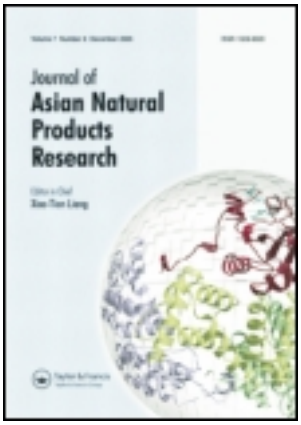

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Isolation and characterization of secondary metabolites from *Plumeria obtusa*

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Chromatographic purification of the methanolic extract of *Plumeria obtusa* yielded two new iridoid obtusadoids A (**1**) and B (**2**), along with eight known compounds plumieridin A (**3**), plumieridine (**4**), 1α-plumieride (**5**), 15-demethylplumieride (**6**), rel-(3*R*,3′*S*,4*R*,4′*S*)-3,3′,4,4′-tetrahydro-6,6′-dimethoxy[3,3′-bi-2H-benzopyran]-4,4′-diol (**7**), glochiflavanoside B (**8**), oleanolic acid (**9**), and methyl coumarate (**10**). The structures of all the isolates (**1**–**10**) were determined by NMR spectroscopy and mass spectrometry. The data of known compounds (**3**–**10**) were further compared with the reported data for these compounds.

Keywords: *Plumeria obtusa*; secondary metabolites; iridoids; characterization

1. Introduction

The genus *Plumeria* of family 'Apocynaceae' is a group of shrubs and trees, commonly known as frangipani (plant of love) [1]. It is constituted of eight species growing in tropical areas of the world [2,3]. In Pakistan, it is represented by two species *Plumeria rubra* and *Plumeria obtusa*, growing as ornamental plants [4]. Various species of genus *Plumeria* are used as medicine for the treatment of diarrhea, gonorrhea, syphilis, venereal sores, and leprosy [5]. *Plumeria* species afford anti-inflammatory, diuretic, emmenagogue, febrifuge, purgative and anti-rubefacient diabetic properties, and are also known for its tonic and expectorant actions [6]. They are used for the treatment of skin diseases, and as antipsychotic and antitumor agents as well as inhibitors of human immunodeficiency virus type-1 [7,8]. The phytochemical literature survey revealed that these plants are rich in iridoids [3,9–11]. The most common are grandines A–C, phoebegrandine B, and fulvoplumeirin, first isolated from *Plumeria acutifolia* as an antibacterial agent [12,13].

In this work, two new iridoid obtusadoids A (**1**) and B (**2**), along with eight known compounds plumieridin A (**3**) [14], plumieridine (**4**) [15], 1-α-plumieride (**5**) [10], 15-demethylplumieride (**6**) [11], rel-(3*R*,3′*S*,4*R*,4′*S*)-3,3′,4,4′-tetrahydro-6,6′-dimethoxy[3,3′-bi-2H-benzopyran]-4,4′-diol (**7**) [16], glochiflavanoside B (**8**) [17], oleanolic acid (**9**) [18], and *p*-methyl coumarate (**10**) [19], have been isolated from the ethyl acetate (EtOAc) fraction of methanolic extract of *P. obtusa* (Figure 1).

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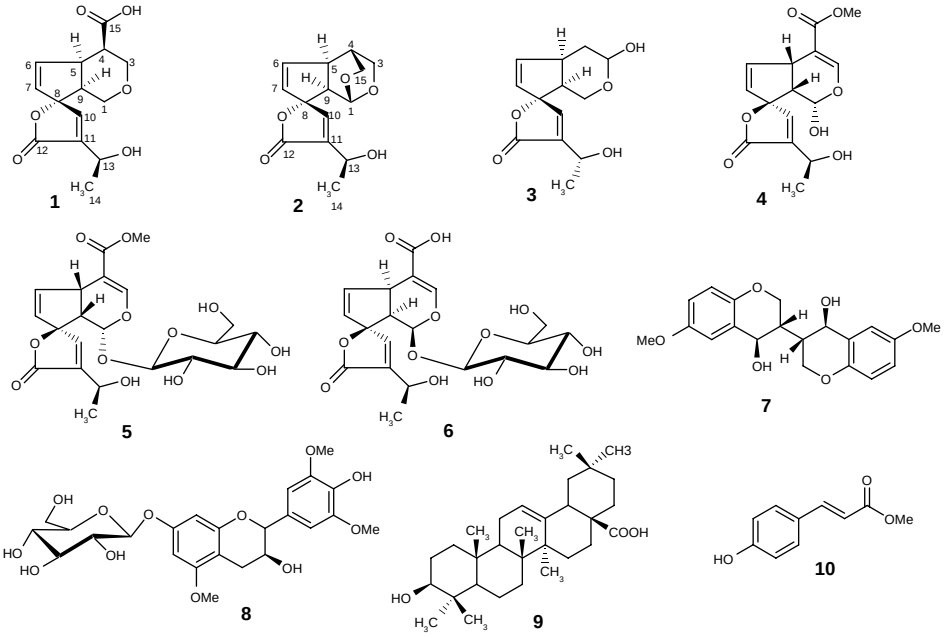

