MONOAMINE OXIDASE INHIBITORS IN SOUTH AMERICAN HALLUCINOGENIC PLANTS: TRYPTAMINE AND β-CARBOLINE CONSTITUENTS OF *AYAHUASCA*

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Summary

Ayahuasca is a hallucinogenic beverage derived by boiling the bark of the Malpighiaceous liana *Banisteriopsis caapi* together with the leaves of various admixture plants, viz. *Psychotria viridis, Psychotria carthagenensis,* or *Diplopterys cabrerana. B. caapi* contains harmine, harmaline, and tetra-hydroharmine while the admixtures contain *N,N*-dimethyltryptamine (DMT). DMT, a potent hallucinogen, is inactive orally due to degradation by visceral monoamine oxidase (MAO). The β-carbolines, however, are highly active reversible inhibitors of MAO and may protect the DMT from deamination by MAO and render it orally active. This mechanism has been proposed to underlie the oral activity of *ayahuasca* but has not been experi-mentally confirmed. In the present study the constituents of the admixture plants and the alkaloids of eight *ayahuasca* samples from Peru were qualita-tively and quantitatively analyzed using two-dimensional thin-layer chro-matography (TLC), high pressure liquid chromatography (HPLC) and gas chromatography/mass spectrometry (GC/MS).

Several *B. caapi* cultivars were quantitatively compared for variations in alkaloid content. Three admixture plants used rarely in the manufacture of *ayahuasca* were also screened for alkaloids. A selected sample of β-car-bolines were screened for activity as MAO inhibitors using an in vitro assay system, and structure/activity relationships were compared. Inhibition observed with single compounds was compared with the activity of selected samples of *ayahuasca* which were screened in the system and also with the activity of mixtures of β-carbolines. The levels of DMT and β-carbolines found in the *ayahuasca* samples examined in the present study were an order of magnitude greater than the levels reported in a previous study. *Ayahuasca* was found to be an extremely effective inhibitor of MAO in vitro and the degree of inhibition was directly correlated with the con-centration of MAO-inhibiting β-carbolines. Inhibition experiments using mixtures of β-carbolines indicated that their effects in combination are

additive, rather than synergistic or antagonistic. Implications of the results in understanding the pharmacology of *ayahuasca* are discussed.

Introduction

The hallucinogenic beverage *ayahuasca* (Quechua for "vine of the souls") is widely used for medicinal, ritual and recreational purposes by the aboriginal and mestizo populations inhabiting the Amazon Basin. While *ayahuasca* is the most common term for the drug in the Peruvian Amazon, in different regions it is known by various vernacular names, including *yage, caapi, natema* and *pinde* (Schultes, 1957). The bitter, coffee-colored beverage is prepared by boiling the bark of the Malpighiaceous jungle liana *B. caapi* (Spruce ex Griseb.) Morton together with the leaves of various admixture plants, the addition of which is believed to intensify or modify the effect (Schultes, 1972). Many of the admixture plants used remain uncharacterized either botanically or chemically, however those used most commonly are *Psychotria viridis* Ruiz and Pavon, *Psychotria carthagenensis* Jacq. and *Diplopterys cabrerana* (Cuatrecasas) Gates; the latter is also a Malpighiaceous liana formerly known as *Banisteriopsis rusbyana* (Gates, 1979; Plowman, pers. commun.).

The most complete study to date of the chemistry of the *ayahuasca* beverage, and of the source-plants and admixture plants used in its manufacture, is that of Rivier and Lindgren (1972); these investigators studied *ayahuasca* samples and vouchered botanical material collected among the Sharanahua and Culina tribes of the upper Rio Purús in Peru. Using GC/MS as the primary analytical tool, Rivier and Lindgren found that the β-carboline alkaloids harmine, harmaline, and tetrahydroharmine (THH), and DMT were the major active constituents of *ayahuasca* (cf. Fig. 2). The β-carbolines are constituents of *B. caapi* (Deulofeu, 1967) while DMT has been reported in the two *Psychotria* spp. (Rivier and Lindgren, 1972) as well as in *D. cabrerana* (Agurell et al., 1968a).

Hashimoto and Kawanishi (1975, 1976) have reported the isolation of six β-carboline bases from the leaves of *B. caapi* in addition to the three main constituents (viz. harmic amide, acetyl norharmine, ketotetrahydronorharmine, harmine *N*-oxide, harmic acid methyl ester and harmalinic acid). However, the extremely low concentrations of these compounds in the plant (0.007—0.0001%) make it unlikely that they contribute significantly to the pharmacological activity of *ayahuasca*.

Although the active alkaloids of *ayahuasca* are now known, certain aspects of the pharmacology of the drug remain to be clarified. DMT is known to be a potent hallucinogen but is also known to be inactive when ingested orally (Shulgin, 1976), probably due to deamination by intestinal and hepatic MAO. Several studies (Ahlborg et al. 1968; Lu et al., 1974, Moore et al., 1975; Shah and Heddon, 1978) have shown that indole-

3-acetic acid (IAA) is a major metabolite of DMT both in vitro and in vivo which suggests that it is a substrate for MAO. Further support for the importance of MAO in the metabolism of DMT is found in the fact that the behavioural effects and tissue levels of DMT in rats are potentiated by pretreatment with the MAO inhibitor iproniazid, a specific inhibitor of MAO-A. However, Barker et al., (1980, 1981) point out that incubation of DMT in rat whole brain homogenates resulted in the formation of *N*-methyltryptamine (NMT), DMT-*N*-oxide, and 2-methyl-tetrahydro-β-carboline as well as IAA. These workers suggest that NMT may be the primary metabolite of DMT, and that IAA is formed secondarily as a result of the oxidative deamination of NMT. NMT is oxidized nine times more rapidly than DMT by MAO, while DMT-*N*-oxide is essentially resistant to metabolism by MAO under aerobic conditions (Smith et al., 1962).

The β-carbolines present in *B. caapi* are known to be highly active reversible inhibitors of MAO (Udenfriend et al., 1958; McIsaac and Estevez, 1966; Buckholtz and Boggan, 1977) and are probably also hallucinogenic. The psychotomimetic activity of the β-carbolines is not well understood because there are relatively few clinical investigations of the effects of β-carbolines on human subjects. Naranjo (1967) reported harmaline to be hallucinogenic at oral doses of 4 mg/kg but could not report similarly unequivocal results for harmine or tetrahydroharmine. Pennes and Hoch (1957) reported harmine was orally inactive at levels approaching 12 mg/kg. It has been suggested (der Marderosian et al., 1968; Agurell et al., 1968b; Schultes, 1972; Shulgin, 1976) that the hallucinogenic properties of the crude *ayahuasca* brew result from a synergistic interaction among the various constituents; specifically, that it results from an oral activation of the DMT through the inhibition of MAO by the β-carbolines. This mechanism would render the DMT orally active by blocking its degradation by visceral MAO. Although this mechanism is reasonable and has long been accepted in the ethnopharmacological literature, the effect of *ayahuasca* on monoamine oxidase has not been experimentally determined. This paper presents the results of our phytochemical and pharmacological investigations of *ayahuasca*, including an evaluation of its effect on MAO in vitro.

Experimental procedures

Field collection of drug samples and plant materials

Herbarium vouchers and preserved plant material

Collection numbers cited throughout this paper refer to the personal collection numbers of D. McKenna, with the exception of Plowman 6040 *(Diplopterys cabrerana)* and Plowman 6041 *(B. caapi* var. "cielo"). Plant material for Plowman 6040 and 6041 was kindly supplied by Dr. Timothy Plowman of the Chicago Field Museum. Authenticated herbarium vouchers for all collection numbers cited have been deposited in the Herbarium of

the Department of Botany, University of British Columbia. Duplicate vouchers of most collections are also on deposit at the Chicago Field Museum. Bark and leaf samples used in the analysis of the admixtures and the *B. caapi* cultivars consisted either of air-dried material or of material preserved in 100% methanol at the time of collection. The section below on tryptamine-containing admixture plants details the work-up procedure used in the analysis of methanol-preserved materials.

Ayahuasca samples

Ayahuasca samples used for analysis are identified in this paper by the name of the *ayahuasquero* from whom they were obtained, and also by a number. In some cases more than one sample was obtained from the same person. Some of the *ayahuasca* samples were diluted approximately 1 : 1 with methanol at the time of collection in order to arrest fermentation and prevent biological transformation of the products. Other samples were not diluted. No obvious difference between the alkaloid content of methanol-diluted samples and undiluted *ayahuasca* samples could be detected by HPLC or TLC.

