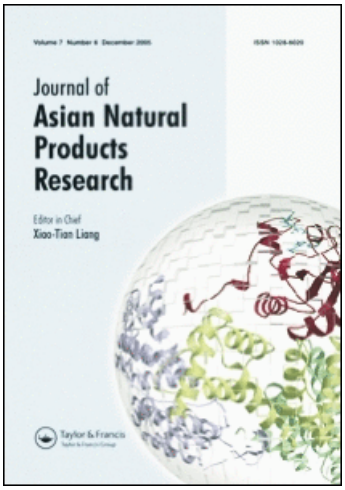

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New salirepin derivatives from *Symplocos racemosa*

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The phytochemical investigation of the *n*-butanol soluble fraction of the bark of stem of *Symplocos racemosa* Roxb. yielded two new phenolic glycosides of salirepin series, symplocuronic acid (**1**) and sympocemoside (**2**), while salirepin (**3**) was isolated for the first time from this plant. The structures of the new compounds were identified by 1D and 2D NMR techniques along with other spectral evidences and by comparison with the published data of closely related compounds.

Keywords: *Symplocos racemosa* Roxb; Symplocaceae; Phenolic glycosides; Salirepin derivatives

1. Introduction

Symplocos racemosa Roxb. (Symplocaceae) is known as Lodhra and is used in the Indian System of Medicine as single drug or in multicomponent preparations (viz. Lodhrasava) [1]. The medicinally bark is useful in eye diseases, for spongy gums and bleeding. It cures "Kapha", disease of the blood, leprosy, dropsy and liver complaints [1]. It is widely used as an Ayurvedic remedy mainly for gynacological disorders and is useful in abortions and miscarriages and for ulcers of the vagina. Unani medicine uses it as an emmenagogue and aphrodisiac. It is a potent remedy for inflammation and cleaning of the uterus. This is used to treat leucorrhoea and menorrhagia [1]. A recent study on the bark of *S. racemosa* Roxb. reveals that its aqueous extract on oral administration significantly stimulates serum FSH level ($p < 0.016$) along with the rise in serum LH level ($p < 0.001$). Moreover, histopathological studies reveal enhanced folliculogenesis, presence of mature follicles and detached oocytes, which are the result of increased FSH and LH levels. All these results are in concordance with the traditional use of this plant for female disorders [1].

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2. Results and discussion

The *n*-butanol fraction of the bark of the stem of *Symplocos racemosa* Roxb. yielded two new phenolic glycosides of salirepin series (**1**, **2**), and their structures were deduced by a detailed analysis of their spectral data and by comparison with the published data of closely resembling compounds.

