol (120 ml). The reaction mixture was extracted with ether (100 ml) and this neutralization-extraction procedure was repeated twice more using sodium bicarbonate solution (100 ml, 0.54 M) and ether (50 ml). The combined ethereal extracts were dried ($MgSO_4$) and the solvent removed <u>in vacuo</u> to give <u>1a</u> in colorless prisms (2.28 g, 90.4%, mp 110-112°).

$^1$H nmr: $\delta^{DMSO}$ (TMS): 3.74 (s, 4H, -C<u>H</u>$_2$-C$_6$H$_5$ x 2), 3.83 (s, 2H, -C<u>H</u>$_2$-C=O), 6.78 (d, 2H, J=8.7 Hz), 7.40 (m, 10H), 7.90 (d, 2H, J=8.7 Hz).

O-Trimethylsilyl derivative of 1a

<u>1a</u> (0.52 g, 1.6 mmole) and BSTFA (1g) were heated at 60° for 30 min under anhydrous conditions with efficient stirring and then the volatile constituents were removed at 80° (2 mm). The resultant yellow solid was decolorized with activated charcoal and recrystallized four times from hexane to give the trimethylsilylated phenol in colorless needles (0.22 g, 35%, mp 79-80°).

Mass spectrum, m/e (% RI): 403 ($M^+$, 0), 212, 211, 210 (0.6, 7.9, 45.6; $(C_6H_5CH_2)_2\overset{+}{N}=CH_2$), 91 (100; $C_6H_5\overset{+}{C}H_2$).

2-Dibenzylamino-2,2-dideutero-1-(4-hydroxyphenyl)ethanone (1b); hydrochloride

a. Basic Medium: Dry nitrogen was bubbled through a stirred solution of 1a.HCl (1.69 g, 4.6 mmole) in $[^2H_2]O$ (25 ml) and dry dioxane (60 ml) for 15 min. A solution of 40% NaO$[^2H_1]$ in $[^2H_2]O$ (1.08 g) was added, dry nitrogen was again passed through the solution for 15 min and the apparatus was sealed under nitrogen. The reaction mixture was stirred at 25° for 24 h, acidified to pH 1 with 38% $[^2H_1]$Cl in $[^2H_2]O$ (1 ml) and the solvent removed in vacuo at ca. 45°. The residue was digested with boiling 95% ethanol (100 ml) and the undissolved sodium chloride removed by filtration. Excess ethanol was removed by evaporation in vacuo to give a saturated solution which was cooled to 0°, filtered and dried to afford 1b.$[^2H_1]$Cl. The entire procedure was repeated (using 10% less of each reagent) to ensure complete deuteration at $C_2$. A total of 1.45 g 1b.$[^2H_1]$Cl (85.2%, mp 228-9° d; lit. mp 232-3° d) was isolated.

b. Acidic Medium: A solution of 1a.HCl (1.69 g, 4.6 mmole) in 38% $[^2H_1]$Cl (2 ml), dioxane (60 ml) and $[^2H_2]O$ (40 ml) was degassed under vacuum, sealed and then heated at 80° for 4 days. The solvent was removed by evaporation in vacuo, fresh reagents were added and the reaction repeated for a further seven days. The product separated from 95% ethanol in colorless prisms (0.86 g, 51%, mp 232-3° d; lit. mp 232-3° d).

The $^1H$ nmr spectrum of each deuterated product showed the absence of a signal due to $-CH_2-C=O$ and this was confirmed by the $^{13}C$ nmr spectrum.

Mass spectrum (of the trimethylsilyl derivative), m/e (% RI): 405 ($M^+$, 0.1), 213, 212, 211, 210 (9.5, 55.8, 24.2, 3.6; $(C_6H_5CH_2)_2\overset{+}{N}=C\ ^2H_2$ ), 91 (100, $C_6H_5\overset{+}{C}H_2$).

