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# INDOLEALKYLAMINES IN *MUCUNA* SPECIES

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## SUMMARY

Before *Mucuna* can be widely promoted as a food and feed crop, the presence of antinutritional components and their potential long-term effects must be appraised. In addition to L-dopa, a number of indolic alkaloids structurally related to serotonin have been reported in various parts of the *Mucuna* plant. Several of these naturally occurring compounds, all of which share tryptamine as a base structure, are known to have hallucinogenic properties of considerable strength. In this study, five related alkaloids (tryptamine, serotonin, *N,N*-dimethyltryptamine, 5-methoxy-dimethyltryptamine, and bufotenine) were assayed in the tissue and/or seed samples of twenty *Mucuna* accessions by liquid chromatography with mass spectral detection. Samples included the roots, stems, leaves, and pods of dried plants, the stems and leaves of fresh-frozen plant material, and raw seeds. In a previous screening study, 5-methoxy-dimethyltryptamine had been confirmed in each of several samples examined, with serotonin and tryptamine found in only a few select samples. The concentrations of these components were estimated to be ~0.001%, low compared to that of L-dopa (4-7% by weight in raw seed), but of concern nonetheless. In the current quantitative study, neither tryptamine nor *N,N*-dimethyltryptamine were detected in any sample (<0.50 µg $g^{-1}$), although ions corresponding to tryptamine were noted periodically as fragmentation products for several of the other compounds, especially serotonin and bufotenine. An unknown compound close to the weight of serotonin, possibly *N*-methyltryptamine, was determined in all plant tissue samples and one seed sample. Although serotonin was not present in any sample tested (<0.50 µg $g^{-1}$), the levels of the unknown indole were estimated against the serotonin standards. In root and pod, 5.96 and 4.03 µg $g^{-1}$ were calculated, respectively, with an average of 8.49 and 3.94 µg $g^{-1}$, respectively, in leaf (n=5) and stem (n=5). Only one seed sample (1.70 µg $g^{-1}$) contained the unknown compound in detectable amounts. Bufotenine was also identified in most samples; in root at a level of 4.14 µg $g^{-1}$, but not in the pod sample. In leaf (n=5), stem (n=4) and raw seed (n=15), concentrations averaged 3.46, 3.09, and 1.48 µg $g^{-1}$, respectively. As expected, 5-methoxy-dimethyltryptamine was quantified in all samples. In root and pod, concentrations of 1.76 and 1.29 µg $g^{-1}$, respectively, were found, with average levels of 1.44, 1.48, and 0.64 µg $g^{-1}$ present in leaf (n=5), stem (n=5), and seed (n=15), respectively. Compared to L-dopa, the detected indoles were present at roughly 0.0001% by weight, lower than had been previously estimated. While ordinary cooking and storage conditions would not be expected to affect the stability of these components, preparation approaches that effectively decrease L-dopa (like boiling) should decrease even further the concentrations of these antinutritional components. It would be unlikely for these low-level alkaloids to have any effect on human and animal consumers, especially as absorption across the gastrointestinal tract is negligible. Serotonin, for instance, is known to be present in common fruits (bananas and pineapples) at levels ten to twenty times the alkaloid concentrations found in *Mucuna* tissues, yet these are considered safe for human and animal consumption. In fact, most tryptamine derivatives are characterized by poor absorption, rapid peripheral metabolism, and for these reasons have little or no recognized oral activity unless ingested in the presence of an oxidase inhibitor. Thus, the presence of low level indolealkylamines is unlikely to affect the potential use of *Mucuna* as a staple crop, providing valuable protein sources for food and feed.

**Key words:** Bufotenine, indolealkylamine, *Mucuna*, serotonin, tryptamine.

## INTRODUCTION

The major drawback of *Mucuna* spp. which has compromised its usefulness as a food source for either humans or livestock, is associated with its chemical content. A review of the available, but limited, literature often shows confusing and conflicting data concerning the chemistry of *Mucuna*. There is some difference of opinion among researchers as to the toxicity of this genus, and subsequently, disagreement

as to the best approach to develop *Mucuna* into a food and feed crop.

In *Mucuna* seeds, as in the beans of many food grain legumes (e.g., common bean and soybean), a large number of anti-nutritional compounds have been identified, including tannins, lectins, phytic acid, cyanogenic glycosides (Siddhuraju *et al.*, 1996; Laurena *et al.*, 1994; Ravindran and Ravindran, 1988), and trypsin and amylase inhibitors (Siddhuraju *et al.*, 1996). Because these factors are initially low in concentration and are inactivated or further reduced in concentration during cooking (Bressani, 1993), they will not be considered in this report. Of much greater concern has been the presence of L-dopa (3,4-dihydroxy-L-phenylalanine or 3-hydroxy-L-tyrosine; Daxenbichler *et al.*, 1971, 1972; Lorenzetti *et al.*, 1998), a compound used in the treatment of Parkinson's disease, and the presence of hallucinogenic indoles such as *N,N*-dimethyl-tryptamine, bufotenine and other tryptamines, including serotonin, in various parts of the *Mucuna* plant (Ghosal *et al*., 1971). While recent research has greatly advanced the current state of knowledge on issues related to L-dopa (Flores *et al*., 2002; Myhrman *et al*., 2002; Szabo and Tebbett, 2002; Eilittä *et al*., 2002), the indolic alkaloids have been largely ignored.

