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# Prehistoric peyote use: Alkaloid analysis and radiocarbon dating of archaeological specimens of *Lophophora* from Texas

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**Abstract**

Two archaeological specimens of peyote buttons, i.e. dried tops of the cactus *Lophophora williamsii* (Lem.) Coulter, from the collection of the Witte Museum in San Antonio, was subjected to radiocarbon dating and alkaloid analysis. The samples were presumably found in Shumla Cave No. 5 on the Rio Grande, Texas. Radiocarbon dating shows that the calibrated 14C age of the weighted mean of the two individual dated samples corresponds to the calendric time interval 3780–3660 BC (one sigma significance). Alkaloid extraction yielded approximately 2% of alkaloids. Analysis with thin-layer chromatography (TLC) and gas chromatography–mass spectrometry (GC–MS) led to the identification of mescaline in both samples. No other peyote alkaloids could be identified.

The two peyote samples appear to be the oldest plant drug ever to yield a major bioactive compound upon chemical analysis. The identification of mescaline strengthens the evidence that native North Americans recognized the psychotropic properties of peyote as long as 5700 years ago.
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*Keywords:* *Lophophora williamsii*; Peyote; Mescal buttons; Mescaline

## 1. Introduction

*"A chemical compound once formed would persist forever, if no alteration took place in the surrounding conditions."* Thomas Henry Huxley (1825–1895) English Biologist/Evolutionists. (cited by Asimov and Schulman, 1988).

The origins of drug use will probably never be fully understood, but some artefacts have survived, such as archaeological samples of drugs, their containers and related paraphernalia. One of the most fascinating, although very minor, approaches for drug research lies in the analysis and interpretation of such remains. Sometimes this field of science has been referred to as archeobotany or archaeoethnobotany (Schultes and von Reis, 1995).

The collections of many ethnographical museums comprise paraphernalia for ritual drug taking, and sometimes the drug itself or its vegetal source is also present. In such cases, botanical examination still may reveal the identity of the drug source, especially if it can be backed up by the results of chemical analysis (De Smet, 1995).

Archaeological investigations in Northeast Mexico and Trans-Pecos Texas have demonstrated that the knowledge of psychotropic drugs in this region goes back to ca. 8500 BCE (De Smet and Bruhn, 2003). The aboriginal inhabitants of this region may have used both the so called "red" or

*Abbreviations:* BC, before Christ; BCE, before Christian era; BP, before present; CE, Christian era

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"mescal bean", from *Sophora secundiflora* (Ort.) Lagasca ex De Candolle and "mescal buttons", dried slices of the peyote cactus, *Lophophora williamsii* (Lem.) Coulter (Adovasio and Fry, 1976; Boyd and Dering, 1996). Unlike peyote, the mescal bean has been used extensively for ornamental purposes (Merrill, 1977), so we cannot know for sure that it has been used for psychoactive effects.

Previously, from one of the archaeological sites in Coahuila, Mexico, a number of "mescal buttons" were retrieved and Carbon-14 dated to 810–1070 CE. Alkaloid analysis revealed the presence of mescaline and four related tetrahydroisoquinoline alkaloids, anhalonidine, pellotine, anhalonine and lophophorine. Compared to freshly prepared "mescal buttons" there was a considerably lower alkaloid content (2.25% compared to ca. 8% in a recent sample) (Bruhn et al., 1978).

Some years ago, one of the authors (De Smet) came across two peyote "buttons" in the exhibition of the Witte Museum in San Antonio, Texas. Although the museum documentation is not very specific, the most likely origin of these "buttons" is one of the Shumla caves, in the lower Pecos region, or another archaeological rock shelter in Southwestern Texas (Boyd and Dering, 1996; Martin, 1937). Previously, these plant remains have been subjected to Carbon-14 dating and their age has been reported as "7000 years". However, all the information we have on that dating is from a book review, where this bare date is given as a personal communication to the reviewer (Furst, 1989).

With the kind help of the museum curators, two samples for phytochemical analysis and renewed Carbon-14 dating were prepared from the buttons in the collection. We here present the full results of these analyses. A preliminary communication of the results has appeared in *The Lancet* (Bruhn et al., 2002). In this paper, the methods are described in full detail.

