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ALKALOIDS FROM THE LEAVES OF *PROSOPIS JULIFLORA*

VIQAR UDDIN AHMAD,* AZRA SULTANA, and SABIHA QAZI

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi 32, Pakistan

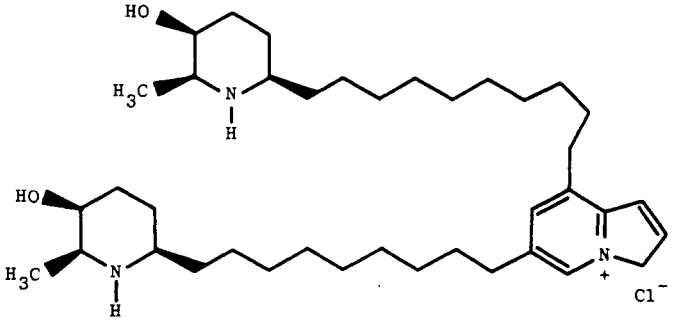
ABSTRACT.—From the leaves of *Prosopis juliflora*, two new piperidine alkaloids, juliprosinene [**1**] and juliflorinine [**2**], were isolated. Their structures were established by modern spectroscopic techniques, particularly nmr.

Prosopis juliflora DC. (mesquite), a member of the Mimosaceae family, is a shrub that grows abundantly in the Sind and Punjab provinces of Pakistan (1). Many plants of the genus *Prosopis* are known to have medicinal properties (2) and are used in folk medicine as astringents, in rheumatism, and as remedies against scorpion stings and snake bites (3–5).

In view of the therapeutic importance attributed to *P. juliflora*, comprehensive investigations on it have been carried out by various groups of workers (6–10). From studies on fresh leaves we reported the isolation and structure elucidation of the new alkaloids juliflorine, julifloricine, and julifloridine (9,10). In continuation of this work we report the isolation and structure determination of two new alkaloids from this plant, which have been named as juliprosinene and juliflorinine. Preliminary screening for antibacterial activity of the alkaloid juliprosinene showed activity against *Escherichia coli* strains 11303 HER 1024 and K-125 HER 1037, *Klebsiella pneumoniae* C_3HER 1111, *Pseudomonas aeruginosa* PA 01 HER 1018, *Staphylococcus aureus* B-827, and *Shigella sonnei* YER HER 1048.

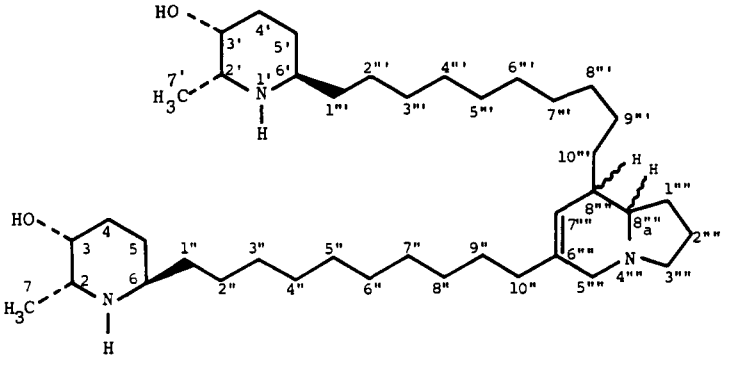
RESULTS AND DISCUSSION

The chloride salt of juliprosinene [**1**] obtained as a gum showed the molecular ion of the cation at *m/z* 624 corresponding to the formula $C_{40}H_{70}N_3O_2$. The predominant ion at *m/z* 114 in the mass spectrum results from the expected cleavage of the C-6 or C-6′ side chain adjacent to nitrogen in the 2-methyl-3-hydroxy-6-alkyl piperidine ring. Its uv spectrum showed absorptions at 208 and 285 nm, while absorption peaks at 3660 and 3350 cm^{-1} in its ir spectrum indicated the presence of NH and OH groups. The molecular formula indicated eight double bond equivalents, two of which were considered to be due to piperidine rings and four to the double bonds in the rings, while the remaining two represented the indolizidine system. The ^{1}H-nmr spectrum of **1** showed



