Jouko J. Vepsäläinen[1]
Seppo Auriola[2]
Mikko Tukiainen[2]
Nina Ropponen[2]
J. C. Callaway[2]

Isolation and Characterization of Yuremamine, a New Phytoindole

Abstract

Yuremamine was isolated and characterized from the stem bark of *Mimosa tenuiflora*. This plant is still used by indigenous peoples in North-eastern Brazil to make *yurema*, a psychoactive beverage that is used for medico-religious purpose (*jurema preta* or *vinho da jurema*, in Portuguese). The characterization of this novel compound by NMR and mass spectrometry introduces a new class of phytoindoles.

Key words
Phytochemistry · ethnopharmacology · tepescohuit · *Mimosa hostilis* · DMT · MAO

Supporting information available online at
http://www.thieme-connect.de/ejournals/toc/plantamedica

Introduction

Yurema is the name of a plant, a deity, certain geographical areas and a sacramental beverage that is made from the root bark of *Mimosa tenuiflora* (Willd.) Poir. This species may be identical to *Mimosa hostilis* (Mart.) Benth. [1]. The beverage, as *jurema preta*, *vinho da jurema* and other names, is still used by people of North-eastern Brazil for medico-religious purposes, and may contain other plant additives [2]. *Yurema* also means 'thorny' in the local Tupi dialect of Pernambuco, Brazil, and this botanical feature is also indicated by the Latin class name of *hostilis*, i. e., a mimosa with thorns. The bark of *M. tenuiflora* is also used in Mexico to prepare *tepescohuit*, a traditional antimicrobial agent that is used to treat burns and other skin problems [3]. An extract of *M. tenuiflora* inhibited the peristaltic reflex of the isolated guinea-pig ileum, which is densely populated with serotonin receptors [4]. Research efforts had already identified both *M. hostilis* and *M. tenuiflora* as a source of *N,N*-dimethyltryptamine (DMT), which is a potent, psychoactive serotonin agonist [5], [6]. Other phytochemical analyses have also identified the presence of triterpenoid glycosides (saponins) in the root bark of *M. tenuiflora*, which may help promote damaged skin growth as a topical ointment in folk medicine [7].

The purpose of the present study was to re-examine the phytochemistry of this species with advanced instrumentation and methodologies, particularly ^{1}H-^{13}C nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS) under mildly acidic pH.

Materials and Methods

Isolation

Stem bark from *Mimosa teniuflora* was identified and collected in Oaxaca, Mexico, by ethnobotanists Rob Montgomery and Jonathan Ott. A voucher specimen (2005 – 7RM) of this sample has been deposited in the University of Kuopio Research Garden her-

Affiliation
[1] Department of Chemistry, University of Kuopio, Kuopio, Finland
[2] Department of Pharmaceutical Chemistry, University of Kuopio, Kuopio, Finland

Correspondence
J.C. Callaway, Ph. D. · Department of Pharmaceutical Chemistry · University of Kuopio · PL 1627 · FIN-70211 Kuopio · Finland · Phone: +358-40-725-2534 · Fax: +358-17-162-456 · E-mail: callaway@uku.fi

Received February 16, 2005 · **Accepted** May 25, 2005

Bibliography
Planta Med 2005; 71: 1053–1057

barium. The dried bark was milled, and 2.265 g were soaked in 250 mL methanol at + 5 °C for 2 weeks in a refrigerator with occasional stirring. Subsequent aliquots were removed and evaporated to dryness under nitrogen at 35 °C as needed. The resulting residue was re-dissolved in an equivalent amount of 15% water and 85% acetonitrile to avoid precipitation. All samples were passed through a 0.2 μm membrane filter before analyses.

