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IRIDOID GALACTOSIDES AND A BENZOFURAN TYPE SESQUITERPENE FROM *BUDDLEJA CRISPA*

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Abstract-Buddlejosides A (**1**) and B (**2**), new iridoid galactosides, and buddlejone (**3**), a benzofuran type sesquiterpene, have been isolated from the EtOAc fraction of *Buddleja crispa* together with β-sitosterol (**4**) and ursolic acid (**5**). Their structures have been assigned on the basis of spectral analyses including 1D and 2D NMR spectral techniques.

INTRODUCTION

The genus *Buddleja*, belonging to the family Buddlejaceae, comprises of 100 species mainly distributed in East Asia, America and South Africa. In Pakistan it is represented by four species.[1] Various species of the genus *Buddleja* are used for the treatment of a variety of ailments such as ulcer, conjunctival congestion, clustered nebulae and skin disorders. Different parts of *B. asiatica* are used as antiinflamatory, abortifaciant, antifungal and also for the treatment of skin diseases.[2,3] The leaves and flowers of *B. globasa* are used for washing wounds and treatment of ulcer.[3] The flowers of *B. officinalis* are used in Chinese medicine for the treatment of conjunctival congestion and clustered nebulae.[3] Previous studies on the genus *Buddleja* have resulted in the isolation of various compounds including glycosides of triterpenes and iridoids,[4-6] flavonoids,[7] phenolic fatty acid esters,[8] diterpenes[9] and sesquiterpenes.[10,11] *B. crispa* Benth. which is a densely tomentose shrub is found in southern part of Pakistan. No phytochemical work has so far carried out on this species. The ethnopharmacological and chemotaxonomic importance of the genus *Buddleja* promoted us to investigate the chemical constituents of *B. crispa*. Herein we report the isolation and structural elucidation of buddlejosides A (**1**) and B (**2**) and benzofuran type sesquiterpene buddlejone (**3**) along with the known compounds β-sitosterol (**4**) and ursolic acid (**5**), respectively.

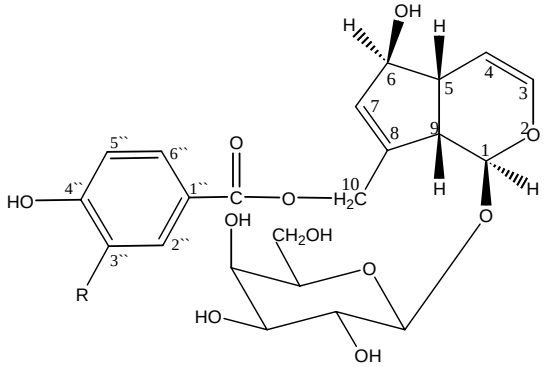
RESULTS AND DISCUSSION

The methanolic extract of shade-dried whole plant material (30 kg) of *B. crispa* was evaporated *in vacuo,* suspended in H_2O, and successively partitioned with *n*-hexane, EtOAc, and BuOH. As a result of a series of column chromatographic techniques compounds (**1–5)** were isolated from the EtOAc fraction as described in the EXPERIMENTAL.

