Journal of Ethnopharmacology, 12 (1984) 179—211
Elsevier Scientific Publishers Ireland Ltd.

MONOAMINE OXIDASE INHIBITORS IN SOUTH AMERICAN HALLUCINOGENIC PLANTS PART 2: CONSTITUENTS OF ORALLY-ACTIVE MYRISTICACEOUS HALLUCINOGENS

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(Accepted May 18, 1984)

Summary

Alkaloid constituents in Myristicaceous bark and leaf samples and in purportedly hallucinogenic preparations derived from Myristicaceous sources were qualitatively and quantitatively analyzed using TLC, GC, alkaloid precipitation tests and GC/MS. Fourteen of the 27 bark and leaf samples analyzed contained detectable amounts of alkaloids. The major bases were *N*,*N*-dimethyltryptamine (DMT) and/or 5-methoxy-*N*,*N*-dimethyltryptamine (5-MeO-DMT); much smaller amounts of tryptamine and/or *N*-methyltryptamine (NMT) were also usually present. β-Carbolines were not detected in the bark or leaf samples. Considerable variation in alkaloid profiles was found, extending to different collections of the same species. Fourteen of the 20 *Virola* samples contained alkaloids; none of the 6 *Iryanthera* species had detectable alkaloids. *Osteophloem platyspermum* contained an indolic base, identified as *N*-methyl-tryptophan methyl ester. Seven samples of an orally-ingested drug made from *Virola* spp. were analyzed. All except one contained substantial amounts of tryptamines; the types and proportions of tryptamines present varied greatly between samples. Samples of Yanomama snuff including various admixtures were analyzed and all components but one contained tryptamines. The drug samples having the highest concentrations of alkaloids contained 15—20 mg/g dry wt while the Myristicaceous bark and leaf samples had much lower concentrations ranging from 0.04 to 0.25 mg/g dry wt. β-Carbolines were detected as trace constituents in only two of the Myristicaceous drug samples.

Four Myristicaceous paste samples were bioassayed in self-experiments. Two of the samples were devoid of detectable hallucinogenic or physiological activity, while some degree of oral activity was detected in two other samples.

The activity of a number of tryptamine derivatives as monoamine oxidase inhibitors (MAOI) was investigated using an in vitro enzyme assay. Activity

0378-8741/84/$10.20

was measured using single compounds and mixtures of compounds and the results were compared to the activity of samples of orally-ingested Myristicaceous pastes. Tryptamine derivatives had significantly less MAOI activity than the activity of β-carboline derivatives measured in a previous study. Some structural correlations for MAOI activity were found for the tryptamine derivatives. Samples of orally-ingested Myristicaceous pastes were assayed for MAOI activity. The inhibition elicited by the paste samples was closely matched by mixtures of tryptamine standards having comparable proportions and concentrations. These observations indicate that the MAOI activity of the pastes is due mainly to the high concentrations of tryptamines; the traces of β-carbolines or non-nitrogenous inhibitors present probably do not contribute significantly to the total inhibition. Thus it appears unlikely that the oral activity of the Myristicaceous pastes is due to the potentiation of the tryptamines via inhibition of MAO by β-carbolines; some mechanism other than MAO inhibition must be sought to account for the oral hallucinogenic activity of the Myristicaceous pastes if they are, in fact, orally active.

Introduction

Various species of the genus *Virola* (Myristicaceae) have been employed by Amazon Indian tribes as the basis of hallucinogenic preparations (Schultes, 1979). The drug obtained from the *Virola* trees is in all cases derived from the "resin", the colorless to reddish exudate of the cambial layer of the bark. Most commonly, the resin is evaporated to dryness over a low fire and ground into a powder, which is ingested in the form of a snuff. The exact method of preparation varies from region to region and tribe to tribe; in some instances the snuff is prepared without the addition of other ingredients, while in others, the powdered leaves or ashes of other plants are added (Schultes and Holmstedt, 1968). Although the use of *Virola* resin as a snuff has the widest geographical and ethnological distribution in the Amazon Basin, a few tribes utilize orally-ingested *Virola* preparations (Schultes, 1979), and there are also reports (Plowman, pers. commun., 1983) that the bark of certain *Virola* spp. may be smoked for hallucinogenic effects. Extensive chemical investigations have been carried out on many *Virola* spp. and related genera (Gottlieb, 1979) and the active hallucinogenic constituents of the *Virola* snuffs have been determined to be indole bases related to tryptamine, e.g. DMT, 5-MeO-DMT and related derivatives (Agurell et al., 1969; Holmstedt et al., 1980). β-Carbolines have also been reported from some species (Holmstedt et al., 1980) and in one species, not known to be used as an hallucinogen, β-carbolines are the major alkaloids present (Cassady et al., 1971). The alkaloidal constituents of the orally-ingested Myristicaceous preparations have not previously been investigated.

The tryptamines DMT and 5-MeO-DMT are potent hallucinogens, but are not orally active, presumably due to deamination by peripheral monoamine oxidase (MAO), the mitochondrially-localized enzyme which catalyzes the oxidative deamination of endogenous biogenic amines. Experimental evidence has been accumulated (Marley and Blackwell, 1970) which indicates that visceral MAO functions as a detoxification mechanism serving to protect the nervous and cardiovascular systems from toxic amines ingested in the diet and formed as a result of aromatic amino acid decarboxylation.

The capacity of β-carbolines to inhibit MAO (Udenfriend et al., 1958; McIsaac and Estevez, 1966; Buckholtz and Boggan, 1977) has been suggested as the mechanism responsible for the oral activity of the orally-ingested drugs prepared from *Virola* spp. (Schultes, 1969; Schultes and Swain, 1976; Schultes et al., 1977). The same mechanism has been postulated (Schultes, 1972; Shulgin, 1976; Der Marderosian et al., 1968; Agurell et al., 1968) to explain the oral activity of *ayahuasca*, another DMT and β-carboline-containing Amazonian hallucinogen derived from *Banisteriopsis caapi* (Malpighiaceae) and *Psychotria viridis* (Rubiaceae). In the presence of the MAO-inhibiting β-carbolines, the tryptamines may be protected from deamination and thus rendered orally active.

We have reported in a previous publication (McKenna et al., 1984) the results of experimental investigations of the activity of *ayahuasca* as an MAO inhibitor (MAOI) in vitro. The present study presents the results of our phytochemical and pharmacological investigations of the orally-active Myristicaceous pastes, including in vitro evaluations of their activity as MAO inhibitors.

Experimental procedures

Detection of alkaloids in Myristicaceous samples

Collection numbers of samples cited in this work refer to the personal collection numbers of D. McKenna. Herbarium voucher specimens for all collections have been deposited in the Herbarium of the Department of Botany, University of British Columbia. Duplicate vouchers of most collections are on deposit at the Field Museum of Natural History, and at the Instituto Pesquisas do Amazonia, Manaus (INPA); duplicates of some collections have also been deposited at the Oakes Ames Economic Herbarium, Harvard University. Herbarium vouchers are available for all of the orally-ingested pastes collected on the Rio Ampiyacu, however no vouchers are available for the La Chorrera paste sample or for the Yanomama snuff samples. Paste samples collected on the Ampiyacu are identified by the name of the informant who prepared the sample, e.g. "Alfredo Moreno no. 1"; collection numbers of the source-plants for the samples are cited in Table 4. All of the bark and leaf samples analyzed in this study were preserved in 100% metha-

nol at the time of collection. The methanol was decanted from the preserved material and the remainder was lyophilized. The freeze-dried material was powdered and extracted with methanol (10—20 ml/g dry wt). The extracts were combined with the original methanol used to preserve the sample, and concentrated under vacuum to a known volume. The crude extracts were sealed and stored at 4°C. For alkaloid screening, aliquots of the extracts equivalent to 3 g dry wt were evaporated to dryness. The residue was shaken with 5 ml 1 N HCl, and filtered. A small aliquot of the acidic filtrate (approx. 0.5 ml) was removed and used for the alkaloid precipitation tests. The remainder was washed with 1 × 5 ml CH_2Cl_2 and the organic layer discarded. The aqueous layer was basified to pH 8—9 with saturated $NaHCO_3$ and extracted with 15-ml portions of CH_2Cl_2; it was further basified to pH 11—12 with 2 N NaOH and extracted until acidified aliquots of the aqueous fraction no longer gave a positive reaction to Meyer's reagent. The organic fractions were combined, dried over anhydrous Na_2SO_4, evaporated to dryness and redissolved in 3 ml methanol. The orally-ingested paste samples were also preserved in methanol and were treated in the same manner as the bark and leaf samples. The Yanomama snuff samples were dried, powdered plant materials; these were extracted directly with methanol and worked up for alkaloids in the same manner as the other samples.

Alkaloid precipitation tests were carried out on small aliquots of the acidic filtrate during the workup procedure. Tests were made with Valser's, Meyer's and Dragendorff's reagents (Martello and Farnsworth, 1962), all of which form precipitates or turbid solutions in the presence of alkaloids. Valser's reagent is approximately an order of magnitude more sensitive than the other two. TLC of the samples was conducted with the purified base extracts which were redissolved in methanol. Three microlitre aliquots were applied to Polygram Silica Gel UV^{254} precoated TLC plates (Brinkmann Instruments) at a point 1.0 cm from the bottom edge. Following application the samples were dried under a stream of air, and developed to a distance of 9 cm in ether/2-butanone/conc. NH_4OH (5:4:1, organic phase). Development was carried out at ambient temperature in an unlined 10 × 23 × 26 cm glass chromatographic tank containing 50 ± 5 ml of solvent. Following development, plates were air-dried, then examined under long and short-wave UV light. Tryptamine bases and tetrahydro-β-carbolines appear as dark UV-absorbing spots under short-wave UV while aromatic and dihydro-β-carbolines have characteristic fluorescences under long-wave UV. Following examination under UV, plates were sprayed with Ehrlich's reagent (Repke et al., 1977) which gives blue to violet colors with tryptamine bases. Tryptamines could be tentatively identified from TLC based on comparison of the R_f values and color reactions with authentic standards. Identities of constituents detected using TLC were confirmed by GC or GC/MS, by comparison with standards. Conditions for the GC/MS analysis were identical to those described previously (McKenna et al., 1984). Conditions of the GC analyses are described below. Following TLC those samples which were positive for alkaloids were evaporated to dryness under nitrogen and stored at −20°C.

Quantitative analysis of tryptamine standards

Those Myristicaceous samples which were alkaloid-positive were quantified using gas chromatography (GC). The instrument used was a Sigma 3B Gas Chromatograph (Perkin Elmer) equipped with a hydrogen flame ionization detector. The column was a 15 m × 0.25 mm SE-30 fused silica capillary column (J & W Scientific). Carrier gas was helium; inlet pressure for the carrier gas was 18 lb/inch^2; inlet pressure for both the hydrogen and air was 30 lb/inch^2; split ratio was 0.67. The chromatograph was temperature programmed, from an initial temperature of 120°C to a final temperature of 200°C. Initial temperature was held for 3 min following injection and then increased at a rate of 10°/min.

