Phytochemistry, Vol. 21, No. 9, pp. 2375–2377, 1982.
Printed in Great Britain.

0031-9422/82/092375-03$03.00/0

TWO SIMPLE TETRAHYDROISOQUINOLINE ALKALOID *N*-OXIDES FROM CACTI*

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(*Received* 11 *January* 1982)

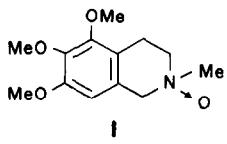
Key Word Index—*Pachycereus pringlei*; *Pterocereus gaumeri*; Cactaceae; cactus alkaloids; tetrahydroisoquinoline *N*-oxides; tehuanine *N*-oxide; deglucopterocereine *N*-oxide.

Abstract—Tehuanine *N*-oxide was isolated from *Pachycereus pringlei*, and deglucopterocereine *N*-oxide was isolated from *Pterocereus gaumeri*. These are apparently the first reported simple tetrahydroisoquinoline *N*-oxides and the first alkaloid *N*-oxides isolated from the Cactaceae.

INTRODUCTION

Over 130 alkaloid *N*-oxides have now been isolated from over a dozen families of higher plants[1, 2]. These compounds represent several alkaloid classes, including the benzylisoquinolines, but to date, no simple tetrahydroisoquinoline alkaloid *N*-oxides have been reported. Previous investigations with cactus alkaloids have yielded β-phenethylamines, simple tetra- and dihydroisoquinolines, and imidazoles[3, 4].

The isolation from cacti of two simple tetrahydroisoquinoline *N*-oxides, tehuanine *N*-oxide (**1**) and deglucopterocereine *N*-oxide (**2**), are reported in this study.



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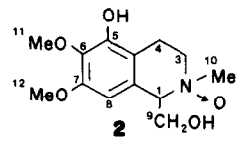

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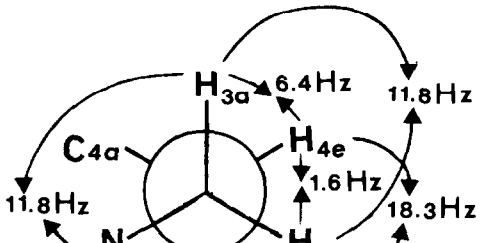

RESULTS AND DISCUSSION

Pachycereus pringlei, a giant cereoid cactus native to Sonora and Baja in Mexico, was previously known to contain four simple tetrahydroisoquinolines (tehuanine, heliamine, lemaireocereine, and weberine)[5, 6]. In a re-investigation of alkaloid fraction A[7] by gradient column adsorption chromatography, **1** was isolated and crystallized as the hydrochloride.

R_f values of **1** on Si gel TLC were lower than those of tehuanine (2-methyl-5,6,7-trimethoxy-1,2,3,4-tetrahydroisoquinoline) and TLC visualization reactions[8] indicated a possible tertiary or quaternary amine. CI- and EI-mass spectra were indicative of tehuanine[9], but the IR spectrum had a shoulder at ν_{max} 1520 cm^{-1}, indicative of N–O stretching[10], which did not appear in the tehuanine spectrum. The ^1H NMR spectrum was also similar to that of tehuanine[9], but with a notable downfield shift (δ 2.55→3.71) of the N-Me proton signals; in addition, the C-1 protons now formed an AB quartet. TLC proved non-identity with a suspected tehuanine isomer, *O*-methylanhalidine (2-methyl-6,7,8-trimethoxy-1,2,3,4-tetrahydroisoquinoline). It is known that alkaloid *N*-oxides often lose oxygen during mass spectral analysis and produce apparent molecular ions at $[M-16]^+$ [11]. Consequently, the *N*-oxide of **1** was prepared by oxidizing tehuanine with *m*-chloroperbenzoic acid. The isolated **1** and reference tehuanine *N*-oxide hydrochlorides were identical (TLC, mp, mmp, IR, ^1H NMR, EI- and CI-MS).

