Phytochemistry, 1968, Vol 7, pp. 2031 to 2036. Pergamon Press. Printed in England

CACTUS ALKALOIDS

STANLEY D. BROWN, JOHN L. MASSINGILL, JR., and JOE E. HODGKINS

Chemistry Department, Texas Christian University, Fort Worth, Texas 76129

(*Received* 3 *April* 1968)

Abstract—Sixteen species of cacti were screened for alkaloids using gas chromatographic analysis in conjunction with Mayer's reagent. Twelve of the species were found to contain extractable alkaloids. Two species apparently contain quarternary amines since the crude extracts gave a positive test with the testing reagent but no bases could be extracted. Two species were found not to contain alkaloids. The gas chromatographic retention times of the principal alkaloids have been correlated with the retention times of twelve known alkaloids to obtain approximate molecular weights of the extracted alkaloids. Using this method three species, *Selenicereus grandiflorus*, *Echinocereus enneacanthus* var. *stramineus* and *E. chloranthus* were found to contain alkaloids whose molecular weights are larger than the known cactus alkaloids. This and other evidence suggests that the plant family Cactaceae contain alkaloids more complicated than the known β-phenylethylamines and tetrahydroisoquinolines.

INTRODUCTION

SINCE the 1890's when cacti were first reported to contain alkaloids, about eighty species have been examined for their presence, and they have been confirmed in approximately fifty of these species.[1-4] The purpose of this present study was to obtain information on the alkaloid content of species of cacti native to the southwestern United States. The plants selected for this study were not collected at random. With one exception, all the species belong to the cactus tribe Cereeae. The criteria used for collecting the plants were the accessibility of the wild plants and the fact that the subtribe was known to contain plants with alkaloids.

Table 1 presents the harvesting data for the plants investigated. The plants were collected from the general vicinity of Fort Worth, Texas; Big Bend National Park (Brewster Co., Texas); and around Phoenix, Arizona (Maricopa Co., Arizona). The plants were identified by professor Lyman Benson, Pomona College, Claremont, California, or W. H. Earle, Director of the Desert Botanical Gardens, Tempe, Arizona.

The dried powdered plant materials were extracted with 95 per cent ethanol. The solvent was removed and water added until *ca.* 300 ml of ethanol-free solution remained. Concentrated hydrochloric acid was added to give 5 per cent concentration and, after extraction with ether, the solution was tested with Mayer's reagent (KHgI).[5] The acidic solution was then made basic and extracted with chloroform, and the chloroform solution was then re-extracted several times with 5 per cent aqueous HCl. The acid-base extraction was repeated and the

[1] L. RETI, *Forschr. Chem. Organ. Naturstoffe*, **6**, 242 (1950), and references cited therein.
[2] C. DJERASSI, *Festschr. Arthur Stoll*, Berkhauser, Basilea, p. 330 (1957).
[3] L. RETI, in *The Alkaloids, Chemistry and Physiology* (edited by R. H. F. MANSKE and H. L. HOLMES), Vol. 4, p. 26, Academic Press, New York (1962).
[4] J. J. WILLAMAN and B. G. SCHUBERT, *U.S. Dept. Agr. Tech. Bull.* No. 1234, p. 57 (1961).
[5] B. T. CROMWELL, in *Modern Methods of Plant Analysis* (edited by K. PAECH and M. V. TRACEY), Vol. 4, p. 373, Springer-Verlag, Berlin (1955).