High-pressure liquid chromatography

The HPLC system consisted of a Shimadzu pump LC-8A Preparative Liquid Chromatograph coupled to the SCL-10A VP System Controller, an SPD-10A UV-Vis diode array detector and the FRC-10A Shimadzu Fraction Collector. The analytical column was a Kromasil 100 C-8, (5 microns 150 × 4.6 mm analytical, or 5 microns 150 × 20 mm for preparative experiments) with the corresponding Kromasil C-8 guard column, using an isocratic mobile phase consisting of 80% water with 0.1% trifluoroacetic acid and 20% acetonitrile. The flow rate was 1 mL/min for analytical experiments, with 20 μL injections, and a 20 mL/min flow rate with 3 – 4 mL injection volumes for preparative experiments. The analytes were detected at a wavelength of 254 nm during 20-minute runs. For the preparative runs, the retention time for the compound of interest (**1**) was 12.9 minutes (k′ = 10.1), and 16.3 minutes (k′ = 13.8) for analytical runs. This peak was collected from 28 preparative chromatographic runs, combined, evaporated, re-dissolved in 3 mL and passed once again as a single solution through the preparative column for the final purification of compound **1**. Eventually, about 12 mg (0.11% w/w) of a dark, red-purple amorphous solid was recovered in pure form, according to HPLC, LC-MS and NMR.

Liquid chromatography-mass spectrometry

HPLC-MS data were recorded on an LCQ quadrupole ion trap mass spectrometer (Finnigan, San Jose, CA, USA) connected to a Rheos 4000 HPLC system (Rheos, Danderyd, Sweden). A Supersphere 60 RP-select B column was used to separate the compounds. The eluent consisted of 0.1% aqueous trifluoroacetic acid in a gradient of 7.5% – 35% acetonitrile over 20 minutes. The spray needle was set to 4.5 kV and the spray was stabilized by a nitrogen sheath flow. The inlet capillary temperature was 225 °C. The MS/MS and MS^3 fragmentations were further studied by a LTQ quadrupole ion trap mass spectrometer (Finnigan, San Jose, CA, USA). In this study HPLC-purified fractions were injected with a manual Rheodyne injector (5 μL loop model 7125, Cotati, CA, USA) using flow injection analysis (without a column). The mobile phase was 20% ACN with aqueous 0.1% trifluoroacetic acid, at a flow rate of 10 μL/min. The full scan mass spectra were recorded in the positive mode over a range of m/z = 150 – 1000. The MS/MS traces were measured using helium as the collision gas, and the collision energy was optimized individually for each parent ion. The accurate mass of the purified phytoindole was measured using an Ultima Autospec high-resolution mass spectrometer (Micromass, UK) in the electrospray ionization mode.

Nuclear magnetic resonance spectroscopy

NMR experiments were performed at 300 K on a Bruker Avance DRX 500 spectrometer equipped with a normal 5 mm QNP probe. The entire sample (ca. 12 mg) was dissolved in 130 μL of CD_3OD and measured in a 2.5 mm microtube, using TMS as an internal standard. Structure assignment was based on normal ^1H proton decoupled ^{13}C and DEPT-135 (where CH and CH_3 are up and CH_2 signals down) NMR spectra, two-dimensional (2D) homonuclear ^1H-^1H correlated COSY (cosygpqf) and long range (LR) ^1H-^1H COSY (cosylrgf), heteronuclear $^1J_{CH}$ correlated ^1H-^{13}C HSQC (hsqcetgpsi) and long range ^1H-^{13}C HMBC (hmbcgpndqf) NMR spectra, measured by standard Bruker pulse programs [8]. Exact chemical shifts and coupling constants for all protons were determined by PERCH software [9]. Assigned chemical shifts and coupling constants for protons and carbons are listed in Table **1** as long-range $^{2-4}J_{CH}$ (HMBC) correlations. Strong correlation signals, such as $^3J_{CH}$ couplings in aromatic systems, are in bold type.

Results and Discussion

Our initial investigations into *M. tenuiflora* bark samples began in late 1994 with analytical HPLC, using diode array detection, which revealed the presence of DMT at a retention time of 7.7 minutes (k′ = 5.2) plus another strong signal of near equivalent response with a retention time of 16.3 minutes (k′ = 13.8) (see Supporting Information Fig. **1**). Subsequently, an authentic sample of *vinho de jurema* [sic] was provided by Prof. Emeritus Bo Holmstedt of the Karolinska in the Spring of 1995. This sample was reportedly prepared from *M. hostilis* in Paraiba, Brazil by traditional methods, and given to Prof. Holmstedt by a *Yurema* priestess in 1983. Analysis of this sample in his laboratory by GC-MS had detected only DMT and no other major alkaloids. Surprisingly similar results were noticed between our earlier HPLC analysis of the *M. tenuiflora* bark sample from Mexico and the authentic sample of *yurema* beverage from Prof. Holmstedt.