Pterocereus gaumeri is an erect, solitary or branched, cactus species native to the Yucatan in Mexico. It has previously yielded two alkaloids: deglucopterocereine [(−)-1-hydroxymethyl-2-methyl-5-hydroxy-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline] and pterocereine its (−)-5-β-*O*-glucopyranoside [12]. In the present study, an ethanol extract of the plant material was partitioned between chloroform and water; the chloroform residue was subjected to gradient column adsorption chromatography and yielded a crystalline compound, **2**.

The R_f value of **2** in normal phase TLC systems was lower than that of deglucopterocereine, but a typical blood-red color with tetrazotized benzidine spray indicated an identical phenolic function[13]. In the CI-mass spectrum the MH^+ at *m/z* 270 suggested the attachment of an oxygen atom to deglucop-

*Part 52 in the series "Cactus Alkaloids". For Part 51 see Pummangura, S., Schifferdecker, R. C. and McLaughlin, J. L. (1982) *J. Nat. Prod.* **45**, (in press).

Table 1. Comparison of ^{13}C NMR chemical shifts (δ) in D_2O of deglucopterocereine hydrochloride and deglucopterocereine *N*-oxide

Carbon assignment	Deglucopterocereine hydrochloride[12] (multiplicity, coupling constant in Hz)	Deglucopterocereine *N*-oxide (multiplicity, coupling constant in Hz)
1	65.1 (*d*, 148.3)	75.7 (*dm*, 149.5-H_1)
3	46.2 (*t*, 145.0)	60.4 (*tm*, 148.4-H_3)
4	17.3 (*t*, 130.5)	21.5 (*tm*, 129.7-H_4)
4a	112.5 (*s*)	112.4 (*m*)
5	147.3 (*s*)	147.1 (*t*, 2.2-H_4)
6	136.2 (*s*)	135.9 (*m*)
7	152.3 (*s*)	152.0 (*p*, 4.0-H_8 and H_{12})
8	103.7 (*d*, 160.2)	103.7 (*dd*, 160.5-H_8, 3.6-H_1)
8a	123.5 (*s*)	126.7 (*m*)
9	62.0 (*t*, 147.4)	63.1 (*td*, 147.1-H_9, 3.7-H_1)
10	40.2 (*q*, 144.0)	56.2 (*qm*, 142.2-H_{10})
11	61.4 (*q*, 145.9)	61.3 (*q*, 145.9-H_{11})
12	57.0 (*q*, 145.9)	56.4 (*q*, 145.6-H_{12})

terocereine (MW 253). In the EI-mass spectrum the dihydroisoquinolinium fragment of deglucopterocereine was observed at *m/z* 252 with a base peak at *m/z* 222 corresponding to the loss of the oxygen and the C-1 hydroxymethyl group. The ^{13}C NMR spectra displayed the close similarity of the aromatic moiety (Table 1); however, C-1, C-3, and C-10 were drastically shifted downfield ($C_1 = \delta\ 65.1 \rightarrow 75.7$; $C_3 = \delta\ 46.2 \rightarrow 60.4$; $C_{10} = \delta\ 40.2 \rightarrow 56.2$), suggesting the presence of the *N*-oxide.

The 470 MHz ^1H NMR spectrum revealed all five spin-spin splittings except those for the H-1 signal because of its partial overlapping with the HDO signal. The peaks at δ 2.87 and 3.01 could be assigned to the resonance signals of H-4a (axial) and H-4e (equatorial). The large geminal coupling (18.3 Hz) for H-4a–H-4e was characteristic for benzylic protons. Considering the Karplus relationship for the dihedral angles and the vicinal coupling constants[14], the following conformation could be deduced. The large vicinal coupling (11.8 Hz) between the H-4a and H-3a was ascribed to their *anti* relationship. This coupling and the small coupling (1.6 Hz) between the H-4e and H-3e permitted the unequivocal assignment of the chemical shifts for H-3a (δ 4.21) and H-3e (δ 3.76). The unusual downfield shift of the axial proton, H-3a, presumably resulted from the *anti* relationship with the $N \rightarrow O$ bond[14] and is indicative of the axial orientation of the $N \rightarrow O$ bond.

