Phenolic Glycosides from *Symplocos racemosa*

Viqar U. Ahmad, Muhammad Zubair, Muhammad A. Abbasi, Farzana Kousar, Farman Ullah, Naheed Fatima, and Muhammad. I. Choudhary

H. E. J. Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi, Karachi-75270, Pakistan

Reprint requests to Prof. V. U. Ahmad. E-mail: vuahmad@cyber.net.pk., zubairbr@yahoo.com

Z. Naturforsch. **60b,** 1101 – 1104 (2005); received May 22, 2005

The re-investigation of the chemical constituents of the bark of the stem of *Symplocos racemosa* Roxb. led to the isolation of two new phenolic glycosides, *Symconoside A* (**1**) and *Symconoside B* (**2**). The structures of the new compounds were determined by 1D and 2D-homonuclear and heteronuclear NMR spectroscopy, chemical evidences, and by comparison with the published data of the closely related compounds. The phenolic glycosides **1** and **2** displayed *in vitro* inhibitory activity against phosphodiesterase-I with the IC_{50} values of 158 ± 0.02 and 900 ± 0.08 μM, respectively.

Key words: Symplocos racemosa, Symplocaceae, Symconoside A, Symconoside B, Phosphodiesterase I

Introduction

Symplocos racemosa Roxb. belongs to a unigenric family Symplocaceae and medicinally it is very important as it is used as a specific remedy for uterine complaints, vaginal and menstrual disorders. Its bark is light, cooling, mild astringent, antidiarrhoeal, antidysenteric, aphrodisiac, and is useful in dropsy, elephantiasis, fillaria, liver complaints, bowel complaints, eye diseases, ulcers, menorrhagia and leucorrhoea [1]. In continuation of our previous work [2, 3] these constituents were also tested against phosphodiesterase-I for their inhibitory activity. Nucleotide pyrophosphatases/phosphodiesterases (NPPI) are type II integral membrane proteins, which also exist, in water-soluble forms that are secreted from the cell as "exo-enzymes". They are widely distributed in mammalian intestinal mucosa, mammalian liver cells, blood serum, snake venom and various plants [4]. The enzymatic activity of NPP involves hydrolysis of nucleotides and nucleotide sugars, with the release of pyrophosphate and / or inorganic phosphate [5]. NPP1 play an important role in the normal and pathological calcification of bone and cartilage. Inhibitors of NPP 1 might be useful as treatment for some forms of arthritis [6].

Results and Discussion

From the ethyl acetate soluble fraction of *Symplocos racemosa* Roxb., two new phenolic glycosides, Symconoside A (**1**) and Symconoside B (**2**) have been isolated and their structures were determined on the basis of spectroscopic studies, chemical evidences and by comparison with the published data of the closely related compounds.

Symconoside A (**1**) was obtained as a colourless gummy solid. Its molecular formula was assigned as $C_{26}H_{32}O_{14}$ on the basis of FAB-HR-MS (M-H, m/z 567.5431) that indicated eleven degrees of unsaturation. Its UV, IR and EI-MS spectra resembled much with those of reported salireposide [2]. The acid hydrolysis of **1** yielded various products, among which the glycone could be separated and identified as glucose on TLC comparison with an authentic sample of this sugar. Its identity was also confirmed by comparing the retention time of the TMS ether of the glycone with a standard sample in gas chromatography (GC) [7] and its optical rotation sign. Its configuration was confirmed as D-glucose by the reported method [8]. The ^{1}H NMR spectrum showed the characteristics signals for ABX spin system of the gentisyl alcohol moiety between $\delta = 6.47$ and 7.02 and a benzoyl residue at $\delta = 7.46$ to 8.01 as in salireposide [2], however the two doublets at $\delta = 4.67$ ($J = 7.6$) and $\delta = 4.65$ ($J = 7.9$) for two anomeric protons revealed the presence of two glucose units in the molecule. The ^{13}C NMR also confirmed the presence of salireposide like moiety, along with an additional glucose unit and its position was deduced through the downfield shift C-6" as compared to the respective signal of reported salireposide [2] indicating that the additional glucose moiety was attached

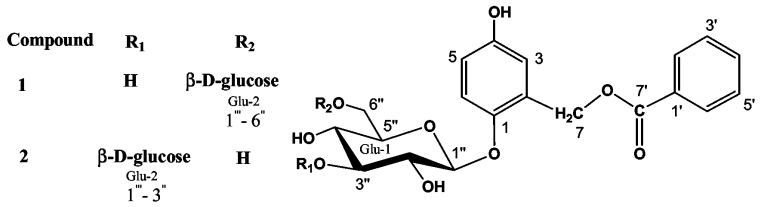

Fig. 1. Structures of compounds **1** and **2**.

