CACTUS ALKALOIDS. XLIX. NEW TRACE ALKALOIDS (DEHYDROSALSOLIDINE AND HELIAMINE) FROM THE SAGUARO, *CARNEGIEA GIGANTEA*, AND CONFIRMATION BY MIKES (MS/MS)

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ABSTRACT.—In a reinvestigation of the alkaloids of the title cactus species, two trace alkaloids were isolated: heliamine (6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) (0.007% yield), previously known from other cereoid cacti, and 1,2-dehydrosalsolidine (1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline) (0.006% yield), a novel fluorescent, tertiary amine. Mikes (ms/ms) analysis of the plant material and extracts suggests that the new alkaloid is not an extraction artifact. This is the first isolation of a dihydroisoquinoline from the Cactaceae; such compounds are speculated to be precursors of the more common tetrahydroisoquinolines; the use of ms/ms to detect traces of potential biosynthetic intermediates is emphasized.

The saguaro cactus, *Carnegiea gigantea* (Engel.) Br. and R., is native to southern Arizona and northern Sonora (1). Its history of folkloric and economic uses has been previously recorded and reviewed (2–4).

Alkaloids were first reported in this species in 1928 (5), and seven have been previously isolated or identified; these are carnegine (1,2-dimethyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) (5, 6); gigantine (1,2-dimethyl-5-hydroxy-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) (7–9); salsolidine (**6**) (norcarnegine; 1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) (10, 11); arizonine (1-methyl-7-methoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline) (11); dopamine (**1**), 3-methoxytyramine (**2**), and 3,4-dimethoxy-β-phenethylamine (**3**) (11). Previous screening of the nonphenolic alkaloids by mass-analyzed ion kinetic energy spectrometry (mikes, ms/ms) detected new trace alkaloids at m/z (MH^+) 194, 238, and 252 (12), so a reinvestigation of the trace alkaloids of this species was initiated.

Use of the usual procedures for the isolation of cactus alkaloids (13), resulted in some carnegine being crystallized directly from fraction B. Fraction A was resolved into phenolic and nonphenolic portions (14), and, after acid-base partitioning, gigantine was crystallized directly from the phenolic portion. The previous isolations of these two compounds have involved more elaborate procedures.

Separation of the nonphenolic portion of fraction A, by column adsorption chromatography on silica gel, yielded salsolidine and additional carnegine. Preparative tlc of combined column fractions 22–31 and 32–36 yielded 1,2-dehydrosalsolidine (**5**), a new fluorescent tertiary alkaloid. Acid-base partitioning of combined column fractions 78–102 yielded heliamine (6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline); this alkaloid is the unknown compound detected equivocally at MH^+ m/z 194 in the previous mikes screen (12). The identifications of the isolated dehydrosalsolidine and heliamine hydrochlorides were based on cims, cochromatography in tlc, mp, mmp, ir, and ^1H nmr and were confirmed by comparisons with respective synthesized and isolated (15) compounds.

Dehydrosalsolidine (**5**) might function as an intermediate in the biosynthesis of salsolidine (**6**). The isolation of this dehydro compound supports the work of Kapadia *et al.* (16), who isolated radioactive dehydroanhalonidine and anhalonidine (1-methyl-6,7-dimethoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline) after slices of peyote cactus were incubated with radioactive peyoruvic acid (1-methyl-1-carboxyl-6,7-dimethoxy-8-hydroxy-1,2,3,4-tetrahydroisoquinoline). A rapid *in*

vivo reduction of dehydrosalsolidine to salsolidine might then account for the relatively low yield (0.006%) of the former from the saguaro plant material.

Because the amount of isolated dehydrosalsolidine was very small, it might have been formed from salsolidine during the extraction process. To demonstrate that this compound was not an extraction artifact and is, indeed, naturally occurring, mikes was employed by a temperature (vaporization) profile technique (17). The powdered plant material, a simple chloroform extract, and the nonphenolic portion of fraction A were analyzed.

