THE SYNTHESIS OF SPECIFICALLY DEUTERATED N-ACETYL-6-HYDROXY-5-METHOXYTRYPTAMINE (6-HYDROXYMELATONIN) FOR USE AS AN INTERNAL STANDARD IN MASS SPECTROMETRY

G. J. Shaw*
Laboratory of Chemistry, National Heart, Lung, and Blood Institute,
National Institutes of Health, Bethesda, Maryland 20014

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SUMMARY

Specifically deuterated N-acetyl-6-hydroxy-5-methoxytryptamine has been synthesised. The mass spectra of the labeled and unlabeled analogs are reported.

Key Words: N-acetyl-6-hydroxy-5-methoxytryptamine, deuterium, synthesis, mass spectra

INTRODUCTION

Using in vivo radiotracer studies N-acetyl-6-hydroxy-5-methoxytryptamine has been shown to be the major metabolic product of the potent melanocyte-contracting substance N-acetyl-5-methoxytryptamine (melatonin) in mice[1,2]. In these animals, N-acetyl-5-methoxytryptamine, when injected intravenously (c.f. intracisternal administration[3]), is rapidly metabolized to the sulphate (60-70%) and glucuronic acid (5%) conjugates of N-acetyl-6-hydroxy-5-methoxytryptamine.

So that the kinetics of this transformation can be followed more closely, a quantitative mass spectrometric assay is being developed. To facilitate accurate quantitative results, it was necessary to synthesis a stable isotopically labeled form of the title compound for use as an internal standard. The results of this work are presented here.

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*N.I.H. Fogarty Fellow, 1976-1977.

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The synthetic sequence of reactions leading to N-acetyl-6-hydroxy-5-methoxy-$\alpha,\alpha,\beta,\beta$-$d_4$-tryptamine (summarised in Figure 1) is based on work presented by Benigni and Minnis[4], Julia and Manoury[6] and on previous studies conducted in this laboratory[6,7]. Treatment of 4-benzyloxy-3-methoxybenzaldehyde[8] (1) with nitromethane in aqueous acetic acid afforded 4-benzyloxy-3-methoxy-β-nitrostyrene(2). Subsequent nitration of 2 gave the corresponding 6,β-dinitro product (3) in good yield. 6-Benzyloxy-5-methoxyindole (4) was prepared from 3 by ring closure in the presence of iron and aqueous acetic acid according to the procedure outlined by Julia and Manoury. C-acylation with oxalyl chloride gave (6-benzyloxy-5-methoxyindole-3-yl)-glyoxyloyl chloride (5) which was readily converted to 6 with ammonia. (6-Benzyloxy-5-methoxyindol-3-yl)-glyoxylamide (6) was reduced with lithium aluminum deuteride in THF to give 6-benzyloxy-5-methoxy-$\alpha,\alpha,\beta,\beta$-$d_4$-tryptamine (7) after only 2 hr (c.f. Ref 1). Acetylation to give 8, followed by catalytic debenzylation proceeded smoothly to 9 which was crystallized from ethanol/hexane.

The final isotopic distribution for 9 was calculated using a program developed by C. F. Hammer[9]. Data were obtained by accurate measurement of peak heights of the ions in the molecular ion region formed by electron ionization of the deuterated and undeuterated compounds. The isotopic distributions (in moles %) were: d_4 = 94.5% (98.8 atoms % D), d_3 = 4.8%, d_3 = 0.7%, d_2 = 0%, d_1 = 0%, d_0 = 0%.

The electron ionization mass spectra of labeled and unlabeled N-acetyl-6-hydroxy-5-methoxytryptamine are shown in Figure 2. The major fragmentation is the same as that found for labeled and unlabeled N-acetyl-5-methoxytryptamine[7]. In the unlabeled compound, the peak at m/e 189 arises from a McLafferty rearrangement with loss of $HNCOHCH_3$ from the molecular ion. Simple cleavage of the side chain gives m/e 176 (M^+-$CH_2NHCOCH_3$). Further fragmentation of this latter ion gives rise to m/e 161. The data for the tetradeuterated analog supports this fragmentation scheme with analogous daughter ion peaks at m/e 192