Of the more than 700,000 plant species identified, fewer than 100 were recognized to contain compounds of psychoactive or hallucinogenic potential in the 1980's (Siegel, 1984). Whether or not *Mucuna* should be included in this group has been uncertain; reports having been contradictory. Serotonin, bufotenine, *N,N*-dimethyltryptamine and other unidentified indoles were first described by Ghosal *et al*. (1971) as present in the pods, seeds, leaves and roots of *M. pruriens*. Compound identification in this study relied primarily on the similarity of melting points, with the melting point of the unknown compound compared to melting points of known compounds; results were supported with UV absorption data when possible. Serotonin was again identified as a *Mucuna* constituent by Duke (1981). Tryptamines were not found, however, in a later study in which Lorenzetti *et al.* (1998) used a sensitive liquid chromatographic analysis procedure, a method considered more reliable than melting point comparison. Finally, Szabo and Tebbett (2002) using liquid chromatography with a mass spectral detector determined that tryptamine derivatives were indeed present in *Mucuna* tissues.

The tryptamines, bufotenine and *N,N*-dimethyl-tryptamine, are naturally occurring compounds and are structurally related to serotonin (5-hydroxytryptamine). Serotonin, a mammalian neuro-transmitter, has a variety of effects on nerves and smooth muscle, respiration, the heart and cardiovascular system, and the gastrointestinal tract. Many plants and insects also produce serotonin; for example, it is present in pineapples (17 μg $g^{-1}$) and bananas (15 μg $g^{-1}$; Feldman and Lee, 1985), in stinging nettles (*Urtica* sp.) and in wasp and scorpion stings. In contact with skin, it causes an intense burning sensation, inflammation, and itching. Although the pods of certain *Mucuna* cultivars are known to cause severe stinging, it is not certain whether this is due to serotonin, to a protein called mucunain (Fairbrothers *et al.*, 1985), or to some other substance.

The purpose of this report is to evaluate the available information concerning the chemistry and toxicology of indolealkylamines present in *Mucuna* spp., to present our own data following chemical analysis of the roots, stems, leaves, and pods of dried plants, the stems and leaves of fresh plant material, and raw seed samples, and to evaluate the implications of the new data in the framework of human health effects.

**Indolealkylamines**

Classical hallucinogens are broadly divided between indolealkylamines and phenylalkylamines. The indolealkylamine category consists of tryptamine derivatives (such as DMT, 5-MeODMT, bufotenine, psilocybin), the ergolines or lysergic acid derivatives (lysergide or LSD), and the β–carbolines (such as ibogaine and the harmala alkaloids). The phenyl-alkylamine category includes phenylethylamines, such as mescaline (e.g., peyote), and phenyl-isopropylamines, such as the amphetamine homologues. Classic hallucinogens are typically similar in structure to one of several neurotransmitter substances, serotonin (5-hydroxytryptamine; 5-HT; Figure 1) being the most common. Of the 70 or so tryptamine analogues, more than 50 have been evaluated for hallucinogenic properties, including serotonin, the mushroom-derived agents psilocybin and psilocin (4-hydroxy-*N,N*-dimethyltryptamine), *N,N*-dimethyltryptamine (DMT), *N*-monomethyl-tryptamine, 5-methoxy-*N,N*-dimethyltryptamine (5-MeODMT), 5-methoxy-*N*-monomethyltryptamine, 5-hydroxy-*N,N*-dimethyltryptamine (bufotenine), and *N,N*-dimethyltryptamine-*N*-oxide (Farnsworth, 1968), among others. While some members of each subgroup are synthetically produced, most were and are available in plant or fungus species.

