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Electron Impact and Chemical Ionization Fragmentation of 5-Methoxytryptamine and Some 6-Methoxy-β-carbolines

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Electron impact (EI) and chemical ionization (CI) mass spectra of 5-methoxytryptamine (5-MT) and seven 6-methoxy-β-carbolines have been recorded and interpreted. Proposed fragmentation pathways are depicted on the basis of spectra of deuterium labelled analogs of 5-MT, 6-methoxy-1,2,3,4-tetrahydro-β-carboline (6-MeO-THBC) and 6-Methoxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (6-MeO-1-Me-THBC).

In the EI mass spectrum of 5-MT, the base peak *m/z* 160 is formed by the expulsion of CH_4N. The positive ion so formed is suggested to have a quinolinium structure. In chemical ionization with CH_4 as the reaction gas, the most abundant ion is produced by cleavage of NH_3.

The ion *m/z* 173 yields the base peak in the EI mass spectrum of 6-MeO-THBC. This ion is formed by a retro Diels-Alder reaction, an important fragmentation pathway of 6-MeO-1-Me-THBC, too.

6-MeO-harmalan easily loses one hydrogen atom in EI fragmentation followed by methyl loss from the methoxyl group, this latter process yielding the base peak, *m/z* 197 in the EI mass spectrum of 6-MeO-harman. M+1 is the base peak in the CI mass spectra of all 6-MeO-β-carbolines.

β-Carbolines occur in many genera of plants[1,2] and they are also found as normal constituents of human and animal tissues[3–7]. Some 1,2,3,4-tetrahydro-β-carbolines (THBCs) possess psychoactive properties affecting the central nervous system by several mechanisms, *e.g.*, by inhibiting 5-hydroxytryptamine uptake[8,9] and monoamine oxidase A.[10,11] They are connected with the etiology of some mental diseases like schizophrenia[12] and alcoholism[4,13–15] but the hypotheses are unsatisfactorily documented.[16]

In human and animal tissues, THBCs are found in trace amounts. Therefore, a highly selective and sensitive method is required for their analysis. Most determinations of THBCs have been performed by gas chromatography-mass spectrometry and the compounds were quantified by selected ion monitoring technique.[3–6,12–14] All measurements were performed by electron impact (EI) ionization and most of the mass spectrometric fragmentations of THBCs were interpreted on the basis of the report by Coutts *et al.*[17]

The aim of present study was to synthesize some THBCs and other compounds (*1–6*, Fig. 1) related to 5-methoxytryptamine and to compare their mass spectrometric fragmentation by EI ionization or chemical ionization (CI) by methane. Deuteriated analogues were synthesized to resolve the mass spectrometric fragmentation of the compounds.

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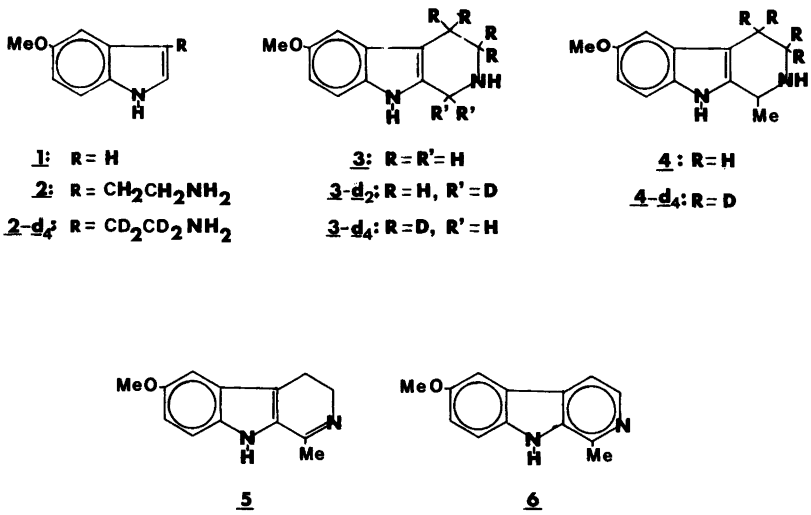


Fig. 1. Structures of the investigated compounds.

MATERIALS AND METHODS

General. All chemicals and solvents were of analytical grade quality. Compounds *3* and *4* were synthesized by the Pictet-Spengler reaction of *2* with glyoxylic acid and acetaldehyde, respectively.[18,19] In the same way, *3-d_4* and *4-d_4* were prepared from *2-d_4*. However, *3-d_2* was synthesized by the Fischer indole synthesis method.[19] Melting points were recorded by a capillary melting point apparatus and are uncorrected.

5-Methoxyindole (*1*) was purchased from EGA Chemie (Steinheim, West Germany). 5-Methoxytryptamine (*2*), 6-methoxyharman (*6*) and 6-methoxyharmalan (*5*) were purchased from Sigma (St. Louis, USA) (Fig. 1).

