CHEMOTAXONOMY OF COLUMNAR MEXICAN CACTI BY MASS SPECTROMETRY/MASS SPECTROMETRY

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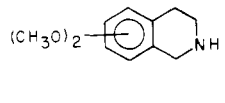
ABSTRACT.—Mass-analyzed ion kinetic energy spectrometry (MIKES) has been used to screen nine related Mexican columnar cactus species for the presence of a related series of tetrahydroisoquinoline alkaloids. The alkaloids can be identified in plant material or, with improved sensitivity, by analysis of an ethanolic extract containing only non-phenolic compounds. However, such solution work-up resulted in N-CH_3 to 1-CH_3 isomerization as determined by MIKES analysis of progressively developed samples (e.g., plant, ethanolic extract, non-phenolic fraction) for one of the tetrahydroisoquinoline alkaloids in three species of cactus. Differentiation between N-CH_3 and 1-CH_3 tetrahydroisoquinoline isomers is provided in the MIKE spectrum, allowing distinguishable classifications of the cacti to be developed (e.g., *C. gigantea* from other species). The MIKES results are compared and contrasted with currently accepted phylogenetic relationships.

Mass-analyzed ion kinetic energy spectrometry (MIKES) (1, 2, 3, 4) has been used to determine the distribution of a set of related tetrahydroisoquinoline alkaloids (**1–9**) in a number of Mexican columnar cactus species. Five *Pachycereus* species were studied along with four closely related species currently classified in other genera. Since alkaloids may be convenient indicators of phylogenetic relationships, the information obtained serves as a chemical guide to taxonomy (5, 6, 7). The results are compared to and generally agree with a recently proposed classification scheme (8).



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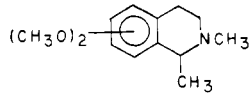

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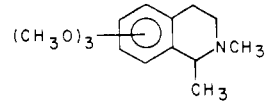

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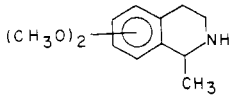

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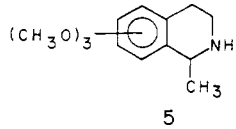

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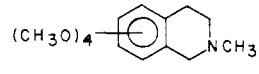

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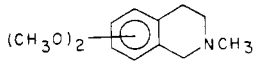

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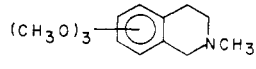

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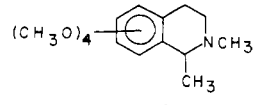

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MIKES is a particular approach to mass spectrometry/mass spectrometry (ms/ms), a method of mixture analysis in which both separation of individual constituents and their subsequent identification are accomplished by mass spectro-

metry (9, 10, 11). Samples are vaporized from the direct insertion probe, ionized by protonation with isobutane chemical ionization, and individual constituents are mass selected in a magnetic field, dissociated (12) by glancing collisions on N_2 (1–2 x 10^{-3} Torr) at 7 keV and characterized by energy analysis of the resulting fragments in an electrostatic field. MIKES offers several advantages to standard methods of alkaloid detection, isolation, and characterization, particularly with regard to eliminating, or at least minimizing, extraction and chemical manipulations; e.g., direct analyses of dry plant material are possible (13).

The possible presence of one or more of the alkaloids in individual cactus samples and extracts was indicated by the profiling of the chemical ionization mass spectrum over the probe temperature interval 80–200°C. A probe temperature of 130° was usually the optimum operating temperature; however, matrix effects were seen to vary this temperature optimum by as much as ±30°C. MIKE spectra of each ion of interest were recorded and compared to those of the authentic compounds to confirm the presence of individual alkaloids. Experiments were performed on powdered *Pachycereus pecten-aboriginum*, on a simple ethanolic extract, and on an extract containing only non-phenolic compounds (14). Significant improvement in the quality of the spectra occurred as sample preparation became more extensive (see figure 1). When the spectral quality