two-proton doublets at δ 1.13 (J = 6.8 Hz, H-7) and δ 1.05 (J = 6.8 Hz, H-7′), indicating they were attached to the two methine groups. There was a triplet at δ 2.68 (J = 7.0) which was assigned to H-10″ and H-10‴ coupled with neighboring methylene protons. There was a broad singlet at δ 3.53 due to H-3 and H-3′. The doublet at δ 6.85 ($J_{1'''',2''''}$ = 10.6 Hz) of H-1‴′ and the double doublet at δ 6.82 of H-2‴′ ($J_{1'''',2''''}$ = 10.6 Hz, $J_{2'''',3''''}$ = 6.2 Hz) showed the presence of double bonds in the indolizidine ring. The former value is shifted downfield as compared to juliprosine (11) due to another double bond conjugated with it. The two signals were coupled with each other, which was confirmed by the cross peaks in the COSY-45 spectrum. There was a double doublet at δ 6.30 (J_{gem} = 14, $J_{3'''',2''''}$ = 6.2 Hz), due to the H_a-3‴′ proton which appeared downfield as compared to juliprosine because of a double bond in juliprosinene. The singlets at δ 9.35 and δ 7.68 were assigned to H-5‴′ and H-7‴′. The ^{1}H-nmr spectrum was similar to that of juliprosine (11), the only difference between juliprosine and juliprosinene being an additional double bond in the structure of the latter, the position of which was confirmed through ^{1}H-nmr and ^{13}C-nmr spectra. The ^{13}C-nmr spectrum of juliprosinene also supported structure **1**, with methine signals at δ 129.5 and 115.5 and a downfield methylene signal at δ 68.2 ppm. The latter signal is assignable to C-3‴′ because this carbon has a double bond on one side and a positively charged nitrogen on the other side. The relative configuration in the piperidine ring is confirmed by comparing the ^{13}C-nmr data with those reported for spectaline (12); the shifts of C-2 and C-2′ at 57.2, C-3 and C-3′ at 67.9, and C-6 and C-6′ at 55.85 were similar to those reported for the corresponding carbons of spectaline (12).

The molecular ion peak in the ms of juliflorinine [**2**] appeared at *m/z* 629 corresponding to the molecular formula $C_{40}H_{75}N_3O_2$, and additional peaks at *m/z* 114, 96, and 70 clearly indicated the presence of a 2-methyl-3-hydroxy piperidine ring system in the compound. Its ir spectrum exhibited absorption bands at 3680 and 3400 cm^{-1} indicating the presence of NH and OH groups, and its uv spectrum showed absorption maxima at 208 and 285 nm.



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In the ^{13}C-nmr spectrum of juliflorinine, signals were observed at δ 51.16, 66.25, 66.87, and 50.29, which indicated that the relative configuration of this compound differs from that of juliprosopine at C-2, C-3, and C-6 in both rings. The ^{13}C assignments presented in Table 1 were made by comparison with the data for the closely related compound juliprosopine (13). It appeared that all the other carbon resonances in the molecule were almost identical to those of the latter compound.

TABLE 1. ^{13}C-nmr spectra of Juliflorinine [**2**] and Juliprosopine (25 MHz, $CDCl_3$).[a]

Carbon	Compound	
	2	Juliprosopine
1‴	33.11	33.2
2‴	22.5	21.5
3‴	55.0	54.5
5‴	55.0	55.3
6‴	135.0	136.3
7‴	124.37	123.8
8‴	41.96	42.6
8a‴	63.75	65.5
2,2′	51.16	57.2
3,3′	66.3, 66.9	67.8
4,4′	30.01	32.2
5,5′	26.25	26.2
6,6′	50.3	55.7
1″,1‴	38.75	37.1
2″,2‴	24.37	25.8
3″,8″	29.95–29.3	30.0–29.4
3‴,8‴	—	—
9″,9‴	26.87	26.6
10″	35.0	35.1
10‴	28.75	28.0
7,7′	11.1, 12.2	18.7

[a]All values are in δ (ppm).