The LC-MS chromatogram obtained from the *M. tenuiflora* extract also showed two major compounds; DMT with a retention time of 8.50 (k′ = 5.0) min and compound **1** with a retention time of 12.9 min (k′ = 7.6) (Fig. **1A**). The mass spectrum of DMT gave a protonated molecular ion at m/z = 189 and a fragment ion at m/z = 144, caused by the immediate loss of $HN(CH_3)_2$ (Fig. **1B**). The mass spectrum of compound **1** also shows a protonated molecular ion at m/z = 477 with no major fragment ions (Fig. **1C**). These two main components from the plant extract were cleanly separated by reversed phase HPLC prior to subsequent off-line electrospray ionization LC-MS. A measured accurate mass of 477.2041 corresponded well with an elemental composition of $C_{27}H_{29}N_2O_6$ (calculated 477.2026). The MS/MS trace of compound **1** gave an m/z = 432 ion as a base peak, indicating a loss of $HN(CH_3)_2$ from the ethylamine side chain, with very few other ions (see Supporting Information Fig. **2A**). The MS^3 trace (Fig. **2**) was used to further verify the molecular structure of compound **1**, showing ions at m/z = 264 and 414 (see Supporting Information Fig. **2B**). A characteristic parent ion at m/z = 189.1380 and fragmentation was consistent with DMT, the other major alkaloid in this plant sample. High resolution LC-MS and NMR experiments eventually led to the structure of compound **1**, which is proposed in Fig. **3**.

The ethylamine substituent was easily identified by ^1H- and ^{13}C-NMR experiments for compound **1**, which was confirmed by results from HMBC correlations (Table **1**). The remaining difficulty

Table **1** ^{1}H-^{13}C NMR assignments from yuremamine (**1**)

Ring	*Atom*	*^{1}H [ppm]*	*^{3}J, ^{4}J [Hz]*	*^{13}C [ppm]*	*$HMBC_{H\rightarrow C}$*
A	3″, 5″	–	–	147.28 s	–
	4	–	–	132.15 s	–
	2″, 6″	6.390 d	0.65	105.89 d	**A3″/5″**, A4″, **A2″/6″**, **A1″**, **D3**
	1″	–	–	133.92 s	–
B	4a	–	–	137.69 s	–
	5	7.319 ddd	8.18, 0.95[1)]	112.33 d	**B7**, **B8a**
	6	7.088 ddd	8.18, 7.05, 1.08	122.63 d	**B4a**, **B8**
	7	7.040 ddd	7.99, 7.05, 0.95	120.21 d	**B5**, **B8a**
	8	7.531 ddd	7.99, 1.08[1)]	118.39 d	**B4a**, **B6**, B8a, C9
	8a	–	–	128.51 s	–
C	9	–	–	107.99 s	–
	9a	–	–	136.54 s	–
D	1	4.255 dd	3.91, 0.98	36.35 d	C9, C9a
	2	4.217 dd	4.87, 3.91	71.84 d	**C9a**, **E1′**
	3	5.170 dt	4.87, 0.65	81.25 d	A4″, A2″/6″, D1, **D2**
E	1′	–	–	113.64 s	–
	2′	–	–	158.88 s	–
	3′	6.482 d	2.46	103.82 d	**E1′**, **E5′**, E4′, E2′
	4′	–	–	156.02 s	–
	5′	6.298 dd	8.38, 2.46	109.70 d	**E1′**, **E3′**
	6′	6.512 dd	8.38, 0.98	131.30 d	D1, **E4′**, **E2′**
Side-	$C^{\alpha}H_2$	3.29 – 3.20[2)]	4)	20.91 t	
chain	$C^{\beta}H_2N$	3.11 – 3.00[3)]	4)	59.52 t	
(Sc)	NMe	2.818 s	–	43.82 q	ScC^{β}, NMe′
	NMe′	2.762 s	–	43.36 q	ScC^{β}, NMe

[1)] $^{5}J_{HH}$ = 0.79 Hz.
[2)] 1H.
[3)] 3H.
[4)] Broad signals due to dynamic effects and *J* values not assigned

was to assign carbon C9a, as C9 had a small $^{4}J_{CH}$ correlation signal with proton B8, while C9a had only a weak correlation to proton D1 and a strong correlation to D2, with no detectable couplings to the CH_2-protons of the indole ethylamine side chain. Following this assignment strategy, chemical shifts for the indole system were found to be in good agreement with literature values [10].