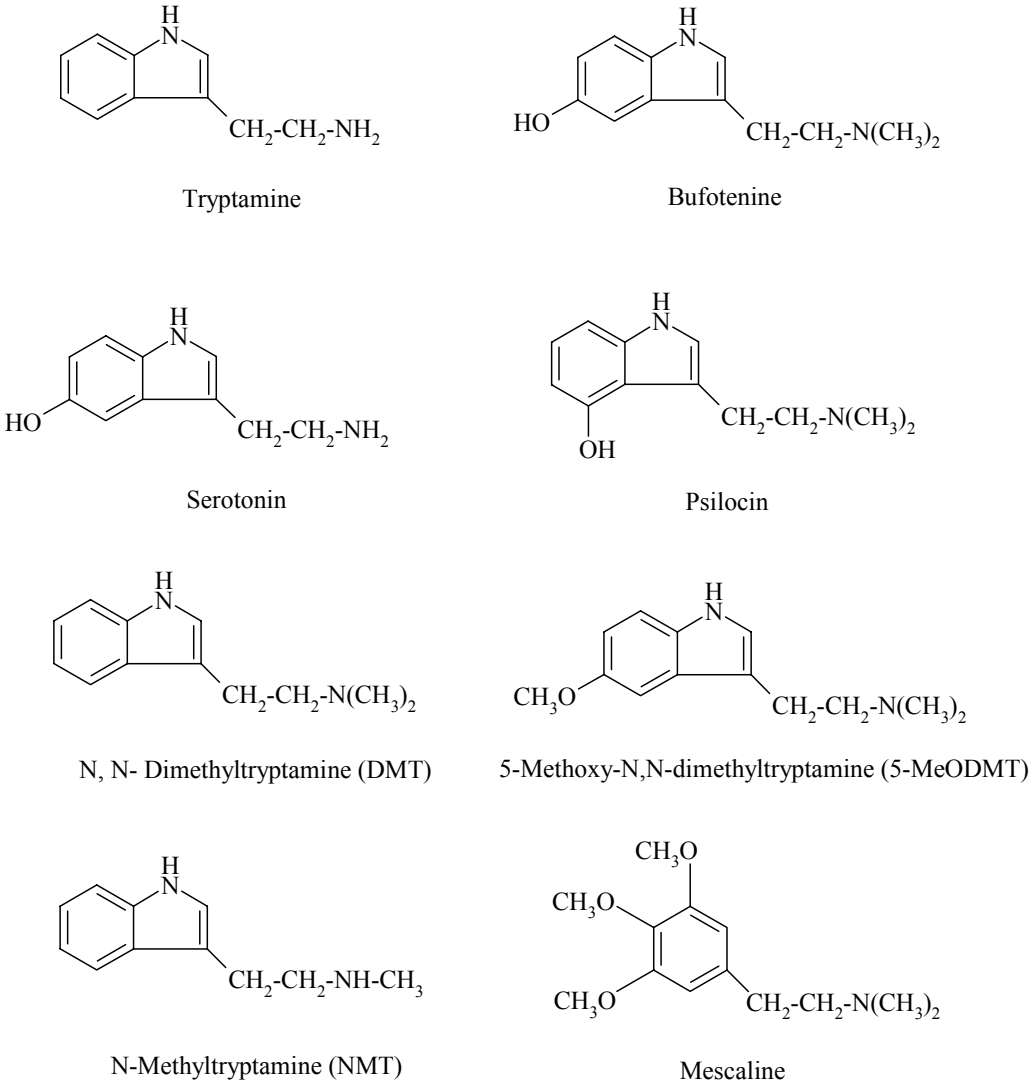


Figure 1. Chemical structures of selected indolealkylamines and mescaline.

The term hallucination finds its origins in the Latin *alucinatio* which means "a wandering in the mind." Hallucinogenic compounds are associated with a change in the content of consciousness, which includes altered perceptions and moods, time distortions, visual hallucinations, thought disorders and frequently euphoria. Although other drugs can also elicit these responses, only hallucinogens can produce these symptoms without diminishing awareness or incurring delirium (Bridger *et al.*, 1978; Hollister, 1968 and 1984). The mechanism by which these changes in perception occur is not well understood, partly because reliable human studies have been extremely limited, partly because animal models do not necessarily correlate to human experience/physiology. Compared to mode of action studies, published material on pharmacodynamics and pharmacokinetics consists of a mixture of science and anecdote, with the science often conducted on animals and the anecodotes often related to human experience.[1] Throughout this paper, all presented information originates from formal studies -- human, unless otherwise indicated. Although most formal studies involving indolealkylamines have focused

[1] For a number of years, the following effectively summarized the pharmacokinetic information available for LSD in human subjects: LSD administered intravenously at a level of 2 µg $kg^{-1}$ indicated a half-life of 103 min in the plasma with a concentration of 6-7 ng $mL^{-1}$ at equilibrium (~30 min after administration; Aghajanian and Bing, 1975; Wagner *et al.*, 1978).

primarily on LSD, DMT and 5-MeODMT have frequently represented the tryptamine subgroup.[2] When no data regarding the indolealkylamines of interest are available, data from studies conducted with the structurally related LSD or mescaline may be inserted as a point of reference.

**Tryptamine and serotonin**

The potency and activity of intoxicating agents are commonly compared to LSD, one of the most potent psychoactive compounds known. Taken orally, 10 µg is reported to induce a mild euphoria in male subjects with true hallucinogenic doses ranging from 50 to 500 µg (Grinspoon and Bakalar, 1979). In comparison, the simplest compound under consideration in this study, tryptamine, has not been reported to possess psychoactive properties, although it can increase blood pressure and cause mild perceptual distortions when peripherally administered (Martin and Sloan, 1970). Although few studies have been conducted on tryptamine, it has been demonstrated in animals that certain physiological effects common to LSD may be produced, but that behavioral effects, used as an indication of human hallucinogenic activity, are not (Martin *et al.*, 1978).

The most studied and best understood indole is the neurotransmitter serotonin (5-hydroxytryptamine, 5-HT). While 5-HT does not itself induce hallucinogenic responses in humans or behavioral responses in animals, it is closely implicated in the hallucinogenic process, as centrally located serotonin receptors in the brain and spinal cord have been repeatedly demonstrated to provide the main pathways of action for psychoactive components. Briefly, excitatory 5-HT receptors have been shown to induce the intoxicating response with inhibitory 5-HT receptors often mediating the effects, the hallucinogens themselves acting as agonists or partial agonists (Glennon, 1990; Burris and Sanders-Bush, 1988). In comparison, biogenic amines such as 5-HT, norepinephrine, and dopamine are considered to commonly act as neuromodulators rather than as excitatory or inhibitory components.