Thin-layer chromatography of ayahuasca brews and DMT-containing admixtures

Two-dimensional TLC was carried out using 10 × 10 cm Polygram Silica Gel G UV[254] precoated plates (Brinkmann Instruments). The origin was marked with pencil in the lower left hand corner of the plate, 1.0 cm from the bottom and left-hand edge. Five microlitre aliquots of the material to be analyzed (consisting either of the crude *ayahuasca* samples or in the case of the admixture plants of a methanol solution of the purified alkaloid fraction) were applied to the origin using a Microcap applicator. The applied sample was dried under a gentle stream of air, and then developed in the first direction using ether/2-butanone/conc. NH_4OH, 5 : 4 : 1 (Solvent 1). Solvent 1 was freshly prepared in a separatory funnel and the upper phase was collected for TLC. Following development in Solvent 1, the plate(s) were removed and allowed to air dry in a fume hood. Development in the second dimension was commenced when the plates were completely free of Solvent 1, indicated by the absence of any solvent odour. Plates were then rotated 90° to the left with respect to their position in Solvent 1, and developed in Solvent 2, consisting of *n*-propanol/1.5% NH_4OH, 9 : 2; this solvent was stable for 2—3 days at room temperature if kept sealed in a ground-glass stoppered flask. Development in both Solvent 1 and 2 was carried out at ambient temperature in an unlined 10 × 30 × 26 cm glass chromatographic tank containing approx. 50 ml of solvent. Following development in Solvent 2, plates were removed and air-dried in a fume hood for 30 min to 1 h or overnight on the laboratory bench. Plates were examined under short- and long-wave UV light to visualize the alkaloids.

DMT and tetrahydro-β-carbolines are visible as dark spots under short-wave UV while the aromatic and dihydro-β-carbolines give characteristic strong fluorescent colours under long-wave UV. Duplicate plates were sprayed with Ehrlich's reagent (Repke et al., 1977) which gives blue to violet colours with DMT and other tryptamine derivatives following exposure to HCl vapours. Aromatic and dihydro-β-carbolines do not react with Ehrlich's reagent, however THH gives a characteristic robin's egg blue colour which develops over 24 h. This slow reaction can be used to conveniently distinguish THH from its more aromatic analogues and also from the tryptamines which give darker blue reactions that appear within 30 min of exposure to HCl vapours. A TLC plate containing aliquots of known β-carboline and tryptamine standards was developed simultaneously with the sample plates; constituents in the samples corresponding to known standards could thus be readily identified by comparison of the sample plates with the "standard" plate (cf. "*ayahuasca* analogue", Fig. 1).

High pressure liquid chromatography

Analytical conditions

A Varian model 5000 HPLC interfaced with a Spectra-physics model SP4100 computing integrator was used for the quantitative analysis of the *ayahuasca* samples, the *B. caapi* cultivars, and the DMT-containing admixture plants. Constituents were detected by UV absorption at 260 nm with a Varian model 634 variable wavelength UV/visible spectrophotometer. Column consisted of a Varian Micropak MCH-10 reverse phase column, 30 cm $\times$ 4 mm i.d. Solvents were methanol/water containing 0.05% triethylamine. A gradient elution program was used for the analysis, from 60—90% methanol at a rate of 1%/min. Solvent flow rate was 2 ml/min. Samples were applied to the column via a Rheodyne model 7125 syringe loading sample injector fitted with a 20-μl sample loop. Samples were loaded onto the sample injector using a 25-μl Hamilton no. 702 microlitre syringe fitted with a no. 22 gauge 90° bevelled needle.

Quantitative methods

Quantitative analyses were carried out using the external standard program supplied with the SP4100 computing integrator. In this method, the column is calibrated with a mixture containing known concentrations of standard compounds. The integrator program calculates the peak area of each standard in the calibration mixture and normalizes the response factor. Calibration at two or more concentrations enables the integrator to generate the coefficients of a linear equation relating sample concentration to peak area. This calibration data is stored in the memory circuits of the integrator and used to calculate the concentration of components of interest in subsequent sample runs by comparison of the peak areas of sample components with the peak areas of standards in the calibration mix.

Stock solutions of harmine, harmaline, tetrahydroharmine and DMT standards were made up to a precise concentration of 1 mg/ml. Equal aliquots from the stock solutions were combined, giving a calibration mixture of 0.25 mg/ml of each component; 1:1 dilution of a portion of this mixture with methanol gave a second calibration mixture in which the concentration of each component was 0.125 mg/ml. The SP4100 integrator was calibrated by making single 20-μl injections of the calibration mixture at each concentration level. The integrator was recalibrated following every five sample injections.

Sample preparation for HPLC quantitation

Ayahuasca samples. The *ayahuasca* samples which had not been diluted with methanol on collection were diluted for analysis so that the alkaloids present were within the concentration range of the calibration standards. A 1.5-ml aliquot of the crude preparation was diluted to 15 ml with chilled methanol and a white, flocculent, proteinaceous precipitate which separated from solution was removed by filtration. This diluted preparation, after filtration through a Pasteur pipette plugged with glass wool, was injected directly into the HPLC. Two replicates of each *ayahuasca* sample were prepared, and each replicate sample was injected twice during separate runs following calibration of the integrator using the standard mixtures. Values reported in Table 1 are the means of these four replicate injections. Values given are 10X the actual value measured since the samples measured were 1/10 the concentration of the undiluted brew.

The *ayahuasca* samples which had been diluted with methanol in the field were quantified for alkaloids in terms of mg/g dry wt of the lyophilized sample. Fifteen millilitres of the methanol-diluted sample was evaporated on a steam bath, frozen, then lyophilized. A portion of the freeze-dried residue was ground to a fine powder and 100 mg was transferred to a 100 ml round-bottom flask. Ten millilitres 100% methanol was added and the solution was extracted over a steam bath for 5 min. The methanol was removed with a Pasteur pipette and filtered through glass wool. The extraction was repeated using a second 10-ml aliquot of methanol. The filtered extracts were combined, and the final volume was adjusted to 10 ml. Twenty microlitre aliquots of this solution were injected onto the HPLC. As with the previous samples, two replicates of each sample were prepared, and each replicate was injected twice. Figures given in Table 2 are the average of these four replicate injections.

B. caapi cultivars. Quantitation of the alkaloid content of the *B. caapi* cultivars was carried out on stem samples which had been dried under low heat (<60°C) in a plant dryer. The air-dried stems were ground to powder in a Wiley mill and 5 g was extracted 2X in 100 ml methanol for 24 h on a rotary shaker. The combined extracts were concentrated under vacuum, filtered through glass wool and the volume adjusted to 25 ml with methanol. Ten microlitre aliquots were injected directly onto the HPLC. Four replicate

injections of each sample were made, and figures given in Table 3 reflect the mean of these four replicate injections.

DMT-containing admixture plants. All of the *Psychotria viridis* samples were analyzed using methanol-preserved leaf material collected in the field; the *Diplopterys cabrerana* sample consisted of freeze-dried leaves derived from a greenhouse propagated clone of Plowman 6040. The methanol was decanted from the methanol-preserved material and the remaining solid matter was frozen, then lyophilized. The freeze-dried leaf material was powdered and extracted overnight on a rotary shaker with methanol (10—20 ml/g dry wt). The *D. cabrerana* leaves were extracted directly with methanol and otherwise treated in the same manner as the *Psychotria* samples. The methanol extracts were filtered, combined with the original methanol used to preserve the samples, and concentrated in a rotary evaporator to a known volume. The crude methanol extracts were sealed and stored at 4°C. For purposes of alkaloid quantitation, an aliquot of the methanolic extract equivalent to 2.0 g dry wt of the freeze-dried leaf material was transferred to a 50 ml round-bottom flask and evaporated to dryness on a rotary evaporator. The residue was shaken with 5 ml of 1 N HCl and filtered. The acidic filtrate 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 2 N NaOH. The basic aqueous fraction was extracted with 3×5 ml CH_2Cl_2. The organic layer was dried over anhydrous Na_2SO_4, evaporated to dryness, redissolved in methanol, and filtered through glass wool. Twenty microlitre aliquots of this purified alkaloid fraction were injected into the HPLC. Concentration of the DMT present was determined by comparison with a standard curve constructed by injecting known amounts of DMT standard. Figures given in Table 4 are means ± S.E. of 2—5 replicate injections.

Gas chromatography/mass spectrometry

DMT-containing admixtures were screened by GC/MS to confirm that the major indole base detected was DMT. The instrument used was a Finnigan model 1020 automated GC/MS interfaced with a Perkin-Elmer Sigma 3B gas chromatograph. The chromatograph was equipped with a 30 m $\times$ 0.25 mm SE-54 fused silica capillary column (J & W Scientific). The chromatograph was temperature-programmed from an initial temperature of 180°C to a final temperature of 250°C. The ramp rate was 3°C/min initiated 3 min after injection of the sample. Injector block temperature was 250°C, detector temperature 260°C. The carrier gas was helium. One microlitre aliquots of DMT standard, or of the purified alkaloid extracts of the leaf samples were injected. Eluted compounds were detected as peaks in the reconstructed ion chromatogram (RIC) generated by the mass spectrometer data system. Under these conditions, DMT had a retention time of 9.5—10 min, base peak: *m/z* 58, M^+ : 188. The indole base present in the *Diplopterys*

cabrerana and *Psychotria* leaf extracts had a mass spectrum and retention time identical with the standard.

