Mass Spectra of some Specifically Deuterated Tryptamines

G. John Shaw,† Graeme J. Wright and G. W. A. Milne
Laboratory of Chemistry, National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

The mass spectra of the four tryptamine derivatives, *N*-acetyl-5-methoxytryptamine (melatonin), *N*-acetyl-5-hydroxytryptamine (*N*-acetyl-serotonin), *N,N*-dimethyl-5-hydroxytryptamine (bufotenine) and *N,N*-dimethyl-5-methoxytryptamine (*O*-methylbufotenine), with specifically labeled [D_4]aminoethyl sidechains have been measured. Comparison of these spectra with those of the unlabeled compounds enable the major fragmentations of the compounds to be defined.

INTRODUCTION

Two previous reports[1,2] have dealt with the fragmentation of substituted tryptamines. One of these[1] characterizes the fragmentation pathways by use of metastable defocusing. The second report[2] uses an alternative approach yielding further unambiguous information on fragmentation processes by studying the mass spectrometric behavior of a stable isotopically enriched analog labeled in the methoxyl group. This method is used to define fragmentations in the indole portion of *N*-acetyl-5-trideuteromethoxytryptamine by studying the loss of deuterium from the labeled molecule. In this paper we report the mass spectra of four tryptamine derivatives *N*-acetyl-5-methoxytryptamine (melatonin), *N*-acetyl-5-hydroxytryptamine (*N*-acetyl-serotonin), *N,N*-dimethyl-5-hydroxytryptamine (bufotenine) and *N,N*-dimethyl-5-methoxytryptamine (*O*-methylbufotenine), specifically tetradeuterated in the methylene groups of the aminoethyl sidechain. Comparison of the spectra of labeled and unlabeled compounds permits verification and extension of earlier observations regarding the sidechain fragmentation and rearrangement processes in the electron impact spectra of these compounds.

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. Precise isotopic enrichments were calculated from the electron ionization mass spectra using the computer program developed by C. F. Hammer.[3]

(5-Benzyloxyindol-3-yl)-glyoxyloyl chloride (4), (5-benzyloxyindol-3-yl)-glyoxylamide (6) and 5-benzyloxy-[α,α,β,β-D_4]tryptamine (8)

These compounds were prepared according to the method described previously.[4]

† Fellow of The Medical Research Council of New Zealand. To whom all correspondence should be addressed.

(5-Methoxyindol-3-yl)-glyoxyloyl chloride (3)

Oxalyl chloride (5.0 g, 39.6 mmole) was added dropwise to a stirred solution of 5-methoxyindole (1, 5.0 g, 34.2 mmole) in dry diethyl ether (100 ml) at 5 °C. The resulting mixture was stirred for an additional 15 min at room temperature before the orange solid was isolated by filtration. This material was washed with 20 ml of ether and air dried to give 7.39 g (92%) of (5-methoxyindol-3-yl)-glyoxyloyl chloride, m.p. 127–128 °C, lit.[5] 126–127 °C. Mass spectrum: 239(3), 237([M]+)(5), 211(7), 210(3), 209(20), 175(15), 174(100), 173(17), 160(3), 159(17), 158(15), 147(3), 146(10), 132(3), 131(11), 130(5), 119(4), 103(6), 102(6), 87(7), 76(6), 75(6), 53(5), 52(4), 51(4), 50(7), 38(8), 36(22), 35(4).

(5-Methoxyindol-3-yl)-glyoxylamide (5)

The glyoxyloyl chloride **3** (7.3 g, 30.8 mmole) was suspended in dry benzene (200 ml) and dry ammonia gas bubbled through the mixture until the color changed from orange to yellow. The solid product (6.4 g, 96%) was filtered off, washed with water and air dried. A sample was crystallized from ethanol as yellow needles, m.p. 243–245 °C, phase change starting at about 227 °C. Mass spectrum: 219(4), 218([M]+)(23), 175(13), 174(100), 173(3), 160(3), 159(16), 158(3), 146(9), 132(2), 131(11), 130(2), 119(3), 104(2), 103(6), 102(2), 76(5), 44(4), 38(8), 36(22).

