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OXIDATIVE METABOLISM OF MESCALINE IN THE CENTRAL NERVOUS SYSTEM—III

SIDE CHAIN DEGRADATION OF MESCALINE AND FORMATION OF 3,4,5-TRIMETHOXY-BENZOIC ACID *IN VIVO*

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Abstract—After intraperitoneal injection of 8-[^{14}C]mescaline.HCl into mice 0·05 per cent of the injected radioactivity was found within 1 hr in the respiratory gases. This observation prompted us to search for mescaline metabolites with a degraded side chain. 3,4,5-trimethoxy-benzoic acid was identified in brain and liver by derivative formation and mass spectrometry. *N*-acetyl-mescaline was also identified by mass spectrometry. Some new cationic mescaline metabolites were detected in brain but the available data did not allow us to establish conclusively the structure of these compounds

ALTHOUGH there are some observations which suggest direct interaction of mescaline with certain receptors[2–7], it is evident from other studies[8–10] that with mescaline metabolic steps, intercalated between resorption and psychotomimetic activity, are more probable than with LSD-25, which has comparable behavioural effects[8,11] and which moreover exhibits cross-tolerance with mescaline.[12,13] Despite these observations there are very few studies of mescaline metabolism,[8] especially studies concerned with mescaline metabolism in brain.[14–17] One of the reasons for this may be that with a few exceptions[18–21] no new mescaline metabolites have been found since the classical work of Slotta and Müller,[22] despite the development of radioactive tracer methods. Interest was focused mainly on mescaline actions on certain metabolic or physiologic processes, and on interactions with supposed receptors[8–10,23–27] Another reason for the small number of metabolic studies in brain may be the lack of sensitive detection methods for mescaline and its metabolites,[8] and the fact that only a very small amount of peripherally administered mescaline penetrates into the brain[15,28,29] so that relatively large amounts of radioactive mescaline are required. The synthesis of 2,6[^{3}H]mescaline[30] facilitates the administration of amounts of radioactivity sufficient for brain metabolic studies. It was established previously that under controlled conditions of tissue extraction, and during the body passage of this compound, no exchange of tritium occurs.[31]

Abbreviations: TLC = thin-layer chromatography; TLE = thin-layer electrophoresis; MS = mass spectrometry; MAO = monoamine oxidase; DAO = diamine oxidase; 3,4,5-TMPAA = 3,4,5-trimethoxy-phenylacetic acid; 3,4,5-TMBA = 3,4,5-trimethoxy-benzoic acid; DANS-Cl = 1-dimethylamino-naphthalene-5-sulphonyl chloride; PPO = 2,5-diphenyloxazol; POPOP = 1,4-bis-[2-(5-phenyloxazolyl)]-benzene; S.E.M. = standard error of the mean value.

In the present paper we describe the *in vivo* formation in mouse brain and liver of 3,4,5-trimethoxybenzoic acid (3,4,5-TMBA), a new mescaline metabolite. The presence in brain of some new and as yet unidentified, cationic mescaline metabolites is demonstrated.

MATERIALS AND METHODS

Radiochemicals. 8[^{14}C]mescaline.HCl (specific activity 4·5 mCi/mmole) was purchased from New England Nuclear Corporation, Boston. 2,6[^{3}H]mescaline.HCl (specific activity 54·1 mCi/mmole) was prepared as described previously.[30]

Reagents. *N*-acetyl-mescaline was prepared by reaction of mescaline.HCl with acetic anhydride in the presence of pyridine. Purification was achieved by ion exchange chromatography on columns of Dowex 50W $\times$8 (100–200 mesh) with methanol as eluant, and recrystallization from toluene. Purity of the compound was checked by TLC and MS.

1,2,3-trimethoxy-4,5,6-tribromobenzene was prepared from pyrogallol trimethylether by bromination in acetic acid solution, 3,4,5-trimethoxy-2,6-dibromophenylacetic acid was obtained by bromination in water of 3,4,5-trimethoxy-phenylacetic acid (3,4,5-TMPAA) at room temperature.

3,4,5-TMPAA was obtained from Fluka A. G., Buchs, 3,4,5-TMBA, mescaline sulphate and other chemicals were from E. Merck, Darmstadt.

1-Dimethylaminonaphthalene-5-sulphonyl chloride (DANS-Cl) was prepared in our laboratory as described previously.[32]

Experimental animals. Male albino mice (NMRI, SPF, Süddeutsche Versuchstierfarm, Tuttlingen) weighing 30–35 g were used. They were housed in standard cages in groups of approximately 10 animals. Food (Altromin standard diet) and water were allowed *ad libitum* until the beginning of the experiments.