Proton assignments for the phenolic E ring of compound **1** were characteristic and straight-forward from ^{1}H chemical shifts. Based on the ^{13}C-NMR shifts, the hydroxy groups on the E ring had to be *meta* to each other, due to large downfield shifts. The pyrogallol ring A was more difficult to recognize, as equivalent carbon signals A3″/5″ and A2″/6″ were only ca. 30 % higher, and not twice as high, when compared to related neighbouring peaks.

Aliphatic methyl and methylene signals were typical and easily assigned from the ^{13}C-NMR DEPT spectrum [10]. The only difficulty remained in the assignment of non-equivalent chemical shifts for the two methyl substituents on the aliphatic nitrogen and NCH_2-protons, which is typically difficult for chiral systems. There is an obvious hydrogen bond in compound **1** from the E2′-OH proton with the lone pair electrons on the aliphatic nitrogen (Fig. **3**), which could protect this aliphatic amine from enzymatic metabolism by monoamine oxidase (MAO).

Based on the 2D ^{1}H-^{1}H- and ^{1}H-^{13}C-NMR correlated experiments, the remaining three aliphatic protons and carbons were observed to form a -CH-CH-CH- chain with the required substituents. Based on long-range 2D ^{1}H-^{13}C correlated results, one end of this chain was found to be adjacent to indole C and phenolic E rings, while the other end was close to the pyrogallol A ring. There were also strong connections between protons D1-E6′ and D3-A3″/5″ in the ^{1}H-^{1}H LR COSY. The $^{3}J_{HH}$ coupling constants for protons D1-D2 and D2-D3 were typical for *trans-trans*-substituted protons in five-membered rings [11].

Initially we applied standard acid-base extraction methods to the stem bark sample, which resulted in a wide variety of complex, unidentified degradation products. Compound **1** is clearly unstable, which may account for the identification of only DMT in previous phytochemical investigations of both *M. hostilis* and *M. tenuiflora* when using basic extraction conditions during sample preparation. The earliest phytochemical analysis of this *Mimosa* species (as *Mimosa hostilis*) identified only one alkaloid, which may or may not have been DMT [2]. The proposed structure in Fig. **3** also explains the chemical instability of this molecule, especially under basic conditions.

Compound **1** is not only a new compound, but also a new class of phytoindoles. According to IUPAC guidelines, this new structure