Symplocuronic acid (**1**) was isolated as an amorphous solid. Its molecular formula $C_{26}H_{30}O_{15}$ was established through the HRFAB-MS(+) showing a quasi-molecular ion $[M + H]^+$ peak at *m/z* 583.5089 which indicated 12 degrees of unsaturation. Its UV absorption band at λ_{max} 284 nm was characteristic of phenolic compounds and its IR spectrum showed specific absorptions at 3364–3025 (br) cm^{-1} and 1733 cm^{-1}, which could be ascribed to a carboxyl group. The intense IR absorption band at 1720 cm^{-1} revealed the presence of ester functionality while the broad C–O stretching band in the region of 1071 cm^{-1} suggested its glycosidic nature. The complete acid hydrolysis of **1** yielded various products and in the hydrolysate separated from the aglycone parts, the two sugars identified by the TLC comparison were found to be glucuronic acid and glucose and these were also supported by the loss of fragments in the FAB-MS from the $[M]^+$ peak at *m/z* 582 to fragment ion peaks at *m/z* 406 [M–glucuronic acid]$^+$ and *m/z* 244 [M–glucuronic acid–glucose]$^+$. Its EI-MS spectrum also exhibited an ion at *m/z* 244 [M–glucuronic acid–glucose]$^+$ and the other characteristic fragments were observed at *m/z* 140 $[C_6H_3(OH)_2CH_2OH]^+$, 123 $[C_6H_3(OH)_2CH_2]^+$, 122 $[C_6H_5CO_2H]^+$, 105 $[C_6H_5CO]^+$ and 77 $[C_6H_5]^+$ which indicated that the aglycone was exactly similar to that of reported salireposide [2–4] and this assignment was thoroughly supported by its ^{1}H NMR data (table 1). However, the remaining signals presumably belonged to two sugar moieties with two anomeric doublets at δ4.72 (H-1″) and δ4.75 (H-1‴) and the evidence for the β-configuration of these sugars was drawn from the coupling constants of $J = 7.3$ and $J = 7.4$ Hz for H-1″ and H-1‴, respectively. The ^{13}C NMR spectrum corroborated the characteristic signals of a benzoyl residue, a substituted gentisyl alcohol unit and a glucose similar to that of known salireposide [2–4] but the additional signals were specific for a glucuronic acid moiety with the carboxy resonance at δ 173.7 [5,6]. Its position in the molecule was deduced through the downfield shift of C-6″ to δ 65.4 as compared to the respective signal of salireposide [2–4] and HMBC correlations of H-1‴ with C-6″ and H-6″ with C-1‴. The important HMBC correlations are shown in figure 1. Since only D-glucose and D-glucuronic acid are known in nature [6], therefore, based upon the above cumulative evidence, **1** was identified as 2-[(benzoyloxy)methyl]-4-hydroxyphenyl-*O*-β-D-glucuronopyranosyl(1 → 6)-β-D-glucopyranoside.

Sympocemoside (**2**) was obtained also as an amorphous solid. It was assigned a molecular formula $C_{19}H_{28}O_{13}$ on the basis of HRFAB-MS (+) (*m/z* 465.4197 $[M + H]^+$, showing 6 degrees of unsaturation. It exhibited UV absorption band (λ_{max} 282 nm) typical of phenolic compounds. The IR absorption bands revealed the presence of hydroxyl groups (3357 br cm^{-1}), methines (2932 cm^{-1}), aromatic ring (1595–1416 cm^{-1}), ether linkage (1266, 1215 cm^{-1}) and the broad (C–O) stretching bands in the regions of 1115–1040 cm^{-1} accounted for its glycosidic nature. The EI-MS spectrum of **2** exhibited the following characteristic fragments: *m/z* 140 $[C_6H_3(OH)_2CH_2OH]^+$, 123 $[C_6H_3(OH)_2CH_2]^+$, 122 $[C_6H_3(OH)_2CH_2OH–H_2O]^+$, which indicated the presence of a gentisyl alcohol moiety in the molecule. In the ^{1}H NMR spectrum, the usual ABX spin system of the gentisyl alcohol group was readily identified by signals observed at δ 7.06 (1H, d, $J = 8.7$ Hz, H-6), 6.77

Table 1. NMR data (MeOD) of compounds **1**–**3**[a]. δ in ppm, *J* in Hz.