The mass spectra of the compounds were recorded using a Jeol JMS D 300 mass spectrometer with JMA 2000 mass analysis system at the resolution of 1000. The ionizing electron beam energy was 70 or 10 eV in the EI, and 300 eV in the CI technique. Methane was used as the reaction gas. The ionization current was 300 μA and ion chamber temperature 230 °C in both techniques. Samples were admitted using a solids insertion probe.

Syntheses. 2,3-Piperidinedione-3-(4-methoxyphenyl) hydrazone. A solution of 2-piperidone-3-carboxylic acid was prepared from its ethyl ester (7.5 mmol) by keeping this at 25 °C in 5.5 ml of 1 M (*aq.*) potassium hydroxide for 2 h. Then a solution of *p*-methoxybenzenediazonium chloride, prepared from *p*-anisidine (5.5 mmol) and sodium nitrite (5.5 mmol) in 15 ml of 3 M hydrochloric acid, was added. The pH of the resulting solution was adjusted to 4 with glacial acetic acid. After stirring at 0 °C for 5 h the yellow precipitate was filtered and washed with cold water, yield 3.3 mmol (55 %), m.p. 165–170 °C.

6-Methoxy-1-oxo-1,2,3,4-tetrahydro-β-carboline. 2.6 mmol of 2,3-piperidinedione-3-(4-methoxyphenyl) hydrazone was refluxed with 5 ml of 90 % formic acid for 1/2 h. After addition of 5 ml of water, an oily phase separated out and was dissolved in hot ethanol. A pale brown precipitate was formed in the cooled solution, yield 1.6 mmol (58 %), m.p. 267–272 °C.

6-Methoxy-1,2,3,4-tetrahydro-β-carboline-1,1-d_2 (3-d_2). 1.4 mmol of 6-Methoxy-1-oxo-1,2,3,4-tetrahydro-β-carboline was suspended in 50 ml of dry tetrahydrofuran, and 23.8 mmol of lithium aluminium deuteride was added. The resulting mixture was refluxed for 10 h. Moistured tetrahydrofuran was added to the suspension to inactivate the unreacted reagent. The organic phase was separated and evaporated. The remaining dark-brown oily mass was dissolved in 10 ml of dichloromethane and washed twice with equal portions of

water. Dichloromethane was evaporated, and the slightly brown crystals were washed with aqueous ethanol, yield 1.2 mmol (83 %), m.p. 219–221 °C. MS, *m/z* (rel. int., %): 204 (40,M), 175 (100), 160 (50). Fig. 4.)

5-Methoxy-3-indoleglyoxylyl chloride. A sample of *1*, 34.2 mmol, was dissolved in 100 ml of sodium-dried diethyl ether, and 39.6 mmol of oxalyl chloride was added to the solution at 5 °C. After the solution had been stirred at room temperature for 15 min, an orange-coloured solid separated. After two ether washings, the yield was 30.2 mmol (88 %), m.p. 123–126 °C. MS, *m/z* (rel.int., %): 237 (5,M), 209 (20), 174 (100), 131 (10).

5-Methoxy-3-indoleglyoxylylamide. 29.5 mmol of 5-Methoxy-3-indoleglyoxylyl chloride was suspended in 200 ml of dry toluene, and a dry ammonia stream was passed through the suspension, until the solid turned yellow. The solid was filtered and washed with water, yield 28.4 mmol (96 %), m.p. 240–244 °C. MS, *m/z* (rel. int., %): 218 (25,M), 174 (100), 131 (10).

5-Methoxytryptamine-α,α,β,β-d_4 (2-d_4). 71.4 mmol of lithium aluminium deuteride was added to the stirred mixture of 22.9 mmol of 5-methoxy-3-indoleglyoxylylamide and 50 ml of dry tetrahydrofuran. This mixture was refluxed for 10 h before the interruption of the reaction with aqueous tetrahydrofuran. After filtration, tetrahydrofuran was evaporated and the oily mass dissolved in dry dichloromethane. After addition of saturated ethanolic hydrogen chloride white *2-d_4* hydrochloride precipitated, yield 7.8 mmol (34 %), m.p. 230–236 °C. The free base was obtained by adding of 0.1 M sodium hydroxide solution to the water solution of *2-d_4* until the free base began to precipitate. The precipitate was filtered and washed with a small amount of hot water, yield 7.1 mmol (91 %), m.p. 118–121 °C. MS, *m/z* (rel. int., %): 194 (27,M), 163 (61), 162 (100), 147 (18). (Fig. 2.)

6-Methoxy-1,2,3,4-tetrahydro-β-carboline-3,3,4,4-d_4 (3-d_4). 4.3 mmol of *2-d_4* hydrochloride was dissolved in 20 ml of water. 4.3 mmol of glyoxylic acid monohydrate was added and the pH of the solution adjusted to 4 using 1 M solution of potassium hydroxide. During 1 hour, a precipitate formed in the stirred solution. The filtered precipitate was suspended in 10 ml of water and 2 ml of concentrated hydrochloric acid added. This suspension was refluxed for 1/2 h, and a clear solution formed. After cooling, *3-d_4* was precipitated by adding of 10 % KOH solution, yield 1.5 mmol (79 %), m.p. 216–219 °C. MS, *m/z* (rel. int., %): 206 (48,M), 205 (18), 176 (14), 175 (100), 174 (14), 160 (47). (Fig. 4.)