Because of the low concentration of dehydrosalsolidine in the plant powder, a corresponding peak at *m/z* 206 was not seen with cims at low direct probe temperature, but at high temperature the two peaks (*m/z* 206 for dehydrosalsolidine and *m/z* 208 for salsolidine) occurred with an intensity ratio (206/208) less than one. In the chloroform extract, peak ratios of greater than one at low temperature decreased to less than one at intermediate temperature; however, if the sample was left in the instrument for ten minutes at this temperature, the peak ratio again became greater than one. The inversion of peak ratio was also observed, without the time delay, at high temperatures. This observation suggested that in the chloroform extract, a second source of ions at *m/z* 206 was present and responsible for delayed volatilization. With the nonphenolic extract, the cims spectra were comparable to those from the chloroform extract except that the peak ratio consistently remained less than one; the second source of ions at *m/z* 206 seemed to have been removed by the anion exchange resin which was used to resolve the phenolic and nonphenolic alkaloids. Figure 1 illustrates the cims temperature profile in the mass range *m/z* 190–225 of the chloroform extract.

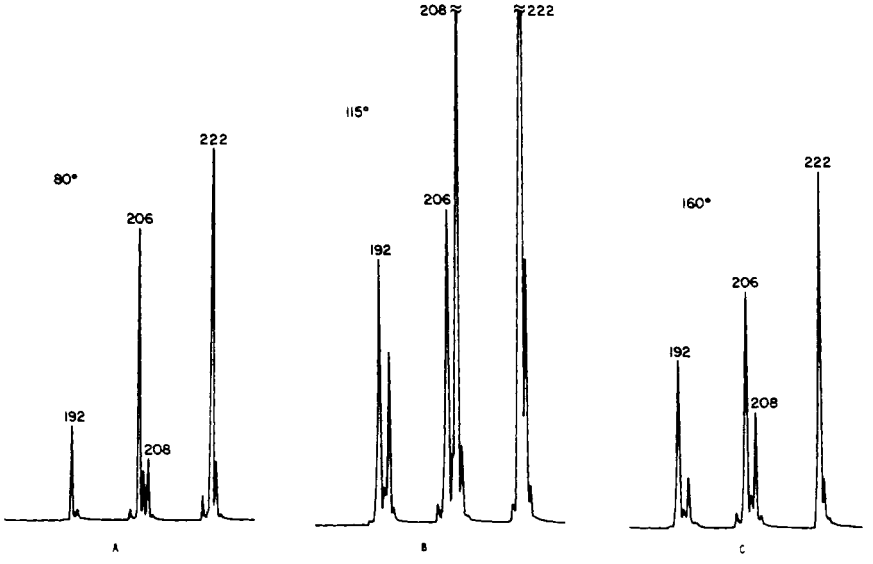

FIG. 1. CIMS temperature profile of chloroform extract of *Carnegiea gigantea* at approximate temperatures of 80° (A), 115° (B), and 160° (C).

To be certain that salsolidine at *m/z* 208 was not being converted to *m/z* 206 during the cims analysis, a temperature profile was determined with pure salsolidine; peaks at *m/z* 206 were insignificant when salsolidine was analyzed.

To insure that the compound detected at *m/z* 206 in the plant material, the chloroform extract, and the nonphenolic fraction was consistently dehydrosalsolidine, mike-cid (collison induced dissociation) spectra of this ion from these samples were obtained at various temperatures. All of the mike spectra of the ions at *m/z* 206, independent of temperature, were identical to those of isolated and synthesized dehydrosalsolidine. Thus, the ions detected at *m/z* 206 were always