## 2. Materials and methods

### 2.1. Plant material

The two peyote samples analyzed are kept in the Witte Museum collection in San Antonio, Texas. They were presumably found in Shumla Cave No. 5 by George Martin in 1933 (Martin, 1937) and identified by him as coming from *Lophophora williamsii* (Lem.) Coulter, but the museum documentation is not very specific. A photograph of the specimens has been published by Boyd and Dering (1996, Fig. 12, p. 269), who also accepted this identification. Only the inner parts of the two samples were scraped out with a fine knife so as not to destroy the appearance of the two specimens. These "inner scrapings" had the form of a coarse, brownish-grey powder. The two samples were analyzed individually for alkaloids and radiocarbon dated separately.

### 2.2. Alkaloid extraction

Two different procedures were employed:

(A) The powdered samples (680 and 416 mg, respectively) were extracted at room temperature three times with EtOH (300 ml) for 48 h each time with stirring. The combined ethanol extracts were filtered and evaporated in vacuo to give 61.2 and 31.2 mg of oily residues, respectively. The residues were dissolved in $H_2O$, made alkaline with conc ammonia (pH 9) and extracted twice with $CHCl_3$ and once with $CHCl_3$:EtOH (3:1). The combined extracts were dried over $Na_2SO_4$ anhydrous, filtered and evaporated to dryness to yield 13.59 mg (2%) and 8.31 mg (2%), respectively, of total alkaloids.

(B) To 100 mg of each of the two samples, 3 ml of 10% HCl was added in an Ehrlenmeyer flask and the flasks immersed in a boiling water bath for 15 min. The solutions were filtered through Whatman No. 1 filter paper, and the residues were washed with 10 ml distilled $H_2O$. The filtrates were then extracted three times with $Et_2O$ (20 ml). The resulting emulsions were centrifuged at 1500 rpm. The ether layers were dried over anhydrous $Na_2SO_4$, filtered and evaporated to dryness to give 1.99 mg (2%) and 1.95 mg (2%), respectively, of the alkaloid fraction.

The alkaloid extracts were tested with Dragendorff's reagent using Whatman No. 1 filter paper.

### 2.3. Thin-layer chromatography

Thin-layer chromatography was carried out on silica gel coated plates (20 cm × 20 cm, 0.25 mm layer) in the system: $CHCl_3$:BuOH:conc $NH_4OH$ (50:50:2.5) according to Lundström and Agurell (1967). After elution, the residue of ammonia was removed by careful drying in a heated oven. The plates were sprayed with ninhydrin reagent (purple colour with mescaline, rf value 0.46) and iodoplatinate—Dragendorff's reagent (brownish-purple colour with mescaline) (Lum and Lebish, 1974).

### 2.4. Gas chromatography–mass spectrometry

The gas chromatography–mass spectrometry (GC–MS) data were obtained using a Voyager quadropole GC–MS instrument (ThermoFinnigan Inc., CA, USA) operating in the full scan electron impact mode. A 2 μl aliquot of the extract was injected by split-less injection into a 30 m HP-5 MS capillary column (0.25 mm i.d. and 0.25 μm film thickness, Agilent Technologies, CA, USA). The injector temperature was 200 °C, ion source 230 °C, column temperature was held at 100 °C for 1 min and increased to 250 °C at a rate of 30 °C/min.

### 2.5. Radiocarbon dating

A simplified chemical pre treatment was applied to the samples (19.8 and 19.2 mg, respectively) by using 1% HCl

below boiling for 6 h. This will mainly eliminate adsorbed $CO_2$ and remove other carbonate fractions of no relevance to the dating. The insoluble fraction was then rinsed in distilled water and dried. Approximately a 60% yield was obtained in this first preparation step. Combustion of the organic fraction was then conducted with CuO at 800 °C for ca. 10 min and the $CO_2$ gas graphitized at 750 °C with an excess of $H_2$ gas and Fe present as a catalyst. A carbon content of ca. 30% was achieved. A small part of the $CO_2$ gas (ca. 0.1 mg) was used for stable isotope analysis, $\delta^{13}C$, in a VG OPTIMA dual inlet mass spectrometer to determine the natural mass fractionation. Radiocarbon was finally measured with the Uppsala new AMS system based on a 5 MV NEC pelletron$^{TM}$ tandem accelerator running in pulsed mode.