	1		***2***		***3***	
No.	*δ (H)*	*δ (C)*[b]	*δ (H)*	*δ (C)*[b]	*δ (H)*	*δ(C)*[b]
1	–	149.8	–	150.3	–	150.0
2	–	133.2	–	133.9	–	133.8
3	6.79 (d, $J = 2.9$)	117.0	6.77 (d, $J = 3.0$)	116.4	6.78 (d, $J = 2.9$)	116.4
4	–	154.2	–	154.2	–	154.0
5	6.49 (dd, $J = 2.9, 8.8$)	116.2	6.65 (dd, $J = 3.0, 8.7$)	115.8	6.65 (dd, $J = 2.9, 8.7$)	115.8
6	7.02 (d, $J = 8.8$)	120.0	7.06 (d, $J = 8.7$)	119.5	7.07 (d, $J = 8.7$)	119.5
7	5.19 (d, $J = 12.5$)	64.3	4.68 (d, $J = 13.0$)		4.51 (d, $J = 13.0$)	61.0
	5.33 (d, $J = 12.5$)		4.50 (d, J=13.0)		4.69 (d, $J = 13.0$)	
1′	–	131.3	4.73 (d, $J = 7.6$)	102.6	4.67 (d, $J = 6.9$)	104.7
2′	8.00 (d, $J = 7.4$)	130.6	3.44 (br t, $J = 7.7$)	77.9	3.43 (br t, $J = 8.8$)	75.1
3′	7.49 (t, $J = 7.6$)	129.6	3.49 m	76.0	3.40 (br t, $J = 8.6$)	78.1
4′	7.62 (t, $J = 7.5$)	134.3	3.42 m	72.5	3.35 (br t, $J = 8.3$)	71.4
5′	7.49 (t, $J = 7.6$)	129.6	3.85 m	77.7	3.46 m	78.0
6′	8.00 (d, $J = 7.4$)	130.6	4.38 m	62.6	3.88 (dd, $J = 7.2, 11.8$)	62.6
			4.46 m		4.23 (dd, $J = 2.8, 11.8$)	
7′	–	167.9	–	–	–	–
1″	4.72 (d, $J = 7.3$)	104.2	4.70 (d, $J = 7.8$)	105.3	–	–
2″	3.45 (br t, $J = 7.8$)	74.4	3.46 (br t, $J = 7.7$)	75.2	–	–
3″	3.49 m	78.3	3.51 m	78.2	–	–
4″	3.41 m	72.0	3.41 m	72.3	–	–
5″	3.86 (ddd, $J = 1.9, 8.9, 11.3$)	75.5	3.85 m	75.9	–	–
6″	4.40 (dd, $J = 7.7, 11.6$)	65.4	4.38 m	62.4	–	–
	4.49 (dd, $J = 1.9, 11.6$)		4.46 m			
1‴	4.75 (d, $J = 7.4$)	104.0	–	–	–	–
2‴	3.47 (br t, $J = 7.7$)	74.4	–	–	–	–
3‴	3.52 m	78.1	–	–	–	–
4‴	3.43 m	72.4	–	–	–	–
5‴	4.02 (d, $J = 9.3$)	75.0	–	–	–	–
6‴	–	173.7	–	–	–	–

a All spectra were recorded at 500 MHz (^{1}H) and 125 MHz (^{13}C); assignment were aided by 2D NMR COSY, HMQC and HMBC experiments.
b ^{13}C NMR multiplicities were determined by DEPT 135°.