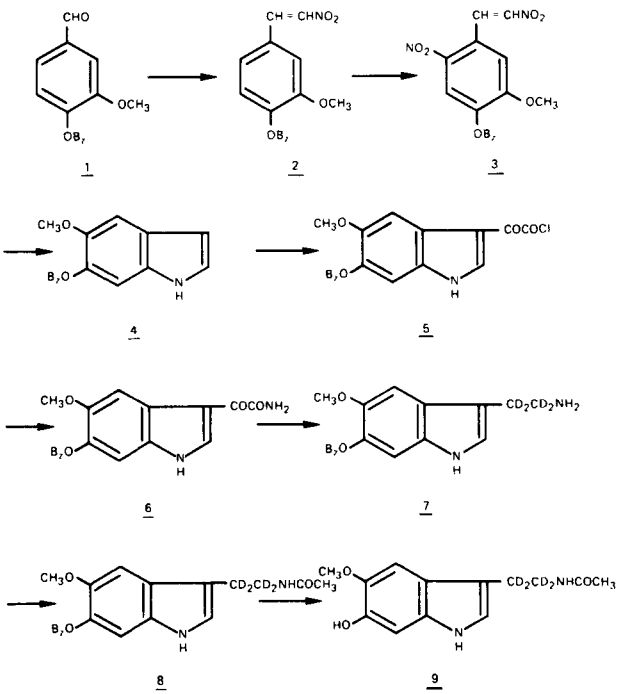

Figure 1. Scheme for the synthesis of N-acetyl-6-hydroxy-5-methoxy-$\alpha,\alpha,\beta,\beta$-$d_4$-tryptamine

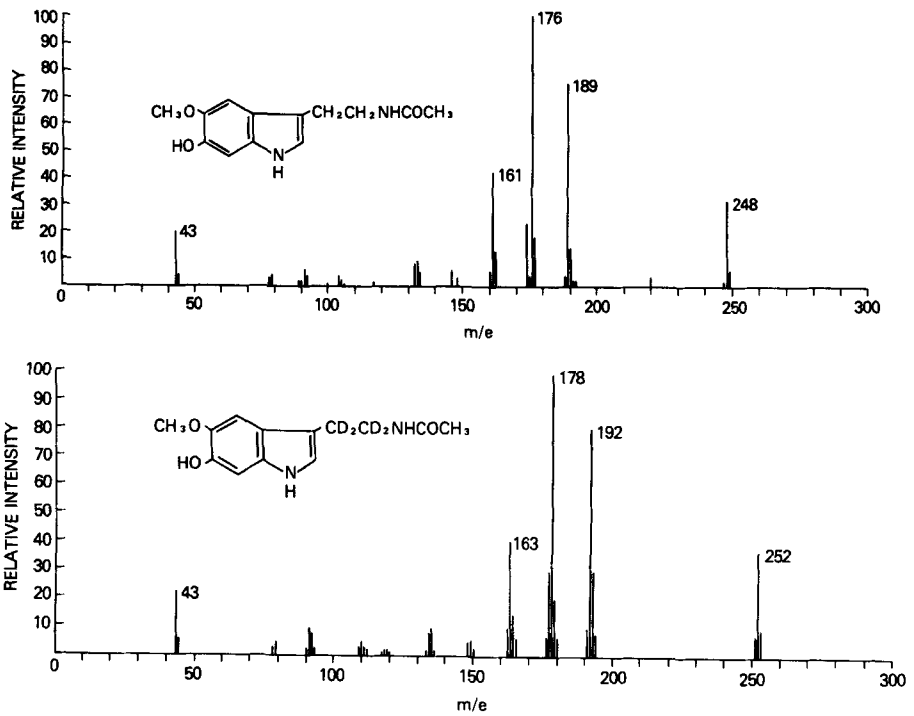

Figure 2. Electron impact mass spectra of N-acetyl-6-hydroxy-5-methoxy-tryptamine (upper trace) and N-acetyl-6-hydroxy-5-methoxy-$\alpha,\alpha,\beta,\beta$-$d_4$-tryptamine (lower trace)

(M^+-$HNCODCH_3$) and m/e 178 (M^+-$CD_2NHCOCH_3$). The ion at m/e 163 clearly represents loss of methyl from the aromatic methyl ether of this ion as 2 deuterium atoms are retained.

From inspection of the same spectrum, it appears that N-acetyl-6-hydroxy-5-methoxy-α,α,β,β-d_4-tryptamine is well suited for use as an internal standard in quantitative mass spectrometry. The spectrum reveals the presence of only a few major ions all of which contain at least two deuterium atoms.

EXPERIMENTAL SECTION

All melting points were measured on a Kofler block and are uncorrected. Low resolution mass spectra were taken with LKB 9000 and LKB 2091 mass spectrometers.

4-Benzyloxy-3-methoxy-β-nitrostyrene (2)- - Nitromethane (29 g, 0.47 mol) was added to 4-benzyloxy-3-methoxybenzaldehyde (1, 38 g., 0.16 mol) and ammonium acetate (24 g, 0.31 mol) in glacial acetic acid (240 cm^3) and the mixture refluxed for 2 hr. A yellow precipitate formed on cooling and was collected, washed with ethanol and air dried. Yield 17.9 g (76%). An analytical sample was crystallized from ethanol. Mp 117.5-118.5°. Lit.[4] 121-122°. Mass spectrum (relative abundance): 285(8)(M^+), 92(10), 91(100).