Alkaloid precipitation tests and TLC of uncommon admixture plants

The uncommon admixture plants were screened for alkaloids following the method of Farnsworth and Euler (1962). Material used in the analysis was preserved in methanol, and this was worked up in a manner identical to that described above for the *Psychotria* samples. Aliquots of the acidic filtrate were tested with 1–2 drops of either Meyer's, Valser's, or Dragendorff's reagent. Appearance of either a marked turbidity or a heavy precipitate on addition of the reagent was interpreted as a positive alkaloid reaction; slight turbidity indicated possible traces of alkaloid. Composition of the reagents used is given in Martello and Farnsworth (1962). After testing with the precipitation reagents, the acidic aqueous solutions were basified to pH 8–9 with saturated $NaHCO_3$ and 2 N NaOH and extracted with 3 X 5 ml CH_2Cl_2. The organic layer was collected, evaporated to dryness under vacuum, and the residue taken up in 2.0 ml methanol. Five microlitre aliquots of this final methanol fraction were applied to precoated Polygram silica gel plates UV[254], and the plate was developed in one direction in butanol/acetic acid/water, 4 : 1 : 1. Following development, the plates were air-dried, examined under short- and long-wave UV light, and sprayed with Dragendorff's modified reagent, (Stahl, 1969).

Monoamine oxidase assays

Preparation of rat-liver cytosol

Rat-liver, derived from mature female Wistar rats, was used as the source of MAO for the assays. The rats were killed by stunning followed by cervical dislocation. The abdominal cavity was opened by removal of the belly skin and peritoneum and the liver perfused with cold 0.1 M sodium phosphate buffer (pH 7.2) (Dawson et al., 1969) by injection into the hepatic vein with a bevelled no. 22 stainless steel syringe. The perfused liver was removed from the abdominal cavity and immediately placed in cold buffer. Portions of liver were transferred to a glass homogenizer and homogenized in cold buffer to a smooth paste (approx. 1 g liver/3 ml buffer). The homogenate was centrifuged at 3000 X g for 20 min to remove nuclear fragments. The supernatant was collected and diluted 1 : 1 with buffer. The diluted cytosol fraction was sonicated for 10 s. This diluted whole cytosol fraction was kept on ice for the duration of the experiment, and was used without further centrifugation.

Preparation of reaction mixture and addition of labelled substrate

The assay was conducted at 37.5°C in 13 X 100 mm disposable test tubes. Volume of the reaction mixture was 0.5 ml. Composition of the reaction

mixture in controls lacking added inhibitor consisted of 375 μl sodium phosphate buffer, 100 μl whole cytosol and 25 μl labelled substrate. The substrate consisted of 5-hydroxy[side-chain-2-^{14}C]tryptamine creatinine sulphate (Amersham). The labelled substrate was diluted with cold carrier 5-hydroxytryptamine (5HT) to a specific activity of 0.835 mCi/mmol and a concentration of 2×10^{-3} M. At this dilution, addition of a 25-μl aliquot to the reaction mixture results in a final volume of 500 μl and a 5HT concentration of 0.1 mM. containing a total of 0.04 μCi of labelled 5HT. All components of the reaction mixture except the labelled substrate were added to the reaction tubes which were maintained at 0°C in an ice bath. Inactivated control blanks, identical in composition to the active controls, were prepared by immersing the tubes in boiling water for 5 min. Prior to initiation of the assay, the reaction tubes were immersed in a 37.5°C water bath and equilibrated for 5 min. The assay was initiated by adding a 25-μl aliquot of the diluted substrate to the tubes, mixing for 1—2 s on a vortex mixer, and replacing in the water bath. Following addition of the substrate the assay tubes were incubated for 30 min at 37.5°C. After 30 min the reaction was terminated by adding 1.0 ml of 1.0 M citrate. This was followed by the addition of 0.5 ml saturated NaCl, and 2.0 ml ethyl acetate to each tube. Tubes were then vortexed for 5 s each and centrifuged at 2000 rev./min for 10 min to separate the organic and aqueous layers. In this method, (cf. Tipton and Youdim, 1976) the labelled reaction products are soluble in the organic (upper) phase while the protonated substrate remains in the lower aqueous phase. Following centrifugation, a 1.0-ml aliquot of the upper phase was transferred to a scintillation vial containing 9.0 ml Aquasol-2 (New England Nuclear). Scintillation vials were counted for 10 min in a Searle Isocap 300 Liquid Scintillation Counter.

Assay with MAO inhibitors

Stock solutions of various β-carbolines, 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. Stock solutions for lower concentrations 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 sodium phosphate buffer 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 replaced the inhibitor. The controls lacking inhibitor represented 0% MAO inhibition;

and the percent inhibition of tubes containing varied concentrations of inhibitor 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.

The *ayahuasca* samples used in the assay were Don Fidel no. 1 and Don Juan no. 2. Five milliliter aliquots of each were diluted to 50 ml with chilled methanol and the white non-alkaloidal precipitate removed by filtration. The filtered solution was then evaporated to dryness under reduced pressure and resolubilized in 5 ml 0.01 N HCl to reconstitute the original volume. A 1 : 1 dilution of this reconstituted solution was used to quantify the alkaloids present in the mixture using the HPLC method described above. This quantification showed the reconstituted samples to contain 3.5 mg/ml and 4.8 mg/ml total alkaloid, respectively. Serial 1 : 10 dilutions of the reconstituted samples, ranging from 0 to 10^{-7}, were made using 0.01 N HCl. Fifty microlitre aliquots of the appropriately diluted *ayahuasca* solutions were added to the reaction mixture in the same manner as the inhibitors, and percent inhibition was calculated relative to the controls (cf. Fig. 3). Inhibitor assays were also conducted using an equimolar mixture of harmine, harmaline and tetrahydroharmine. A mixture of these compounds in which each constituent was present in the same proportion as found in the *ayahuasca* samples but in which the overall concentration was approximately equivalent to the concentration of the equimolar solution was also assayed (cf. Fig. 4). Points plotted in Figs. 3 and 4 are means ± S.E. of four replicate determinations. Standard errors less than 1% are not shown. See the Results and Discussion for an evaluation and comparison of these various inhibitor assays.

Results and discussion

The alkaloidal constituents of a number of *ayahuasca* brews, cultivars of *B. caapi* and a variety of admixture plants were qualitatively and quantitatively investigated using two-dimensional TLC and HPLC as the primary analytical tools. Admixture samples were also analyzed using gas chromatography/mass spectrometry (GC/MS). Some admixture plants were screened for alkaloids using precipitation tests and TLC. In vitro MAO inhibition was studied using selected β-carboline standards, mixtures of β-carboline standards, and *ayahuasca* preparations. Details of the analytical and experimental methods are presented in the Experimental section.
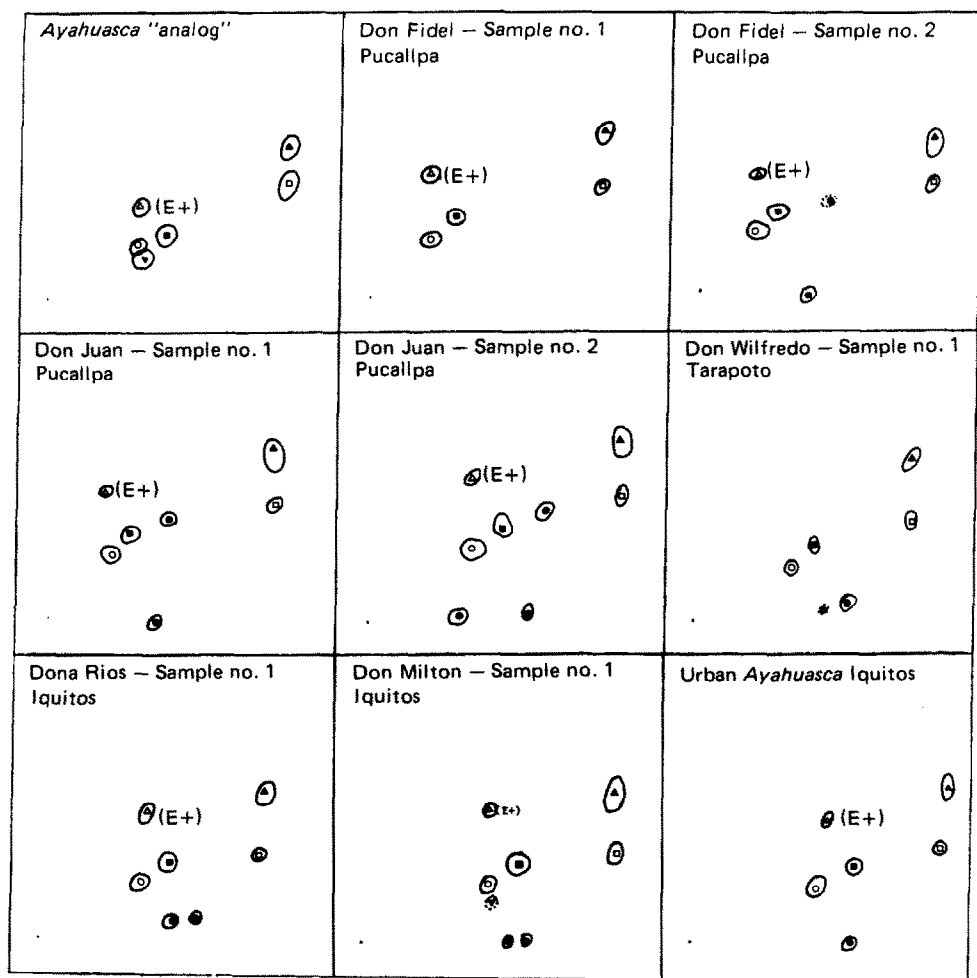
Ayahuasca brews

During the course of ethnobotanical fieldwork in the spring of 1981, eight samples of *ayahuasca* preparations were obtained from *ayahuasqueros*

living on the outskirts of the Peruvian towns of Iquitos, Pucallpa and Tarapoto. These samples were qualitatively analyzed using two-dimensional TLC and quantified using HPLC. Identification of alkaloids was based on comparison with authentic standards.