Calibration of column using tryptamine standards

Retention times, peak heights, and peak height ratios of tryptamine derivatives were determined using standards (Table 1). Retention times were calculated as the mean ± S.E. of a minimum of 10 injections. Mixtures of tryptamine standards were used to construct calibration curves used in the quantitative analyses of the Myristicaceous samples. Concentration of the

TABLE 1

TRYPTAMINE STANDARDS: GC ANALYTICAL DATA

Tryptamine standard	Retention time (min)	Concentration (mg/ml)				
		0.0625	0.125	0.25	0.5	1.0
Tryptamine	6.6 ± 0.15					
Peak height (cm)		n.d.[a]	n.d.	1.6	7.5	—
PHR[b]		—	—	0.6	2.8	—
5-MeO-tryptamine	9.3 ± 0.15	n.d.	n.d.	n.d.	2.1	9.3
Peak height		—	—	—	0.8	3.5
PHR						
NMT	7.1 ± 0.21					
Peak height		n.d.	0.42	1.7	3.2	—
PHR		—	0.11	0.43	0.82	—
DMT	7.3 ± 0.13					
Peak height		0.94	2.9	6.7	17.7	—
PHR		0.24	0.74	1.7	4.5	—
5-MeO-DMT	9.7 ± 0.13					
Peak height		0.65	1.9	4.3	10.3	—
PHR		0.16	0.48	1.1	2.6	—

[a] n.d. = not detected.

[b] PHR = peak height ratio. PHR = height of standard peak at concentration x/height of gramine peak at 0.25 mg/ml. Value of denominator was 3.91 for n = 42 injections.

tryptamine calibration mixtures ranged from 0.0625 to 0.5 mg/ml for DMT, 5-MeO-DMT and NMT, and from 0.25 to 1.0 mg/ml for tryptamine and 5-methoxy-tryptamine. Standards were dissolved in methanol/pyridine 9 : 1 and aliquots of 1 μl were injected. Tryptamine and 5-MeO-tryptamine, being polar and readily ionized compounds, could not be reliably detected if less than 250 ng was injected; well below 50 ng of the less polar methylated tryptamines could be detected, however. Ten replicate injections of each calibration mixture were made at each concentration level. Gramine was included as an internal standard in all of the calibration mixtures, at a constant concentration of 0.25 mg/ml. The mean peak height ratio for each standard at each concentration, with respect to the internal standard, was determined (cf. Table 1). A linear relationship was observed over the concentration ranges stated, when peak height ratio was plotted against concentration.

Preparation of Myristicaceous samples for GC quantitation

The base fractions of the Myristicaceous bark and leaf samples had been previously evaporated to dryness and stored at $-20°C$. Each sample represented the alkaloids extracted from a known amount of dry weight of plant material, usually 3.0 g. One ml of methanol/pyridine (9 : 1) was added to the samples, which were heated briefly over a steam bath and filtered. The filtrate was collected, and adjusted to a final volume of 1.5 ml. An aliquot equivalent to 1.0 g dry wt was removed and adjusted to 0.5 ml; this sample was further diluted to 1.0 ml by adding 0.5 ml of solvent containing gramine at a concentration of 0.5 mg/ml. Thus the final concentration of the sample was 1.0 g dry wt/ml, and the final concentration of the gramine internal standard was 0.25 mg/ml. One microlitre aliquots were injected into the GC. Five replicate injections of each sample were run. Concentrations of tryptamines in the samples were calculated based on their peak height ratios with respect to the internal standard, using the calibration curves constructed using tryptamine standards.

The Myristicaceous paste and snuff samples had not been evaporated to dryness and therefore the alkaloid fractions from known dry weights of plant material were dissolved in approximately known volumes of methanol. These samples were heated briefly over a steam bath, filtered, and brought to a known volume with additional methanol. Thus the concentration of the sample could be expressed in terms of g dry wt/ml, e.g. "2.0 g in 4 ml". At this point 1 μl of sample was injected into the GC to estimate the dilution required to bring the sample within the range of the calibration curves. An aliquot representing an appropriate portion of the total dry wt of the sample was diluted with an equal aliquot of methanol/pyridine (9 : 1) containing gramine (0.5 mg/ml) to give a final concentration of gramine of 0.25 mg/ml. Peak height ratios with respect to the internal standard were calculated, and the concentrations of tryptamines in the samples were estimated as described above. Duplicate samples were prepared as described and 5 replicate injections (1 μl) of each were made.

Bioassay of Myristicaceous paste samples

In an effort to confirm the oral hallucinogenic activity of the Myristicaceous paste samples, portions of four of the six paste samples listed in Table 5 were ingested in self-experiments. The ingestion of all samples was preceded by several hours' fasting. The amount of paste sample ingested varied from approximately 1.5 g to 10 g; in the trials in which larger amounts were ingested, the total amount was divided into 3—4 smaller doses, which were ingested over a period of several hours. This procedure was followed in an effort to determine the physiologically active dose, after the initial dose(s) had proven inactive. The following paste samples were bioassayed for oral activity (the collection number, identity of the source-plant, and approximate amount ingested are given in parentheses): La Chorrera "*Oo'-koey*" (no voucher available, probable *Virola* spp., 5—10 g ingested); Jorge Churay no. 1 (DMK-34, *V. pavonis*, approx. 10 g ingested); Alfredo Moreno no. 1 (DMK-40, *V. sebifera*, 3—4 g ingested); Don Marcos Flores no. 1 (DMK-59, *V. elongata*, 1.5—2 g ingested). Results of these bioassay trials are reported in the Results and Discussion.

In vitro assay of rat-liver MAO

The Myristicaceous pastes and a selected sample of tryptamine standards were assayed for activity as MAOI in vitro using rat-liver cytosol as the source of enzyme and 5-hydroxy[^{14}C]tryptamine (Amersham) as substrate. The procedures followed in the preparation of the rat-liver cytosol and the enzyme reaction mixture have been previously described (McKenna et al., 1984).

Assays using tryptamine standards

Stock solutions of various tryptamine derivatives, dissolved in 0.1 N HCl, were prepared so that addition of a 50-μl aliquot to a reaction mixture would result in an inhibitor concentration of 10^{-3} M and a total volume of 500 μl. The tryptamine derivatives 5-methoxy-diisopropyl tryptamine and 3-[2(2,5-dimethyl)pyrrolyl-ethyl]indole were poorly soluble in 0.1 N HCl so these compounds were dissolved in 10% Tween-80. Stock solutions were prepared by making serial 1 : 10 dilutions, over a concentration range from 10^{-3} to 10^{-10} M. Assay mixtures containing inhibitor consisted of 325 μl buffer, 100 μl whole cytosol, 50 μl inhibitor solution of the appropriate concentration and 25 μl substrate. The inhibitor solution was added just before the assay tubes were transferred to the warm water bath, so that the incubation time of the cytosol plus inhibitor at 37.5°C was 5 min. Boiled blanks containing denatured enzyme were included in each run. In addition controls containing active enzyme but no inhibitor were also assayed simultaneously (inhibitor was replaced either with buffer, 10% Tween-80, or with 50 μl 0.1 N HCl). There was no appreciable difference between controls in which buffer replaced the inhibitor and those in which 0.1 N HCl or 10%

Tween-80 replaced the inhibitor. The controls lacking inhibitor represented 0% MAO inhibition; and the % inhibition of tubes containing inhibitors was calculated relative to these controls, by dividing the amount of labelled reaction products recovered (measured as cpm) by the amount recovered from the control tubes. The background count represented by the activity detected in the boiled blanks (usually 200—300 cpm) was subtracted from both control counts and inhibitor counts.

Preparation and quantitation of Myristicaceous paste samples for MAO assay

The Myristicaceous paste samples used for the assay were Alfredo Moreno sample no. 1, prepared from *Virola sebifera*, (DMK-40), Don Marcos sample no. 1, (*V. elongata*, DMK-59) and the "oo'-koey" from La Chorrera, Colombia (no voucher). Aliquots of the methanol extracts of each paste sample equivalent to 0.2—0.4 g dry wt of the original sample were evaporated to dryness and resolubilized in 2 ml methanol. One millilitre was removed, diluted 1 : 1 with methanol, and the alkaloid concentration was quantitatively determined using HPLC. The quantitative methods and analytical conditions were identical to those described previously (McKenna et al., 1984). The molar concentration of tryptamines in each sample was calculated from the quantitative data (Table 2). An "analogue" of each sample was prepared using tryptamine standards. Each paste "analogue" consisted of a mixture of tryptamine standards in the same proportions and concentrations as were measured in the paste samples. Each paste sample and its analogue were simultaneously assayed in the monoamine oxidase system. If the MAOI activity of the paste sample and the analogue were equivalent, this would indicate that enzyme inhibition by the pastes was attributable solely to the

TABLE 2

ALKALOID CONCENTRATION IN MYRISTICACEOUS PASTE SAMPLES ASSAYED FOR MAOI ACTIVITY[a]

Sample name	Alkaloids detected	Alkaloid concentration (mg/ml)	Molar concentration (M)
La Chorrera	NMT	1.38	7.9×10^{-4}
"oo'-koey"	DMT	0.1	0.53×10^{-4}
(no voucher)	Total	1.48	8.43×10^{-4}
Don Marcos no. 1 (DMK-59)	5-MeO-DMT	2.03	9.3×10^{-4}
Alfredo Moreno no. 1	5-MeO-DMT	1.19	5.48×10^{-4}
(DMK-40)	DMT	0.3	1.54×10^{-4}
	Total	1.49	7.02×10^{-4}

[a] HPLC quantitation carried out on samples resolubilized in 10% Tween-80. See Materials and Methods.

tryptamines, which are competitive substrates of 5-hydroxy-tryptamine (5HT) for MAO-A. If the MAOI activity of the paste exceeded the activity of the analogue, this would indicate that some constituents in addition to the tryptamines were contributing to the over-all inhibitory activity. Following HPLC quantitation, the paste samples were diluted with an equivalent amount of 10% Tween-80 and the methanol was removed under nitrogen. If necessary additional Tween-80 was added to restore the sample to its original volume. The paste "analogues" were prepared from stock solutions of tryptamine standards dissolved in 10% Tween-80. An alkaloid-free paste sample (derived from DMK-34, *V. pavonis*) and a lignan fraction from *V. elongata* (DMK-59) were also prepared in 10% Tween-80. Concentrations of the undiluted stock solutions of each was 3.0 g dry wt/ml and 10 mg/ml, respectively.

