CACTUS ALKALOIDS. XXXIX. A GLUCOTETRAHYDROISOQUINOLINE FROM THE MEXICAN CACTUS, *PTEROCEREUS GAUMERI*

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ABSTRACT.—An unusual glucoalkaloid has been crystallized from the title cactus plant. Acid employed in the usual alkaloid extraction schemes decomposed the new nonphenolic alkaloid (pterocereine) into a new phenolic alkaloid (deglucopterocereine) and glucose. β-Glucosidase also hydrolyzed the glucoalkaloid. Spectral analysis of the structures indicated that pterocereine is (−)-1-hydroxymethyl-2-methyl-5-β-*O*-glycosyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline. Further structural conformation was accomplished by carbon-13-hydrogen long-range coupling analysis. Neither compound exhibited significant cytotoxicity in the 9 KB system.

Most cactus alkaloids are either β-phenethylamines or the related simple tetrahydroisoquinolines. In a continuing chromatographic screening of cacti for new alkaloids, unknown compounds were detected in *Pterocereus gaumeri* (Br. & R.) MacDoug. & Mir., a species that is native to the Yucatan in Mexico (1–3). The plant apparently has no folkloric uses but is commercially available in the United States as an ornamental.

Following our usual procedures for extraction of cactus alkaloids (4), a large amount of plant material (520 g) was defatted, basified, and extracted by chloroformic percolation. The chloroform residue was extracted with 1 N hydrochloric acid which was, in turn, extracted with chloroform and ethyl ether to remove nonalkaloidal impurities (extract B). The aqueous solution was then basified and re-extracted with the organic solvents to produce extracts A (alkaloids) and C (water soluble alkaloids). Anion exchange chromatography was performed on the combined extracts A and C to resolve the alkaloids into phenolic and nonphenolic fractions.

A new alkaloid (pterocereine) crystallized as the free base from the nonphenolic fraction and also from the water rinse of the ion exchange column (0.062% yield). A new phenolic alkaloid (subsequently identified as deglucopterocereine) was crystallized as the hydrochloride from the phenolic fraction. Column chromatography of the mother liquor from the nonphenolic fraction resolved additional alkaloids but these were decomposed in attempts to crystallize them as their hydrochlorides. Preparative thin-layer chromatography (tlc) of the mother liquor from the phenolic fraction resulted only in a small additional quantity of deglucopterocereine (0.164% total yield).

Pterocereine gave unusually low R_f values, indicating unusually high polarity, in tlc systems designed for cactus alkaloids (5, 6). The chemical ionization ms (low resolution) gave an M+1 peak at 416 with a base peak at 254 suggesting loss of $C_6H_{10}O_5$ (possibly a glucose unit); minor peaks at 398 and 384 indicated respective loss of water and methanol. Electron impact ms (high resolution) failed to detect the molecular ion, but the peak at 384 sufficed for an exact mass measurement of the molecular ion minus CH_2OH; the molecular formula was, thus, established to be $C_{19}H_{29}O_9N$. A positive Molish test supported the sug-

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gested presence of a sugar moiety in the molecule. Tlc of hydrolysis products revealed that both β-glucosidase and acid hydrolyzed pterocereine to yield glucose and the phenolic alkaloid. The phenolic alkaloid was thus identified as deglucopterocereine—the glucose being bonded in β-linkage to a phenolic hydroxyl.