2-Dibenzylamino-2,2-dideutero-1-(3,5-dideutero-4-hydroxyphenyl)ethanone (1c); deuterobromide

A mixture of 1a.HCl (9.9 g, 25 mmole), 45% $[^2H_1]$ Br (9 ml), dry dioxane (freshly distilled, 28 ml) and C $[^2H_3]$ COO $[^2H_1]$ (5 ml) was stirred magnetically under nitrogen for 48 h with exclusion of moisture and then allowed to cool to room temperature. The solvent was partially removed in vacuo and the resultant solid separated from methanol as colorless fluffy crystals (0.67 g, 71%, mp 232-3° d; lit. mp 232-3° d).

Mass spectrum, m/e (% RI): 331 ($M^+$, 0), 212, 211, 210 (11.8, 64.8, 21.2; $(C_6H_5CH_2)_2\overset{+}{N}=CR_2$), 91 (100, $C_6H_5\overset{+}{C}H_2$).

The products from two such reactions (1.35 g) were combined and reacted as described above with $[^2H_1]Br$ (14 ml), dioxane (25 ml) and $C[^2H_3]COO[^2H_1]$ (7.3 ml) to give colorless fluffy crystals (0.95 g, 70%, mp 232° d; lit. mp 232-3° d).

The $^1H$ nmr spectrum showed that the signal due to $-C\underline{H}_2-C=O$ and the hydrogens attached to $C_3$ and $C_5$ were absent: this was confirmed by the $^{13}C$ nmr spectrum.

Mass spectrum, m/e (% RI): 335 ($M^+$, 0), 212, 211, 210 (4.5, 24.2, 4.5), 91 (100).

2-Amino-2,2-dideutero-1-(3,5-dideutero-4-hydroxyphenyl)ethanol (2b); deuterobromide

A mixture of 1c.$[^2H_1]Br$ (0.69 g, 1.88 mmole), palladium on charcoal (157 mg, 10%), ethanol (7.8 ml), $[^2H_2]O$ (1.6 ml) and $C[^2H_3]COO[^2H_1]$ (1.6 ml) was stirred in an atmosphere of hydrogen at room temperature for 44 h until the absorption of hydrogen ceased. The reaction mixture was filtered through Celite, which was then washed several times with ethanol. The solvent was removed from the combined filtrate and washings in vacuo and the product crystallized from ethanol as a pale beige microcrystalline solid (40 mg (8%), mp 154-6° d; lit.[13] mp 170° ).

The isotope composition of $M^+$ of the PFP derivative was as follows: m/e (% RI): 591 (0), 592 (1.6), 593 (1.4), 594 (12.8), 595 (74.5), 596 (9.6).

2-Methylamino-2,2-dideutero-1-(3,4-dihydroxyphenyl)ethanone (3b); hydrochloride

a. Basic medium: This was prepared from 3a.HCl in a manner similar to that described for 1b.HCl. Thus 3a.HCl (0.4 g, 1.7 mmole), $[^2H_2]O$ (17 ml), dioxane (8 ml) and 40% $NaO[^2H_1]$ in $[^2H_2]O$ (0.43 g) were allowed to react for 6 h (without replenishing the reagents) to give 3b.HCl (0.29 g, 72%; mp 224.5-226.5°;lit.[14] mp 243° d).

b. Acidic medium: This was prepared from 3a.HCl in a manner similar to that described for 1b.HCl. Thus 3a.HCl (1.0 g, 4.24 mmole), 38% $[^2H_1]Cl$ (2 ml), $[^2H_2]O$ (40 ml) and dioxane (20 ml) were reacted together as before to afford 3b.HCl (0.55 g, 55%, mp 237-238.5° ; lit.[14] mp 243° d).

In each case the $^1H$ and $^{13}C$ nmr spectra showed the absence of signals attributable to the $-C\underline{H}_2-C=O$ group.

The corresponding free base (14 mg, 94%, mp 231-2° d; lit.[14] mp 235-6° d) was prepared by adding one drop of concentrated aqueous ammonia to a solution of the hydrochloride salt (15 mg) in $[^2H_2]O$ (0.3 ml).