The ^{1}H-nmr spectrum, which was very different from that of juliprosopine (13), strongly supported the above structure. A quintet at δ 3.66 was assigned to H-3 protons that resembled the H-3 of iso-6-cassine (14) at δ 3.66, while in spectaline (12) and in juliprosopine the same protons appeared at δ 3.55 as a broad singlet. The H-2 in iso-6-cassine resonated at δ 3.06 as an octet, and in spectaline and juliprosopine it was observed at δ 2.90 and δ 2.74 as a multiplet and q × d, respectively. In juliflorinine this proton was observed at about the same position as an octet but was distorted due to the appearance of the signal of H-3‴ at δ 3.17. A doublet at δ 1.03 ($J = 7.0$ Hz) due to the methyl group attached to the piperidine ring was similar to that of juliprosopine (13) and julifloridine (9). No other methyl peaks were present. The peak due to methylene protons of the two side chains appeared at a singlet at δ 1.20. The protons of the indolizidine ring were observed at similar positions as in juliprosopine.

From ^{13}C-nmr data of 2-hydroxy 3-methyl-6-alkyl piperidine alkaloids the signals of C-2, C-3, and C-6 were observed, respectively, at δ 57.0, 67.6, and 55.7 in spectaline and at 57.2, 67.8, and 55.7 ppm in juliprosopine. The chemical shifts of these carbon atoms in iso-6-cassine and julifloridine (9,15) were at δ 50.4, 68.9, and 49.5 and at 50.1, 69.3, and 49.7 ppm. The chemical shifts of these carbon atoms were observed in juliflorinine at δ 51.16 (C-2), 66.3 and 66.9 (C-3), and 50.3 (C-6) (Table 1). It was observed that the values were closer to those of iso-6-cassine and julifloridine than to those of juliprosopine and spectaline. Methyl carbons in juliflorinine appeared at δ 11.1 and 12.2 for 2-Me and 2′-Me while the same carbon signal was observed at 18.9 ppm in juliprosopine and at δ 19.0 ppm in spectaline. The methyl carbon signal of iso-6-cassine appeared at δ 15.7.

From ^{13}C assignments it was proposed that juliflorinine possesses the same configuration in both the rings.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ir spectra (in $CHCl_3$) were recorded on a Jasco IRA-I and uv spectra (in MeOH) were measured on Pye Unicam SP-800 spectrometer. Mass spectra were recorded on Finnigan MAT 312 double focusing mass spectrometer coupled with PDP 11/34 computer system. The ^{1}H-nmr (300 MHz) and ^{13}C-nmr (75 MHz) spectra were recorded on a Bruker AM-300 spectrometer in $CDCl_3$. Merck Kieselgel 60 PF 254 coated on glass plates was used for analytical (thin layer) and preparative (thick layer) chromatography.

Nutrient broth medium was prepared by dissolving 8 g of nutrient broth (Merck) in 1000 ml of warm distilled H_2O. Nutrient agar medium was prepared by dissolving 8 g of nutrient broth powder (Merck) in 1000 ml of distilled H_2O, adding 20 g of agar (Fluka), and boiling for 2 min.

ANTIMICROBIAL ACTIVITY.—Inocula were prepared by incubating one loopful of stock culture in 10 ml of nutrient broth for 24 h at 37°. Nutrient agar plates were swabbed with 0.1 ml of the overnight inocula. Wells of 0.6 cm were dug with a sterile borer in the inoculated agar. Into the well was placed 0.1 ml of the test solution. A control was maintained by using 0.1 ml of DMSO. The plates were incubated at 37° for 24 h. All tests were done in duplicate.

PLANT MATERIAL.—The leaves of *P. juliflora* were collected from the grounds of Karachi University and identified by the Department of Botany, University of Karachi. A voucher specimen has been deposited in the Herbarium of the Botany Department, University of Karachi.

EXTRACTION AND ISOLATION.—The leaves of *P. juliflora* (20 kg) were extracted exhaustively with MeOH. The residue obtained on evaporation of the MeOH extract was partitioned between EtOAc and H_2O. The aqueous layer was basified with NH_4OH (pH 9) and extracted repeatedly with $CHCl_3$. The alkaloid-containing $CHCl_3$ layers were combined and evaporated at reduced pressure to afford a gummy residue; this was treated with C_6H_6, and C_6H_6-soluble and C_6H_6-insoluble portions were obtained. The C_6H_6-soluble portion was selected for investigation and chromatographed on a neutral alumina column. The polar fractions were rechromatographed. Juliflorinine was eluted from the column with $CHCl_3$-MeOH (93:7). Purity was checked by tlc [Si gel, $CHCl_3$-MeOH-diethylamine (90:10:10)]. Juliprosinene was eluted with $CHCl_3$-MeOH (73:27). Juliprosinene was further purified by tlc [Si gel, $CHCl_3$-MeOH-NH_3 (70:30:1)].

