Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology & Therapeutics 102 (2004) 111–129

Pharmacology & Therapeutics

www.elsevier.com/locate/pharmthera

Associate editor: B.L. Roth

Clinical investigations of the therapeutic potential of ayahuasca: rationale and regulatory challenges

Dennis J. McKenna*

Center for Spirituality and Healing, Academic Health Center, University of Minnesota, C592 Mayo Memorial Building, Mayo Mail Code 505, 420 Delaware Street Southeast, Minneapolis, MN 55455, USA

Abstract

Ayahuasca is a hallucinogenic beverage that is prominent in the ethnomedicine and shamanism of indigenous Amazonian tribes. Its unique pharmacology depends on the oral activity of the hallucinogen, *N*,*N*-dimethyltryptamine (DMT), which results from inhibition of monoamine oxidase (MAO) by β-carboline alkaloids. MAO is the enzyme that normally degrades DMT in the liver and gut. Ayahuasca has long been integrated into mestizo folk medicine in the northwest Amazon. In Brazil, it is used as a sacrament by several syncretic churches. Some of these organizations have incorporated in the United States. The recreational and religious use of ayahuasca in the United States, as well as "ayahuasca tourism" in the Amazon, is increasing. The current legal status of ayahuasca or its source plants in the United States is unclear, although DMT is a Schedule I-controlled substance. One ayahuasca church has received favorable rulings in 2 federal courts in response to its petition to the Department of Justice for the right to use ayahuasca under the Religious Freedom Restoration Act. A biomedical study of one of the churches, the Uñiao do Vegetal (UDV), indicated that ayahuasca may have therapeutic applications for the treatment of alcoholism, substance abuse, and possibly other disorders. Clinical studies conducted in Spain have demonstrated that ayahuasca can be used safely in normal healthy adults, but have done little to clarify its potential therapeutic uses. Because of ayahuasca's ill-defined legal status and variable botanical and chemical composition, clinical investigations in the United States, ideally under an approved Investigational New Drug (IND) protocol, are complicated by both regulatory and methodological issues. This article provides an overview of ayahuasca and discusses some of the challenges that must be overcome before it can be clinically investigated in the United States.

Keywords: Ayahuasca; Hoasca; β-carbolines; DMT; Serotonin transporters; IND; Clinical studies; Alcoholism; Substance abuse; Immune modulation

Abbreviations: DMT, *N*,*N*-dimethyltryptamine; 5-HT, 5-hydroxytryptamine; IND, Investigational New Drug (application); IRB, International Review Board; MAO, monoamine oxidase; MAOI, monoamine oxidase inhibitor; THH, tetrahydroharmine; UDV, Uñiao do Vegetal.

Contents

1. Introduction . . . 112
2. What is ayahuasca? . . . 112
3. Prehistorical origin of ayahuasca . . . 112
4. Traditional and indigenous use of ayahuasca . . . 113
5. Syncretic religious use of ayahuasca . . . 113
6. Chemistry of ayahuasca and its source plants . . . 114
7. Pharmacological actions of ayahuasca and its active alkaloids . . . 115
8. Recent biomedical investigations of ayahuasca . . . 117
   8.1. The "Hoasca Project" . . . 117
   8.2. Assessment of acute and long-term psychological effects of hoasca teas . . . 118
   8.3. Assessment of the acute physiological effects of hoasca tea . . . 119
   8.4. Characterization of the pharmacokinetics of hoasca alkaloids in human subjects . . . 119
   8.5. Assessment of serotonergic functions in long-term users of hoasca . . . 120

* Tel.: 612-624-0112; fax: 612-626-5280.
E-mail address: mcken031@umn.edu (D.J. McKenna).

0163-7258/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pharmthera.2004.03.002

9. Current legal and regulatory status of ayahuasca . . . 121
10. The rationale for clinical studies of ayahuasca . . . 122
11. Ayahuasca clinical research to date . . . 122
11.1. Key findings from the "Hoasca Project" . . . 122
12. Potential therapeutic applications of ayahuasca . . . 122
12.1. Treatment of alcoholism and substance abuse . . . 122
12.2. Treatment of serotonergic deficits . . . 123
12.3. Immune modulation . . . 123
12.4. Clinical studies by Spanish investigators . . . 124
13. The pathway to clinical studies . . . 124
13.1. Step I: Preclinical study—phytochemistry and pharmacology . . . 124
13.2. Step II: Investigational New Drug application . . . 125
13.3. Step III: Initial clinical studies—safety assessment . . . 125
13.4. Step IV: Subsequent clinical studies—efficacy of ayahuasca therapy for alcoholism . . . 126
14. Conclusion . . . 126
References . . . 127

1. Introduction

Of the numerous plant psychotropics utilized by indigenous populations of the Amazon Basin, perhaps none is as interesting or complex, botanically, chemically, or ethnographically, as the beverage known variously as ayahuasca, caapi, or yage. The beverage is most widely known as *ayahuasca*, a Quechua term meaning "vine of the souls," which is applied both to the beverage itself and to one of the source plants used in its preparation, the malpighiaceous jungle liana, *Banisteriopsis caapi* (Schultes, 1957). In Brazil, transliteration of this Quechua word into Portuguese results in the name, hoasca. Hoasca, or ayahuasca, occupies a central position in mestizo ethnomedicine, and the chemical nature of its active constituents and the manner of its use make its study relevant to contemporary issues in neuropharmacology, neurophysiology, and psychiatry.

2. What is ayahuasca?

In a traditional context, ayahuasca is a beverage prepared by boiling—or soaking—the bark and stems of *B. caapi* together with various admixture plants. The admixture employed most commonly is the Rubiaceous genus *Psychotria*, particularly *Psychotria viridis*. The leaves of *P. viridis* contain alkaloids, which are necessary for the psychoactive effect. Ayahuasca is unique in that its pharmacological activity is dependent on a synergistic interaction between the active alkaloids in the plants. One of the components, the bark of *B. caapi*, contains β-carboline alkaloids, which are potent monoamine oxidase-A (MAO-A) inhibitors; the other component, the leaves of *P. viridis* or related species, contains the potent short-acting hallucinogenic agent *N*,*N*-dimethyltryptamine (DMT). DMT is not orally active when ingested by itself, but can be rendered orally active when ingested in the presence of a peripheral MAO inhibitor, such as the β-carbolines. This interaction is the basis of the psychotropic action of ayahuasca (McKenna et al., 1984). There are also reports (Schultes, 1972) that other *Psychotria* species are similarly utilized in other parts of the Amazon. In the northwest Amazon, particularly in the Colombian Putumayo and Ecuador, the leaves of *Diplopterys cabrerana*, a jungle liana in the same family as *Banisteriopsis*, are added to the brew in lieu of the leaves of *Psychotria*. The alkaloid present in *Diplopterys*, however, is identical to that in the *Psychotria* admixtures, and pharmacologically, the effect is similar. In Peru, various admixtures in addition to *Psychotria* or *Diplopterys* are frequently added, depending on the magical, medical, or religious purposes for which the drug is being consumed. Although a virtual pharmacopoeia of admixtures are occasionally added, the most commonly employed admixtures (other than *Psychotria*, which is a constant component of the preparation) are various Solanaceous genera, including tobacco (*Nicotiana* sp.), *Brugmansia* sp., and *Brunfelsia* sp. (Schultes, 1972; McKenna et al., 1995). These Solanaceous genera are known to contain alkaloids, such as nicotine, scopalamine, and atropine, which have effects on both central and peripheral adrenergic and cholinergic neurotransmission.

3. Prehistorical origin of ayahuasca

The origins of the use of ayahuasca in the Amazon Basin are unknown. It is uncertain where the practice may have originated, and about all that is certain is that it was already widespread among numerous indigenous tribes throughout the Amazon Basin by the time ayahuasca came to the attention of Western ethnographers in the mid-19th century (McKenna, 1999). This fact alone argues for its antiquity; beyond that, little is known. Plutarco Naranjo, the Ecuadorian ethnographer, has summarized what little information is available on the prehistory of ayahuasca (Naranjo, 1979, 1986). There is abundant archeological evidence, in the form of pottery vessels, anthropomorphic figurines,

snuffing trays and tubes, etc. that plant hallucinogen use was well established in the Ecuadorian Amazon by 1500–2000 BC (Naranjo, 1979, 1986). Unfortunately, most of the specific evidence, in the form of vegetable powders, snuff trays, and pipes, is related to the use of psychoactive plants *other* than ayahuasca, such as coca, tobacco, and the hallucinogenic snuff derived from *Anadenanthera* species and known as *vilka* and various other names. There is nothing in the form of iconographic materials or preserved botanical remains that would unequivocally establish the prehistorical use of ayahuasca. It is probable that these pre-Colombian cultures, sophisticated as they were in the use of a variety of psychotropic plants, were also familiar with ayahuasca and its preparation. Recent archaeological evidence has come to light (Heckenberger et al., 2003) indicating the existence of a complex, technologically sophisticated riverine/agrarian civilization in the upper Xingu region of Brazil dating at least to 1200 AD. While these discoveries do not directly address the question of the antiquity of knowledge of ayahuasca, they do support the supposition that a complex civilization capable of impacting and actively managing its forest environment for agricultural purposes, would also be likely to be similarly knowledgeable regarding the uses of medicinal species occurring in the ecosystem. The lack of data is frustrating, however, particularly with respect to a question that has fascinated ethnopharmacologists since the late 1960s when its importance was first brought to light through the work of Schultes and his students.

As mentioned above, ayahuasca is unique among plant hallucinogens in that it is prepared from a combination of 2 plants: the bark or stems of *Banisteriopsis* species, together with the leaves of *Psychotria* species or other DMT-containing admixtures. The beverage depends on this unique combination for its activity. There seems small likelihood of "accidentally" combining the 2 plants to obtain an active preparation when neither is particularly active alone, yet we know that at some point in prehistory, this fortuitous combination was discovered. At that point, ayahuasca was "invented." Just how this discovery was made, and who was responsible, we may never know, though several charming myths address the topic. Mestizo *ayahuasqueros* in Peru will, to this day, say that this knowledge comes directly from the "plant teachers" (Luna, 1984a, 1984b). The mestres of the Brazilian syncretic sect, the Uñiao do Vegetal (UDV), say with equal conviction that the knowledge came from "the first scientist," King Solomon, who imparted the technology to the Inca king during a little publicized visit to the New World in antiquity. In the absence of data, these explanations are all that we have. All that we can say with confidence is that the knowledge of the techniques for preparing ayahuasca, including knowledge of the appropriate admixture plants, had diffused throughout the Amazon Basin by the time the use of ayahuasca came to the attention of any modern researcher (Anonymous, 1855).

4. Traditional and indigenous use of ayahuasca

The use of ayahuasca under a variety of names is a widespread practice among various indigenous aboriginal tribes endemic to the Amazon Basin (Schultes, 1957). Such practices undoubtedly were well established in pre-Columbian times, and in fact, may have been known to the earliest human inhabitants of the region. Considerable genetic intermingling and adoption of local customs followed in the wake of European contact, and ayahuasca, along with a virtual pharmacopoeia of other medicinal plants, gradually became integrated into the ethnomedical traditions of these mixed populations. Today, the drug forms an important element of ethnomedicine and shamanism as it is practiced among indigenous mestizo populations in Peru, Colombia, and Ecuador. The sociology and ethnography of the contemporary use of ayahuasca in mestizo ethnomedicine has been extensively described (Dobkin de Rios, 1972, 1973; Luna, 1984a, 1984b, 1986).

5. Syncretic religious use of ayahuasca

From the perspective of the sociologist or the ethnographer, discussion of the use of ayahuasca or hoasca can conveniently be divided into a consideration of its use among indigenous aboriginal and mestizo populations, and its more recent adoption by contemporary syncretic religious movements, such as the UDV, Barquinia, and Santo Daime sects in Brazil. It is within the context of acculturated groups such as these that questions regarding the psychological, medical, and legal aspects of the use of ayahuasca become most relevant, and also, most accessible to study.

The use of ayahuasca in the context of mestizo folk medicine closely resembles the shamanic uses of the drug as practiced among aboriginal peoples. In both instances, the brew is used for curing, for divination, as a diagnostic tool, and a magical pipeline to the supernatural realm. This traditional mode of use contrasts from the contemporary use of ayahuasca tea within the context of Brazilian syncretic religious movements. (Note: The hoasca religions are "syncretic" in that they represent a fusion of indigenous religious practices with elements of Christianity.) Within these groups, the members consume ayahuasca tea at regular intervals in a ritual manner that more closely resembles the Christian Eucharist than the traditional aboriginal use. The individual groups of the UDV, termed *nucleos*, are similar to a Christian Hutterite sect, in that each group has a limited membership, which then splits to form a new group once the membership expands beyond the set limit. The *nucleo* consists of the congregation, a group leader or mestre, various acolytes undergoing a course of study and training to become mestres, and a temple. These structures, often circular in layout and beautifully decorated, are the sites where the sacrament is prepared and consumed at prescribed times, usually the first and third Saturday of each month.