TABLE 1. HARVESTING DATA*

Tribe	Subtribe	Genera	Species	Area harvested	Month of harvest
Cereeae	Cereanae	*Carnegiea*	*gigantea*	Maricopa Co. Arizona	June
		Lemaireocereus	*prunosis*	Desert Botanical Gardens, Phoenix, Arizona	June
		Trichocereus	*pachanoi*	Desert Botanical Gardens, Phoenix, Arizona	June
	Hylocereanae	*Selenicereus*	*grandiflorus*	Desert Botanical Gardens, Phoenix, Arizona	June
	Echinocereanae	*Echinocereus*	*enneacanthus var. stramineus*	Brewster Co. Texas	November
		Echinocereus	*chloranthus*	Brewster Co. Texas	November
		Echinomastus	*dasyacanthus*	Brewster Co. Texas	April, November
		Echinocactus	*caespitosus*	Jack Co. Texas	October, April
		Echinocactus	*horizonthalus*	Brewster Co. Texas	November, April
		Echinocactus	*texensis*	Guadalupe Co. Texas	November, April
		Echinocactus	*polycephalus var. xeranthoide*	Desert Botanical Gardens, Phoenix, Arizona	June
		Ferrocactus	*wislizeni*	Maricopa Co. Arizona	June
	Coryphanthanae	*Neomammillaria*	*meiacantha*	Brewster Co. Texas	November
		Coryphantha	*macromeris*	Brewster Co. Texas	November
		Coryphantha	*vivipara*	Jack Co. Texas	October, April
Opuntieae		*Opuntia*	*schottii*	Brewster Co. Texas	November

* The system of Britton and Rose is used for the classifications.[6]

[6] N. L. BRITTON and N. J. ROSE, *The Cactaceae*, Vols. I–IV, Carnegie Institution of Washington, Washington, D.C. (1919–1923).

final chloroform solution analyzed by gas chromatography. If the gas chromatographic analysis after successive purifications contained peaks whose retention times represented compounds with molecular weights greater than 125, alkaloids were assumed to be present.

RESULTS AND DISCUSSIONS

Utilization of gas chromatography in a survey of this type has been shown to be an efficient method for the detection, separation, isolation, and identification of alkaloids.[7–9] In using a gas chromatograph, one is limited to compounds that are readily vaporized in the instrument and care must be taken to insure that there are no non-basic materials present in the sample. To insure that only organic bases were being chromatographed, the acid-base

TABLE 2. SCREENING RESULTS

Species	Mayer's test of crude extract	Mayer's test of pure bases	GLC retention times in min	Mol. wt (± 25)[7]
Carnegia gigantea	+	+	5·67, 5·45, 5·07, 9·58	205*, 218†
Lemaireocereus prunosis	+	+	2·58, 4·62, 5·30, 7·50	
Trichocereus pachanoi	+	+	2·67, 4·58, 5·00, 6·97 8·53, 10·37	
Selenicereus grandiflorus	+	+	2·75, 4·00, 4·58, 5·50 5·92, 10·08, 11·75	202, 204 290
Echinocereus enneacanthus var. *stramineus*	+	+	1·08, 1·25, 1·33, 1·83 2·08, 3·28, 4·28, 5·25 5·70, 13·25	130, 140 200, 312
E. chloranthus	+	+	4·75, 6·25, 6·58, 7·50 9·42, 10·42, 12·00 13·92, 17·50	375
Echinomastus dasyacanthus	+	+	2·58	130
Echinocactus caespitosus	+	+	5·32	199
E. horizonthalus	−	−		
E. texenis	+	−		
E. polycephalus var. *xeranthoide*	+	+	5·42, 6·42, 7·00	
Ferrocactus wislizeni	+	+	3·75, 4·08, 5·25, 5·83	200, 210
Neomammillaria meiacantha	+	+	5·50	202
Coryphantha macromeris	+	+	1·42, 2·08, 2·50, 3·67 4·00, 4·50, 5·83, 6·75 7·30, 11·58, 13·83	180, 190‡
C. vivipara	+	−		
Opuntia schottii	−	−		

* Carnegine; mol. wt. 221.[10]
† Gigantine; mol. wt. 237.[11]
‡ Macromerine; mol. wt. 225.[11]

[7] J. L. MASSINGILL, JR., and J. R. HODGKINS, *Phytochem.* **6**, 977 (1967).
[8] J. L. MASSINGILL, JR., and J. E. HODGKINS, *Anal. Chem.* **37**, 952 (1965).
[9] H. M. FALES and J. PISANO, in *Biomedical Applications of Gas Chromatography* (edited by H. A. SZYMANSKI), p. 62, Plenum Press, New York (1964).
[10] H. HEYL, *Arch. Pharm.* **266**, 668 (1928).
[11] J. E. HODGKINS, S. D. BROWN and J. L. MASSINGILL, *Tetrahedron Letters* **14**, 1321 (1967).