Results and discussion

Detection and identification of alkaloids in Myristicaceous samples

Bark and leaf samples

The base composition of the Myristicaceous bark and leaf samples varies considerably (Table 3); the variation extends both to different parts of the same plant, and to different collections of the same species. These observations are consistent with a similar recent survey of Myristicaceous species (Holmstedt et al., 1980) whose authors also remarked upon the anatomical and individual variability found in the base composition of the samples. In the present study, alkaloids were detected in 14 of the 27 collections. Among those which were negative for alkaloids were several collections of *V. pavonis*, and 6 *Iryanthera* spp. Of the *Iryanthera* species examined in the present survey, one (*I. ulei*) was included in the survey of Holmstedt et al. (1980). These investigators detected a trace (0.000013%) of 5-MeO-DMT in *I. ulei*. The *V. pavonis* samples, of which 4 collections were examined, were all negative for alkaloids with the exception of DMK-30 which contained a low level (0.07 mg/g dry wt) of DMT in the leaves and shoot tips. This collection has been tentatively determined (W. Rodrigues 1983) as *V. pavonis* on the basis of sterile material, however the presence of DMT casts doubt on this determination. An indeterminate *Virola* species from Brazil (Plowman 12,218) the bark of which reportedly is smoked by witch doctors as an additive to tobacco (Plowman, pers. commun., 1983) was also negative for alkaloids. Six collections of *V. elongata* were analyzed and all were positive for alkaloids. Five of these contained tryptamine bases, viz. DMT, 5-MeO-DMT, NMT; all three compounds were detected in some collections while others contained only one or two out of the three. All samples in which DMT was detected also contained lesser amounts of NMT, an observation which probably reflects the position of this compound as the penultimate step in the biosynthesis of DMT. No similar association

TABLE 3

DETECTION OF ALKALOIDS IN MYRISTICACEOUS BARK AND LEAF SAMPLES

Name and collection no.	Alkaloid reagent tests[a]			TLC[b]		Ehrlich's reaction[c]	Compounds detected[d]
	D	M	V	UV fluor.	UV abs.		
DMK-30 *Virola pavonis*							
Leaves (3.0 g dry wt.)	++	+/–	++	–	+	+(2)	DMT, NMT
Twigs (3.0 g)	++	+	++	–	+	+(2)	DMT, NMT
DMK-32 *V. pavonis*							
Leaves (3.0 g)	–	–	–	–	–	–	–
Twigs (3.0 g)	–	–	–	–	–	–	–
DMK-34 *V. pavonis*							
Leaves (3.0 g)	+/–	+/–	+/–	–	+	–	–
Twigs (3.0 g)	–	–	–	–	+	–	–
Bark (3.0 g)	+/–	+/–	+/–	+	–	–	–
DMK-35 *Virola* sp.							
Leaves (3.0 g)	++	–	++	–	+	+(2)	DMT, NMT
DMK-36 *Virola* sp.							
Leaves (3.0 g)	+/–	–	–	–	+	+(2)	DMT, NMT
DMK-37 *Virola* sp.							
Leaves (3.0 g)	++	–	++	–	+	+(2)	DMT, NMT
DMK-40 *V. sebifera*							
Leaves (3.0 g)	+	–	+	+	–	+(1)	NMT
Bark (3.0 g)	++	+	++	–	+	+(3)	DMT, NMT, 5MeO-DMT
DMK-41 *V. elongata*							
Bark (3.0 g)	++	+	++	–	+	+(2)	NMT, DMT
DMK-44 *Iryanthera longiflora*							
Bark (3.0 g)	–	–	–	–	+	–	–
DMK-45 *V. elongata*							
Bark (3.0 g)	++	–	++	++	+	+/–(1)	–
DMK-46 *V. callophylla*							
Bark (6.8 g)	+	+/–	+	–	+	+(3)	NMT, 5-MeO-DMT, tryptamine
DMK-47 *Iryanthera macrophylla*							
Bark (3.0 g)	–	–	–	–	+	–	–
DMK-48 *I. ulei*							
Bark (3.0 g)	–	–	–	–	+	–	–

DMK-49 *I. crassifolia*							
Bark (2.3 g)	—	—	—	—	—	—	—
DMK-50 *I. juruensis*							
Bark (3.0 g)	—	—	—	—	+	—	—
DMK-51 *I. paraensis*							
Bark (3.0 g)	—	—	—	—	—	—	—
DMK-52 *Virola multinervia*							
Bark (3.0 g)	—	—	—	—	—	—	—
DMK-56 *V. callophylla*							
Seeds and fruits (3.0 g)	++	+	++	—	+	+(2)	DMT, NMT
DMK-59 *V. elongata*							
Bark (3.0 g)	++	+	++	—	+	+(2)	NMT, 5-MeO-DMT
Leaves (3.0 g)	+	—	+	+	—	+(1)	NMT, DMT
DMK-63 *V. pavonis*							
Bark (3.0 g)	—	—	—	—	+	—	—
DMK-67 *V. elongata*							
Leaves (3.0 g)	++	+	++	+	+	+(1)	5-MeO-DMT
Bark (3.0 g)	+/—	—	+/—	—	—	—	—
DMK-68 *V. elongata*							
Leaves (3.0 g)	++	+	++	+	+	+(1)	5-MeO-DMT
Bark (3.0 g)	+/—	—	+/—	—	—	—	—
DMK-69 *V. elongata*							
Leaves (3.0 g)	++	+	++	+	+	+(1)	5-MeO-DMT
Bark (3.0 g)	+/—	—	+/—	—	—	—	—
DMK-75 *V. loretensis*							
Leaves (3.0 g)	—	—	—	—	—	—	—
DMK-78 *Osteophloem platysperumum*							
Leaves (3.0 g)	++	+	++	—	+	+(1)	N-Methyltryptophan methyl ester
DMK-82 *V. albidiflora*							
Bark (3.0 g)	—	—	—	—	—	—	—
Plowman 12218 *Virola* sp							
Bark (2.0 g)	—	—	—	—	+	—	—

[a] Reaction to alkaloid precipitation reagents. D = Dragendorff's reagent; M = Meyer's reagent: V = Valser's reagent. Composition of reagents is according to Martello and Farnsworth (1962).

[b] Results of TLC of basic fractions: presence/absence of UV-fluorescent or UV-absorbing spots is indicated.

[c] Presence/absence of Ehrlich-positive spots (diagnostic for tryptamines) on TLC plate is indicated. Numbers in parentheses indicate no. of Ehrlich-positive spots detected.

[d] Indicates major compounds identified in sample, using a combination of methods including TLC, GC, HPLC, and in some cases GC/MS.

of either DMT or NMT with 5-MeO-DMT was found. DMK-35, 36, 37 were all collected at the same site, and all had a similar base composition; although the voucher material for these collections is sterile, they probably represent *V. elongata*. DMK-45, determined as *V. elongata*, possessed a completely anomalous alkaloid profile; only traces of Ehrlich-positive compounds could be detected by TLC. The major bases are apparently fluorescent compounds similar to β-carbolines. The fluorescent constituents detected did not match the R_f values and mass spectra of the available β-carboline standards, however. The limited amount of plant material precluded structural characterization. DMK-45 may represent a genetic variant of *V. elongata* in which tryptamine biosynthesis has been re-directed to the synthesis of β-carbolines, or it may represent a misidentification of the collection. Cassady et al. (1971) reported the isolation of 6-MeO-tetrahydroharman, 6-MeO-harmalan and 6-MeO-harman as the major bases of *Virola cuspidata;* these investigators did not detect tryptamines in this species. The most recent taxonomic monograph of the genus *Virola* (Rodrigues, 1980) does not recognize *V. cuspidata* as a legitimate species, considering it equivalent to *V. elongata*.

This is not the only instance in which confusion has arisen in the phytochemical and ethnobotanical literature as a result of the ill-defined species concepts in the genus *Virola*. Numerous publications (Schultes, 1969, 1979; Schultes and Holmstedt, 1968; Schultes and Hoffmann, 1980; Agurell et al., 1969; Holmstedt et al., 1980; Schultes and Swain, 1976) refer to the use of *Virola theiodora* in the preparation of hallucinogenic snuffs and orally-ingested pastes; yet this species is not recognized by Rodrigues (1980) and is treated as equivalent to *V. elongata* or *V. calophylla*. The species list cited in Holmstedt et al. (1980) contains two entries for *V.cuspidata*, five entries for *V. elongata*, and two entries for *Virola theidora;* if Rodrigues' revision of the genus is followed, all of these are properly designated by the binomial *V. elongata*. Inasmuch as the majority of the Myristicaceous voucher collections examined in the present study were determined by Dr. Rodrigues, the species concepts established in his revision have been followed.

Holmstedt et al. (1980) examined three collections of *Virola multinervia*, two of which contained DMT in the bark while the third was alkaloid negative. A single specimen of this species was examined in the present survey and was alkaloid negative. Holmstedt et al. (1980) did not detect alkaloids in the leaves of *V. sebifera* and this was the only tissue examined; in our survey, this species contained substantial amounts of DMT, 5-MeO-DMT and NMT in the bark and traces of NMT in the leaves (cf. Table 5). Our collection of this species (DMK-40) was the source-plant for one of the orally-ingested paste samples obtained at Puco Urquillo. Corothie and Nakano (1969) reported the isolation of DMT as the sole base from the bark of *V. sebifera*. Holmstedt et al. (1980) examined three collections of *Osteophloem platyspermum* (incorrectly cited in Table I of their study as *Osteophloem platyphyllum*). DMT, 5-MeO-DMT and 5-hydroxy-DMT were detected in

one of the samples, while the others were alkaloid-negative. *O. platyspermum* was represented in the present survey by a single sample of leaves (DMK-78) which contained a single Ehrlich positive base not corresponding to any of the available tryptamine standards. This compound gave $R_f = 0.6$ in the TLC solvent used, and a GC retention time of 10.1 min. The compound was isolated by collecting the peaks from HPLC runs. UV spectral analysis was typical of unsubstituted indoles (Fig. 1) with absorption maxima at 289, 279 and 273 nm and a minimum at 286. The mass spectrum (Fig. 1) matched that of a reference spectrum of *N*-methyl-l-tryptophan methyl ester; the base peak was 130 mass units and the parent ion was *m/z* 232. The compound *N,N*-dimethyl-l-tryptophan has an identical molecular weight and base peak, however *N*-methyl-l-tryptophan methyl ester can be distinguished from it by the presence of a peak at 173 mass units which re-

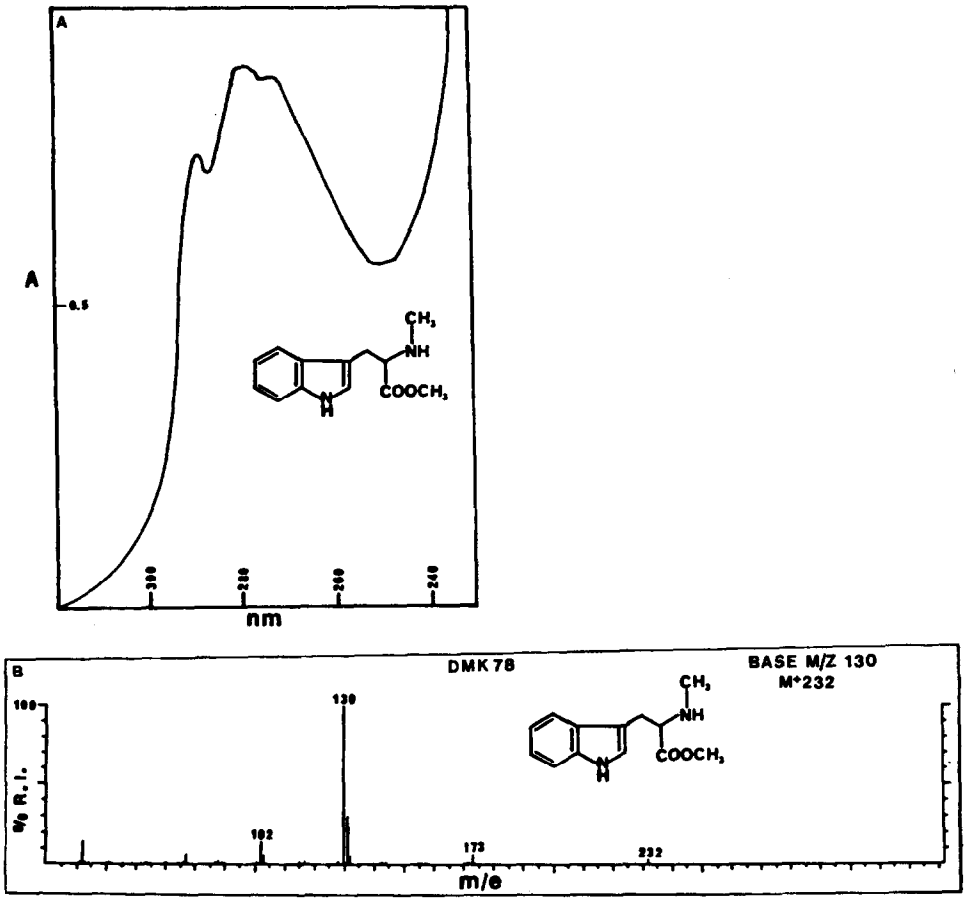

Fig. 1. (A) UV and (B) mass spectra of *N*-methyl-l-tryptophan methyl ester from *Osteophloem platyspermum*.

presents the loss of the methyl carboxylate fragment (COOMe) from the parent ion (M-59). This peak is absent from the mass spectrum of *N,N*-dimethyl-l-tryptophan. The compound in *O. platyspermum* clearly shows an M-59 peak at *m/z* 173, thus providing strong evidence that it is in fact *N*-methyl-l-tryptophan methyl ester. This compound has not been reported in the Myristicaceae although it has been found in several species in the Leguminosae (Johns et al., 1971; Mandava et al., 1974), and in *Sida cordifolia* (Malvaceae) (Ghosal et al., 1975). The mass spectral data for our sample is consistent in all respects with that reported by Mandava et al. (1974). Identification of an *N*-methylated tryptophan in a Myristicaceous genus is interesting from a biosynthetic standpoint as it indicates that the pathway to tryptamines in this family may involve methylation of the side chain nitrogen prior to decarboxylation.