Mass spectrum, m/e (% RI): 181-8 (M+, < 0.4), 137-141 (0.4, 2.0, 4.3, 2.1, 0.5; $(HO)_2C_6H_3CO^+$), 152-7 (0, 0.1, 0.9, 3.1, 5.4, 1.8; $C_8$ $[^1H_4]$ $[^2H_4]$ $[^2H_4]O_3$), 47 (100; $CH_3\overset{+}{N}$ $[^2H_1]$=C $[^2H_2]$ ).

Isomeric 2-(N-trideuteromethyl)-1-(hydroxyphenyl)ethanols (Synephrines)

These were prepared by the general method described by Bergmann and Sulzbacher[15] and modified by Teng and Bruce[16].

5-[2-(Benzyloxy)phenyl]-3-N-trideuteromethyl-2-oxazolidone (4b): 5-[2-(Benzyloxy)phenyl]-2-oxazolidone[10] (4a, 10 g, 0.037 mole) was added to a solution of sodium (1.28 g) in dry methanol (40 ml). The solvent was removed, the residue suspended in dry toluene (100 ml) and reacted with hexadeuterodimethyl sulfate (7.1 g, 0.053 mole) in the manner previously described[10] for the undeuterated isotopomer. The product separated from methanol in plates (9.4 g, 88.5%, mp 104-104.5°; lit.[10] mp 102-4°).

2-(N-Trideuteromethylamino)-1-[2-(benzyloxy)phenyl] ethanol (7a): The alkylated oxazolidone (4b, 5g) was heated under reflux with aqueous potassium hydroxide (3N, 55 ml) and methanol (55 ml) for 5 h. Work-up as previously described[10] afforded 7a which crystallized in needles (4.3 g, 93.8%, mp 75-6°; lit.[10] mp 81-3°) from ether. The hydrochloride salt was isolated as a glass.

2-(N-Trideuteromethylamino)-1-(2-hydroxyphenyl)ethanol (7b): benzoate: A solution of the hydrochloride salt of the benzyl derivative (7a, 1.3 g) in methanol (60 ml) was shaken with palladium on charcoal (10%, 0.2 g) in an atmosphere of hydrogen as previously described[10]. The resultant pale yellow gum did not form a crystalline hydrochloride salt but was instead converted to the benzoate salt which was decolorized with charcoal and crystallized from ethanol/ether in small prisms (0.7 g, 47.9%, mp 136-7°; lit.[10] mp 133-5°).

The $^1H$ nmr spectrum showed the absence of any signal attributable to the N-$CH_3$ group.

The isotope composition of $M^+$ of the PFP derivative was as follows: m/e (% RI): 605 (0.2), 606 (0.7), 607 (18.4), 608 (70.5), 609 (10.1).

The following compounds were prepared in a manner similar to that described above for their analogs:

5-[3-(Benzyloxy)phenyl]-3-N-trideuteromethyl-2-oxazolidone (5b) was obtained in pasty crystals (98%; lit.[15] mp 65-6°) which were used without further purification.

2-(N-Trideuteromethyl)-1-[3-(benzyloxy)phenyl]ethanol (8a) was a colorless solid (96%, mp 94-7°; lit.[17] mp 102.5-103.5°). The corresponding hydrochloride salt separated from ethanol/ether in tiny colorless needles (72.7%, mp 144-6°; lit.[16] mp 148-150°). 2-(N-Trideuteromethyl)-1-(3-hydroxyphenyl)ethanol (8b) hydrochloride separated from ethanol/ether as colorless microcrystals (69%, mp 140-144°; lit.[16] mp 142-3°)

The $^1H$ nmr spectrum showed the absence of any signal attributable to the N-$CH_3$ group.

The isotope composition of $M^+$ of the PFP derivative was as follows; m/e (% RI): 605 (0.4), 606 (1.8), 607 (19.7), 608 (68.3), 609 (9.8).

5-[4-(Benzyloxy)phenyl]-3-N-trideuteromethyl-2-oxazolidone (6b) was obtained in 90% yield (mp 77-9°; lit.[15] mp 101-2°) and was employed without further purification.