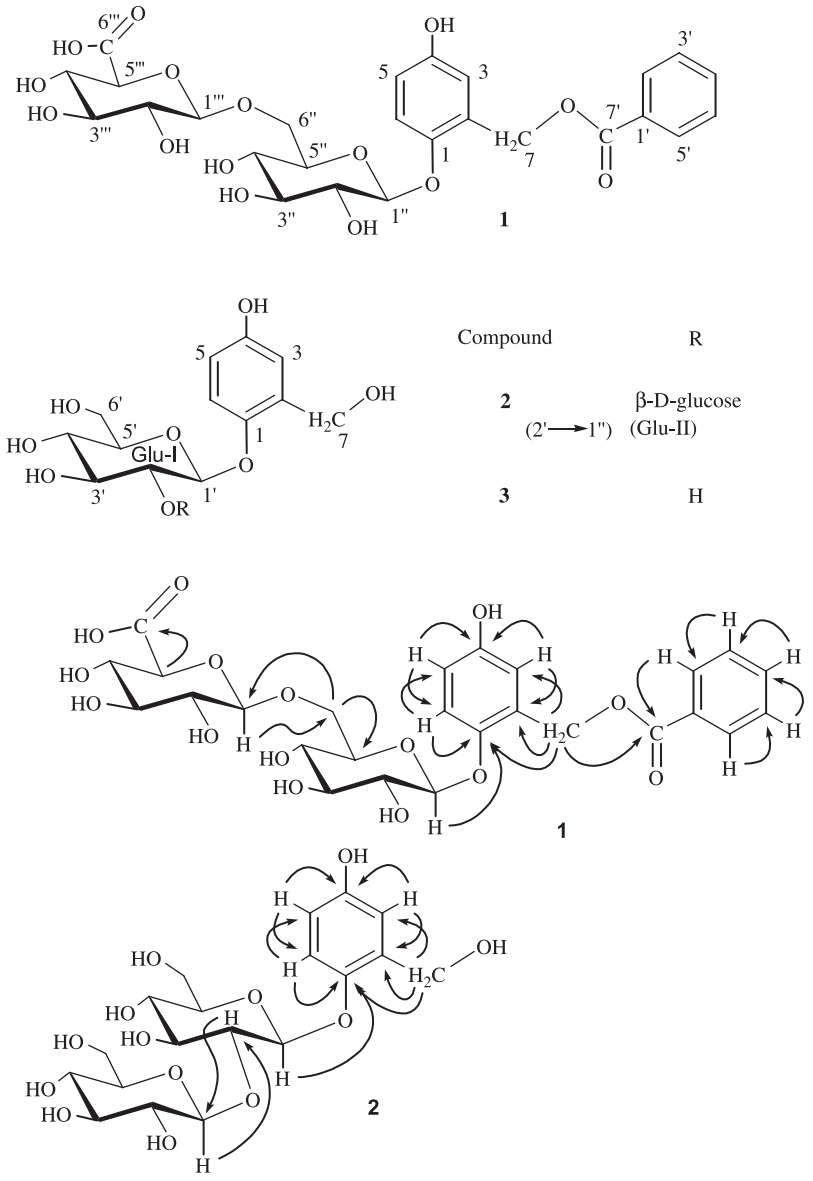

Figure 1. Structures of compounds **1**–**3**, and key HMBC correlations of **1** and **2**.

(1H, d, $J = 3.0$ Hz, H-3), and 6.65 (1H, dd, $J = 8.7$, 3.0 Hz, H-5) and the two anomeric doublets at δ 4.73 ($J = 7.6$ Hz, H-1′) and 4.70 ($J = 7.8$ Hz, H-1″) clearly ascertained the presence of two β-glucose moieties in the molecule. In addition to the similar signals of reported salirepin [7], its ^{13}C NMR spectrum also revealed the signals for an additional glucose unit and its position in the molecule was determined through the downfield shift of C-2′ (δ77.9) and up-field shifts of C-1′ (δ102.6) and C-3′ (δ76.0) as compared to the respective signal of known salirepin (table 1). In the ^{1}H–^{1}H COSY spectrum of **2**, a broad triplet signal at δ3.44 was assigned to H-2′ on the basis of a cross-peak with the anomeric proton H-1′ (δ4.73) and thereafter the HMBC correlations of H-2′ with C-1″ and H-1″ with C-2′ confirmed that the additional glucose(II) was linked glycosidically to C-2′ of first

glucose(I). The important HMBC correlations are shown in figure 1. Since only D-glucose is known in nature, the structure of **2** was deduced as 2-(oxymethyl)-4-hydroxyphenyl-*O*-β-D-glucopyranosyl (1 → 2)-β-D-glucopyranoside.

Salirepin (**3**) was isolated for the first time from our investigated source and its structure was established by mass spectrometry, 1D and 2D NMR techniques and by comparison with the published data [7].

3. Experimental

3.1 General experimental procedures

For column chromatography (CC), silica gel (70–230 mesh) and for flash chromatography (FC), silica gel (230–400 mesh) was used. TLC was performed on pre-coated silica gel G-25-UV_{254} plates. Detection was carried out at 254 nm, and by ceric sulphate and aniline phthalate reagents. For recycling HPLC (LC 908 W) a semi-preparative (M-80) reverse phase was used. Purity was checked on TLC with different solvent systems using methanol, acetic acid, water and $CHCL_3$ giving single spot. The optical rotations were measured on a Jasco-DIP-360 digital polarimeter. The UV and IR spectra were recorded on Hitachi-UV-3200 and Jasco-320-A spectrophotometer, respectively. ^{1}H NMR, ^{13}C NMR, COSY, HMQC and HMBC spectra were run on Bruker spectrometers operating at 500, 400 and 300 MHz. EI-MS and FAB-MS spectra were recorded on a JMS-HX-110 spectrometer, with a data system.