Comparison of the TLC profiles of the eight samples (Fig. 1) shows that the major constituents vary little from sample to sample; different batches made by the same *ayahuasquero* (cf. Don Fidel nos. 1 and 2, and Don Juan, nos. 1 and 2) are generally similar, and there is also little variation in the constituents of brews made by different *ayahuasqueros*. Harmine, harmol, harmaline and THH were found to be the major β-carbolines present in all of the samples, while harmalol was not detected in any samples save one (Don Milton no. 1). DMT was found in all samples except that from Tarapoto. No other significant Ehrlich-positive spots were detected. Known constituents were identified by comparison with a mixture of authentic standards (*ayahuasca* "analogue", upper left in Fig. 1). Traces of fluorescent compounds not corresponding to any of the β-carboline standards were also detected in most samples; it is assumed that these represent β-carbolines of undetermined structure. Absence of DMT in the sample from Tarapoto is significant, since this is the only sample in which *Psychotria carthagenensis* was employed as the admixture rather than the more commonly used *P. viridis*.

The quantitative HPLC analysis of five undiluted samples prepared by two *ayahuasqueros* in Pucallpa shows little variation from batch to batch, either in total alkaloid content or in the proportions of constituents (Table 1). The methanol-diluted samples were lyophilized and their alkaloid contents compared on a dry weight basis (Table 2). These samples showed considerable differences in alkaloid content (expressed as mg alkaloid/g dry wt). The sample from Pucallpa had the highest total alkaloid content (75.7 mg/g dry wt) of which 76% was harmine, 10.6% was THH, and 7.6% was DMT. The samples from Iquitos and Tarapoto generally had lower total alkaloid levels, and also differed in the proportions of different constituents. The reason for the difference in relative proportion of harmine and THH in the Pucallpa samples and those from other regions of Peru could be related to the type of *B. caapi* cultivar employed to prepare the drink, or to the method of preparation. Environmental variables, such as the type of soil in which the cultivar is grown, or different conditions of exposure to sunlight, may also be involved. Table 1 shows, however, that all of the *ayahuasca* samples from Pucallpa consistently show approximately the same relative proportions of harmine to THH to harmaline. This may indicate that all of these samples were prepared either from the same *B. caapi* cultivar or clones of the same cultivar. This would not be surprising since Don Juan is the uncle of Don Fidel and they often collaborate in the preparation of their brews.

Summarizing the results of their quantitative studies, Rivier and Lindgren (1972) state that a typical 200 ml dose of *ayahuasca* contains a total of 65

Ayahuasca "analog"

Don Fidel — Sample no. 1
Pucallpa

Don Fidel — Sample no. 2
Pucallpa

(E+)

(E+)

(E+)

Don Juan — Sample no. 1
Pucallpa

Don Juan — Sample no. 2
Pucallpa

Don Wilfredo — Sample no. 1
Tarapoto

(E+)

(E+)

Dona Rios — Sample no. 1
Iquitos

Don Milton — Sample no. 1
Iquitos

Urban Ayahuasca Iquitos

(E+)

(E+)

(E+)

△ DMT
▲ Harmine
▪ Harmaline
▫ Harmol
○ THH
▼ Harmalol
● Unknown
(E+) — positive to Erlich's reagent

Solvents:
(1) Ether/2-butanone/conc.
NH$_4$OH, 5:4:1 (upper phase)
(2) n-Propanol/1.5% NH$_4$OH, 9:2

Plates:
Polygram silica gel GF$_{254}$
(Brinkmann Instruments)

Fig. 1. Two-dimensional TLC of *ayahuasca* samples. See Experimental section for details of methods used for TLC.

TABLE 1

PERUVIAN *AYAHUASCA* — HPLC QUANTITATION OF UNDILUTED SAMPLES

Name of sample	Alkaloid concentration (mg/ml)[a]									
	Harmol	Harmine	%	THH	%	Harmaline	%	DMT	%	Total
Don Fidel no. 1	tr tr	3.85 (0.01)	66	1.06 (0.05)	18	0.3 (0.1)	5	0.61 (0.01)	10	5.85 (0.19)
Don Fidel no. 2	tr	4.64 (0.3)	62	1.77 (0.1)	24	0.45 (0.1)	6	0.6 (0.1)	8	7.48 (0.16)
Don Fidel no. 3	tr	3.4 (0.2)	53	1.94 (0.06)	30	0.34 (0.2)	5	0.7 (0.2)	11	6.38 (0.1)
Don Juan no. 1	tr	5.3 (0.36)	66	1.73 (0.1)	21	0.51 (0.16)	6	0.51 (0.16)	6	8.05 (0.36)
Don Juan no. 2	tr	5.51 (0.26)	67	1.67 (0.07)	20	0.41 (0.14)	5	0.6 (0.2)	7	8.19 (0.28)
Average alkaloid content[b]		4.67 (0.2)	65	1.60 (0.08)	22	0.41 (0.06)	6	0.6 (0.06)	8	7.28 (0.23)

[a]Figures given are mg alkaloid/ml of undiluted sample, ± S.E., shown in parentheses; percentages are % total alkaloid.
[b]Average based on $n = 18$ replicate injections.

mg alkaloid, of which 30 mg is harmine, 10 mg is THH and 25 mg DMT. This is some 10—30 times less than the dosage at which the β-carbolines are hallucinogenically active in the pure form (cf. Introduction), although it is well within the range at which they are effective as MAO inhibitors. Twenty five milligrams is just below the threshold dose for DMT when this compound is injected intramuscularly (Szara, 1957) but it is possible that the threshold may be lowered under conditions of MAO inhibition. Commenting on their findings, Rivier and Lindgren (1972, p. 127) conclude: "In view of these results, new pharmacological experiments for a better understanding of the hallucinogenic action of *ayahuasca* seem necessary".

The alkaloid levels found in the five samples from Pucallpa (Table 1) exceed the levels reported by Rivier and Lindgren (1972) in samples collected on the upper Rio Purús by at least an order of magnitude. Thus, (based on the average of the five samples) a 100-ml dose of the Pucallpa *ayahuasca* (cf. Table 1) contains 728 mg total alkaloid, of which 467 mg is harmine, 160 mg is THH, 41 mg is harmaline and 60 mg is DMT. This is well above the threshold dose for DMT but is still considerably below the hallucinogenic dose level for the β-carbolines. In practice the typical dose ingested

TABLE 2

PERUVIAN *AYAHUASCA* — HPLC QUANTITATION OF LYOPHILIZED SAMPLES

Sample and origin	Alkaloid concentration (mg/g d. wt.)[a]								
	Harmine	%	THH	%	Harmaline	%	DMT	%	Total
Don Fidel no. 1 Pucallpa	57.6 (0.33)	76	8.0 (0.53)	10.5	4.2 (0.22)	5.5	5.8 (0.5)	8	75.6 (3.3)
Urban Ayahuasca Iquitos	28.3 (1.3)	42	25.5 (1.4)	38	5.8 (0.9)	9	7.2 (1.7)	11	66.8 (3.4)
Don Wilfredo Tarapoto	14.4 (0.44)	50	10.5 (1.2)	36	4.2 (0.3)	14	n.d.[b]	—	29.1 (1.6)
Don Milton Iquitos	10.2 (0.32)	33	10.2 (1.7)	33	5.2 (1.7)	17	5.7 (1.6)	8	31.3 (5.6)
Dona Rios Iquitos	8.6 (0.3)	27	9.6 (1.8)	30	6.3 (1.8)	20	7.0 (2.1)	2	31.5 (5.6)
Average alkaloid content[c]	23.8 (4.2)		11.1 (1.9)		5.1 (0.5)		6.4 (0.74)		46.9 (4.9)

