Available online at www.sciencedirect.com
SCIENCE DIRECT®
Phytochemistry 65 (2004) 587–591
PHYTOCHEMISTRY
www.elsevier.com/locate/phytochem

Growth inhibitory alkaloids from mesquite (*Prosopis juliflora* (Sw.) DC.) leaves

Hiroshi Nakano[a,*], Eri Nakajima[b], Syuntaro Hiradate[b], Yoshiharu Fujii[b], Kosumi Yamada[c], Hideyuki Shigemori[c], Koji Hasegawa[c]

[a]*Doctoral Program in Agricultural Sciences, University of Tsukuba, Tsukuba, Ibaraki, 305-8572, Japan*
[b]*National Institute for Agro-Environmental Sciences, Tsukuba, Ibaraki, 305-8604, Japan*
[c]*Institute of Applied Biochemistry, University of Tsukuba, Tsukuba, Ibaraki, 305-8572, Japan*

Received 29 July 2003; received in revised form 11 December 2003

Abstract

Plant growth inhibitory alkaloids were isolated from the extract of mesquite [*Prosopis juliflora* (Sw.) DC.] leaves. Their chemical structures were established by ESI-MS, ^{1}H and ^{13}C NMR spectra analysis. The I_{50} value (concentration required for 50% inhibition of control) for root growth of cress (*Lepidium sativum* L.) seedlings was 400 μM for 3‴′-oxo-juliprosopine, 500 μM for secojuliprosopinal, and 100 μM for a (1:1) mixture of 3-oxo-juliprosine and 3′-oxo-juliprosine, respectively. On the other hand, the minimum concentration exhibiting inhibitory effect on shoot growth of cress seedlings was 10 μM for 3‴′-oxo-juliprosopine, 100 μM for secojuliprosopinal, and 1 μM for a (1:1) mixture of 3-oxo-juliprosine and 3′-oxo-juliprosine, respectively. Among these compounds, a (1:1) mixture of 3-oxo-juliprosine and 3′-oxo-juliprosine exhibited the strongest inhibitory effect on the growth of cress seedlings.

Keywords: *Prosopis juliflora*; Leguminosae; Mesquite; Plant growth inhibitors; Alkaloid; 3-Oxo-juliprosine; 3′-Oxo-juliprosine; 3‴′-Oxo-juliprosopine; Secojuliprosopinal

1. Introduction

Mesquite (*Prosopis juliflora* (Sw.) DC.), Leguminosae is widespread in Saudi Arabia, the United States of America and India (Sankhla et al., 1965; Bragg et al., 1978; Pandit et al., 1995; Al-Humaid and Warrag, 1998). Recently, L-tryptophan, syringin, and (−)-lariciresinol were isolated from mesquite leaf leachates as candidates for allelopathy in mesquite (Nakano et al., 2001, 2002). Among these inhibitors, L-tryptophan exhibited the strongest activity on the growth of lettuce and barnyard grass seedlings and is believed to be a major allelochemical in mesquite (Nakano et al., 2001, 2002, 2003). Since most of these studies concentrated on leachates and exudates from leaves, the plant growth inhibitor(s) from leaf extracts of mesquite have not yet been reported. In this study, we report the isolation and identification of growth inhibitory alkaloids from the leaf extracts of mesquite and their biological activities on the growth of cress seedlings.

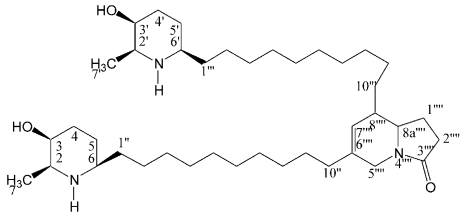
2. Results and discussion

2.1. Determination of isolated compounds

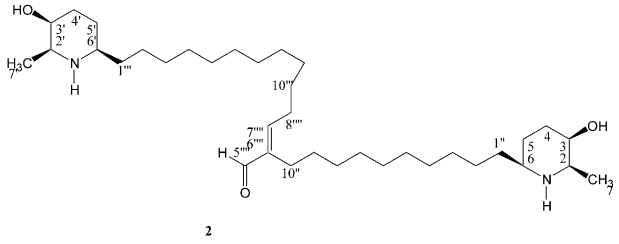
Fresh leaves of mesquite (*Prosopis juliflora* (Sw.) DC.) were extracted with MeOH with the extract then partitioned between H_2O and EtOAc. The aqueous layer was next basified with NH_4OH (pH 9) and partitioned with $CHCl_3$. The $CHCl_3$-soluble materials were subjected to repeated chromatographic steps on a Sep-Pak ODS cartridge column. Further purification using reversed-phase HPLC afforded 3‴′-oxo-juliprosopine (**1**), secojuliprosopinal (**2**), and a (1:1) mixture of 3-oxo-juliprosine (**3a**) and 3′-oxo-juliprosine (**3b**). It was unsuccessful to separate pure component from the mixture (**3a** and **3b**). Compound **1** was isolated as a colorless gum [$[\alpha]_D^{23}$