(5-Benzyloxyindol-3-yl)-*N,N*-dimethylglyoxylamide (14)

Dry dimethylamine, prepared by the reaction of dimethylamine hydrochloride and concentrated sulfuric acid and dried by passage through a column of potassium hydroxide pellets, was passed through a suspension of (5-benzyloxyindol-3-yl)-glyoxyloyl chloride (**4**, 6.0 g, 19.1 mmole) in dry benzene (200 ml). The resulting yellow solid (5.6 g, 91%) was filtered, washed with water and air dried. A sample, crystallized from ethanol, had m.p. 183–184 °C, lit.[6] 179–180 °C. Mass spectrum: 323(4), 322([M]+)(14), 252(3), 251(19), 250(100), 194(3), 160(11), 159(24), 132(4), 131(15), 103(6), 92(3), 91(38), 72(12), 65(8), 44(8).

(5-Methoxyindol-3-yl)-*N,N*-dimethylglyoxylamide (13)

(5-Methoxyindol-3-yl)-glyoxyloyl chloride (**3**, 6.2 g, 26.1 mmole) was suspended in dry benzene (250 ml) and reacted with dry dimethylamine. The product (5.89 g, 92%) was filtered, washed with water and air dried. A sample, crystallized from ethanol had m.p. 221–222 °C, lit.[6] 221.5–222 °C. Mass spectrum: 247(3), 246([M]+)(18), 175(13), 174(100), 173(3), 160(2), 159(10), 158(2), 146(5), 132(2), 131(8), 130(2), 119(2), 103(4), 76(3), 72(9).

5-Methoxy-[α,α,β,β-D_4]tryptamine (7)

The glyoxylamide **5** (3.1 g, 14.2 mmole) was added to a stirred mixture of lithium aluminum deuteride[7] (2.5 g, 59.5 mmole, >99% D) in dry tetrahydrofuran (distilled from lithium aluminum hydride) over 3 min. This mixture was refluxed for 2 h before quenching the excess lithium aluminum deuteride with wet tetrahydrofuran. The resulting mixture was further refluxed for 15 min before filtering. The filtrate was concentrated *in vacuo* and the resulting oil was taken up in chloroform, washed successively with sodium hydroxide solution and water and dried over sodium sulphate. The solvent was removed giving a light brown oil. Final traces of water were removed by azeotropic distillation with benzene. The oil was then taken up in chloroform and the product precipitated with dry hydrogen chloride giving the hydrochloride salt (m.p. 235–240 °C, lit.[8] 247.5–248.5 °C, 1.66 g, 51%). Moles percent deuterated species: [D_4] = 88.2%, [D_3] = 11·7%. Mass spectrum: shown in Fig. 1.

5-Benzyloxy-*N,N*-dimethyl-[α,α,β,β-D_4]tryptamine (16)

The glyoxylamide **14** (0.5 g, 1.5 mmole) was added to a stirred mixture of lithium aluminum deuteride (0.4 g, 9.5 mmole) in dry tetrahydrofuran over 3 min. The mixture was refluxed and worked up as above. The resulting oil was dissolved in dry chloroform and the hydrochloride salt m.p. 158–161 °C, (0.26 g, 50%) precipitated with hydrogen chloride gas. Mass spectrum: 299(1), 298([M]+)(4), 238(1), 147(1), 119(1), 92(1), 91(4), 61(4), 60(100), 59(3).