[a] Figures given are mg alkaloid/g dry wt of lyophilized sample; standard errors ($n = 4$) are given in parentheses. Percentages given are % total alkaloid.
[b] n.d. = not detected.
[c] Based on $n = 16$ replicate injections.

in the Pucallpa ceremonies rarely exceeds 75 ml and is usually closer to 55—60 ml. The relatively large differences in the alkaloid content of the upper Purús *ayahuasca* analyzed by Rivier and Lindgren and the Pucallpa *ayahuasca* analyzed in the present study may be readily explained by the differences in the method of preparation in the two regions. In the upper Purús method, stems of *B. caapi* totalling about 900 cm in length and 1—4 cm diameter are cut into short sections, crushed, and packed in a 15-ℓ metal vessel together with alternating layers of leaves of *Psychotria* sp. Ten litres of water are added and the mixture is boiled for 1 h, strained and cooled. The mixture is then consumed without further processing. The method employed in Pucallpa starts out similarly but the mixture is boiled for a much longer time, approximately 10—15 h. The water may be drained off and replaced with fresh water several times during this boiling process. The separate batches are combined, allowed to cool, and filtered through a strainer or cheesecloth. The plant material is removed from the cooking pot and discarded, and then the strained *ayahuasca* is poured back into the pot and simmered over a low fire until it has been concentrated to about half its original volume. The 5 or 6 ℓ of *ayahuasca* obtained from

this process are transferred to wine or beer bottles and stoppered with corks. In this form the brew may be kept for up to 6 months without refrigeration.

Alkaloid content of B. caapi cultivars

All of the *ayahuasqueros* that we interviewed during our field studies in Peru recognized several different "kinds" of *ayahuasca* which were claimed to vary in their psychological effect. The differentiation of these varieties of *ayahuasca* was based in part on the types of admixture plants which were added, and in part on the type of *B. caapi* which was utilized. Several types of *B. caapi* were generally recognized by these practitioners and were distinguished by different adjectives e.g. "cielo" *ayahuasca*, "lucero" *ayahuasca*, "rumi" *ayahuasca*. Some claimed to distinguish as many as 10 kinds of *Bani steriopsis* vine (the term *ayahuasca* is indiscriminately applied either to the *B. caapi* vine or to the beverage made from it) but most were familiar with only two or three kinds. Presumably these "kinds" of *B. caapi* are referable to different cultivars, races, or chemical or morphological varieties of *B. caapi*. There were no outstanding morphological differences between the three or four kinds of *B. caapi* which we collected, and the relevant voucher specimens have all been determined as *B. caapi* by taxonomic specialists in the Malpighiaceae (W.R. Anderson and B. Gates, University of Michigan). HPLC analysis of the alkaloid levels in the dried stems of three of the recognized varieties plus one specimen (DMCK no. 125) for which the vernacular name is unknown has shown that there is considerable variation between samples (Table 3). The lowest level was found in DMCK no. 126 which

TABLE 3

HPLC QUANTITATION OF *BANISTERIOPSIS CAAPI* CULTIVARS

Collection no.:	DMCK no. 110	DMCK no. 124	DMCK no. 125	DMCK no. 126	DMCK no. 128	Plowman 6041
Name: Origin:	"cielo" Tara-poto	"Pucahuasca" Pilluana — R. Huallaga	— Pto. Almendras	"cielo" Iquitos	"rumi" Iquitos	"cielo" Tarapoto — 1976 UBC — 1982
Alkaloids detected (mg/g dry wt)						
Harmine	5.3	5.9	6.35	0.57	4.4	1.0
THH	0.95	3.3	1.95	0.25	1.45	1.32
Harmaline	1.1	3.2	3.8	0.75	2.07	0.5
Harmol	0.05	0.06	1.2	0.1	0.65	0.01
Harmalol	n.d.[a]	trace	0.35	n.d.	n.d.	n.d.
Total alkaloids	7.4.	12.46	13.6	1.7	8.6	2.8

[a]n.d. = not detected.

contained 1.8 mg/g total alkaloid, while DMCK no. 125 contained the highest level, 13.6 mg/g. There appears to be no consistent correlation of alkaloid content with particular recognized cultivars, however. The variation observed probably has more to do with the age of the plant, and the soil, light, water, and other environmental conditions affecting the growth of the particular specimen. The amounts of alkaloids are in the same general range as those detected in the *B. caapi* samples analysed by Rivier and Lindgren (1972). Further clarification of this question of possible chemical or morphological differences between recognized types of *B. caapi* cultivars would require a systematic sampling of as many different individuals of each type as possible; climatic, edaphic and other environmental factors should also be considered.

Alkaloid content of ayahuasca admixture plants

DMT-containing admixtures

In Peru, the admixture plant employed most frequently in the preparation of *ayahuasca* appears to be *Psychotria viridis* R. & P. We encountered only one *ayahuasquero* during our fieldwork who preferred to use another species of *Psychotria*, tentatively identified as *Psychotria carthagenensis* Jacq. Interestingly, no alkaloids of any kind were detected in this collection (DMCK no. 109, Tarapoto) however, all of the *Psychotria viridis* collections contained DMT as the single major base. Identity of the compound was confirmed by GC/MS and comparison of its HPLC retention time, TLC R_f and Ehrlich's colour reaction with that of an authentic standard (Table 4). The *Psychotria viridis* samples analyzed contained fairly substantial amounts of DMT, between 1 and 1.6 mg/g dry wt in the leaves. No alkaloid was detected in fruits or stems of *P. viridis*. No other alkaloids were detected in any of the *Psychotria* samples with the exception of DMCK no. 139, in which a trace constituent with a mass spectrum corresponding to that reported (Rivier and Lindgren, 1972) for 2-methyl-tetrahydro-β-carboline was detected. A single sample of *Diplopterys cabrerana* (Plowman 6040), the Malpighiaceous admixture, was available for analysis and this also contained DMT together with an extremely trace amount of 5-hydroxy-DMT. The alkaloid extract of dried leaves of the original (1976) collection of Plowman 6040 had an ion chromatogram that was essentially identical to leaf extracts of greenhouse propagated clones of this specimen. Plowman 6040 contained slightly higher levels of DMT (1.74 mg/g dry wt) than the *Psychotria viridis* samples, but otherwise was indistinguishable in terms of alkaloid content. Although Plowman 6040 was collected in Tarapoto where it was being utilized as an *ayahuasca* admixture, this use of *Diplopterys cabrerana* in Peru is uncommon; this species is the usual admixture in Southern Colombia and Ecuador (Pinkley, 1969) and in fact Plowman 6040 was originally brought to Tarapoto as a live cutting from the Rio Pastaza in Ecuador (Plowman, pers. commun.). *Psychotria viridis*, or less frequently, *Psychotria carthagenensis* are the ad-

TABLE 4

DMT CONTAINING ADMIXTURE PLANTS: ANALYSIS BY TLC, HPLC AND GC/MS

Collection no.:	DMCK	DMCK	DMCK	DMCK	Plowman 6040
Name:	no. 21	no. 108	no. 109	no. 139	*Diplopterys*
Origin:	*Psychotria viridis* "chacruna" Iquitos	*Psychotria viridis* "suija" Tarapoto	*Psychotria carthagenensis* "yage-chacruna" Tarapoto	*Psychotria viridis* "chacruna" Pucallpa	*cabrerana* "chagro-panga" Tarapoto
TLC					
Solvent I hR_f[a]	42	42	n.d.[c]	38	42
Solvent II hR_f[b]	23	24	—	27	25
Reaction to Erlich's reagent	+(blue)	+(blue)	—	+(blue)	+(violet)
HPLC					
Retention time (min)	18.4 (0.1)[d]	18.3 (.03)	—	18.7(0.05)	18.4 (0.15)
mg/g dry wt	1.58(0.3)	1.02(.04)	—	1.2(0.17)	1.74(0.41)
GC/MS					
Trace constituents	n.d.	n.d.	n.d.	2-Me-THBC	5-OH-DMT

[a]Solvent I: ether/2-butanone/conc. NH$_4$OH, 5:4:1 (upper phase); $hR_f = R_f \times 100$.
[b]Solvent II: n-propanol/1.5% NH$_4$OH 9:2.
[c]n.d. = not detected.
[d]Figures in parentheses are standard errors.

mixtures of choice in Peru and few of our informants in Peru were familiar with *D. cabrerana* under its common names, *chagro-panga* or *ocoyage*.

