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Mescaline concentrations in small regrowth crowns vs. mature adult crowns of *Lophophora williamsii* in a South Texas population.

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Abstract

A phytochemical analytical study was conducted to address quantitatively the question of whether the mescaline concentration in *Lophophora williamsii* (peyote) plants is dependent on the maturity and/or size of the plant. Tissue samples (4 grams each) biopsied from 10 mature peyote cacti and 14 whole small regrowth buttons (2−4 grams each) were collected from the same population in the Tamaulipan Thornscrub ecoregion of South Texas. For each of the two groups (mature and small), the individual tissue samples were pooled, desiccated, and ground to powder with mortar and pestle. The alkaloids were extracted from 2 g of the powdered tissue from each group with 100 mL methanol in a closed beaker at 25°C with daily manual swirling for a week, followed by evaporation of the methanol to dryness in a ventilated hood at 25°C, then acid-base extraction with dichloromethane. The mescaline concentration in each of the extracts was then determined by HPLC. Though we did not analyze each individual plant for statistical analysis, inspection of the results of the analyses of the pooled samples clearly indicates that the mature plants contain more mescaline than the small buttons in the population studied.

Introduction

Fig. 1. An adult peyote cactus (diameter ca. 6 cm) in habitat.

Peyote (*Lophophora williamsii*) is a small (up to ca. 8 cm diameter), spineless, globular cactus of northeastern Mexico and adjacent Texas (Fig. 1). It is of cultural and economic importance for its use as a religious sacrament, based largely on the psychoactive properties of its principal alkaloid, mescaline, in the Native American Church (NAC), where the harvested crowns of peyote plants are traditionally known as "buttons" because of the fact that the crowns become hard, discoid, and reduced in diameter as they desiccate over a period of months.

Over the past few decades peyote has become scarce in many parts of its historical geographic range. While the largest part of the reduction in peyote population size is clearly habitat destruction associated with urban sprawl and adverse agricultural practices (notably root-plowing), another major cause is overharvesting of the plant for ceremonial use by the NAC. Overharvesting of peyote has several different—but additive—adverse effects on the wild populations: (1) It reduces the harvestable population size, and selectively removes the largest crowns first, as these are most valued in the peyote market. (2) That reduces the reproductive output of the population, as a direct consequence of the removal of the largest crowns that produce most of the seed in an unharvested population. (3) The phenomenon of regrowth of new crowns from areoles of the subterranean stem (Terry & Mauseth 2006) temporarily increases the number of crowns in the population but severely decreases the average size of crowns in the population (Terry et al. 2011). (4) The decreased size of peyote buttons available to the NAC means that an individual in an NAC peyote ceremony must consume more buttons to equal the weight of the smaller number of buttons that would be consumed if mature crowns were available. This leads to a vicious circle of more frequent harvesting to supply the demand for greater numbers of smaller buttons, which leads to yet smaller buttons—but now fewer, as the overharvested plants exhibit signs of decreased energy reserves for the production of more new crowns following repeated harvesting (Terry et al., in press). (5) Looking at the quality of peyote in purely pharmacological terms, there would appear to be yet another disadvantage to regrowth buttons for ceremonial use, apart from their small size, and that is the possibility that the dry-weight concentration of mescaline in the small regrowth buttons is substantially lower than in mature peyote crowns that are now uncommon in the regulated market. If the small regrowth buttons were weaker in mescaline concentration than mature buttons, that would encourage consumption of still larger numbers of buttons to attain the desired spiritual state which each participant in an NAC meeting reaches by consuming "enough" peyote. That would further exacerbate the vicous circle of increased harvesting leading to increased consumption leading to increased harvesting. The purpose of the current study was to address the hypothesis that small regrowth peyote crowns have lower concentrations of mescaline (on a dry-weight basis) than control mature crowns from plants in the same population.