The membership of these newer syncretic groups spans a broad socioeconomic range and includes many educated, middle-class, urban professionals (including a number of physicians and other health professionals). Some older members have engaged in the practice for 30 or more years without apparent adverse health effects.

The UDV and the Santo Daime sects are the largest and most visible of several syncretic religious movements in Brazil that have incorporated the use of ayahuasca into their ritual practices. Of the 2 larger sects, it is the UDV that possesses the strongest organizational structure as well as the most highly disciplined membership. Of all the ayahuasca churches in Brazil, the UDV has also been the most pivotal in convincing the government to remove ayahuasca from its list of banned drugs. In 1987, the government of Brazil approved the ritual use of hoasca tea[1] in the context of group religious ceremonies. This ruling has potentially significant implications, not only for Brazil, but for global drug policy, as it marks the first time in over 1600 years that a government has granted permission to its nonindigenous citizens to use a psychedelic substance in the context of religious practices.

6. Chemistry of ayahuasca and its source plants

The chemical constituents of ayahuasca and the source plants used in its preparation have been well characterized (Rivier & Lindgren, 1972; McKenna et al., 1984). *B. caapi* contains the β-carboline derivatives harmine, tetrahydroharmine (THH), and harmaline as the major alkaloids (Callaway et al., 1996). Trace amounts of other β-carbolines have also been reported (Rivier & Lindgren, 1972; Hashimoto & Kawanishi, 1975, 1976; McKenna et al., 1984), as well as the pyrrolidine alkaloids shihunine and dihydroshihunine (Kawanishi et al., 1982) (Fig. 1). The admixture plant, *P. viridis*, contains a single major alkaloid, DMT, while *N*-methyl tryptamine and methyl-tetrahydro-β-carboline have been reported as trace constituents (Rivier & Lindgren, 1972; McKenna et al., 1984). The admixture plant *Psychotria carthagenensis* has been reported to contain the same alkaloids (Rivier & Lindgren, 1972) but a subsequent investigation could not confirm the presence of DMT in the single collection examined (McKenna et al., 1984). The concentrations of alkaloids reported in *B. caapi* range from 0.05% dry weight to 1.95% dry weight; in *Psychotria*, the concentration of alkaloids ranged from 0.1% to 0.66% dry weight (Rivier & Lindgren, 1972; McKenna et al., 1984). Similar ranges and values were reported by both groups of investigators.

The concentrations of alkaloids in the ayahuasca beverages are, not surprisingly, several times greater than in the source plants from which they are prepared. Based on a quantitative analysis of the major alkaloids in several samples of ayahuasca collected on the upper Rio Purús, Rivier and Lindgren (1972) calculated that a 200-mL dose of ayahuasca contained an average of 30 mg of harmine, 10 mg THH, and 25 mg DMT. Callaway et al. (1996) determined the following concentrations of alkaloids in the hoasca tea utilized in the biomedical study with the UDV (in mg/mL): DMT, 0.24; THH, 1.07; harmaline, 0.20; and harmine 1.70. A typical 100-mL dose of hoasca thus contains (in mg): DMT, 24; THH, 107; harmaline, 20; and harmine, 170. Interestingly, these concentrations are above the threshold of activity for intravenous administration of DMT (Strassman & Qualls, 1994).

McKenna et al. (1984) reported somewhat higher values for the alkaloid content of several samples of Peruvian ayahuasca. These investigators calculated that a 100-mL dose of these preparations contained a total of 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 within the range of activity for DMT administered intramuscularly (Szara, 1956) or intravenously (Strassman & Qualls, 1994) and is also well within the range for harmine to act effectively as a monoamine oxidase inhibitor (MAOI). In vitro, these β-carbolines function as MAOI at ~10 nM (e.g., harmine's IC_{50} for MAOI is $\sim 1.25 \times 10^{-8}$ M; cf. Buckholtz & Boggan, 1977; McKenna et al., 1984). In mice, harmaline administered intraperitoneally (5 mg/kg) causes 100% inhibition by 2 hr postinjection, the activity falling off rapidly thereafter (Udenfriend et al., 1958). This dose corresponds to ~375 mg in a 75-kg adult, but, based on the measured concentration of harmine in the liver, it is likely that one-half this dose or less would also be effective. The reasons for the discrepancy in alkaloid concentrations between the samples examined by Rivier and Lindgren (1972) and those examined by McKenna et al. (1984) are readily explained by the differences in the methods of preparation. The method employed in preparing ayahuasca in Pucallpa, Peru, where the samples analyzed by McKenna et al. (1984) were collected, results in a much more concentrated brew than the method employed on the upper Rio Purús, the region which was the source of the samples examined by Rivier and Lindgren. The concentrations and proportions of alkaloids can vary considerably in different batches of ayahuasca, depending on the method of preparation, as well as the amounts and proportions of the source plants.

β-Carbolines, by themselves, may have some psychoactivity and thus may contribute to the overall psychotropic activity of the ayahuasca beverage; however, it is probably inaccurate to characterize the psychotropic properties of β-carbolines as "hallucinogenic" or "psychedelic" (Shulgin et al., 1997). As MAO inhibitors, β-carbolines can increase brain levels of serotonin, and the primarily sedative effects of high doses of β-carbolines are thought to result from their blockade of serotonin deamination. The primary action of β-carbolines in the ayahuasca beverage is their inhibition of

[1] In the parlance of the UDV, the tea is sometimes called *hoasca*, which is a Portuguese transliteration of *ayahuasca*. The term as used here applies specifically to the tea used within the UDV, while *ayahuasca* is used to denote non-UDV sources of the brew.

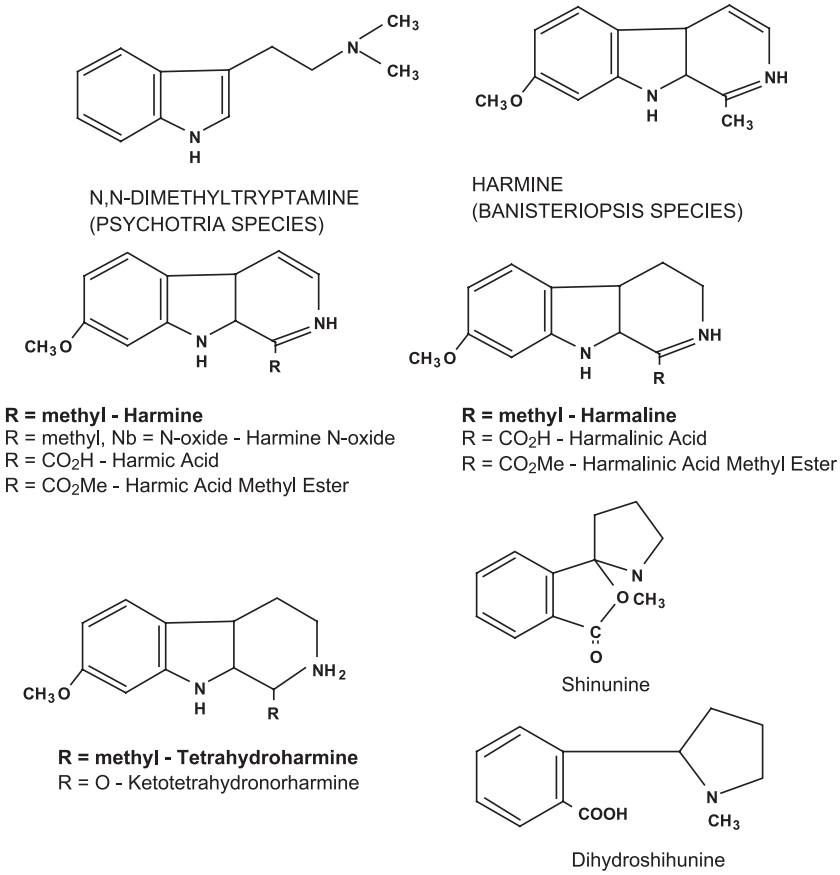

Fig. 1. Structures of ayahuasca alkaloids.

peripheral MAO, which protects the DMT in the brew from peripheral degradation and thus renders it orally active. There is some evidence, however, that THH, the second most abundant β-carboline in the beverage, acts as a weak 5-hydroxytryptamine (5-HT) uptake inhibitor and MAOI. Thus, THH may prolong the half-life of DMT by blocking its intraneuronal uptake, and hence, its inactivation by MAO, localized in mitochondria within the neuron. On the other hand, THH may block serotonin uptake into the neuron, resulting in higher levels of 5-HT in the synaptic cleft; this 5-HT, in turn, may attenuate the subjective effects of orally ingested DMT by competing with it at postsynaptic receptor sites (Callaway et al., 1999).

7. Pharmacological actions of ayahuasca and its active alkaloids

The psychotropic activity of ayahuasca is a function of the inhibition of peripheral MAO by the β-carboline alkaloids in the mixture. This action prevents the peripheral oxidative deamination of the DMT, which is the primary psychotropic component, rendering it orally active and enabling it to reach its site of action in the central nervous system in an intact form (Schultes, 1972; McKenna et al., 1984). DMT alone is inactive following oral administration at doses up to 1000 mg (Shulgin, 1982; Nichols et al., 1991). DMT is active by itself following parenteral administration starting at around 25 mg (Szara, 1956; Strassman & Qualls, 1994). Because of its oral inactivity, users have employed various methods of parenteral administration. For example, synthetic DMT is commonly smoked as the freebase; in this form, the alkaloid volatilizes readily and produces an immediate, intense psychedelic episode of short duration (5–15 min), usually characterized by multicolored, rapidly moving visual patterns behind the closed eyelids (Stafford, 1977). The Yanomamo Indians and other Amazonian tribes prepare a snuff from the sap of various trees in the genus *Virola*, which contain large amounts of DMT and the related compound, 5-methoxy-DMT, which is also orally inactive (Schultes & Hofmann, 1980; McKenna & Towers, 1985). The effects of the botanical snuffs containing DMT, while not as intense as smoking DMT freebase, are similarly rapid in onset and of limited duration. The ayahuasca beverage is unique in that it is the only traditionally used psychedelic where the enzyme-inhibiting principles in one plant (β-carbolines) are used to facilitate the oral activity of the psychoactive principles in another plant (DMT). The psychedelic experience that follows ingestion of ayahuasca differs markedly from the effects of parenterally ingested DMT; the time of onset is ~35–40 min after ingestion, and the effects, which are less intense than parenterally administered synthetic DMT, last

~4 hr. The subjective effects of ayahuasca include phosphene imagery seen with the eyes closed, dream-like reveries, and a feeling of alertness and stimulation. Peripheral autonomic changes in blood pressure, heart rate, etc., are also less pronounced in ayahuasca than parenteral DMT. In some individuals, transient nausea and episodes of vomiting occur, while others are rarely affected in this respect. When ayahuasca is taken in a group setting, vomiting is considered a normal part of the experience and allowances are made to accommodate this behavior (Callaway et al., 1999).

The amounts of β-carbolines present in a typical dose of ayahuasca are well above the threshold for activity as MAOI. It is likely that the main contribution of the β-carbolines to the acute effects of ayahuasca results from their facilitation of the oral activity of DMT, through their action as MAOI at peripheral sites. It is worthy of note that β-carbolines are highly selective inhibitors of MAO-A, the form of the enzyme for which serotonin, and presumably other tryptamines including DMT, are the preferred substrates (Yasuhara et al., 1972; Yasuhara, 1974). This selectivity of β-carbolines for MAO-A over MAO-B, combined with their relatively low affinity for liver MAO, may explain why no reports have appeared of hypertensive crises or peripheral autonomic stimulation associated with the ingestion of ayahuasca in combination with foods containing tyramine (Callaway et al., 1999). On the other hand, Suzuki et al. (1981) have reported that DMT is primarily oxidized by MAO-B; it is possible, therefore, that high concentrations of β-carbolines, partially inhibit MAO-B as well as MAO-A; but the greater affinity of tyramine for MAO-B enables it to compete for binding to the enzyme and displace any residual β-carbolines.

