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Phenolic glycosides from *Symplocos racemosa*: natural inhibitors of phosphodiesterase I

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Abstract

One new phenolic glycoside named benzoylsalireposide (**1**) along with one known phenolic glycoside named salireposide (**2**) have been isolated from *Symplocos racemosa*. Four other known compounds i.e. β-amyrin (**3**), oleonolic acid (**4**), β-sitosterol (**5**) and β-sitosterol glycoside (**6**) were also isolated from this plant. The structure elucidation of the isolated compounds was based primarily on 1D- and 2D-NMR analysis, including COSY, HMQC, and HMBC correlations. The compound **1** and **2** showed inhibitory activity against snake venom phosphodiesterase I.

Keywords: Symplocos racemosa Roxb; Symplocaceae; Phenolic glycosides; Phosphodiesterase I

1. Introduction

Symplocos racemosa Roxb. is a medicinal plant widely used by the traditional practitioners against various diseases as single or in compound drug. It is known as Lodh tree or Lodh Pathani. The bark tastes sweetish and astringent. Its color is agreeable and aromatic (Khory, 1986). In indigenous system of medicine, Lodh is used for management of menstrual disorders and to provide firmness to spongy and bleeding gums. Its decoction is also used for the treatment of bowel complaints and ulcers (Dhaon et al., 1989). In Europe it was formerly looked upon as a substitute for cinchona bark and has been known at various times as 'Ecorce de latour', 'China californica', China Brasilensis', and 'China Paragua tan' (Watt, 1972). Compound **1** and **2** showed activity against snake venom phosphodiesterase I. Nucleotide pyrophosphatases/phophodiesterase (NPPI) or plasma cell antigen 1(PC-1) successively hydrolyses 5′-mononucleotides from nucleotides and their derivatives. They exist both as membrane proteins with an extracellular active site and as soluble proteins in body fluid. They are widely distributed in mammalian intestinal mucosa, mammalian liver cells, blood serum, snake venom and various plants (Mathieu et al., 2000). NPPs are believed to be involved in a wide variety of processes, such as bone formation, insulin resistance and metastasis of cancer cells. Inhibitors of PC-1 might be useful as treatment for some forms of arthritis (Mathieu et al., 2000; Kristen et al., 2001; Frank et al., 2001).

2. Results and discussion

From the ethyl acetate extract of *S. racemosa*, a new compound (**1**) was obtained with one known compound: salireposide (**2**), which has been isolated for the first time from this plant. Salireposide (**2**) was isolated as a white powder. It gave a FAB MS $[M-1]^-$ ion at m/z 405 and calculated for $C_{20}H_{22}O_9$. The usual ABX spin system of the gentisyl alcohol group was readily identified between δ 6.47 and 7.00. Another set of peaks appearing between δ 7.49 and 8.02 were characteristic of benzoyl protons as in benzoic acid. The structure of salireposide (**2**) was further confirmed by the comparison of ^1H and ^{13}C NMR spectral data with reported literature data (Ekabo et al., 1993; Dommisse et al.,

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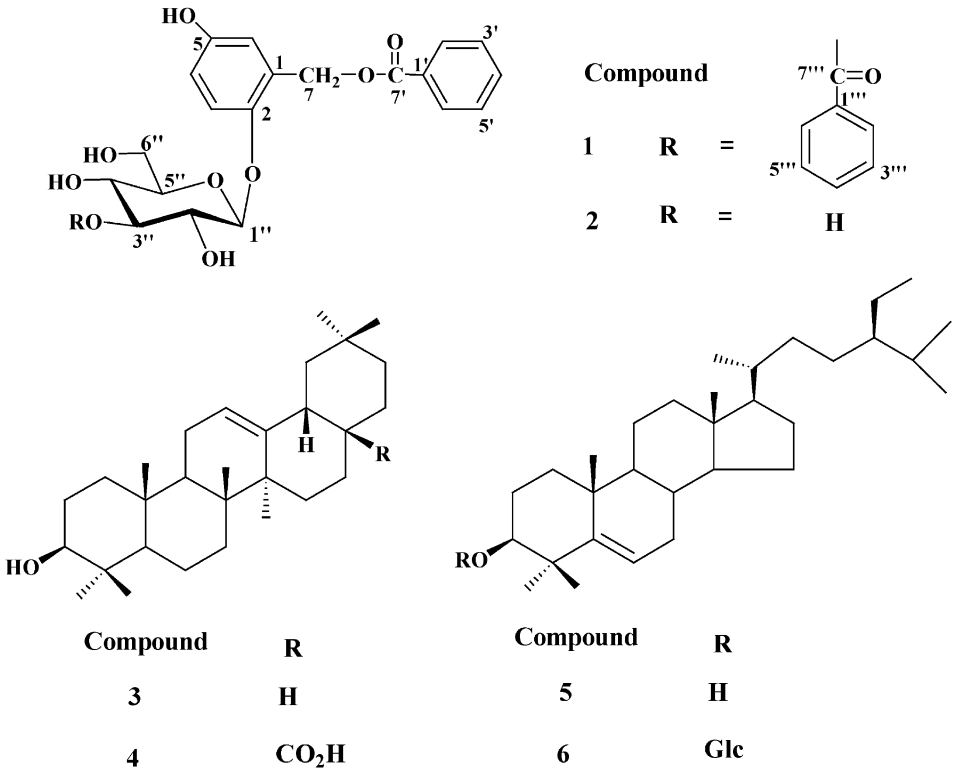