Determination of respiratory ^{14}C after 8[^{14}C]mescaline administration. Two groups of animals were injected with a total of 0·11 mCi 8[^{14}C]mescaline.HCl and 2·9 mCi 2,6[^{3}H]mescaline.HCl. (The mescaline dose was the same as in all other experiments, namely 120 mg/kg body weight.) The animals were placed into a Perspex tube through which a stream of washed air was pumped at 1·5 l/hr. Respiratory water was adsorbed in a tube containing silica gel, and CO_2 was absorbed in a column filled with glass beads, containing 50 ml 1 N KOH. After 1 hr the animals were killed by decapitation and different organs were removed as quickly as possible for the identification of mescaline metabolites (see below). The KOH absorption solution was transferred quantitatively into a three-necked bottle and acidified by dropwise addition of a total of 15 ml of 5 N H_2SO_4 through a funnel. The CO_2 liberated by the acid was flushed by a slow stream of pure nitrogen into a stirred, ice cold solution of 2 ml of β-phenylethylamine dissolved in 2 ml methanol. Ten ml of a liquid scintillator (containing 4·2 g PPO and 0·05 g POPOP/l toluene) were added to the β-phenylethylamine solution after 30 min, and radioactivity was then determined by liquid scintillation spectrometry at a window width suitable for $^{14}C/^{3}H$-measurement. Control groups of mice (without injected radioactive material) were treated in the same manner. The β-phenylethylamine solutions obtained from these experiments were used for the determination of the background.

Administration of mescaline and preparation of tissue extracts. Four mg of radioactive mescalines HCl was injected intraperitoneally in 0·2 ml solution. After decapi-

tation (normally 1 hr after mescaline administration) the brain, liver and kidneys were quickly removed and immediately homogenized with 5 or 10 parts of ice cold 0·2 N perchloric acid. The homogenates were stored for 12 hr at +3° and were then centrifuged for 30 min at approximately 800 *g*. The supernatant was used as tissue extract.

Separation of the tissue extract and isolation of the mescaline metabolites

Isolation of the neutral, anionic and cationic mescaline metabolites was achieved by a combination of several methods, starting with ion exchange chromatography or with solvent extraction of the tissue extract. A diagrammatic survey of the methods used is given in Fig. 1.

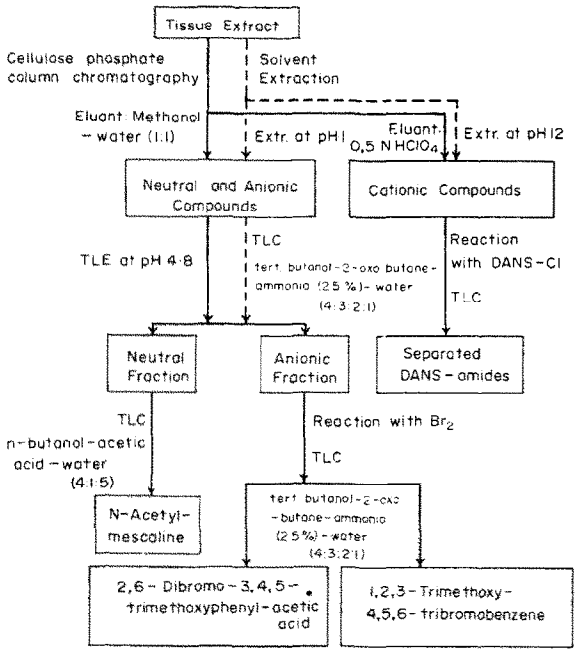

FIG. 1. Diagrammatic survey of the methods used for the isolation of the mescaline derivatives from tissue extract.

(a) *Ion exchange chromatography and TLE.* Cellulose phosphate (H^+-form; Whatman P11, W. & R. Balston, Maidstone) slurry in methanol–water (1 : 1) (by volume) was put into 150 × 25 mm columns. The combined extracts of 10 brains were neutralized with K_2CO_3. Ethanol was added to give a 30% ethanol solution. After removal of the precipitated $KClO_4$, the supernatant was applied to the column at a flow rate of 1 ml/cm^2/hr. The neutral plus anionic compounds were eluted from the column with a total of 300 ml of a methanol–water mixture (1 : 1). The cationic fraction was obtained by elution with 200 ml of 0·5 N $HClO_4$. After neutralization of the perchloric acid with K_2CO_3 and removal of $KClO_4$ the fractions were evaporated almost to dryness *in vacuo* and redissolved in 1 ml of ethanol. The ethanol solutions were applied to thin-layer plates (200 μm layer of silica gel G_{F254}; E. Merck, Darmstadt) in 12 cm long streaks; distance from the plate edge 10 cm. The plates were

sprayed with pyridine acetate buffer pH 4·8,[33] and then submitted to TLE at 40 V/cm (3°) in a Camag TLE chamber (Camag, Muttenz). The neutral plus anionic compounds were run for 4 hr, the cationic compounds for 2 hr. By scanning with a radiochromatogram scanner (LB 2720, Berthold, Erlangen) two radioactive zones were discernible on the electrophoretograms of the anionic plus neutral compounds, one near the origin and one moving towards the anode. A thorough examination of a TLE for radioactive compounds by scraping out 1 cm wide zones and measurement of the radioactivity in the methanol extracts of these zones by liquid scintillation counting yielded the same radioactivity profile along the length of the electrophoretogram as the radioactivity scanning. The neutral fraction corresponded in its electrophoretic mobility to *N*-acetyl-mescaline, the anionic fraction to 3,4,5-TMPAA and 3,4,5-TMBA. The localization of the reference compounds on the TLE and TLC was achieved by fluorescence quenching after thorough drying at 70°. The radioactive zones were scraped off and eluted from the silica gel with 3 × 2 ml of methanol. These extracts were submitted to further procedures suitable for the identification of the labelled compounds (see below).