DMT and its derivatives and β-carboline derivatives are widespread in the plant kingdom (Smith, 1977; Allen & Holmstedt, 1980) and both classes of alkaloids have been detected as endogenous metabolites in mammals, including man (Barker et al., 1980; Airaksinen & Kari, 1981; Bloom et al., 1982). Methyl transferases, which catalyze the synthesis of DMT, 5-methoxy-DMT, and bufotenine, have been characterized in human lung, brain, blood, cerebrospinal fluid, liver, and heart, and also in rabbit lung, toad, mouse, steer, guinea pig, and baboon brains, as well as in other tissues in these species (McKenna & Towers, 1984). Endogenous psychotogens have been suggested as possible etiological factors in schizophrenia and other mental disorders, but the evidence remains equivocal (Fischman, 1983). Although the occurrence, synthesis, and degradative metabolism of DMT in mammalian systems has been the focus of scientific investigations (Barker et al., 1980, 1981), the candidacy of DMT as a possible endogenous psychotogen essentially ended when experiments showed comparable levels in both schizophrenics and normal subjects (Uebelhack et al., 1983). At present, the possible neuroregulatory functions of this "psychotomimetic" compound are incompletely understood, but Callaway (1988) has presented an interesting hypothesis regarding the possible role of endogenous DMT and β-carbolines in regulating sleep cycles and rapid eye movement states.

β-Carbolines are tricyclic indole alkaloids that are closely related to tryptamines, both biosynthetically and pharmacologically. They are readily synthesized via the condensation of indoleamines with aldehydes or α-keto acids and their biosynthesis probably also proceeds via similar reactions (Callaway et al., 1994; Fig. 2). β-Carbolines have also been identified in mammalian tissue including human plasma and platelets and rat whole brain, forebrain, arcuate nucleus, and adrenal glands (Airaksinen & Kari, 1981). 6-Methoxy-tetra-

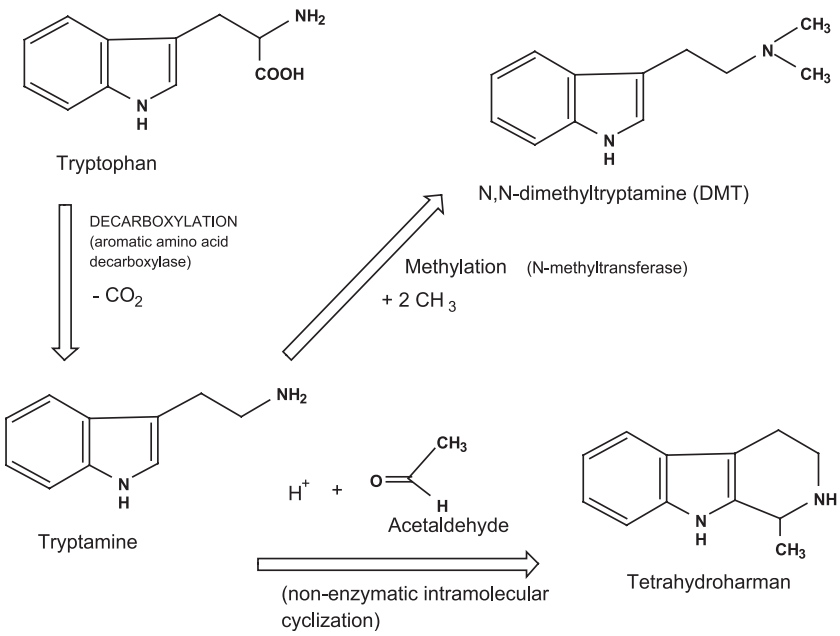

Fig. 2. Biosynthesis of DMT and a tetrahydro-β-carboline from tryptophan.

hydro-β-carboline has been recently identified as a major constituent of human pineal gland (Langer et al., 1984). This compound inhibits the high-affinity binding of [^{3}H]-imipramine to 5-HT receptors in human platelets (Langer et al., 1984), and also significantly inhibits 5-HT binding to 5-HT$_1$ type receptors in rat brain; the compound has a low affinity to 5-HT$_2$ receptors, however (Taylor et al., 1984). 2-Methyltetrahydro-β-carboline and harman have been detected in human urine following ethanol loading, (Rommelspacher et al., 1980) and it was once speculated that endogenous β-carbolines and other amine-aldehyde condensation products might be related to the etiology of alcoholism (Rahwan, 1975). At least one β-carboline has been identified as a by-product of the oxidative metabolism of DMT in rat brain homogenates (Barker et al., 1980).

β-Carbolines exert a variety of neurophysiological and biological effects (McKenna & Towers, 1984). β-Carboline derivatives are selective, reversible, competitive inhibitors of MAO-A (Buckholtz & Boggan, 1976, 1977). Other neurophysiological actions of β-carbolines include competitive inhibition of the uptake of 5-HT, dopamine, epinephrine, and norepinephrine into synaptosomes (Buckholtz & Boggan, 1976; Pahkla et al., 1997), inhibition of Na^+-dependent membrane ATPases (Canessa et al., 1973), interference with biosynthesis of biogenic amines (Ho, 1979), and vasopressin-like effects on sodium and water transport in isolated toad skin (de Sousa & Grosso, 1978). β-Carboline-3-carboxylate and various esterified derivatives have been implicated as possible endogenous ligands at benzodiazepine receptors (Lippke et al., 1983). β-Carboline ligands of these receptors can induce epileptiform seizures in rats and in chickens homozygous for the epileptic gene (Johnson et al., 1984; Morin, 1984); this proconvulsant action can be blocked by other receptor ligands, including diazepam and β-carboline-carboxylate propyl ester (Johnson et al., 1984; Morin, 1984).

β-Carbolines also exhibit other biological activities in addition to their effects on neurophysiological systems. For instance, Hopp et al. (1976) found that harmine exhibited significant anti-trypanosomal activity against *Trypanosoma lewisii*. This finding may explain the use of ayahuasca in mestizo ethnomedicine as a prophylactic against malaria and internal parasites (Rodriguez et al., 1982). Certain β-carbolines are known to exert mutagenic or co-mutagenic effects and the mechanism responsible may be related to their interactions with nucleic acids (Hayashi et al., 1977; Umezawa et al., 1978). The UV light-activated photocytotoxic and photogenotoxic activity of some β-carbolines has also been reported (McKenna & Towers, 1981; Towers & Abramosky, 1983).

8. Recent biomedical investigations of ayahuasca

Although achieving some notoriety in North American literature through the popular press and the writings of Burroughs and Ginsberg (1963), the psychological and physiological phenomena induced by ayahuasca have received little rigorous study. Various travelers to the Amazon have reported their own first hand experiences with ayahuasca (Weil, 1980; Davis, 1996), while both formal and informal ethnographic narratives have excited the public imagination (Lamb, 1971; Luna & Amaringo, 1991). Interest in the exotic origins and effects of ayahuasca have attracted a steady stream of North American tourists, often enticed by articles and advertisements in popular and New Age magazines (Krajick, 1992; Ott, 1993). Concern over possible adverse health effects resulting from the use of ayahuasca by such naive travelers has recently been expressed by a noted authority on mestizo ayahuasca use (Dobkin de Rios, 1994). These concerns are in marked contrast to testimonials of improved psychological and moral functioning by the adherents of the syncretic hoasca churches in Brazil.

The individuals who are attracted to the UDV seem to belong to a somewhat more educated socioeconomic class than those who join the Santo Daime (Grob et al., 1996). Of the ~7000 members of the UDV in Brazil, perhaps 5–10% are medical professionals, among them physicians, psychiatrists, psychologists, chiropractors, and homeopathic physicians. Most of these individuals are fully aware of the psychologically beneficial aspects of the practice and evince a great interest in the scientific study of hoasca, including its botany, chemistry, and pharmacology. The medically educated members can discuss all of these aspects with sophistication equal to that of any US-trained physician or other medical professional. At the same time, they do have a genuine spiritual reverence for the hoasca tea and the experiences it evokes. The UDV places a high value on the search for scientific truth and sees no conflict between science and religion; most members of the UDV express a strong interest in learning as much as possible about how the tea acts on the body and brain. As a result of this unique circumstance, the UDV presents an ideal context in which to conduct a biomedical investigation of the acute and long-term effects of hoasca/ayahuasca.

8.1. The "Hoasca Project"

Due to a fortunate combination of circumstances, my colleagues and I were invited to conduct such a biomedical investigation of long-term hoasca drinkers by the Medical Studies section of the UDV (Centro de Estudos Medicos). This study, which was conducted by an international consortium of scientists from Brazil, the United States, and Finland, was financed through private donations to various nonprofit sponsoring groups, notably Botanical Dimensions, which provided major funding, the Heffter Research Institute, and the Multidisciplinary Association for Psychedelic Studies. Botanical Dimensions is a nonprofit organization dedicated to the study and preservation of ethnomedically significant plants, and the Multidisciplinary Association for Psychedelic Studies (http://www.MAPS.org) and the Heffter

Research Institute (http://www.Heffter.org) are nonprofit organizations dedicated to the investigation of the medical and therapeutic uses of psychedelic agents. The field phase of the study was conducted during the summer of 1993 at one of the oldest UDV temples, the Nucleo Caupari located in the Amazonian city of Manaus, Brazil. Subsequent laboratory investigations took place at the respective academic institutions of some of the principle investigators, including the Department of Psychiatry, Harbor UCLA Medical Center, the Department of Neurology, University of Miami School of Medicine, the Department of Psychiatry, University of Rio de Janeiro, Department of Internal Medicine, University of Amazonas Medical School, Manaus, and the Department of Pharmaceutical Chemistry, University of Kuopio, Finland. This study has since become anecdotally known as "the Hoasca Project" in subsequent presentations of the results in various public venues.

Because this study was the first of its kind, there was virtually no preexisting data on the objective measurement of the physical and psychological effects of ayahuasca in human subjects. As a result, this study was in some respects a pilot study; its primary objectives were modest, representing an effort to collect a basic body of data, without attempting to relate the findings to either possible detrimental effects of ayahuasca, or to possible therapeutic effects. The study had 4 major objectives:

- Assessment of acute psychological and physiological effects of hoasca in human subjects;
- Assessment of serotonergic functions in long-term users of hoasca tea;
- Quantitative determination of active constituents of hoasca teas in plasma;
- Quantitative determination of active constituents of hoasca teas.

Most of these objectives were achieved, and the results have been published in various peer-reviewed scientific journals (Callaway et al., 1994, 1996, Grob et al., 1996; Callaway et al., 1999). Some key findings are summarized briefly below.

8.2. Assessment of acute and long-term psychological effects of hoasca teas

The subjects in all of the studies consisted of a group of 15 healthy, male volunteers, all of whom had belonged to the UDV for a minimum of 10 years, and who ingested hoasca once every 2 weeks, on average, in the context of the UDV ritual. None of the subjects actively used tobacco, alcohol, or any drugs other than hoasca. For some comparative aspects of the study, a control group of 15 age-matched males was also used; these individuals were recruited from among the friends and siblings of the volunteer subjects, and like them, were local residents of Manaus having similar diets and socioeconomic status. None of the control subjects were members of the UDV, and none had ever ingested hoasca tea.

The psychological assessments, administered to both groups, consisted of structured psychiatric diagnostic interviews, personality testing, and neuropsychological evaluations. Measures administered to the UDV hoasca drinkers, but not to the hoasca-naive group, included semistructured and open-ended life story interviews. A phenomenological assessment of the altered state elicited by hoasca was quantified using the Hallucinogen Rating Scale developed by Dr. Rick Strassman in his work with DMT and psilocybin in human subjects (Strassman et al., 1994).

The UDV volunteers showed significant differences from the hoasca-naive subjects in the Tridimensional Personality Questionnaire and the WHO-UCLA Auditory Verbal Learning Test. The Tridimensional Personality Questionnaire assesses 3 general areas of behavior, viz., novelty-seeking, harm avoidance, and reward dependence. With respect to novelty-seeking behaviors, UDV members were found to have greater stoic rigidity versus exploratory excitability, greater regimentation versus disorderliness, and a trend toward greater reflection versus impulsivity; but there was no difference between the groups on the spectrum between reserve and extravagance. On the harm reduction scale, UDV subjects had significantly greater confidence versus fear of uncertainty and trends toward greater gregariousness versus shyness and greater optimism versus anticipatory worry. No significant differences were found between the 2 groups in criteria related to reward dependence.

The 15 UDV volunteers and the control subjects were also given the WHO-UCLA Auditory Learning Verbal Memory Test. Experimental subjects performed significantly better than controls on word recall tests. There was also a trend, although not statistically significant, for the UDV subjects to perform better than controls on number of words recalled, delayed recall, and words recalled after interference.

The Hallucinogen Rating Scale, developed by Strassman et al. (1994) for the phenomenological assessment of subjects given intravenous doses of DMT, was administered to the UDV volunteers only (since control subjects did not receive the drug). All of the clinical clusters on the HRS were in the mild end of the spectrum compared with intravenous DMT. The clusters for affect, intensity, cognition, and volition were comparable to an intravenous DMT dose of 0.1–0.2 mg/kg, and the cluster for perception was comparable to 0.1 mg/kg iv DMT; the cluster for somatesthesia was less than the lowest dose of DMT measured by the scale, 0.05 mg/kg.