1986). The signals assignments of all protons and carbons in their spectra were thoroughly supported by ^1H–^1H COSY, HMQC and HMBC.

Compound **1** was isolated as an amorphous powder. Its FABMS showed a $[M]^+$ ion at m/z 510, which established that the molecular formula was $C_{27}H_{26}O_{10}$ and indicated 15° of unsaturation. Compounds **1** exhibited a UV band (λ_{max} 282 nm) typical of phenolic compounds. There was an intense IR absorption at 1710 cm^{-1} in indicating the presence of an ester function. Other strong bands were observed at 3420 (OH), 1600, 1500 (C=C, Ar), 1277 and 1070 (C–O–C) cm^{-1}. The EIMS spectrum of **1** exhibited an anion at m/z 244 [M−Benzoyl glucose]$^+$. This ion disintegrated further to the following characteristic fragments: $[C_6H_5CO_2H]^+$ (m/z 122, 83%), $[C_6H_3(OH)_2CH_2]^+$ (m/z 123, 52%), $[C_6H_5CO]^+$ (m/z 105, 100%), and $[C_6H_5]^+$ (m/z 77, 66.8%).

The ^1H NMR spectrum of **1** had a doublet at δ 4.88 ($J=7.86$ Hz) for an anomeric proton in the α-orientation. In the ^1H NMR spectrum, the presence of an *ortho*- and *meta*-substituted phenolic residue [exhibited by the following signal: δ 6.47 (dd, $J=3.0$, 8.7 Hz, H-4), 6.78 (d, $J=2.9$ Hz, H-6), 7.04 (d, $J=8.7$ Hz, H-3) and two benzoyl residue, observed as 10-proton signals at δ 7.54–8.08, were observed and suggested a structure similar to that of **2** except for the presence of an additional benzoyl esterifying group. This was placed at C-3″ of the glucose on the basis of NMR data. The ^1H NMR and ^{13}C NMR spectra showed resonance that was readily attributed to β-glucose and two benzoate esters. However, the carbon signals due to a glucose moiety were different from those of **2**. A downfield shift at C-3″ (+ 1.3 ppm) (Table 1 and 2), and upfield shifts at C-2″ (−1.7 ppm) and C-4″ (−1.7 ppm) were observed by comparison with similar signals of **2** which indicated that the additional benzoyl residue was attached to C-3″ of the glucosyl moiety (Mizuno et al., 1991). In the ^1H–^1H COSY spectrum of **1**, a broad triplet signal at δ 3.86 assigned to H-2″ caused a cross peak with the anomeric proton (δ 4.88 d, $J=7.8$ Hz) and further caused a cross peak with a broad triplet at δ 5.33. Therefore, the triplet was assigned to a proton at H-3″, the chemical shift of which was at a lower field than the usual one, indicating that the position was acylated with a benzoyl residue (Mizuno et al., 1991).

In addition to the compound **1** and **2**, some known constituents such as β-amyrin (Ahmad and Atta-ur-Rahman, 1994a). Oleonolic acid (Ahmad and Atta-ur-Rahman, 1994b), β-sitosterol (Greca et al., 1990) and β-sitosterol glycoside (Seo et al., 1978) have also been isolated from our investigated source. Their structures were established by comparing their spectral data and physical constants.

