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The Synthesis of α,α,β,β-d_4-Serotonin

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Abstract—α,α,β,β-d_4-Serotonin (94% d_4, 6% d_3) has been synthesized for use as an internal standard in mass spectrometric determinations of serotonin in biological systems.

Introduction

TISSUE and blood concentrations of hydroxyindoles such as serotonin have been measured using absorption spectroscopy,[1] colorimetric determinations[2,3] and more importantly, a histofluorescence technique.[4] Each of these methods lacks specificity for individual components and so can only be semiquantitative. Recent investigations[5] leading to an assay for biogenic amines have resulted in the development of a g.c.m.s. technique[6,7] which not only offers a high degree of specificity but also a marked increase in sensitivity (10^{-10}–10^{-11} mol). As an extension of this technique, selected ion monitoring decreases this lower limit of detectability to 10^{-12} or 10^{-13} mol.[8]

The g.c.m.s. methods require the use of an internal standard, ideally a stable isotope enriched compound of identical structure to the compound under investigation. No supplier currently offers serotonin modified in this way and we have, therefore, synthesized deuterated serotonin and will make it available to other scientists for this purpose until it becomes commercially available.

To our knowledge, only two methods for the synthesis of deuterated serotonin have been published. The first relies on selective exchange of nuclear protons.[9] This, however, leads to a mixture of isotopically enriched species. The second involves a chemical synthesis via 5-benzyloxygramine.[10]

In this paper we report an alternative synthesis leading to α,α,β,β-d_4-serotonin in 33% overall yield from 5-benzyloxyindole.

Results and discussion

The synthesis of 5-(benzyloxy)indole-3-glyoxyloyl chloride was carried out, after Speeter and Anthony,[11] by acylation of 5-benzyloxyindole with oxalyl chloride. Reaction in anhydrous diethyl ether[12] gave a readily isolable product in 94% yield. In the mass spectrum of the glyoxyloyl chloride, the molecular ion is of low abundance (1%) at *m/e* 313. The major fragments are the benzyl ion as the base peak at *m/e* 91, and an ion at *m/e* 159 (14%) resulting from α-cleavage of the glyoxyloyl chloride sidechain and benzyl loss.

The glyoxyloyl chlorides of substituted indoles have been shown by Misztal[12] to react readily with a series of amines under various reaction conditions. In this case, 5-(benzyloxy)indole-3-glyoxylamide was prepared by a modification of the procedure of Benigni and Minnis[13] for the synthesis of 5,6-dihydroxyindole derivatives. Dry ammonia gas was bubbled through a well-stirred suspension of the acid chloride in benzene giving the glyoxylamide in 96% yield. The mass spectrum, again, has as its base peak the benzyl ion (*m/e* 91) and a fragment ion at *m/e* 250 (50%) resulting from loss of $CONH_2$ from the molecular ion.

Transformation of the glyoxylamide to the fully deuterated β-aminoethyl sidechain was achieved by reduction with lithium aluminium deuteride. In this reduction, variable yields of *O*-benzyl-d_4-serotonin hydrochloride were obtained depending upon the solvent and reaction time. Use of tetrahydrofuran as a solvent and a reaction time of 2 h after final addition of substrate was sufficient to give a 50% yield for this step. The resulting oil was isolated and characterized as a hydrochloride salt. The molecular ion (*m/e* 270) in the electron ionization spectrum gives rise to major fragments at *m/e* 238 and *m/e* 239 due to a McLafferty rearrangement involving carbon 2 of the indole nucleus and simple cleavage of the sidechain, respectively. Daughter ions at *m/e* 147 and *m/e* 148 are derived from the above ions by benzyl loss. The ion at *m/e* 91 is also present.

In the final step, the *O*-benzyl-d_4-serotonin hydrochloride salt was hydrogenated in absolute ethanol in the presence of 10% palladium on charcoal catalyst. The product was crystallized from an acetone + water mixture as the creatinine sulphate salt, in 74% yield.