Most intoxicating indolealkylamines exert a stronger effect on the central serotonin system than on the central catecholamine system (Bridger *et al.*, 1978). Like LSD, DMT decreases the breakdown and transport removal of 5-HT (measured as an increase in brain 5-HT and a decrease in brain 5-HIAA, 5-hydroxyindoleacetic acid, the major metabolite of 5-HT) even when synthesis of 5-HT has been inhibited, but exhibits no significant effect on brain dopamine levels or on dopamine turnover after synthesis inhibition. Unlike LSD, DMT also exhibits no effect on brain norepinephrine levels. Although tryptamine is itself a neurotransmitter active in the central nervous system (CNS), hallucinogenic tryptamine derivatives are believed to adhere more to a serotonergic mechanism than to a mode of action utilizing tryptamine receptors. Due to the complexity of the serotonergic system, precise modes of action are not well understood. While phenylalkylamines have been shown to selectively prefer only certain classes of 5-HT receptor sites, indolealkylamines have been shown to bind nonselectively and with high affinity to various populations of 5-HT receptor sites. A significant correlation ($r > 0.9$) between receptor affinities and both discrimination-derived $ED_{50}$ values and human hallucinogenic potency has been determined (Glennon, 1990; Glennon *et al.,* 1992; Titeler *et al.*, 1988). For detailed descriptions regarding neuronal and behavioral studies and their resulting impacts on biological models of activity, please consider Davis *et al.*, 1984; Appel and Rosencrans, 1984; Aghajanian, 1984; Jacobs, 1984; Geyer and Krebs, 1994; and Winter, 1994.

**DMT and 5-MeODMT**

The most well-known of the hallucinogenic tryptamines, DMT, is endogenous to the seeds of *Anadenanthera peregrina*, *Anadenanthera colubrina*, and *Mimosa hostilis*, the bark of *Virola calophylla*, and the leaves of *Banisteriopsis rusbyana* and *Psychotria viridis*, all species native to the Orinoco basin, Amazonian regions, and Mexico. Intoxicating snuffs, containing DMT and frequently 5-MeODMT (by up to 11%; Siegel, 1984) as the primary active ingredients, have been made from these materials since at least 1500 B.C. Intranasal inhalation is the typical means of administration, as these compounds are not orally active on their own. For DMT or 5-MeODMT to be active after ingestion, an amine oxidase inhibitor must be mixed into the preparation. Naturally occurring β-carbolines are sometimes used for this purpose. Up to 350 mg of DMT has been taken orally with no resulting hallucinogenic effect (Turner and Merlis, 1959).

When DMT was evaluated for dose-response in human trials (Strassman and Qualls, 1994; Strassman *et al.*, 1994), intravenously administered levels of 0.05 mg $kg^{-1}$ or less were frequently mistaken for placebo by the male subjects. Although 0.1 mg $kg^{-1}$ could be reliably distinguished from placebo, feelings of tenseness did not progress to a psychoactive state.

[2] It is important to note that not all members of these structurally related groups are necessarily psychoactive; LSD has the non-hallucenogenic analogue 2-bromo-LSD, as DMT has 7-hydroxy-DMT.

The hallucinogenic threshold requires that 15-35 mg of DMT be inhaled or injected (~0.2 - 0.5 mg $kg^{-1}$); for intentional intoxication, 50-100 mg is usually taken (Gillin *et al.*, 1976; Grinspoon and Bakalar, 1979).

When compared with LSD (often taken orally), the onset of effect is rapid, as inhaled/injected DMT takes a more direct route into the bloodstream and by-passes the gastrointestinal system. First effects begin in a matter of seconds after exposure, peak between 5 and 20 min (the acute phase), then diminish to a normal level over an additional 30 or so minutes (the secondary phase; Kaplan *et al.*, 1974). In comparison, the duration of symptoms for LSD (having an oral/intravenous threshold of 30-50 μg) is ~4 hr, acute, and ~6 hr, secondary. Oxidative deamination of DMT to the inactive indole acid occurs quickly; within 70 min tissue (brain, liver, kidney, and blood) concentrations were not measurable in rats after a 10 mg $kg^{-1}$ i.p. dose (Sitaram and McLeod, 1990). Clearance of parent (<2%) and metabolites is primarily through the urine. Due to a lack of detailed studies in humans, it is not known how well this elimination pathway correlates between species, although it is in agreement with observations made thus far (Kaplan *et al.*, 1974).

Physiologically, the symptoms induced by DMT are similar to those of LSD, but more intense – pupils dilate, blood pressure elevates, pulse rate increases. Psychologically, the rate at which thoughts take place is perceived to increase, a sense of timelessness often occurs, along with the perceived feeling that solid objects may melt into vibratory patterns (a mixing of the senses). Intensely colored geometric patterns/ objects may form, undulate, and reform. Visions or hallucinations are often quickly paced, one upon another. A sense of heightened awareness or enlightenment may occur. The experience may be either pleasant or unpleasant, depending in part on the setting and mental perspective of the subject prior to taking the compound. Even when ranked unpleasant, DMT is not known for inducing psychotic reactions, as are LSD and mescaline (Bowers, 1972). With frequent use, tolerance (a decrease in or elimination of effect) can occur in a matter of days. Cross-tolerance with LSD (both physiological and psychological) has also been documented and indicates a common mechanism of action (Koravic and Domino, 1976). To date, there have been no reports of lethal overdose for the tryptamine derivatives.