(b) *Solvent extraction and TLC.* 20–50 ml of tissue extract (brain or liver) were saturated with Na_2SO_4 and then extracted with 3 × 100 ml ethyl acetate. The combined extracts (acidic plus neutral compounds) were washed with 50 ml of 0·2 N $HClO_4$, dried and evaporated to dryness in vacuo, and subsequently submitted to TLC.

TLC of this fraction was carried out using tert. butanol-2-oxobutane–ammonia (25 per cent)–water (4:3:2:1) as solvent. The cationic compounds were obtained from the residual tissue extract after saturation with $Na_2CO_3 . 1OH_2O$, adjustment to pH 12 with NaOH, and extraction with 3 × 100 ml of ethyl acetate.

(c) *Isolation of the individual mescaline derivatives.* The *neutral fractions* (see Fig. 1) were rechromatographed using the organic layer of the solvent mixture *n*-butanol–acetic acid–water (4:1:5). The anionic fractions were spread out over zones of 10 × 30 mm on the thin-layer plates. Samples of 3,4,5-TMPAA, 3,4,5-TMBA and 2,3,4-TMBA were also applied to the plates as reference compounds. The plates were placed into Br_2 vapour saturated chromatographic tanks for 5 min. After removal of excess bromine by storage of the plates under an exhaustor for 5 min, the chromatograms were developed with *tert.*-butanol–2-oxo-butane–ammonia (25 per cent)–water (4:3:2:1). Again the spots were visualized by means of fluorescence quenching under a u.v. lamp (254 nm). The corresponding zones were scraped off, and the brominated derivatives eluted from the silica gel with ethanol, in the case of the anionic fractions obtained by TLE. The residues of these extracts were submitted to MS. The anionic fractions obtained by solvent extraction and TLC (see Fig. 1) were rechromatographed first with 96% ethanol as solvent, and rechromatographed on a second plate with methanol–acetone (95:5).

The *cationic compounds* (see Fig. 1) were immediately submitted to reaction with DANS-Cl under the usual reaction conditions[32,34] in 5 ml of acetone–water (3:1) containing 5 mg of DANS-Cl. The DANS-amides were extracted with 2 × 5 ml of toluene. The residue of the washed toluene phase was applied to a thin-layer plate in a 10 cm long streak. The plate was developed twice with tetrachloromethane–triethylamine (5:1). (In some experiments the DANS-amides were separated bi-dimensionally using the solvents benzene–methanol (9:1) (1st dimension) and benzene–triethylamine

(5:1) (2nd dimension), instead of unidimensional separation with tetrachloromethane–triethylamine.) Three radioactive zones were discernible on these chromatograms by scanning. The radioactive zones were eluted with ethyl acetate, and the extracts were reapplied to thin-layer plates and separated two-dimensionally using the solvents cyclohexane–butyl acetate (3:8) (1st dimension) and tetrachloromethane–triethylamine (5:1) (2nd dimension).[34] All radioactive spots discernible on these chromatograms were fluorescent. After elution of the radioactive spots, one part of the extract was used for the determination of the $^3H/^{14}C$-ratio by liquid scintillation spectrometry, the other part was submitted to MS.

Radioactivity measurement. Radioactivity of the samples dissolved in 1 ml of methanol was determined by liquid scintillation on a Packard TriCarb (model 3375) using 10 ml of PPO in toluene (5g/l) as scintillator solution. Double isotope measurements were carried out by the two channel method. Counting efficiency was determined by internal standards (New England Nuclear Corporation, Boston). Tritium values were corrected for ^{14}C-counts in the ^3H-channel.

Mass spectrometry. The mass spectra were prepared with the CH5 mass spectrometer (Varian, MAT, Bremen) at an electron beam energy of 70 eV. Temperature of the electron source was 250°.

RESULTS

In vivo *formation of* $^{14}CO_2$ *from* 8[^{14}C]*mescaline*

In two independent experiments with two groups of five mice $^{14}CO_2$ formation was measured for 1 hr after the injection of a mixture of 0·11 mCi 8[^{14}C]mescaline·HCl and 2·9 mCi 2,6[^3H]mescaline·HCl. During this time 0·05 per cent of the injected ^{14}C was expired as $^{14}CO_2$.

