Antibiotic Activity of an Extract of Peyote (Lophophora Williamii (Lemaire) Coulter)[1]

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Attempts to obtain antibiotics from diverse groups of plants have been numerous. These attempts have included such varied taxa as bacteria, lichens, mosses (1) and flowering plants (2).

The Arizona State Department of Liquor Control recently confiscated several sacks of peyote, *Lophophora williamsii* (Lemaire) Coulter, and turned a number of the plants over to the authors for research purposes.

The use of peyote in religious rites by many Indian tribes is common knowledge (3). In addition, curative properties for such varied ailments as toothache, pain in childbirth, fever, breast pain, skin diseases, rheumatism, diabetes, colds, and blindness, among other things, have been claimed for this plant by the same peoples (4). It is listed in the *Farmocopia Mexicana* where incorporation of the name into a native term, the verb "empeyotizarse," meaning self medication for the relief of a hangover after over indulgence in alcoholic beverages, is reported (5). The *U. S. Dispensatory* (6) lists peyote under the name *Anhalonium* and indicates its use to some extent in various forms of neurasthenia and hysteria and also in cases of asthma.

The alkaloid composition of this plant has been intensively studied (7). However, peyote is often listed under a synonym, *Anhalonium,* as *Anhalonium williamsii* or *Anhalonium lewinii.* In addition, the common name is also applied to plants which taxonomists place in different genera such as *Ariocarpus, Astrophytum, Pelecyphora, Stromboformis, Aztekium, Obregonia, Dolichothele,* and *Solisia,* so that some question may be raised as to the validity of many reports (8).

Methods

Extracts of whole peyote plants were prepared in various solvents and screened for antimicrobial activity. Ninety-five % ethanol yielded an extract exhibiting the best inhibition against bacterial growth. A 25% mixture (w/v) of plant material to ethanol was macerated for 15 minutes in a Waring blendor and filtered through coarse filter paper in a Buchner funnel to remove the dense pulp. Precipitation of water-insoluble material occurred following removal of the ethanol *in vacuo* in a 60° C water bath. A volume of distilled water equal to the volume of liquid remaining in the flask was then added and the precipitate removed by filtration. This crude supernatant was tested for antibiotic activity and showed positive microbial inhibition.

In an attempt to remove the organic acids and alkaloids, the following technic was employed. The supernatant was adjusted to pH 2 with 1 M HCl and maintained at 5° C for 24 hours during which time a fine precipitate developed. The material was filtered, the supernatant adjusted to pH 12 with 1 N NaOH, refrigerated for 24 hours, and a second precipitate removed by filtration. All precipitates, including the one recovered

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from *in vacuo* evaporation, were redissolved, adjusted to pH 7 and tested for antibiosis. The supernatant at this point was dark yellow and, in an attempt to remove the color, the extract was adjusted to pH 7, adsorbed with activated charcoal and filtered with the aid of Celite. The remaining colorless supernatant was evaporated to dryness, leaving a crystalline residue. The residue was dissolved by shaking for 2 hours in purified absolute methanol. Insoluble contaminating salts were removed by filtration and the supernatant methanol extract was evaporated to dryness. The crystalline substance obtained in this manner was considered to contain the principal active antibiotic substance and was given the name *Peyocactin*. The crystals were then dissolved in distilled water, adjusted to pH 7, and tested for antibiosis.

Antimicrobial Assay

Antibiotic activity was determined by placing 0.05 ml of the extract in Oxford cups set in Petri dishes containing 12 ml of Penassay Agar (Difco) seeded with 0.1 ml of a 24-hour broth culture of the test organisms (9, 10). The test organisms used in screening the peyocactin and the other extracts included *Staphylococcus aureus* (USDA 209), *Sarcina lutea* (USDA 1001), *Bacillus subtilis* (USDA 220), *Neisseria catarrhalis,* and *Escherichia coli* (ATCC 8677). The seeded plates were usually incubated for 18 hours and presence or absence of zones of inhibition recorded. Plates inoculated with enteric bacteria were read after only 12 hours of incubation. In an effort to obtain a more nearly complete antimicrobial spectrum of the peyocactin, numerous other bacteria and one pathogenic fungus were tested using the method described above (Table 1).