## 3. Results

### 3.1. Radiocarbon dating

The results for the two individual peyote samples Ua-12433 and Ua-12434 are given in Figs. 1 and 2.

A calibrated age (computer code OxCal v.3.9) for the weighted mean age ($4952 \pm 44$ BP) of the two dated samples ($5030 \pm 65$ BP($\delta^{13}C = -16.1$‰ VPDB), Ua-12433) and $4885 \pm 60$ BP ($\delta^{13}C = -22.3$‰ VPDB), Ua-12434) corresponds to the following time intervals: (1σ, 68.2% probability) 3780–3690 (57.8%) and 3680–3660 (10.4%) calender age BC; (2σ, 95.4% probability) 3910–3870 (4%) and 3800–3640 (91.4%) calender age BC (Fig. 3).

### 3.2. Alkaloid analysis

Standard alkaloid extraction procedures carried out on the samples gave residues that tested positive for alkaloids (orange colour) with the Dragendorff reagent. The alkaloid yield was approximately 2% in both samples. The extracts were then analyzed by thin-layer chromatography and gas chromatography–mass spectrometry. Mescaline could be identified in both samples, based on identical retention times (GC) (Fig. 4) and rf values (TLC) and mass spectrum as authentic mescaline (Fig. 5).

The samples were also checked for the possible presence of the major peyote tetrahydroisoquinoline alkaloids: lophophorine, anhalonine, pellotine and anhalonidine. There was no trace of these alkaloids in either of the two samples.

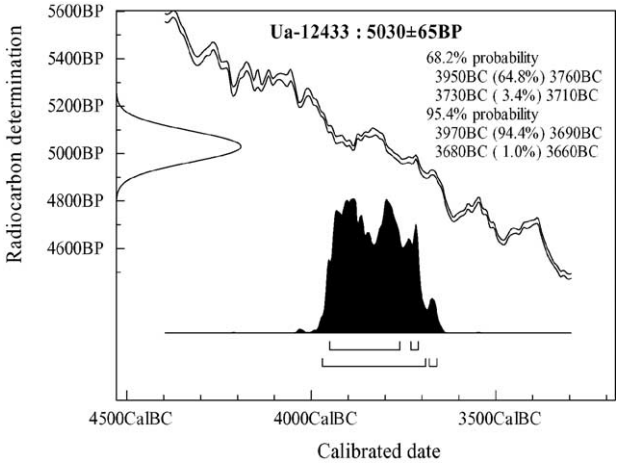



Fig. 1. Graphical presentation of the radiocarbon dating results according to the OxCal v.3.9 computer code. Sample Ua-12433.

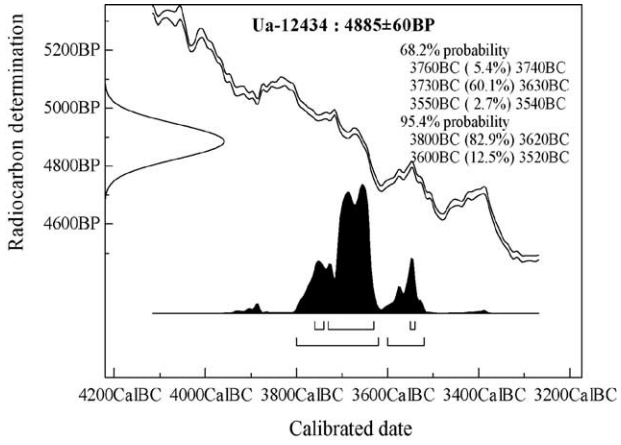



Fig. 2. Graphical presentation of the radiocarbon dating results according to the OxCal v.3.9 computer code. Sample Ua-12434.