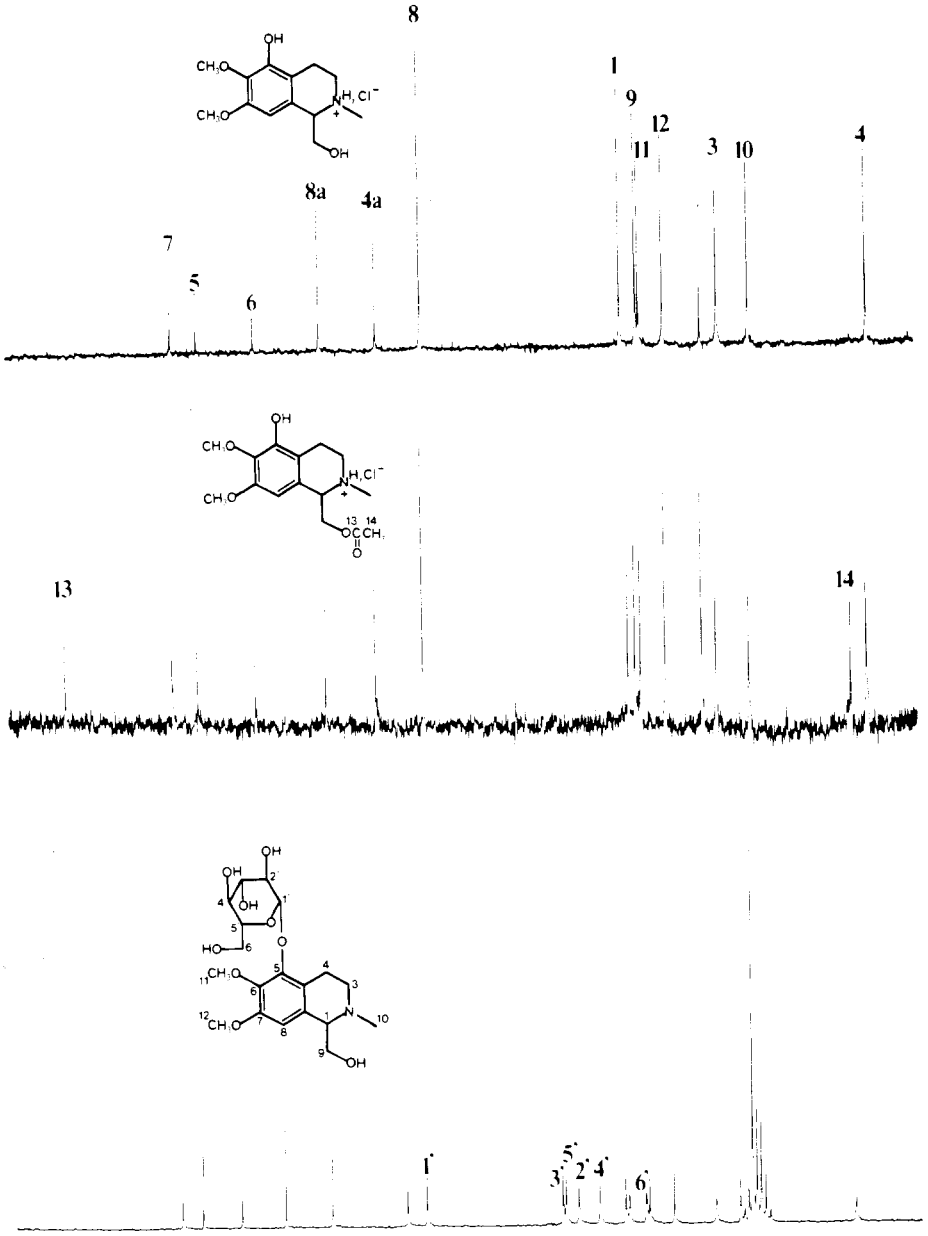

FIG. 1. *Proton-decoupled Carbon-13 Natural Abundance Spectra.*
Top: Deglucopterocereine HCl (in D_2O)
Middle: Acetyldeglucopterocereine HCl (in D_2O)
Bottom: Pterocereine (in DMSO-d_6)

CI ms of deglucopterocereine gave an M+1 peak at 254; EI ms failed to reveal the molecular ion, but high resolution analysis of a peak minus $-CH_2OH$ gave 222.113 which exactly matched the calculated mass for the $C_{12}H_{16}O_3N$ fragment; the empirical formula was thus established to be $C_{13}H_{19}O_4N$. A small peak at 236 represented loss of water from the alcoholic group.