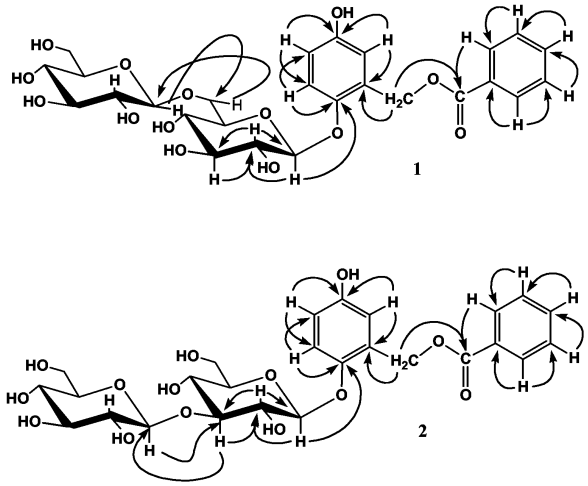

Fig. 2. Important HMBC correlations of compounds **1** and **2**.

with C-6". This assignment was further confirmed by the long-range HMBC correlation of H-1"' with C-6" and H-6" protons with C-1"'. The important HMBC correlations are shown in Fig. 2.

Symconoside B (**2**) was also obtained as a colourless gummy solid and had a different R_f value on TLC [MeOH: $CHCl_3$; 2: 8] when compared with the pure sample of **1**. The acid hydrolysis provided various products, among which the glycone could be separated and identified as D-glucose by the similar methods as described for **1**. Its UV, IR, FAB-HR-MS (M-H) and ^{1}H NMR spectra were almost similar to those of **1**, but the ^{13}C NMR spectrum revealed different signals for the glucose-I moiety, which indicated that **2** was a regio-isomer of **1**. The downfield shift of C-3" and upfield shifts of C-2" and C-4" as compared to the respective signals of **1** as well as the reported salireposide [2], indicated that the glucose-II moiety in this molecule was linked to C-3" of the glucose-I, which was also confirmed by the long range HMBC correlations of the H-1"' with C-3"and H-3" with C-1"' as shown in the Fig. 2.

A very limited number of natural inhibitors of phosphodiesterase I has been reported so far. We have already reported the inhibitory activity of some of the

Table 1. ^{1}H and ^{13}C NMR data (MeOD) of compounds **1** and **2**.

	1		**2**	
	δ (H)	δ (C)[b]	δ (H)	δ (C)[b]
1	–	149.8	–	149.6
2	–	134.3	–	134.5
3	6.78 (d, $J = 2.8$)	116.6	6.79 (d, $J = 2.7$)	116.3
4	–	154.3		154.5
5	6.47 (dd, $J = 3.0, 8.1$)	115.5	6.48 (dd, $J = 3.0, 8.2$)	115.1
6	7.02 (d, $J = 8.7$)	119.4	7.01 (d, $J = 8.8$)	119.7
7	4.52 (d, $J = 13.0$)	65.4	4.53 (d, $J = 13.0$)	65.7
	4.66 (d, $J = 13.0$)		4.67 (d, $J = 13.0$)	
1'	–	131.1	–	131.4
2', 6'	8.01 (dd, $J = 3.0, 7.5$)	130.6	8.00 (dd, $J = 3.1, 7.5$)	130.4
3', 5'	7.46 (t, $J = 7.4$)	129.6	7.47 (t, $J = 7.6$)	129.8
4'	7.61 (br t, $J = 7.6$)	134.7	7.60 (br t, $J = 7.7$)	134.5
7'	–	167.5	–	167.1
1"	4.67 (d, $J = 7.6$)	104.9	4.65 (d, $J = 7.8$)	104.8
2"	3.74 (brt, $J = 7.4$)	75.1	3.86 (brt, $J = 7.5$)	73.4
3"	3.85 (brt, $J = 7.8$)	78.0	3.47 (brt, $J = 7.2$)	82.5
4"	3.83 (brt, $J = 7.3$)	72.3	3.87 (brt, $J = 7.4$)	70.6
5"	3.44 m	75.6	3.78 m	75.8
6"	4.11 (dd, $J = 7.3, 11.8$)	65.4	4.01 m	61.2
	4.32 (dd, $J = 1.9, 11.8$)		4.21 m	
1"'	4.72 (d, $J = 7.8$)	105.1	4.76 (d, $J = 7.6$)	104.1
2"'	3.76 (brt, $J = 7.5$)	75.4	3.84 (brt, $J = 7.3$)	75.4
3"'	3.47 (brt, $J = 7.9$)	78.0	3.48 (brt, $J = 7.8$)	77.3
4"'	3.89 (brt, $J = 7.2$)	72.0	3.90 (brt, $J = 7.5$)	71.8
5"'	3.88 m	75.5	3.75 m	75.6
6"'	4.20 m	62.0	4.18 m	61.5
	4.25 m		4.35 m	