The most striking findings of the psychological assessment came from the structured diagnostic interviews and the semistructured open-ended life story interviews. The Composite International Diagnostic Interview was used for the structured diagnostic interview. None of the UDV subjects had a current psychiatric diagnosis, whereas 2 of the control subjects had an active diagnosis of alcohol misuse and

hypochondriasis. Only 1 subject among the controls had a past psychiatric disorder that was no longer present; an alcohol misuse disorder that had remitted 2 years previously. However, prior to membership in the UDV, 11 of the UDV subjects had diagnoses of alcohol misuse disorders, 2 had past major depressive disorders, 4 had past histories of drug misuse (cocaine and amphetamines), 11 were addicted to tobacco, and 3 had past phobic anxiety disorders. Five of the subjects with a history of alcoholism also had histories of violent behavior associated with binge drinking. All of these pathological diagnoses had remitted following entry into the UDV. All of the UDV subjects interviewed reported the subjective impression that their use of hoasca tea within the context of the UDV had led to improved mental and physical health and significant improvements in interpersonal, work, and family interactions.

8.3. Assessment of the acute physiological effects of hoasca tea

The major focus of the biochemical and physiological measurements carried out for the study was on the acute effects subsequent to consuming hoasca tea. One of the objectives was simply to measure the effects of hoasca on standard physiological functions, such as heart rate, blood pressure, and pupillary diameter, subsequent to ingestion (Callaway et al., 1999). We found that all of these responses were well within normal parameters. Hoasca, not surprisingly, caused an increase in pupillary diameter from baseline (predose) levels of 3.7 to ~4.7 mm at 40 min, which continued to 240 min after ingestion, at which point measurements were discontinued. Respiration rate fluctuated throughout the 240 min, from a low of 18.5 at baseline to a high of 23 at 100 min. Temperature rose from a baseline low of 37 °C at baseline to a high of 37.3 °C at 240 min (although the ambient temperature also increased comparably during the course of the experiments, which were conducted from 10:00 to 16:00 h). Heart rate increased from 71.9 bpm at baseline to a maximum of 79.3 bpm by 20 min, decreased to 64.5 bpm by 120 min, then gradually returned toward basal levels by 240 min. There was a concomitant increase in blood pressure; both systolic and diastolic pressure increased to maxima at 40 min (137.3 and 92.0 mm Hg, respectively) over baseline values (126.3 and 82.7 mm Hg, respectively) and returned to basal values by 180 min. We also measured neuroendocrine response for plasma prolactin, cortisol, and growth hormone; all showed rapid and dramatic increases over basal values from 60 (cortisol) to 90 (growth hormone) to 120 min (prolactin) after ingestion. The observed response is typical of serotonergic agonists, and is comparable to the values reported by Strassman and Qualls (1994) in response to injected DMT. In our study, the neuroendocrine response to oral DMT was delayed by a factor of 4 or 5 compared with the almost immediate (<15 min) response to injected DMT.

8.4. Characterization of the pharmacokinetics of hoasca alkaloids in human subjects

The fourth objective of the study was to measure pharmacokinetic parameters of the hoasca alkaloids in plasma following ingestion of hoasca tea and to correlate this to the amounts of alkaloids ingested (Callaway et al., 1996, 1999). The UDV collaborators held a special "preparo" to prepare the sample of hoasca that was used for all subjects in the study. The mestres confirmed the activity in the usual manner, via ingestion, and pronounced it active and suitable for use in the study. Subsequent analysis by high-performance liquid chromatography found the tea to contain (in mg/mL): harmine, 1.7; harmaline, 0.2; THH, 1.07; and DMT 0.24. Each subject received an aliquot of tea equivalent to 2 mL/kg body weight, which was consumed in a single draught. Based on the average body weight (74.2 ± 11.3 kg), the average dose of tea was 148.4 ± 22.6 mL, containing an average of 35.5 mg DMT, 158.8 mg THH, 29.7 mg harmaline, and 252.3 mg harmine. These doses are above the threshold level of activity for DMT as a psychedelic, and for harmine and THH as MAO inhibitors; harmaline is essentially a trace constituent of hoasca tea (Callaway et al., 1996, 1997).

Only 12 of the 15 volunteers had sufficient plasma levels of DMT to permit pharmacokinetic measurements, possibly due to early emesis during the course of the session. Of these, the maximum plasma concentration (C_{max}; 15.8 ng/mL) occurred at 107 min after ingestion, while the half-life ($T_{1/2}$) was 259 min. THH was measured in 14 of the 15 subjects; the C_{max} was 91 ng/mL, which was reached at 174 min. This compound displayed a prolonged half-life of 532 min, in contrast to harmine, which had a half-life of 115.6 min. The C_{max} for harmine and harmaline was 114.8 and 6.3 ng/mL, respectively, and time of maximum concentration (T_{max}) was 102 and 145 min, respectively. The $T_{1/2}$ for harmaline could not be measured (Callaway et al., 1999).

This study was conceived because of the need to collect some basic data on the physiological and pharmacokinetic characteristics of hoasca, since none had existed previously. The conclusions to be drawn from the results, if any, are interesting and potentially significant, particularly in that these findings may offer a physiological rationale for the marked improvements in psychological health that are correlated with long-term hoasca use. Not surprisingly, the highest plasma concentrations of DMT correlated with the most intense subjective effects; however, the psychological measurement (Hallucinogen Rating Scale) indicated that comparable plasma levels of injected DMT in Strassman and Qualls' (1994) study resulted in a more intense subjective experience than the effects reported from the hoasca tea. One possible explanation is that THH, by acting as a 5-HT reuptake inhibitor, may have resulted in a greater availability of 5-HT at the synapse, and this may have competed with DMT for occupancy at serotonergic synapses. Alternatively, the rapid increase in brain levels of DMT following intra-

venous administration may result in a more intense subjective effect compared with that experienced when DMT is absorbed more slowly following oral administration.

Another point worthy of remark is that the activity of THH in hoasca is apparently more a function of its inhibition of 5-HT uptake than to its MAOI action. THH is a poor MAOI compared with harmine ($EC_{50} = 1.4 \times 10^{-5}$ M for THH vs. 8×10^{-8} M for harmine), and while the plasma levels for harmine are well above the EC_{50} values, those for THH are well below the EC_{50} value for this compound as an MAOI.

8.5. Assessment of serotonergic functions in long-term users of hoasca

Another objective of the study was to investigate whether long-term use of hoasca resulted in any identifiable "biochemical marker" that was correlated with hoasca consumption, particularly with respect to serotonergic functions, since the hoasca alkaloids primarily affect functions mediated by this neurotransmitter. Ideally, such a study could be carried out on postmortem brains; since this was not possible, we settled on looking at serotonin transporter sites in blood platelets, using [^{3}H]-citalopram to label the transporters in binding assays (Table 1). The up- or down-regulation of peripheral platelet recognition sites has been proposed to be indicative of similar biochemical events occurring in the brain, although there is some controversy about the correlation between platelet transporter changes and changes in central nervous system transporters in patients receiving antidepressant medications (Stahl, 1977; Pletscher & Laubscher, 1980; Rotman, 1983);. However, platelet-binding assays were deemed suitable for the purposes of our study, as our objective was not to resolve this controversy but simply to determine if some kind of long-term biochemical marker could be identified. Neither did we postulate any conclusions about the possible "adverse" or "beneficial" implications of such a marker, if detected. We conducted the assays on platelets collected from the same group of 15 volunteers after they had abstained from consuming the tea for a period of 1 week. We also collected platelet specimens from the age-matched controls who were not hoasca drinkers. We were surprised to find a significant up-regulation in the density of the citalopram binding sites in the hoasca drinkers compared with control subjects. Although the hoasca drinkers had a higher density of transporters (B_{max} = 993 fmol/mg protein in hoasca drinkers vs. 724 fmol/mg protein in matched controls; cf. Table 1), there was no change in their affinity for the labeled citalopram binding site. The significance of this finding, if any, is unclear. There is no other pharmacological agent that is known to cause a similar up-regulation, although chronic administration of 5-HT uptake inhibitors has been reported to *decrease* both B_{max} (the density of binding sites) (Hrinda, 1987) and 5-HT transporter mRNA in rat brain (Lesch et al., 1993). Increases in B_{max} for the uptake site in human platelets have been correlated with old age (Marazziti et al., 1989) and also to the dark phase of the circadian cycle in rabbits (Rocca et al., 1989). It has been speculated (Marazziti et al., 1989) that up-regulation of 5-HT uptake sites in the aged may be related to the natural course of neuronal decline. Although our sample size was limited, we found no correlation with age, and the mean age of the sample was 38 years. Also, none of our subjects showed evidence of any neurological or psychiatric deficit. In fact, in view of their exceptionally healthy psychological profiles, one of the investigators speculated that perhaps the serotonergic up-regulation is associated, not simply with age, but with "wisdom"—a characteristic often found in the aged, and in many hoasca drinkers.

Another interesting self-experiment related to this finding was carried out by one of the investigators, Jace Callaway, following his return to Finland after the field phase of the study was completed. Dr. Callaway has access to single photon emission computerized tomography scanning facilities in the Department of Pharmacology at the University of Kuopio. Suspecting that the causative agent of the unexpected up-regulation might be THH, Dr. Callaway took single photon emission computerized tomography scans of his own brain 5-HT uptake transporters prior to beginning a 6-week course of daily dosing with THH, repeating the scan after the treatment period. He found that the density of central 5-HT receptors in the prefrontal cortex had increased; when he discontinued THH, their density gradually returned to previous levels over the course of several weeks. While this experiment only had 1 subject, if it is indicative of a general effect of THH that can be replicated and confirmed, the implications are potentially significant. A severe *deficit* of 5-HT uptake sites in the frontal cortex has been found to be correlated with aggressive disorders in violent alcoholics (Tiihonen et al., 1997; Hallikainen et al., 1999); if THH is

Table 1
Age and kinetic parameters of platelet 5-HT uptake activity as measured by [^{3}H]-citalopram for tea drinkers and controls (from Callaway et al., 1994)

Tea drinkers			Controls		
Age (years)	B_{max} (fmol/mg protein)	K_d (nM)	Age	B_{max} (fmol/mg protein)	K_d (nM)
28	1179	2.91	21	1022	2.29
30	914	3.01	24	458	1.63
35	971	2.97	24	593	2.39
36	1153	3.05	29	653	2.15
38	831	2.73	29	856	2.96
38	1470	4.88	36	888	2.89
39	688	1.48	38	718	2.46
40	790	2.17	39	674	3.38
40	847	3.11	43	766	2.17
40	1096	3.42	45	614	2.37
43	855	1.53			
46	771	2.70			
48	1346	3.05			
Mean = 38.5	993*	2.84	32.8	724*	2.47
SEM = 1.6	69	0.25	2.8	55	0.16

Unpaired student's *t*-test.
*P = 0.006.

able specifically to reverse this deficit, it may have applications in the treatment of this syndrome. These findings are especially interesting when viewed in the context of the psychological data collected in the hoasca study (Grob et al., 1996). The majority of the subjects had had a previous history of alcoholism, and many had displayed violent behavior in the years prior to joining the UDV; virtually all attributed their recovery and change in behavior to their use of hoasca tea in the UDV rituals. While it can be argued that their reformation was due to the supportive social and psychological environment found within the UDV, the finding of this long-term change in precisely the serotonin system that is deficient in violent alcoholism argues that biochemical factors may also play a role.

9. Current legal and regulatory status of ayahuasca

In recent decades, ayahuasca has been integrated into the religious practices of several syncretic religions in Brazil, where it is consumed as a sacrament in large group rituals, sometimes involving up to several hundred people. There are 3 primary Brazilian religions that employ ayahuasca as a sacrament: the UDV, the Santo Daime, and the Barquinia. The Santo Daime and UDV churches include about 10,000 members each throughout Brazil. The Barquinia is a smaller group, consisting of only a few hundred members, primarily in the Rio Branco area of the Amazon. The religious practices of these groups, and their sacramental use of ayahuasca, are sanctioned and legally permitted by CONFEN, the Brazilian regulatory agency that fulfills a role similar to the FDA and DEA in this country.