The uv absorption bands at 214 and 268 nm and ^{13}C NMR spectrum in deuterio chloroform solution (fig. 1) suggested the basic skeleton of tetrahydroisoquinoline (7, 8). Two *O*-methyl and one *N*-methyl groups were shown by the ^{13}C signals at 60.7 (OCH_3; q, 144.7 Hz), 56.0 (OCH_3; q, 144.6 Hz) and 41.7 (NCH_3; q, 133.1 Hz) ppm, and the corresponding ^1H signals at 3.94, 3.90 and 2.55 ppm (table 1). From the ^{13}C NMR studies of other tetrahydroisoquinoline derivatives (8, 9), the methylene carbon peaks at 44.5 (t, 136.1 Hz) and 17.0 (t, 128.8 Hz) ppm can be assigned to C–3 and C–4, respectively. Then the only methine carbon peak at

TABLE 1. *Summary of physical data used for structure elucidation.*

	Pterocereine	Deglucopterocereine	Acetyldeglucopterocereine
UV: λmax			
log ε	(free base)	(hydrochloride)	(hydrochloride)
	(H_2O) 213–215 sh., 268; 3.29, 2.60	(H_2O) 214–215 sh., 268; 3.15, 2.16	(MEOH) no sh., 258 —, 2.70
MP:	(free base)	(hydrochloride)	(hydrochloride)
	198–199°	247–248°	192–193°
$[\alpha]^{25}D$	(free base)	(hydrochloride)	
	−4.51° (1.35%, H_2O)	−1.04° (2.20%, H_2O)	—
M WT:	(free base)	(hydrochloride)	(hydrochloride)
CI ms	415	253	295
EI ms (high resol.)	384.164 on M^+ minus CH_2OH	222.113 on M^+ minus CH_2OH	—
IR: (KBr)	(free base)	(hydrochloride)	(hydrochloride)
	3400, 3210, 2850, 1660, 1590, 1490, 1150, 850, 750	3290, 2620, 1600, 1490, 1110, 960, 810, 750	3580, 3400, 1740, 1640, 1510, 1380, 860, 380
^1H NMR (60 MHz, δ)	(free base, DMSO-d_6)	(hydrochloride, $CDCl_3$)	(hydrochloride, D_2O)
	2.40, 3H, s (NCH_3)	2.55, 3H, s (NCH_3)	2.52, 3H, s ($OCOCH_3$)
	3.77, 3H, s (OCH_3)	3.90, 3H, s (OCH_3)	3.42, 3H, s (NCH_3)
	3.80, 3H, s (OCH_3)	3.94, 3H, s (OCH_3)	4.23, 3H, s (OCH_3)
	6.74, 1H, s (=CH)	6.30, 1H, s (=CH)	4.50, 3H, s (OCH_3)
		5.0, 1H, bs (OH)	
^{13}C NMR, 25 MHz			
(δ, Hz)	(free base, DMSO-d_6)	(free base, $CDCl_3$)	(hydrochloride, D_2O)
	151.2(s), 147.1(s), 139.6(s), 122.1(s), 107.6(d, 157.5), 103.8 (d, 163.0), 77.1(d, 140.4), 76.5(d, 140.0), 74.1(d, 144.4), 70.1 (d, 142.8), 65.1(d, 133.1), 64.4(t, 140.4), 61.2(t, 141.0), 60.5(q, 145.3), 55.8(q, 144.7), 47.5(t, ca. 145), 42.9 (q, ca. 144), 20.6 (t, 129.4)	150.4(s), 146.4(s), 129.4(s), 114.4(s), 102.0(d, 156.3), 64.0 (d, 139.2), 63.5(t, 144.7), 60.7(q, 144.7), 56.0(q, 144.6), 44.5 (t, 136.1), 41.9(q, 133.1), 17.0(t, 128.8)	173.2(s), 152.2(s), 147.3(s), 136.1(s), 122.5(s), 112.7(s), 103.3(d), 63.7(d), 62.4(t), 61.3(q), 56.4(q), 46.6(t), 40.3(q), 20.3(q), and 17.4(t)
		(hydrochloride, D_2O) 152.3(s), 147.3(s), 136.2(s), 123.5(s), 112.5(s), 103.7(d, 160.2), 65.1(d, 148.3), 62.0(t, 147.4), 61.4(q, 145.9), 57.0(q, 145.9), 46.2(t, 145.0), 40.2(q, 144.0), 17.3(t, 130.5)	