Buddlejoside A (**1)** was isolated as a gummy solid. The HR negative FAB-MS established the molecular formula $C_{22}H_{26}O_{11}$ showing a $[M-H]^-$ peak at *m/z* 465.4327 (calcd for $C_{22}H_{25}O_{11,}$ 465.4329). The IR spectrum showed the absorption bands due to hydroxyl (3410 cm^{-1}), ester carbonyl (1715 cm^{-1}) and aromatic groups (1450 cm^{-1}). The ^{13}C NMR spectra of **1** showed 22 carbon signals including two methylene, sixteen methine and four quaternary carbons. The signal at δ 167.8 was due to an ester moiety while the oxymethine carbon at δ 100.1 could be ascribed to an anomeric carbon of a sugar unit. Further oxymethine carbon signals were observed at δ 97.9, 82.8, 77.9, 76.2, 74.8 and 71.4. The two oxymethylene carbons were observed at δ 63.6 and 62.7, respectively. The signals at δ 142.3, 141.7, 132.4 and 105.5 showed the presence of two olefinic bonds. The ^1H NMR spectrum (in CD_3OD) showed signals of oxymethylene protons at δ 4.92 and δ 5.11 (1H each, d, *J* = 15.2 Hz). Two oxymethine protons resonated at δ 4.79 (d, *J* = 7.3 Hz) and δ 4.47 (1H, br s), while the signals of olefinic protons were observed at δ 6.34 (1H, dd, *J* = 6.0, 1.8 Hz), 5.82 (1H, br s) and 5.12 (1H, dd, *J* = 6.0, 3.9 Hz). The presence of *p*-hydroxy benzoyl moiety was evident from the symmetrical doublets at δ 7.92 (2H, d, *J* = 8.7 Hz) and 6.85 (2H, d, *J* = 8.7 Hz) and further confirmed by electron impact mass spectrum (EI-MS) showing intense peaks at *m/z* 138, 121, 93 and 65. A large coupling constant of the anomeric proton signal at δ 5.43 (d, *J* = 7.7 Hz) confirmed the glycoside linkage. The above data particularly for those of aglycone showed closely resemblance to those of iridoid glucoside, agnuside.[12] The sugar moiety could be identified as galactose by comparing its ^1H and ^{13}C chemical shifts with those reported in literature and further confirmed through acid hydrolysis of **1** which provided various products, among which the glycone could be separated and identified as galactose by comparing the retention time of its trimethylsilyl (TMS) ether with that of the standard in gas chromatography (GC). The sign of optical rotation allowed us to assign D-configuration to galactose. Thus structure of **1** was assigned as formula (**1**).

The structure was confirmed by HMQC and HMBC experiments. The anomeric proton at δ 4.67 (H-1´) showed cross peaks with δ 97.9 (C-1) and 74.8 (C-2´) while methylenic protons at δ 4.92 and 5.11 (H-10) showed HMBC interactions with carbonyl carbon at δ 167.8, δ 142.3 (C-8), 132.4 (C-7) and 49.3 (C-9) confirmed the position of benzoyloxy group and olefinic bond at C-8 and C-7, respectively. Further important HMBC correlations are illustrated in Fig. 1. The stereochemistry at various stero centers of the iridoid unit was assigned on the basis of similarity of spectral data with related compound

and further confirmed through NOE as previously reported in literature.[13]



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2: R = OH

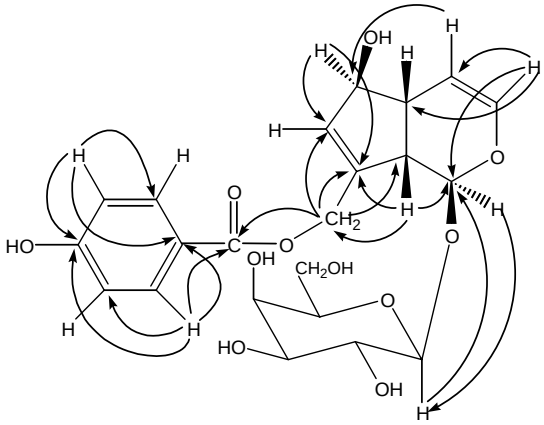


Figure 1: Important HMBC correlations of **1**

Table 1. ^{1}H (400 MHz) and ^{13}C NMR (100 MHz) assignments (δ/ppm) of buddlejosides **(1)** and **(2)**