Ayahuasca and its source plants are not internationally prohibited under the 1971 Convention on Psychotropic Substances although one of them contains a Schedule I controlled substance, DMT. The source plant *B. caapi* contains β-carboline alkaloids, which are not listed as controlled substances either internationally or in the United States, so the controversy centers around the occurrence of DMT in the admixture plant, *P. viridis*, or other DMT-containing admixtures that may occasionally be substituted for *P. viridis*. Pure DMT is a Schedule I controlled substance under US law and is listed as a controlled substance under the International Convention on Psychotropic Substances. However, neither *P. viridis* nor any of the numerous other plant species that are known to contain DMT (cf. Smith, 1977; Ott, 1993) are specifically controlled or regulated as controlled substances. Many of these plants are freely and legally available from Internet web sites and elsewhere (Halpern & Pope, 2001). A recent opinion letter was issued by Herbert Schaepe, the Secretary of the Board for the United Nations International Narcotics Control Board (INCB) that has been helpful in clarifying at least the international legal status of plants containing DMT and other hallucinogens (Schaepe, 2001). In response to an inquiry by Mr. Lousberg, Chief, Inspectorate for Health Care of the Ministry of Public Health in the Netherlands regarding whether plant materials and their decoctions are covered by the Convention on Psychotropic Substances. The inquiry was sent by Mr. Lousberg. The opinion letter (dated 17 January 2001) states:

> No plants (natural materials) containing DMT are at present controlled under the 1971 Convention on Psychotropic Substances. Consequently, preparations (e.g., decoctions) made of these plants, including *ayahuasca*, are not under international control, and, therefore, not subject to any of the articles of the 1971 convention.

A copy of this letter was submitted in support of a suit filed by the North American chapter of the UDV seeking a preliminary injunction against attempts by the Department of Justice to suppress the group's religious use of hoasca (see below).

The UDV and Santo Daime churches that originated in Brazil now have legally incorporated American chapters that use *ayahuasca* in religious ceremonies. In November 2000, the US chapter of the UDV, based in Santa Fe, petitioned the Department of Justice in the US District Court for the District of New Mexico for the return of some 30 gal of its sacramental hoasca. This material had been seized by the DEA from the home of the UDV leader in May 1999. The UDV appealed for a special exemption to allow for the religious use of ayahuasca under the provisions of the Religious Freedom Restoration Act (see http://www.nvo.com/cd/nss-folder/pubfiles/PltnfsComplaint.pdf) (O Centro Espirita Beneficiente UDV et al., 2000). A favorable ruling by that court in 2002 allowed for the religious use of hoasca tea by the UDV Church (US District Court for the District of New Mexico: No CV 00-1647 JP/LRP (see "Federal Court Rules in Favor of Ayahuasca-Using Church," http://www.cognitiveliberty.org/dll/udv_pj_granted.htm; Center for Cognitive Liberty and Ethics, 2002a, 2002b). The government filed an emergency stay, and the favorable ruling was temporarily contravened (see http://www.nvo.com/cd/nss-folder/pubfiles/emerstay.htm) (Westlaw Citation # 31862699, 2002). In September 2003, a 3-judge review panel in the 10th US Circuit Court of Appeals in Denver again returned a ruling in favor of the UDV. The ultimate resolution of this case remains pending and may eventually require review by the US Supreme Court. A complete chronology of this case and links to relevant public documents filed in the process can be found on the web site of the Center for Cognitive Liberty and Ethics (http://www.cognitiveliberty.org/dll/udv_index.htm).

Although the ultimate outcome of the UDV's efforts to secure legal sanction for their religious use of ayahuasca may entail years of litigation, it is clear that increasing numbers of people in the United States are using ayahuasca on a regular basis in rituals and in religious ceremonies. The growing use of ayahuasca for religious (and recreational) purposes in the United State has public health implications. Additionally, the growth of "ayahuasca tourism" in the Amazon is attracting increasing numbers of American and

foreign travelers (Dobkin de Rios, 1994). The uncertain legal and regulatory status under US law of ayahuasca, or more specifically, its DMT-containing component, *P. viridis*, also may pose challenges for the eventual pursuit of clinical investigations of ayahuasca under an Investigational New Drug (IND) application.

10. The rationale for clinical studies of ayahuasca

There are currently 2 primary rationales supporting the need for controlled clinical investigations of ayahuasca:

1. Ayahuasca is becoming increasingly popular in this country for both religious and recreational purposes. The currently pending legal challenges to the religious use of ayahuasca, described above, have so far resulted in rulings favorable to the UDV, the religious organization that has petitioned the government for the legal right to employ ayahuasca as a sacrament under the Religious Freedom Restoration Act. This raises the possibility that religious use of ayahuasca may eventually become legally sanctioned in the United States. Yet, increasing use of ayahuasca, whether ritually or recreationally, has public health implications. Despite a long history and tradition of indigenous use that indicates that the preparation can be used safely, very little actual clinical data have been accumulated, which provides a scientific basis for the notion that ayahuasca is safe for human use. Such studies are needed to inform the currently pending legal challenges to its use for religious purposes. In addition, such studies would provide health professionals with a body of information as to its safety, possible side effects, potential drug interactions, potential for adverse reactions, and possible toxicity. Such data may be essential for health providers in the event they are required to treat individuals who have ingested ayahuasca.
2. A second rationale is that ayahuasca may have therapeutic applications, and these require investigation within the context of well-designed clinical studies. Ideally, such studies should be conducted under an FDA-approved IND protocol, together with whatever regulatory permissions are required from the DEA and an institutional IRB affiliated with the institution where the study is to be conducted.

11. Ayahuasca clinical research to date

11.1. Key findings from the "Hoasca Project"

Grob et al. (1996) studied the short- and long-term toxicity profile of hoasca use in the Brazilian UDV. They reported that there was no evidence of acute toxicity during the sessions or of long-term toxicity or other adverse health effects. Hoasca, in the context of the UDV, is consumed regularly by men and women ranging in ages from 13 to 90 and appears to be safe. Many of the older members of the UDV, who are now well into their 80s, have used hoasca regularly since their teenage years and are remarkable for their mental acuity, lack of serious disease history, and physical vigor (Callaway et al., 1999). Psychological screening tests and evaluations have found no evidence of long-term mental or cognitive impairment in long-term hoasca drinkers (UDV members). In fact, most members performed slightly better than control subjects on measures of cognitive function, verbal facility and recall, mathematical ability, motivation, and emotional well-being and personality adjustment (Grob et al., 1996). The study protocol included the psychological assessment of 15 long-term UDV members utilizing hoasca as a legal, psychoactive sacrament, as well as 15 matched controls with no prior history of hoasca ingestion. Measures administered to both groups included structured psychiatric diagnostic interviews, personality testing, and neuropsychological evaluation. Overall assessment revealed high functional status. Implications of these findings and the need for further investigation are discussed (Grob et al., 1996). In addition to psychological parameters, the study included pharmacokinetic measurements of the major active alkaloids (Callaway et al., 1999). Three key findings have emerged from the study, which has come to be known as the "Hoasca Project." These have been described in detail in the previous sections.

12. Potential therapeutic applications of ayahuasca

Two kinds of evidence argue that ayahuasca may have therapeutic applications. A considerable body of anecdotal evidence, coupled with a long history of ethnomedical use, indicates that ayahuasca may be useful for the treatment of abuse disorders, such as alcoholism and substance abuse, as well as for physical maladies such as cancer. In addition, the results of a 1993 biomedical study of long-term members of the UDV in Brazil have provided data that may indicate directions for the future direction of clinical studies of ayahuasca.

12.1. Treatment of alcoholism and substance abuse

In the right circumstances, meaning within appropriate supportive settings and social milieus such as the Brazilian UDV, regular and long-term hoasca use may result in profound, lasting, and positive behavioral and lifestyle changes. The most dramatic example is the finding that, prior to their joining the UDV church, most members that were interviewed had histories of alcoholism, substance abuse, domestic violence, and other maladaptive behaviors and lifestyles. These dysfunctional behaviors resolved themselves on subsequent induction into the UDV and regular use of the hoasca sacrament (Grob et al., 1996).

Dr. Micheal Winkelman coined the term *psychointegrator plants* for describing the psychological benefits of ayahuasca administration (Winkelman, 1995). There is increasing interest in shamanistic forms of healing and ayahuasca's reputed psychological, medical, and spiritual benefits have stirred interest among North American scientists, physicians, and the intellectual lay public. Many North Americans and Europeans travel to the Amazon to participate in ayahuasca ceremonies led by traditional shamans (also called *ayahuasqueros*) (Dobkin de Rios, 1994). A recent report on BBC radio (http://news.bbc.co.uk/2/hi/programmes/crossing_continents/3243277.stm) discusses the successful use of ayahuasca to treat cocaine addition at a Peruvian clinic. The results of the Hoasca study, described above, also argue that ayahuasca treatment, within an appropriate psychotherapeutic context, may also be applicable to the treatment of alcoholism.

12.2. Treatment of serotonergic deficits

Coupled with these positive psychological and behavioral changes, was an unexpected finding. Apparently, regular ayahuasca use results in a long-term modulation of serotonin systems in the brain; specifically, that populations of serotonin transporters exhibit an elevated density in platelets and in the brain, an effect that may be due to one of the β-carbolines in the ayahuasca mixture (see Table 1). The parameter measured in the hoasca study was an elevation in the density of 5-HT transporters in platelets, and did not directly measure brain levels. However, psychopharmacologists have long-used platelets as a peripheral marker of similar biochemical events occurring within the brain (Stahl, 1977; Pletscher & Laubscher, 1980). The serotonin transporter is the membrane bound protein in serotonin containing neurons that manages the reuptake of this neurotransmitter from the synapse, and is the site of action of Prozac and other selective serotonin reuptake inhibitors. Hence, the 5-HT transporter is intimately involved with syndromes such as depression and other mood disorders, suicidal behavior, etc. (Coccaro et al., 1989; Roy et al., 1991; Tiihonen et al., 1997). Callaway et al. (1994) have hypothesized that the elevation of 5-HT transporters seen with long-term ayahuasca use and the positive behavioral changes are linked. Work by other investigators have found severe deficits in the density of these transporters in people with behavioral disorders, especially patients with a history of alcoholism linked with violence, and in those prone to suicidal behavior (Tiihonen et al., 1997; Hallikainen et al., 1999). Based on our finding that 5-HT transporters are significantly elevated in long-term users of ayahuasca, we speculate that ayahuasca may be able to reverse these deficits over time (Callaway et al., 1994).

Serotonergic deficits have been linked to a variety of functional, behavioral, and neurodegenerative disorders, ranging from alcoholism to depression, autism, schizophrenia, attention deficit hyperactivity disorder, and senile dementias. Recent advances in the cloning of neurotransmitter transporter genes and the development of transporter-deficient knockout mice have given neurobiologists powerful new tools for investigating the role of monoamine transporters in the regulation of neurotransmitter functions and their potential links to neurobiological and behavioral disorders (Blakely & Bauman, 2000). Genetic polymorphisms in transporter genes and their expression have been linked to anxiety states (Lesch et al., 1996), autism (Cook et al., 1997), affective disorders (Sher et al., 2000), and alcohol and cocaine abuse (Little et al., 1998). The results from the UDV study indicate that one or more constituents in ayahuasca may be able to modulate gene expression for the serotonin transporters and that the long-term changes observed are correlated with positive behavioral changes. If these preliminary findings are borne out by more rigorous clinical studies neurobiologists may have a new compound that may be applied to the study of serotonin transporter expression and regulation. Eventually, compounds developed form this work may yield new treatments for substance abuse and psychiatric disorders.

12.3. Immune modulation

The third piece of evidence that has emerged is more anecdotal than scientific, but is nonetheless intriguing. This is that ayahuasca may have significant immunomodulatory effects. A number of users of ayahuasca in North America have reported that they have experienced remissions of cancers and other serious illnesses in conjunction with regular use of the tea (Topping, 1998). Additionally, the longevity, physical vigor, and mental acuity evidenced by many *ayahuasqueros* in Peru has long been noted as remarkable. Many of these shamans living in developing nations are well into their 70s, 80s, and 90s and yet appear to live out their years in a state of physical and mental health that would be the envy of many in the so-called developed countries. Certainly, some of this is due to dietary factors and a physically vigorous and demanding lifestyle; but some of it may be the result of exceptional immune functions due to their years of working with ayahuasca. Many of these practitioners are accustomed to consuming it several times a week in the performance of their healing practices and have done so most of their adult lives (Luna, 1984a, 1984b, 1986). In the context of mestizo folk medicine and indigenous shamanic practice, ayahuasca has long enjoyed a reputation as a healing medicine, with properties that transcend its acute, psychological effects. These properties may be attributable to its immune potentiating effects, if they are found to exist. Although the evidence at this point is anecdotal and speculative, this hypothesis can be easily resolved empirically. Many plants are known to possess immune-potentiating properties and both in vivo and in vitro methodologies have been developed to measure the immune-modulating properties of plant extracts (Wilasrusmee et al., 2002a, 2002b; Klein et al., 2000). A recent study characterized the immunopotentiating and antitumor properties of another

well-known indigenous hallucinogen, the peyote cactus (Franco-Molina et al., 2003).