N,N-Dimethyl-5-methoxy-[α,α,β,β-D_4]tryptamine (15)

The glyoxylamide **13** (1.0 g, 4.0 mmole) was added to a mixture of lithium aluminum deuteride (1.0 g, 23.8 mmole) in dry tetrahydrofuran. The mixture was worked up as above to give a light brown oil which on crystallizing from hexane gave the deuterated tryptamine (**15**) as a solid, m.p. 67 °C, lit.[6] 67–67.5 °C, 461 mg, 52%. Moles percent deuterated species: [D_4] = 90.1%, [D_3] = 9.9%. Mass spectrum: shown in Fig. 2.

N-Acetyl-5-methoxy-[α,α,β,β-D_4]tryptamine (10)

5-Methoxy-[α,α,β,β-D_4]tryptamine hydrochloride (**7**, 45 mg, 0.2 mmole) was dissolved in pyridine (15 ml) and acetic anhydride (15 ml) and stirred for 1 h. The mixture was poured onto ice, extracted with chloroform and washed with hydrochloric acid and water before drying over sodium sulfate. The solvent was removed giving a yellow oil which crystallized from benzene to give *N*-acetyl-5-methoxy-[α,α,β,β-D_4]tryptamine (m.p. 115–117 °C, lit.[9] 116–118 °C, 35 mg, 76%). Moles percent deuterated species: [D_4] = 89.1%, [D_3] = 10.9%. Mass spectrum: shown in Fig. 3.

N-Acetyl-5-benzyloxy-[α,α,β,β-D_4]tryptamine (11)

The hydrochloride of 5-benzyloxy-[α,α,β,β-D_4]tryptamine (**8**, 50 mg, 0.16 mmole) was dissolved in pyridine (15 ml) and acetic anhydride (15 ml) and the solution stirred for 1 h. The reaction was worked up as before to yield a gum (45 mg, 88%). Mass spectrum: 313(9), 312([M]+)(29), 253(31), 252(58), 239(9), 238(42), 221(10), 179(50), 178(11), 162(24), 161(73), 91(100), 65(19), 43(19).

N-Acetyl-5-hydroxy-[α,α,β,β-D_4]tryptamine (12)