Only a few inhibitors of Phosphodiesterase I have been reported so far and the majority of them are synthetic in origin. Compounds **1**–**6** were tested for their inhibitory activity against snake venom Phosphodiesterase I. Compound **1** showed strong inhibitory potential with IC_{50} of 171±0.002 μM as compared to Cystein (IC_{50} 748±0.015 μM) and EDTA (IC_{50} 274±0.007

Table 1
NMR data (in $CDCl_3$) for compound **1**[a]

Position	δ_H	δ_c[b]
1	–	134.1
2	–	149.7
3	7.04 (*d*, *J* = 8.7 Hz)	119.6
4	6.47 (*dd*, *J* = 3.0, 8.7 Hz)	115.4
5	–	154.3
6	6.78 (*d*, *J* = 2.9 Hz)	116.0
7	4.54 (*d*, *J* = 13.0 Hz)	65.1
	4.69 (*d*, *J* = 13.0 Hz)	
1′	–	131.2
2′/6′	8.03 (*dt*, *J* = 1.4, 6.1 Hz)	130.6
3′/5′	7.47 (*br d*, *J* = 7.5 Hz)	129.4
4′	7.61 *m*	134.2
7′	–	167.7
1″	4.88 (*d*, *J* = 7.8 Hz)	104.4
2″	3.86 (*br t*, *J* = 7.5 Hz)	73.4
3″	5.33 (*t*, *J* = 9.3 Hz)	79.3
4″	3.88 (*br t*, *J* = 7.5 Hz)	70.3
5″	3.78 (*ddd*, *J* = 1.6, 7.7, 9.6 Hz)	75.4
6″	4.48 (*dd*, *J* = 7.2, 11.6 Hz)	60.7
	4.72 (*dd*, *J* = 7.2, 11.6 Hz)	
1‴	–	131.6
2‴/6″	8.10 (*dt*, *J* = 1.4, 7.0 Hz)	130.7
3‴/5″	7.51 (*br d*, *J* = 7.7 Hz)	129.6
4‴	7.61 *m*	134.4
7‴	–	167.8

[a] All spectra were recorded at 400 MHz (^1H) and 100 MHz (^{13}C); assignment were aided by 2D NMR COSY, HMQC and HMBC experiments.
[b] ^{13}C NMR multiplicities were determined by DEPT 135°.

Table 2
NMR data (in $CDCl_3$) for compound **2**[a]

Position	δ_H	δ_c[b]
1	–	134.3
2	–	149.9
3	7.00 (*d*, *J* = 8.8 Hz)	119.6
4	6.46 (*dd*, *J* = 3.0, 8.3 Hz)	115.6
5	–	154.1
6	6.75 (*d*, *J* = 3.0 Hz)	116.3
7	4.47 (*d*, *J* = 13.1 Hz)	65.3
	4.47 (*d*, *J* = 13.1 Hz)	
1′	–	131.3
2′/6′	8.02 (*tt*, *J* = 3.0, 8.0 Hz)	130.6
3′/5′	7.49 (*br t*, *J* = 7.4 Hz)	129.6
4′	7.62 *m*	134.0
7′	–	167.8
1″	4.78 (*d*, *J* = 2.9 Hz)	104.5
2″	3.40–3.51 *m*	75.1
3″	3.40–3.51 *m*	78.0
4″	3.40–3.51 *m*	72.0
5″	3.70 (*dd*, *J* = 2.2, 7.3 Hz)	75.6
6″	4.42 (*dd*, *J* = 7.3, 11.7 Hz)	61.0
	4.42 (*dd*, *J* = 7.3, 11.7 Hz)	

[a] All spectra were recorded at 400 MHz (^1H) and 100 MHz (^{13}C); assignment were aided by 2D NMR COSY, HMQC and HMBC experiments.
[b] ^{13}C NMR multiplicities were determined by DEPT 135°.

μM). Compound **2** showed moderate inhibitory activity with IC_{50} of 544 ± 0.0021 μM while **6** showed weak activity and **3**, **4** and **5** were inactive against this enzyme.

3. Experimental

3.1. General

Column chromatography (CC): silica gel, 70–230 mesh. Flash chromatography (FC): silica gel 230–400 mesh. TLC: pre-coated silica gel G-25-UV_{254} plates: detection at 254 nm, and by ceric sulphate reagent. Optical rotations: Jasco-DIP-360 digital polarimeter. UV and IR Spectra: Hitachi-UV-3200 and Jasco-320-A spectrophotometer, respectively. ^1H and ^{13}C NMR, COSY, HMQC and HMBC Spectra: Bruker spectrometers operating at 500 and 400 MHz; chemical shifts δ in ppm and coupling constants in Hz. EI-, CIMS: JMS-HX-110 with a data system.