Liver and brain of the animals used in the respiration experiments were extracted and used for the identification of mescaline metabolites. In similar experiments in which mescaline mixtures with $^3H/^{14}C$-ratio of 20 ± 1 were injected, different organs of the animals were used for the determination of $^3H/^{14}C$-ratios in the acidic, neutral and cationic mescaline metabolites. As can be seen from Table 1, significant ^{14}C losses were observable only in the brain fractions containing the anionic mescaline metabolites, and to a smaller extent in the neutral fraction, whereas no changes in the $^3H/^{14}C$-ratios were observed in the fractions isolated from liver and kidney.

TABLE 1. $^3H/^{14}C$-RATIOS OF THE NEUTRAL, ANIONIC AND CATIONIC FRACTIONS OF THE PERCHLORIC ACID EXTRACTS OF DIFFERENT MOUSE ORGANS 1 hr AFTER THE INTRAPERITONEAL ADMINISTRATION OF 120 mg/kg OF A MIXTURE OF 8[^{14}C] AND 2,6[^3H]-MESCALINE.HCl

Organ	Neutral fraction $^3H/^{14}C$	Anionic fraction $^3H/^{14}C$	Cationic fraction $^3H/^{14}C$
Brain	22·7 ± 0·7	24·1 ± 0·8	21·0 ± 0·1
Liver	20·7 ± 0·6	20·8 ± 0·1	20·4 ± 0·1
Kidney	21·3 ± 0·4	19·8 ± 0·3	20·0 ± 0·3

0·2 ml of the injected solution contained 4 mg mescaline.HCl; the $^3H/^{14}C$-ratio of this solution was 20·0 ± 1. (The figures in the table are the mean values of triplicate determinations of the organs of three animals ± S.E.M.)

Identification of mescaline metabolites in brain and liver

(a) *Anionic metabolites.* In addition to the well known mescaline metabolite 3,4,5-TMPAA[22,35] we were able to show the formation of 3,4,5-TMBA in mouse brain and liver after the intraperitoneal injection of mescaline. Several methods were applied to establish the formation of this compound.

The perchloric acid extracts prepared from brain and liver of mice 1 hr after the intraperitoneal injection of 2,6[^{3}H]mescaline (or of mixtures of 8[^{14}C] and [^{3}H] mescaline, see above) were separated on cellulose phosphate columns described in detail under Materials and Methods. The anionic plus neutral compounds were further separated by TLE at pH 4·8. Two radioactive zones were discernible on the electrophoretograms. The zone moving towards the anode had a mobility identical with that of 3,4,5-TMPAA. The other zone remained near the origin. These zones were scraped out and eluted. TLC separations of 3,4,5-TMPAA and 3,4,5-TMBA were incomplete (Fig. 2). Therefore it was not possible to identify unambiguously 3,4,5-TMBA by MS,

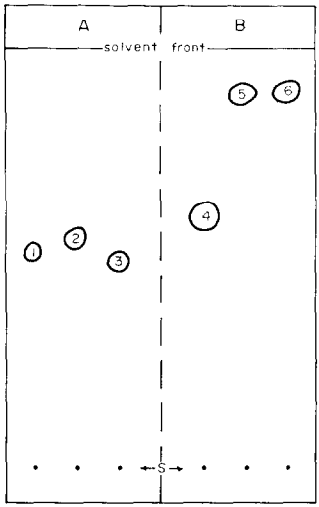

FIG. 2. Thin-layer chromatographic separation of 3,4,5-TMPAA (1), 3,4,5-TMBA (2), 2,3,4-TMBA (3), and of their bromination products 2,6-dibromo-3,4,5-TMPAA (4) and 1,2,3-trimethoxy-4,5,6-tribromo-benzene (5 and 6). Solvent: tert. butanol–2-oxobutane–25% ammonia–water (4:3:2:1); 200 μm silica gel G_{F254}; S = origin.

because the mass spectra of the molecular ion of 3,4,5-TMBA and the fragments formed from these compounds by electron impact are with one exception not characteristically different enough to allow the detection of small amounts of 3,4,5-TMBA in the presence of 3,4,5-TMPAA (Fig. 3), especially if background is considerable due to impurities extracted from the chromatographic plates. On these grounds it was necessary to work out a suitable method for the identification of 3,4,5-TMBA in the presence of 3,4,5-TMPAA.