[a] All spectra were recorded at 500 MHz (^{1}H) 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°.

isolated constituents from this plant against the enzyme phosphodiesterase-I and in continuation of our previous work [2, 3], here we report the inhibitory activity of some more new phenolic glycosides against the same enzyme, to establish a structure-activity relationship. The glycoside **1** exhibited a strong inhibitory potential against phosphodiesterase-I with an IC_{50} value of 158 ± 0.02 while **2** displayed a weak inhibitory activity with an IC_{50} value of 900 ± 0.08 μM as compared to the moderate inhibitory potential of reported salireposide 544 ± 0.021 μM [2], relative to cystein (IC_{50} 748 ± 0.015 μM) and EDTA (IC_{50} 274 ± 0.007 μM). Thus, in terms of structure-activity

relationship, the presence of an additional glucose unit at C-6" in **1** significantly enhances its inhibitory potential against phosphodiesterase-I as compared to the reported salireposide however its presence at C-3" in **2** results in a decrease of inhibitory activity against the enzyme phosphodiesterase-I.

Experimental Section

General

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 precoated silica gel G-25-UV_{254} plates. Detection was carried out at 254 nm, and by ceric sulphate/ aniline phthalate reagent. Purity was checked on TLC with solvent systems using methanol, 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 spectrophotometers respectively. GC was performed on Shimadzu 9-A, column 25% carbowax 20M on chromosorb W 80/100 mesh, 145°, FI detector. ^{1}H NMR, ^{13}C NMR, COSY, HMQC and HMBC spectra were run on Bruker spectrometers operating at 500, 400 and 300 MHz. The chemical shifts (δ) are given in ppm and coupling constants in Hz. EI-MS and FAB-MS spectra were recorded on a JMS-HX-110 spectrometer, with a data system.

Plant material

The plant *Symplocos racemosa* (Symplocaceae) was collected from Abbottabad, Pakistan, in August 2002, 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.

Extraction and isolation

The shade-dried ground bark of stem (40 kg) was exhaustively extracted with methanol ($50\,\mathrm{l} \times 4$) at room temperature. The extract was evaporated to yield the residue (1.4 kg), which was dissolved in water (2.5 l) and partitioned successively with *n*-hexane, chloroform, ethyl acetate, *n*-butanol and water (3.0 l of each solvent) yielding after solvent removal, 250 g (17.85%) of *n*-hexane fraction, 275 g (19.64%) of choloform, 282 g (20.14%) of ethyl acetate, 350 g (25%) of *n*-butanol fraction and 243 g (17.35%) of water fraction. The ethyl acetate fraction was subjected to VLC (40×20 cm i.d., 1500 g) over plate-silica with gradient of hexane and chloroform ($0 : 100$) and followed by methanol up to (0 – 100%). Twelve fractions (Fr. 1 – 12) were collected. The Fr. 11 (20 g) from VLC was loaded on flash silica gel column (60×4 cm i.d., 150 g) and eluted with MeOH: $CHCl_3$ ($20 : 80$) to get four sub-fractions ($Fr_{sb}11.1$ – 11.4). The $Fr_{sb}11.4$ (0.5 g) was subjected to repeated flash silica column (60×2 cm i.d., 25 g) chromatography. Based on the difference in TLC spots, compounds **1** and **2** were purified from this column.