6-Methoxy-1,2,3,4-tetrahydro-β-carboline (3). This was synthesized analogously to *3-d_4*, but from *2* hydrochloride, yield 1.5 mmol (75 %), m.p. 216–219 °C. MS, *m/z* (rel. int., %): 202 (49,M), 201 (16), 174 (16), 173 (100), 158 (50). (Fig. 4)

(±)-6-Methoxy-1-methyl-1,2,3,4-tetrahydro-β-carboline-3,3,4,4-d_4 (4-d_4). 4.3 mmol of *2-d_4* hydrochloride was dissolved in 25 ml of water. The pH was adjusted to 4 with 0.1 M hydrochloric acid, and 31.8 mmol of acetaldehyde in 15 ml of mixture of water and ethanol (15/85 v/v) was added. This solution was stirred under a nitrogen atmosphere for 2 days at 20 °C. During a few days in the refrigerator in a neutralized solution, a white precipitate was formed, yield 0.7 mmol (15 %), m.p. of the base 222–225 °C. MS, *m/z* (rel. int., %): 220 (52,M), 219 (25), 218 (15), 206 (15), 205 (100), 204 (13), 189 (40), 188 (44), 174 (14). (Fig. 4.)

(±)-6-Methoxy-1-methyl-1,2,3,4-tetrahydro-β-carboline (4). This was synthesized similarly to *4-d_4* but from *2* hydrochloride, yield 0.8 mmol (19 %), m.p. of the base 224–226 °C. MS, *m/z* (rel. int., %): 216 (54,M), 215 (22), 202 (14), 201 (100), 187 (42), 186 (15), 172 (14). (Fig. 4.)

RESULTS AND DISCUSSION

Synthesis of deuterium labelled compunds. The deuteriated compounds were prepared by reduction of corresponding carbonyl compounds; 5-methoxy-3-indoleglyoxylylamide and 6-methoxy-1-oxo-1,2,3,4-tetrahydro-β-carboline. The reduction of THBC derivatives with a carbonyl group at C_1 with lithium aluminium deuteride in dry tetrahydrofuran gave a good yield (80 %) of *3-d_2*. In addition, the deuterium atoms were incorporated into the correct positions. When preparing *2-d_4*, an intractable suspension was formed after the addition of