3.2. Plant material

The plant *S. racemosa* (Symplocaceae) was collected from Abbot Abad, 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 air-dried ground plant (30 kg) was exhaustively extracted with methanol at room temperature. The extract was evaporated to yield the residue (818 g). The whole residue was extracted with hexane, chloroform, ethyl acetate and butanol. The ethyl acetate extract (106.2 g) was subjected to CC over a silica gel column using hexane with gradient of $CHCl_3$ upto 100% and followed by methanol. Ten fractions were collected. Fraction no. 8 of the first column was loaded on silica gel (flash silica 230–400 mesh) and eluted with MeOH:$CHCl_3$ (5:95) to purify compound **1**. Fraction no. 9 was subjected to column chromatography and eluted with MeOH:$CHCl_3$ (7:93) to purify compounds **2** and **6**. Fraction nos. 3 and 4 were loaded on silica gel (flash silica 230-mesh) and eluted with EtOAc:hexane (20:80) to purify compounds **3** and **5** and **4** respectively.

3.3.1. Benzoylsalireposide (1-benzoylmethyl-5-hydroxyphenyl-β-D-(3′-benzoyl) gluco-pyranoside (1)

Amorphous powder (12.5 mg): $C_{27}H_{26}O_{10}$; $[\alpha]_D^{23}$ −7.69 (*c* 0.182, MeOH); UV λ_{max} nm (log ϵ) (MeOH): 282.5 (2.95), 229 (3.50), 205 (3.01) nm; IR ν_{max} ($CHCl_3$): 3420 (OH), 2927 (C-H), 1710 (C=O, ester) 1600,1500

(C=C, Ar), 1277, 1070 (C–O–C), 804, 710, 627 cm^{-1}; ^1H and ^{13}C NMR: Table 1; FAB-MS (Pos. ion mode) *m/z* 511 $[M+1]^+$; FAB-MS (Neg. ion mode) *m/z* 509 $[M-1]^-$; EIMS *m/z* (rel. int.): 244 [M−Benzoyl glucose]$^+$ (46.7), 122 $[C_6H_5CO_2H]^+$ (83), 123 $[C_6H_3(OH)_2CH_2]^+$ (52), 105 $[C_6H_5CO_2]^+$ (100), 77 $[C_6H_5]^+$ (66.8).

3.3.2. Salireposide (1-benzoylmethyl-5-hydroxyphenyl-α-D-glucopyranoside) (2)

White powder (2.0 g): $C_{20}H_{22}O_9$; $[\alpha]_D^{23}$ −9.1 (*c* 0.21, MeOH); UV λ_{max} nm (log ε) (MeOH): 290 (3.06), 227 (3.50), 205 (3.03) nm; IR ν_{max} ($CHCl_3$): 3410 (OH), 2975 (C–H), 1704 (C=O, ester),1510, 1430, 1210 cm^{-1}; ^1H and ^{13}C NMR: Table 2; FABMS: *m/z* 405 $[M-1]^-$; EIMS *m/z* (rel. int.): 140 [$C_6H_3(OH)_2CH_2OH$, gentisyl alcohol]$^+$ (78), 122 [gentisyl alcohol–H_3O]$^+$ (82), 105 $[C_6H_5CO_2]^+$ (100), 77 $[C_6H_5]^+$ (52).

3.4. Enzyme inhibition assay

Activity against snake venom phosphodiesterase I (Sigma p 4631) (EC 3.1.4.1) was assayed by using the reported method (Mamillapalli et al., 1998) with the following modifications. 33 mM Tris–HC1 buffer pH 8.8, 30 mM Mg–acetate with 0.000742 U/well final concentration using a microtiter plate assay and 0.33 mM *bis*-(*p*-nitropheny1) phosphate (Sigma N-3002) as substrate. From Merck Cystein and EDTA (Gomez et al., 1998; Nakabayashi et al., 1994; Razzell, 1963; Razzell and Khorana, 1959) were used as positive controls ($1C_{50}$ = 748 μM ± 0.015, 274 μM ± 0.007, respectively). After 30 min pre-incubation of the enzyme with the test samples, enzyme activity was monitored spectrophotometrically at 37 °C on a microtitre plate reader (SpectraMax, Molecular Devices) by following the rate (change in O.D/min) of release of *p*-nitrophenol from *p*-nitrophenyl phosphate at 410 nm. All assays were conducted in triplicate.

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