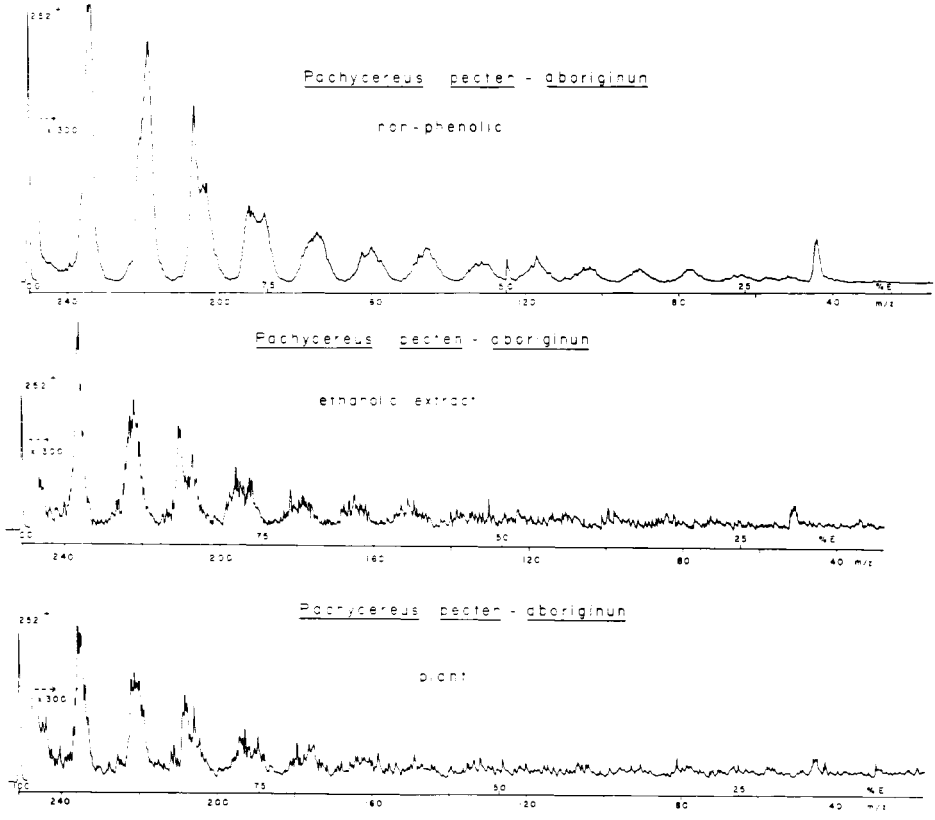

FIG. 1. MIKE spectra of m/z 252 for various samples (plant ethanolic extract, and non-phenolic extract) of *P. pecten-aboriginum* illustrating significant improvement in spectral quality with sample preparation.

was satisfactory for positive identification, the pure plant material was the sample of choice. Sample preparation was unnecessary, and avoidance of solution work-up eliminated the induction of *N*-methyl to l-methyl isomerization (see below).

The MIKES data are collected in table 1. In the genus *Pachycereus*, three different trends regarding alkaloid type and/or content can be clearly delineated: (1) a group containing a complete or nearly complete set of biogenetically related alkaloids [$(M+H)^+$ 194–282], as in the case of *P. weberi*, *P. pecten-aboriginum*, and *P. pringlei*; (2) species completely devoid of alkaloids, e.g., *P. hollianus*; (3) species having only the simplest alkaloids [$(M+H)^+$ 194 and 208], e.g., *P. marginatus*. It appears that *P. weberi* belongs in the genus *Pachycereus*, is on a common branch with *P. pecten-aboriginum* and *P. pringlei*, and is remote from *P. marginatus* and even further removed from *P. hollianus* (8). *P. weberi* and *P. hollianus* were formerly classified in the artificial genus *Lemairecereus* Br. and R. and were later moved to *Pachycereus* (15).

TABLE 1. Chemotaxonomy by MIKES.[a]

Species	Compound $(M+H)^+$	**1** 194	**2** 208	**3** 208	**4** 222	**5** 238	**6** 238	**7** 252	**8** 268	**9**[b] 282
Pachycereus pecten-aboriginum		+	−	+	+	−	+	+	+	+[c]
Pachcereus weberi		+	+[e]	+	+	−	+	+[d]	+	−
Pachycereus pringlei		+	−	+	+	−	+		+	−
Carnegea gigantea		[c]	+	−	+	+	−	+	−	−
Backebergia militaris		+	+[e]	+	−	−	−	−	−	−
Pachycereus marginatus		−	+[e]	+	−	−	−	−	−	−
Lophocereus schottii		+	−	+	−	−	−	−	−	−
Pachycereus hollianus		−	−	−	−	−	−	−	−	−
Neobuxbaumia euphorbioides		−	−	−	−	−	−	−	−	−

[a]Results are for a non-phenolic fraction (14) from an ethanolic extract of plant material. Key: +, positive identification of alkaloid; −, alkaloid not present.

[b]No reference compound was available for **9**. Its presence was inferred by interpretation of the MIKE spectrum.

[c]This alkaloid may be present, however, it is not definitely assignable at the current detection limit.

[d]This alkaloid appears to be present, however, interference from another ion of the same m/z shows a fragment at 179^+ in the MIKE spectrum.

[e]Probably an artefact of the extraction, see text.