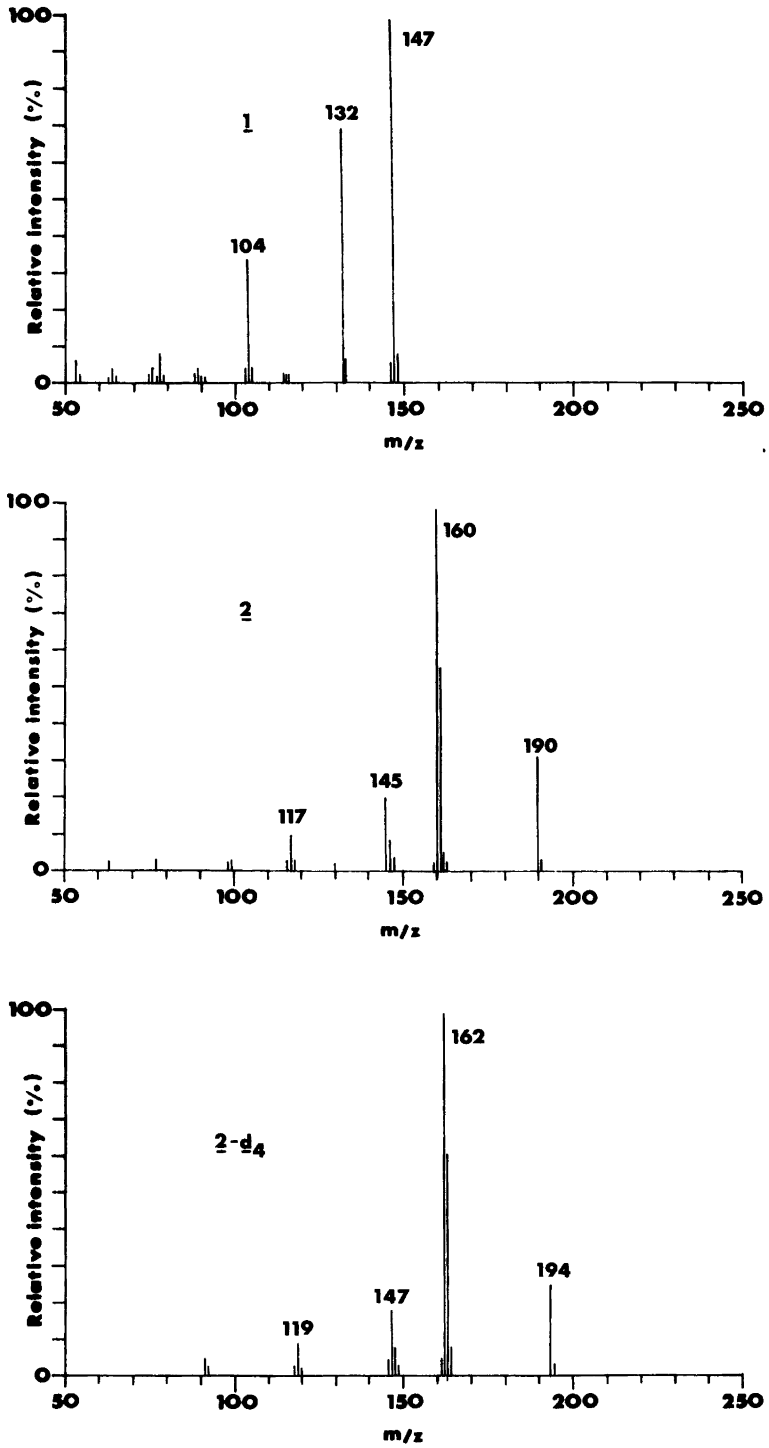


Fig. 2. The EI mass spectra of compounds *1*, *2*, and *2*-d_4.

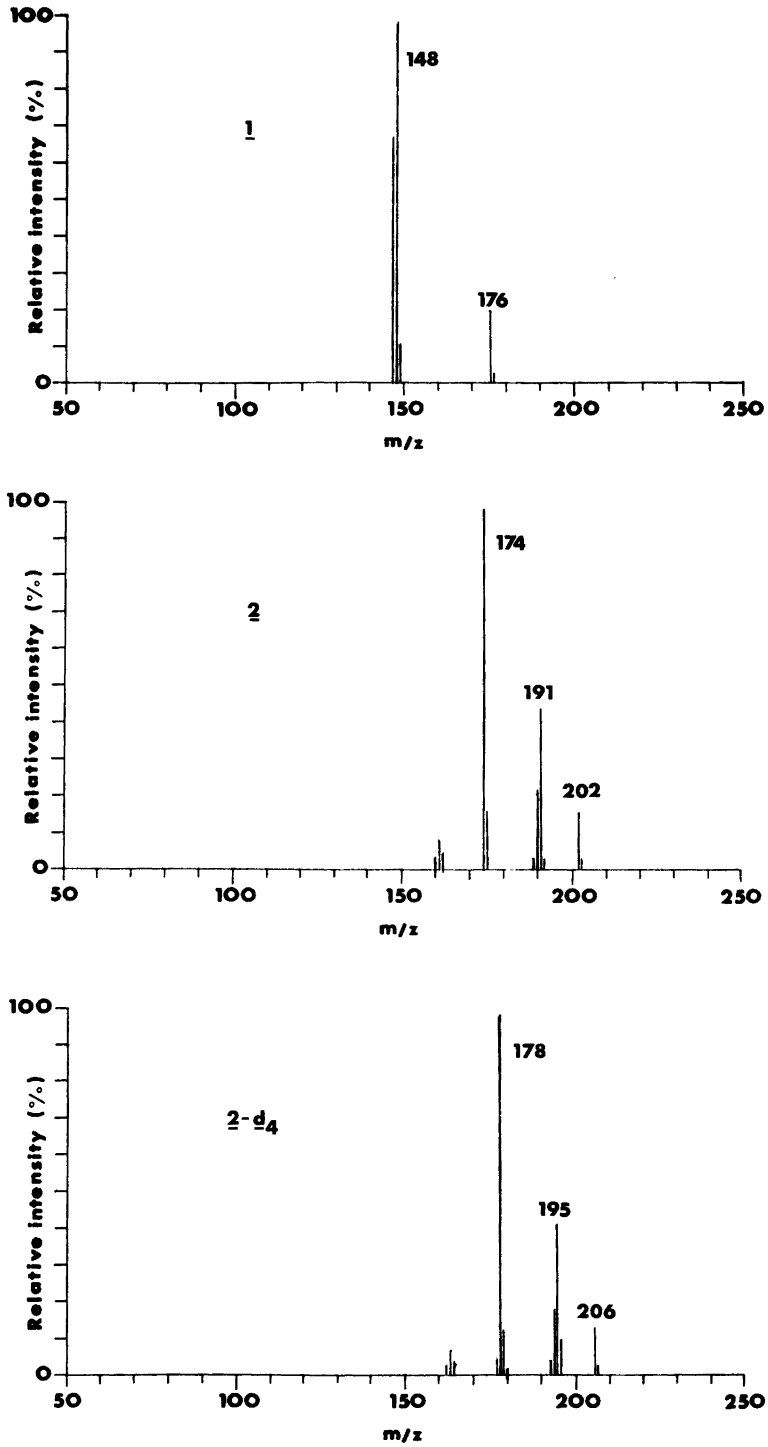


Fig. 3. The CI mass spectra of compounds *1*, *2* and *2-d_4*.