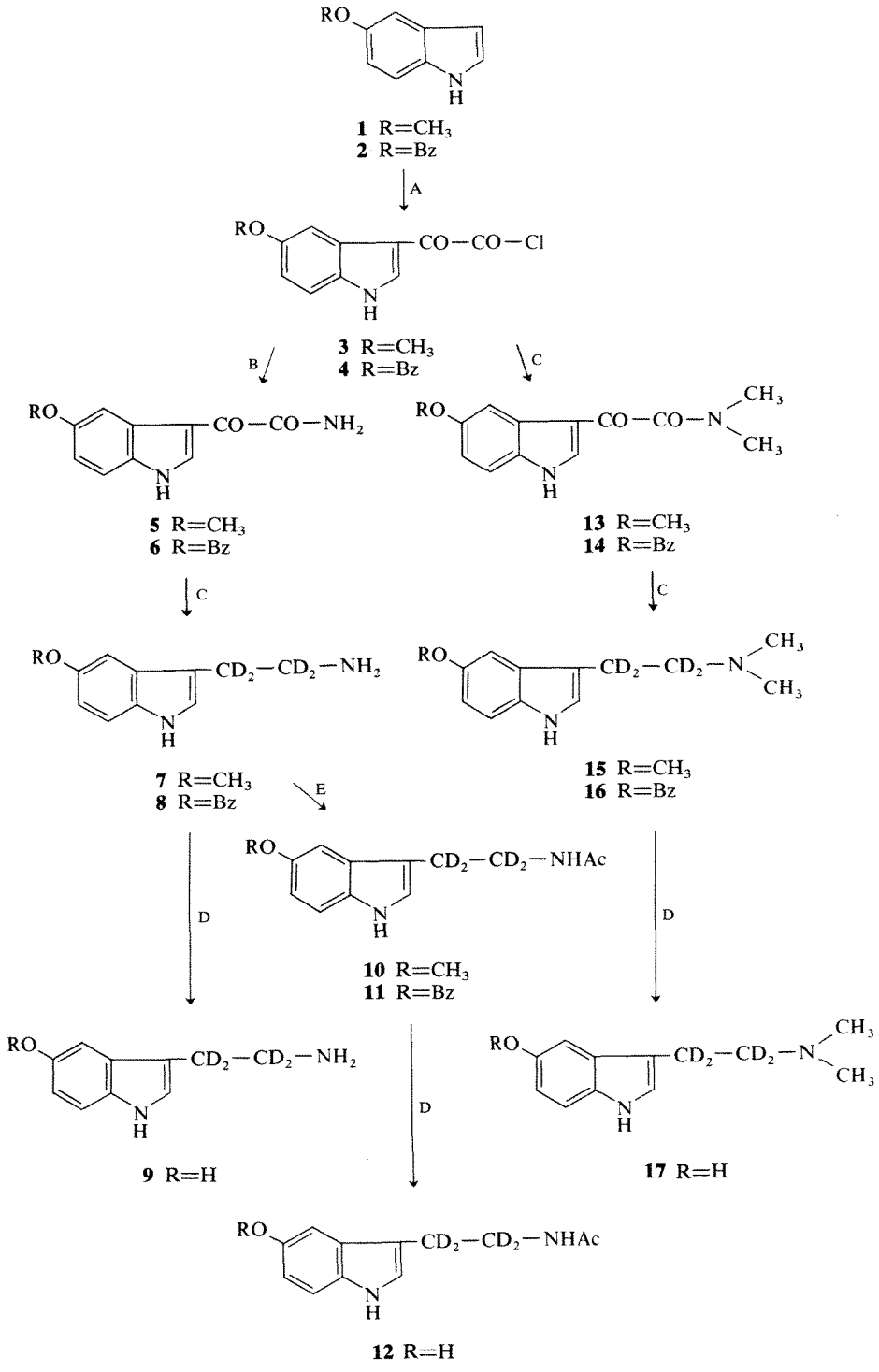
N-Acetyl-5-benzyloxy-[α,α,β,β-D_4]tryptamine (**11**, 0.6 g, 1.9 mmole) was dissolved in ethanol (250 ml) and stirred for 4 h under hydrogen at 3 atmospheres. The mixture was filtered and the charcoal washed with additional ethanol. The combined filtrates were reduced under vacuum to give the deuterated compound as a light brown gum (350 mg, 83%). Moles percent deuterated species: [D_4] = 89.5%, 5%, [D_3] = 10.5%. Mass spectrum: shown in Fig. 3.

N,N-Dimethyl-5-hydroxy-[α,α,β,β-D_4]tryptamine (17)

The hydrochloride of *N,N*-dimethyl-5-benzyloxy-[α,α,β,β-D_4]tryptamine (**16**, 30 mg, 0.09 mmole) was dissolved in ethanol (250 ml) and stirred for 4 h under hydrogen at 3 atmospheres. The product was worked up as before and the resulting gum crystallized from ethyl acetate as a solid, m.p. 142–144 °C, lit.[10] 146–147 °C, (15.7 mg, 84%). Moles percent deuterated species: [D_4] = 91.0%, [D_3] = 9.0%. Mass spectrum: shown in Fig. 2.