The alkaloid pattern of *C. gigantea* somewhat resembles those of the more evolved species of *Pachycereus*; a striking difference, however, is the ring closure unit, which in the case of *Carnegiea* always produces a l-methyl isomer. The isomers **2** and **3** (fig. 2) are probably formed by quite different biogenetic pathways, the former arising by condensation of a C_2 unit (or pyruvic acid with subsequent decarboxylation) with a phenylethylamine unit. The latter probably involves two successive C_1 unit elaborations, viz., glyoxalic acid with subsequent decarboxylation for the ring cyclization and an independent *N*-methylation step (16). It is apparent that *Carnegiea gigantea* might have been grouped with *P. pringlei* were a method such as MIKES, which is capable of distinguishing isomers, not employed. In the case of *B. militaris*, the chemical information shows distinct differences (less complex alkaloids) as compared to the *Pachycereus* species. Segregation of both *Carnegiea* and *Backebergia* into monotypic genera are thus supported by the alkaloid chemotaxonomy. For *L. schottii* the alkaloid content was similar to that of *P. marginatus* and suggests a closer relationship than is now accepted between these two species. The single *Neobuxbaumia* species studied was com-

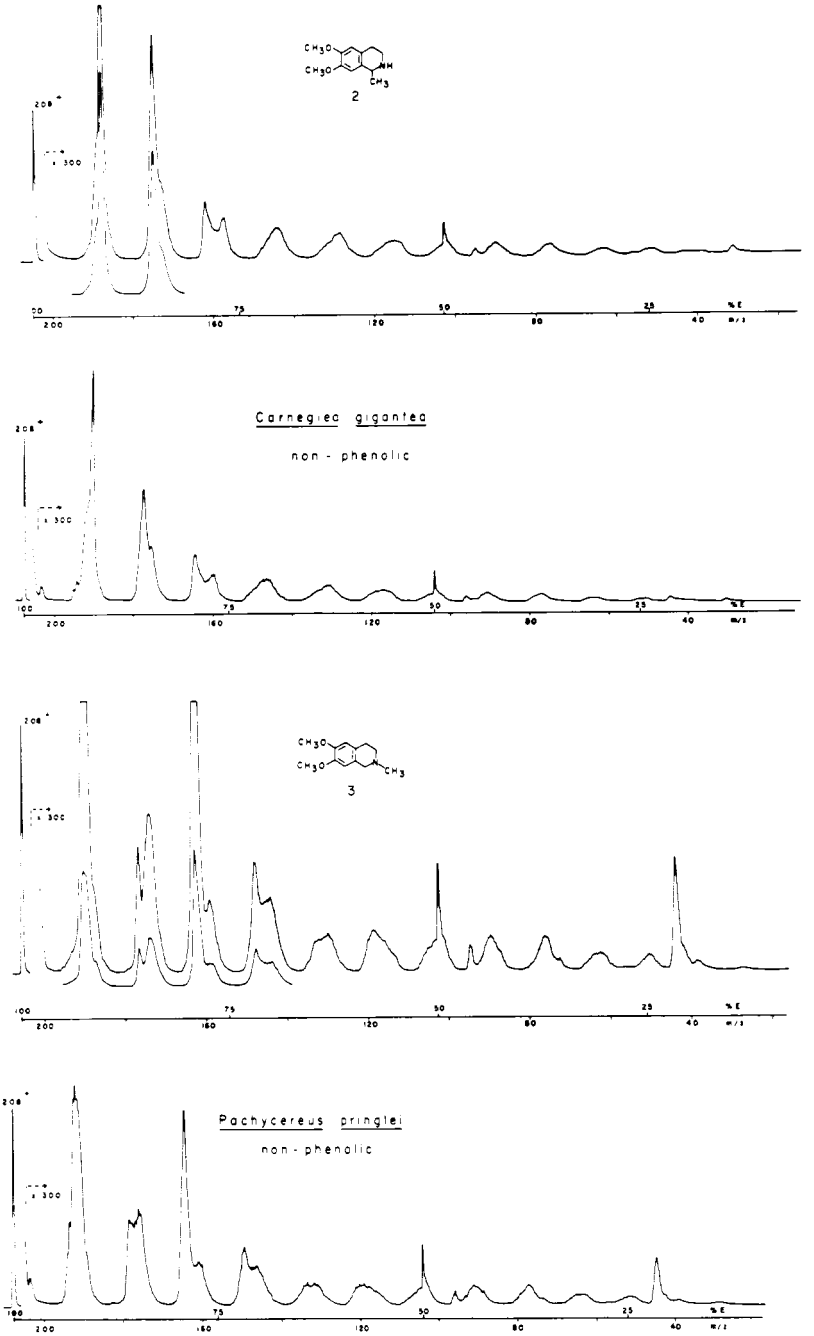

FIG. 2. MIKE spectra of *m/z* 208 (protonated dimethoxymethyl tetrahydroisoquinoline) showing that the 1-methyl and N-methyl isomers **2** and **3** give substantially different spectra and that extracts of *C. gigantea* and *P. pringlei* can be identified as containing compounds **2** and **3**, respectively.

pletely devoid of these alkaloids and would seem remote from the *Pachycereus* species.