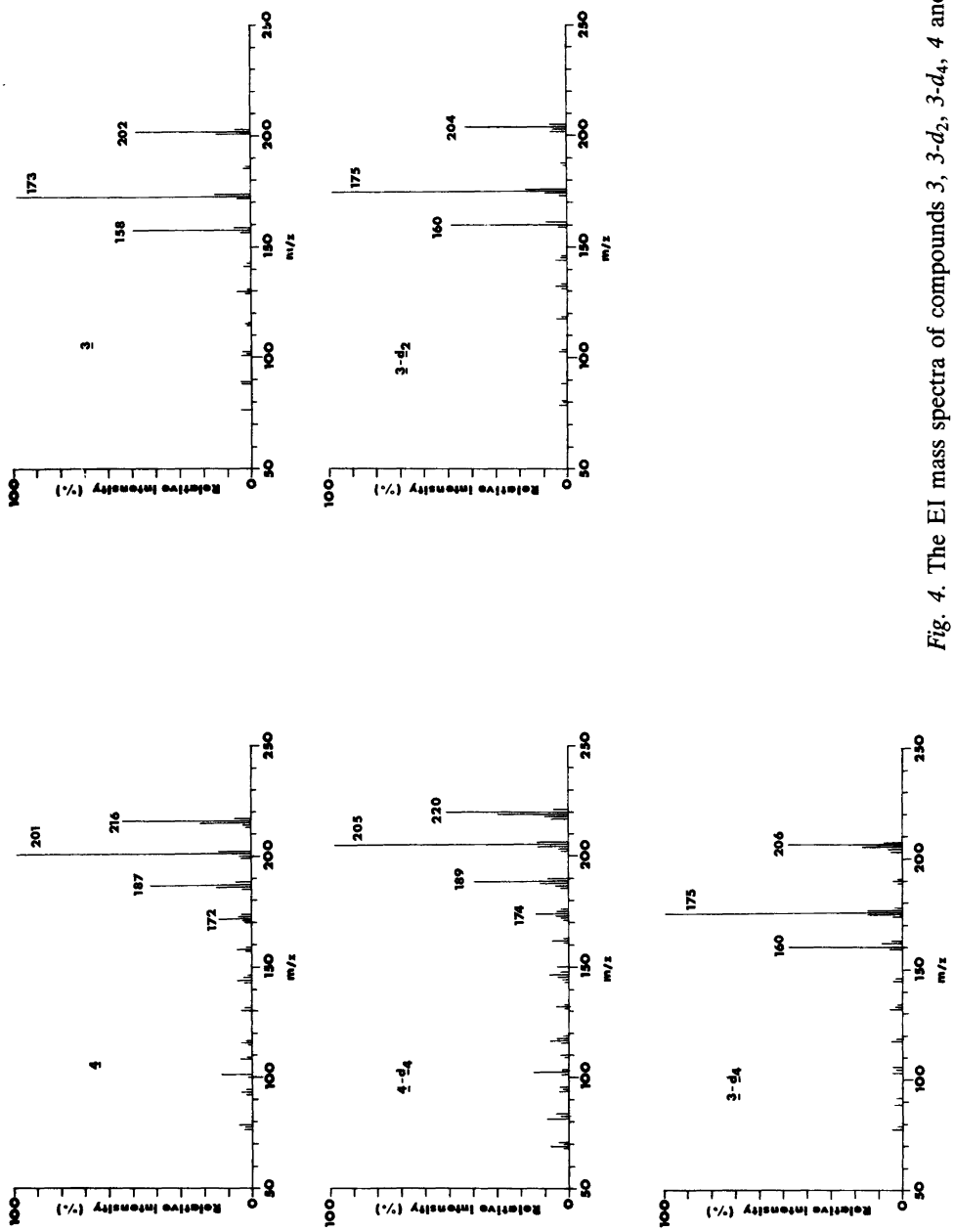


Fig. 4. The EI mass spectra of compounds *3*, *3*-d_2, *3*-d_4, *4* and *4*-d_4.