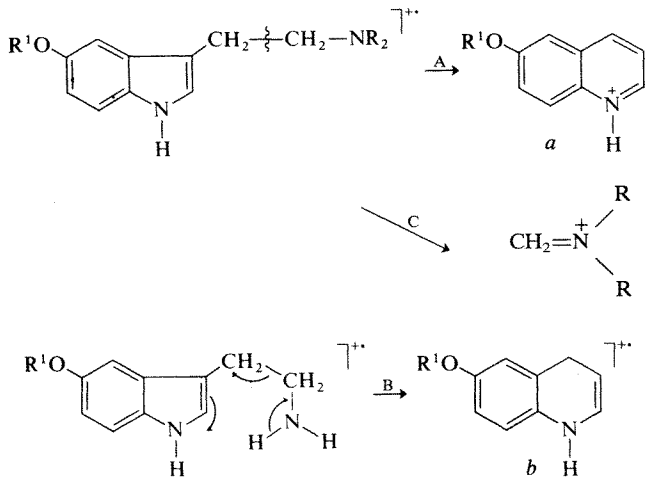
RESULTS AND DISCUSSION

The labeled compounds were synthesized by modifications of the method developed for the synthesis of specifically labeled serotonin.[4] The synthetic scheme is outlined in Scheme 1 and is described in detail in the Experimental section. The mass spectra of compounds **7**, **10**, **15** and **17** (Scheme 1) together with the mass spectra of their undeuterated analogs are shown in Figs. 1–3. Scheme 2 shows three major cleavages (A, B and C) previously thought to be significant in the tryptamine series. Metastable ion evidence[1] suggests that cleavage between the alpha and beta carbon atoms of the aminoethyl sidechain (path A) gives rise to an ion at *m/e* 162 in compounds **7** and **15**, and *m/e* 148 in **17**.



Scheme 1. Syntheses of specifically deuterated tryptamines. (A) $(COCl)_2$/ether, (B) NH_3, (C) $LiAlD_4$, (D) 5% Pd/C, (E) $(CH_3CO)_2O$/pyridine, (F) $N(CH_3)_2$/ether.

These ions, $(M-CD_2NH_2)$ and $(M-CD_2CMe_2)$, due to loss of 32 a.m.u. and 60 a.m.u. respectively, and for which a suitably substituted quinolinium structure (*a*, Scheme 2) has been proposed,[1] appear 2 a.m.u. higher than in the spectra of the undeuterated analogs demonstrating that this process is indeed a straightforward cleavage. The alternative fragmentation process which again occurs with charge retention on the indole moiety is found only in the primary amines (path B, Scheme 2), and gives rise to a relatively intense ion at *m/e* 163 in the spectrum of **7** (Fig. 1). This cleavage results from the fragmentation of the sidechain with loss of 31 a.m.u. (CD_2NH) from the molecular ion. A possible skeletal rearrangement could lead to a 2,3-dehydroquinolinium ion structure (*b*, Scheme 2) in which two deuterium atoms are retained on carbon 4. It has been suggested,[1] without any experimental support, that in the unlabeled compounds, ion *b* is an alternative source of ion *a*. Such a process is reasonable in that it involves a transition from an odd electron state (*m/e* 163) to an even electron state (*m/e* 162) with concomitant loss of H. The driving force for such a transition would be augmented



Scheme 2. Fragmentation processes in methoxytryptamines.

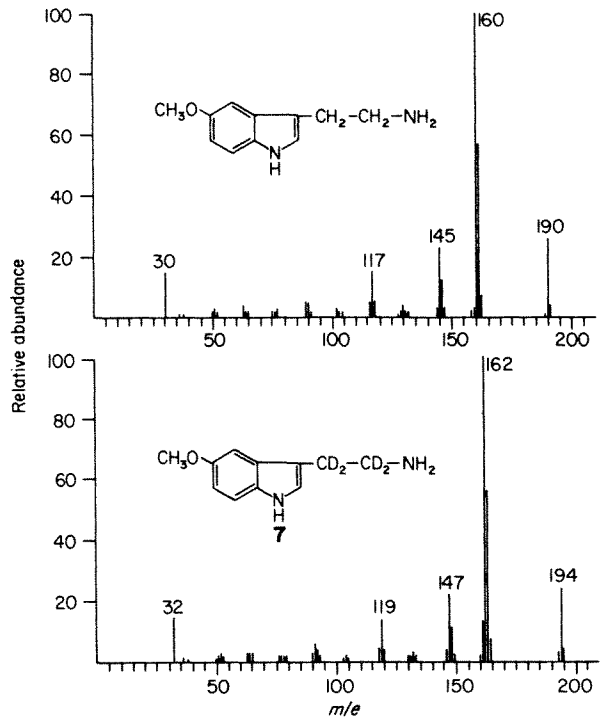


Figure 1. Mass spectra of deuterated and undeuterated methoxytryptamines.