	Compound (1)		**Compound (2)**	
C. No.	^{1}H-NMR (CD_3OD)	^{13}C-NMR (CD_3OD)	^{1}H-NMR (CD_3OD)	^{13}C-NMR (CD_3OD)
1	4.79 (d, 7.3)	97.9	4.78 (d, 7.4)	98.0
3	6.34 (dd, 6.0, 1.8)	141.7	6.35 (dd, 6.0, 1.8)	141.8
4	5.12 (dd, 6.0, 3.9)	105.5	5.13 (dd, 6.0, 3.9)	105.3
5	2.70 (1H, m)	46.2	2.68 (1H, m)	46.4
6	4.47 (br s)	82.8	4.49 (br s)	82.6
7	5.82 (br s)	132.4	5.80 (br s)	132.2
8		142.3		142.5
9	2.98 (br t, 7.4)	49.3	3.00 (br t, 7.4)	48.5
10	4.92 (d, 15.2)	63.6	4.95 (d, 15.2)	63.8
	5.11(d, 15.2)		5.14(d, 152)	
1´	4.67 (d, 7.8)	100.1	4.80 (d, 7.8)	100.3
2´	3.20-3.41 (m)	74.8	3.26-3.48 (m)	74.9
3´	3.20-3.41 (m)	77.9	3.26-3.48 (m)	77.6
4´	3.29 (t, 1.6)	71.4	3.32 (t, 1.6)	71.2
5´	320-3.41 (m)	76.2	3.26-3.48 (m)	76.4
6´	3.60 (dd 11.9, 5.6)	62.7	3.58 (12.0, 5.8)	62.5
	3.80 (dd 11.9, 2.3)		3.79 (12.0, 2.4)	
1´´		122.0		122.6
2´´	7.92 (d, 8.7)	132.9	7.56 (1H, d, 2.0)	114.2
3´´	6.85 (d, 8.7)	116.2		154.2
4´´		163.6		149.6
5´´	6.85 (d, 8.7)	116.2	6.87 (1H, d, 8.4)	117.1
6´´	7.92 (d, 8.7)	132.9	7.62 (1H, dd, 2.0, 8.4)	124.8
C=O		167.8		167.9

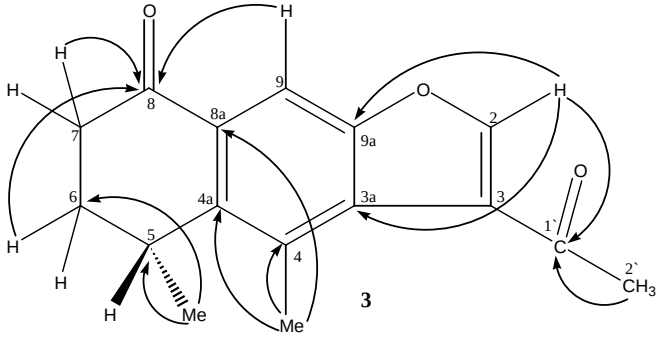
Figures in parentheses denote J values (Hz). δ in ppm from TMS.

Buddlejoside B (**2)** was isolated as a gummy solid. The HR negative FAB-MS established the molecular formula $C_{22}H_{26}O_{12}$ showing a $[M-H]^-$ peak at *m/z* 481.4320 (calcd for $C_{22}H_{25}O_{12,}$ 481.4323). The IR spectrum showed the absorption band due to hydroxyl (3414 cm^{-1}), ester carbonyl (1717 cm^{-1}) and aromatic groups (1448 cm^{-1}). The IR, ^{1}H and ^{13}C NMR spectra of **2** were almost identical to those of **1** except the difference due to 3′′,4′′-dihydroxybenzoyl moiety. The substitution pattern was confirmed by ^{1}H NMR which showed a ABX system at δ 7.62 (1H, dd, *J* = 2.0, 8.4 Hz, H-6′′), 7.56 (1H, d, *J* = 2.0 Hz, H-2′′) and 6.87 (1H, d, *J* = 8.4 Hz, H-5′′).[14] The ^{13}C NMR spectra of **2** also confirmed the 3′′,4′′-

dihydroxybenzoyl group showing downfield signals at δ 154.2 (C-3″) and 149.6 (C-4″).[14] The positions of hydroxyl and D-galactose moieties were further confirmed by HMBC correlations.

Buddlejone (**3)** was isolated as an amorphous solid. The molecular composition $C_{16}H_{16}O_3$ was determined by M^+ at *m/z* 256.3004 (calcd for $C_{16}H_{16}O_3$, 256.3006) in the HR EI-MS, suggesting a tricyclic compound. The IR spectrum revealed the presence of carbonyl (1698 cm^{-1}) and aromatic ring (1624, 1595, 1470 and 1444 cm^{-1}).