2-(N-Trideuteromethyl)-1-[4-(benzyloxy)phenyl]ethanol (9a) was prepared (89%, mp 102-3°; lit.[15] mp 104-5°) and used, without purification, to prepare the corresponding hydrochloride salt which was crystallized from ethanol/ether (80%, mp 152-3°; lit.[15] mp 149°).

2-(N-Trideuteromethyl)-1-(4-hydroxyphenyl)ethanol (9b); hydrochloride. Upon hydrogenation 9a.HCl gave the amine hydrochloride as a microcrystalline colorless solid (58%, mp 152-3°; lit.[15] mp 150-2°) from ethanol/ether.

The $^1H$ nmr spectrum showed the absence of any resonance due to N-$CH_3$.

The isotope composition of $M^+$ of the PFP derivative was as follows: m/e (% RI): 606 (0.2), 607 (22.2), 608 (71.8), 609 (5.9).

2-Amino-1-(2,4,6-trideutero-3-hydroxyphenyl)ethanol (m-octopamine, 10a) and its N-methyl derivative (m-synephrine, 10b)

A solution of the compound (50-400 mg) in $[^2H_1]$Cl (1.5 ml, 20% w/w) and $[^2H_2]O$ (1.5 ml) was placed in a 5 ml amber colored ampule, sealed and heated in an oil bath at ca. 80° for 20-24 h. The solvent was removed by evaporation in vacuo to give 10a.$[^2H_1]$Cl (90%, mp 158-9°; lit.[18] mp 159-160°) and 10b.$[^2H_1]$Cl (85%, mp 140-4°; lit.[15] mp 140-5°).

Isomeric 2-hydroxy-2-hydroxyphenylethanoic acids (hydroxymandelic acids)

2-Hydroxy-2-(2-hydroxyphenyl)ethanoic acid.

a. 3',4',5',6'-tetradeutero derivative. $[^2H_5]$-Phenyl acetate (7.1 g, 98%), obtained by the reaction of $[^2H_6]$-phenol (5 g) with acetyl chloride (4.1 g), was converted to $[^2H_4]$-2-hydroxyacetophenone (33%) by treatment[19] with anhydrous aluminum chloride at 165° for 3 h. The resultant pale yellow liquid (2.1 g) was oxidized with selenium dioxide (1.78 g)

in dioxane (10.5 ml) and water (0.33 ml) as described by Howe et al.[20] to give [$^2H_4$]-2-hydroxyphenylglyoxylic acid (1.4 g, 56%) as an orange oil. Reduction[21] of the latter with sodium borohydride afforded [$^2H_4$]-2-hydroxymandelic acid as a viscous yellow oil which was crystallized as the corresponding bis-(piperazinium) salt (34%, mp 170° d; lit.[22] mp 161-2° d).

The isotopic composition of $M^+$ of the PFP-Me derivative was as follows; m/e (% RI): 474 (1.5), 475 (0), 476 (23.4), 477 (39.6), 478 (35.5).

b. 2,3',5'-trideutero derivative. 2-Hydroxyphenylglyoxylic acid was reduced[20] with sodium borodeuteride to give [$^2H_1$]-2-hydroxymandelic acid in almost quantitative yield. This gum (50 mg) was reacted with [$^2H_1$]Cl, [$^2H_2$]O and C[$^2H_3$]COO [$^2H_1$]as previously described[22] to give 44 mg (87%) of a gum which contained 25% [$^2H_3$]-2-hydroxymandelic acid as determined by GC-MS-SIM of its PFP-Me derivative.

The isotopic composition of $M^+$ of the PFP-Me derivative was as follows; m/e (% RI): 474 (0), 475 (1.7), 476 (22.3), 477 (69.8), 478 (6.1).