4-Benzyloxy-3-methoxy-6,β-dinitrostyrene (3)- - To a stirred mixture of 4-benzyloxy-3-methoxy-β-nitrostyrene (2, 31.0 g, 0.1 mol) in glacial acetic acid (350 cm^3) was added fuming nitric acid (d = 1.5, 40 cm^3). During this addition the temperature rose to 40° and solution resulted; at 45° the product precipitated out of solution. The reaction was cooled and stirred for an additional 30 min. The yellow precipitate was poured onto ice, filtered and washed with ice cooled water. The product was air dried and crystallized from ethanol. Yield 30.0 g (86%). Mp 163-164°. Lit.[5]. 167°. Mass spectrum: 330(1)(M^+), 269(5), 92(11), 91(100).

6-Benzyloxy-5-methoxyindole (4)- - To a stirred solution of 80% acetic acid (50 cm^3) was added iron powder (63.0 g). The temperature was raised to 90° and 4-benzyloxy-3-methoxy-6,β-dinitrostyrene (3, 15 g, 45 mmol) suspended

in glacial acetic acid (50 cm^3) was added over 10 min. The mixture was refluxed for 1 hr and then filtered, while hot, into a saturated solution of sodium bisulphite (500 cm^3) . The mixture was extracted with chloroform and dried before filtering. Evaporation of the filterate in vacuo gave a solid which was washed in ethanol and filtered. Yield 7.1 g (62%). An analytical sample was crystallized from ethanol. Mp 146-147°. Lit.[4] 148-150°. Mass spectrum: 254(8), 253(39)(M^+), 163(12), 162(100), 134(29), 119(19), 116(10), 106(8), 104(8), 91(48), 65(10), 64(5), 63(6).

(6-Benzyloxy-5-methoxyindol-3-yl)-glyoxyloyl chloride (5) - - Excess oxalyl chloride (4.0 cm^3, 40 mmol) was added dropwise to a well-stirred solution of 6-benzyloxy-5-methoxyindole (4, 5 g, 19 mmol) in dry diethyl ether (550 cm^3) at 5-6°. Following the development of a dark orange-red solution, the reaction was stirred for an additional 15 min before filtering and evaporating to dryness. Yield 5.7 g (85%). Mass spectrum: 315(5), 279(8), 251(7), 126(9), 92(10), 91(100), 65(11).

(6-Benzyloxy-5-methoxy-3-yl)-glyoxylamide (6) - - The acid chloride residue (5, 5 g, 14 mmol) was suspended in toluene (100 cm^3) and dry ammonia bubbled through the stirred mixture for 30 min. The resulting light yellow solid was filtered, washed well with water and air dried. Yield 4.3 g (92%). Crystallized from 50% dioxan/water. Mp 238-239°. Lit.[4] 242-244°. Mass spectrum: 325(11), 324(54)(M^+), 280(9), 234(15), 233(100), 188(13), 161(20), 160(10), 91(30).

6-Benzyloxy-5-methoxy-α,α,β,β-tryptamine (7)- - The glyoxylamide (6, 3 g, 9.2 mmol) was added over 5 min to a stirred suspension of lithium aluminum deuteride (3 g, 73 mmol, 99 atoms % D) in dry THF (400 cm^3). The mixture was gently refluxed, with stirring, for an additional 2 hr. The excess LAD was destroyed with wet THF and the mixture filtered. The salts were washed with fresh THF and the solvent removed from the combined filtrates. The oily residue was taken up in ethyl acetate, washed with dilute sodium hydroxide, water and then dried. Evaporation in vacuo gave a light brown oil which was

further dried by azeotropic distillation with benzene. The product was precipitated as the hydrochloride salt from a benzene solution, with dry hydrogen chloride gas. The product was filtered giving a light grey powder. Yield 1.2 g (44%). Mass spectrum: 301(13), 300(39)(M^+), 268(17), 210(28), 209(100), 179(15), 178(42), 177(20), 163(15) 90(40).

N-acetyl-6-hydroxy-5-methoxy-α,α,β,β-d_4-tryptamine (9) - - The hydrochloride salt (7, 0.5 g, 1.5 mmol) was dissolved in 50% pyridine/acetic anhydride (30 cm^3) and allowed to stand at 4° for 12 hr before pouring onto ice and was worked up in the usual way. The resulting gum (8), without further purification, was dissolved in absolute ethanol (10 cm^3) and stirred with 10% palladium on charcoal for 4 hr under hydrogen at 3 atmospheres. At the end of this time the mixture was filtered and the solvent removed under vacuum. The light brown oily residue was crystallized from ethanol/hexane. Yield 228 mg (61%). Mp = 172-174°, Lit.[4] 174-175°. Mass spectrum: Fig. 2.

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9. This program, LABDET, is a component of the NIH/EPA Computer-based Chemical Information System. For further details contact G. W. A. Milne in this Laboratory