An ethnobotanically interesting aspect of folk taxonomy related to the identification of the "proper" *Psychotria* spp. for use as admixtures came to light during the course of fieldwork. Some *Psychotria* species, including *Psychotria viridis*, possess tiny spine-like extensions of the mid-rib on the abaxial surface of the leaf. These appear to be slightly swollen glandular structures which may be equivalent to the "dolmatia" found in some *Psychotria* spp. (Gentry, pers. commun.). A dolmatium is a part of a leaf, petiole, stem or other plant part that is inhabited by ants or other insects. The dolmatia-like structures of the *Psychotria viridis* specimens we examined appeared to be much too small to accomodate ants of any visible size, but may be inhabited by mites (Prance, pers. commun.). These tiny dolmatia-like structures were pointed out by the *ayahuasqueros* we interviewed as the key feature used to identify the *chacrunas* suitable for use as admixtures. "Chacruna" is the vernacular term for *Psychotria*. All of the *ayahuasqueros* except one insisted that these structures — termed by them

espinas (spines) — had to be present; plants lacking *espinas* were regarded as false *chacrunas* and were considered to have no value as admixtures. Indeed, all of the *Psychotria viridis* collections which we analyzed (cf. Table 4) possessed these structures, and all contained DMT; the single specimen which lacked these structures also contained no tryptamines or other alkaloids. This specimen (DMCK no. 109) may correspond to *Psychotria carthagenensis* Jacq., although the collection is sterile and the identification therefore tentative; further doubt is cast on the identification by the fact that Rivier and Lindgren (1972) reported DMT in *Psychotria carthagenensis,* while none was detected in DMCK no. 109.

Alkaloids in uncommon admixture plants

   Ayahuasca is usually prepared using one of the DMT-containing admixture plants mentioned above, either *Diplopterys cabrerana* or a *Psychotria* sp., less commonly, however, other admixtures are utilized, either in conjunction with the tryptamine-containing admixtures, or in place of them. Many of these admixtures have been identified in the ethnobotanical literature (Pinkley, 1969; Schultes, 1972, 1979; Rivier and Lindgren, 1972) although virtually nothing is known of their chemical or biodynamic properties. This would appear to be a promising area for further research. During our fieldwork in Peru three collections were made of plants which were stated by informants to be used as admixtures to *ayahuasca* (Table 5). One of these, *Teliostachya lanceolata,* has been discussed by Schultes (1979) as an admixture, but the other two, *Abuta grandifolia* (Menispermaceae) and *Cornutia odorata* (Verbenaceae) have not previously been reported as admixtures. Plant material from these collections, preserved in methanol, were screened for alkaloids using alkaloid precipitation tests and TLC (Table 5). Insufficient material was available to permit further chemical characterization. The only collection giving an unambiguously positive test was *Abuta grandifolia* (DMCK no. 74). This species has recently been reported (Setor de Fitoquimica, 1971) to contain palmatine, a typical quaternary base of the benzylisoquinoline family which characterizes the Menispermaceae. Although palmatine is one of the commonest alkaloids in nature, investigations of its pharmacology are surprisingly sparse. One study (Ch'en and Ch'i, 1965) found that palmatine inhibited the effect of epinephrine on blood pressure of rabbits, on the isolated rat seminal vesicle and on the toad hind-leg; its derivative *dl*-tetrahydropalmatine inhibited the effect of 5HT on isolated rat uteri, colon and stomach. Palmatine also exhibited anticholinesterase activity. Both alkaloids had ACTH and bactericidal activity. Some of these properties may be antagonistic to the effects of the β-carbolines while others may be synergistic. For instance, harmaline causes an increase in 5HT concentration in the whole brain, while harmine causes a significant decrease in acetylcholine in brain; on the other hand, harmine strongly inhibits the ATP-Mg^{2+} dependent uptake of norepinephrine into isolated adrenal medullary vesicles (Ho, 1977). Whether the

TABLE 5

TEST FOR ALKALOIDS IN UNCOMMON ADMIXTURE PLANTS

Coll. no:	DMCK no. 74	DMCK no. 22	DMCK no. 119	DMCK no. 1
Genus:	*Abuta*	*Teliostachya*	*Cornutia*	*Justicia*
Species:	*grandifolia*	*lanceolata*	*odorata*	*pectoralis*
Family:	Menispermaceae	Acanthaceae	Verbenaceae	Acanthaceae
Part used (g):	Bark (2)	Leaves (2.7)	Leaves (5.7)	Leaves (1.2)
Reaction to				
Meyer's reagent	+ +	—	±	—
Valser's reagent	+ +	—	±	—
Dragendorff's	+ +	—	—	—
TLC				
Dragendorff's[a]	+ +	—	—	—
Fluorescent				
Spots detected:				
Long wave UV	+	—	—	+
Short wave UV	+	—	—	+

[a]Modified for TLC according to Stahl (1969).

overall effect of palmitine is agonistic or antagonistic to the action of the β-carbolines, there seems little doubt that addition of the bark of *Abuta grandifolia* to *ayahuasca* could modify its effect. Further investigations of the pharmacology of this and many other admixture plants are needed in order to clarify their contribution to the effects of *ayahuasca.*

Justicia pectoralis (Acanthaceae) is also included in Table 5. *Justicia pectoralis* is not used as an *ayahuasca* admixture but has been reported as an admixture to the *Virola* snuffs (Chagnon et al., 1971), and these authors have suggested that it may be used by itself as an hallucinogenic snuff. No tryptamines or alkaloids of any other type were detected in our collection of this species. GC/MS analysis of extracts of *Justicia pectoralis* indicate that it contains the coumarin derivative umbelliferone and the quaternary nitrogen derivative betaine (MacRae, unpublished data).

Effects of selected β-carbolines and ayahuasca extracts on MAO

Function and properties of MAO
The mitochondrially-localized enzyme, MAO, plays an important role in mammalian metabolism. MAO catalyzes the oxidative deamination of biogenic monoamines including tyramine, tryptamine, serotonin, norepinephrine, dopamine and other monoamines, according to the general reaction equation (Yasunobu et al., 1976):

$$RCH_2NH_2 + O_2 + H_2O \xrightarrow{MAO} RCHO + NH_3 + H_2O_2$$

The enzyme is widely distributed throughout various tissues in verte-
brates and invertebrates, including the liver, brain, small intestine, blood
plasma and platelets, heart and lungs (Marley and Blackwell, 1970). The
most significant metabolic function of MAO is related to its ability to
inactivate endogenously produced monoamines such as serotonin or dop-
amine, which function as CNS neurotransmitter substances (Iverson, 1979).
Considerable experimental evidence has been accumulated (Marley and
Blackwell, 1970) which indicates that the visceral MAO system functions
as a detoxification mechanism serving to protect the nervous and cardio-
vascular systems from toxic biogenic amines ingested in the diet and
formed as a result of aromatic amino acid decarboxylation. Evidence
based on substrate specificity and selective sensitivity to certain MAO
inhibitors indicates that MAO consists of two species, designated MAO-A
and MAO-B (Fuller et al., 1970; Houslay and Tipton, 1974; Donnelly
et al., 1976, 1977). Houslay and Tipton (1974) carried out a kinetic
evaluation of the two species using mixed substrate assays and concluded
that the substrates for species A activity were substituted β-phenylethyl-
amines and β-phenylethanolamine derivatives having a free p-hydroxyl
group; and 5HT. Substrates for species B activity included substituted
benzylamine and β-phenylethylamine derivatives, but not substituted
β-phenylethanolamines. Tryptamine, 5-methoxytryptamine, dopamine
and the m-O-methyl derivatives of dopamine are substrates for both
MAO-A and MAO-B. Neff and Yang (1974) point out that substances
which interact with MAO-A such as serotonin and norepinephrine have
more polar aromatic rings than substances which interact with MAO-B
(e.g. β-phenylethylamine, benzylamine). In general adding a polar hy-
droxyl group to β-phenylethylamines (e.g. tyramine) or removing one
from serotonin to form tryptamine results in a common substrate. The
specific metabolism of DMT has not been investigated, but if it conforms
to the above rule it would be a substrate for both MAO-A and MAO-B
since it lacks aromatic substituents. Further evidence for the existence
of two species of MAO comes from experiments using selective MAO
inhibitors (Fuller et al., 1970).