64.0 (d, 139.2 Hz) ppm must be designated to the C-1, which is attached to the hydroxymethyl carbon (62.0 ppm; t, 144.7 Hz). The existence of the hydroxymethyl group was confirmed by the formation of a monoacetate in acetic anhydride-glacial acetic acid (table 1).

Only one aromatic proton at 6.30 (singlet) ppm and one methine carbon at 102.0 (dd, 156.3 and 1.8 Hz) ppm in the NMR spectra revealed the presence of a pentasubstituted benzene system. The small splitting (1.8 Hz) resulting from the long-range coupling with H-1 (the only aliphatic methine proton) suggested that the aromatic proton was attached to C-8. Then the remaining two methoxy and one phenolic groups have to be at the C_7, C_6, and C_5 positions. The exact locations of these substituents were determined by ^{13}C–^{1}H long-range coupling analyses of this compound and other related compounds (9). Three downfield signals at 150.4, 146.4 and 133.8 ppm can be assigned to C_7, C_5 and C_6, respectively, based on simple chemical shift theory. In the proton-coupled ^{13}C NMR spectrum (fig.

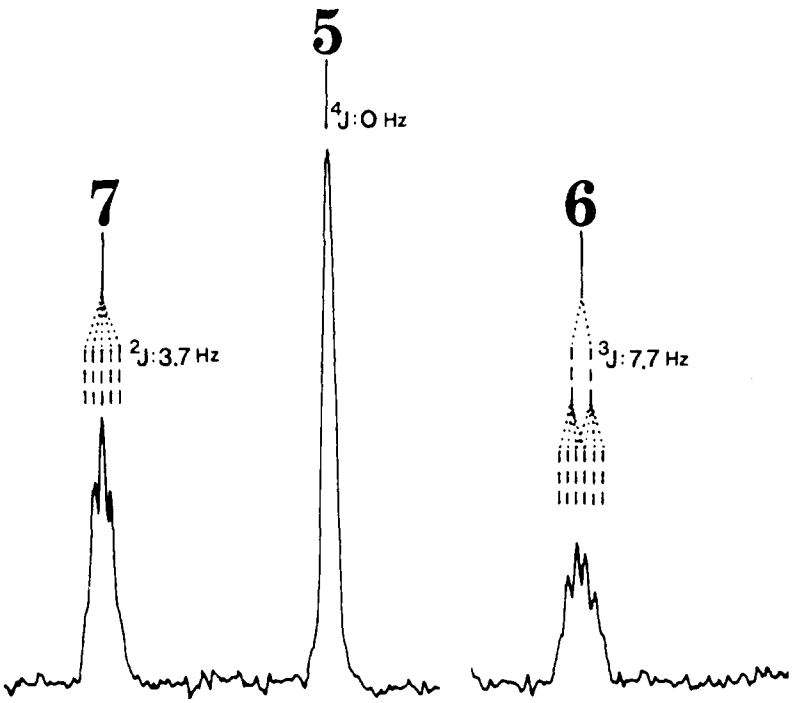

FIG. 2. *Proton-coupled Carbon-13 Spectrum of Deglucopterocereine in Deuteriochloroform Solution.* Only the Signals of C-5, C-6 and C-7 are shown (J indicates the coupling constants with H-8).

2), there are distinct couplings between the *O*-methyl protons and the C_7 and C_6, which further couple with the H_8 [$^{2}J(C_7$–$H_8)$:3.7 Hz and $^{3}J(C_6$–$H_8)$:7.7 Hz]. However, no significant coupling can be seen between the C_5 and H_8, indicating their 1,4-relationship based on the general ^{13}C–^{1}H long-range coupling patterns (10–12). This assignment is consistent with the tetrazotized benzidine color reaction (orange-red) which has been observed with 5 or 8 hydroxy tetrahydroisoquinolines, whereas 6 or 7 hydroxy compounds produce yellow-brown chromophores (13).