Deglucopterocereine *N*-oxide (**2**) was then prepared by oxidation of deglucopterocereine with *m*-chloroperbenzoic acid. Two products were observed (TLC), major and minor, and the major product crystallized. This product was identical to the isolated **2** (TLC, mp, mmp, IR, ^1H NMR, CI- and EI-MS). In addition, reduction of **2** with activated zinc dust yielded deglucopterocereine. The attack of *m*-chloroperbenzoic acid from the axial side of deglucopterocereine is attributed to the anchemeric assistance of the hydroxymethyl group by forming an intramolecular hydrogen bond. This intramolecular hydrogen bonding could also account for the retention of the oxygen atom in the mass spectrum, in clear contrast to the spectrum of tehuanine *N*-oxide where such hydrogen bonding is not possible. The absolute configuration of deglucopterocereine remains in question, but the oxygen atom of the *N*-oxide is obviously *cis* with the hydroxymethyl at C-1.

To ascertain that both **1** and **2** are natural and not extraction artifacts, solutions of tehuanine and deglucopterocereine were stored in basic chloroform and monitored periodically by TLC; there were no traces of *N*-oxide formation even after several days. Re-extraction of samples of the original plant material with basic methanol–chloroform, followed by direct TLC of the concentrated extracts, revealed the respective *N*-oxides. Thus, the *N*-oxides are believed to be natural compounds and not extract artifacts.

EXPERIMENTAL

General. Mps are uncorr. IR spectra were recorded in KBr. ^1H NMR spectra were measured in $CDCl_3$ and D_2O at 80 MHz or at the Purdue University Biological Magnetic Resonance Laboratory at 470 MHz. ^{13}C NMR spectra (25 MHz) were recorded in D_2O. Micro analyses were performed in the Chemistry Department, Purdue University.

Plant material. Fresh cuttings of *Pachycereus pringlei* (S. Wats.) Br. and R. and *Pterocereus gaumeri* (Br. and R.) MacDoug. and Mir. [*Anisocereus gaumeri* (Br. and R. Backbg.)] were obtained commercially from Grigsby Cactus Garden, 2534 Bella Vista, Vista, CA 92083, U.S.A., in August 1980 and February 1977 and 1978, respectively. The plants conformed to published descriptions[15] and reference photographs are on file. After slicing, freezing and freeze-drying, the plant materials were pulverized through a 2 mm screen in a Wiley Mill.

Extractions. With *P. pringlei*, 500 g plant material was defatted with petrol (30–60°) in a Soxhlet extractor for 72 hr. The air-dried marc was then percolated with $CHCl_3$ and the $CHCl_3$ extract (24 l.) evaporated and processed to yield fraction A (alkaloids, 1.22 g)[7].

With *P. gaumeri*, 200 g plant material was exhaustively percolated with EtOH. The residue was dissolved in $CHCl_3$, filtered and evaporated to obtain extract Y (10 g).

Isolation and identification of tehuanine n-oxide

(1). Fraction A (1.22 g) was subjected to CC on Si gel (133 g, 0.062–0.22 mm, E. Merck) packed in a C_6H_6 slurry in a 3 × 60 cm column. The column was eluted with a gradient of C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$, $CHCl_3$–MeOH–58% NH_4OH, and MeOH. Fractions of 100 ml were collected and combined on the basis of TLC analysis in solvent C[7].

Combined fraction 136–145 contained **1** as a single major compound at low R_f which crystallized as the hydrochloride from EtOH–Et_2O (72 mg, 0.014% yield): mp 185°; EI-MS, *m/z* (%): 237 (66), 236 (78), 206 (38), 194 (100), and 179 (76); ^{1}H NMR (80 MHz, $CDCl_3$): δ 6.38 (1H, *s*, C=CH), 4.9 (2H, *q*, CH_2-1), 3.9 (3H, *s*, OMe), 3.7 (3H, *s*, NMe), centered at 3.1 (4H, *m*, CH_2CH_2-3,4); IR similar to that of tehuanine hydrochloride[9], but with a notable shoulder at 1520 cm^{-1}.