The ^{13}C NMR spectra of **3** showed the signals of 16 carbon atoms in agreement with the molecular formula. These included three methyl, two methylene, three methine and eight quaternary carbons. The downfield signals at δ 202.1 and 198.3 could be assigned to carbonyl groups. Two methyl groups were observed at δ 19.0 and 17.2 and eight sp^2 carbons were also observed at δ 160.4, 155.2, 143.6, 131.3, 130.5, 127.1, 125.4 and 109.0. The above data showed **3** to be a benzofuran type tricyclic sesquiterpene. The ^1H NMR spectrum showed a signal at δ 8.35 due to C_2-H of a 3-substituted benzofuran moiety and the aromatic proton peri to carbonyl group was observed at δ 8.11. The presence of acetyl group was evident by the singlet of methyl at δ 2.98 while another secondary methyl was observed at δ 1.32 (d, *J* = 7.0 Hz). The spectral data showed close agreement to those of viteralone,[15] the only notable difference being the replacement of formyl group at C_3 by the acetyl moiety. The presence of secondary methyl and acetyl moieties was further confirmed by successive losses of 15 mass units from the molecular ion peak in EI-MS, resulting in peaks at m/z 241 and 226, respectively. The other fragmentation patterns were similar to furanoeremophilane.[16] The structure of **3** was fully supported by HMBC correlations illustrated in figure 3. The relative configuration of C_5-methyl must be quas-axial (*J* values of C_5-H are 7.0, 5.4 and 2.6 Hz).[17] Irradiation of methyl signal at δ 1.32 simplified the signal of C_5-H into a double doublet (*J* = 5.0 and 2.5 Hz) in conformity to its being in pseudoequatorial orientation. The chemical shift values and coupling constants exactly matched to viteralone in which quas-axial disposition of secondary methyl has already been proved. The absolute configuration at C-5 was determined by CD data. Snatzke et al. previously reported that *R* and *S* enantiomers of methyl-4 tatralone-1 differ in having positive or negative Cotton effects[18]. This concept was later applied to determine the absolute configuration of viteralone[15]. In case of **3** the UV spectrum was more complex then tetralones but the Cotton effect at 335 nm (+ 15193) allowed us to assign the *R* configuration at C-5.



3

Figure 2: Structure and important HMBC correlation of **3**

Table 2. ^{1}H (400 MHz) and ^{13}C NMR(100 MHz) Data (δ/ppm) of buddlejone **3**

C. No.	^{1}H-NMR ($CDCl_3$)	^{13}C-NMR ($CDCl_3$)
2	8.35 (1H,s)	160.4
3		125.4
3a		127.1
4		143.6
4a		131.3
5	3.51 (br m)	29.0
6	2.08 (dt, 13.4, 2.6), 2.32 (tt, 9.9, 5.4)	28.9
7	2.66 (ddd, 18.1, 9.9, 2.6), 2.91 (ddd, 18.1, 13.4, 5.4)	32.9
8		198.3
8a		130.5
9	8.11 (1H, s)	109.0
9a		155.2
1´		202.1
2´	2.98 (3H, s)	23.8
4 -CH_3	2.89 (s)	17.2
5 -CH_3	1.32 (d, 7.0)	19.0

Figures in parentheses denote *J* values (Hz). δ in ppm from TMS

Conclusion

The stereostructures of two new iridoid galactosides and a benzofuran type sesquiterpene have been elucidated with the help of spectroscopic techniques.

EXPERIMENTAL

General: Optical rotations were taken on a JASCO DIP-360 digital polarimeter. IR spectral data were measured on a JASCO 302-A spectrophotometer in $CHCl_3$. UV spectra were obtained on a Hitachi UV-3200 spectrophotometer. NMR spectra were run on a Bruker instrument. Chemical shifts δ are shown in ppm relative to TMS as internal standard and coupling constant *J* are described in Hz. EI-, FAB-, and HREI-MS were recorded on a JEOL JMS-HX-110 and JMS-DA-500 mass spectrometers, *m/z* (relative. int). Silca gel 60, 200-440 mesh (Merck) were used for column chromatography, respectively. Silica gel plates (Si 60 F_{254}, Merck) were used for TLC.

Plant Material: The whole plant material was collected in March 2003 from Baluchistan and identified as *Buddleja crispa* by Prof. Rasool Bakhsh Tareen, Department of Botany, University of Baluchistan, Pakistan. A voucher specimen (BBU-101) is deposited in the herbarium of the Department of Botany, University of Baluchistan, Quetta, Pakistan.