Figure 2 shows that the MIKE spectra provide unambiguous identifications of the isomers l-methyl and *N*-methyltetrahydroisoquinoline and that the plant extracts can be clearly assigned to one or the other isomer. Since MIKES can also directly analyze plant samples, it can be used to avoid artifact-forming reactions occurring in solution prior to the actual analysis. Isomerization of *N*-methyl to l-methyltetrahydroisoquinolines in solution could be followed from MIKE spectra of several samples, particularly, *P. weberi*, *P. marginatus*, and *B. militaris*. For example, a non-phenolic extract of *Pachycereus weberi* showed in the MIKE spectrum at *m/z* 208, $(M+H)^+$, for **2** and **3** (fig. 2), fragments at *m/z* 191–193, 176–179, and 165 of the intensity ratio 7:5:4. However, the authentic l-methyltetrahydroisoquinoline gives fragments at *m/z* 191, 179, and 165 of intensity 7:4:1, and the N-methyl isomer yields fragments at *m/z* 192–193, 176, and 165 of intensity 3:1:4. These results indicate an approximately 50:50 mixture of the two isomers in this extract. MIKE spectra of the pure plant material indicated only the presence of the *N*-methyl isomer. The isomerization represents a particularly insidious case of artifact formation, since both series of alkaloids, i.e., l-methyl and *N*-methyl, were found to be naturally occurring in these closely related cactus species.

These observations reinforce the potentially important complementary role for MIKES to standard methods of natural products chemistry. While gas chromatography/mass spectrometry might also have been successfully applied, the fact that a solution would have had to be studied would have limited its value. MIKES advantages include: (1) high information content and specificity, *N*-methyl and l-methyl isomers are readily distinguished; (2) low detection limits, 1 g of plant material was used for the extraction; (3) short analysis time; and (4) minimization of sample preparation. The future usefulness of MIKES to chemotaxonomic screening studies is quite apparent.

EXPERIMENTAL

PLANT MATERIALS.—Field-dried specimens of shredded *Pachycereus weberi* (Coult.) Br. and R. were received from the Medicinal Plant Resources Laboratories, Agricultural Research Center, U.S. Department of Agriculture, Beltsville, Maryland. Specimens of *Carnegia gigantea* (Engelm.) Br. and R. were purchased from El Paso Cactus Gardens, El Paso, Texas. Specimens of *Pachycereus pringlei* (S. Wats.) Br. and R. were purchased from Grigsby Cactus Gardens, Bella Vista, California. Specimens of *Neobuxbaumia euphorbioides* (Haw.) Buxb. were purchased from Abbey Garden, Reseda, California. Specimens of *P. marginatus* (DC.) Br. and R. were obtained from C. M. Fitzpatrick, The Cactus Gardens, Edinburg, Texas. Specimens of *P. hollianus* (Web.) Buxb. were obtained from Modlin's Cactus Gardens, El Corto Visto, California, and Huntington Botanical Gardens, San Marino, California.

Specimens of *Backebergia militaris* (Audot.) Bravo ex Sanchez Mejorada were obtained from Dr. A. Gibson, University of Arizona, Tucson, Arizona, and specimens of *P. pecten-aboriginum* (Engelm.) Br. and R. were obtained from Mr. R. S. Felger, Arizona-Sonora Desert Museum, Tucson, Arizona.

Heliamine (6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline), *oxymethylcorypalline* (2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline), *salsolidine* (1-methyl-7,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline), *carnegine* (1,2-dimethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline), *tehuanine* (2-methyl-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline), *weberine* (2-methyl-5,6,7,8-tetramethoxy-1,2,3,4-tetrahydroisoquinoline), and *oxymethyl pellotine* (1,2-dimethyl-7,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline) hydrochlorides, which have been either synthesized or isolated in previous work (17), were used as reference alkaloid materials.

The fresh plants were photographed, checked for taxonomic verification, and freeze-dried in a Virtis freeze dryer model VSM-15. The dried plant materials were ground through a 2 mm screen in a Wiley mill.

EXTRACTION PROCEDURES.—One gram of the pulverized plant material was extracted by maceration for 12–18 hours in 95% ethanol. The ethanolic extract was concentrated to dryness *in vacuo*.

The non-phenolic fraction of the ethanolic extract was prepared on an Amberlite IRA 401S (Mallinkrodt) anion exchange column in the hydroxide form. The column effluent was collected (14) and concentrated to dryness *in vacuo*.

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