12.4. Clinical studies by Spanish investigators

Subsequent to the completion of the "Hoasca Project" and the publication of its results, a group of investigators at the Universitat Autonoma de Barcelona in Spain have conducted clinical investigations examining various aspects of the pharmacology of ayahuasca in healthy volunteers. Using a freeze-dried, preparation of Brazilian ayahuasca standardized to contain known quantities of DMT and β-carboline alkaloids, these investigators initially conducted safety assessments and dose-response studies in a small sample of healthy volunteers (Riba et al., 2001a, 2001b). After documenting physiological and subjective changes induced by the lyophilized ayahuasca preparation, the investigators concluded that it was well tolerated and presented an acceptable side-effect profile. Subsequent investigations focused on characterization of the pharmacokinetic profiles and effects on cardiovascular parameters (Yritia et al., 2002; Riba et al., 2003). Electrophysiological and psychological effects were investigated using topographic pharmaco-EEG mapping, (Riba et al., 2002a), while effects on the startle reflex and sensory and sensorimotor gating were assessed using suppression of the P50 auditory evoked potential and prepulse inhibition of the startle reflex (Riba et al., 2002b). In contrast to studies with other psychedelic agents, such as psilocybin, these investigators found a decremental effect on sensory gating as measured by the auditory evoked potential and a lack of effect on sensorimotor gating measured by prepulse inhibition. More relevant, perhaps, to the immediate concerns regarding the safety of using ayahuasca in humans are that no serious adverse reactions, evidence of toxicity, or lack of tolerability was experienced by the subjects over the series of studies.

13. The pathway to clinical studies

There exists both a need and an opportunity to investigate these questions in a more rigorous scientific framework that conforms to currently accepted medical paradigms for conducting clinical research. The evidence from the Brazilian UDV study is intriguing, but not compelling; the sample of subjects studied was small, and given the exigencies of conducting this kind of research in a foreign country and a nonclinical venue, inevitably many factors could not be controlled. Additionally, the question of immune potentiation has yet to be investigated in any manner beyond anecdotal reports and casual observations. The safety and efficacy of ayahuasca has not been definitively verified, and safety concerns in the United States grow as use of ayahuasca as a spiritual sacrament grows. The American medical community will insist, rightly so, on more rigorous investigations in the form of controlled clinical trials before any possible therapeutic investigations can proceed.

A number of hurdles must be overcome before ayahuasca can be studied in a clinical context in the United States. First of all, a clarification of its legal status is needed to determine what kinds of permissions, if any, are needed from the DEA and other relevant law-enforcement agencies to pursue clinical studies. Although the opinion issued by the Secretary for the UN Narcotics Control Board (Schaepe, 2001) indicates that neither the preparation nor the source plants are internationally regulated, the status of ayahuasca under US law remains in limbo. The UDV's case for an exemption for the sacramental use of hoasca under the Religious Freedom Restoration Act has received favorable rulings in 2 Federal courts and appeal to the Supreme Court is likely. It is to be hoped that a favorable resolution in this case will simplify the regulatory challenges to biomedical studies of ayahuasca.

13.1. Step I: preclinical study—phytochemistry and pharmacology

Assuming the regulatory dilemmas are eventually resolved, it remains unlikely that the FDA will approve an initial IND application for a clinical study without an extensive body of data derived from preclinical investigations. Although the clinical studies by Riba et al. (2001a, 2001b, 2002a, 2002b, 2003) and Yritia et al. (2002) have produced evidence that ayahuasca can be used safely in a clinical setting, FDA regulators may insist on generating preclinical data within the United States.

Ayahuasca has been poorly studied in animal models, and such a requirement on the part of the FDA could contribute to better quality research in subsequent clinical phases. Many of the questions relating to the understanding of ayahuasca's pharmacology can and should be addressed first in appropriate in vitro and animal models. The results of those preclinical studies will then be useful in terms of framing and focusing subsequent clinical studies.

Ayahuasca, like all botanical medicines, is a complex combination of hundreds, or perhaps even thousands, of biologically active compounds. Although there have been extensive phytochemical investigations of ayahuasca over the years, almost all of them have focused exclusively on the alkaloids, since they are the psychoactive constituents. Not even all of the alkaloids have been investigated thoroughly; for example, the pharmacology of the pyrrolidine alkaloids shihunine and dihydroshihunine in *B. caapi* remains a black box although the compounds were isolated over 30 years ago. Ayahuasca and its source plants are likely to contain flavonoids, tannins, lignans, saponins, volatile oils, and many of the other classes of secondary metabolites that are widespread in plants. All of these have enormous molecular diversity and all have been shown to posses an equally wide spectrum of pharmacological activities. None of those in ayahuasca, however, have been investigated from this perspective. Ad-

ditionally, much of the early phytochemical work and preclinical pharmacology on *Banisteriopsis* extracts was conducted over 30 years ago (Deuloufeu, 1967; O'Connell, 1969; Stull et al., 1971; Ferguson, 1972). There have been important advances in both phytochemical analysis methods and preclinical pharmacology since this work was carried out, and there is a need to revisit these questions using more current methodologies. A thorough phytochemical characterization of ayahuasca and its source plants then becomes the first order of business for any preclinical studies. Extracts of each plant should be made and fractionated using standard phytochemical procedures. The biological activities of the fractions can be further characterized using appropriate in vitro assays. These investigations will provide guidance for further studies in animal models and eventually in clinical trials; it may also detect activities, even toxicity, that is currently unsuspected or uncharacterized. It is important that these investigations be conducted using source plants of known provenance, documented by herbarium voucher collections, so that the data will be applicable to the development of a standardized preparation for eventual use in IND-authorized clinical studies.

Preclinical studies will also enable investigators to examine aspects of ayahuasca's pharmacology that may be relevant to its suspected therapeutic activity. For example, a recent in vitro investigation reported activities in *Banisteriopsis* extracts potentially relevant to the treatment of Parkinson's disease (Schwarz et al., 2003) in which the β-carboline components alone did not sufficiently account for all of the observed activity. Techniques, such as in vitro receptor binding and autoradiography, can be applied to characterize the effects of ayahuasca alkaloids and extracts on modulation of the serotonin transporter and other neurobiological functions. Similarly, questions about the possible immune-potentiating effects of ayahuasca can be initially addressed in animal or in vitro test systems. Relatively standard in vitro and in vivo techniques for measuring immune response have been applied to a variety of medicinal plants (cf. Klein et al., 2000; Wilasrusmee et al., 2002a, 2002b).

13.2. Step II: investigational new drug application

The phytochemical profiling and preclinical pharmacological and toxicological data generated in the preclinical phase will lay the foundation for the IND application.

The FDA has issued a draft of guidelines, still under review, for the development of botanical drugs (FDA/CDER, 2002). The current draft can be viewed at http://www.fda.gov/cder/guidance/1221dft.htm. The study medication to be developed must conform to these guidelines, in whatever form may be current at the time the application is submitted.

Ayahuasca as prepared and traditionally consumed in the Amazon is a decoction prepared from fresh plant materials that is consumed orally as a beverage. The suggested preparation under the IND protocol differs from the traditional preparation in that it will be a freeze-dried, aqueous extract made from fresh plant materials and administered in capsules. Although not "traditional," this preparation can be more easily standardized and is likely to have longer term stability than an aqueous decoction. Moreover, use of a freeze-dried, encapsulated, standardized preparation will make the clinical and preclinical results more readily comparable with the work of Riba et al. (2001a, 2001b). These investigators have set a standard of sorts since they are the only investigators so far to conduct clinical studies with ayahuasca. This extract can be standardized to contain a quantified amount of 3 of the known active alkaloids, viz., DMT, harmine, and THH. The methods will follow those described by Riba et al. (2001b) in the preparation of a freeze-dried extract for clinical study. These investigators reported that 611 g of a freeze-dried, yellowish powder was obtained from a 9.6-L batch of Brazilian ayahuasca, which was used in that study. This represents a solid matter content of 63.6 g/L of soluble solutes from the decoction process. In the present study, we suggest a slightly different methodology be adopted from that followed by Riba et al., in that freeze-dried decoctions of each plant should be prepared separately and quantitatively analyzed. The separate extracts can then be combined in appropriate proportions based on the analytical data, such that the final extract will contain known concentrations of the key alkaloids, harmine, THH, and DMT. This method should minimize the inherent variability created by generating different batches of ayahuasca and then lyophilizing the whole extract. Final volumes and alkaloid concentrations of the combined extracts can be adjusted, if necessary, using inert excipient materials such as lactose.

Advances in phytopharmaceutical technology over the past decade make the preparation of a standardized version of ayahuasca, in which the levels of active constituents are well-characterized, a relatively straightforward proposition. The challenge is to use methods that are similar to traditional indigenous methods of preparation, but carried out under a consistent and replicable protocol. To develop a replicable extraction and standardization procedure, some range-finding work will be needed using small batches of test material to determine the appropriate extraction parameters prior to scaling up to produce the test batch to be used in the study. Specifically, parameters such as the initial and final volumes of solvent (water) to be used, and the effect of boiling time, on the amount of soluble extractives and the alkaloid levels of the final, freeze-dried extract of each plant will need to be determined. Analytical methods, primarily thin-layer chromatography, and high-performance liquid chromatography can be used for the qualitative and quantitative characterization of each freeze-dried extract (McKenna et al., 1984; Callaway et al., 1996).

13.3. Step III: initial clinical studies—safety assessment

Once IND and IRB approvals have been obtained, the initial clinical study should focus on assessing safety in

normal, healthy volunteers. Specific therapeutic outcomes must be deferred to a subsequent, Phase II study in an appropriate patient population. The therapeutic applications to be addressed will in part be determined by the data generated in the animal models and in the initial clinical study in healthy volunteers. In many respects, the objective of this initial study will be to replicate the results of the study of Riba et al. (2001a, 2001b) to establish the safety and tolerability of the standardized ayahuasca preparation. An open-label (i.e., nonplacebo) dose escalation study design will be employed in a minimum of 12 subjects over a 12-week period, for a total of 6 doses per subject at 2-week intervals. In this respect, the proposed dosing protocol differs from that employed by Riba et al. (2001a, 2001b). In that study, subjects received a total of 4 doses of standardized extract ranging from 0 (placebo) to 1.0 mg/kg DMT/kg. Administration was single-blind, in that the doses were known to the investigators for safety reasons, but subjects were told they would receive 1 of 4 doses at random.

In the IND study, we are proposing to administer a total of 6 doses at 2-week intervals over the 12-week course of the study. As with Riba et al.'s study, randomized low, medium, and high doses will be used in a single-blind design. This time course and dosing regimen will be used because it corresponds more closely to the anticipated therapeutic dosing regimen in a subsequent efficacy trial. Table 2 summarizes the proposed clinical guidelines and parameters to be assessed in this initial Phase I safety study.

Table 2
Proposed clinical guidelines and assessment parameters for a Phase I safety study of ayahuasca

Number of subjects	12–15 evaluable subjects (15–25 subjects initially screened)
Subject characteristics	Normal, healthy volunteers, male and female, ages 20–50
Preparation	Subject education, informed consent
Inclusion criteria	Naïve to effects of ayahuasca but prior experience with hallucinogens. No exposure to hallucinogens within previous 6 months; abstinence from alcohol, cannabis, or other illegal drugs for minimum 2 weeks prior to initiation of the study
Exclusion criteria	Evidence of psychological or neurological abnormalities; evidence of abnormal liver, GI, or renal functions or disease; evidence of hypertension or abnormal CV function; evidence of HIV or other infections; pregnancy; evidence of concomitant use of prescription medications
Compliance monitoring	Periodic random urinalysis applied at intervals throughout the study
Dosing regimen	Six total doses administered at 2-week intervals; randomized low, medium, and high doses will be administered in a single-blind model
Controls	Control values will be baseline values for measured parameters, prior to initiation of the study
Measured parameters	Adverse events; CBC; chemistry panel (SMAC-24); EKG; blood pressure; heart rate; temperature; EEG spectral analysis; SF-36 Medical Outcomes Survey; psychological evaluations (see below); platelet 5-HT transporter radioligand binding profiles; plasma levels of DMT, THH, harmine, and harmaline; T/B/NK cell panel; NK cell activity
Psychological evaluations	Hallucinogen rating scale scored at 8 hr postsession; structured psychiatric evaluations

13.4. Step IV: subsequent clinical studies—efficacy of ayahuasca therapy for alcoholism

If the safety and tolerability of ayahuasca is confirmed in the initial study as we anticipate it will be, then the efficacy trial should focus on a clear therapeutic objective. Based on the data generated by the Hoasca study, the treatment of alcohol dependence would appear to be the most logical target. In the UDV subjects in that study (Grob et al., 1996), cessation of alcohol use was linked to ingestion of hoasca tea in a supportive social context and to long-term elevations in the density of platelet 5-HT transporters. Ideally, a clinical efficacy study would seek to replicate these conditions to the extent possible. Candidates with a history of alcohol dependence should be selected for the study. In addition, neuroimaging technologies could be applied to prescreen the subjects for the existence of central serotonin transporter deficits. Subjects found to display the deficits, along with a behavioral history of alcohol dependence, could then be selected for inclusion in the study. The subject's response over the course of the treatment could be assessed by monitoring several parameters, including (1) rates of relapse with respect to drinking; (2) changes in personality, attitudes, and behavior as assessed in psychiatric interviews and by the application of assessment instruments such as the Tridimensional Personality Questionnaire, and (3) long-term modulation (presumably, elevation) of serotonin transporter densities measured with single photon emission computerized tomography or similar appropriate neuroimaging methodology. If supported by the preclinical and initial clinical data, secondary outcome measures might also include the assessment of immune response.