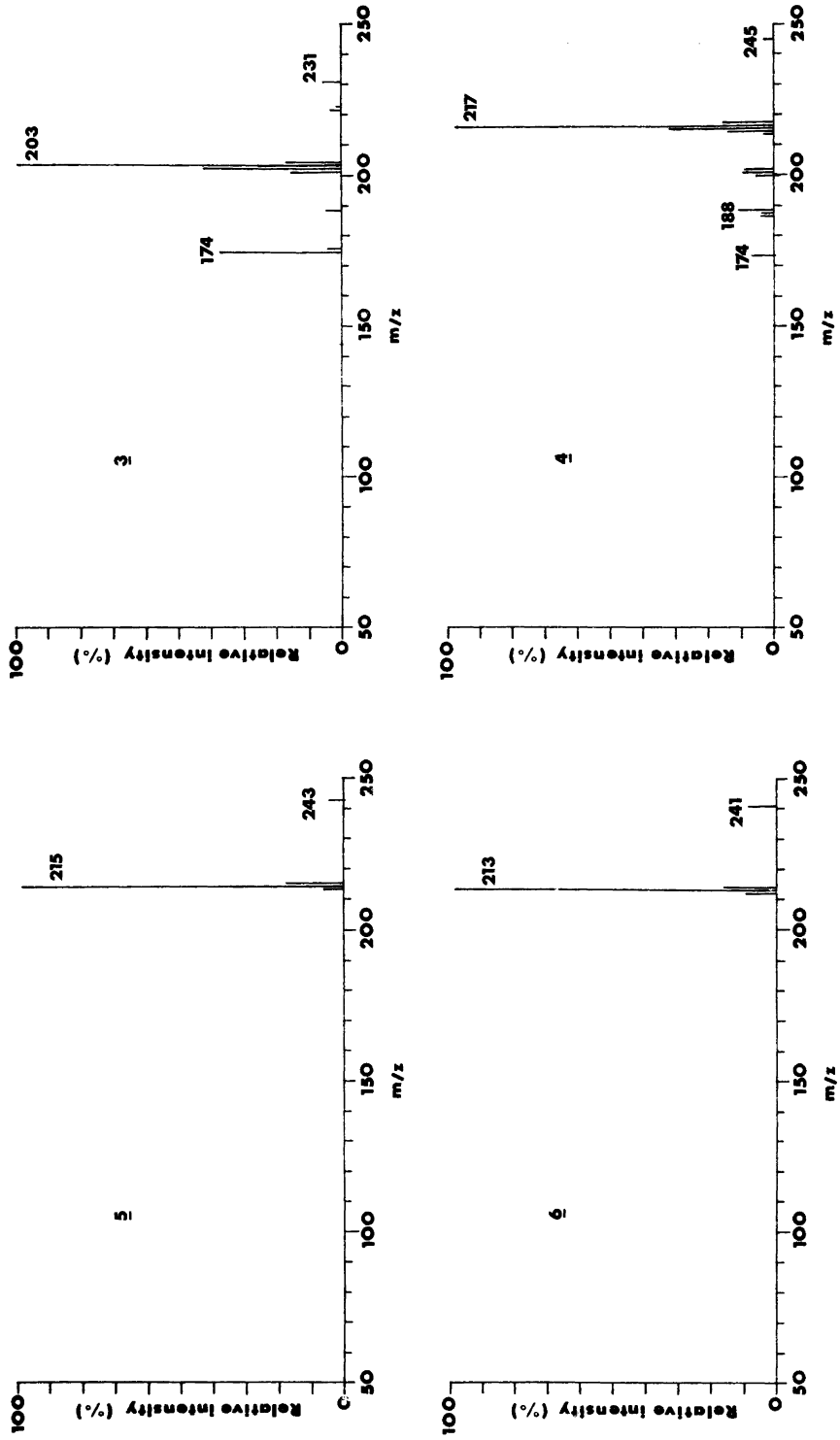


Fig. 5. The CI mass spectra of compounds *3-6*.

Table 1. The isotopic purity of the deuterium labelled compounds. Results have been corrected for $(M-H)^+$ peaks.

Compound	D_0	D_1	D_2	D_3	D_4
2-d_4	6.5	0	0	0	93.5
3-d_2	4.6	1.9	93.5	0	0
3-d_4	1.6	0	5.7	4.4	88.3
4-d_4	3.6	1.3	19.4	11.4	64.3

aqueous tetrahydrofuran. This made the extraction difficult and the poor crystallization tendency of the compund diminished the yield (34 %).

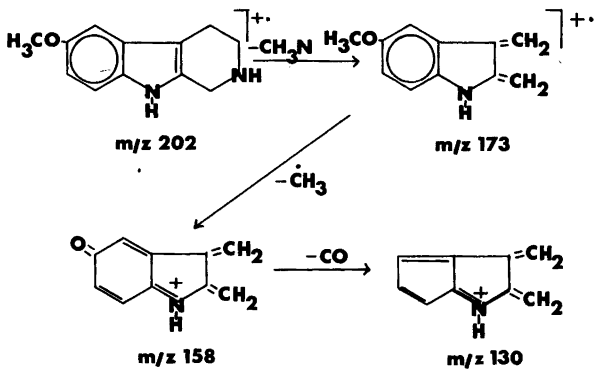
The isotopic purity of the compounds was determined by EI mass spectrometry, monitoring the ratios of molecular ions of labelled and unlabelled compunds at an electron energy of 10 eV (Table 1). The compounds which crystallized immediately after reduction (*2-d_4* and *3-d_2*) showed a higher isotopic purity than the copounds (*3-d_4* and *4-d_4*) synthesized from *2-d_4*. Deuterium exchange during synthesis in an acidic solution had taken place, although the deuterium atoms in *2-d_4* were at positions where proton exchange under neutral conditions is inhibited.