It would have been an attractive method to convert the two acids into the corresponding amines with one carbon less in the side chains by a Schmidt-reaction,[36] and to identify the amines after dansylation by TLC and MS.[34,37] However, due to the splitting off of methyl groups under the reaction conditions of the Schmidt-reaction, the yields of amines obtained are very low, as was demonstrated previously.[38]

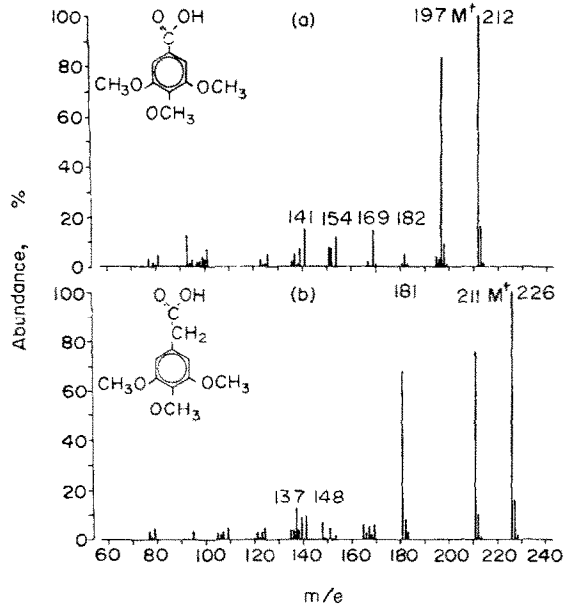

FIG. 3. Mass spectra of 3,4,5-TMBA (a) and of 3,4,5-TMPAA (b). Electron beam energy 70 eV

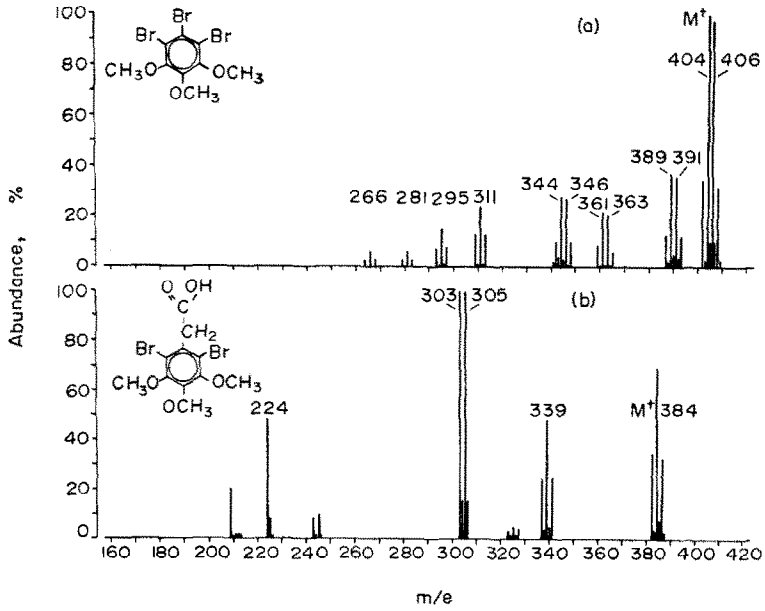

FIG. 4. Mass spectra of 1,2,3-trimethoxy-4,5,6-tribromo-benzene (a) and of 2,6-dibromo-3,4,5-TMPAA (b). Electron beam energy 70 eV.

The problem was solved by bromination at room temperature of the two acids, which were adsorbed to silica gel on a normal thin-layer plate. Under these conditions 3,4,5-TMPAA formed 2,6-dibromo-3,4,5-trimethoxy-phenylacetic acid, while 3,4,5-TMBA was converted into 1,2,3-trimethoxy-4,5,6-tribromo-benzene. The structures of

these compounds were confirmed by comparison with authentic material and by MS (Fig. 4) and by chromatography (Fig. 2).

Although the amounts of 3,4,5-TMBA found in mouse brain and liver 1 hr after the mescaline administration were low, it was nevertheless possible to identify this compound unambiguously by its very characteristic mass spectrum, in several experiments.

The bromination method did not allow us to distinguish between 3,4,5-TMBA and its structural isomer, 2,3,4-TMBA. Since we had some reason to discern between these isomers (see below) a further attempt was made to establish the structure of the new mescaline metabolite. Two-dimensional thin-layer chromatography using the solvent tert. butanol–2-oxo-butane–25% ammonia–water (4:3:2:1) in both directions separated 2,3,4-TMBA from 3,4,5-TMBA. The trimethoxybenzoic acid isolated from brain and liver exhibited chromatographic behaviour identical with that of authentic 3,4,5-TMBA.

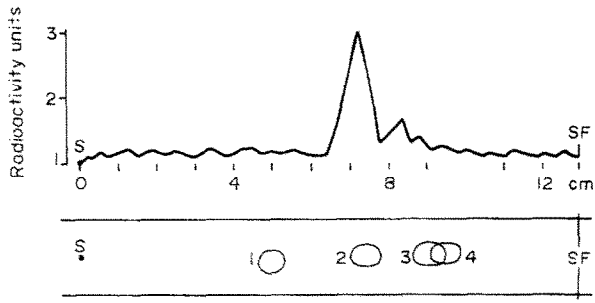

FIG. 5. Thin-layer chromatogram and radioactivity scan of the "neutral fraction" obtained by TLE Solvent: *n*-butanol–acetic acid–water (4:1:5). Reference compounds: 1 = mescaline.HCl, 2 = *N*-acetyl-mescaline, 3 = 3,4,5-TMPAA, 4 = 3,4,5-TMBA. S = origin, SF = solvent front. 200 μm silica gel G_{F254}.