by the increased stability of the aromatic quinolinium ion. Support for such a theory can be found by inspection of the mass spectra of 5-methoxy-[D_4]tryptamine (**7**, Fig. 1), 5-hydroxy-[D_4]tryptamine[4] and [D_4]tryptamine.[11] A comparison of the labeled and unlabeled spectra in each case shows that in the labeled spectra the ion at m/e [M−33], resulting from loss of CD_2NH+D has a higher relative abundance than the corresponding ion (at m/e [M−31]) in the spectra of the unlabeled compounds. This suggests that the 2,3-dehydroquinolinium ion, by loss of deuterium from the carbon-4 position could give the quinolinium ion in which only one deuterium atom is retained. An alternative explanation, that some H/D randomization has occurred in the 2,3-dehydroquinolinium ion, so that either H or D could be lost in the same conversion is not precluded. Further fragmentation of the quinolinium ion in the deuterated methoxytryptamines (**7** and **15**) giving rise to an ion at m/e 147 clearly represents a loss of methyl from the aromatic methyl ether as the two deuteriums are retained in both cases. In both spectra this ion gives rise to peaks at m/e 119 (cf. unlabeled analogs, m/e 117) due to further loss of the ring oxygen as CO. Loss of CO is not observed in the electron impact spectrum of **17**. Another important fragmentation, path C, is again initiated by β cleavage but in this case charge is retained by the sidechain nitrogen (Scheme 2). This mode of fragmentation is more strongly favored in the tertiary amines than in the primary amines presumably because of the increased stability of the dimethyliminium ion, giving rise to the base peak at m/e 60 for