Having about the same potency as psilocybin, 5-MeODMT is a little less than 1% as potent as LSD, but considerably more potent than DMT. When administered intranasally, the threshold dose is 4-6 mg (Grinspoon and Bakalar, 1979). Physiological and psychological effects are similar to those experienced with DMT. Like DMT, 5-MeODMT is inactive orally unless a MAOI is present. Metabolism of 5-MeODMT dosed to rats is also rapid and clearance of parent (<0.5%) and metabolites (indole acids) is through the urine (Sitaram and McLeod, 1990).

**Bufotenine**

Although bufotenine is found in many of the same plants as the previously mentioned tryptamines and is known to be produced in the parotid glands of toads (*Bufo* spp), it is not clear whether or not bufotenine possesses any psychoactive properties (reviewed by Lyttle *et al*., 1996). Structurally, bufotenine is incapable of passing the blood-brain barrier, but can elicit behavioral effects in animals when the dose is administered directly into the CNS (Glennon *et al.*, 1980) or when the hydroxyl group is modified by acylation for easier passage into the CNS (Gessner and Dankova, 1975). Reported levels of effect in humans, as well as the nature of such effects, are strongly anecdotal in nature and will not be repeated here.

Despite the fact that bufotenine (5-hydroxy-*N,N*-dimethyltryptamine), an isomer of psilocin (4-hydroxy-*N,N*-dimethyltryptamine), is classified as a Schedule I controlled substance in the United States (DEA registry # 74333), a review of the scientific literature indicates that bufotenine is a pressor agent (affecting cardiovascular function, especially heart rate and blood pressure) at high doses administered intravenously or intramuscularly, but not an hallucinogenic (Chern *et al.*, 1991; McLeod and Sitaram, 1985; Shulgin, 1981; Fozard and Ali, 1978). That alteration of cardiac function can in turn affect the transport of oxygen to the brain may be the source of belief in bufotenine's psychedelic aspects. Visual distortions were reported, along with significant changes to the sinus rhythm when 10 mg of bufotenine were administered intramuscularly (Turner and Merlis, 1959). As a pressor, serotonin is about twice as active as bufotenine.

In the toad genus *Bufo*, hallucinogenic activity may actually originate with 5-MeODMT, an O-methylated analog of bufotenine, found in only a few of the known two hundred *Bufo* species. Although bufotenine is present in the venom secreted by the parotid gland of all toad species, it is far more likely to contribute to a potentially lethal toxicity than to induce any psychoactive effect (Lyttle, 1993). In the venom, bufotenine is a minor constituent. Other components include phenylethylamines such as epinephrine (which may act to increase penetrability across the blood-brain barrier, but this has not been proven), norepinephrine, and dopamine, other tryptamine

derivatives such as serotonin, and bufodienolides (steroidal derivatives that affect the heart in a manner similar to digitalis or act as potent vasoconstrictors). Neither bufotenine nor 5-MeODMT have shown activity when taken orally (Horgan, 1990; McKim, 1986) which leaves reported hallucinogenic highs due to "toad-licking" in a questionable state.

**Structure-activity considerations**

Structure-activity studies have attempted to explain variations in potency, specific symptoms, duration of effects, and observed differences in mode of action on the neuronal level as a function of minor structural differences between related drugs (Nichols and Glennon, 1984; Jacob and Shulgin, 1994). With regard to structure, minor differences can have profound effects. For instance, 5-HT, which is centrally active in the brain and spinal cord, does not usually affect activity in the CNS when administered peripherally, as the compound is unable to enter the CNS from the periphery. This limitation is directly related to its chemical structure, especially to polar functional groups (hydroxyl group at ring position number 5 in 5-HT, also in bufotenine; Shulgin and Nichols, 1978). Formation of an ether (a modification that decreases polarity) will often disallow this limitation, as is shown by the recognized psychoactive effects of 5-MeO-DMT. In contrast, DMT has no overtly polar functional groups and also exhibits intoxicating symptoms.

On consumption, the enzyme monoamine oxidase rapidly metabolizes most tryptamine derivatives into inactive by-products, often into indole acids, before the agents reach the CNS, frequently while still in stages of absorption. Serotonin, for example, is so poorly absorbed and has such a short half life in the periphery that L-tryptophan, the serotonin precursor, must be administered at levels of 3-6 g daily rather than serotonin itself to compromised individuals. For these reasons, intoxicating preparations intended for oral application are generally combined with a naturally occurring inhibitor like the harmala alkaloids or with one of the commercially available MAOI (monoamine oxidase inhibitors), medically prescribed antidepressants known to elevate brain amines. This recognized limitation has led to the purposeful design of chemical structures to avoid enzymatic inactivation. The synthetically produced α-methyltryptamine and 5-methoxy-α-methyltryptamine differ from DMT and 5-MeODMT in that the synthetic structures posses a single methyl group attached to the carbon preceding the amine group which lacks the dimethyl functionality; this difference allows for oral activity at about the same levels of potency as the natural analogs when inhaled or injected (Shulgin and Nichols, 1978).

## EXPERIMENTAL CONSIDERATIONS

This study is a continuation of earlier research by Szabo and Tebbett (2002). Previously, the presence of certain indolic alkaloids was ascertained in the tissues and seeds of various *Mucuna* accessions. As a step toward determining whether or not these components are of potential concern in food, feed, or fodder produced from *Mucuna*, the components were quantified in fresh and dried plant material.