Synthesis of tehuanine N-*oxide* (**1**). Following the method of ref. [16], 90 mg of tehuanine hydrochloride [9] was converted to the free base, dissolved in 1 ml $CHCl_3$ and cooled to 0–5°. A soln of 100 mg *m*-chloroperbenzoic acid in 1 ml $CHCl_3$ was added dropwise with stirring which continued for 3 hr while the temp. was raised slowly to ambient. The reaction mixture was added to a 1 × 40 cm chromatography column packed with 16 g alkaline Al_2O_3 (A 540, Fisher) in a $CHCl_3$ slurry. The column was eluted with $CHCl_3$ (100 ml) and then with $CHCl_3$–MeOH (3:1, 200 ml). The residue from the $CHCl_3$–MeOH eluates yielded 68 mg (88% yield) **1** hydrochloride: mp 186–187°; elemental analysis calc. for $C_{13}H_{20}NO_4$ HCl: C, 53.88%; H, 6.90%; and N, 4.83%; found: C, 53.65%; H, 6.84%; and N, 4.99%. This material was identical with the isolated **1** (mmp, TLC, IR, ^{1}H NMR, CI- and EI-MS).

Isolation and identification of deglucopterocereine N-*oxide* (**2**). Extract Y was separated by CC on Si gel (300 g, 4 × 100 cm column) eluted with a gradient of C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$ and $CHCl_3$–EtOH. Material eluted with 15% EtOH in $CHCl_3$ migrated at a lower R_f on TLC (solvent B[7]) than deglucopterocereine. The residue crystallized (mp 210°, 65 mg, 0.038% yield) from EtOH–Et_2O. The CI-MS gave strong peaks at *m/z* 270 and 254. EI-MS *m/z* (%): 269 (5), 252 (8), 222 (100), 210 (27), and 180 (4); IR ν_{max}^{KBr} cm^{-1}: 3480, 2920, 1600, 1580, 1490, 1440, 1410, 1360, 1305, 1240, 1180, 1140, 1105, 1048, 1000, and 908; ^{1}H NMR (80 MHz, D_2O): δ 6.4 (1H, *s*, C=CH), 3.7 (3H, *s*, OMe), 3.7 (3H, *s*, OMe), 3.5 (3H, *s*, NMe), 3.3 (4H, *m*, CH_2–CH_2-3,4), signals for CH_2-1 and CH_2OH obscured by D_2O peak; ^{1}H NMR (470 MHz, D_2O): δ 6.42 (1H, *s*, C=CH), 4.63 (1H, *m*, CH-1), 4.35 (1H, *dd*, *J* = 13.8, 2.1 Hz), 3.95 (1H, *dd*, *J* = 13.8, 4.2 Hz, CH_2OH), 3.70 (3H, *s*, OMe-6), 3.63 (3H, *s*, OMe-7), 4.21 (1H, *td*, *J* = 11.8, 6.4 Hz, CH_2-3a), 3.76 1H, *ddd*, *J* = 11.8, 7.4, 1.6 Hz, CH_2-3e), 3.33 (3H, *s*, NMe), 3.01 (1H, *ddd*, *J* = 18.3, 6.4, 1.6 Hz, CH_2-4e), 2.87 (1H, *ddd*, *J* = 18.3, 11.8, 7.4 Hz, CH_2-4a). ^{13}C NMR (25 MHz, D_2O): see Table 1.