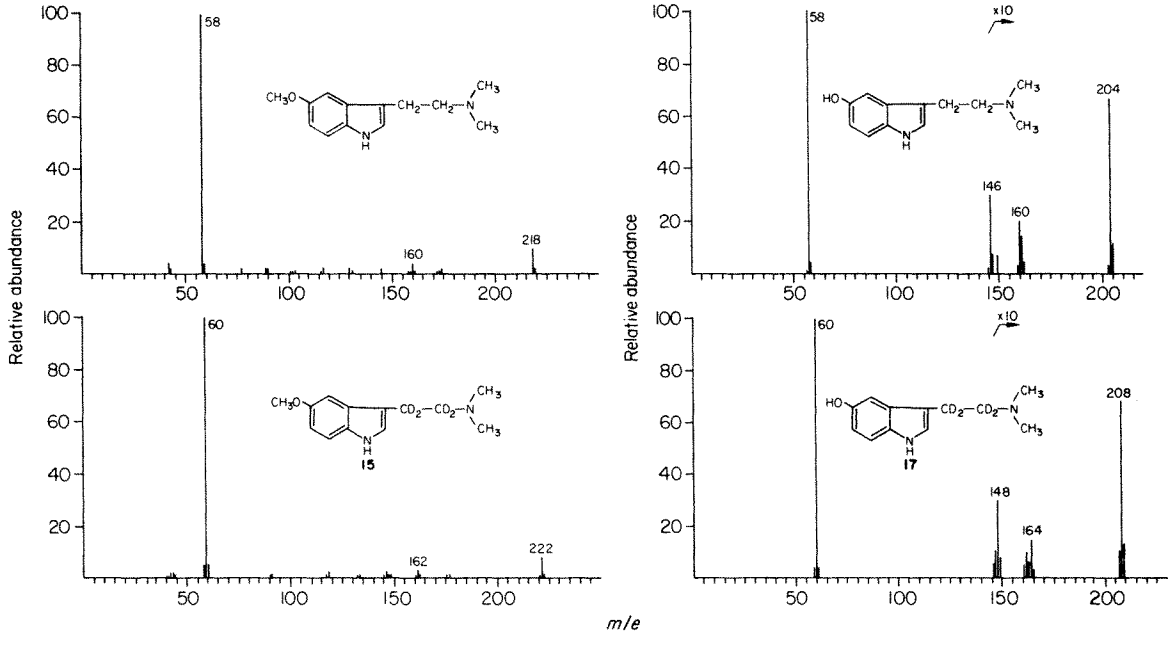


Figure 2. Mass spectra of deuterated and undeuterated *N,N*-dimethyltryptamines.

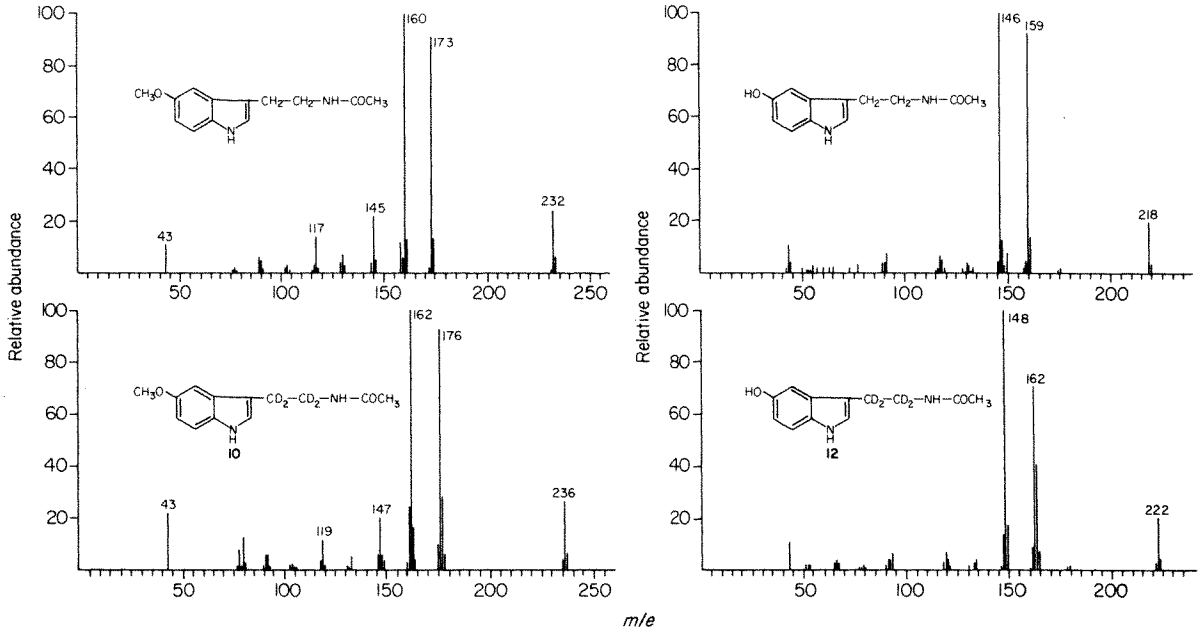
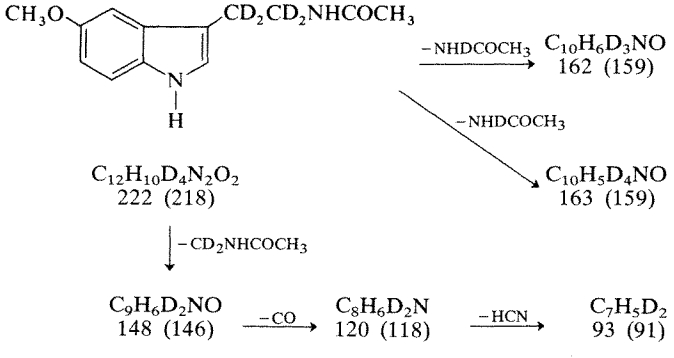
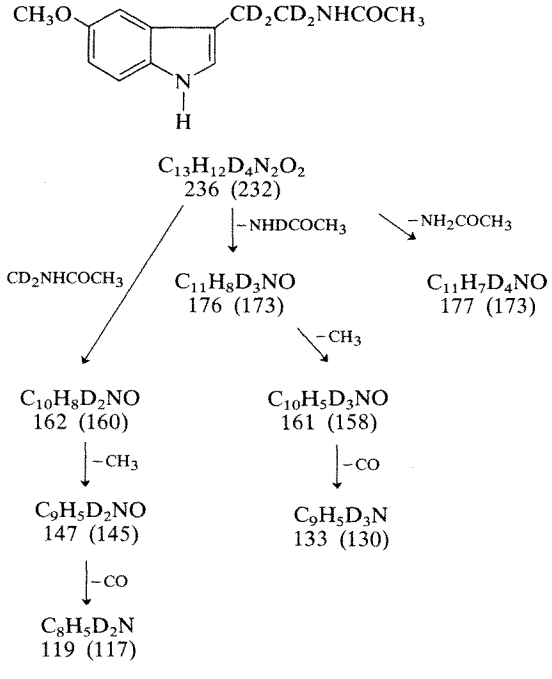