**Sample materials**

The plant materials used in this study originated from various sources. Most had previously been assayed for L-dopa content and screened for selected indolealkylamines (Szabo and Tebbett, 2002). In preparation for extraction, all seed samples were ground in a Wiley mill with a 1-mm stainless steel screen. Initial preparations for other sample types are defined with the source description: (1) Raw seed from a local variety of *Mucuna pruriens* found in Malawi was supplied by the Rockefeller Foundation-Malawi. (2) Leaves, stems, pods, and roots from *Mucuna pruriens* var. *utilis* grown in the Rebublic of Benin were collected, dried, and ground prior to shipment by CIEPCA (Center for Cover Crops Information and Seed Exchange in Africa, Cotonou, Benin). Two whole seed samples (Nord 98 and IITA 98) of the same variety grown in different locations were also supplied. (3) Raw seed was also sent from the Agronomy Department, Auburn University, Alabama. These samples had not previously been screened for the presence of indolic alkaloids. (4) Fresh leaf and stem samples of four *Mucuna* accessions were planted, grown in greenhouses, and harvested at the University of Florida, Gainesville, Florida, from seed supplied by the Agronomy Department, University of Florida and by ECHO (Educational Concerns for Hunger Organization, Inc., Ft. Myers, Florida). The samples, designated mottled-Gainesville, ECHO 92023 991E, ECHO 91080 991E, and ECHO 60002 PG1 were chopped by hand in preparation for analysis.

**Sample extraction**

The extraction method was modified from the technique described by Brain (1976). Approximately 0.5 g of dried plant or seed material was weighed into a culture tube. (For fresh plant and dried pod samples, ~1 g of material was used with all solvent volumes adjusted accordingly.) The sample was mixed with 3 mL of 0.1 N hydrochloric acid, heated in a boiling water bath for 5 min, and allowed to cool to room temperature. Three milliliters of ethanol were then added; the sample was shaken for 10 min by hand and

centrifuged at 2000 rpm for 10 min. The supernatant was removed by pipette to a fresh vial, and the residue was re-extracted with the supernatants being collected together. Ethanol was added to the combined extracts to a final volume of 5 mL. A 1-mL aliquot was then removed and filtered through a syringe packed with glass wool coupled to a 0.45-μm PTFE syringe filter. Just prior to analysis, caffeine was added as an internal standard (20 μg $mL^{-1}$). All solvents used were of reagent grade. All water was distilled and deionized. Suitability of the extraction method for the indolic alkaloids (~95% recovery) had previously been determined through a recovery study in which a mixed standard containing all analytes was added to ground seed samples at a level of 0.05% dry wt.

**Sample analysis**

Alkaloid content was determined by liquid chromatography with mass spectrometric detection using electrospray ionization. (Table 1 contains a detailed description of the instrumentation and parameters.) Stock solutions of tryptamine hydrochloride (99%; Aldrich Chemical, Milwaukee, WI), serotonin (96.7%; Sigma), *N,N*-dimethyl-tryptamine (Alltech), 5-methoxy-dimethyltryptamine (Sigma), and bufotenine (Radian Analytical Products, Austin, TX) were prepared individually. Bufotenine and DMT were dissolved in methanol; tryptamine in ethanol; and 5-HT and 5-MeODMT in water acidified to a pH of 3.2 in acetic acid with a few drops of methanol to assist dilution. A five-point curve ($R^2 \geq 0.998$) of mixed standards was freshly prepared in ethanol from the individual stocks each day; each contained 20 μg $mL^{-1}$ of caffeine to match the sample extracts.

Qualitatively, the positive confirmation of a compound's identity requires comparison of the retention time and the pattern of fragmentation ions of the sample and the standard. These must all agree within some limit of acceptability, generally, 0.2-0.3 min for the retention time and 5-10% for the ion proportions. Due to the similarity in structures among this particular group of compounds, close retention times and the sharing of fragmentation ions were not uncommon. There was also the reasonable concern that one of the many previously unrecognized analogues could appear in the extracts. To ensure identification of any target compound believed to be present, that sample extract was spiked to a level of 20 μg $mL^{-1}$ with a standard of the suspect analyte and re-analyzed. The unspiked and spiked spectra were then compared against each other, as well as against the corresponding standard. While identification depended on multiple ions, mass spectra of the standards and samples were also collected in selected-ion mode (single-ion monitoring) to maximize sensitivity for quantification. The $[M + H]^+$ ions at m/z 161 for tryptamine, the $[M + H]^+$ ions at m/z 177 for 5-HT, the $[M + H]^+$ ions at m/z 205 for bufotenine, the $[M + H]^+$ ions at m/z 189 for DMT, and $[M + H]^+$ ions at m/z 219 for 5-MeODMT were selected for quantification.