2-Hydroxy-2-(2,4,6-trideutero-3-hydroxyphenyl)ethanoic acid (10c). The undeuterated isotopomer (200 mg) was reacted with [$^2H_1$]Cl in [$^2H_2$]O by the general procedure described above for compounds 10a and 10b. The resultant viscous oil was decolorized with charcoal and afforded colorless needles (0.16 g, 78%, mp 127-9°; lit.[23] mp 131-2°) on trituration with petroleum ether (60/80 fraction).

The isotopic composition of $M^+$ of the PFP-Me derivative was as follows; m/e (% RI): 474 (1.0), 475 (10.6), 476 (36.5), 477 (50.6), 478 (1.3).

Isomeric hydroxyphenylethane-1,2-diols (phenylethylene glycols)

1,2,2-Trideutero-1-(2-hydroxyphenyl)ethane-1,2-diol (11). A solution of 2,3-dioxo-2,3-dihydrobenzofuran[24] (3.0 g, 0.02 mole) in dry tetrahydrofuran (50 ml) was added dropwise with stirring to a suspension of lithium aluminum deuteride (2g, 0.048 mole) in dry ether (20 ml) under an atmosphere of nitrogen. The reaction mixture was heated under reflux for 1.5 h and then stirred at room temperature overnight. Work-up as previously described[11] afforded a colorless microcrystalline solid (2.5 g, 78.3%, mp 84-5°; lit.[11] mp 77-81°).

The $^1H$ nmr spectrum showed that signals due to -CHOH-$CH_2$OH were absent.

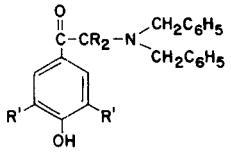
The isotope composition of $M^+$ of the PFP derivative was as follows; m/e (% RI): 592 (0.3), 593 (1.0), 594 (21.8), 595 (69.0), 596 (8.0).

2,2,2',4',6'-Pentadeutero-1-(3-hydroxyphenyl)ethane-1,2-diol (12). A solution of 2-hydroxy-2-(2,4,6-trideutero-3-hydroxyphenyl)ethanoic acid (10c, 0.58 g) in dry tetra-hydrofuran (20 ml) was added dropwise with stirring to a mixture of lithium aluminum deuteride (0.95 g) in dry tetrahydrofuran (30 ml). The mixture was refluxed for 3 h and processed as before to give an oil[11] which failed to crystallize.

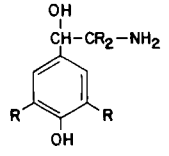
The isotope composition of $M^+$ of the PFP derivative was as follows; m/e (% RI): 592 (0), 593 (0.9), 594 (5.6), 595 (17.1), 596 (37.9), 597 (34.7), 598 (3.8).

## RESULTS AND DISCUSSION

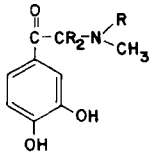
It is well established that α-keto protons of aryl ketones may be exchanged for deuterium under acidic or basic conditions.[25,26] However, exchange of the aromatic protons may also occur if they are sufficiently activated by substituents. We have found that protons attached to the α-carbon atom of an aromatic ketone may be exchanged completely and specifically by deuterium under acidic or basic conditions. The compounds employed were 2-dibenzylamino-1-(4-hydroxyphenyl)ethanone hydrochloride (1a.HCl) and 2-methylamino-1-(3,4-dihydroxyphenyl)ethanone hydrochloride (3a.HCl) which are precursors of p-octopamine and epinephrine, respectively. Thus, the treatment of 1a.HCl or 3a.HCl with $[^2H_1]$ Cl in $[^2H_2]$O-dioxane or with NaO$[^2H_1]$, $[^2H_2]$O-dioxane afforded the corresponding dideutero derivatives (1b, 3b). The use of $^1H$ and $^{13}C$ nmr[27] showed that complete exchange of the α-hydrogens had occurred in each compound by both routes.