Most of the MAO inhibitors which have been the focus of biochemical
and clinical investigations belong to one of four classes, viz. hydrazine
derivatives, phenylcyclopropylamine derivatives, N-benzyl-N-methyl pro-
pargylamine, or 2-methyl-3-piperidinopyrazine (Pletscher, 1966). These
are synthetic compounds which are not known to have corresponding
analogues in nature. The major exception is the β-carboline derivatives,
a group of tricyclic indole alkaloids which are widespread in nature (Allen
and Holmstedt, 1980) (cf. Fig. 2). These compounds are biosynthetically
closely related to tryptamine and its derivatives and can be readily syn-
thesized from tryptamine derivatives (Ho et al., 1967). Udenfriend et al.
(1958) were the first to demonstrate that harmaline and related compounds

R¹ = H, R² = OCH₃, R³ = CH₃   Harmine
$R^1 = H, R^2 = OCH_3, R^3 = CH_3$ Harmine
$R^1 = OCH_3, R^2 = H, R^3 = CH_3$ 6-MeO-Harman
$R^1 = H, R^2 = OH, R^3 = CH_3$ Harmol
$R^1 = R^2 = H, R^3 = CH_3$ Harman
$R^1 = R^2 = R^3 = H$ Norharman

Aromatic β-carbolines

$R^1 = H, R^2 = OCH_3$ Harmaline
$R^1 = OCH_3, R^2 = H$ 6-MeO-Harmalan

Dihydro-β-carbolines

$R^1 = H, R^2 = OCH_3, R^3 = CH_3, R^4 = H$ Tetrahydro-harmine

$R^1 = OCH_3, R^2 = R^3 = H, R^4 = CH_3$ 2-Methyl-6MeO-tetrahydro-β-carboline

Tetrahydro-β-carbolines

N,N-Dimethyltryptamine

Fig. 2. Structures of DMT and of selected β-carbolines tested for MAO inhibition (cf. Table 6). Harmine, harmaline, THH and DMT are the major alkaloids of *ayahuasca*.

are extremely potent reversible inhibitors of MAO. These investigators showed that the fully aromatic β-carbolines were the most effective inhibitors, and that activity decreased with increasing saturation of the piperidine ring; tetrahydro-β-carbolines still showed significant activity, however. Subsequently, experiments by Fuller et al. (1970) showed that harmaline selectively inhibited oxidation of serotonin, indicating that it was a specific inhibitor of MAO-A.

The capacity of β-carbolines to inhibit MAO has been suggested to be the mechanism responsible for the oral activity of *ayahuasca* (der Marderosian et al., 1968; Agurell et al., 1968b; Schultes, 1972; Shulgin, 1976) and possibly also for the activity of other orally ingested DMT-containing drugs derived from *Virola* spp. (Schultes, 1969; Schultes and Swain 1976; Schultes et al., 1977). The capacity of *ayahuasca* to inhibit MAO has not been experimentally investigated prior to the present study. A similar experimental investigation of the constituents and pharmacological activity of the orally-active preparations derived from *Virola* spp will be the subject of a subsequent publication.

β-Carbolines as inhibitors of MAO: structure/activity relationships of selected derivatives

In order to assess the influence of structural variation of the β-carboline skeleton (cf. Fig. 2) on the MAO inhibitory activity of these compounds, a selected group of standards (Table 6) was assayed in vitro using a rat-liver cytosol fraction as the source of enzyme and ^{14}C-labelled 5-hydroxy-tryptamine creatinine sulphate as substrate. Table 6 lists I_{50} values for the present experiment and also includes the values reported by McIsaac and Estevez (1966) and Buckholtz and Boggan, (1977) for comparison. The I_{50} value corresponds to the molar concentration of inhibitor at which enzyme activity is 50% inhibited with respect to controls lacking inhibitor. McIsaac and Estevez (1966) used a calf-liver mitochondrial fraction and [^{14}C]-tyramine as substrate, while Buckholtz and Boggan (1977) utilized mouse whole brain homogenate and [^{14}C]tryptamine as substrate. Since the degree of MAO inhibition measurable in vitro is partially determined by the tissue source and workup of the enzyme preparation, and partially

TABLE 6

RAT-LIVER MAO: INHIBITION BY β-CARBOLINES AND *AYAHUASCA*

Inhibitor	I_{50}[a]	I_{50} McIsaac et al.[b]	I_{50} Buckholz et al.[c]
Harmine	1.26×10^{-8}	1.5×10^{-8}	8.0×10^{-8}
Harmaline	1.58×10^{-8}	1.0×10^{-6}	6.0×10^{-8}
THH	1.77×10^{-6}	Not tested	1.4×10^{-5}
6MeO-harmalan	1.20×10^{-6}	4.5×10^{-7}	1.8×10^{-5}
6MeO-harman	7.08×10^{-7}	1.5×10^{-8}	3.1×10^{-5}
Harmol	5.0×10^{-7}	2.7×10^{-8}	5.8×10^{-6}
Harman	4.47×10^{-7}	5.0×10^{-9}	3.3×10^{-6}
Norharman	3.55×10^{-6}	7.5×10^{-10}	2.0×10^{-5}
2Me-6MeO-THBC	3.98×10^{-7}	Not tested	Not tested
Harmine + THH + harmaline equimolar mix	3.16×10^{-7}	Not tested	Not tested
Ayahuasca "analogue"[d]	3.98×10^{-7}	Not tested	Not tested
Ayahuasca samples[e]			
Don Fidel no. 1	1.58×10^{-5}	—	—
Don Juan no. 2	$<1.0 \times 10^{-7}$	—	—

[a] I_{50} = molar concentration of inhibitor equal to 50% inhibition of activity with respect to controls (present experiment). Values given are means of at least three separate determinations.

[b] Results reported by McIsaac and Estevez (1966) using calf-liver mitochondria and tyramine as substrate.

[c] Results reported by Buckholtz and Boggan (1977) using mouse whole brain homogenate and tryptamine as substrate.

[d] Mixture of β-carbolines approximating proportions found in *ayahuasca*, viz. 69% harmine, 26% THH and 4.6% harmaline.

[e] Figures given for *ayahuasca* represent dilution factor with respect to undiluted samples.

by the particular substrate used, the I_{50} values reported in Table 6 for the present study are not directly comparable to those reported by McIsaac and Estevez (1966) or Buckholtz and Boggan (1977). In general, however, the conclusions suggested by the present study regarding the structure/activity relationships of β-carbolines as MAO inhibitors are in agreement with the previous studies. Thus, for example, the fully aromatic and di-hydro-β-carbolines are significantly more potent inhibitors than analogues in which the piperidine ring is fully saturated (cf. harmine, harmaline, and THH). Inhibitory potency is roughly equivalent between the 7-methoxylated fully aromatic and dihydro derivatives harmine and harmaline, but of the 6-methoxylated analogues the fully aromatic compound is more potent. The 7-substituted β-carbolines were generally more potent inhibitors than the corresponding 6-substituted analogues in our study and that of Buckholtz and Boggan (1977) but McIsaac and Estevez (1966) found 6-methoxy-harman was equipotent with harmine while 6-methoxy-harmalan was a more active inhibitor than harmaline. Hydroxyl substitution on the aromatic ring results in a less active compound than the corresponding methoxy-substituted compound (cf. harmine and harmol). Buckholtz and Boggan (1977) and the present study found that harman, lacking substituents on the aromatic ring, was less active than the 7-methoxylated analogue but approximately equipotent to the 6-methoxylated fully aromatic compounds. McIsaac and Estevez (1966) reported harman to be somewhat more active than either harmine or 6-methoxy-harman. Lack of a methyl group at C1 (cf. harman and norharman) resulted in reduced activity in our assays and in those of Buckholtz and Boggin (1977), but McIsaac and Estevez (1966) reported somewhat greater activity for nor-harmane than for harmane. We found 2-methyl-6-methoxy-tetrahydro-β-carboline (2Me-6MeO-THBC), a compound reported from *Virola* sp. (Agurell et al., 1968b) to be in the same general range of potency as harman and harmol. Thus 2-Me-6-MeO-THBC exhibited the greatest inhibitory activity of any of the 6-methoxylated β-carbolines tested, and was approximately an order of magnitude more active than THH. The greater activity of this compound with respect to THH and the other 6-methoxylated analogues may be due primarily to the methylation of the piperidine nitrogen. This compound was not investigated by either McIsaac and Estevez (1966) or Buckholtz and Boggan (1977); the former authors, however, reported that acetylation of the piperidine nitrogen resulted in a compound lacking inhibitory activity.

MAO inhibition by ayahuasca samples and by combinations of β-carbolines
Once the relative inhibitory activity of a representative sample of β-carbolines had been evaluated in our assay system, the next step was to evaluate the activity of appropriately diluted *ayahuasca* samples. The activity exhibited by the *ayahuasca* samples was compared to that shown by a mixture of harmine, harmaline and THH, which are known to be the

major β-carbolines found in *ayahuasca*. Two types of standard mixtures were used for comparison. The first mixture was an equimolar mixture of harmine, harmaline and THH; the second contained approximately the same molar concentration of total alkaloid as the first mixture, but in this case the proportion of each constituent reflected the proportions present in the *ayahuasca* brews; these proportions and concentrations had previously been determined by quantitative HPLC. Thus, this "*ayahuasca* analogue" (as the second mixture was termed) contained 69% harmine, 26% THH and 4.6% harmaline; DMT, which comprised 6.1% of the total alkaloids, was not included in the "analogue" mixture.