Table 1. Description of instrumentation and parameters.

| | |
|---|---|
| HPLC System | |
| Instrumentation: | Hewlett-Packard HP1100 system with autosampler, degasser, binary pump modules, and variable wavelength UV detector (Hewlett-Packard Company, Wilmington, DE) |
| Column: | Adsorbosil C8 5 μm (4.6 mm ID, 150 mm length; Serial #97032088; Alltech) |
| Mobile Phase: | Solvent A: 20 mM ammonium acetate in water<br>Solvent B: methanol<br><br>Gradient: 13% Solvent B for 2 min; 13% increased to 100% Solvent B over 14 min; hold 8 min; 100% decreased to 13% Solvent B over 2 min; equilibrate 5 min |
| Column Temp: | 25 °C |
| Flow Rate: | 1 mL/min |
| Injection Vol.: | 50 μL |
| UV Detection: | 280 nm |
| Mass Spectrometer | |
| Instrumentation: | Finnigan LCQ Ion Trap Mass Spectrometer (Finnigan MAT, San Jose, CA) |
| Range: | 100-500 m/z full scan mode |
| Source: | Electrospray Ionization |
| Spray Volt: | 4.20 kV |
| Capillary Volt: | 20 V |
| Capillary Temp: | 220 °C |

## RESULTS AND DISCUSSION

In the previous screening study (Szabo and Tebbett, 2002), 5-MeODMT had been identified in each of several samples examined, but 5-HT and tryptamine found only in a few select samples. The concentrations of these components were estimated to be ~0.001%, low compared to that of L-dopa (~5% by weight in the

seeds and 0.1-0.5% in plant tissues), but of concern nonetheless. The earlier screening results along with the current measured alkaloid concentrations for dry weights of the various samples are presented in Table 2.

Table 2. Summary of assay results.

| Sample description | Screen results | Estimated concentration (μg $g^{-1}$ dry wt.) | | |
|---|---|---|---|---|
| | | Unknown | Bufotenine | 5-MeODMT |
| Malawi, local variety: | | | | |
| Whole seed | 5-MeODMT, Tryptamine | 1.70 | 1.20 | 0.63 |
| Benin samples, *M. pruriens*: | | | | |
| Roots | 5-MeODMT | 5.96 | 4.14 | 1.76 |
| Stems | 5-MeODMT | 5.05 | 7.56 | 2.04 |
| Leaves | 5-MeODMT, Tryptamine | 9.39 | 8.29 | 2.73 |
| Pods | 5-MeODMT | 4.03 | <0.50 | 1.29 |
| Whole seed (Nord 98 ) | 5-MeODMT | <0.50 | 1.46 | 0.34 |
| Whole seed (IITA 98) | 5-MeODMT | <0.50 | 1.25 | 0.39 |
| Gainesville fresh plants: | | | | |
| ECHO 92023 991E | | | | |
| stems | 5-MeODMT, 5-HT | 1.73 | 1.92 | 1.05 |
| leaves | 5-MeODMT, 5-HT | 6.41 | 1.94 | 0.73 |
| ECHO 91080 991E | | | | |
| stems | 5-MeODMT, 5-HT | 6.91 | 3.09 | 2.79 |
| leaves | 5-MeODMT, 5-HT | 9.37 | 2.82 | 1.41 |
| ECHO 60002 PG1 | | | | |
| stems | 5-MeODMT, 5-HT | 3.24 | 2.88 | 1.00 |
| leaves | 5-MeODMT, 5-HT | 9.55 | 2.09 | 1.17 |
| Mottled GNV | | | | |
| stems | 5-MeODMT, 5-HT | 2.80 | <0.50 | 0.52 |
| leaves | 5-MeODMT, 5-HT | 7.72 | 2.15 | 1.17 |
| Auburn seed: | | | | |
| 90 Day runner white, 024-W | NA | <0.50 | 1.24 | 0.64 |
| 90 Day runner speckled, 024-S | NA | <0.50 | 1.79 | <0.50 |
| Bella Mina speckled, 025 S-1 | NA | <0.50 | 1.30 | <0.50 |
| Bella Mina speckled, 025 S-2 | NA | <0.50 | 1.26 | 0.64 |
| Bella Mina speckled , 025 S-4 | NA | <0.50 | 1.99 | 0.55 |
| Bella Mina light black, 025-LB | NA | <0.50 | 1.45 | 0.80 |
| Edgar farm white, 023-W | NA | <0.50 | 1.53 | 0.62 |
| PI364362, 004 | NA | <0.50 | 0.92 | 086 |
| PI365411, 005 | NA | <0.50 | 2.25 | 0.83 |
| PI365414, 006 | NA | <0.50 | 1.76 | 0.72 |
| USA black, 022-B | NA | <0.50 | 1.58 | 0.63 |
| USA white, 022-W | NA | <0.50 | 1.24 | <0.50 |

Abbreviations: NA, not available; 5-MeODMT, 5-methoxy dimethyltryptamine; 5-HT, serotonin

In the current study, neither tryptamine nor DMT were detected in any sample (< 0.5 μg $g^{-1}$). Although ions corresponding to tryptamine were noted at a number of retention times as fragmentation products for other compounds, especially serotonin and bufotenine, tryptamine, itself, was excluded as a possible constituent at levels ≥0.5 μg $g^{-1}$ in *Mucuna* samples due to discrepancies between observed retention times for the samples and the tryptamine standard. An unknown compound close to the weight of serotonin, possibly *N*-methyltryptamine, was determined to be present in all plant tissue samples and one seed sample. Although serotonin was not identified in any sample tested (<0.5 μg $g^{-1}$), the levels of the unknown indole were estimated against the serotonin standards. In root and pod, 5.96 and 4.03 μg $g^{-1}$ were calculated, respectively, with an average of 8.49 and 3.94 μg $g^{-1}$, respectively, in leaf (n=5) and stem (n=5). Only one seed sample (1.70 μg $g^{-1}$) contained the unknown in detectable amounts. Bufotenine was also identified in

most samples, in root at a level of 4.14 μg $g^{-1}$, but not in the pod sample. In leaf (n=5), stem (n=4) and raw seed (n=15) concentrations averaged 3.46, 3.09, and 1.48 μg $g^{-1}$, respectively. As expected, 5-MeODMT was detected in all samples. In root and pod, concentrations of 1.76 and 1.29 μg $g^{-1}$, respectively, were found with average levels of 1.44, 1.48, and 0.45 μg $g^{-1}$ present in leaf (n=5), stem (n=5), and seed (n=15), respectively. Compared to L-dopa, the detected indoles were present at roughly 0.0001% by weight, lower than had been previously hypothesized.