14. Conclusion

The reputation of ayahuasca as a jungle medicine and shamanic psychedelic plant concoction is nearly legendary, but scientific investigations by a variety of investigators over several decades have succeeded in elucidating much of the botany, chemistry, pharmacology, and ethnography that underlies the legend. Clinical investigations of its possible applications in psychiatric practices or in other healing modalities have been few, and formidable legal and technical challenges must be faced and overcome before further studies of its potential as a medicine can be investigated in appropriate clinical settings. It is to be hoped that the completion of recent, albeit preliminary, clinical studies in Brazil and Spain, and the possible approval of its use in a religious context, will open the door to further rigorous scientific investigations of ayahuasca's potential to heal.

References

Airaksinen, M. M., & Kari, I. (1981). β-Carbolines, psychoactive compounds in the mammalian body. *Med Biol 59*, 21–34.

Allen, J. R. F., & Holmstedt, B. (1980). The simple β-carboline alkaloids. *Phytochemistry 19*, 1573–1582.

Anonymous (1855). Journal of a voyage up the Amazon and Rio Negro by Richard Spruce, San Carlos del Rio Negro, June 27, 1853. *Hooker J Bot Kew Garden Misc 6/7*.

Barker, S. A., Monti, J. A., & Christian, S. T. (1980). Metabolism of the hallucinogen *N*,*N*-dimethyltryptamine in rat brain homogenates. *Biochem Pharmacol 29*, 1049–1057.

Barker, S. A., Monti, J. A., & Christian, S. T. (1981). *N*,*N*-Dimethyltryptamine: an endogenous hallucinogen. *Int Rev Neurobiol 22*, 83.

Blakely, R. D., & Bauman, A. L. (2000). Biogenic amine transporters: regulation in flux. *Curr Opin Neurobiol 10*, 328–336.

Bloom, F., Barchus, J., Sandler, M., & Usdin, E. (Eds.). (1982). *β-Carbolines and Tetrahydroisoquinolines*. New York: Alan R. Liss.

Buckholtz, N. S., & Boggan, W. O. (1976). Effects of tetrahydro-β-carbolines on monoamine oxidase and serotonin uptake in mouse brain. *Biochem Pharmacol 25*, 2319–2321.

Buckholtz, N. S., & Boggan, W. O. (1977). Monoamine oxidase inhibition in brain and liver produced by B-carbolines: structure-activity relationships and substrate specificity. *Biochem Pharmacol 26*, 1991–1996.

Burroughs, W. S., & Ginsberg, A. (1963). *The Yage Letters*. San Francisco, CA: City Lights Books.

Callaway, J. C. (1988). A proposed mechanism for the visions of dream sleep. *Med Hypotheses 26*, 119–124.

Callaway, J. C., Airaksinen, M. M., McKenna, D. J., Brito, G. S., & Grob, C. S. (1994). Platelet serotonin uptake sites increased in drinkers of ayahuasca. *Psychopharmacology 116*, 385–387.

Callaway, J. C., Raymon, L. P., Hearn, W. L., McKenna, D. J., Grob, C. S., Brito, G. S., & Mash, D. C. (1996). Quantitation of *N*,*N*-dimethyltryptamine and harmala alkaloids in human plasma after oral dosing with Ayahuasca. *J Anal Toxicol 20*, 492–497.

Callaway, J. C., McKenna, D. J., Grob, C. S., Brito, G. S., Raymon, L. P., Poland, R. E., Andrade, E. N., Andrade, E. O., & Mash, D. C. (1999). Pharmacokinetics of hoasca alkaloids in healthy humans. *J Ethnopharmacol 65*, 243–256.

Canessa, M., Jaimovich, E., & de la Fuente, M. (1973). Harmaline: a competitive inhibitor of Na^+ ion in the (Na^+/K^+)ATPase system. *J Membr Biol 13*, 263–282.

Center for Cognitive Liberty and Ethics (2002a). *Federal court rules in favor of ayahuasca-using church* [Press release]. Available at: http://www.cognitiveliberty.org/dll/udv_pj_granted.htm.

Center for Cognitive Liberty and Ethics (2002b). *Uniao do Vegetal (UDV) v. United States. Case Chronology as of Dec. 2002.* Available at: http://www.cognitiveliberty.org/dll/udv_index.htm.

Coccaro, E. F., Siever, L. J., Klar, H. M., Maurer, G., Cochrane, K., Cooper, T. B., Mohs, R. C., & Davis, K. L. (1989). Serotonergic studies in patients with affective and personality disorders. Correlates with suicidal and impulsive aggressive behavior. *Arch Gen Psychiatry 46*, 587–599 (Erratum in: Arch Gen Psychiatry).

Cook, E. H. Jr., Courchesne, R., Lord, C., Cox, N. J., Yan, S., Lincoln, A., Haas, R., Courchesne, E., & Leventhal, B. L. (1997). Evidence of linkage between the serotonin transporter and autistic disorder. *Mol Psychiatry 2*, 247–250.

Davis, W. (1996). *One River: Explorations and Discoveries in the Amazon Rainforest*. New York: Simon & Schuster.

de Sousa, R. C., & Grosso, A. (1978). Vasopressin-like effects of a hallucinogenic drug—harmaline—on sodium and water transport. *J Membr Biol 40*, 77–94.

Deuloufeu, V. (1967). Chemical constituents isolated from *Banisteriopsis* and related species. *Psychopharmacol Bull 4*, 17–18.

Dobkin de Rios, M. (1972). Visionary vine: psychedelic healing in the Peruvian Amazon. *Int J Soc Psychiatry 17*, 256–269.

Dobkin de Rios, M. (1973). Curing with *ayahuasca* in an urban slum. In M. Harner (Ed.), *Hallucinogens and Shamanism* (pp. 67–85). London: Oxford Univ. Press.

Dobkin de Rios, M. (1994, January). Drug tourism in the Amazon. *Omni*, 20.

FDA/CDER Center for Drug Evaluation and Research (2002). *Guidance for industry: botanical drug products.* Available at: http://www.fda.gov/cder/guidance/1221dft.htm.

Ferguson, G. G. (1972). Action of alkaloidal extract of *Banisteriopsis quitensis* on rabbit ileum. *J Pharm Sci 61*, 23–234.

Fischman, L. G. (1983). Dreams, hallucinogenic drug states, and schizophrenia: a psychological and biological comparison. *Schizophr Bull 9*, 73–94.

Franco-Molina, M., Gomez-Flores, R., Tamez-Guerra, P., Tamez-Guerra, L., Castillo-Leon, L., & Rodriguez-Padilla, C. (2003). In vitro immunopotentiating properties and tumour cell toxicity induced by *Lophophora williamsii* (Peyote) cactus methanolic extract. *Phytother Res 17*, 1076–1081.

Grob, C. S., McKenna, D. J., Callaway, J. C., Brito, G. S., Neves, E. S., Oberlender, G., Saide, O. L., Labigalini, E., Tacla, C., Miranda, C. T., Strassman, R. J., & Boone, K. B. (1996). Human pharmacology of hoasca, a plant hallucinogen used in ritual context in Brazil. *J Nerv Ment Dis 184*, 86–94.

Hallikainen, T., Saito, T., Lachman, H. M., Volavka, J., Pohjalainen, T., Ryynanen, O. P., Kauhanen, J., Syvalahti, E., Hietala, J., & Tiihonen, J. (1999). Association between low activity serotonin transporter promoter genotype and early onset alcoholism with habitual impulsive violent behavior. *Mol Psychiatry 4*, 385–388.

Halpern, J. H., & Pope, H. G. Jr. (2001). Hallucinogens on the Internet: a vast new source of underground drug information. *Am J Psychiatry 158*, 481–483.

Hashimoto, Y., & Kawanishi, K. (1975). New organic bases from Amazonian *Banisteriopsis caapi*. *Phytochemistry 14*, 1633–1635.

Hashimoto, Y., & Kawanishi, K. (1976). New alkaloids from *Banisteriopsis caapi*. *Phytochemistry 15*, 1559–1560.

Hayashi, K., Nagao, M., & Sugimura, T. (1977). Interactions of harman and norharman with DNA. *Nucleic Acids Res 4*, 3679–3685.

Heckenberger, M. J., Kuikuro, A., Kuikuro, U. T., Russell, J. C., Schmidt, M., Fausto, C., & Franchetto, B. (2003). Amazonia 1492: pristine forest or cultural parkland?. *Science 301*, 1710–1714.

Ho, B. T. (1979). Pharmacological and biochemical studies with β-carboline analogs. In W. B. Essman, & L. Vazelli (Eds.), *Current Developments in Psychopharmacology*, *vol. 4* (pp. 215–247). New York: Spectrum Press.

Hopp, K. H., Cunningham, L. V., Bromel, M. C., Schermeister, L. J., & Kahlil, S. K. W. (1976). In vitro antitrypanosomal activity of certain alkaloids against *Trypanosoma lewisi*. *Lloydia 39*, 375–377.

Hrinda, P. D. (1987). Regulation of high- and low-[^{3}H]-imipramine sites in rat brain by chronic treatment with antidepressants. *Eur J Pharmacol 138*, 159–168.

Johnson, D. D., Fisher, T. E., Tuchek, J. M., & Crawford, R. D. (1984). Pharmacology of methyl- and propyl-B-carbolines in a hereditary model of epilepsy. *Neuropharmacology 23*, 1015–1017.

Kawanishi, K., Uhara, Y., & Hasimoto, Y. (1982). Shinunine and dihydroshihunine from *Banisteriopsis caapi*. *J Nat Products 45*, 637–638.

Klein, C., Sato, T., Meguid, M. M., & Miyata, G. (2000). From food to nutritional support to specific nutraceuticals: a journey across time in the treatment of disease. *J Gastroenterol Suppl 12*, 1–6.

Krajick, K. (1992, June 15). Vision quest. *Newsweek*, 44–45.

Lamb, F. B. (1971). *Wizard of the Upper Amazon: The Story of Manuel Cordova-Rios*. Boston: Houghton-Miflin.

Langer, S. Z., Lee, C. R., Segnozac, A., Tateishi, T., Esnaud, H., Schoemaker, H., & Winblad, B. (1984). Possible endocrine role of the pineal gland for 6-methoxytetrahydro-*β*-carboline, a putative endogenous neuromodulator of the [^{3}H]imipramine recognition site. *Eur J Pharmacol 102*, 379–380.

Lesch, K. P., Aulakh, C. S., Wolozin, B. L., Tollliver, T. J., Hill, J. L., &

Murphy, D. L. (1993). Regional brain expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. *Mol Brain Res 17*, 31–35.

Lesch, K. P., Bengel, D., Heils, A., Sabol, S. Z., Greenberg, B. D., Petri, S., Benjamin, J., Muller, C. R., Hamer, D. H., & Murphy, D. L. (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science 274*, 1527–1531.

Lippke, K. P., Schunack, W. G., Wenning, W., & Muller, W. E. (1983). *β*-Carbolines as benzodiazepine receptor ligands: 1. Synthesis and benzodiazepine receptor interaction of esters of *β*-carboline-3-carboxylic acid. *J Med Chem 26*, 499–503.

Little, K. Y., McLaughlin, D. P., Zhang, L., Livermore, C. S., Dalack, G. W., McFinton, P. R., DelProposto, Z. S., Hill, E., Cassin, B. J., Watson, S. J., & Cook, E. H. (1998). Cocaine, ethanol, and genotype effects on human midbrain serotonin transporter binding sites and mRNA levels. *Am J Psychiatry 155*, 207–213.

Luna, L. E. (1984). The concept of plants as teachers among four mestizo shamans of Iquitos, northeast Peru. *J Ethnopharmacol 11*, 135–156.

Luna, L. E. (1984). The healing practices of a Peruvian shaman. *J Ethnopharmacol 11*, 123–133.

Luna, L. E. (1986). *Vegitalismo: Shamanism Among the Mestizo Population of the Peruvian Amazon*. Stockholm: Almqvist & Wiksell.

Luna, L. E., & Amaringo, P. (1991). *Ayahuasca Visions: The Religious Iconography of a Peruvian Shaman*. Berkeley, CA: North Atlantic Books.