EI and CI mass spectrometry of 5-methoxytryptamine (2). The base peaks in the EI mass spectra of *2* and *2-d_4* were *m/z* 160 and *m/z* 162, respectively, indicating the elimination of CH_2NH_2 or CD_2NH_2 fragments. A quinolinium structure has been suggested for these ions.[20] Ions *m/z* 161 and 163 were of great importance too, having relative intensity of 60 % for *2* and 61 % for *2-d_4*. The mass difference between the ions indicated that they were formed by a McLafferty rearrangement. A further fragmentation of those ions yielded the ions *m/z* 145 and 147 for *2* and *2-d_4*, respectively, resulting from the methyl loss from the 5-methoxy substituent. An additional expulsion of CO gives ions *m/z* 117 (*2*) and 119 (*2-d_4*). (Fig. 2).

The expulsion of a CH_2NH_2 fragment from *2* by CI was less important when compared with the loss of the same fragment by EI. The cleavage of ammonia led to the base peak formation. The methoxy group of *2* remained unaffected by CI although the loss of methanol in mass spectra of some alkaloids with methoxy substituent forms a prominent peak.[21] The intensity of the quasimolecular $(QM)^+$ ion $(M+H)^+$ was unexpectedly low, not more than 50 %, indicating the lability of a molecule with an aliphatic amino group. Another interesting peak was found in the CI mass spectrum of *2*. This peak $(M+12)^+$ (rel. int. 15 %) most probably indicated an immonium ion formation of the primary amino group with the reaction gas. The peak was not found in the CI mass spectrum of *1*, indicating that its formation cannot be connected with the indole structure of *2* (Fig. 3.)

The EI and CI framgmentation of some 6-methoxy-β-carbolines. The tetrahydropyridine ring of *3* and *4* was very easily broken by EI, whereas the aromatic pyridine ring of *6* remained unaffected. The most intense ion $(M-29)^+$ *m/z* 173 in the mass spectrum of *3* was formed by a retro Diels-Alder reaction. Further fragmentation produced ions $(M-44)^+$ *m/z* 158 and $(M-72)^+$ *m/z* 130 after the expulsion of the methyl radical and the CO molecule, respectively, from the methoxy substituted benzene ring (Scheme 1). The molecular ion and those three ions together accounted for 60 % of the total ion current.

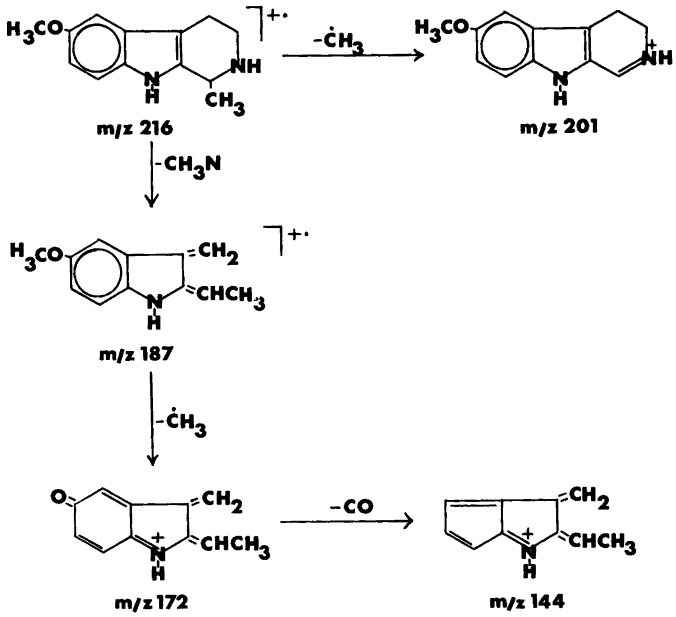
The fragmentation of *4* occurred via the expulsion of the fragment CH_3N, forming the ion $(M-29)^+$ *m/z* 187 with relative intensity of 42 %. The loss of CHD_2N from *4-d_4*