(b) *Neutral metabolites.* The radioactive zone remaining near the origin of the electrophoretogram, mentioned in the previous section was called neutral fraction. TLC of this fraction yielded two radioactive spots, one of which contained at least 80 per cent of radioactivity and was identified as *N*-acetyl-mescaline. The criteria were identical chromatographic behaviour (Fig. 5) and identical mass spectra (Fig. 6). The spot containing the smaller part of radioactivity was not identified.

(c) *Cationic metabolites.* The cationic fractions were reacted with DANS-Cl and the DANS-amides separated by TLC. In addition to DANS-mescaline three radioactive DANS-derivatives were purified by TLC and were obtained in sufficient amounts as to allow us to prepare mass spectra. The position of these spots on two-dimensional TLC can be seen in Fig. 7. Spot B co-chromatographed with DANS-2,3,4-trimethoxy-β-phenylethylamide. The $^{3}H/^{14}C$-ratio of this compound was the same as that of the administered mescaline.HCl, which indicates an intact side chain of this molecule. The mass spectrum of spot B resembled very closely those of DANS-2,3,4-trimethoxy-β-phenylethylamide and DANS-mescaline (Fig. 8). (It has to be emphasized that the substance amount of spot B was in the range of 0·1–1 nmole, so that the spectrum had to be prepared by flash evaporation, and the concentration of the substance vapour

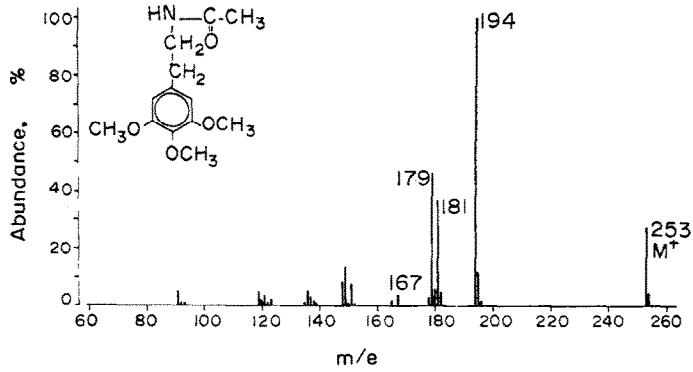

FIG. 6. Mass spectrum of *N*-acetyl-mescaline. Electron beam energy 70 eV.

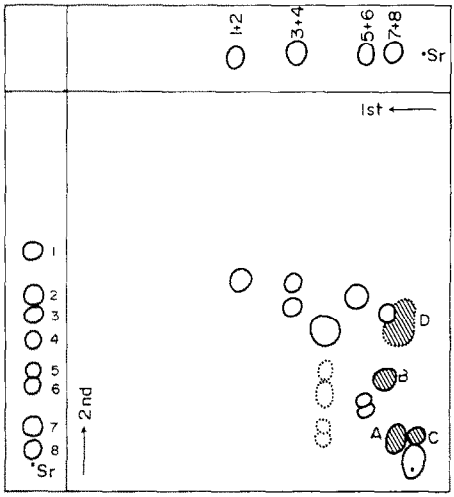

FIG. 7. Two-dimensional thin-layer chromatogram of the DANS-derivatives of the cationic fraction. Hatched spots (A–D): radioactive compounds. Solvents: 1st dimension: cyclohexane–butyl acetate (8:3) (two runs); 2nd dimension: tetrachloromethane–triethylamine (5:1) (two runs). Reference compounds: 1 = DANS-benzylamide; 2 = DANS-β-phenylethylamide; 3 = 4-methoxy-benzylamide; 4 = DANS-4-methoxy-β-phenylethylamide; 5 = DANS-2,3,4-trimethoxy-benzylamide; 6 = DANS-2,3,4-trimethoxy-β-phenylethylamide; 7 = DANS-3,4,5-trimethoxy-benzylamide; 8 = DANS-3,4,5-trimethoxy-β-phenylethylamide (DANS-mescaline).

was presumably not the same throughout the recording of the mass spectrum). 2,3,4 trimethoxy-β-phenylethylamine was not present in detectable amounts in the injected mescaline. Spots C and D (Fig. 7) could not be identified. The mass spectra showed clearly that these spots were not uniform. However, since characteristic fragments of trimethoxyphenyl-derivatives were observed in the spectra of these compounds (*m/e* 194, 181; see Fig. 8), it is most probable that simple, although hitherto unknown mescaline derivatives were formed *in vivo* from mescaline. From the ions at *m/e* 458 and 514, which are most probably the molecular ions of the main constituents of spots C and D, we assume the respective formations of 2-oxo-2-(3,4,5-trimethoxyphenyl)-ethylamine and of the enol propionate of this compound, although it must be emphasized that other structures are equally probable on the grounds of the available