The mass spectrum of α,α,β,β-d_4-serotonin (as the creatinine sulphate salt) is shown in Fig. 1. The parent ion (*m/e* 180) is small, and shows the material to be 94% d_4 and 6% d_3. The intense ions arising from loss of CD_2NH_2 (*m/e* 148) and CD_2NH (*m/e* 149) still contain two deuterium atoms. Therefore, these ions can be used to monitor the tetradeuterated material in

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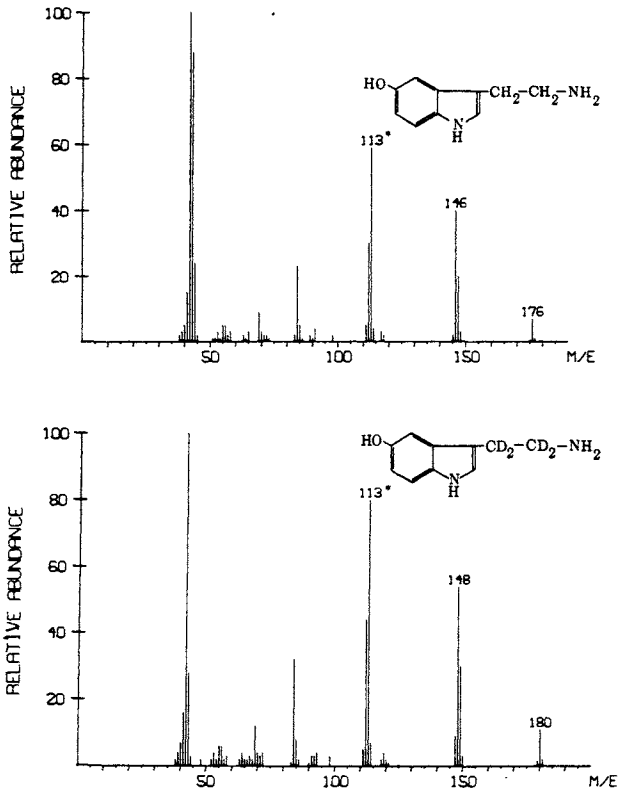

FIG. 1. Mass spectra of serotonin and α,α,β,β-d_4-serotonin (* denotes creatinine sulphate molecular ion).

the presence of undeuterated serotonin, whose spectrum is also shown in Fig. 1.

Experimental

All melting points (m.p.) were measured on a Kofler hot-stage and are uncorrected. Low-resolution electron ionization mass spectra were recorded with an LKB 9000 mass spectrometer and chemical ionization mass spectra with a Finnigan 3200 quadrupole mass spectrometer. Accurate mass measurements were made on an A.E.I. MS-9 instrument at a resolving power of 10 000. Precise isotopic enrichment was calculated from the electron ionization mass spectra using a computer program developed by C. F. Hammer. (This program, LABDET, is a component of the NIH/EPA computer-based Chemical Information System. For further details contact G.W.A.M.)

5-(BENZYLOXY)INDOLE-3-GLYOXYLOYL CHLORIDE

Excess oxalyl chloride (4 cm^3, 0.04 mol) was added dropwise to a well-stirred solution of 5-benzyloxyindole (5 g, 0.02 mol) in dry diethyl ether (250 cm^3) at 5–10 °C. Following the development of an orange precipitate, the mixture was stirred for an additional 15 min at room temperature. The solid was filtered and washed with ether (50 cm^3) and allowed to air dry, giving 5-(benzyloxy)indole-3-glyoxyloyl chloride (6.6 g, 94%), m.p. 143–147 °C, Lit.[14] 146–150 °C. Mass spectrum: 313(1), 287(2), 286(1), 285(6), 251(1), 250(7), 249(8), 194(2), 160(2), 159(14), 158(2), 132(1), 131(5), 130(1), 103(3), 102(2), 92(9), 91(100), 77(1), 76(2), 75(2), 65(10), 51(2), 50(2), 39(3), 38(5), 36(12).