1a. R = R' = H
b. R = D; R' = H
c. R = R' = D



2a. R = H
b. R = D



3a. R = H
b. R = D

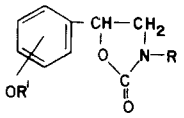
Moreover, except in the case of 1a in acidic medium, it was apparent that the exchange of aromatic hydrogens was not appreciable. From the nmr data it was estimated that compound 1b prepared in an acid medium contained approximately 20% of the corresponding tetradeuterated derivative (1c). Exchange in basic medium was convenient as well as specific: reaction was complete within 6h with little trace of oxidation products. Exchange under acidic conditions required several days and replenishment of reagents.

The mass spectra of compound 1a.HCl and its corresponding O-trimethylsilyl derivative showed the absence of a molecular ion, a moderately abundant peak due to $CH_2{=}\overset{+}{N}(C_6H_5CH_2)_2$ (m/e 210, arising from cleavage of the $C_\alpha$-$C_\beta$ bond) and a base peak at m/e 91 ($C_6H_5\overset{+}{C}H_2$). As expected, the mass spectra of compounds 1b and 1c each exhibited an ion at m/e 212, thereby reflecting the conclusions reached from the nmr data. It was not possible to confirm the extent of deuteration on the ring in compound 1c by mass spectrometry since no fragment of appreciable abundance contained the p-hydroxyphenyl group. Compounds 3a and 3b also fragmented by α-cleavage under electron impact to give $CH_2{=}\overset{+}{N}HCH_3$ (m/e 44) and $C[^2H_2]{=}\overset{+}{N}[^2H_1]CH_3$ (m/e 47), respectively. The α, α, β-trideuterated o- and p-octopamines were obtained commercially. Fragmentation of the corresponding PFP derivatives afforded $M^+$ of low abundance together with two relatively abundant structure-specific ions $(M-[^2H_2][^1H_1]NCOC_2F_5)^+$, m/e 430 and $(M-C[^2H_2]{=}NHCOC_2F_5)^+$, m/e 416. The intensity of the latter ion has a substantial contribution from the corresponding non-deuterated ion and, since two structure-specific ions are required[28] for unambiguous identification (and subsequent quantification) of compounds by GC-MS-SIM, it was desirable to synthesize octopamines bearing deuterium atoms on the aromatic ring.

Thus the tetradeuterated p-octopamine (2b) was prepared from 1a by the general route developed by Kalir and co-workers[29] for the synthesis of α-methylnorepinephrine and norepinephrine. Dibenzylamino-1-(4-hydroxyphenyl)ethanone hydrochloride (1a.HCl) was reacted with concentrated $[^2H_1]Br$ to give the tetradeuterated derivative (1c.$[^2H_1]Br$). The extent of deuteration was assessed by examination of the nmr spectrum for intensity of the signals due to hydrogens attached to $C_3$, $C_5$ and the carbon atom adjacent to the carbonyl group. Reduction of the carbonyl group in 1c was achieved by catalytic hydrogenation to afford 1c.$[^2H_1]Br$. The isotopic distribution in $M^+$ of the tris-PFP derivative of 1c.$[^2H_1]Cl$ was 75% and 0% of the $[^2H_4]$and $[^2H_0]$ species, respectively

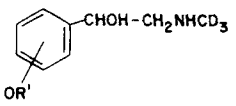
and hence this compound was most useful for the estimation of p-octopamine in biological fluids and tissues.[30]

Isomeric N-trideuteromethyl synephrines could be conveniently and efficiently prepared by the procedure[10,15,16] of Bergmann and Sulzbacher. Thus, the oxazolidones[15]