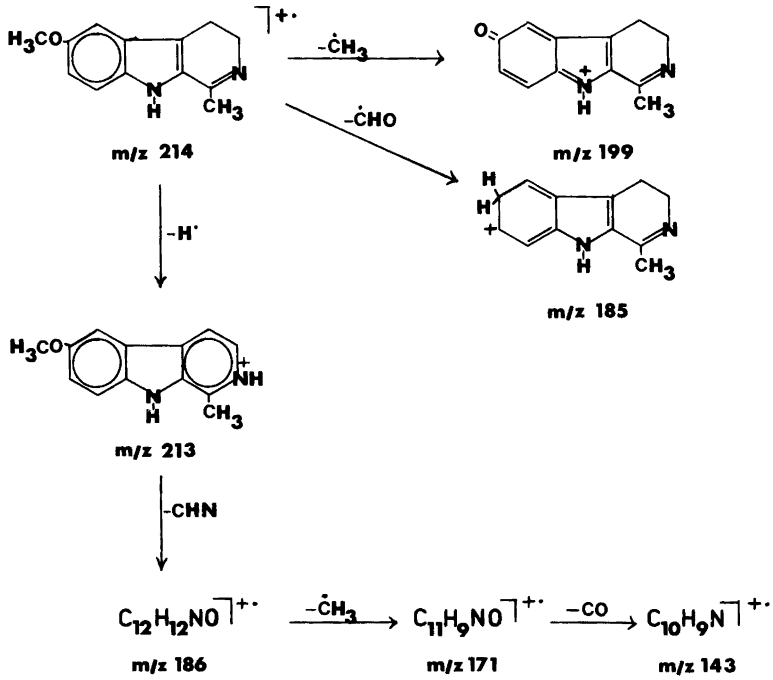
Scheme 1. EI fragmentation pattern of compound *3*.

confirmed this retro Diels-Alder fragmentation pathway. However, the base peak of *4* was formed by the expulsion of the methyl radical from C_1. The ion $(M-29)^+$ after the retro Diels-Alder reaction was further fragmented by the successive losses of CH_3 and CO from the ring methoxy group (Scheme 2, Fig. 4.). Compound *5* easily underwent hydrogen loss by EI. The ion $(M-H)^+$ was the base peak although the relative intensity of the molecular ion was 90 % reflecting a good stability of the compound against EI (Scheme 3). Further fragmentation occurred via the expulsion of the methyl radical from 6-methoxy group. Other ions formed had relative intensities less than 10 %.

Dehydrogenation of *4* to *6* greatly stabilized the molecule against EI. The aromatic pyridine ring in *6* in contrast to the tetrahydropyridine ring in *4* was very stable. The



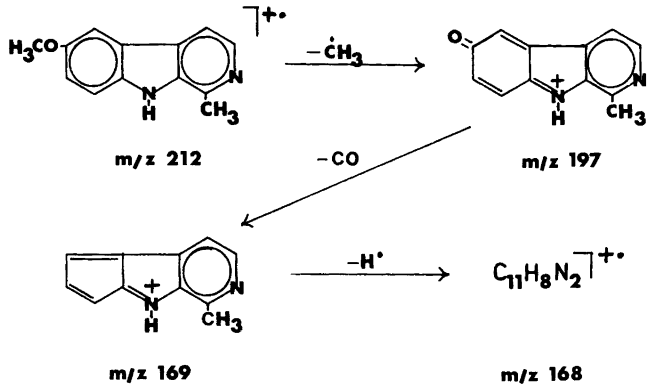
Scheme 2. EI fragmentation pattern of compound *4*.



Scheme 3. EI fragmentation pattern of compound *5*.

molecular ion of *6* was not, however, the base peak, since the expulsion of the methyl radical from the 6-methoxy substituent easily occurred, forming the most abundant ion $(M-15)^+$ *m/z* 197. Further expulsion of CO followed with a low intensity. (Scheme 4).

The degree of aromatization of β-carboline alkaloids greatly influenced the EI mass spectra. The retro Diels-Alder reaction was the main fragmentation pathway of 6-methoxy-THBCs (Schemes 1 and 2), while this mechanism of *5* was unimportant, and the loss of one hydrogen atom from the parent molecule formed the base peak (Scheme 3). On the other hand, the expulsion of the methyl radical from the 6-methoxy group of *6* gave the most



Scheme 4. EI fragmentation pattern of compound 6.

abundant ion (Scheme 4). These results agree with the earlier report based on accurate mass measurements.[16] Because of the mild ionization technique, the CI mass spectra of *3–6* had few peaks, the $(M+H)^+$ ions forming the base peaks. The relative intensities of the M^+ ions varied from 30 % to 50 % in the CI mass spectra of *3* and *4*, while the relative intensities of M^+ of *5* and *6* were less than 10 % (Fig. 5). The tetrahydro derivatives (*3* and *4*) only underwent further fragmentation. The proton affinity of the reaction gas was strong enough to break down the tetrahydropyridine ring. The ion $(M-29)^+$ in the CI mass spectrum of *3* was formed by a retro Diels-Alder reaction. The fragmentation pathway was verified using deuterium labelled analogues (*3*-d_2 and *3*-d_4).

The methyl group from C_1 of *4* or *4*-d_4 was also loosened by the reaction gas. However, the relative intensities of the ions so formed were 10 %. The tetrahydropyridine ring of *4* was affected by the methane, too, causing the loss of CH_3N by a retro Diels-Alder mechanism. The $(QM)^+$ ion $(M+29)^+$ was only seen in the CI mass spectrum of *6* with low intensity. This indicated its ability to add the ion $(C_2H_5)^+$ during the ionization.

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