Differences in constituent identifications between the earlier study (Szabo and Tebbett, 2002) and this one are possibly due to loss or to metabolism during storage, although differences are most likely due to the low concentrations, structural similarities, and to an earlier mobile phase that had not been as finely optimized for separations of these closely related structures. The ions associated with tryptamine were also among the major fragments formed for the other indoles, most strongly for 5-HT and bufotenine. In addition, the ions of 5-HT and bufotenine strongly overlapped in profile. Due to the structural similarities among the derivatives, this was not surprising. With the improved separation program and with a larger presence of analyte in each sample, differentiating the analogues was simplified – sufficiently so that an unknown tryptamine could be recognized, quantities estimated, and an identification suggested. Based on the probable molecular weight, as estimated from the likely molecular ion (the largest ion present at a high level after the molecules had been fragmented in the detector) in the fragmentation pattern, and retention time in relation to the other indole derivatives, the unknown may be *N*-methyltryptamine. In at least some plant species where DMT and/or 5-MeODMT are endogenous, *N*-methyltryptamine has also been identified (Holmstedt and Lindgren, 1979).

Now that it has been determined *Mucuna* beans and plant tissues do indeed contain psychoactive indolic alkaloids in measurable amounts, what does this mean for humans and animals consuming food and feed products? Two factors should immediately be considered: (1) threshold limits of effect for oral administration, and (2) effects of preparation and cooking processes. Because of structural similarities, these agents share the same mechanism of action, which means that additive levels must be evaluated in addition to levels of the individual components. To simplify this approach, one can consider only the tryptamine derivative (5-MeODMT) with the lowest threshold (4-6 mg) when administered intranasally. Like most naturally occurring tryptamines, it is inactive orally. However, for the sake of argument, one can assume 4 mg in a 70 kg human male (0.05 mg $kg^{-1}$) would induce symptoms. The highest measured presence of any tryptamine derivative in a seed sample was 2.25 μg $g^{-1}$ d.wt. for bufotenine. This correlates to 0. 00225 mg $g^{-1}$ in dried seed. Assuming 150 g of dried seed would be consumed by the 70 kg subject, he would consume 0.0048 mg $kg^{-1}$, a level more than 10 times below the inhaled threshold, and much further below any ingested threshold. This amount of tryptamine derivative would not be psychoactive on ingestion. To address the additive effect, one can take the seed sample with the highest total indole content, 3.53 μg $g^{-1}$ in seed, and repeat the above assumptions. In this case, the consumed concentration would be 0.008 mg/kg body weight, about twice the level calculated for the individual alkaloids, but still below a level of concern.

Although indolic alkaloids are generally stable to freezing, drying and the elevated temperatures of cooking, they are water-soluble, and so can be removed by boiling. For example, the psychoactive mushrooms of the *Psilocybe* genus are often frozen or dried for storage. Occasionally they are boiled prior to use, in which case the water is ingested rather than the mushroom as the potency of the mushrooms is decreased during processing (Spoerke and Hall, 1990). Psilocin, psilocybin, and other tryptamine derivatives are contained in the tissues at total levels around 15 mg per 30 g of mushroom, much higher than the concentrations of tryptamine derivatives in *Mucuna*.

Regarding the obvious concern of chromosomal damage and the possibility of elevated levels of birth defects over time due to exposure of indolealkylamines, there is no information related to the tryptamines directly. However, a multi-generational study of routine mescaline (peyote) users has indicated no adverse effect (Dorrance *et al.*, 1975). When considering the implications of this information, one should note that both men and women partake and that exposure levels are true hallucinogenic levels, and not ones below threshold. Unfortunately, there has been no such study conducted among the snuff-users of South America.

Does food preparation affect indolic alkaloid levels? Are these levels sufficiently high to be of concern to healthy adults and children, and to health-compromised persons? Although reliable information on these topics is limited, the detected levels of indolealkylamines are well below any known threshold level for oral activity even before one takes food preparation into account. Based on chemical properties, boiling or cracking and boiling the seed should certainly decrease the alkaloid content even further, along with the L-dopa content. Regarding healthy adults, pregnant women, and children, again,

the best evidence for use is likely found in the sub-threshold levels of the components and in the multi-generational study of traditional peyote users, which showed no effect. For anyone taking monoamine oxidase inhibitors *Mucuna* consumption should probably be limited until a physician can be consulted or until additional research clarifies any potential for adverse effect. To secure the future of *Mucuna* as a food for human consumption, the evaluation of prepared food products for indolealkylamine content should allay any remaining concerns.

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