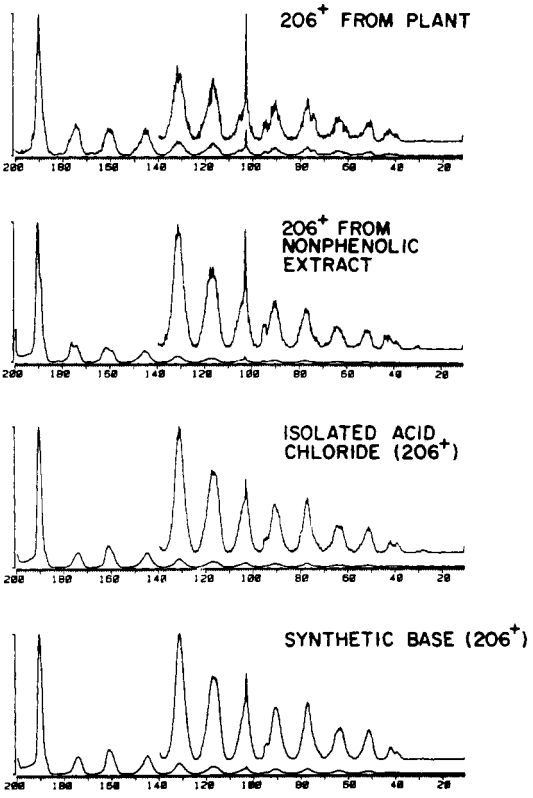

FIG. 2. MIKE spectra of compound at *m/z* 206 (dehydrosalsolidine) from the plant powder, the nonphenolic fraction, isolated compound (hydrochloride salt), and the synthetic compound as the free base.

from dehydrosalsolidine. Some of these data are illustrated in figure 2. Closer examination of the cims spectra of the crude extracts shows evidence for the presence of additional dihydroisoquinolines, e.g., note the peak at *m/z* 192 (dehydroheliamine) which accompanies heliamine at *m/z* 194 (figure 1). Note, too, that carnegine at *m/z* 222, being *N*-methylated, is not accompanied by a corresponding dihydro compound at *m/z* 220.

The compound (second source) which produced additional ions at *m/z* 206 at higher temperature from the chloroform extract merits some further discussion. Considering the proposal of Kapadia *et al.* (16) for tetrahydroisoquinoline biosynthesis, this compound might have a structure similar to peyoruvic acid. Such an acid might be expected to decarboxylate at high temperatures and would have been eliminated from the nonphenolic portion by the anion exchange resin. These data suggest a logical scheme for salsolidine (**6**) biogenesis (figure 3); all of the illustrated compounds in this scheme have at least some evidence for their natural occurrence in *C. gigantea;* alternative sequences may also be proposed, e.g., dopamine (**1**) and/or 3-methoxytyramine (**2**) may, in actuality, be condensed with the pyruvate moiety. The power of the mikes technique in detecting small amounts of potential biosynthetic intermediates, such as dehydrosalsolidine (**5**) and the indirect evidence for its precursor acid (**4**), is especially emphasized.

The nonphenolic alkaloid detected at *m/z* 238 in large abundance in the original cims analysis (12) was essentially absent in the nonphenolic portions assayed in this current study; this peak is now attributed to contamination of the original nonphenolic extract with the abundant phenolic alkaloid, gigantine (MH^+ 238).

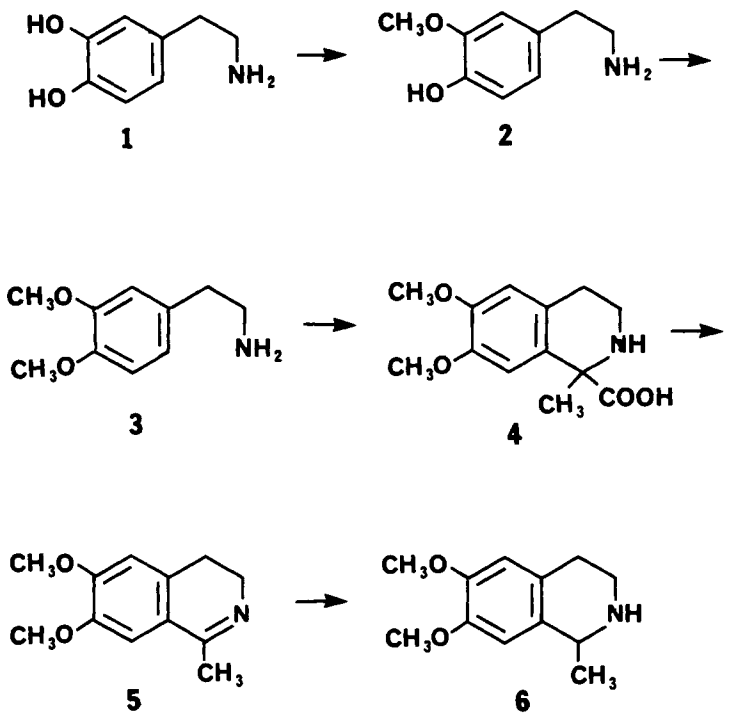

FIG. 3. Proposal for the biogenesis of salsolidine.