Two of the *ayahuasca* samples from Pucallpa, Don Juan sample no. 2 and Don Fidel sample no. 1, were analyzed using quantitative HPLC in order to determine the concentration of alkaloids in the undiluted samples. Total alkaloid concentration was determined to be 4.8 mg/ml and 3.5 mg/ml, respectively. The samples were then subjected to serial 1:10 dilutions so that the most dilute solution was 1×10^{-7} as concentrated as the undiluted sample. Fifty microlitre aliquots of the appropriate dilutions from each sample were assayed for MAO inhibitory activity using the in vitro rat-liver cytosol system (Fig. 3, Table 6). Figure 3 shows clearly that both *ayahuasca* samples are extremely effective MAO inhibitors; Don Fidel's no. 1 still showed >40% inhibition of the enzyme at 10^{-5} full strength, while Don Juan's no. 2 exceeded 50% inhibition even at one ten-millionth (10^{-7}) the concentration of the undiluted brew. These in vitro results indicate that *ayahuasca* is active as an MAO inhibitor even when diluted by many orders of magnitude. These observations constitute the first empirical demonstration of the effect of *ayahuasca* on MAO and provide evidence for the hypothesis that the hallucinogenic properties of *ayahuasca* are due to its inactivation of visceral MAO and consequent oral potentiation of the DMT in the preparation. It should be emphasized, however, that the present in vitro study represents only the first step toward understanding the pharmacology of *ayahuasca*. Further investigations are required, particularly in vivo studies of the action of *ayahuasca* in both animals and humans, before the pharmacology of this Amazonian drug is fully elucidated. Another point worth mentioning is the fact that on occasion *ayahuasca* is prepared from *B. caapi* alone without the addition of any admixture plants; in these instances, DMT would be absent from the preparation. This would alter its pharmacology and presumably the hallucinogenic effects, if present, would be due to the β-carbolines alone. In this case concentrations of β-carbolines considerably greater than those measured in our samples would be required if a non-synergistic mechanism is assumed.

It is informative to compare the % MAO inhibition produced by an equimolar mixture of harmine, harmaline, and THH with the inhibition elicited by single components of this mixture (Fig. 4, Table 6). The equimolar solution inhibited 50% of the enzyme at a concentration of 3.16×10^{-7} M (cf. Table 6) (in this case, molarity of the solution is based on the
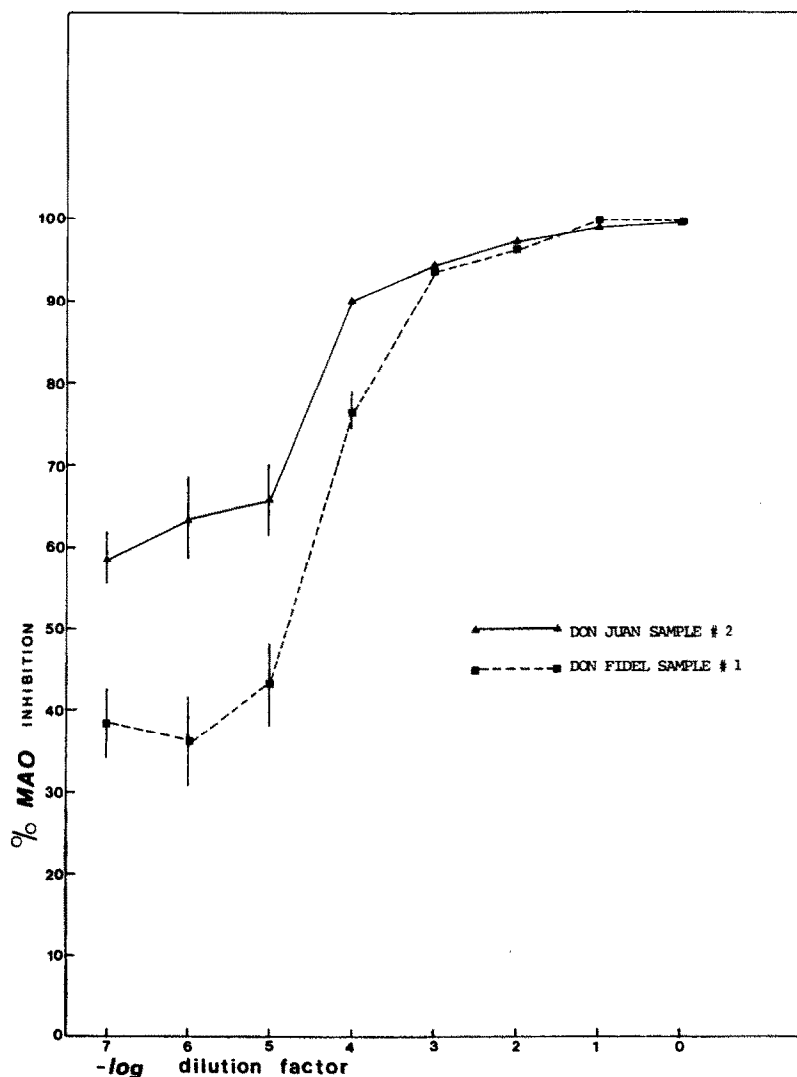
Fig. 3. In vitro inhibition of rat-liver MAO by *ayahuasca* samples. See text for details.

average of the molecular weights of the three compounds). This value is approximately intermediate between the I_{50} value of the most active constituent of the mixture (harmaline, $I_{50} = 1.58 \times 10^{-8}$ M) and the least active (THH, $I_{50} = 1.77 \times 10^{-6}$ M). This observation indicates that these compounds do not interact synergistically with respect to their inhibition of MAO. A synergistic potentiation would result in an I_{50} value considerably lower than the I_{50} values of any one constituent by itself; such a synergism is not observed, indicating that the inhibitory activity of the three com-
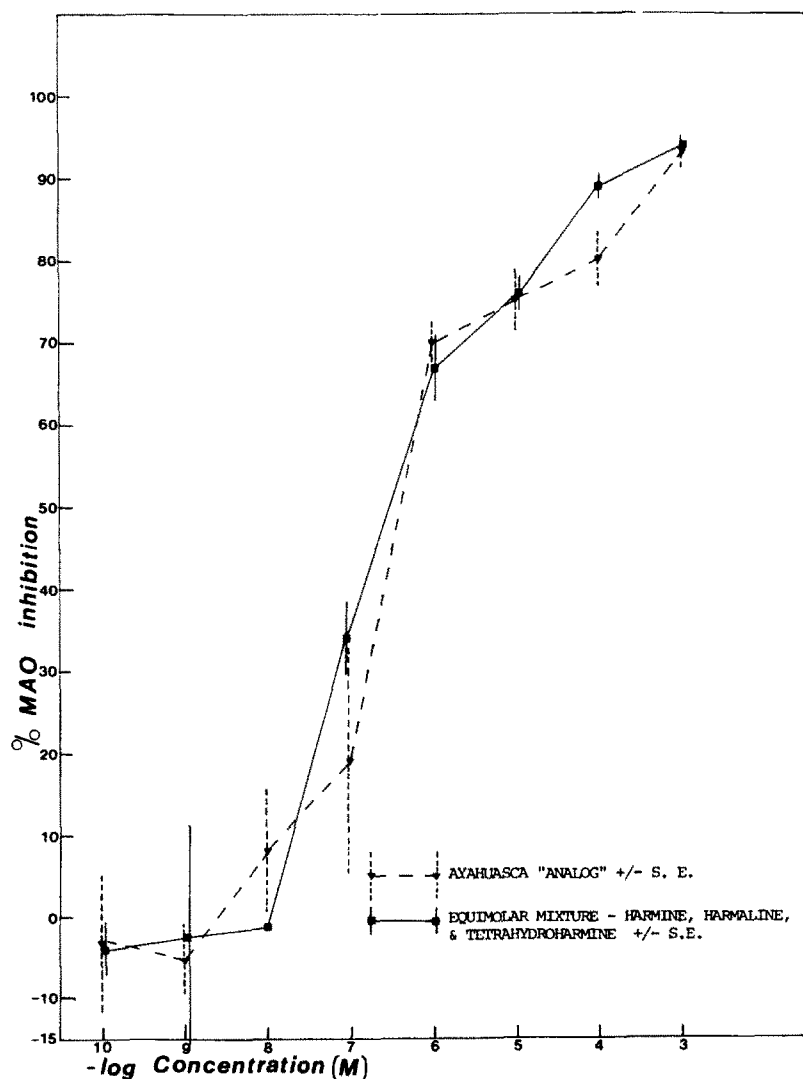
Fig. 4. In vitro inhibition of rat-liver MAO by mixtures of harmine, harmaline and THH.
See text for details.

pounds collectively is not greater than the activity of the most active compound of the group.

This supposition is further supported by the assay of the *"ayahuasca analogue"* (Fig. 4, Table 6) in which the total alkaloid concentration is equal to the concentration of the equimolar mixture, but the proportions of harmine, THH, and harmaline approximate those found in Don Juan sample no. 2, i.e. 69% harmine, 26% THH and 4.6% harmaline. I_{50} values

for the *ayahuasca* "analogue" are nearly identical with those of the equimolar mixture (3.98×10^{-7} and 3.16×10^{-7}, respectively) indicating that the combination of harmine and THH alone can account for most of the MAO inhibition exhibited by *ayahuasca*. While harmaline is equivalent to or slightly stronger than harmine, it is essentially a trace component in *ayahuasca* and probably does not contribute significantly to the MAO inhibition which this drug elicits.

Acknowledgements

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