3.2 Plant material

The plant *Symplocos racemosa* (Symplocaceae) was collected from Abbottabad, Pakistan, in January 2001, and identified by Dr Manzoor Ahmed (Taxonomist) at the Department of Botany, Post-Graduate College, Abbottabad, Pakistan. A voucher specimen (no. 6453) has been deposited at the herbarium of the Botany Department of Post-Graduate College, Abbottabad, Pakistan.

3.3 Extraction and purification

The shade-dried ground bark (30 kg) was exhaustively extracted with methanol at room temperature. The extract was evaporated to yield a residue (818 g), which was dissolved in water and partitioned with hexane, chloroform, ethyl acetate and *n*-butanol successively. The *n*-butanol extract (23 g) was subjected to column chromatography over silica gel using $CHCl_3$ with gradient of methanol up to 100%. Eleven fractions (Fr. 1–11) were collected. Fraction 5 was submitted to repeated FC (230–400 mesh) and eluted with MeOH/$CHCl_3$ (18:82) to get purified **3** (42.5 mg). Fraction 6 was loaded on flash silica gel and eluted with MeOH/$CHCl_3$ (19:81) to get two sub-fractions (Fr_{sb}. 6.1 and Fr_{sb}. 6.2). Sub-fraction 6.2 was then submitted to Sephadex LH-20 and eluted with pure water and the resulting impure **1** was finally purified on recycling HPLC (LC 908 W) using a reverse phase semi preparative (M-80). Elution was carried out at a flow rate of 4 ml/min. under isocratic conditions with MeOH/H_2O (1:1). The peaks were detected by UV and RI detectors. The peak obtained at a retention time of 46 min resulted in purified **1** (12.9 mg). Similarly, the fraction 10 was

subjected to FC and eluted with MeOH/$CHCl_3$ (24:76) to get two sub-fractions (Fr_{sb}. 10.1 and Fr_{sb}. 10.2). Sub-fraction 10.2 was then passed through Sephadex LH-20 and eluted with pure water and the resulting impure **2** was finally purified on recycling HPLC using a semi-preparative reverse phase (M-80), eluting at a flow rate of 4 ml/ min under isocratic conditions with MeOH/H_2O (1:1) and the peak obtained at a retention time of 20 min yielded the purified **2** (14.1 mg).

3.4 Identification

3.4.1 Acid hydrolysis of 1–3. A solution of (**1**–**3** separately) (8 mg) in MeOH (5 ml) containing 2 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 inseparable mixture of products. The aqueous phase was neutralized with Ag_2CO_3, filtered and evaporated *in vacuo*. The residue obtained showed the presence of glucose and glucuronic acid in **1** and only glucose in **2** and **3**, when compared with the authentic samples of these sugars on TLC (EtOAc/MeOH/AcOH/H_2O 11:2:2:2) by way of visualising the spots with aniline phthalate reagent.