Deglucopterocereine is thus proposed to be (−)-1-hydroxymethyl-2-methyl-5-

hydroxy-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline, and pterocereine is its (−)-5-β-*O*-glucopyranoside. Both pterocereine and deglucopterocereine are new natural compounds. The hydroxymethyl group and the phenolic glucoside are both uncommon among tetrahydroisoquinoline alkaloids (14). In the 9KB cytotoxicity assay, neither pterocereine nor deglucopterocereine exhibited significant activity ($ED_{50} > 100\mu g/ml$ and $16\mu g/ml$, respectively).

EXPERIMENTAL[2]

PLANT MATERIAL.—Fresh whole plants and cuttings were purchased commercially from Grigsby Cactus Gardens, 2326 and 2354 Bella Vista, Vista, California 92083 in February of 1977 and 1978. The plants conformed to the description of *Pachycereus (?) gaumeri* Br. and R. (3), a provisional name which has been changed to *Pterocereus gaumeri* (Br. and R.) MacDoug. and Mir. (1); the synonym, *Anisocereus gaumeri* (Br. and R.) Backbg., has also been proposed (2). Reference photographs are on file and sample plants are being maintained in our greenhouse. The plants were sliced, frozen, freeze-dried, and pulverized through a 2 mm screen in a Wiley mill.

EXTRACTION AND ISOLATION OF PHENOLIC AND NONPHENOLIC ALKALOID FRACTIONS.—Preliminary tlc screening of 10 g of the plant material and fractionation via anion-exchange chromatography showed two major spots corresponding to a phenolic and a nonphenolic alkaloid. Subsequently, 520 g of the plant material was defatted, basified, and extracted via chloroformic percolation (20 liters) (4). The chloroform extract was condensed to 4 liters and left overnight to form 2.63 g of a deposit. The filtrate was condensed to 1 liter and was processed, essentially as previously described (4), to yield extracts A (alkaloids), B (non-alkaloidal material), and C (water soluble alkaloids). Tlc of extracts A and C showed that they were nearly identical, so they were combined to give 6.85 g of total alkaloids which were resolved via anion-exchange chromatography (13) on 110 g of Amberlite IRA 401 resin (hydroxide form) into nonphenolic and phenolic fractions.

ISOLATION OF PTEROCEREINE.—The nonphenolic alkaloid fraction totaled 2.5 g of residue which produced crystals upon concentration in ethanol; two recrystallizations gave 0.236 g of a crystalline nonphenolic alkaloid having a constant mp, after repeated recrystallization at 198–199°, and showing homogeneity on tlc. This new alkaloid was named pterocereine. The mother liquor (1.65 g of residue), in which tlc revealed several additional alkaloids, was chromatographed on a silica gel column to produce 0.09 g of the same alkaloid from a 30% ethanol in chloroform eluate and other crystalline alkaloid fractions which were decomposed in attempts to prepare their hydrochlorides. The water rinse (2 liters) from the anion exchange column (13), upon condensation under rotary vacuum evaporation, furnished an additional 0.021 g of the same alkaloid.

ISOLATION OF DEGLUCOPTEROCEREINE.—The phenolic alkaloid fraction gave 2.45 g of dark brown residue which was dissolved in absolute ethanol and acidified (pH paper) by dropwise addition of 5% hydrochloric acid in absolute ethanol. The crystalline mass that formed was recrystallized five times from ethanol (yielding 0.796 g) to give a constant mp at 247–248° and homogeneity on tlc. Preparative tlc of the mother liquor in solvent B furnished an additional 0.06 g of the same alkaloid and a small amount of minor alkaloids which failed to produce crystalline salts. This new phenolic alkaloid was subsequently named deglucopterocereine.