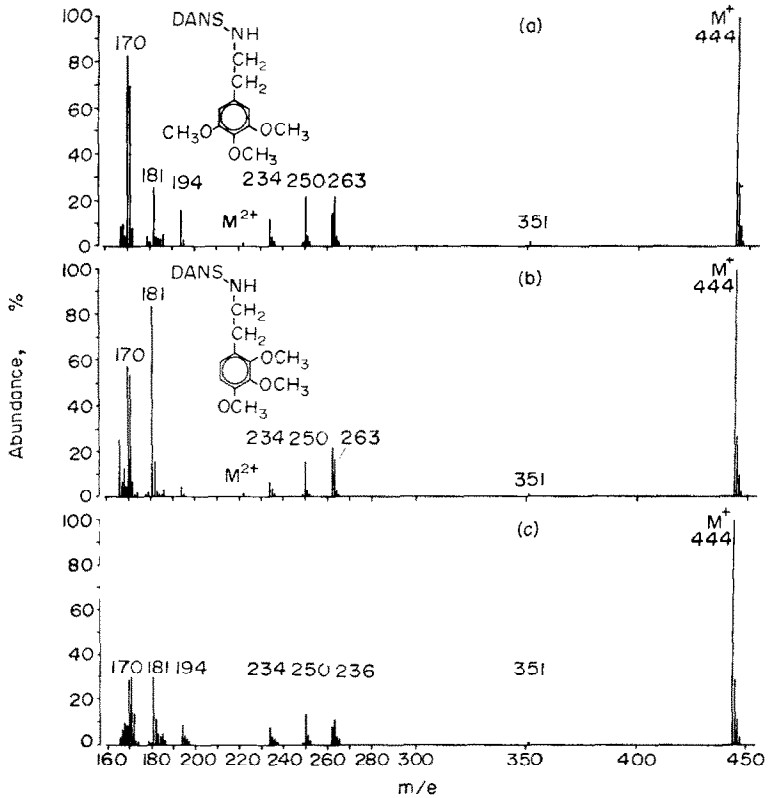

FIG. 8. Mass spectra of DANS-3,4,5-trimethoxy-β-phenylethylamide (DANS-mescaline) (a), DANS-2,3,4-trimethoxy-β-phenylethylamide (b), and of the substance eluted from spot B of the thin-layer chromatogram (c), (Fig. 7). Electron beam energy 70 eV.

information. The identity of spot C with DANS-*N*-methyl-mescaline (mol. wt 458) could be excluded on the grounds of chromatographic behaviour and of MS. In the spectrum of spot C the fragment ion at *m/e* 277, which is typical of *N*-methyl- and α-methyl-β-phenylethylamines, among others,[37] could not be observed.

DISCUSSION

Two metabolic pathways of mescaline may exist in brain: *N*-acetylation and oxidative deamination to 3,4,5-TMPAA. *N*-acetyl-mescaline and its demethylation product, *N*-acetyl-β-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine have been found in human urine.[20] The occurence of *N*-acetyl-mescaline in brain, cerebrospinal fluid and urine, however, was presumed mainly on chromatographic evidence.[15,20]

The confirmation of the presence of *N*-acetyl-mescaline in brain after mescaline administration by unequivocal methods may perhaps suggest a more intense engagement with an apparently general phenomenon of amine metabolism, the *N*-acetylation of biogenic amines.[39–42] It may be mentioned in this connection that acetylation of aromatic amines by acetyl-CoA:arylamine-*N*-acetyltransferase (EC 2.3.1.5) played a considerable role in the discovery of acetyl-CoA.[43] The smaller portion of radioactivity in the neutral fraction (see Fig. 5) could not be identified; the amounts of this

compound were too small to allow us to prepare a mass spectrum. Whether 3,4,5-trimethoxyphenylethanol or a demethylation product of *N*-acetyl-mescaline was formed in brain *in vivo* remains therefore undecided.

It seemed to be a well established fact that the only anionic metabolite of mescaline was 3,4,5-TMPAA. This acid was identified in the urine of dogs after mescaline ingestion by Slotta and Müller.[22] Its occurrence in cat brain was established by Neff and coworkers,[14] and it was identified in our laboratory as the main reaction product of mescaline oxidation by brain homogenates and brain mitochondria.[44] Identification of 3,4,5-TMPAA in brain was also based on R_f. Three observations prompted us to look thoroughly for a mescaline metabolite with a shortened side chain:

(1) The apparent loss of ^{14}C after administration of a mixture of 8[^{14}C] and 2,6[^{3}H] mescaline·HCl, especially in the anionic fraction of brain extracts (Table 1).
(2) The expiration of $^{14}CO_2$ after 8[^{14}C]mescaline administration.
(3) A small but well measurable *in vitro* oxidation of mescaline by brain and liver homogenates in the presence of MAO inhibitors.[1,44]