## 4. Discussion

*"The deliberate seeking of the psychoactive experience is likely to be at least as old as anatomically (and behaviourally) modern humans: one of the characteristics of Homo sapiens sapiens."* Andrew Sherratt (1995).

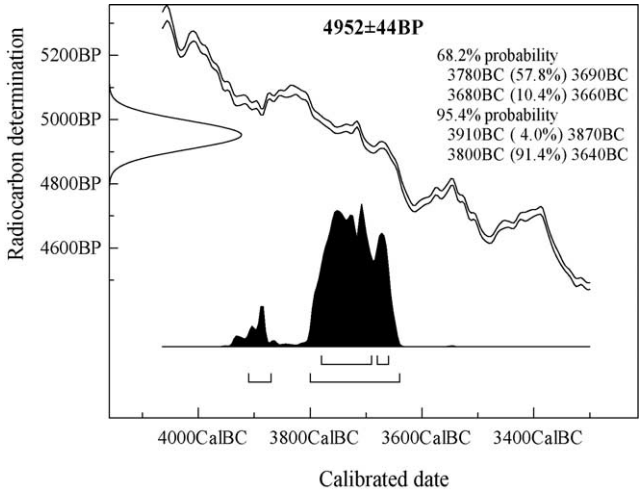



Fig. 3. Graphical presentation of the radiocarbon dating results according to the OxCal v.3.9 computer code. Weighted mean age of the two samples: Ua-12433 and Ua-12434.

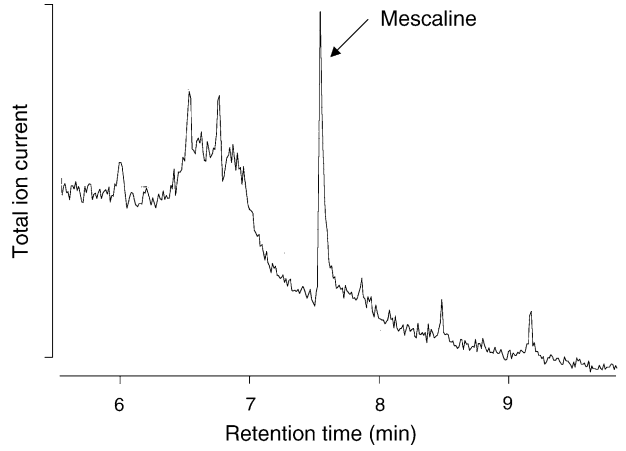



Fig. 4. GC–MS analysis (total ion chromatogram) of peyote alkaloid extract (sample Ua-12433).

The detection of mescaline in both of the two investigated samples, both analyzed by two methods based on different principles, is reliable evidence for the presence of this hallucinogenic drug. Recently dried "mescal buttons" can contain up to about 8% of total alkaloids, of which about 30% is mescaline (Bruhn and Holmstedt, 1974). In the present analysis, alkaloid content was approximately 2% and the only peyote alkaloid we could identify was mescaline. There was no trace of any of the tetrahydroisoquinoline alkaloids usually found in peyote (Kapadia and Fayez, 1973). In a previously studied 1000-year old specimen of peyote the alkaloid content was slightly higher, about 2.25%, and four tetrahydroisoquinoline alkaloids could be identified by GC–MS (Bruhn et al., 1978).

The age of the two specimens of peyote "buttons" that we have now dated is to be found in the calendric time interval 3780–3660 BC. The earlier reported radiocarbon date of 7000 years BP has not been formally published, only as a personal communication in a book review (Furst, 1989).

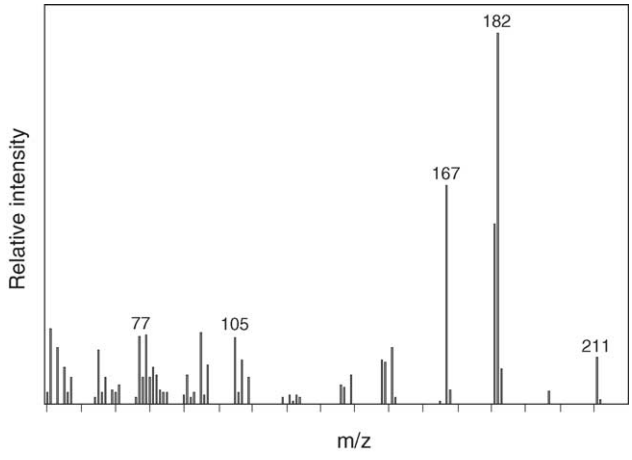



Fig. 5. Mass spectrum of mescaline peak in peyote alkaloid extract (sample Ua-12433).