Snuff and paste samples

The alkaloid constituents of a number of samples of Myristicaceous drug preparations were also investigated (Table 4). These samples included one sample of orally-ingested paste collected at La Chorrera, Colombia, in 1971, and six paste samples collected in 1981 at the Witoto/Bora villages of Puco Urquillo and Brillo Nuevo on the Rio Ampiyacu in Peru. Four samples of Yanomama snuff from Venezuela were also screened.

All but one of the Myristicaceous paste samples contained substantial concentrations of alkaloid (Tables 4 and 5). In most cases the base composition of the pastes reflected the base composition of the source plants used to prepare them; the concentrations of tryptamines in the pastes were usually one to two orders of magnitude greater than the concentration in the crude plant material. β-Carbolines were found in only two of the samples and in both instances were present in such low concentrations that mass spectral analysis was the only method capable of detecting them. The second sample prepared from DMK-67 (*V. elongata*) contained low concentrations of DMT, NMT and a trace constituent with a mass spectrum closely matching that published (Agurell et al., 1969) for 2-methyl-tetrahydro-β-carboline. In this sample the β-carboline component was so close to the limit of detection that a poor spectrum was obtained. However the sample from La Chorrera, for which no voucher is available, contained high levels of NMT and DMT, and traces of two β-carbolines; one of these could be unequivocally established as 2-methyl-tetrahydro-β-carboline based on its identity with the published mass spectrum (Agurell et al., 1969), (M^+ = 186, *m/z* 143 = 100%, additional peaks at *m/z* 130, 115, 89, 77) while the other, eluting immediately after 2-methyl-tetrahydro-β-carboline, had M^+ = 200, *m/z* 185 = 100%, and strong peaks at *m/z* 157, 144, 129, 118, 92, 77 (Fig. 2). The fragmentation pattern is similar to 2-methyl-tetrahydro-β-carboline except that all major peaks are 14—15 mass units higher. Like 2-methyl-tetrahydro-β-carboline, this compound also shows a prominent peak at M-43, corresponding to the loss of the $CH_3—N{=}CH_2$ fragment,

although the base peak at *m/z* 185 (M-15) probably represents the facile loss of a methyl from C_1 and the formation of a stabilized intermediate with positive charge on the piperidine N. The prominent peak at *m/z* 157 is produced by loss of the piperidine N and the adjacent carbon 3, a typical fragmentation pattern for tetrahydro-β-carbolines (Coutis et al., 1970). A reasonable structure can be postulated for this compound, viz. 1,2-dimethyl tetrahydro-β-carboline. A similar compound, 6-methoxy-1,2-dimethyl-tetrahydro-β-carboline, was isolated by Agurell and coworkers (Agurell et al., 1968) from the seeds of *Anadenanthera peregrina* (Leguminosae).

The failure to detect β-carbolines in all of the paste samples and the extremely low levels found in two samples raises some interesting questions with regard to the postulated mechanism of oral activity of these pastes. The hallucinogenic activity of the Myristicaceous pastes almost certainly results from the high concentrations of psychotomimetic tryptamines which they contain, yet these compounds are not orally active by themselves, requiring an MAO inhibitor to protect them from intestinal and hepatic degradation. The β-carbolines are MAO inhibitors and theoretically could orally potentiate the tryptamines in the pastes, provided they were present in sufficient concentration to effectively inhibit MAO. This mechanism has been postulated (Agurell et al., 1968; Schultes, 1969; Schultes and Swain, 1976) to account for the oral activity of the Myristicaceous pastes as it probably also does for *ayahuasca* (cf. Agurell et al., 1968; Schultes, 1972; Der Marderosian et al., 1968; McKenna et al., 1984). The aromatic β-carbolines found in high concentration in *ayahuasca* (McKenna et al., 1984) are considerably more effective as MAO inhibitors than the tetrahydro-β-carbolines detected in the *Virola* pastes (cf. McKenna et al., 1984; Buckholtz and Boggan, 1976, 1977). Since not all of the paste samples contain β-carbolines, and those which do contain trace amounts of the less effective tetrahydro-β-carbolines, it seems extremely unlikely that these constituents could have any pharmacological significance in terms of orally potentiating the tryptamines in the pastes. In fact, the question of whether the Myristicaceous pastes are orally effective as hallucinogens remains open; bioassay of some of the paste samples collected on the Rio Ampiyacu by one of the authors (D.M.) produced equivocal results, in that some samples exhibited physiological activity, while others were apparently physiologically inert. None of the samples exhibited the hallucinogenic activity that is characteristic of tryptamines when ingested parenterally (cf. Szara, 1957). In any case, it appears that their oral activity, if present, must depend on some mechanism other than β-carboline mediated inhibition of MAO. The low levels of β-carbolines detected in the pastes, and the failure to detect similar compounds in the source-plants used for the drugs, also leaves open the possibility that they may be formed as artifacts either during the cooking and concentration of the *Virola* resin or during the work-up of the alkaloid extract. This possibility has also been raised by Holmstedt et al. (1980). Tetrahydro-β-carbolines of the type found in the paste samples can be

TABLE 4

DETECTION OF ALKALOIDS IN MYRISTICACEOUS PASTE AND SNUFF SAMPLES

Name and collection no. of source plant	Amount extracted (g dry wt.)	Alkaloid reagent test[a]			TLC[b]		Ehrlich's reaction[c]	Compounds detected[d]
		D	M	V	UV fluor.	UV abs.		
A. Orally active paste samples[e]								
1. DMK-40 *V. sebifera* Alfredo Moreno no. 1 Puco Urquillo	2.0	+++	++	+++	—	+	+(2)	NMT, DMT, 5-MeO-DMT, 5-MeO-NMT
2. DMK-67 *V. elongata* Alfredo Moreno no. 2 Puco Urquillo	2.0	+++	++	+++	—	+	+(1)	NMT, DMT, methyl-tetrahydro-β-carboline (GC/MS)
3. DMK-68 *V. elongata* Alfredo Moreno no. 3 Puco Urquillo	2.0	+++	++	+++	—	+	+(1)	NMT
4. DMK-69 *V. elongata* Alfredo Moreno no. 4 Puco Urquillo	2.0	+++	++	+++	—	+	+(1)	NMT
5. DMK-34 *V. pavonis* Jorge Churay Puco Urquillo	2.0	—	—	—	—	—	—	—
6. DMK-59 *V. elongata* Marcos Flores Brillo Nuevo	5.4	+++	++	+++	—	+	+(2)	5-MeO-DMT, 5-MeO-NMT
7. No voucher *"oo'-koey"* La Chorrera, Colombia (1971)	2.0	+++	++	+++	—	+	+(2)	DMT, NMT, methyl-tetrahydro-β-carboline, dimethyl tetrahydro-β-carboline (GC/MS)

B. Yanomamo snuffs (Venezuela, 1972)[f]								
1. No voucher *"buhenak + mashahara"* *Justicia pectoralis* + unknown species?	1.7	++	—	++	+	+	—	Coumarin, umbelliferone *(TLC, GC/MS)*
2. No voucher *"mashahari"* *Justicia pectoralis*	3.0	++	++	++	+	+	+(3)	DMT, 5-MeO-DMT, NMT, umbelliferone, coumarin, (TLC, GC/MS)
3. No voucher *"caraknak"* *Virola* sp. carbonized resin?	3.0	++	++	++	+	+	+(2)	DMT, 5-MeO-DMT
4. No voucher *"yakuana-sagona"* *Virola* sp. or complete snuff	4.0	+++	+++	+++	+	+	+(2)	NMT, 5-MeO-DMT

[a] Reaction to alkaloid precipitation reagents. D = Dragendorff's reagent; M = Meyer's reagent; V = Valser's reagent. Composition of reagents according to Martello and Farnsworth (1962).

[b] Results of TLC of basic fractions. UV fluor. indicates presence/absence of fluorescent spots; UV abs. indicates presence/absence of UV-absorbant spots.

[c] Presence/absence of Ehrlich-positive spots (diagnostic for tryptamines) on TLC. Numbers in parentheses indicate no. of Ehrlich-positive spots detected.

[d] Indicates major compounds identified in sample, using combination of methods including TLC, GC, HPLC and GC/MS.

[e] First line indicates collection no. and identity of source-plant, where known; second line indicates name of person manufacturing sample; third line, village of origin.

[f] No vouchers available for Yanomama snuff samples. Second line lists Yanomama name of sample; third line, probable botanical identity based on names cited in Schultes and Holmstedt (1968).

TABLE 5

QUANTITATIVE ANALYSIS OF TRYPTAMINES IN MYRISTICACEOUS SAMPLES

Sample name and origin	Species and collection no.	Alkaloids detected	mg/g dry wt	%
A. Orally active Myristicaceous pastes				
"oo'-koey"	No voucher	DMT	1.58	14
La Chorrera,		NMT	9.43	86
Colombia		Total	11.01	
"oo'-koey"	DMK-40	DMT	3.8	20
Puco Urquillo	*V. sebifera*	5-MeO-DMT	13.2	70
Alfredo Moreno				
no. 1		NMT	1.78	10
		Total	18.78	
"oo'-koey"	DMK-67	DMT	0.065	7
Puco Urquillo	*V. elongata*	NMT	0.86	93
Alfredo Moreno				
no. 2		Total	0.92	
"oo'-koey"	DMK-68	DMT	0.25	10
Puco Urquillo	*V. elongata*	NMT	2.2	90
Alfredo Moreno				
no. 3		Total	2.45	
"oo'-koey"	DMK-69	DMT	0.164	10
Puco Urquillo	*V. elongata*	NMT	1.43	90
Alfredo Moreno				
no. 4		Total	1.59	
"ku'-ru-ku"	DMK-59	5-MeO-DMT	15.72	100
Brillo Nuevo	*V. elongata*			
Marcos Flores				
no. 1				
B. Yanomama snuffs and snuff admixtures				
"mashahari"	*Justicia pectoralis*	DMT	0.09	15
Venezuela	(no voucher)	5-MeO-DMT	0.52	85
		Total	0.61	
"caraknak"	*Virola* sp.	DMT	0.064	17
Venezuela	carbonized resin	5-MeO-DMT	0.32	83
	(no voucher)	Total	0.384	
"yakuana-sagona"	*Virola* sp. (snuff)	5-MeO-DMT	19.7	100
Venezuela	(no voucher)			
C. Virola bark & leaf samples				
Virola sp.	DMK-30			
	Leaves	DMT	0.08	100
		NMT	Trace	
	Twigs	DMT	0.085	100
		NMT	Trace	
Virola sp.	DMK-35			
	Leaves	DMT	0.097	100
		NMT	Trace	
Virola sp.	DMK-36			
	Leaves	DMT	0.106	100
		NMT	Trace	

TABLE 5 (*Continued*)

Sample name and origin	Species and collection no.	Alkaloids detected	mg/g dry wt	%
Virola sp.	DMK-37			
	Leaves	DMT	0.097	100
		NMT	Trace	
Virola sebifera	DMK-40			
	Leaves	NMT	Trace	
	Bark	DMT	0.078	30
		5-MeO-DMT	0.181	70
		NMT	Trace	
		Total:	0.259	
Virola elongata	DMK-41			
	Bark	DMT	0.063	38
		NMT	0.102	62
		Total:	0.165	
Virola calophylla	DMK-46			
	Bark	DMT	0.56	100
Virola calophylla	DMK-56			
	Seeds and fruit	DMT	0.185	100
		NMT	Trace	
Virola elongata	DMK-59			
	Bark	5-MeO-DMT	0.23	100
		NMT	Trace	
	Leaves	DMT	0.17	100
		NMT	Trace	

readily formed from tryptamine derivatives such as NMT by aldehyde condensation followed by cyclization (Taborsky and McIsaac, 1964).