Marazziti, D., Falcone, M., Rotondo, A., & Castrogiovanni, P. (1989). Age related differences in human platelet 5-HT uptake. *Naunyn-Schmiedeberg's Arch Pharmacol 340*, 593–594.

McKenna, D. J. (1999). Ayahuasca: An ethnopharmacologic history. In R. Metzner (Ed.), *Ayahuasca: Hallucinogens, Consciousness, and the Spirit of Nature* (pp. 187–213). New York: Thunder's Mouth Press.

McKenna, D. J., & Towers, G. H. N. (1981). Ultra-violet mediated cytotoxic activity of β-carboline alkaloids. *Phytochemistry 20*(5), 1001–1004.

McKenna, D. J., & Towers, G. H. N. (1984). Biochemistry and pharmacology of tryptamine and β-carboline derivatives: a minireview. *J Psychoact Drugs 16*, 347–358.

McKenna, D. J., & Towers, G. H. N. (1985). On the comparative ethnopharmacology of the malpighiaceous and myristicaceous hallucinogens. *J Psychoact Drugs 17*, 35–39.

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

McKenna, D. J., Luna, L. E., & Towers, G. H. N. (1995). Biodynamic constituents in Ayahuasca admixture plants: an uninvestigated folk pharmacopoeia. In S. von Reis, & R. E. Schultes (Eds.), *Ethnobotany: Evolution of a Discipline* (pp. 349–361). Portland: Dioscorides Press.

Morin, A. M. (1984). β-Carboline kindling of the benzodiazepine receptor. *Brain Res 321*, 151–154.

Naranjo, P. (1979). Hallucinogenic plant use and related indigenous belief systems in the Ecuadorian Amazon. *J Ethnopharmacol 1*, 121–145.

Naranjo, P. (1986). El *ayahuasca* in la arqueología ecuatoriana. *Am Indig 46*, 117–128.

Nichols, D. E., Oberlender, R., & McKenna, D. J. (1991). Stereochemical aspects of hallucinogenesis. In R. R. Watson (Ed.), *Biochemistry and Physiology of Substance Abuse*, *vol. III* (pp. 1–39). Boca Raton, FL: CRC Press, chap. 1.

O Centro Espirita Beneficiente Uñiao do Vegetal, et al., v. Janet Reno, et al. (2000). Case No. CV 00-1647 in the US District Court for the District of New Mexico Plaintiffs' Original Complaint for Declaratory and Injunctive Relief.

O'Connell, F. D. (1969). Isolation of caffeine from *Banisteriopsis inebrians* (Malpighiaceae). *Naturwissenschaften 56*, 139.

Ott, J. (1993). *Pharmacotheon: Entheogenic Drugs, Their Plant Sources and History*. Kennewick, WA: Natural Products.

Pahkla, R., Rago, L., Callaway, J. C., & Airaksinen, M. M. (1997). Binding of pinoline on the 5-hydroxytryptamine transporter: competitive interaction with [^{3}H] citalopram. *Pharmacol Toxicol 80*, 122–126.

Pletscher, A., & Laubscher, A. (1980). Use and limitations of platelets as models for neurons: amine release and shape change reaction. In A. Rotman (Ed.), *Embo Workshop on Platelets: Cellular Response Mechanisms and Their Biological Significance* (pp. 267–276). Wiezmann Institute of Science.

Rahwan, R. G. (1975). Toxic effects of ethanol—possible role of acetaldehyde, tetrahydroisoquinolines, and tetrahydro-*β*-carbolines. *Toxicol Appl Pharmacol 24*, 3–27.

Riba, J., Rodriguez-Fornells, A., Strassman, R. J., & Barbanoj, M. J. (2001a). Psychometric assessment of the Hallucinogen Rating Scale. *Drug Alcohol Depend 62*, 215–223.

Riba, J., Rodriguez-Fornells, A., Urbano, G., Morte, A., Antonijoan, R., Montero, M., Callaway, J. C., & Barbanoj, M. J. (2001b). Subjective effects and tolerability of the South American psychoactive beverage Ayahuasca in healthy volunteers. *Psychopharmacol (Berl) 54*, 85–95.

Riba, J., Anderer, P., Morte, A., Urbano, G., Jane, F., Saletu, B., & Barbanoj, M. J. (2002a). Topographic pharmaco-EEG mapping of the effects of the South American beverage *ayahuascain* healthy volunteers. *Br J Clin Pharmacol 53*, 613–628.

Riba, J., Rodriguez-Fornells, A., & Barbanoj, M. J. (2002b). Effects of *ayahuascaon* sensory and sensorimotor gating in humans as measured by P50 suppression and prepulse inhibition of the startle reflex, respectively. *Psychopharmacol (Berl) 165*, 18–28.

Riba, J., Valle, M., Urbano, G., Yritia, M., Morte, A., & Barbanoj, M. J. (2003). Human pharmacology of ayahuasca: subjective and cardiovascular effects, monoamine metabolite excretion, and pharmacokinetics. *J Pharmacol Exp Ther 306*, 73–83.

Rivier, L., & Lindgren, J. (1972). *Ayahausca*, the South American hallucinogenic drink: ethnobotanical and chemical investigations. *Econ Bot 29*, 101–129.

Rocca, P., Galzin, A. -M., & Langer, S. A. (1989). Light-dark differences in [^{3}H]-paroxetine binding in rabbit platelet membranes. *Naunyn-Schmiedeberg's Arch Pharmacol 340*, 41–44.

Rodriguez, E., Cavin, J. C., & West, J. E. (1982). The possible role of Amazonian psychoactive plants in the chemotherapy of parasitic worms: a hypothesis. *J Ethnopharmacol 6*, 303–309.

Rommelspacher, H., Strauss, S., & Lindemann, J. (1980). Excretion of tetrahydroharmane and harmane into the urine of man after a load with ethanol. *FEBS Lett 109*, 209–212.

Rotman, A. (1983). Blood platelets in psychopharmacological research. *Prog Neuro-psychopharmacol Biol Psychiatry 7*, 135–151.

Roy, A., Adinoff, B., DeJong, J., & Linnoila, M. (1991). Cerebrospinal fluid variables among alcoholics lack seasonal variation. *Acta Psychiatr Scand 84*, 579–582.

Schaepe, L. (2001). *International control of the preparation "ayahuasca."* United Nations International Control Board. Opinion letter submitted to Mr. Lousberg, Chief, Inspectorate for Health Care, Ministry of Public Health, Den Haag, the Netherlands. Introduced in evidence in O Centro Espirita Beneficiente Uñiao do Vegetal et al. v. Janet Reno et al., Case No. CV 00-1647 JP/RLP (application for preliminary injunction) in the US District Court for the District of New Mexico to the Hon. James A. Parker Chief Judge.

Schultes, R. E. (1957). The identity of the malpighiaceous narcotics of South America. *Bot Mus Leaf Harv Univ 18*, 1–56.

Schultes, R. E. (1972). Ethnotoxocological significance of additives to New World hallucinogens. *Plant Sci Bull 18*, 34–41.

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

Schwarz, M. J., Houghton, P. J., Rose, S., Jenner, P., & Lees, A. D. (2003). Activities of extract and constituents of *Banisteriopsis caapi* relevant to parkinsonism. *Pharmacol Biochem Behav 75*, 627–633.

Sher, L., Greenberg, B. D., Murphy, D. L., Rosenthal, N. E., Sirota, L. A., & Hamer, D. H. (2000). Pleiotropy of the serotonin transporter gene for seasonality and neuroticism. *Psychiatr Genet 10*, 125–130.

Shulgin, A. T. (1982). Psychotomimetic drugs: structure-activity relationships. In L. L. Iversen, S. D. Iversen, & S. H. Snyder (Eds.), *Handbook of Psychopharmacology*, *vol. 11* (pp. 243–333). New York: Plenum.

Shulgin, A., Perrine, D. M., & Shulgin, A. (1997). *TIHKAL: The Continuation*. Berkeley, CA: Transform Press.

Smith, T. A. (1977). Tryptamine and related compounds in plants. *Phytochemistry 16*, 17–175.

Stafford, P. (1977). *Psychedelics Encyclopedia*. Berkeley, CA: And/Or Press.

Stahl, S. M. (1977). The human platelet: a diagnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. *Arch Gen Psychiatry 34*, 509–516.

Strassman, R. J., & Qualls, C. R. (1994). Dose-response study of *N*,*N*-dimethyltryptamine in humans: I. neuroendocrine, autonomic, and cardiovascular effects. *Arch Gen Psychiatry 51*, 85–97.

Strassman, R. J., Qualls, C. R., Uhlenhuth, E. H., & Kellner, R. (1994). Dose-response study of N,N-dimethyltryptamine in humans. II. Subjective effects and preliminary results of a new rating scale. *Arch Gen Psychiatry 51*, 98–108.

Stull, R. E., Ferguson, N. M., & Ferguson, G. G. (1971). Selected pharmacological studies of an alkaloidal extract of *Banisteriopsis quitensis*. *J Pharm Sci 60*, 1121–1123.

Suzuki, O., Katsumata, Y., & Oya, M. (1981). Characterization of eight biogenic indoleamines as substrates for type A and type B monoamine oxidase. *Biochem Pharmacol 30*, 1353–1358.

Szara, S. (1956). Dimethyltryptamine: its metabolism in man; the relation of its psychotic effect to the serotonin metabolism. *Experientia 12*, 411–441.

Taylor, D. L., Silverman, P. B., & Ho, B. T. (1984). Effects of 6-methoxytetrahydro-β-carboline on 5-hydroxytryptamine binding in rat brain. *J Pharm Pharmacol 36*, 125–127.

Tiihonen, J., Kiukka, J. T., Bergstrõm, K. A., Karhu, J., Viinamaki, H., Lehtonen, J., Hallikainen, T., Yang, J., & Hakola, P. (1997). Single-photon emission tomography imaging of monoamine transporters in impulsive violent behavior. *Eur J Nucl Med 24*, 1253–1260.

Topping, D. M. (1998). Ayahuasca and cancer: one man's experience. *Bull Multidiscip Assoc Psychedelic Stud 8*, 22–26 (Available at: http://www.maps.org/news-letters/v08n3/08322top.html).

Towers, G. H. N., & Abramosky, Z. (1983). UV-mediated genotoxicity of furanoquinoline and of certain tryptophan-derived alkaloids. *J Nat Products 46*, 576–581.

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

Uebelhack, R., Franke, L., & Seidel, K. (1983). Methylated and unmethylated indolarmine in the cisternal fluid in acute endogenous psychosis. *Biomed Biochem Acta 42*, 1343–1346.

Umezawa, K., Shirai, A., Matsushima, T., & Sugimura, T. (1978). Comutagenic effect of harman and norharman with 2-acetyl-aminoflourine derivatives. *Proc Natl Acad Sci USA 75*, 928–930.

Weil, A. T. (1980). In the land of yage. *The Marriage of the Sun and Moon: A Quest for Unity in Consciousness* (pp. 99–131). Boston: Houghton-Mifflin.

Westlaw Citation # 31862699 (2002). Available at: http://nvo.com/cd/nss-folder/pubfiles/emerstay.htm.

Wilasrusmee, C., Kittur, S., Siddiqui, J., Bruch, D., Wilasrusmee, S., & Kittur, D. S. (2002a). In vitro immunomodulatory effects of ten commonly used herbs on murine lymphocytes. *J Altern Complement Med 8*, 467–475.

Wilasrusmee, C., Siddiqui, J., Bruch, D., Wilasrusmee, S., Kittur, S., & Kittur, D. S. (2002b). In vitro immunomodulatory effects of herbal products. *Am Surg 68*, 860–864.

Winkelman, M. (1995). Psychointegrator plants: their roles in human consciousness, cultures, and health. In M. Winkelman, & W. Andritsky (Eds.), Sacred plants, consciousness, and healing. Cross-cultural and interdisciplinary perspectives. *Yearb Cross-Cult Med Psychother*, 9–53.

Yasuhara, H. (1974). Studies on monoamine oxidase (report XXIV). Effect of harmine on monoamine oxidase. *Jpn J Pharmacol 24*, 523–533.

Yasuhara, H., Sho, S., & Kamijo, K. (1972). Differences in the actions of harmine on the oxidations of serotonin and tyramine by beef brain mitochondrial MAO. *Jpn J Pharmacol 22*, 439–441.

Yritia, M., Riba, J., Ortuno, J., Ramirez, A., Castillo, A., Alfaro, Y., de la Torre, R., & Barbanoj, M. J. (2002). Determination of *N*,*N*-dimethyltryptamine and beta-carboline alkaloids in human plasma following oral administration of ayahuasca. *J Chromatogr B Anal Technol Biomed Life Sci 779*, 271–281.