* Corresponding author at present address: National Agricultural Research Center for Kyushu Okinawa Region, Chikugo, Fukuoka, 833-0041, Japan. Tel.: +81-942-52-0670; fax: +81-942-53-7776.
E-mail address: nakanohr@affrc.go.jp (H. Nakano).

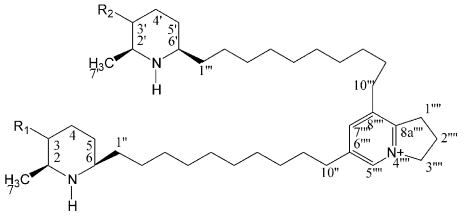
0031-9422/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.phytochem.2004.01.006



1



2



3a R_1=O, R_2=β-OH
3b R_1=β-OH, R_2=O

+4.0° (*c* 1.0, MeOH)]. The positive ion ESI-MS spectrum of **1** displays a pseudomolecular ion peak at *m/z* 645 $[M+H]^+$ and its molecular formula was inferred as $C_{40}H_{73}N_3O_3$ from the HRESIMS data [*m/z* 644.5728 $(M+H)^+$, Δ_H −0.3 mmu]. The IR spectrum implied the presence of hydroxyl, amino (3420 cm^{-1}) and lactam carbonyl (1680 cm^{-1}) groups. The structure of **1** was deduced from detailed analysis of the ^1H and ^{13}C NMR spectral data (Table 1) aided with 2D NMR experiments (^1H–^1H COSY, HMQC, and HMBC). The ^{13}C NMR spectral data indicated that the molecule possessed one lactam carbonyl carbon, one trisubstituted olefin, two oxymethines, five methines bearing with a nitrogen atom, one methine, two methyls, and several methylenes. The NMR spectral data were similar to those of juliprosopine (Ott-Longoni et al., 1980) except for a pyrrolidine ring (C-1‴-N-4‴ and C-8a‴). The ^1H-^1H COSY connectivities of C-2 to C-6 and C-7 and C-2′ to C-6′ and C-7′ and the ^{13}C NMR resonances at δ_C 15.9 (2-Me, 2′-Me), δ_C 57.6 (C-2, C-2′), δ_C 65.8 (C-3, C-3′), and δ_C 58.8 (C-6, C-6′) (Christofidis et al., 1977) indicated the presence of two 2-methyl-3-hydroxypiperidine rings. HMBC correlations of H-7‴ (δ_H 5.46, *s*) to C-5‴ (δ_C 43.4), C-8a‴ (δ_C 59.9), and C-10″ (δ_C 35.7), H-1‴ to C-2‴ (δ_C 30.1) and C-3‴ (δ_C 176.6), and H_{ax}-5‴ (δ_H 4.06) to C-6‴ (δ_C 135.1) and ^1H–^1H COSY connectivities of C-7‴ to C-8a‴ and C-1‴ to C-2‴ revealed that **1** possessed an indolizidine-3(2*H*)-one moiety. Thus, the structure of **1** was assigned to be 3‴-oxo-juliprosopine.

Compound **2** was obtained as a colorless gum ($[\alpha]_D^{28}$ +5.0° (*c* 1.0, MeOH)) and the molecular formula, $C_{36}H_{69}N_2O_3$, of **2** was established by its positive ion HRESIMS [*m/z* 577.5336 $(M+H)^+$, Δ_H +2.7 mmu]. The ^1H NMR spectrum (Table 1) of compound **2** indicated the presence of one olefinic proton (H-7‴, δ_H 6.54, *t*, *J* = 7.7 Hz) and one aldehyde (H-5‴, δ_H 9.28, *s*). ^1H–^1H COSY connectivities of C-2 to C-6 and C-7 and an HMBC correlation between H-2 and C-6 (δ_C 135.1) indicated the presence of two 2-methyl-3-hydroxypiperidine rings. HMBC correlations of H-5‴ to C-10‴ (δ_C 25.5), C-6‴ (δ_C 145.2), and C-7‴ (δ_C 157.6), H-10‴ (δ_H 2.19, *t*, *J* = 7.3 Hz) to C-5‴ (δ_C 197.3), C-6‴, and C-7‴, and H-8‴ (δ_H 2.34, *dt*, *J* = 7.3, 7.3 Hz) to C-6‴ and C-7‴ revealed the presence of a α, β-unsaturated aldehyde functionality (C-5‴ to C-7‴) connected between C-10″/C-6‴ and C-8‴/C-7‴, which was supported by the IR (1681 cm^{-1}) and UV [λ_{max} 224 nm (ϵ 4700), MeOH] spectra. The geometry of the trisubstituted olefin at C-6‴ was elucidated to be *E* from NOESY correlations of H-5‴ to H-7‴ and H-10″ to H-8‴. Thus, structure **2** was determined to be secojuliprosopinal.