Materials and Methods

Fourteen regrowth peyote crowns weighing 2−4 grams each (fresh weight) were collected from 14 individual plants in a South Texas population in March 2012. (This group of samples will henceforth be referred to as the "Small Regrowth" group.) Biopsy samples of crown tissue (ca. 4 g fresh weight from each individual) were field-collected from 10 mature (eight-ribbed, 5−6 cm in diameter) individual peyote plants in the same population. (This group of samples will henceforth be referred to as the "Mature" group.) The cactus tissue from each group was cut into small slices, which were set to dry for a week on a drying rack. Once desiccated, the tissue of each group was ground to a fine powder with a mortar and pestle. At that point the individual plant samples in each group were pooled and mixed to constitute a single homogeneous sample to represent each group. A sample of 2.0 g of the ground cactus tissue from each group was then placed in a 200-mL beaker to which 100 mL of HPLC-grade methanol was added and the beaker for each sample was sealed with Parafilm® to prevent evaporation of the methanol and . kept at room temperature with daily swirling for a week to effect alkaloid extraction. The methanol extract was evaporated to dryness under the hood at room temperature, and the residue of each extract was dissolved in 200 mL HPLC-grade water. The pH was lowered to approximately 3 with concentrated HCl. The acidified aqueous extract was poured into a 500-ml separatory funnel, to which 50 mL of dichloromethane was added. The mixture was shaken gently to mix the aqueous and dichloromethane phases thoroughly, and the aqueous and dichloromethane layers were then allowed to separate overnight. The dichloromethane layer (containing fats and other nonpolar compounds) was drained out of the separatory funnel and set aside. The rationale is that protonated mescaline and related alkaloids (phenethylamines and tetrahydroisoquinolines) remain in the acidified aqueous layer. Two additional defatting extractions of the remaining aqueous phase in the separatory funnel were done, each with 50 mL of dichloromethane, and in each case the aqueous and organic layers were allowed to separate, whereupon the dichloromethane phase was drained from the separatory funnel and set aside.

After the third defatting extraction of the acidified aqueous phase, the aqueous phase was drained from the separatory funnel into a beaker and the pH raised to about 12 with sodium hydroxide (5 M), in order to deprotonate essentially 100% of the mescaline (pK_a = 9.5). The alkalinized aqueous extract was then poured into a separatory funnel, and 50 mL of dichloromethane was added. The rationale is that the deprotonated alkaloids would now be more soluble in the nonpolar dichloromethane than in water. The organic and aqueous phases were mixed well and left to separate. The dichloromethane layer was drained into a 200-mL beaker and set aside. Another 50 mL of dichloromethane was then added to the remaining aqueous phase in the separatory funnel for the second dichloromethane extraction of the alkalinized aqueous phase. The organic phase was drained into the same labeled beaker as with the first dichloromethane extraction of the alkalinized aqueous phase. The combined dichloromethane extracts were then left under a hood at room temperature, and the dichloromethane was evaporated to dryness. The residue, containing the mescaline and related alkaloids, was then redissolved in 10.0 mL of methanol and stored in the freezer in a sealed vial. This acid-base extraction procedure was done on the Soxhlet tissue extract of each of the four cactus populations. The samples were run on an Agilent 1260 Infinity HPLC, using 70% methanol (HPLC grade) as the mobile phase, and a Phenomenex Gemini 5μ C18 column as the stationary phase. Samples of 1.0 μL were injected with a flow rate of 1.2 mL/min and run for 30 minutes. Each of the two samples of alkaloid extract, after appropriate dilution to obtain on-scale HPLC peaks, was run three times, and the values of area under the curve (AUC) of the three runs per sample were averaged. Appropriate dilutions were made of mescaline standard and run on the HPLC three times under the same conditions described above, to create a standard curve (Fig. 2). The mean AUC value for each sample of alkaloid extract was then interpolated on the standard curve to yield the corresponding concentration of mescaline in the HPLC sample of alkaloid extract. From the latter value (one such mean concentration value per sample group), we calculated the original concentration of mescaline in the desiccated tissue sample from the Small Regrowth group and the Mature group

Confirmation of the identity of mescaline in these samples was achieved with GC/MS. A sample methanol extract of *L. williamsii* from this study was evaporated to dryness under an N_2 stream at room temperature, and the residue dissolved in dichloromethane for analysis. The instrumentation was an Agilent 6890 GC equipped with a DB-5ms (0.25 mm I.D. x 30 m) column, splitless injection (250°), and an Agilent 5972 MSD (transfer line at 275°), operated in full scan mode. Helium was used as the carrier, and the oven program was 70° (with a 1-min hold), then 20°/min to 250, with a final hold. The mescaline peak had an identical retention time to an authentic reference sample, and the mass spectrum matched a spectrum in the NIST database (Stein et al., 2005) with 93.5% probability of best match.