Four non-Myristicaceous collections utilized as admixtures to the orally-ingested pastes were screened for alkaloids using precipitation tests. All were alkaloid-negative. These admixtures consisted of an unidentified white crustose lichen, on the bark of a small tree, *Rinora racemosa* (Mart. & Zucc.) Kuntze (Violaceae, DMK-38); the leaves of a fern, *Anemia* sp. (DMK-39), which were steeped in the water used to cook the *Virola* paste; and the stems of *Philodendron nervosum* (Schult. & Schult.) Kunth. (DMK-64), which were crushed and then wrung into the water used to cook the *Virola* resin. In addition to these admixtures, the resin was usually mixed with ashes made from the dried fruit husks of *Theobroma bicolor* H. & B. (DMK-65), or *T. subinacum* Mart (DMK-43).

In addition to the orally-ingested Myristicaceous samples, four snuff samples collected among the Yanomama Indians of Venezeula were analyzed. These samples represent various components used in the manufacture of the snuffs, but are not accompanied by voucher specimens. They were collected by Dr. Ernesto Migliazza, an ethnolinguist who conducted field research on Yanomama language and grammar from 1970—1972. They were given to the

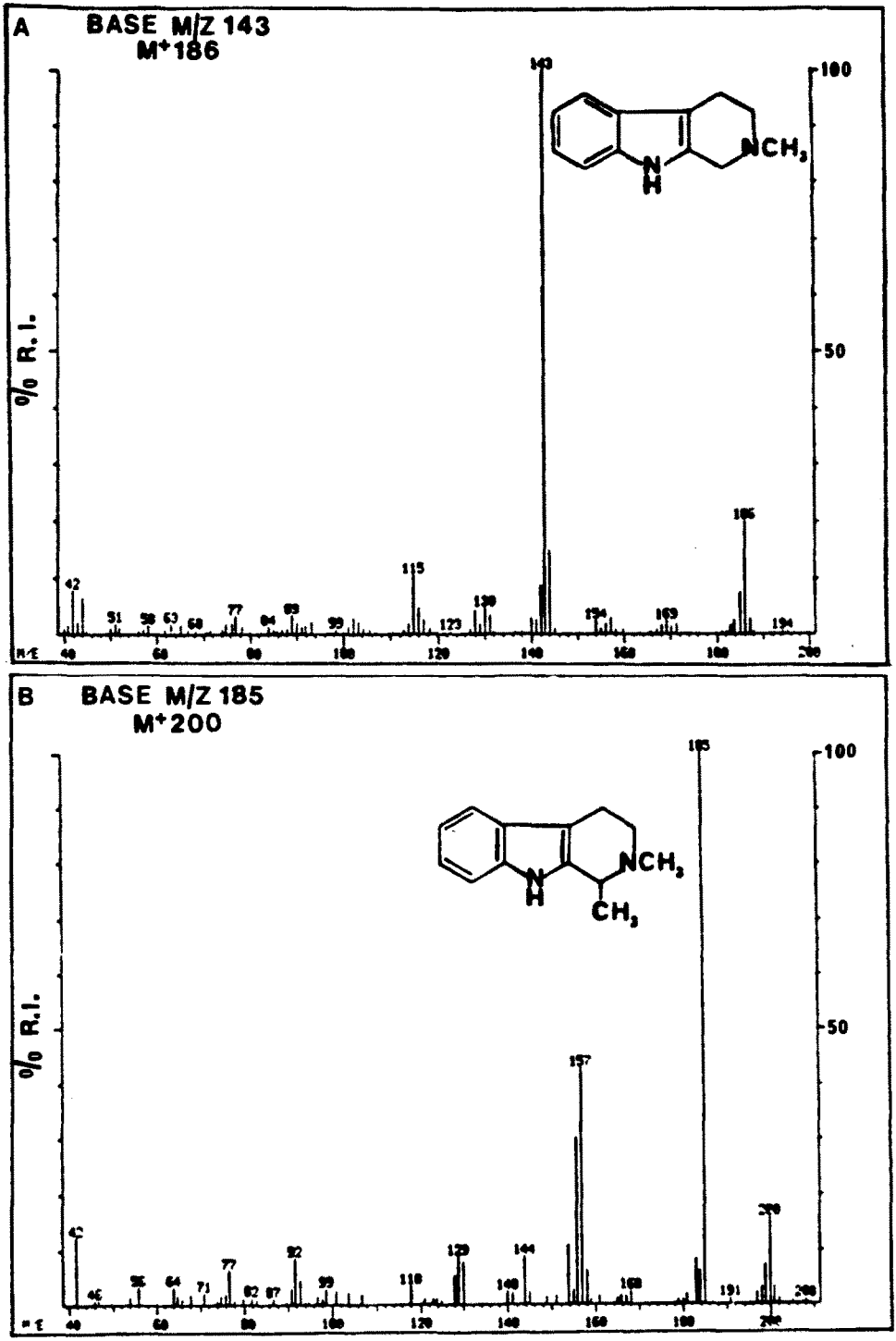

Fig. 2. Mass spectra of (A) methyl-tetrahydro-β-carboline and (B) dimethyl tetrahydro-β-carboline from La Chorrera *Oo'-koey*.

author in 1974 by Dr. Migliazza, of the Department of Anthropology at the University of Maryland, College Park. Dr. Migliazza collected the samples in connection with his liguistic studies; although no vouchers were collected, the correct native name of each of the samples was noted by Dr. Migliazza. By comparing these names with those reported by Schultes and Holmstedt (1968), we were able to arrive at the probable botanical identity of each sample. Subsequent chemical analyses tended to confirm these tentative identifications. Two of the samples are aromatic powdered leaves of a herbaceous plant which is almost certainly *Justicia pectoralis* var. *stenophylla* (Acanthaceae). One of these samples is labelled "mashahari" while the other is labelled "buhenak + mashahara". Schultes and Holmstedt (1968) state that the Karauetari Indians, a Yanomama subgroup on the Rio Cauaburi in Brazil, apply the name *mashahari* to *Justicia pectoralis* var. *stenophylla*, while Indians of the Rio Tototobi, Brazil, have two names for *Justicia pectoralis: masha-hara-hanak* (*hanak* means "leaf") and *boo-hanak*; this latter term is sufficiently close to "buhenak" that the terms are probably synonymous. Another possibility is that the Yanomama may recognize more than one kind or variety of *Justicia pectoralis* and apply different terms to each type. Chemical analyses of the samples lends support to this latter supposition. The sample labelled "mashahara + buhenak" contained no alkaloids (cf. Table 4) but did contain the benzopyran derivatives coumarin and umbelliferone. These compounds were found in the Peruvian collections of *Justicia pectoralis** made by the author (DMK-1, cf. MacRae and Towers, 1984a); their identity has been established by comparison of their mass spectra and TLC R_f values with authentic standards. Based on this evidence, it seems reasonable to speculate that the sample labelled "buhenak + mashahara" consists of powdered *Justicia pectoralis* leaves without any admixtures. The situation is less clear with respect to the sample labelled "mashahari". Although the label indicates that only one ingredient (*Justicia pectoralis*) is present, chemical analysis (Tables 4 and 5) has detected DMT, 5-MeO-DMT and NMT in this sample in addition to the coumarin derivatives. Several explanations may account for this difference but unfortunately the issue cannot be decided in the absence of botanical voucher specimens. One explanation is that the labels have been exchanged on the two samples, and the sample labelled "mashahari" is really "buhenak + mashahara"; if this is the case, then the ingredient "buhenak" may not represent *Justicia pectoralis*, but instead may be a term applied to another ingredient, probably a *Virola* species, which does contain tryptamines and is used in the manufacture of Yanomama snuff. An alternative explanation is that this sample does in fact represent *Justicia pectoralis* or a related species, which synthesizes tryptamines as well as coumarins. There exist reports (Prance, pers.

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*Note: Schultes (pers. commun. to D. MacRae, 1982) states that *Justicia pectoralis* var. *stenophylla* is probably a growth form of *Justicia pectoralis* rather than a genetic variant; in keeping with Prof. Schultes' suggestion, the varietal epithet is not used in subsequent citations of *Justicia pectoralis*.

commun., 1983; Chagnon et al., 1971) that occasionally the Yanomama do prepare an intoxicating snuff using *Justicia pectoralis* as the sole ingredient; these reports need to be corroborated by further ethnobotanical and chemical investigations. A third sample consists of black, partially charred and carbonized plant material, labelled with the Yanomama term "caraknak". This term does not correspond to any of those reported by Schultes and Holmstedt (1968), but the sample may consist of partially carbonized *Virola* resin. Schultes and Holmstedt (1968), describing the preparation of the snuff on the Rio Tototobi, mentions that a portion of the resin becomes carbonized in the process of boiling; this carbonized material is powdered separately from the rest of the resin to form a blackish brown powder. The remainder of the uncarbonized resin is ground into a light coffee-colored powder, which is then mixed with the carbonized material to produce the final snuff. Based on this description, it seems probable that the partly charred material labeled "caraknak" consists of carbonized *Virola* resin. The low levels of DMT and 5-MeO-DMT which this sample contained (cf. Table 5) support this identification. The fourth sample is labelled "yakuana-sagona" and consists of dark reddish brown powdered bark material. Both its appearance and its high alkaloid content (cf. Table 5) make it virtually certain that this sample consists either of powdered, concentrated *Virola* resin or of the finished snuff containing *Virola* resin plus other admixtures. According to Schultes and Holmstedt (1968), the term applied to the snuff on the Rio Tototobi is *nyakwana*, which is reasonably close to *yakuana*. Determining the exact equivalence of these native terms is complicated by the fact that the Yanomama linguistic family includes at least four major languages, each with numerous dialects; many of these dialects are mutually unintelligible (Migliazza, 1972). In view of this it is remarkable that the names recorded by Migliazza for the various snuff components can be matched so closely with those recorded by Schultes and Holmstedt.