Figure 3. Mass spectra of deuterated and undeuterated *N*-acetyltryptamines.



Scheme 3. Fragmentation processes in *N*-acetyl-5-hydroxytryptamines (numbers are ion *m/e* values; those in parentheses refer to the analogous undeuterated ions).



Scheme 4. Fragmentation processes in *N*-acetyl-5-methoxytryptamines (numbers are ion *m/e* values; those in parentheses refer to the analogous undeuterated ions).

compounds **15** and **17** and *m/e* 58 for the undeuterated analogs. In the primary amine, 5-methoxytryptamine, the corresponding ions at *m/e* 32 and *m/e* 30 for the labeled and unlabeled species, respectively, are only about 20% of the base peak. It is interesting to note that the small peak at *m/e* 59 in **15** appears due to label randomization prior to fragmentation. The results obtained for the acetylated amines (Fig. 3) are summarized in Schemes 3 and 4. Comparison of the ratios of the intensities of the ions $[M-NH_2COCH_3+1]^+/[M-NH_2COCH_3]^{\ddagger}$ in the spectra of unlabeled *N*-acetyl-5-methoxytryptamine (*m/e* 173, 174) and unlabeled *N*-acetyl-5-hydroxytryptamine (*m/e* 159/160) with the corresponding ions in the appropriate deuterated compounds **10**(*m/e* 176, 177) and **12**(*m/e* 162, 163), shows that the previously reported (Scheme 4) loss of NH_2COCH_3 from the molecular ions is accompanied in the deuterated compounds by loss of $NHDCOCH_3$ the two processes being indistinguishable in the undeuterated molecules. This suggests that loss of NH_2COCH_3 occurs by rearrangement which involves a H from either the 2-position of the indole ring or the carbon atom of the sidechain adjacent to it. Either process can proceed via a 6-membered cyclic transition state. Although partial H/D scrambling in the parent ions could also account for these changes in intensities on deuteration, this is not supported by a comparison of the $[M-CH_2NHCOCH_3]^+$ ion region in the spectra of **10**(*m/e* 162) and **12**(*m/e* 148) and of the associated unlabeled analogs, namely *m/e* 160 and *m/e* 146, respectively. If significant scrambling had taken place in the moleucular ions, loss of $CD_2NHCOCH_3$ in the

spectra of the deuterated compounds should also be accompanied by loss of $CDHNHCOCH_3$ and $CH_2NHCOCH_3$, neither of which is observed to any significant extent.

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