Results and Discussion

Mean mescaline concentrations for the two groups sampled are presented in Table 1. The GC-MS results confirming the identity of mescaline in the samples are presented in Figs. 3a and 3b. These findings clearly support the hypothesis that mescaline concentration is reduced in small regrowth crowns of peyote in comparison to the concentration in mature crowns from unharvested plants. This confirms the validity of the widespread belief among NAC members (T. Herrera, pers. obs.) that small regrowth buttons are "weaker" than larger, mature buttons. It also adds another dimension to the damage done by the too frequent harvesting of peyote that is currently taking place in South Texas.

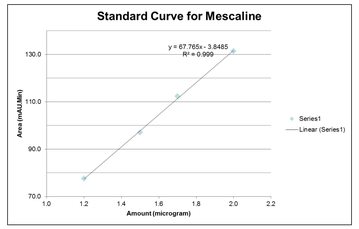

Fig. 2. Standard curve of area under the curve (AUC) of mescaline of the mescaline HPLC peak as a function of concentration of mescaline standard. Correlation coefficient *r* = 0.999.

Crown Type	Mean Mescaline Concentration (% by dry tissue weight)
Mature	3.80
Small Regrowth	2.01

Table 1. Mean Mescaline Concentration as percentage of as a constituent of peyote crown tissue (dry-weight basis) in Mature peyote crowns and Small Regrowth crowns sampled in situ.

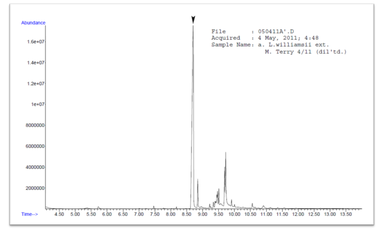

Fig. 3a. Gas chromatograph of peyote extract showing prominent peak of the predominant alkaloid. 93.5%.

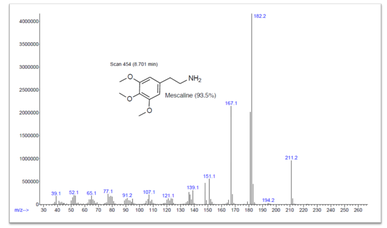

Fig. 3b. Mass spectrum of the prominent GC peak in Fig. 3a. This spectrum matches that of mescaline with *P* = 93.5%.

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References

Stein S, Mirokhin Y, Tchekhovskoi D, Mallard G, Mikaia A, Zaikin V, Little J, Zhu D, Clifton C, Sparkman D. 2005. The NIST Mass Spectral Search Program for the NIST/EPA/NIH Mass Spectral Library. Version 2.0 d. FairCom Corp., ChemSW, Fairfield, CA.

Terry M, Mauseth JD. 2006. Root-shoot anatomy and post-harvest vegetative clonal development in *Lophophora williamsii* (Cactaceae: Cacteae): Implications for conservation. *Sida, Contributions to Botany* **22**: 565−592.

Terry M, Trout K, Williams, B, Herrera T, Fowler N. 2011. Limitations to natural production of *Lophophora williamsii* (Cactaceae) I. Regrowth and survivorship two years post harvest in a South Texas population. Journal of the Botanical Research Institute of Texas 5: 661−675.

Terry M, Trout K, Williams B, Herrera T, Fowler N. Limitations to natural production of *Lophophora williamsii* (Cactaceae) II. Effects of repeated harvesting at two-year intervals in a South Texas population. Journal of the Botanical Research Institute of Texas 6: xxx−xxx. In press.