Figure 1. Structures of compounds **1**–**10**.

2. Results and discussion

The EI-MS of **1** displayed the molecular ion at *m/z* 280, whereas the HR-EI-MS analysis of the same ion gave the molecular formula $C_{14}H_{16}O_6$ (*m/z* 280.0938) with seven double bond equivalents (DBE). The ^{1}H NMR spectrum of **1** displayed signals for three olefinic protons at δ 6.23, 5.60, and 7.29, whereas their corresponding carbons resonated in the ^{13}C NMR spectrum (Table 1) at δ 139.2, 133.1, and 149.1. In addition, a quaternary olefinic carbon displayed its position at δ 140.8, indicating the presence of at least two double bonds. The carbon signals at δ 172.6 (C═O), 149.1 (C—H), and 140.8 (C) were attributed to a five-member α,β-unsaturated γ-lactone system, which was further confirmed through IR spectrum that displayed a strong absorption band at 1755 cm^{-1} for lactone carbonyl. The IR spectrum also showed bands for hydroxyl function, carboxylic group, and double bond. The ^{1}H NMR spectrum displayed an oxygenated methine at δ 4.54 (dq, J = 6.5, 1.3 Hz). The cross peaks of this methine in COSY spectrum (Figure 1) with a methyl doublet (δ 1.43, J = 6.5 Hz) and an olefinic methine (δ 7.29), together with the HMBC correlation of H-10 (δ 7.29) with C-13 (δ 63.8) revealed that the hydroxyethyl group was attached with α,β-unsaturated lactone system. These data were an indication of iridoid nucleus [11,14]. The olefinic methines at δ 6.23 (H-6) and 5.60 (H-7) were assigned to the double bond of the cyclopentene based on COSY and HMBC spectral data. The HMBC correlations observed for H-5 (δ 3.94) with C-7 (δ 133.1) and C-8 (δ 100.4), H-6 (δ 6.23) with C-8 (δ 100.4), and H-7 (δ 5.60) with C-5 (δ 45.3) and C-9 (δ 45.6) established the presence of the cyclopentene unit in **1**. The *spiro*-center of cyclopentene and α,β-unsaturated lactone was fixed at C-8. These whole data accommodated six DBE and the remaining one could be attributed to a hydrogenated pyran ring. The signals for two oxygenated methylene protons appeared in the spectrum at δ 4.46

Table 1. ^{1}H and ^{13}C NMR spectral data of compounds **1** and **2** (CD_3OD, 500 and 125 MHz).

	1		**2**	
Position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1	4.46 dd (J = 12.5, 3.5) 4.26 m	67.9	4.71 d (J = 7.5)	96.1
3	4.02 dd (J = 11.5, 2.5) 3.72 m	60.1	4.14 dd (J = 12.5, 5.0) 3.41 dd (J = 12.5, 2.0)	57.7
4	3.18 ddd (J = 7.0, 6.5, 2.5)	46.4	3.26 m	38.7
5	3.94 ddt (J = 9.0, 6.5, 2.0)	45.3	3.09 m	42.4
6	6.23 dd (J = 5.5, 2.0)	139.2	6.30 m	143.8
7	5.60 dd (J = 5.5, 2.0)	133.1	5.33 d (J = 6.0)	130.6
8	–	100.4	–	98.8
9	3.07 ddd (J = 9.0, 6.5, 3.5)	45.6	2.54 m	45.9
10	7.29 d (J = 1.0)	149.1	7.36 d (J = 1.5)	150.3
11	–	140.8	–	138.8
12	–	172.6	–	173.0
13	4.54 q (J = 6.5)	63.8	4.56 m	63.8
14	1.43 d (J = 6.5)	22.3	1.41 d (J = 6.5)	22.5
15	–	174.8	3.76 dd (J = 12.0, 6.2) 3.70 dd (J = 12.0, 2.6)	62.5