In each test, controls of physiological saline and the crude extract from which ethanol and precipitates had been removed were used. Because of the high level of activity exhibited by peyocactin against *S. aureus,* 18 penicillin-resistant strains of this organism were obtained and tested. All of the strains tested were found to be inhibited by peyocactin to approximately the same degree as the *S. aureus* (USDA 209) originally used. Ten of the resistant strains of *S. aureus* were known phage types[4] and the other 8 were coagulase positive and mannitol positive strains isolated from patients.[5]

[4]Kindly supplied by Dr. Griffith, V. A. Staphylococcus Ref. Lab., Batavia, N. Y.

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TABLE I

RESULTS OF ANTIMICROBIAL ASSAY OF PEYOCACTIN

Organism	Activity of* Peyocactin	Organism	Activity of* Peyocactin
Agrobacterium tumefaciens	++	*Proteus vulgaris*	0
Bacillus cereus	++	*Pseudomonas aeruginosa*	0
B. subtilis (USDA 220)	+++	*Salmonella pullorum*	0
Diplococcus pneumoniae	0	*S. typhimurium*	0
Escherichia coli (ATCC 8677)	+++	*Sarcina lutea* (USDA 1001)	++++
Klebsiella pneumoniae	0	*Shigella flexneri*	++
Micrococcus flavus	+++	*Staphylococcus aureus* (USDA 209)	+++
M. rubens	+++	*S. epidermitis*	+++
Mycobacterium phlei	++	*Streptococcus pyogenes*	++++
Neisseria catarrhalis	+++	*S. salivaris*	+++
Phytomonas campestris	+++	*Candida albicans*	+

* 0 — no activity noted
\+ — zone of inhibition only under Oxford cup
++ — idem. 8 to 10 mm diameter
+++ — idem. 11 to 15 mm diameter
++++ — idem. larger than 15 mm diameter

In Vivo Studies

Swiss-Webster white mice were used for preliminary animal toxicity tests and protection studies to indicate the degree of inhibitory action of peyocactin against fatal staphylococcal infection. In every case the protected animals survived while those in the control group succumbed within 60 hours after infection with *S. aureus*.[6]

Discussion

Results of *in vitro* studies with the ethanol extract of peyote indicate the presence of an antimicrobial agent. Attempts to purify this ethanol extract have resulted in the isolation of an unidentified substance appearing as amorphous crystals after evaporation of the methanol. The crystals are very soluble in water and other polar solvents. In spite of its solubility in water, however, the antimicrobial agent cannot be separated from the plant material by simple water extraction. Methanol acts as an effective solvent, showing little difference in the final activity from that of the ethanol extract. Peyocactin exhibits no solubility in hexane or other non-polar solvents.

Limited animal studies were performed with dissolved peyocactin so that an indication of toxicity and possible *in vivo* usefulness might be obtained. The material injected into the peritoneal cavity of mice was shown to be toxic in certain concentrations, but because different batches of the extract showed differing ranges of toxicity and antimicrobial activity, the toxicity could not be expressed in weight/volume relationship. Levels of non-toxic administrations were found that would protect mice against staphylococcal infection fatal to control mice. These results are considered to be only a presumptive preliminary study. With successful isolation of peyocactin in pure form, complete toxicity and *in vivo* protection studies will be undertaken.

[6]We appreciate the assistance of Dr. Kenneth F. Wertman, other members of Dept. of Bacteriology, and Univ. of Arizona for facilities and mice to carry on these preliminary tests.

Summary

A water-soluble crystalline substance, separated from an ethanol extract of *Lophophora williamsii* (Lemaire) Coulter, exhibited antibiotic activity against a wide spectrum of bacteria and a species of the imperfect fungi. The name peyocactin has been given to the principal antimicrobial component contained in this partially purified substance. Of particular interest was its inhibitory action against 18 strains of penicillin-resistant *Staphylococcus aureus*. Preliminary protection studies with mice suggest the *in vivo* effectiveness of peyocactin.

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