Compounds **3a** and **3b** were obtained as colorless gum ($[\alpha]_D^{28}$ +3.0° (*c* 1.0, MeOH)) and displays a pseudomolecular ion peak at *m/z* 624 (M^+) in the positive ion ESIMS. HRESIMS analysis revealed the molecular formula to be $C_{40}H_{70}N_3O_2$ [*m/z* 624.5446 (M^+), Δ −2.2 mmu], indicating an oxidized form of juliprosine (Dätwyler et al., 1981). The ^1H and ^{13}C NMR spectra (Table 1) of **3a** were similar to those of juliprosine except for signals due to a 2-methyl-3-hydroxypiperidine ring (N-1 to C-7). The ^1H–^1H COSY connectivities of C-2 to C-7 and C-4 to C-6 and HMBC correlations of H-2 (δ_H 3.91), H-4 (δ_H 2.92), and H-5 (δ_H 1.88 and 2.19) to C-3 (δ_C 195.7) indicated the presence of 2-methyl-3-oxopiperidine ring. The ^1H and ^{13}C NMR spectra (Table 1) of **3b** were similar to those of juliprosine except for signals due to the 2′-methyl-3′-hydroxypiperidine ring (N-1′ to C-7′). The ^1H-^1H COSY connectivities of C-2′ to C-7′ and C-4′ to C-6′ and HMBC correlations of H-2′ (δ_H 3.91), H-4′ (δ_H 2.92), H-5′ (δ_H 1.88 and 2.19) to C-3′ (δ_C 195.7) indicated the presence of a 2′-methyl-3′-oxopiperidine ring. HMBC correlations of H-5‴ (δ_H 8.65) to C-3‴ (δ_C 61.3), C-6‴ (δ_C 143.7), C-7‴ (δ_C 146.6), C-8a‴ (δ_C 157.3), and C-10‴ (δ_C 34.0), H-7‴ (δ_H 8.20) to C-5‴ (δ_C 139.6), C-8a‴, C-10″, and C-10‴ (δ_C 34.0), and H-1‴ (δ_H 3.50) to C-8‴, C-8a‴, C-2‴ (δ_C 22.9), and C-3‴ revealed that **3a** and **3b** possessed the dihydroindolizinium moiety connected between C-6‴/C-10″ and C-8‴/C-10‴, which was supported by UV spectrum [λ_{max} 276 (ϵ 3400) and 284 (2800) nm, MeOH]. Thus, the structures of **3a** and **3b**

were assigned to be 3-oxo-juliprosine and 3′-oxo-juliprosine, respectively.

2.2. Biological activity

The effect of the three isolated alkaloids on root and shoot growth of cress seedlings was examined (Fig. 1). They showed inhibitory effects on both root and shoot growth of cress in a dose-dependent manner. The calculated I_{50} value (concentration required for 50% inhibition of control) for root growth was 400 μM for **1** and 600 μM for **2**, whereas both compounds exhibited lower inhibition on shoot growth at the same concentrations used. On the other hand, a (1:1) mixture of **3a** and **3b** showed a much stronger inhibition on both the root and shoot growth of cress seedlings. The I_{50} value was 100 μM for the root and 200 μM for the shoot tissue, respectively. Therefore, a (1:1) mixture of **3a** and **3b** seemed to have the strongest activity among these compounds. Although the isolation and identification of analogous alkaloids described here has been previously reported in mesquite leaf extracts (Ott-Longoni et al., 1980; Dätwyler et al., 1981; Ahmad et al., 1989), the biological activity on the plant growth has not been reported. Thus, the study of structure-activity relationship of these compounds described here and their analogs would be important to investigate in the future.