The trace alkaloid at *m/z* 252 was not detected in the current isolation study and remains unidentified; however, it is likely a methoxylated derivative of carnegine, possibly O-methylgigantine. Further studies to detect and isolate additional fluorescent dihydroisoquinolines in cacti are now indicated.

EXPERIMENTAL[1]

PLANT MATERIAL.—Whole plants, 0.5–2 m in height, were purchased from El Paso Cactus Gardens, Box 133, Anthony, New Mexico 88021, in July 1969; identifications were made by Mr. Clark Champie of El Paso, Texas, and the material conformed to published descriptions (1); reference photographs are on file. The plants were sliced, oven dried at 40–50°, and pulverized through a 2 mm screen in a Wiley Mill.

ALKALOID EXTRACTION AND FRACTIONATION.—The powdered plant material (1.0 kg) was defatted in a Soxhlet-type extractor with petroleum ether (30–60°) for 72 hr. The dried defatted material was then moistened with chloroform-methanol-conc. ammonium hydroxide (2:2:1) and packed into a large percolator. After maceration with chloroform-methanol-conc. ammonium hydroxide (90:9:1) for 8 hr, slow percolation with chloroform (15 liters) was performed. The residue from the chloroform extract was processed through acid-base partitioning to prepare fraction A (31.6 g), fraction B (3.5 g), and fraction C (8.3 g) (13).

ISOLATION OF CARNEGINE.—Fraction B was dissolved in a small volume of absolute ethanol and left overnight. Crystals of carnegine base formed and were recrystallized from absolute ethanol yielding 600 mg [0.06% yield, tlc systems A,C,E,F, and G (13, 18)]; the hydrochloride was identical to carnegine hydrochloride [ir, mp and mmp 210°, lit. mp 209–211° (9)].

ISOLATION OF GIGANTINE HYDROCHLORIDE.—A portion of fraction A (5.5 g) was dissolved in ethanol and resolved into phenolic and nonphenolic portions; 65 g of Amberlite IRA-400

[1]Melting points were determined with a Mel-Temp apparatus and are uncorrected. Ir spectra were obtained by use of KBr pellets in a Beckman IR-33 spectrophotometer. Mass spectra were determined on a Hitachi RMU-6 spectrometer; the mike spectrometery system was used as previously described (12, 17). ^{1}H nmr were determined on a Varian FT-80 with $CDCl_3$ with TMS as internal standard. Analytical tlc plates were Bakerflex SG IB2-F, and preparative tlc plates (20 x 20 cm) were prepared with a 1–2 mm thickness of SG 60 F-254 (Brinkman); the tlc solvent systems (A–G) and spray reagents have been previously described (13, 18). Reference heliamine hydrochloride was isolated from *Pachycereus weberi* (15); reference dehydrosalsolidine hydrochloride was made previously as an intermediate product in the synthesis of carnegine (19).

(hydroxide form) (14) was used. The acidic column eluates containing the phenolic fraction were basified with sodium hydroxide to pH 9.5 (pH paper) and extracted two times each with equal volumes of chloroform and ethyl ether. The combined chloroform-ethyl ether layers were dried over anhydrous sodium sulfate and reduced to dryness under rotary vacuum evaporation. Dissolution in absolute ethanol, acidification with 5% hydrogen chloride in absolute ethanol, and addition of anhydrous ethyl ether produced a crude alkaloid hydrochloride. Recrystallization from absolute ethanol yielded 326 mg of gigantine hydrochloride [0.18% yield, tlc in systems A,C,E,F, and G (13, 18), ir, mp and mmp 223°, lit. mp 221.5–222.5° (9)].

ISOLATION AND IDENTIFICATION OF DEHYDROSALSOLIDINE (5).—The nonphenolic portion of fraction A (3.5 g) showed at least four different alkaloids (tlc in solvent C). Two of these alkaloids were secondary amines and the other two were either tertiary or quaternary amines (18). One of the secondary amines was identified (tlc) as salsolidine, and one of the tertiary amines was identified (tlc) as carnegine. Crystallization of the hydrochloride (absolute ethanol-ethyl ether) of the entire nonphenolic portion yielded a mixture (1.14 g) of carnegine and salsolidine hydrochlorides. The residue (2.0 g) from the mother liquor was obtained, and a portion (1.3 g) was subjected to column adsorption chromatography (3 x 60 cm column, 140 g of silica gel 60, 0.063–0.22 mm, E. Merck). A mixture of chloroform-methanol-conc. ammonium hydroxide (500:50:1) was used as a mobile phase; a total of 107 fractions of 30 ml each were collected.