Quantitative analyses of Myristicaceous samples

The quantitative data for the tryptamines found in the various drug samples (Table 5) show that the Yanomama snuff ("yakuana-sagona") and the various paste samples contain similar amounts of alkaloids on a dry wt basis. The snuff sample contained 18.82 mg/g 5-MeO-DMT, a figure of the same order of magnitude as that reported by Agurell et al. (1969) who found 7.15 mg/g in a sample of "epena" snuff from the Rio Cauaburi. A sample of *nyakwana* snuff from the Rio Tototobi was reported by Agurell et al. (1969) to contain more than 110 mg/g DMT and 5-MeO-DMT, or more than 11% alkaloids. In light of the other quantitative work reported in this and other studies (Agurell et al., 1969; Holmstedt et al., 1980), this figure is questionable. It is possible that an error was made either in the measurement of the alkaloid content of the sample, or in the calculation of the experimental data. The other components of the Yanomama snuffs

which were found to contain tryptamines ("mashahari" and "caraknak") contained quite low levels compared to the "yakuana" sample. Tryptamine concentrations found in the orally-ingested Myristicaceous drugs were generally of the same order of magnitude as those in the Yanomama snuff. For instance, the first batch of paste (designated *oo'-koey* in Witoto) obtained from Alfredo Moreno at Puco Urquillo, contained 18.1 mg/g alkaloid, 65% of which was 5-MeO-DMT. The second, third, and fourth batches obtained from Alfredo Moreno contained anomalously low levels of alkaloid. These batches were derived from DMK-67, 68 and 69, respectively, all of which have been determined as *V. elongata*. Comparison of the voucher specimens, and the qualitative and quantitative similarities of the alkaloid profiles of the bark, leaf and paste samples obtained from these three specimens (cf. Tables 3, 4, 5) make it clear that these three paste samples were all derived from the same tree.

The variation seen in the levels of alkaloid in the orally ingested samples (Tables 4 and 5) ranged from no alkaloid to substantial concentrations of alkaloid. This quantitative variation is accompanied by considerable variation in the kind and number of tryptamine bases present. For example the sample prepared from DMK-40 contained measurable levels of DMT, 5-MeO-DMT and NMT, while that prepared from DMK-59 contained only 5-MeO-DMT in any significant quantity. Similar differences in composition were found in the snuff samples. The base composition of the pastes reflected that of the source-plants, at least in those instances where the source-plants were available for examination (Tables 3 and 4). The alkaloid concentrations in the prepared pastes were usually 60—200 times greater than in the source plants (Table 5). Therefore it seems that the technology of preparing the drug, which basically involves extracting, cooking and concentrating the resin, does not result in a drastic change in the base composition compared to the source-plants; however minor constituents, such as the tetrahydro-β-carbolines, may be artifacts of the cooking process. The fact that these compounds have also been isolated from "raw" untreated *Virola* bark (Agurell et al., 1968) argues against this, however.

Disregarding for the moment the question of whether the orally-ingested Myristicaceous drugs require a MAOI in order to potentiate their activity, we can use the quantitative data from Table 5 to estimate the amount of drug which would be required to elicit psychotomimetic effects typical of tryptamines. Szara (1957) reported in self-experiments that the lower threshold dose for DMT administered i.m. was 30 mg, i.e. 0.4 mg/kg for a 75 kg adult, with optimal effects reached at 0.7—1.0 mg/kg, i.e. between 50 and 75 mg. Thus from Table 5 it is clear that for samples with the highest alkaloid content a threshold dose would require the ingestion of somewhat less than 2 g of paste or snuff, while an "optimal" dose would require the ingestion of 4—5 g dry wt of plant material. However if 5-MeO-DMT instead of DMT were the major constituent then approximately one-tenth as much plant material would be an adequate dose, as the threshold dose for this com-

pound is 1/10 that of DMT (cf. Kantor et al., 1980). Ingestion of several grams of the orally-ingested pastes would not be a problem but the absorption of 4 g of Yanomama snuff through the mucous lining of the nose and throat might present some mechanical difficulties. The most active pastes and snuffs may be those that contain a relatively greater proportion of 5-MeO-DMT; the two paste samples which showed the greatest activity in the bioassays did have a higher proportion of 5-MeO-DMT. In fact, the actual pharmacological activity of these preparations is probably determined by more than a simple dose-response relationship; the presence or absence of MAO inhibitors, the route of ingestion, the diet, body weight, and general health of the person ingesting the drug, the frequency of use and whether or not tolerance is developed, are all factors which could affect the physiological action of these drugs.

Bioassays of Myristicaceous paste samples

Two of the four Myristicaceous paste samples which were bioassayed in self-experiments in an effort to confirm or disconfirm their activity as orally-active hallucinogens proved to be without detectable physiological or psychological effects. This apparent lack of activity was confirmed in repeated trials involving the ingestion of amounts far in excess of those recommended by native informants. One of the inactive samples (Jorge Churay no. 1, derived from DMK-34, *V. pavonis*) proved on subsequent analysis to be devoid of detectable alkaloids of any kind (cf. Table 4), while the other inactive sample (La Chorrera *Oo'-koey*, no voucher) was found to contain primarily NMT, smaller amounts of DMT, and traces of two β-carbolines (cf. Table 5). The oral inactivity of these two samples can thus be rationalized on the basis of the chemical findings. The remaining two paste samples both exhibited some degree of oral activity. In the case of Alfredo Moreno's sample no. 1 (derived from DMK-40, *V. sebifera*) the activity consisted of the elicitation of hypnagogic imagery behind closed eyelids. No other psychological or physiological symptoms were noted, and the activity was ephemeral and easily disrupted by external stimuli. Subsequent analysis of this sample showed it to contain substantial concentrations of DMT and 5-MeO-DMT, but no β-carbolines (cf. Table 5). The fourth sample assayed (Marcos Flores no. 1, prepared from DMK-59, *V. elongata*) contained high levels of 5-MeO-DMT and a trace of NMT (cf. Table 5). This sample exhibited the greatest degree of oral activity of any of the four tested. Ingestion of 1.5—2.0 g of this paste elicited a rapid and profound response in one of the authors (D.M.) characterized by considerable physiological distress rather than the perceptual and psychological disturbances usually typical of hallucinogens. The initial effects, which manifested within 10 min following ingestion, consisted of a strong burning sensation in the mouth and throat, which quickly developed into a feeling of numbness in the lips, tongue and throat. Swallowing was difficult and breathing was impaired. The numbness gradually spread throughout the body, with a tingling sensation in the extre-

mities. The subject felt chilled, and stated that his body felt heavy and inert. He became somewhat alarmed at the rapidity of onset of the symptoms, and stated that he could now understand why *Virola* resin was used as an arrow poison. His breathing was irregular and shallow, and he felt motivated to concentrate on maintaining a regular, deep rhythm of breathing. The subject experienced a greatly enhanced acuity of hearing, but otherwise reported no perceptual changes, hypnagogic images, or hallucinations. After about 45 min the symptoms of physiological distress gradually subsided; this was followed by drowsiness and the subject fell into a light sleep for about 15 min. Most of the unpleasant physical sensations had disappeared by this time, but the feeling of being chilled continued for the rest of the evening. In later accounts of the experiment, the subject compared the effect of the paste sample to that of a pressor amine or general anaesthetic rather than to a hallucinogen. However, the bioassay left no doubt that this particular sample was highly orally active, although the activity was not typical of that usually associated with psychotomimetic tryptamines.

Activity of tryptamine derivatives and orally-active pastes as MAO inhibitors

Tryptamines

Although the structure/activity relationships of the β-carboline derivatives as MAO inhibitors has been systematically investigated (McIsaac and Estevez, 1966; Buckholtz and Boggan, 1977; McKenna et al., 1984), similar investigations of the MAOI activity of tryptamine derivatives have not been conducted. Barlow (1961) investigated the effects of DMT and several related methylated tryptamines as inhibitors of amine oxidase in guinea-pig liver homogenates were found a greater degree of inhibition when tyramine and 5HT were used as substrates than when tryptamine was the substrate. In a more recent study (Ho et al., 1970) MAO inhibition by 5-substituted dimethyltryptamines, α-methyltryptamines and gramines was examined. These investigators could find no correlation of inhibitory activity with the steric and electronic characteristics of the 5-position substituents. The DMT derivatives were more active than the gramine derivatives as MAOI and both were generally less active than the α-methyltryptamines. Of the four DMT derivatives tested, greatest inhibitory activity was shown by DMT, a 1 mM concentration giving 50% inhibition; 5-MeO-DMT was 3.4 times less active on a molar basis. In the present study, various tryptamine derivatives were assayed as MAOI in order to assess the influence of different indole ring substituents and modifications of the alkyl side-chain on the inhibitory activity. Presumably the activity exhibited by the various derivatives is also a reflection of their activity as competitive substances of MAO-A.

Most of the tryptamines assayed (Table 6) were one to two orders of magnitude less effective than β-carbolines as inhibitors of MAO (cf. McKenna et al., 1984). The major exception is *N,N*-dimethyltryptamine; with the lowest I_{50} value of all tryptamine derivatives tested, this compound has an I_{50} comparable to tetrahydroharmine and 6-MeO-harmalan (McKenna

TABLE 6
MAOI ACTIVITY OF TRYPTAMINE DERIVATIVES AND MYRISTICACEOUS PASTES

Compound or sample assayed	I_{50}[a]	% Inhibition at 1×10^{-3} M
Tryptamine derivatives		
5-Hydroxy-tryptophan	6.31×10^{-4}	56
Tryptamine	2.82×10^{-5}	89
5-MeO-tryptamine	3.98×10^{-5}	89
5-MeO-*N*-acetyl-tryptamine (melatonin)	3.98×10^{-4}	70
N-Methyl-tryptamine	2.24×10^{-5}	90
N,N-Dimethyltryptamine	1.58×10^{-6}	99
5-MeO-*N,N*-dimethyltryptamine	4.47×10^{-5}	
4-Hydroxy-*N,N*-dimethyl tryptamine (psilocin)	2.82×10^{-5}	96
4-Phosphoryl-*N,N*-dimethyltryptamine (psilocybin)	$>1.00 \times 10^{-3}$	35
5-MeO-*N,N*-diisopropyltryptamine	$>1.00 \times 10^{-3}$	40
2,5-Dimethyl-pyrrollyltryptamine (3-[2(2,5-dimethyl)pyrrolyl-ethyl]-indole)	2.51×10^{-5}	91
		% Inhibition at highest concentration (M)
Myristicaceous pastes and paste analogues		
La Chorrera *"oo'-koey"*	1.51×10^{-5}	96 (8.43×10^{-4})[b]
La Chorrera analogue	1.90×10^{-5}	94 (8.43×10^{-4})
Alfredo Moreno sample no. 1	8.91×10^{-6}	96 (7.02×10^{-4})
A. Moreno analogue	6.30×10^{-6}	97 (7.02×10^{-4})
Marcos Flores sample no. 1	2.95×10^{-5}	92 (9.30×10^{-4})
Marcos Flores analogue	2.34×10^{-5}	92 (9.30×10^{-4})
Non-alkaloidal samples		
Jorge Churay sample no. 1 (DMK-34. *V. pavonis*)	1200[c]	88 (15×10^{3}[c])
Crude lignan fraction (DMK-59, *V. elongata*)	20[c]	64 (50[c])

[a] Molar concentration at 50% inhibition of enzyme activity.
[b] Figures given at % inhibition at highest molar concentration of tryptamines in sample or analogues.
[c] Figures given are concentrations expressed as μg dry weight/ml.

et al., 1984). All other tryptamine derivatives are one to several orders of magnitude less effective than DMT as MAOI, and this reduction in activity may be related to the relative size and polarity of ring and side-chain substituents and also to the basicity of the side-chain N. Thus 5-MeO-DMT is some one and one-half orders of magnitude less active than DMT as MAOI, and the corresponding 5-MeO-diisopropyl analogue is essentially without significant MAOI activity at the highest concentration used. The influence of bulky ring substituents is well illustrated by comparison of psilocin and

psilocybin; the 4-hydroxy substituted compound psilocin shows reduced but still significant MAOI activity compared to DMT, but substitution with the large phosphoryl ester (psilocybin) essentially abolishes the activity. The effect of various alkyl side-chain substituents appears to be more complex than simply relative size; for instance 3-[2(2,5-dimethyl)pyrrolyl-ethyl]-indole exhibits approximately the same inhibitory activity as tryptamine, even though it has by far the largest alkyl-N-substituent; in this case the lipophilic nature of the N-substituent may permit a considerable degree of interaction with lipophilic residues of the enzyme despite its size. A certain degree of basicity of the side chain nitrogen appears to be a requirement for MAOI activity, presumably because the protonated amine interacts with an anionic moiety at the active site of the enzyme. Substitutions which abolish this basic character, e.g. the zwitterionic 5-hydroxy-tryptophan, and the N-acetylated compound melatonin, appear also to abolish the MAOI activity. Presence of non-polar substituents on the basic nitrogen may enhance interactions with a secondary lipophilic site; this would explain why DMT is a more active MAOI than tryptamine or NMT. In the case of the diisopropyl and higher homologs, however, activity may be diminished due to steric factors even though the substituents are non-polar.