Synthesis of deglucopterocereine N-*oxide* (**2**). Deglucopterocereine hydrochloride[12], 76 mg, was converted to the free base, 57 mg, which was dissolved in 1 ml $CHCl_3$. The method of ref. [16], using 39 mg *m*-chloroperbenzoic acid in $CHCl_3$, followed by CC was used as described above. TLC in solvents A and C[7] indicated two products as major and minor isomers. Homogeneous **2**, the major isomer, crystallized and was recrystallized as the base from EtOH–Et_2O, 19.5 mg (34% yield); mp 210–213°; elemental analysis calc. for $C_{13}H_{19}NO_5$: C, 57.78%; H, 7.04%; N, 5.18%; found: C, 57.44%; H, 7.42%; N, 5.18%. This product was identical with the isolated **2**: mmp; TLC, IR, ^{1}H NMR, CI- and EI-MS.

Reduction of deglucopterocereine N-*oxide* (**2**). **2** (20 mg) was dissolved in 10 ml 1 N H_2SO_4. 500 mg activated Zn dust was added and the mixture stirred at room temp. for 30 min. The basified filtrate was extracted with $CHCl_3$ and Et_2O, and the extract residue yielded crystalline deglucopterocereine hydrochloride which was identical (TLC, IR) with authentic deglucopterocereine hydrochloride[12].

Natural occurrence of **1** *and* **2**. Small amounts of tehuanine and deglucopterocereine were stable (TLC) after several days of dissolution in the solvent mixtures used for plant extraction with no evidence of *N*-oxide formation. In addition, re-extraction of the plant materials with $CHCl_3$–MeOH–conc. NH_4OH (2:2:1) produced simple extracts in which the respective *N*-oxides were detected by co-chromatography in TLC solvents A and C[7].

Acknowledgments—Partial research support is acknowledged in grants from the Cactus and Succulent Society of America and NIH BRSG RR-05586. S. Pummangura acknowledges fellowship support from Chulalongkorn University, Bangkok, Thailand. We thank Dr. W. E. Scott, Hoffmann–LaRoche, Inc., for a sample of *O*-methylanhalidine hydrochloride (Ro 1–2057).

REFERENCES

1. Phillipson, J. D. and Handa, S. S. (1978) *J. Nat. Prod.* **41**, 385.
2. Phillipson, J. D. (1971) *Xenobiotica* **1**, 419.
3. Mata, R. and McLaughlin, J. L. (1982) *Rev. Latinoam. Quim.* **12**, 95.
4. Pummangura, S., McLaughlin, J. L., Davis, D. V. and Cooks, R. G. (1982) *J. Nat. Prod.* **45** (in press).
5. Mata, R. and McLaughlin, J. L. (1980) *Planta Med.* **38**, 180.
6. Unger, S. E., Cooks, R. G., Mata, R. and McLaughlin, J. L. (1980) *J. Nat. Prod.* **41**, 288.
7. Ranieri, R. L. and McLaughlin, J. L. (1976) *J. Org. Chem.* **41**, 319.
8. Ranieri, R. L. and McLaughlin, J. L. (1975) *J. Chromatogr.* **111**, 234.
9. Mata, R. and McLaughlin, J. L. (1980) *Phytochemistry* **19**, 673.
10. Silverstein, R. M., Bassler, G. C. and Morrill, T. C. (1974) *Spectrometric Identification of Organic Compounds* 3rd edn. John Wiley, New York.
11. Bryce, T. A. and Maxwell, J. R. (1965) *J. Chem. Soc. Chem. Commun.* 206.
12. Mohamed, Y. A. H., Chang, C.-j. and McLaughlin, J.L. (1979) *J. Nat. Prod.* **42**, 197.
13. Smith, I. (1969) *Chromatographic and Electrophoretic Techniques* Vol. 1, p. 404. Interscience, New York.
14. Jackman, L. M. and Sternhell, S. (1969) *Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry*. Pergamon Press, Oxford.
15. Backeberg, C. (1977) *Cactus Lexicon* (in English) 3rd edn, pp. 67 and 383. Blandford Press, Poole.
16. Craig, J. C., Dwyer, F. P., Glazer, A. N. and Hornington, E. C. (1961) *J. Am. Chem. Soc.* **83**, 1871.