In our opinion 3,4,5-TMBA seemed to be one of the most probable metabolites which fitted into the concept of side chain degradation of mescaline. A comparison of the chromatographic and electrophoretic mobility of 3,4,5-TMPAA and 3,4,5-TMBA showed us that these metabolite would not have been detected by the methods applied hitherto for the identification of mescaline metabolites. 3,4,5-TMPAA and 3,4,5-TMBA exhibit similar chromatographic features, and in addition, 3,4,5-TMBA derived from 8[^{14}C]mescaline is not radioactive, so that its sensitive detection was not possible at all. It should be noted that all metabolic studies carried out in brain[8,14,15,45,46] with the exception of some experiments in our laboratory[1,16,17,28] were performed with the use of 8[^{14}C]mescaline.HCl. Since, as mentioned above, the mass spectra of 3,4,5-TMPAA and 3,4,5-TMBA did not differ sufficiently to allow the unambiguous identification of small amounts of 3,4,5-TMBA in a mixture with 3,4,5-TMPAA (Fig. 3), it was necessary to form other derivatives from the two compounds in order to allow their chromatographic separation. Bromination on an active silica gel surface was suitable, since under these conditions derivatives are formed from the two acids, which differ very considerably not only in their chromatographic behaviour (Fig. 2) due to the loss of the carboxyl group of 3,4,5-TMBA, but which render typical mass spectra (Fig. 4). Although it was not possible to isolate 1,2,3-trimethoxy-4,5,6-tribromo-benzene, the reaction product of 3,4,5-TMBA, in a pure form from tissue extracts, we were nevertheless able to obtain mass spectra of the spot co-chromatographing with 1,2,3-trimethoxy-4,5,6-tribromo-benzene, which exhibited the typical pattern of peaks at *m/e* 402,404,406,408 and 387,389,391,393, indicating the presence of 3 Br atoms in the molecule.

The *in vivo* formation of 3,4,5-TMBA from mescaline raises a number of questions concerned with the enzymatic mechanism, the subcellular localization of the enzyme (or enzymes) involved in this reaction, and its functional implications.

It is evident from prior observations that mitochondria can oxidize mescaline to 3,4,5-TMPAA. It is presumed that mescaline is oxidized in liver both by DAO and by MAO,[47] while in brain MAO most probably deaminates mescaline.[1,44] It could be expected that the 3,4,5-TMBA formation was catalysed by microsomal rather than

mitochondrial enzymes. It will be shown in a following paper that this expectation is valid.

The mechanism of 3,4,5-TMBA formation is not yet clarified. It is quite possible that some of the radioactive compounds observed in the cationic fraction of the brain extracts are intermediates of the conversion of mescaline to 3,4,5-TMBA.

As was shown previously, one of the major differences between mescaline and its non-hallucinogenic isomer 2,3,4-trimethoxy-β-phenylethylamine, was that the latter compound is more susceptible to oxidative deamination, both *in vitro* and *in vivo*.[1,16,17] Especially in this respect there is a close analogy between mescaline and the hallucinogenic amphetamines. This analogy is now extended to metabolic fates. It is known that amphetamines can be oxidatively degraded by microsomal enzymes. From this reaction conjugates of the benzoic acids with glycine result.[48,49] Although it is not established, we may presume that suitable substrates of mitochondrial MAO will not be subject to microsomal oxidation under normal conditions, while mescaline as a poor substrate of MAO is submitted to some extent to oxidative side chain degradation. It may well be possible, however, that side chain degradation of phenylethylamines occurs under certain circumstances *in vivo*. At least the work of Kveder and Iskric[50,51] seems to indicate this. Interaction of mescaline with structural components of the endoplasmic reticulum is suggested by the observations of Datta and Ghosh, who found a destabilizing effect of mescaline on brain ribosomal particles,[52,53] and an inhibitory effect of mescaline on the amino acid incorporating ability of brain cortex ribosomes.[54]

The cationic metabolites of mescaline observed after dansylation and TLC are presumably not demethylation products of mescaline. This can be derived from their greenish-yellow fluorescence colour, which is typical of DANS-derivatives of aliphatic amines, while DANS-phenol esters exhibit a deep yellow to orange fluorescence on thin-layer plates, under the experimental conditions.[32,43] Furthermore, the mass spectra showed no signs of O-DANS-derivatives.[37] It should be mentioned that Neff and coworkers[14] were looking carefully for mescaline demethylation products in cat brain, without success.

Our results are puzzling on several grounds. First of all, there is no doubt that the DANS-derivative of one of the metabolites (spot B, Fig. 7) has and identical R_f at least in two solvent systems, and a mass spectrum similar to that of authentic DANS-2,3,4-trimethoxy-β-phenylethylamine and DANS-mescaline (Fig. 8). Since we found no trace of the isomer in the injected mescaline, we were compelled to believe that the compound behaving like 2,3,4-trimethoxy-β-phenylethylamine was formed from mescaline *in vivo*.

The data obtained from the other radioactive DANS-derivatives of the cationic fractions are not clear enough as to allow the unambiguous identification of the compounds. A more detailed study of the cationic mescaline metabolites will be necessary.

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