Activity of Myristicaceous pastes and paste "analogues" as MAO inhibitors

The three Myristicaceous paste samples which quantitative GC analysis had previously shown to have the highest concentrations of tryptamines (Table 5) were assayed for MAOI activity in the in vitro rat-liver enzyme system. The concentrations and proportions of tryptamines in the paste samples were determined by HPLC after the samples had been prepared for use in the assay. Paste "analogues", consisting of mixtures of tryptamine standards in the same concentrations and proportions as in the pastes themselves were run simultaneously with each paste sample (cf. Materials and Methods). The results of these assays (Table 6, Fig. 3) show that the MAOI activity exhibited by the orally-ingested pastes is paralleled almost exactly by the activity of the paste analogues, containing only tryptamines. In all cases, the I_{50} of the paste sample is quite close to that of the analogue mixture indicating that the MAOI activity of the pastes is due solely to the presence of the tryptamines; it seems unlikely, therefore, that the presence of trace amounts of β-carbolines in addition to the tryptamines (as in, e.g. the La Chorrera sample) has any significance in terms of enhancing the MAOI action of the pastes or of orally potentiating the tryptamines present. The relative MAOI activity of the pastes appears to be a function of the types and amount of tryptamines present, e.g. the La Chorrera sample had an I_{50} only slightly lower than the Don Marcos sample; the former sample contained NMT + DMT, both of which are more active MAOI than 5-MeO-DMT which is the single major tryptamine in the Don Marcos sample. Alfredo Moreno's sample no. 1 had the lowest I_{50} value of the three, which is consistent with expectations since this sample contained proportionately

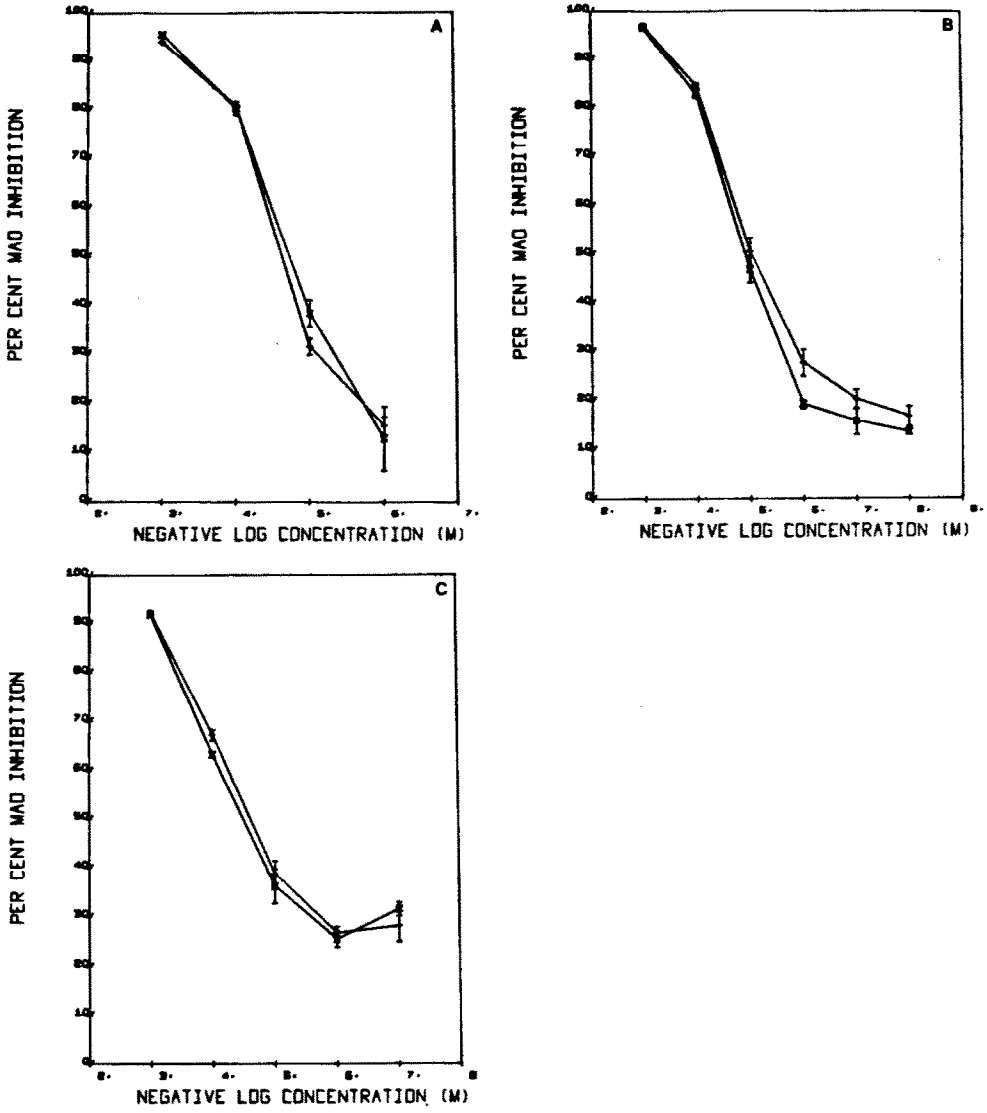

Fig. 3. MAOI activity in Myristicaceous pastes (A) La Chorrera *Oo'-koey*. +, paste sample; X, paste "analogue". (B) Alfredo Moreno *Oo'-koey* sample no. 1. X, paste sample; +, paste "analogue". (C) Marcos Flores *Ku'-ru-ku* sample no. 1. X, paste sample; +, paste "analogue".

the highest concentration of DMT, the most effective MAOI of the tryptamine derivatives tested. Although in vivo experiments would be required to confirm these results, the in vitro evidence indicates that these pastes do not exhibit MAOI activity beyond what is due to the tryptamine bases alone. Therefore, if these pastes are orally active hallucinogens, it appears that

some mechanism other than MAO inhibition must be sought to explain their oral activity.

The MAOI activity of the paste samples may also be partially due to the presence of non-nitrogenous MAOI in addition to the tryptamines and traces of β-carbolines. In order to investigate this possibility, a paste sample (derived from DMK-34, *V. pavonis*) which was alkaloid-free (cf. Table 4) was assayed in the system; a crude lignan fraction obtained from *V. elongata* (DMK-59) was also assayed. Some degree of inhibition was observed with both samples but was significant only at the highest concentrations. For the paste sample, this was 15,000 μg dry wt/ml of crude extract, and for the lignan sample, the highest concentration was 50 μg dry wt/ml of the crude lignan fraction. By contrast, the tryptamine-containing paste samples showed comparable amounts of inhibition at dry wt concentrations on the order of 3—4 μg dry wt/ml of crude extract. The inhibition shown by the alkaloid-free samples therefore appears to be non-specific and probably is due to a general denaturing of the proteins in the enzyme preparation due to the high phenolic content of the samples. The possibility that the Myristicaceous pastes may contain some highly specific non-nitrogenous MAO inhibitor seems somewhat remote.

What alternative mechanism might account for the oral activity of some samples? There are basically two possibilities worth consideration; both would require further experimental investigations to confirm. The first is that the oral activity of the Myristicaceous pastes is not due to the tryptamines at all, but rather to some other biologically active constituents, such as lignans. Certainly this would help to explain why the oral activity observed in self-experiments was atypical for hallucinogens. It is equally possible, however, that tryptamines taken orally differ significantly in their effects from tryptamines administered parenterally. That *Virola* resin (including the sample assayed) does contain biologically active lignans has been established (MacRae and Towers, unpublished data); but whether these lignans are capable of eliciting the spectrum of biological responses observed in the bioassay, is not known. The second possibility, which also calls for further experimental investigation, is that the oral inactivation of DMT, 5-MeO-DMT, and related derivatives is not due to oxidative deamination by peripheral MAO; alternative metabolic pathways may be relatively more significant in the degradation of these compounds in the periphery. Both in vitro and in vivo studies of DMT metabolism (Szara and Axelrod, 1959; Barker et al., 1980) suggest that 6-hydroxylation and/or N-oxidation of DMT occurs more readily in peripheral tissues than deamination by MAO. 6-Hydroxylation has been shown to occur in peripheral tissues but apparently not in brain (Szara and Axelrod, 1959; Barker et al., 1980); significantly, 6-hydroxy derivatives of DMT and related compounds are inactive as hallucinogens (Shulgin, 1976). Little is known of the hallucinogenic action of DMT-NO, but if it follows the general pattern for tertiary amine N-oxides (Bickel, 1969), it would either be completely inactive, or ten to

one hundred times less active than DMT. Other studies of in vivo DMT metabolism in the presence of MAO inhibitors and microsomal mixed function oxidase (MFO) inhibitors (Shah and Hedden, 1977) have found that while the MAOI iproniazid prolongs plasma and tissue half-life of DMT, the MFO inhibitor SKF-525A does not; the authors interpret these results as support for the hypothesis that DMT is metabolized mainly by MAO in vivo. A problem with most in vivo studies of the type reported by Shah and Hedden (1977), is that the DMT is administered to the animal i.p. rather than orally; thus the compound reaches the circulation directly and avoids "intestinal/hepatic-portal shunt" metabolism. It is possible that the metabolism of DMT via the intestinal/hepatic-portal shunt may differ in important respects from its metabolism when introduced directly into the bloodstream or body cavity. In the hepatic shunt 6-hydroxylation, and/or N-oxidation, may be relatively more important than MAO as a catabolic route for the compound. In vivo metabolic studies involving i.p. or other parenteral routes of administration actually shed little light on DMT metabolism following oral administration. The fact is that our understanding of the peripheral metabolism of DMT and related compounds is far from complete; all that is known for certain is that more than one type of oxidative reaction is involved. MAO may be partially responsible for the degradation of tryptamines in the periphery, but microsomal MFOs, which are involved in both the 6-hydroxyl and N-oxide pathways, are possibly even more important. Interestingly, if orally-administered DMT is a substrate for microsomal oxidases, then a mechanism can be proposed to explain the oral activity of some *Virola* pastes, even though they lack β-carbolines and are not significantly effective as MAOI. This alternative mechanism postulates that some of the non-alkaloid constituents in the pastes may possess anti-oxidant activity and/or exhibit activity as specific inhibitors of microsomal MFOs. In the former case, presence of high concentrations of non-specific anti-oxidants could scavange a high proportion of the molecular oxygen in the vicinity, thus making less of it available as co-substrate for the microsomal enzyme catalyzing DMT N-oxidation and 6-hydroxylation. In the latter case, constituents in the *Virola* resin may specifically inhibit hepatic microsomal MFO and thus block the oxidation(s) of DMT caused by these enzymes. In either case the compound could be protected from oxidative transformation in the intestinal/hepatic shunt and thus be taken up into the CNS in the form of the unchanged tertiary amine. A number of lignans have been characterized which exhibit protective activity against hepatotoxins (cf. MacRae and Towers, 1984b); inhibition of hepatic MFO has been proposed as the probable mechanism. All of the active compounds possessed a methylenedioxyphenyl moiety, but analogs lacking this configuration did not have hepatoprotective properties. The methylenedioxyphenyl group has been implicated in other studies as the main pharmacophore responsible for mixed function oxidase inhibition (Brattsten, 1977). The Myristicaceous genera *Virola* and *Iryanthera* are both rich in constituents incorporating the methylenedioxy-