o: 4
m: 5
p: 6

a. R = H, R' = $C_6H_5CH_2$–
b. R = $CD_3$, R' = $C_6H_5CH_2$–




o: 7
m: 8
p: 9

a. R' = $C_6H_5CH_2$–
b. R' = H

(4a, 5a, 6a), obtainable in good yield by a four stage process from the corresponding hydroxybenzaldehydes, were N-trideuteromethylated by treatment with hexadeuterodimethyl sulfate and base. The resulting alkylated derivatives (4b, 5b, 6b) were hydrolysed with potassium hydroxide in aqueous methanol. Hydrogenolysis of the protecting group afforded the desired N-trideuteromethyl compounds (7b, 8b, 9b) in good overall yield. The PFP derivatives of these three isomers afforded very similar mass spectra which were analogous to those already reported by Midgley et al.[31] for the corresponding TFA derivatives of the synephrines. The spectra exhibited few ions of significant abundance: the base peak ($CH_2=\overset{+}{N}(C[^2H_3])COC_2F_5$, m/e 193) arises by α-cleavage but this ion is not useful for biological investigations since the corresponding m/e 190 ion is not structure-specific. The PFP derivatives of 7b, 8b and 9b afford less than 1% of a molecular ion upon electron impact. However, in each case, $M^+$ contains negligible amounts of the undeuterated and 70% of the $[^2H_3]$ species and this structure-specific ion has proved to be highly suitable for the estimation of synephrines in biological media.[31,32]

A more convenient procedure for the preparation of ring-labeled octopamines was sought and it was found that 2,4,6-$[^2H_3]$-m-octopamine.$[^2H_1]$Cl (10a) could be readily obtained in high yield by treatment of m-octopamine with 10% $[^2H_1]$Cl at 80° for 24 h in a sealed ampule. Similar treatment of o-and p-octopamine resulted in unsatisfactory distribution of deuterium, poor yields and polymeric by-products (Table 1). The isotopic composition of these compounds was determined by the GC-MS-SIM analysis of appropriate

TABLE 1

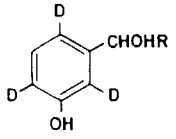
Isotope Composition of the Product of $[^1H]$-$[^2H]$ Exchange

| Starting Compound | Reaction Time[a] (h) | Ions Monitored (m/e of $[^2H_0]$ species) | Percent Incorporation $[^2H_0]$ | $[^2H_1]$ | $[^2H_2]$ | $[^3H_3]$ | $[^2H_4]$ | $[^2H_5]$ |
|---|---|---|---|---|---|---|---|---|
| o-Octopamine | 24 | 415[d] | 4.7 | 33.7 | 61.7 | | | |
| | 48 | 415[d] | - | 35.8 | 64.3 | | | |
| m-Octopamine | 24 | 415[d] | 0.2 | 4.8 | 37.2 | 57.0 | 0.8 | |
| | | 591($M^+$)[d] | 0.6 | 5.4 | 39.0 | 53.8 | 1.1 | |
| p-Octopamine | 24 | 415[d] | 6.6 | 37.5 | 17.7 | 36.0 | 2.2 | |
| o-Synephrine | 24 | 605($M^+$)[d] | 4.4 | 30.6 | 61.9 | 1.2 | 1.8 | 0.1 |
| | 48 | 605[d] | 2.1 | 26.1 | 65.5 | 5.9 | 0.3 | 0.1 |
| m-Synephrine | 24 | 605[d] | 0.1 | 5.3 | 28.3 | 57.6 | 8.7 | - |
| p-Synephrine | 22 | 605[d] | 10.2 | 37.8 | 46.9 | 4.6 | 0.3 | 0.1 |
| m-Synephrine-C $^2H_3$ | 21 | 608[d] | - | 2.3 | 35.7 | 55.5 | 6.2 | 0.2 |
| OHMA | 0.75[b] | 474($M^+$)[e] | - | 1.7 | 22.3 | 69.8 | 6.1 | |
| MHMA | 24 | 474($M^+$)[e] | 1.0 | 10.6 | 36.5 | 50.6 | 1.3 | |
| | | 415[e] | - | 9.3 | 38.0 | 51.6 | 1.2 | |
| PHMA | 21 | 474($M^+$)[e] | 2.4 | 22.4 | 73.0 | 0.9 | 1.3 | |
| | 24[c] | 415[e] | 36.8 | 50.0 | 13.2 | - | - | |
| | 48[c] | 415[e] | 16.1 | 41.9 | 40.3 | 1.5 | 0.2 | |