extraction was repeated until the gas chromatograms were essentially constant for a particular plant. This method also prevents the gas chromatographic detection of betaines, quarternary compounds and certain non-quarternary substances that might be regarded as alkaloids (psilocybin, lysergic acid). These compounds would be soluble in the alkaline-aqueous phase and would be eliminated by the acid-base extractions.

Table 2 presents the results of screening sixteen species of cacti. Of the cacti examined, twelve contained alkaloids that gave both positive Mayer's test and signals when the basic fraction was analyzed by gas chromatography. A rough approximation of the molecular weights of the alkaloids extracted can be obtained from Fig. 1, which is a plot of the retention

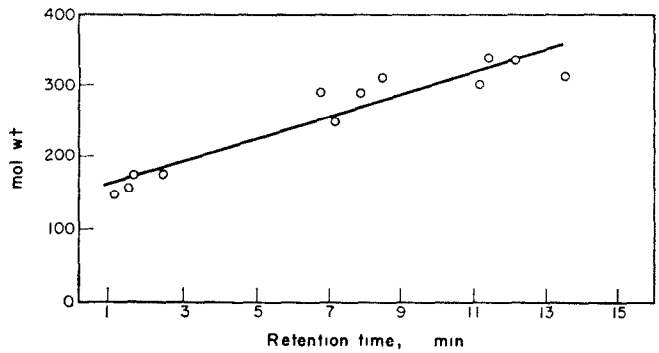
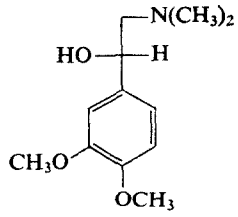


FIG. 1. PLOT OF THE RETENTION TIMES OF SOME KNOWN ALKALOIDS VS. THEIR MOLECULAR WEIGHTS ADAPTED FROM REF. 6.



(I)

times of twelve known alkaloids vs. their molecular weights.[6] The retention time of the principal compound or compounds is underlined in the table where such compounds were evident from the gas chromatograms. The extracts from three species (*Lemaireocereus prunosis*, *Trichocereus pachanoi* and *Echinocactus polycephalus*) did not contain any predominant peaks. The approximate molecular weights from the principal compounds of the other species are given in Table 2.

The cactus *Carnegiea gigantea* was extracted to obtain carnegine for a standard.[10] In doing this, we found that it contained another alkaloid which we have isolated and identified as an isomer of pellotine[1] and not, 1,2-dimethyl-4-hydroxy-6,7-dimethoxytetrahydroisoquinoline as previously proposed.[11]

Selenicereus grandiflorus has been reported to contain the unidentified alkaloid "cactine".[12] When we analyzed the basic extracts by gas chromatography on a 1 per cent JXR

[12] T. W. SULTAN, *N Y. Med. J.* p. 681 (1891).

column, we were not able to obtain good separation. The chromatogram on this column contained two broad bands in which the various minor constituents were shoulders, however, when this extract was chromatographed on a 1 per cent QF-1 column, separation of three major and four minor constituents was achieved.

Coryphantha macromeris contained the largest amount of pure bases. We have separated the principal alkaloid from this plant, macromerine, and proved the structure to be I by synthesizing the optically active alkaloid.[13] Macromerine has also been found in the related cactus *C. runyonii*.[14]

It is interesting to note that one species, *Echinomastus dasyacanthus*, when harvested in the spring contained one major alkaloid, but when harvested in the fall no bases were present. The extracts from three other species were the same when they were harvested during the spring and fall. One of these, *Echinocactus horizonthalus*, did not contain any basic material, nor did the crude extract give a positive test with Mayer's reagent. The crude extract of the other two, *E. texensis* and *C. vivipara*, gave a positive test with the alkaloidal testing reagent but did not yield any bases after the acid-base extractions, indicating these two plants contain quarternary alkaloids. Quarternary bases are known to occur in other species of cacti.[1]