phenyl group, including fatty acid derivatives, neolignans, flavans and diarylpropanoids (Gottlieb, 1979). Several novel lignans having this substitution were characterized in the bark of DMK-59 (*V. elongata*) (MacRae and Towers, unpublished data) which was the source-plant for Marcos Flores' paste sample; this sample showed the greatest degree of oral activity in self-experiments. A number of other phenolic compounds, including flavans, flavanoids, isoflavonoids, diarylpropanoids, and neolignans, have been isolated from a number of *Virola* and *Iryanthera* spp.; some of these compounds could act as antioxidants and could contribute to the inactivation of MFOs via this non-specific mechanism. In any case the peripheral metabolism of DMT and related compounds, following oral administration, may be significantly altered in the presence of antioxidants and/or specific MFO inhibitors; under these conditions the compounds might well reach the CNS in the form of the unchanged tertiary amine. Further in vivo and in vitro experiments would be required to confirm or disconfirm this alternative mechanism of oral activity. In view of the phytochemical and pharmacological data accumulated in the present study, it appears that this alternative hypothesis is at least as probable, if not more probable, than MAO inhibition as the mechanism responsible for the oral activity of the Myristicaceous pastes.

Acknowledgements

We thank Felipe Balza for excellent technical assistance with the GC/MS analyses, and Dr. G. Bellward, Faculty of Pharmaceutical Sciences, University of British Columbia, for the use of radioisotope laboratory facilities in the development of the MAO assay. Mr. Wade Davis, of the Harvard Botanical Museum, helped with our collecting efforts on the Rio Ampiyacu, and we appreciate his contribution. The herbarium voucher specimens were determined by Dr. W. Rodrigues, INPA, Manaus, and Dr. T. Plowman, Chicago Field Museum, and we thank them for their contributions to this work. Partial financial support for this research was provided by the Natural Science and Engineering Research Council of Canada and we acknowledge their support. Financial support for one of the authors (D.M.) was provided by a UBC Graduate Fellowship.

References

Agurell, S., Holmstedt, B., Lindgren, J.E. and Schultes, R.E. (1968) Identification of two new β-carboline alkaloids in South American hallucinogenic plants. *Biochemical Pharmacology* 17, 2487—2488.

Agurell, S., Holmstedt, B. and Lindgren, J.E. (1969) Alkaloids in certain species of *Virola* and other South American plants of ethnopharmacologic interest. *Acta Chemica Scandinavica* 23, 903—916.

Barker, S.A., Monti, J.A. and Christian, S.T. (1980) Metabolism of the hallucinogen N,N-dimethyltryptamine in rat brain homogenates. *Biochemical Pharmacology* 29, 1049—1057.

Barlow, R.B. (1961) Effects on amine oxidase of substances which antagonize 5-hydroxytryptamine more than tryptamine on the rat fundus strip. *British Journal of Pharmacology* 16, 153—162.

Bickel, M.H. (1969) The pharmacology and biochemistry of N-oxides. *Pharmacological Reviews* 21, 325—358.

Brattsten, L.B. (1977) Biochemical defense mechanisms in herbivores against plant allelo-

Buckholtz, N.S. and Boggan, W.O. (1967) Effects of tetrahydro-β-carbolines on monoamine oxidase and serotonin uptake in mouse brain. *Biochemical Pharmacology* 25, 2319—2321.

Buckholtz, N.S. and Boggan, W.O. (1976) Effects of tetrahydro-β-carbolines on monoliver produced by β-carbolines: structure-activity relationships and substrate specificity. *Biochemical Pharmacology* 26, 1991—1996.

Cassady, J.M., Blair, G.E., Raffauf, R.F. and Tyler, V.E. (1971) The isolation of 6-methoxyharmalan and 6-methoxyharman from *Virola cuspidata. Lloydia* 34, 161—162.

Chagnon, N., LeQuesne, P. and Cook, J.M. (1971) Yanomamo hallucinogens: anthropological, botanical, and chemical findings. *Current Anthropology* 12, 72—74.

Corothie, E. and Nakano, T. (1969) Constituents of the bark of *Virola sebifera. Planta Medica* 17, 184—188.

Coutis, R.T., Locock, R.A. and Slywka, G.W.A. (1970) Mass spectra of selected β-carbolines [β-9H-pyrido(3,4-b)indoles]. *Organic Mass Spectrometry* 3, 879—889.

Der Marderosian, A.H., Pinkley, H.V. and Dobbins, M.F. IV. (1968) Native use and occurrence of N,N-dimethyltryptamine in the leaves of *Banisteriopsis rusbyana. American Journal of Pharmacy* 140, 137—147.

Ghosal, S., Ballav, R., Chauhan, P.S. and Mehta, R. (1975) Alkaloids of *Sida cordifolia. Phytochemistry* 14, 830—832.

Gottlieb, O.R. (1979) Chemical studies on medicinal Myristicaceae from Amazonia. *Journal of Ethnopharmacology* 1, 309—323.

Ho, B.T., McIsaac, W.M., An, R., Harris, R.T., Walker, K.E. and Kralik, P.M. (1970) Biological activities of some 5-substituted N,N-dimethyltryptamines, α-methyltryptamines, and gramines. *Psychopharmacologia* 16, 385—394.

Holmstedt, B., Lindgren, J.E., Plowman, T., Rivier, L., Schultes, R.E. and Tovar, O. (1980) Indole alkaloids in Amazonian Myristicaceae: field and laboratory research. *Harvard Botanical Museum Leaflets* 28, 215—234.

Johns, S.R., Lamberton, J.A. and Sioumis, A.A. (1971) The major alkaloid of *Aotus subglauca* (Leguminoseae), S-(+)-N-methyltryptophan methyl ester. *Australian Journal of Chemistry* 24, 439—440.

Kantor, R.E., Dudlettes, S.D. and Shulgin, A.T. (1980) 5-Methoxy-α-methyl-tryptamine (α-O-dimethylserotonin), a hallucinogenic homolog of serotonin. *Biological Psychiatry* 15, 349—352.

MacRae, D. and Towers, G.H.N. (1984a) *Justicia pectoralis,* a study of the basis for its use as a *Virola* snuff admixture. *Journal of Ethnopharmacology,* 12, 93—111.

MacRae, W.D. and Towers, G.H.N. (1984b) Biological activities of lignans. *Phytochemistry,* in press.

Mandava, N., Anderson, J.D. and Dutky, S.R. (1974) Indole plant-growth inhibitor from *Abrus precatorius* seeds. *Phytochemistry* 13, 2853—2856.

Marley, E. and Blackwell, B. (1970) Interactions of monoamine oxidase inhibitors, amines and foodstuffs. *Advances in Pharmacology and Chemotherapy* 8, 185—239.

Martello, R. and Farnsworth, N. (1962) Observations on the sensitivity of several common alkaloid precipitating reagents. *Lloydia* 25, 176—185.

McIsaac, W.M. and Estevez, V. (1966) Structure-action relationships of β-carbolines as mono-amine oxidase inhibitors. *Biochemical Pharmacology* 26, 1625—1627.

McKenna, D.J., Towers, G.H.N. and Abbott, F.S. (1984) Monoamine oxidase inhibitors in South American hallucinogenic plants: tryptamine and β-carboline constituents of *Ayahuasca. Journal of Ethnopharmacology* 10, 195—223.

Migliazza, E.C. (1972) *Yanomama Grammar and Intelligibility*. PhD. Thesis, Indiana University.

Repke, D.B., Leslie, D.T. and Guzman, G. (1977) Baeocystin in *Psilocybe, Conocybe*, and *Panaeolus*. *Lloydia* 40, 566—578.

Rodrigues, W.A. (1980) Revisao taxonomica das especies de *Virola* Aublet (Myristicaceae) do Brasil. *Acta Amazonica* 1, 1—123.

Schultes, R.E. (1969) *Virola* as an orally-administered hallucinogen. *Harvard Botanical Museum Leaflets* 22, 229—240.

Schultes, R.E. (1972) Ethnotoxicological significance of additives to New World hallucinogens. *Plant Science Bulletin* 18, 34—41.

Schultes, R.E. and Hofmann, A. (1980) *The Botany and Chemistry of Hallucinogens* (2nd edn.), Charles C. Thomas, Springfield, IL.

Schultes, R.E. and Swain, T. (1976) Further notes on *Virola* as an oral hallucinogen. *Journal of Psychedelic Drugs* 8, 317—324.

Schultes, R.E. and Holmstedt, B. (1968) The vegetal ingredients of the Myristicaceous snuffs of the Northwest Amazon. *Rhodora* 70, 113—160.

Schultes, R.E., Swain, T. and Plowman, T. (1977) *Virola* as an oral hallucinogen among the Boras of Peru. *Harvard Botanical Museum Leaflets* 25, 259—272.

Schultes, R.E. (1979) Evolution of the identification of the Myristicaceous hallucinogens of South America. *Journal of Ethnopharmacology* 1, 211—239.

Shah, N.S. and Hedden, M.P. (1977) Behavioural effects and metabolic fate of N,N-dimethyltryptamine in mice pretreated with β-diethylaminoethyl-diphenylpropyl-acetate (SKF-525-A), iproniazid, and chlorpromazine. *Pharmacology, Biochemistry and Behaviour* 8, 351—356.

Shulgin, A.T. (1976) Psychotomimetic agents. In: Maxwell Gordon (Ed.), *Psychopharmacological Agents*, Vol. IV, Academic Press, New York, London, Chap. 4.

Szara, S. (1957) The comparison of the psychotic effect of tryptamine derivatives with the effects of mescaline and LSD-25 in self-experiments. In: S. Garattini and V. Ghetti (Eds.), *Psychotropic Drugs*, Elsevier, Amsterdam, pp. 460—467.

Szara, S. and Axelrod, J. (1959) Hydroxylation and N-demethylation of N,N-dimethyltryptamine. *Experientia* 15, 216—217.

Taborsky, R.G. and McIsaac, W.M. (1964) The synthesis and preliminary pharmacology of some 9H-pyrido[3,4-b]indoles (β-carbolines) and tryptamines related to serotonin and melatonin. *Journal of Medicinal Chemistry* 7, 135—141.

Udenfriend, S., Witkop, B., Redfield, B.G. and Weissbach, H. (1958) Studies with reversible inhibitors of monoamine oxidase: harmaline and related compounds. *Biochemical Pharmacology* 1, 160—165.