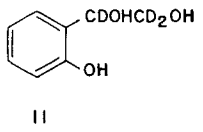
[a]200 mg compound, $[^2H_2]O$ (1.5 ml), $[^2H_1]Cl$ (1.5 ml, 20%), heated at 80°.
[b]$[^2H_2]O$, $[^2H_1]Cl$, 190°.
[c]200 mg compound, $[^2H_2]O$ (1.5 ml), $[^2H_1]Cl$ (1.5 ml, 20%), heated at 60°.
[d]PFP derivative
[e]PFP-Me derivative

ion(s) of the corresponding PFP derivatives. Compound 10a has been extremely valuable in the assay of m-octopamine in biological media.[30]

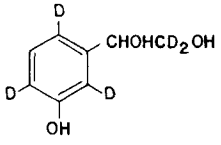
Similar treatment of the isomeric synephrines (N-methyl octopamines) afforded comparable results (Table 1): 2,4,6-[$^2H_3$]-m-synephrine (10b), which was obtained in 90%




10a. R = $CH_2NH_2$
b. R = $CH_2NHCH_3$
c. R = COOH




11




12

yield, contained approximately 60% and 0.1% of the [$^2H_3$] and the corresponding undeuterated species, respectively. The results with o- and p-synephrine were not satisfactory since, particularly in the case of the latter, low yields of deuterated products containing significant amounts of starting materials were obtained.

The isomeric octopamines and synephrines are metabolized largely to the corresponding hydroxymandelic acids which are excreted in the free form in urine.[10,22,32-39] Their ring-deuterated analogs have been prepared by Midgley et al.[22] by heating the non-deuterated species with [$^2H_1$]Cl at ca. 180° for several hours. This procedure gave poor yields of the desired product which nevertheless did contain less than 1% of the corresponding non-deuterated isotopomers. However, using this reaction [$^2H_3$]-o-hydroxymandelic acid (OHMA) was prepared from [$^2H_1$]-OHMA.

When the acids were subjected to the conditions (see Table 1) which had been used to insert deuterium into the aromatic nucleus of m-octopamine and m-synephrine, crystalline 2,4,6-[$^2H_3$]-m-hydroxymandelic acid (10c) (MHMA) was obtained in excellent yield. Analysis of the isotopic composition showed that $M^+$ of the PFP-Me derivative contained ca. 1% and 50% of the undeuterated and [$^2H_3$] species respectively. This exchange reaction was not successful with OHMA and PHMA (p-hydroxymandelic acid): exchange of the aromatic hydrogens occurred to a limited extent and, in the case of PHMA, was accompanied by the formation of a pink, water-insoluble product. [$^2H_4$]-OHMA (as the crystalline bis-(piperazinium salt) was prepared from [$^2H_6$]-phenol via Fries' rearrangement of the acetate, oxidation of the resultant [$^2H_2$]-2-hydroxyaceto-

phenone with selenium dioxide and reduction of the 2-hydroxyphenylglyoxylic acid with sodium borohydride. The PFP-Me derivatives of these acids fragmented under electron impact in a manner similar to that reported by Midgley et al.[22] for the corresponding TFA-Me derivatives of the undeuterated HMA's. Several structure-specific ions result and the appreciable intensity of $M^+$ has rendered this particularly useful for quantitative work in biological media.[22,30,32]

The second major metabolite of octopamines and synephrines is the corresponding hydroxyphenylglycol (HPG).[11,33,35] [$^2H_3$]-OHPG (11) was readily prepared in high yield by reduction of 2,3-dioxo-2,3-dihydrobenzofuran[24] (or 2-hydroxyphenylglyoxylic acid[20]) with lithium aluminum deuteride. Similarly, reduction of [$^2H_3$]-MHMA (10c) afforded [$^2H_5$]-MHPG (12) but thus far it has not been possible to obtain deuterated PHPG by these means. The PFP derivatives of these compounds afford analogous mass spectra to those already reported by us[11,32] for such compounds. In each case $M^+$ is sufficiently abundant to serve as the ion of choice in quantitative determinations by GC-MS-SIM in biological media.

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