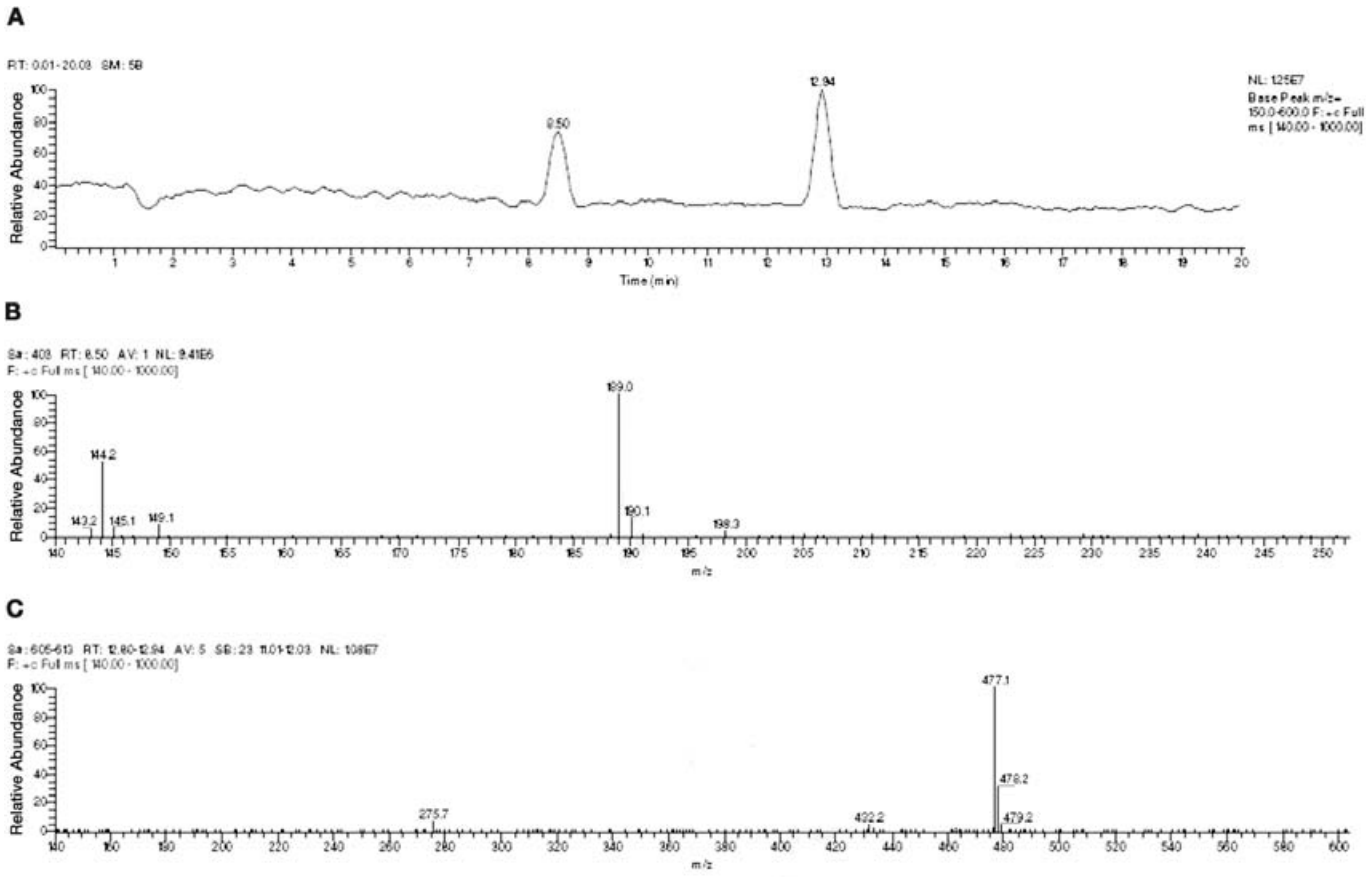

Fig. **1** LC-MS of *M. tenuiflora* extract (**A**) showing the fragmentation of DMT at 8.50 min (k′ = 5.0) with a base peak of *m/z* = 189 (**B**) and compound **1** at 12.9 min (k′ = 7.6) with a base peak of *m/z* = 477 (**C**).

can be named as 1″-[1-(2′,4′-dihydroxyphenyl)-9-(2-dimethylaminoethyl)-2-hydroxy-2,3-dihydro-1*H*-3a-azacyclopenta[*a*]inden-1-yl]benzene-3″,4″,5″-triol, with a suggested common name of yuremamine. The structural assignment was based on mass spectral data and both one and two dimensional ^{1}H- and ^{13}C-NMR experiments.

Yurema is still used for its psychoactive properties in traditional religions of Brazil [1]. However, a pharmacological explanation for the visionary effects from this sacred beverage is lacking, as DMT is not orally active because of its rapid enzymatic metabolism by MAO. *Ayahuasca*, another sacred visionary beverage from Brazil, typically contains DMT (from the leaves of *Psychotria viridis*) but another plant is required to render DMT orally active; *Banisteriopsis caapi*, which contains the potent MAO inhibitor harmine [12], [13]. It is suggested herein that intramolecular hydrogen bonding of the tertiary aliphatic nitrogen of *yuremamine* protects it from metabolism and could allow it to act as an inhibitor of MAO, thus facilitating the oral activity of DMT in this single-plant formulation. Presently, the putative pharmacology of purified *yuremamine* is unknown.

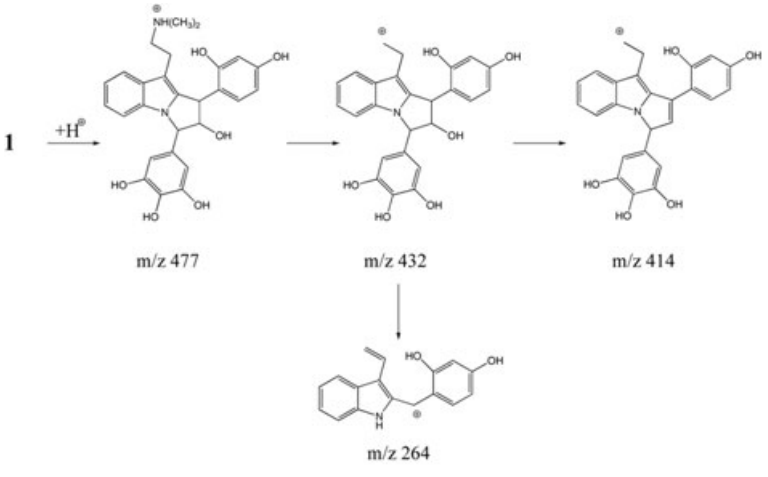

Fig. **2** Probable MS/MS and MS^3 fragments for compound **1**.