After analytical tlc (solvent C), the column fractions were combined according to their similar alkaloid content. Fractions 22–31 showed a major alkaloid (carnegine) and a minor alkaloid (an unknown fluorescent tertiary or quaternary amine). Preparation of the hydrochloride of the mixture yielded 353 mg of additional carnegine hydrochloride. The mother liquor was then separated by preparative tlc (5 plates, single development in solvent C). Chloroform-methanol (1:1) was used to elute the band containing the minor alkaloid. Preparation of the hydrochloride yielded 3 mg from this fraction; an additional 4 mg was obtained by similar preparative tlc of column fractions 32–36 (8 plates, single development in solvent C). This gave 7 mg (0.006% yield) of the new, minor, fluorescent alkaloid.

The cims of the new alkaloid hydrochloride showed MH^+ m/z 206; the known alkaloid salsolidine has MH^+ 208, and, because the fluorescence indicated a possible double bond conjugated with the aromatic ring, 1,2-dehydrosalsolidine was suspected. The ^1H nmr spectrum was similar to that of salsolidine but with a down-field chemical shift and small long range coupling ($J=1.5$) for the 1-methyl protons: δ 6.96 (1 H, s, =CH), δ6.66 (1 H, s, =CH), δ 3.89 (3 H, s, OCH_3), δ 3.88 (3 H, s, OCH_3), δ 3.61 (2 H, m, 3-CH_2), δ 2.62 (2 H, m, 4-CH_2), and δ 2.34 (3 H, t, $J=1.5$, 1-CH_3). The ir spectrum gave peaks at V max (cm^{-1}): 2920, 2820, 1615, 1590, 1560, 1500, 1440, 1400, 1315, 1225, 1190, 1135, 1040, 990, 850, and 800. Tlc cochromatography (systems A, C, E, F, and G) with reference synthetic compound identified the isolated compound as dehydrosalsolidine, **5**, (1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline); mp 195–197°, reference mp 201–202° (19), mmp no depression; ir and mikes identical with reference.

ISOLATION OF SALSOLIDINE (**6**).—Combined fractions 42–61 from the chromatography column yielded 120 mg (absolute ethanol-ethyl ether) (0.096% yield) of salsolidine hydrochloride [tlc systems A,C,E,F, and G (13, 18), ir].

ISOLATION AND IDENTIFICATION OF HELIAMINE.—Tlc indicated that combined fractions 78–102 from the chromatography column contained a nonphenolic secondary amine, other than salsolidine, which had to be new to this species. An attempt to crystallize the hydrochloride failed, so the acidic residue was dissolved in a small amount of water, extracted with chloroform and ethyl ether, basified with sodium hydroxide, and reextracted with chloroform-ethyl ether. The alkaloid residue from the second chloroform-ether extracts then yielded 9 mg (0.007% yield) of crystalline hydrochloride. Cims indicated an m/z 194 for MH^+ corresponding to a dimethoxytetrahydroisoquinoline. The isolated salt was identical to heliamine hydrochloride: tlc solvents A, C, E, F, and G; mp, mmp, 243°, lit. mp 248° (15); ir, ^1H nmr, and eims spectra were essentially identical to those of the reference.

MIKES ANALYSES.—Powdered plant material, the residue from a basic chloroform extract (13), and the nonphenolic portions of fraction A (13) were analyzed by isobutane ionization in the mikes instrument (12, 17) at various temperatures (50–275°) (e.g., see figure 1). The hydrochloride and free base of the isolated dehydrosalsolidine and reference dehydrosalsolidine base were analyzed at 80° and gave identical collision-induced dissociation (cid) fragmentations (air, 10^{-3} Torr, 7 keV) as the m/z 206 peak in the crude extracts (figure 2). Analyses of reference salsolidine hydrochloride (80–220°) gave no significant peak at m/z 206, eliminating the possibility of this origin for the m/z 206 peak.

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