3. Experimental

3.1. General

ESI-MS was recorded on a Platform LC (Waters) and the ^1H and ^{13}C NMR spectra were on JNM α-600 spectrometer (JEOL) and AVANCE-500 (Bruker) spectrometers.

3.2. Isolation of alkaloids

Fresh leaves (1 kg fresh wt) of mesquite (*Prosopis juliflora* (Sw.) DC.) were soaked in MeOH (6 l) for 2 weeks at room temp with the extract filtered and concentrated to dryness in vacuo at 35 °C, with the concentrate (20 g) was then partitioned between H_2O and EtOAc with the aqueous layer was basified with NH_4OH (pH 9) and extracted repeatedly with $CHCl_3$. The combined $CHCl_3$ layers were concentrated to dryness in vacuo at 35 °C with the concentrate (5 g) separated by C18 cartridge chromatography (Sep-Pak Vac 35 cc

Table 1
^1H and ^{13}C NMR spectral data in CD_3OD (^1H; 600 MHz, ^{13}C 150.8 MHz) for **1**, **2**, **3a**, and **3b**

	1			**2**			**3a**			**3b**		
No.	δ_H		δ_C	δ_H		δ_C	δ_H		δ_C	δ_H		δ_C
1	–		–	–		–	–		–	–		–
2	3.19–3.25	(*m*)	57.6	3.15–3.21	(*m*)	57.7	3.24–3.30	(*m*)	58.0	3.85–3.95	(*m*)	58.3
3	3.83	(*br.s*)	65.8	3.79	(*br.s*)	65.9	3.85	(*br.s*)	195.7	–		66.6
4	1.71–1.80	(*m*)	23.6	1.66–1.75	(*m*)	23.7	*		39.5	2.63–2.77	(*m*)	*
5	1.55–1.65	(*m*)	34.8	1.50–1.61	(*m*)	34.8	*		*	*		*
6	3.00–3.08	(*m*)	58.8	2.96–3.04	(*m*)	58.9	3.05–3.15	(*m*)	64.9	4.05–4.10	(*m*)	59.5
1′	–		–	–		–	–		–	–		–
2′	3.19–3.25	(*m*)	57.6	3.15–3.12	(*m*)	57.7	3.85–3.95	(*m*)	58.3	3.24–3.30	(*m*)	58.0
3′	3.83	(*br.s*)	65.8	3.79	(*br.s*)	65.9	–		66.6	3.85	(*br.s*)	195.7
4′	1.71–1.80	(*m*)	23.6	1.66–1.90	(*m*)	23.7	2.63–2.77	(*m*)	*	*		39.5
5′	1.55–1.65	(*m*)	34.8	1.50–1.61	(*m*)	34.8	*		*	*		*
6′	3.00–3.08	(*m*)	58.8	2.96–3.04	(*m*)	58.9	4.05–4.10	(*m*)	59.5	3.05–3.15	(*m*)	64.9
7″	1.91–1.99	(*m*)	*	1.86–1.94		*	*		*	*		*
10″	2.05	(*t*, $J=7.3$ Hz)	35.7	2.19	(*t*, $J=7.3$ Hz)	25.5	2.80	(*t*, $J=7.3$ Hz)	34.0	2.80	(*t*, $J=7.3$ Hz)	34.0
1‴	1.91–1.99	(*m*)	*	1.86–1.94		*	*		*	*		*
10‴	*		*	*		*	2.80	(*t*, $J=7.3$ Hz)	34.0	2.80	(*t*, $J=7.3$ Hz)	34.0
1⁗	2.40	(*m*)	*	–		–	3.50	(*t*, $J=7.7$ Hz)	32.4	3.50	(*t*, $J=7.7$ Hz)	32.4
2⁗	*		*	–		–	2.50–2.60	(*m*)	22.9	2.50–2.60	(*m*)	22.9
3⁗	*		*	–		–	4.90	(*t*, $J=7.9$ Hz)	61.3	4.90	(*t*, $J=7.9$ Hz)	61.3
4⁗	–		–	–		–	–		–	–		–
5⁗	H_{ax}; 3.40	(*d*, $J=18.3$ Hz)	43.2	9.28	(*s*)	197.3	8.65	(*s*)	139.6	8.65	(*s*)	139.6
	H_{eq}; 4.06	(*d*, $J=18.3$ Hz)										
6⁗	–		103.1	–		145.2	–		141.5	–		141.5
7⁗	5.46	(*s*)	124.7	6.54	(*t*, $J=7.7$ Hz)	157.6	8.20	(*s*)	146.6	8.20	(*s*)	146.6
8⁗	–		135.1	2.34	(*dt*, $J=7.3$, 7.3 Hz)	*	–		143.7	–		143.7
8a⁗	–		176.6	–		–	–		157.3	–		157.3
2–Me	*		15.9	*		16.0	*		16.1	*		16.1
2′–Me	*		15.9	*		16.0	*		16.1	*		16.1

* Signals overlapped.