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[2]Melting points were determined on a Laboratory Apparatus Mel-Temp melting point apparatus and were uncorrected. Uv spectra were determined on a Cary model 17 recording spectrometer. Ir spectra were determined on a Beckman model IR-33 recording spectrophotometer using KBr pellets. ^{1}H-nmr spectra were obtained on a Varian EM-360 using Me_4Si as internal or external standard and ^{13}C-nmr spectra were obtained on a Jeol PFT-100 using deuterated solvent or methanol as internal standard. EI high resolution mass spectra were determined on a Hitachi RMU-6, and EI and CI low resolution mass spectra were determined on a DuPont 21-492B. Optical rotations were obtained using a Perkin Elmer 141 Polarimeter. Analytical tlc plates were purchased from Merck (silica gel 60, kieselguhr F 254, and cellulose F 254). Analytical tlc separations and cochromatography of cactus alkaloids were performed using solvents A, B, D, G, and H and visualization reagents as previously described (5). *n*-Butanol-pyridine-water, 6:4:3 on cellulose; ethyl acetate-65% isopropanol, 65:35 and *n*-butanol-acetone-water, 4:5:1 on kieselguhr; and acetone-water, 9:1 and acetone-water-chloroform-methanol, 75:5:10:10 on silica gel were solvents employed to identify glucose in hydrolysates using sprays of aniline acid phthalate, sulfuric acid, and anisaldehyde-sulfuric acid reagent with heat to visualize the sugars. 9 KB cytotoxicity testing was performed at the Purdue Cancer Center following protocols set by the National Cancer Institute.

STRUCTURAL STUDIES WITH PTEROCEREINE.—This new nonphenolic alkaloid (0.062% yield) occurred as white crystals sparingly soluble in chloroform, absolute ethanol and acetone, slightly soluble in ethanol, and moderately soluble in water. On tlc plates it reacted immediately with iodine vapor and Dragendorff's reagent, slowly with iodoplatinate, and not at all with tetrazotized benzidine, fluorescamine, or dansyl chloride, indicating a nonphenolic tertiary amine (6, 13). It responded strongly to the general test for carbohydrates with Molish's reagent suggesting the presence of a sugar moiety. A developed tlc plate was sprayed with 5% hydrochloric acid, warmed at 40° for 45 min, and then sprayed with tetrazotized benzidine; an orange-red chromophore was then produced indicating hydrolysis to a phenolic aglycone.

To confirm the presence of a glycosidic function, pterocereine was hydrolyzed by β-glycosidase (Calbiochem) at pH 4, 40°C, for 48 hr. Testing with a series of reference sugars and five different tlc chromatographic systems with visualization reagents specific for sugars easily identified glucose in the hydrolysate as the only glycone. The aglycone was readily detected on the same chromatograms by spraying with tetrazotized benzidine reagent. Similar results were obtained after hydrolysis of pterocereine with both sulfuric and hydrochloric acids. Tlc revealed that the phenolic aglycone from pterocereine was identical to the major phenolic alkaloid that had been previously isolated; the phenolic alkaloid was consequently named deglucopterocereine.

STRUCTURAL DETERMINATION OF DEGLUCOPTEROCEREINE.—This new phenolic alkaloid (0.164% yield) crystallized from ethanol as a white hydrochloride salt. The base was freely soluble in chloroform and ethanol and insoluble in water. On tlc plates, the alkaloid reacted positively with iodine vapor, iodoplatinate, Dragendorff's reagent, and tetrazotized benzidine (6, 13); the orange-red chromophore produced by tetrazotized benzidine was indicative of either a 5 or 8 hydroxy tetrahydroisoquinoline (13). No visualization reaction was produced with fluorescamine, indicating a tertiary amine (6). Silicotungstic acid solution formed a characteristic precipitate, and a crystalline picrate (mp 195–196°) and monoacetate hydrochloride (mp 192–193°) were readily obtained. The alkaloid was negative in the Molish's test for carbohydrates. The physical data used for the structural elucidation are summarized in table 1.

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