3.4.2 Symplocuronic acid. (= 2-[(Benzoyloxy)methyl]-4-hydroxyphenyl-*O*-β-D-glucurono pyranosyl(1 → 6)-β-D-glucopyranoside; **1**). Amorphous powder (12.9 mg): $C_{26}H_{30}O_{15}$; $[\alpha]_D^{23}$ + 16.66 (*c* 0.028, MeOH); UV λ_{max} nm (log ε) (MeOH): 389.4 (2.81), 366.7 (2. 45), 343.2 (2.73), 339.3 (2.52), 284.1 (3.44), 256.6 (3.04), 227.4 (3.98) nm; IR ν_{max} (KBr): 3364–3025 br. (OH), 2928 (C–H), 1733 (C═O, acid), 1720 (C═O, ester), 1501–1453 (C═C, Ar), 1280 (C–O–C), 1071 (C–O), 894, 807, 715, 659 cm^{-1}; ^1H NMR and ^{13}C NMR: table 1 ; HRFAB-MS (+): *m/z* 583.5089 $[M + H]^+$ (calcd for $C_{26}H_{31}O_{15}$, 583.5073); FAB-MS (Positive mode) *m/z* 583 $[M + H]^+$, *m/z* 407 [M–glucuronic acid]$^+$, *m/z* 245 [M–glucuronic acid–glucose]$^+$; FAB-MS (Negative mode) *m/z* 581 $[M - H]^-$, *m/z* 405 [M–glucuronic acid]$^-$, *m/z* 243 [M–glucuronic acid–glucose]$^-$; EI-MS: *m/z* (rel. int.): 244 [M–glucuronic acid–glucose]$^+$ (46.1), 140 $[C_6H_3(OH)_2CH_2OH]^+$ (32.3), 123 $[C_6H_3(OH)_2CH_2]^+$ (41.7), 122 $[C_6H_5COOH]$ (70.8), 105 $[C_6H_5CO]^+$ (100), 77 $[C_6H_5]^+$ (61.3).

3.4.3 Sympocemoside. (= 2-(Oxymethyl)-4-hydroxyphenyl-*O*-β-D-glucopyranosyl(1 → 2)-β-D-glucopyranoside; **2**). Amorphous powder (14.1 mg): $C_{19}H_{28}O_{13}$; $[\alpha]_D^{23}$ − 57.89 (*c* 0.038, MeOH); UV λ_{max} nm (log ε) (MeOH): 282 (3.51), 260 (3.25) nm; IR ν_{max} (KBr): 3357 br. (OH), 2932 (C–H), 1595–1416 (C═C, Ar), 1266, 1215 (C–O–C), 1115–1040 (C–O), 862, 663 cm^{-1}; ^1H NMR and ^{13}C NMR: table 1 ; HRFAB-MS (+): *m/z* 465.4197 $[M + H]^+$ (calcd for $C_{19}H_{29}O_{13}$, 465.4185); FAB-MS (Positive mode): *m/z* 465 $[M + H]^+$, *m/z* 303 [M–glucose]$^+$, *m/z* 141 [M–glucose–glucose]$^+$; FAB-MS (Negative mode): *m/z* 463 $[M - H]^-$, *m/z* 301 [M–glucose]$^-$, *m/z* 139 [M–glucose–glucose]$^-$; EI-MS: *m/z* (rel. int.): 140 $[C_6H_3(OH)_2CH_2OH]^+$ (88.1), 123 $[C_6H_3(OH)_2CH_2]^+$ (65.9), 122 $[C_6H_3(OH)_2CH_2OH–H_2O]^+$ (100).

3.4.4 Salirepin. (= 2-(Oxymethyl)-4-hydroxyphenyl-β-D-glucopyranoside; **3**). White powder (42.5 mg): $C_{13}H_{18}O_8$; $[\alpha]_D^{23}$ − 45.1 (*c* 0.0368, MeOH); UV λ_{max} nm (log ε) (MeOH): 285.8 (2.92), 251.2 (2.30), 225.6 (3.36) nm; IR ν_{max} (KBr): 3408 (OH), 2921 (C–H), 1665–1443, (C═C, Ar), 1268, 1215 (C–O–C), 1084, 1040 (C–O) 992, 671 cm^{-1};

^{1}H NMR and ^{13}C NMR: table 1 ; FAB-MS (Positive mode): *m/z* 303 $[M + H]^+$; FAB-MS (Negative mode): *m/z* 301 $[M - H]^-$; EI-MS: *m/z* (rel. int.): 140 $[C_6H_3(OH)_2CH_2OH]^+$ (77.6), 123 $[C_6H_3(OH)_2CH_2]^+$ (36.5), 122 $[C_6H_3(OH)_2CH_2OH-H_2O]^+$ (100).

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