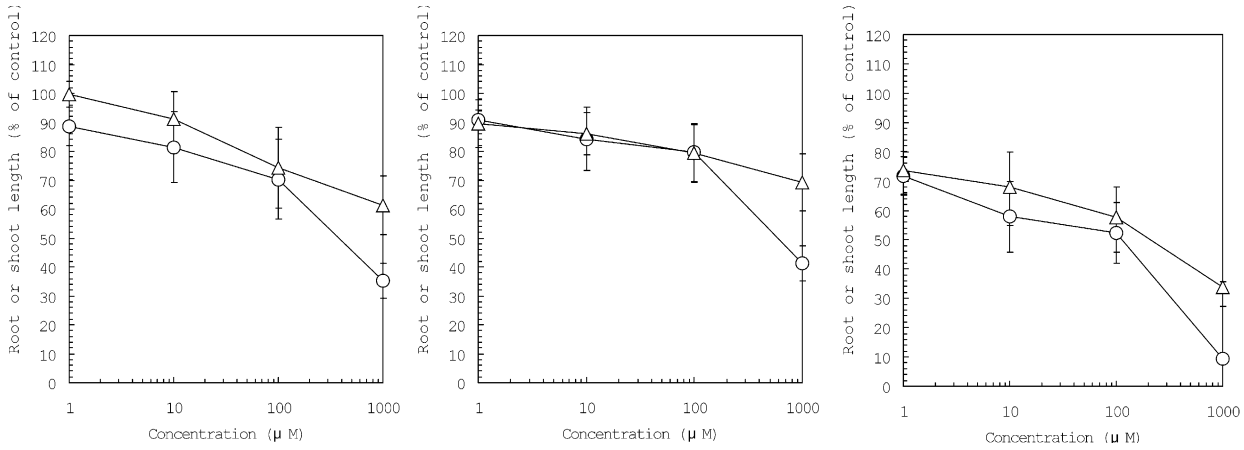


Fig. 1. Effects of **1**,**2**, and a (1:1) mixture of **3a** and **3b** on the root (○) and shoot (△) growth of cress seedlings. Means±SE of results from 3 replicates of 10 plants.

(10 g), Waters) into three fractions of MeOH- H_2O-TFA (trifluoroacetic acid) (80: 20: 0.1, v/v), MeOH-TFA (100: 0.1, v/v) and CH_3Cl–TFA (100: 0.1, v/v), respectively. The MeOH–H_2O–TFA fraction was concentrated to dryness in vacuo at 35 °C. The concentrate (1.25 mg) was subjected to HPLC (ODS-80Ts, 4.6 × 250 mm, Tosoh, MeOH:H_2O:TFA = 75:25:0.1, v/v, 0.8 ml/min, with detection at 214 nm). The fraction, with a retention time of 20–35 min, yielded 3′′′′-oxo-juliprosopine (**1**, 1.0 mg) and secojuliprosopinal (**2**, 2.0 mg). The fraction, with a retention time of 0–10 min, was concentrated to dryness in vacuo at 35 °C. The concentrate (323.3 mg) was further purified by HPLC (ODS-80Ts, 4.6 × 250 mm, Tosoh, MeOH:H_2O:TFA = 56:44:0.1, v/v, 0.8 ml/min, detected at 254 nm), with the fraction, with a retention time of 24–27 min, affording a (1:1) mixture of 3-oxo-juliprosine and 3′-oxo-juliprosine (**3a** and **3b**, 7.2 mg).

3.2.1. 3′′′′-Oxo-juliprosopine (*1*)

Colorless gum, $[\alpha]_D^{23}$ +4.0° (*c* 1.0, MeOH), HRE-SIMS: *m*/*z* 644.5728 $(M+H)^+$, Δ_H −0.3 mmu (calc. for $C_{40}H_{73}N_3O_3$). IRν_{max} (KBr) cm^{-1}: 3420, 2928, 2855, 1680, 1458, 1203, 1136, 837, 722. For ^1H and ^{13}C NMR spectral analysis, see Table 1.