Extraction and Isolation: The shade-dried plant (30 kg) were chopped and extracted thrice with MeOH (60 L) at rt for 96 h. The methanolic extract was evaporated *in vacuo* to give a dark greenish residue (800 g), which was partitioned between *n*-hexane and water. The water fraction was further extracted with EtOAc and *n*-BuOH. The EtOAc fraction (40 g) was subjected to column chromatography eluting with *n*-hexane-EtOAc in increasing order of polarity to give eight fractions. The fraction obtained from *n*-hexane-EtOAc (3:7) was rechromatographed over flash silica using *n*-hexane-EtOAc (6:4-2:8) as solvent system to give two successive fractions. The second fraction was a mixture of two compounds which were separated by preparative TLC using solvent system chloroform-MeOH (9:1) to yield compounds (**1)** (12 mg) and (**2)** (10 mg). The fraction obtained from *n*-hexane-EtOAc (7:3) was further purified by column chromatography on silica gel using *n*-hexane-EtOAc (9:1) as a solvent system to afford compound (**3)** (14 mg). Compounds (**4**) (9 mg) and (**5)** (11 mg) were obtained through elution with *n*-hexane-EtOAc (8:2) followed by further purification through column chromatography over silica gel using *n*-hexane-EtOAc (9:1) as the eluent.

Acid Hydrolysis of Compounds (1) and (2)

A solution of **1** or **2** (8 mg) in MeOH (5 mL) containing 1 N HCl (4 mL) was refluxed for 4 h, concentrated under reduced pressure, and diluted with H_2O (8 mL). It was extracted with EtOAc and the residue recovered from the organic phase was found to be an inseperatable mixture of products. The aqueous phase was concentrated and D-galactose was identified by the sign of its optical rotation ($[\alpha]_D^{20}$ + 79.9^o for **1** and 80.1^o for **2**, c in each case 0.02, H_2O). It was also confirmed based on the comparison of retention time of its TMS ether (α-anomer 3.8 min, β-anomer 5.2 min) with that of standard sample.

Buddlejoside A (1): Gummy solid; HRFAB-MS *m/z*: 465.4327 (calcd for $C_{22}H_{25}O_{11,}$ 465.4329) $[M-H]^-$; UV λ_{max} (MeOH) nm (log ε) 258 (4.40), 202 (4.46); IR ν_{max} cm^{-1}: 3410, 1715, 1450; EI-MS *m/z* 466 (8), 138 (43), 121 (100), 93 (22), 73 (38), 65 (25); $[\alpha]_D^{25}$ -76.7^o (c = 0.02 MeOH); ^1H and ^{13}C NMR are shown in Table 1.

Buddlejoside B (2): Gummy solid; HRFAB-MS *m/z*: 481.4320 (calcd for $C_{22}H_{25}O_{12,}$ 481.4323) $[M-H]^-$; UV λ_{max} (MeOH) nm (log ε) 260 (4.43), 203 (4.47); IR ν_{max} cm^{-1}: 3414, 1717, 1448; EI-MS *m/z* 482 (10), 154 (40), 137 (100), 109 (20), 65 (23); $[\alpha]_D^{25}$ -85^o (c = 0.02 MeOH); ^1H and ^{13}C NMR are shown in Table 1.

Buddlejone (3): Amorphous solid; HREI-MS *m/z*: 256.3004 (calcd for $C_{16}H_{16}O_{3,}$ 256.3006); HR FAB-MS m/z: 255.2925 (calcd for $C_{16}H_{15}O_3$ 255.2927) $[M-H]^-$, UV λ_{max} (MeOH) nm (log ε) 286 (4.30), 221

(4.36); IR ν_{max} cm^{-1}: 1698, 1624, 1595, 1470, 1444 cm^{-1}; EI-MS *m/z:* 256 (80), 241 (100), 226 (50), 199 (20), 128 (13), 115 (20), 91 (6), 77 12); $[\alpha]_D^{25}$ -46° (c = 0.02 MeOH); ^1H and ^{13}C NMR are shown in Table 2. CD ($[\theta]_{nm}$ MeOH, c=1.0), 380 (0), 335 (+15193), 315 (0), 299 (-9102), 275 (-4290), 254 (-5303), 245 (-3596), 215 (-24100), 203 (0).

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