(1H, dd, J = 12.5, 3.5 Hz, H_{eq}-1), 4.26 (1H, m, H_{ax}-1), 4.02 (1H, dd, 11.5, 2.5 Hz, H_{eq}-3), and 3.72 (1H, m, H_{ax}-3) indicated that the pyran ring of iridoid nucleus must be saturated. The cross peak of H-3 (δ 4.02, 3.72) of pyran ring with H-4 (δ 3.18) in COSY spectrum and HMBC correlations (Figure 2) of H-3 and H-4 with the carbon resonated at δ 174.8 helped to fix the carboxylic function at C-4.

The relative stereochemistry at various centers in **1** could be established through NOESY data, molecular model, and in comparison with the reported compounds from the same genus [11,14]. The equatorial position of carboxylic function at C-4 could be established due to NOESY correlations of H-3_{eq} (δ 4.02) with H-4 (δ 3.18), which was further confirmed through NOESY interactions of H-4 with *cis*-hydrogens (H-5 and H-9) of cyclopentene ring. The dihedral angle between these hydrogens in molecular model and various coupling constants associated with them also supported the idea. The stereochemistry on the remaining centers was

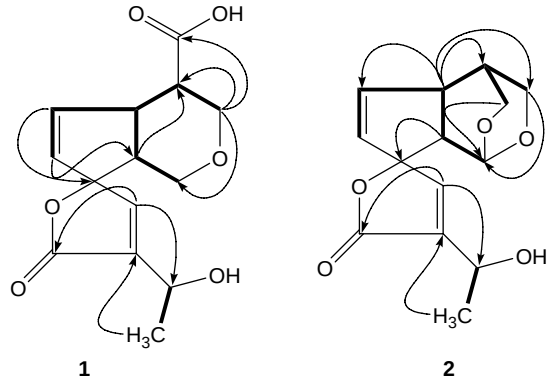

Figure 2. HMBC ( ) and COSY (—) correlations of **1** and **2**.

exactly similar as established in iridoids reported from various *Plumeria* species [11,14].

Compound **2** was also found to be an iridoid whose EI-MS showed the molecular ion at *m/z* 264 and the formula was determined by HR-EI-MS as $C_{14}H_{16}O_5$ with seven DBE. The IR spectrum showed the absorption bands for hydroxyl function and five-membered lactone moiety, but the bands for carboxylic group was missing as observed in **1**. The NMR spectral data (Table 1) were almost similar to that of **1** as an evidence of only two olefinic systems in the molecule. The ^{13}C NMR spectrum of **2** displayed signals for 14 carbons, among them, four (δ 150.3, 143.8, 138.8, and 130.6) were assigned to two double bonds, while one at δ 173.0 was attributed to the lactone unit. These data justified three DBE and the remaining four DBE could only be attributed to four cyclic systems as cyclopentene, pentacyclic lactone, and two pyran rings. The two oxymethylenes of two pyran systems were found to resonate in the ^{1}H NMR spectrum at δ 4.14 (1H, dd, $J = 12.5$, 5.0 Hz, H-3$_{eq}$), 3.41 (1H, dd, $J = 12.5$, 2.0 Hz, H-3$_{ax}$), 3.76 (dd, $J = 12.1$, 6.2 Hz, H-15a), and 3.70 (dd, $J = 12.1$, 2.6 Hz, H-15b) with their corresponding carbons at δ 57.7 and 62.5, respectively. The methine resonating at δ 4.71 (d, $J = 7.5$ Hz, H-1) showed HMBC correlations with oxygenated methylenes (δ 57.7 and 62.5), methines [C-5 (δ 42.4) and C-9 (δ 45.9)], and with the *spiro*-carbon C-8 (δ 98.8). Furthermore, the methylene protons (δ 4.14, 3.41) displayed their HMBC correlations with C-1 (δ 96.1). The above-discussed data and COSY correlations (Figure 1) revealed that the carboxylic function in **1** has been reduced to alcohol and is involved in second pyran ring formation, changing the conformation of first pyran ring (in **1**) from chair into boat form (in **2**). The relative stereochemistry at various centers could be established based on coupling constants, NOESY interaction, and molecular model. The NOESY interactions of H-4 (δ 3.26) with H-3$_{ax}$ and H-3$_{eq}$ confirmed H-4 as equatorial and α, and bridged bond to be axial and β in orientation. This information could also be confirmed through molecular model as the bridge formation was only possible between axial —OH at C-1 and axial —CH_2OH at C-4. The remaining part of the molecule was exactly similar to that of **1**.