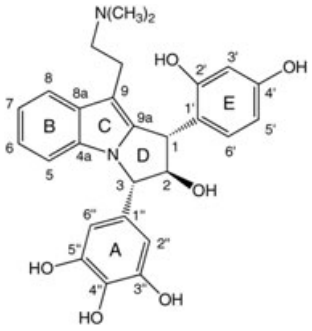

Fig. **3** Proposed chemical structure for compound **1**; yuremamine.

Acknowledgements

Our thanks to Anita Hemmilä for linguistic contributions, Prof. Ulysses Paulino de Albuquerque for cultural information on *yurema/jurema* and Jukka Leppänen, Ph.D. for technical assistance with HPLC. This work is dedicated to the memory of Prof. Emeritus Bo Holmstedt. This work was partly supported by grant number 204755 from the Academy of Finland.

References

[1] Paulino de Albuquerque U. The use of medicinal plants by the cultural descendents of African people in Brazil. Acta Farm Bonaerense 2001; 20: 139 – 44

[2] Gonçalves de Lima O. Archivos do Instituto de Pesquisas. Agronomicas 1946; 4: 45

[3] Lozoya X, Navarro V, Arnason JT, Kourany E. Experimental evaluation of *Mimosa tenuiflora* (Willd.) Poir. (Tepescohuite) I. Screening of the antimicrobial properties of bark extracts. Arch Invest Med (Méx) 1989; 20: 87 – 93

[4] Meckes-Lozoya M, Lozoya X, Gonzales JL, Martinez M. Effect produced by the alkaloid fraction of *Mimosa tenuiflora* (tepescohuite) on the peristaltic reflex of the guinea pig ileum. Arch Invest Med (Méx) 1990; 21: 171 – 4

[5] Pachter IL, Zacharias DE, Ribeiro O. Indole alkaloids of *Acer saccharinum* (the silver maple), *Dictyoloma incanescens*, *Piptadenia colubrine*, and *Mimosa hostilis*. J Org Chem 1959; 24: 1285 – 7

[6] Meckes-Lozoya M, Lozoya X, Marles RJ, Souchy-Breau C, Varasen A, Arnason JT. *N,N*-Dimethyltryptamine alkaloid in *Mimosa tenuiflora* bark (tepescohuite). Arch Invest Med (Méx) 1990; 21: 175 – 7

[7] Jiang Y, Massiot G, Lavaud C, Teulon J-M, Guéchot C, Haag-Berrurier M et al. Triterpenoid glycosides from the bark of *Mimosa tenuiflora*. Phytochemistry 1991; 30: 2357 – 60

[8] Parella T. In: Pulse program catalogue for Topspin *v*1.2. NMR Quide *v* 4.0. Barcelona: Bruker BioSpin GmbH, 2004

[9] Laatikainen R, Niemitz M, Weber U, Sundelin J, Hassinen T, Vepsäläinen J. General strategies for total-lineshape-type spectral analysis of NMR spectra using integral-transform iterator (NMR). J Magn Reson A 1996; 120: 1 – 10

[10] Pretsch E, Bühlmann P, Affolter C. In: Structure Determination of Organic Compounds. Heidelberg: Springer Verlag, 2000

[11] Padwa A, Fryxell GE, Gasdaska JR, Venkatramanan MK, Wong GSK. A dipolar cycloaddition approach to pyrrolo[1,2-*a*]indoles using *N*-[(trimethylsily1)methyl]-substituted indoles. J Org Chem 1989; 54: 644 – 53

[12] Callaway JC, McKenna DJ, Grob CS, Brito GS, Raymon LP et al. Pharmacokinetics of *Hoasca* alkaloids in healthy humans. J Ethnopharmacol 1999; 65: 243 – 56

[13] Ott J. Ayahuasca analogues – Pangæan Entheogens. Kennewick, WA, USA: Natural Products, 2004