5-(BENZYLOXY)INDOLE-3-GLYOXYLAMIDE

The acid chloride (6.6 g, 0.02 mol) was suspended in dry benzene (Baker Analyzed Reagent) (400 cm^3) and dry ammonia gas bubbled through the mixture for 30 min. The yellow solid was filtered, washed well with water and air dried giving 5-(benzyloxy)indole-3-glyoxylamide (5.8 g, 95%). A portion of the product was recrystallized from ethanol, m.p. 275–278 °C. *m/e*, 294.1014 (calc. for $C_{17}H_{14}N_2O_3$, 294.1004). Mass spectrum: 295(6), 294(21), 251(1), 250(50), 203(6), 175(3), 160(7), 159(21), 147(4), 132(5), 131(18), 103(10), 92(10), 91(100), 81(5), 77(3), 76(6), 65(41), 57(3), 55(3), 53(5), 52(3), 51(4), 50(4), 44(8), 43(5), 41(5), 39(7), 28(12).

O-BENZYL-α,α,β,β-d_4-SEROTONIN HYDROCHLORIDE

The glyoxylamide (10 g, 0.34 mol) was added over 2 min to a stirred suspension of lithium aluminium deuteride (10 g, 0.24 mol, >99 atom % D)[19] in tetrahydrofuran (700 cm^3) which had been distilled from lithium aluminium hydride. The mixture was gently refluxed, with stirring, for an additional 2 h. The excess lithium aluminium deuteride was destroyed by adding wet tetrahydrofuran cautiously and the resulting mixture was filtered. The salts were washed with fresh tetrahydrofuran and the solvent was removed from the filtrates. The residue was taken up in ether, washed with dilute aqueous sodium hydroxide solution and water, and then dried over sodium sulphate and evaporated *in vacuo* to give a light yellow oil which was further dried by azeotropic distillation with benzene. The residual oil was taken up in a small volume of chloroform and precipitated as the hydrochloride salt with dry hydrogen chloride gas. Yield 4.9 g (48%). The light brown solid was washed with hot ethanol and allowed to cool before filtering, m.p. 252–255 °C, Lit. 242–247 °C,[10] 263–264 °C,[15] 265 °C.[16] *m/e*, 270.1670 (calc. for $C_{17}H_{14}D_4N_2O$, 270.1670). Mass spectrum: 271(11), 270(36), 240(20), 239(91), 238(95), 237(7), 180(7), 179(22), 178(4), 162(9), 161(10), 149(22), 148(100), 147(50), 134(4), 133(9), 132(5), 120(10), 119(24), 93(7), 92(40), 91(74), 90(5), 65(18), 38(12), 36(34), 32(35).

α,α,β,β-d_4-SEROTONIN CREATININE SULPHATE MONOHYDRATE

O-Benzyl-α,α,β,β-d_4-serotonin hydrochloride (2 g, 6.5 mmol) was suspended in ethanol (400 cm^3) and stirred with 10% palladium on charcoal (0.8 g) for 4 h under hydrogen gas 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 taken up in a solution of creatinine sulphate (1.4 g) in hot water (15 cm^3) and hot acetone (100 cm^3) added to precipitate the crude product. The suspension was heated for 5 min, cooled, filtered and the precipitate washed with acetone to give[17] α,α,β,β-d_4-serotonin creatinine sulphate monohydrate (1.7 g, 74%) as a

pale tan solid, m.p. 214–215 °C. This material was recrystallized from ethanol + water in 80% yield, m.p. 215–216 °C, Lit. 214–216 °C.[18] Mol percent deuterated species (determined on the TFA derivative) $^2H_4$ = 94%, $^2H_3$ = 6%. *m/e* 180.1205 (calc. for $C_{10}H_8D_4N_2O$, 180.1201).

AUTHORS' NOTE

We wish to make the deuterated serotonin prepared during this investigation freely available for nonclinical research purposes until such time as it becomes commercially available. Therefore, we will forward 50 mg quantities of the creatinine sulphate salt to those requesting a sample. Please contact G.W.A.M.

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