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a Jasco DIP-360 digital polarimeter. The IR spectra were recorded on Shimadzu IR-460 spectrophotometer (ν in cm^{-1}). The ^{1}H NMR spectra were recorded on a Bruker AMX-400 MHz instrument using TMS as an internal reference. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (J) are in Hz. The ^{13}C NMR spectra were recorded at 100 MHz on the same instrument. EI-MS and HR-EI-MS were recorded on a Jeol JMS-HX 110 spectrometer with data system. Column chromatography was carried out using silica gel of 70–230 and 230–400 mesh. Aluminum sheets pre-coated with silica gel 60 F_{254} (20 × 20 cm, 0.2 mm thick; E-Merck (Darmstadt, Germany); purchased from a local authorized dealer of E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm), followed by ceric sulfate as spraying reagent.

3.2 Plant material

The aerial parts of *P. rubra* were collected in April 2009 from the lawns of Science Faculty, Baghdad-ul-Jadeed Campus, The Islamia University of Bahawalpur, Pakistan, and were identified by Dr Muhammad Arshad, Cholistan Institute

for Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan, where a voucher specimen (No. PL-09-273) has been deposited.

3.3 Extraction and isolation

The shade dried and ground plant material (10 kg) was extracted twice with methanol at room temperature for 1 week. The concentrated methanolic extract (400 g) was suspended in water and extracted with *n*-hexane and EtOAc. The EtOAc-soluble fraction (60 g) was subjected to silica gel column chromatography using *n*-hexane, *n*-hexane:EtOAc, EtOAc, and EtOAc:MeOH in an increasing polarity order to get six fractions A–F. Fractions A–B on further silica gel column chromatography eluting with an isocratic *n*-hexane:CH_2Cl_2 (1:1) yielded **1** (6 mg), **2** (11.5 mg), **3** (8 mg), **4** (12 mg), and **9** (20 mg), respectively. Fraction C on further purification on silica gel column eluting with *n*-hexane:EtOAc (6:4) yielded **7** (12 mg) and **10** (5 mg). Fraction E was cleaned on Sephadex LH-20 eluted with MeOH and then subjected to RP-8 flash column chromatography eluting with 50% aqueous methanol to get **5** (22 mg). Fraction F was also cleaned on Sephadex LH-20 with MeOH and was purified on RP-8 flash column eluting with 40% aqueous methanol to get **6** (13 mg) and **8** (9 mg).

*3.3.1 Obtusadoid A (**1**)*

White amorphous powder (6 mg); $[\alpha]_D^{25} + 31.7$ ($c = 0.01$, MeOH); IR ν_{max} (KBr): 3321, 3231–2624, 1755, 1719, 1605, 1110 cm^{-1}; UV λ_{max} (MeOH): 211 nm; the ^1H and ^{13}C NMR spectral data (see Table 1); EI-MS: *m/z* 280 (8), 262 (12), 262 (12), 214 (34), 185 (45), 115 (15), 91 (19), 44 (100); HR-EI-MS: *m/z* 280.0938 $[M]^+$ (calcd for $C_{14}H_{16}O_6$, 280.0946).

*3.3.2 Obtusadoid B (**2**)*

White amorphous powder (11.5 mg); $[\alpha]_D^{25} + 62.3$ ($c = 0.012$, MeOH); IR ν_{max} (KBr): 3440, 3005, 1755, 1603, 1094 cm^{-1}; UV λ_{max} (MeOH): 211 nm; the ^1H and ^{13}C NMR spectral data (see Table 1); EI-MS: *m/z* 264 (4), 235 (65), 217 (21), 188 (29), 160 (100), 137 (55), 119 (65), 109 (75), 91 (90); HR-EI-MS: *m/z* 264.0990 $[M]^+$ (calcd for $C_{14}H_{16}O_5$, 264.0997).

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Note

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