Furst gives the following information: *"A new radiocarbon date has unexpectedly added six millennia to the cultural history of Lophophora williamsii,- - - the new C-14 assay was obtained by the isotope laboratory at UCLA from one of the two well-preserved plants that had languished, their historical significance unsuspected, for many years in the archaeological collections of the Witte Museum in San Antonio. The two plants were excavated in 1933 with other Desert Culture remains in one of the Rio Grande rock shelters known as the Shumla Caves. I would like to thank Rainer Berger, Director of the Isotope Laboratory in the Institute of Geophysics and Planetary Physics at UCLA, for the C-14 date on the Witte Museum's peyote sample,- - -"* (Furst, 1989). It has not been possible to obtain more information regarding that radiocarbon dating, which has been seriously questioned (Dering, personal communication).

Earlier, nicotine and caffeine have been identified in plant remains from a medicineman's tomb in Bolivia, 1600 years old (Bruhn et al., 1976; Holmstedt and Lindgren, 1972), and morphine has been found in a 3500-year-old ceramic container from Cyprus (Bisset et al., 1996).

The preservation of plant remains in archaeological sites varies greatly, depending upon the environmental setting. There are many changes that can take place in plant tissues during drying and/or processing, but under appropriate conditions of preservation alkaloids can obviously persist in plant material for extended periods of time (Raffauf and Morris, 1960). Thus, dry cave deposits in arid areas, such as Texas or Coahuila, are ideal for the recovery of plant materials (Willey, 1995). Dry, non-powdered plant tissues and cells may actually be regarded as containers that can help to protect the enclosed phytochemicals.

Interestingly, some South American mummies have been shown to contain cocaine metabolites, indicative of coca chewing. Coca leaves were chewed by many Andean pre-Columbian Indian groups, and the cocaine metabolite benzoylecgonine has been found in the scalp hair of 8 Chilean mummies with dates ranging from 2000 BC to 1500 AD (Cartmell et al., 1991). Baez et al. (2000) also studied hair of Chilean mummies for traces of cocaine, opiates and cannabis, but revealed exclusively negative results in all 19 samples. As discussed by Wischmann et al. (2002), the investigation of archaeological human remains for active substances from drugs requires specific analytical strategies that incorporate also their persistent metabolites.

The question how long humans have used psychoactive plants is impossible to answer (Schultes, 1998). In the Western hemisphere the above-mentioned findings of the seeds of *Sophora secundiflora* (Ort.) Lag. ex DC., now known as the red bean or mescal bean, seem to be the oldest (Naranjo, 1995). These seeds are found in the same and similar caves as the now analyzed cactus samples, but in much deeper strata and radiocarbon dated to 8440–8120 BC (Adovasio and Fry, 1976). However, there are some doubts as to the actual ingestion of these beans, which also have an important place as ornamental beads (Merrill, 1977).

Items of material culture recovered from the Shumla Cave excavations are similar to the paraphernalia used in peyote ceremonies by various Indian groups, and include rasping sticks made from bone or wood, a rattle made from deer scapula, a pouch and reed tubes containing cedar incense, and feather plumes (Martin, 1937; Stewart, 1987). Also, interpretation of the rock art pictographs from the Lower Pecos cultural area adds evidence indicating great antiquity for the use of peyote (Boyd and Dering, 1996).

From a scientific point of view, the now studied "mescal buttons" appears to be the oldest plant drugs which ever yielded a major bioactive compound upon phytochemical analysis. From a cultural perspective, our identification of mescaline strengthens the evidence that native North Americans already recognized and valued the psychotropic properties of the peyote cactus 5700 years ago.

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