3.2.2. Secojuliprosopinal (*2*)

Colorless gum, $[\alpha]_D^{28}$ +5.0° (*c* 1.0, MeOH), HRE-SIMS: *m*/*z* 577.5336 $(M+H)^+$, Δ_H +2.7 mmu (calc. for $C_{36}H_{69}N_2O_3$). IRν_{max} (KBr) cm^{-1}: 3423, 2927, 2855, 1681, 1458, 1203, 1137, 1011, 838, 801. For ^1H and ^{13}C NMR spectral analysis, see Table 1.

3.2.3. 3-Oxo-juliprosine and 3′-oxo-juliprosine (*3a* and *3b*)

Colorless gum, $[\alpha]_D^{28}$ +3.0° (*c* 1.0, MeOH), HRE-SIMS: *m*/*z* 624.5446 (M^+), Δ_H −2.2 mmu (calc. for $C_{40}H_{70}N_3O_2$). IRν_{max} (KBr) cm^{-1}: 3423, 2929, 2857, 1682, 1508, 1457, 1202, 1133, 834, 801, 721. For ^1H and ^{13}C NMR spectral analysis, see Table 1.

3.3. Bioassay

Ten seeds of cress (*Lepidium sativum* L.) were placed on a filter paper (No. 1, Toyo) moistened with 500 μl of test solution in a 3.3 cm Petri dish and kept for 2 days at 25 °C in the dark, after which the lengths of their roots and shoots were measured.

Acknowledgements

We thank Banyu Pharmaceutical Co., Ltd. for measurement of ESIMS.

References

Ahmad, V.U., Sultana, A., Qazi, S., 1989. Alkaloids from the leaves of *Prosopis Juliflora*. J. Nat. Prod. 52, 497–501.

Al-Humaid, A.I., Warrag, M.O.A., 1998. Allelopathic effects of mesquite (*Prosopis juliflora*) foliage on seed germination and seedling growth of bermudagrass (*Cynodon dactylon*). J. Arid. Env. 38, 237–243.

Bragg, L.H., Bacon, J.D., McMillan, C., Mabry, T.J., 1978. Flavonoid patterns in the *Prosopis Juliflora* complex. Biochem. Syst. Ecol. 6, 113–116.

Christofidis, I., Welter, A., Jadot, J., 1977. Spectaline and iso-6 cassine, two new piperidine 3-ol alkaloids from the leaves of *Cassia spectabilis*. Tetrahedron 33, 977–979.

Dätwyler, P., Ott-Longoni, R., Schöpp, E., Hesse, M., 1981. Über Juliprosin, ein weiteres Alkaloid aus *Prosopis juliflora* A. DC. Helv. Chim. Acta 64, 1959–1963.

Nakano, H., Fujii, Y., Suzuki, T., Yamada, K., Kosemura, S., Yamamura, S., Suzuki, T., Hasegawa, K., 2001. A growth-inhibitory substance exuded from freeze-dried mesquite (*Prosopis juliflora* (Sw.) DC.) leaves. Plant Growth Regul. 33, 165–168.

Nakano, H., Fujii, Y., Yamada, K., Kosemura, S., Yamamura, S., Hasegawa, K., Suzuki, T., 2002. Isolation and identification of plant

growth inhibitors as candidate(s) for allelopathic substance(s), from aqueous leachate from mesquite (*Prosopis juliflora* (Sw.) DC.) leaves. Plant Growth Regul. 37, 113–117.

Nakano, H., Nakajima, E., Fujii, Y., Yamada, K., Shigemori, H., Hasegawa, K., 2003. Leaching of allelopathic substance, L-tryptophan from the foliage of mesquite (*Prosopis juliflora* (Sw.) DC.) plants by water spraying. Plant Growth Regul. 40, 49–52.

Ott-Longoni, R., Viswanathan, N., Hesse, M., 1980. Die Konstitution des Alkaloides Juliprosopin aus *Prosopis juliflora* A. DC. Helv. Chim. Acta 63, 2119–2129.

Pandit, B.R., Mahesh, K.R., Kotiwar, O.S., 1995. Effect of *Prosopis juliflora* (Sw.) DC. extracts on root and shoot growth of bajra seedlings. Geobios 22, 145–148.

Sankhla, N., Baxi, M.D., Chatterji, U.N., 1965. Eco-physiological studies on arid zone plants. 1. Phytotoxic effects of aqueous extract of mesquite, *Prosopis juliflora* DC. Current Science 21, 612–614.