JULIPROSINENE [**1**].—Compound **1** was isolated as a gum: $[\alpha]_D$ +9.5° (c = 0.04, $CHCl_3$); uv λ max (MeOH) 208 and 285 nm; ir ν max ($CHCl_3$) 3660, 3350, 2935, 1625, 1505, 965, 877 cm^{-1}; eims *m/z* (rel. int.) $[M]^+$ 624 (3.63), 608 (3.67), 389 (21.76), 114 (100), 92 (12.30), 70 (96.68); hrms *m/z* $[M+1]^+$ 625.55329 (calcd for $C_{40}H_{71}N_3O_2$, 625.55316), 389.355 (calcd for $C_{25}H_{45}N_2O$, 389.351); ^{1}H-nmr (300 MHz, $CDCl_3$) δ 9.35 (1H, s, H-5′′′′); 7.68 (1H, s, H-7′′′′), 6.30 (2H, dd, $J_{3''',2'''}$ = 6.2, J_{gem} = 14 Hz, H_a-3′′′′), 3.53 (2H, br s, H-3, H-3′), 6.85 (1H, d, $J_{1''',2'''}$ = 10.6 Hz, H-1′′′′), δ 6.25 (1H, dd, $J_{3b''',2'''}$ = 6.2 Hz, J_{gem} = 14 Hz, H_b-3′′′′), 2.75–2.97 (2H, m, H-2, H-2′), 2.68 (4H, t, J = 7.0 Hz, H-10″, H-10‴), 1.25 (32H, s, CH_2 of side chain), 6.82 (1H, dd, $J_{1''',2'''}$ = 10.6 Hz, $J_{2''',3'''}$ = 6.2 Hz, H-2′′′′), 2.65–2.55 (2H, m, H-6, H-6′), 1.13, 1.05 (2d, J = 6.8 Hz, 2-Me, 2′-Me); ^{13}C-nmr (75 MHz, $CDCl_3$), δ 129.5 (C-1′′′′), 115.5 (C-2′′′′), 68.2 (C-3′′′′), 139.49 (C-5′′′′), 139.14 (C-6′′′′), 143.98 (C-7′′′′), 141.9 (C-8′′′′), 160.73 (C-8a′′′), 57.26 (C-2, C-2′), 67.9 (C-3, C-3′), 32.09 (C-4, C-4′), 25.84 (C-5, C-5′), 55.85 (C-6, C-6′), 36.10–35.86 (C-1″, C-1‴), 24.98, 25.00 (C-2″, C-2‴), 32.09–30.93 (C-3″–C-10″, C-3‴–C-10‴), 18.17, 18.10 (C-7, C-7′).

JULIFLORININE [**2**].—$[\alpha]_D$ +3.9 (c = 0.03, $CHCl_3$); uv λ max (MeOH) 208, 285 nm; ir ν max ($CHCl_3$) 3680, 3400, 2920, 2840, 2485, 1600, 1360, 1140 cm^{-1}; ms *m/z* (rel. int.) $[M]^+$ 629 (12), 611 (7), 574 (7), 389 (100), 333 (8), 248 (18), 187 (59), 114 (76), 70 (88), 55 (92); ^{1}H-nmr ($CDCl_3$, 100 MHz) δ 5.275 (1H, s, H-7′′′′), 3.66 (quintet, H-3), 3.33 (1H, d, J = 15.5 Hz, H_{eq}-5′′′′), 3.06 and 3.17 (octet, distorted, H-2 and H-3′′′′), 2.7 (1H, d, J = 15.5 Hz, H_{ax}-5′′′′), 2.57 (2H, m, H-6, H-6′), 1.202 (s, methylene protons of side chains), 1.0275 (d, J = 7 Hz, 2-Me, 2′-Me); ^{13}C-nmr see Table 1.

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