From the previous work on cactus alkaloids, it has appeared that these plants contain bases of the same general pattern, β-phenylethylamines or tetrahydroisoquinolines.[1] The lone exception to this pattern is pilocereine[15] which is a trimer of three tetrahydroisoquinolines. With the exception of three species in this survey, the principle compounds all fall in the molecular weight range one would expect for these types of alkaloids. The remaining three plants, *S. grandiflorus*, *Echinocereus enneacanthus* var. *stramenius* and *E. chloranthus* contain principal alkaloids of molecular weights 330 ± 25, 380 ± 25, and 490 ± 25, respectively. Even though this is a very small sampling of the species of these two subtribes, it is interesting to note that no alkaloids have been previously identified from the two subtribes. We feel that a larger number of species from these two subtribes should be examined to determine if more of these plants contain alkaloids of this molecular weight range, and these identified to determine if there are more complicated alkaloids than β-phenylethylamines and tetrahydroisoquinolines in the plant family *Cactaceae*.

EXPERIMENTAL

Preparation of Plants for Extraction

The plants extracted are listed in Table 1. After the plants were harvested, the entire plant was cut into 1–2 cm^3 pieces and dried at 50–60°. The oven was attached to a water pump to create a weak current of air. The dried plants (*ca.* 10 per cent of the wet weight) were ground to a powder in a Waring blender and stored until extracted.

Extraction

500 g of dried plant material was extracted in a modified soxhlet extractor with alcohol and 0·5 per cent acetic acid for 4 days or until fresh extracts were colorless. The alcohol was removed on a steam bath at reduced pressure and water added and evaporated until an alcohol-free aqueous solution was obtained. Conc. HCl was added until the solution contained 5 per cent HCl. The aqueous solution was then tested with Mayer's reagent.[5]

[13] Publication pending on the absolute configuration.
[14] L. E. Below, A. Y. Leung, J. L. McLaughlin and A. G. Paul, *J. Pharm. Sci.* **57**, 515 (1968).
[15] Carl Djerassi, H. W. Brewer, Catherine Clarke and Lois J. Durham, *J. Am. Chem. Soc.* **84**, 3210 (1962).

Separation of the Bases

The acidic solution was extracted with ether until the etheral extracts were colorless. The acid solution was made basic with Na_2CO_3 or K_2CO_3 and extracted with $CHCl_3$ in a continuous liquid–liquid extractor. The $CHCl_3$ extracts were dried (Na_2SO_4), concentrated at reduced pressure, and analyzed by gas chromatography. The bases were extracted with aqueous 5 per cent HCl, the combined acidic solutions were made basic (K_2CO_3 or Na_2CO_3) and extracted several times with $CHCl_3$. The $CHCl_3$ extracts were dried (Na_2SO_4), concentrated at reduced pressure and gas chromatographed. This process was repeated until the last two gas chromatograms were essentially the same. The final solution was tested with Mayer's reagent.[5]

A Barber-Colman Model 5000 gas chromatograph equipped with a linear-temperature programmer and a flame-ionization detector was used. The column used was a 180 × 0·3 cm o.d. aluminium tubing packed with 1 per cent JXR on 100/200 mesh silanized Gas Chrom P (Applied Science Laboratories, State College, Pa.). The column used to separate the alkaloids of *Selenicererus grandiflorus* was 1 per cent QF-1 on 80/100 mesh silanized Diaport S. The flow rate of N_2 carrier gas was 60 ml/min. The column temperature was programmed from 100 to 300° (100–250° with QF-1) at 12° min; the injection port and detector bath were kept at 300°. The retention times were recorded from the time of injection to the top of the alkaloid peak.

Acknowledgements—This investigation was supported by a grant from the Texas Christian University Research Foundation (C6561). The authors wish to thank Professor Lyman Benson and Mr. W. H. Earle for the classification of our species of cacti.