Acid hydrolysis of **1** *and* **2**

A solution of (**1** – **2** 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 in **1** to **2**, when compared with an authentic sample of this sugar on TLC (EtOAc: MeOH: AcOH: H_2O: 11 : 2 : 2 : 1) *via* visualizing the spots with aniline phthalate reagent. The identity in **1** to **2** was also confirmed by comparing the retention time of the TMS ether of the glycone with a standard sample (retention time α-anomer 4.1 min and β-anomer 7.8 min) and by the sign of optical rotation ($[\alpha]_D^{20} + 52$).

Trimethylsilyl (TMS) derivative

Pyridine (1 μl) and *N*, *O-bis*(trimethylsilyl) trifluoroacetamide (BSTFA, 10 μl) were added to the dried sample (about 100 μg) and heated at 80 °C for 10 min. Aliquots of the reaction mixture were injected directly into the gas chromatograph [9].

Configuration of D*-glucose*

A solution in pyridine (100 μl) of the hydrolyzed sugar from **1** – **2** (separately) (0.04 mol/l) and L-cysteine methyl ester hydrochloride (0.06 mol/l) were mixed, and the solution was warmed at 60 °C for 1 h. Acetic anhydride (150 μl) was then added, and each mixture was warmed at 90 °C for another 1 h. After evaporation of pyridine and acetic anhydride *in vacuo*, each residue was dissolved in acetone (350 μl) and the solution (1 μl) was subjected to GLC. A peak for a peracetylated thiazolidine derivative with retention time at 9.39 min was observed for all three samples, which was identical to the derivative of authentic D-glucose prepared in the same manner [8].

In vitro phosphodiesterase-I inhibition assay

Activity against snake venom phosphodiesterase I (Sigma p 4631) (EC 3.1.4.1) was assayed by using the reported method (10) 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. Cystein and EDTA (-) of Merck were used as positive controls ($1C_{50} = 748\ \mu M \pm 0.015$, $274\ \mu M \pm 0.007$, 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.

Symconoside A (***1***) (= *2-[benzoyloxymethyl-4-hydroxyphenyl*-1-oxy-O-β-D-glucopyranosyl-(1 → 6)-1-*O*-β-D-glucopyranoside). Colourless gummy Solid (20 mg): $C_{26}H_{32}O_{14}$; $[\alpha]_D^{23} + 85.1$ ($c = 0.01$, MeOH); UV λ_{max} nm ($\log\varepsilon$) (MeOH): 290 (3.03), 227 (3.25), 205 (3.09) nm; IR ν_{max} (KBr): 3415 (OH), 2970 (C-H), 1706 (C=O, ester),1520, 1425, 1215 cm^{-1}; ^1H and ^{13}C NMR: Table 1; FAB-HR-MS [M-H]: 567.5431; EI-MS *m/z* (rel. int.): 244 [M – (2 × glucose)]$^+$ (68), 140 [$C_6H_3(OH)_2CH_2OH$, gentisyl alcohol]$^+$ (82), 123 [$C_6H_3(OH)_2CH_2$]$^+$ (55), 122 [gentisyl alcohol - H_3O]$^+$ (84), 122 [$C_6H_5CO_2H$]$^+$ (84), 105 [$C_6H_5CO_2$]$^+$ (100), 77 [C_6H_5]$^+$ (50).

Symconoside B (= *2-[benzoyloxymethyl-4-hydroxyphenyl*-1-oxy-*O*-β-D-glucopyranosyl-(1 → 3)-1-*O*-β-D-glucopyranoside) (**2**). Gummy solid (17 mg): $C_{26}H_{32}O_{14}$; $[\alpha]_D^{23} - 15.0$ ($c = 0.040$, MeOH); UV λ_{max} nm ($\log\varepsilon$) (MeOH): 290 (3.06), 227 (3.55), 205 (3.07) nm; IR ν_{max} (KBr): 3410 (OH), 2973 (C-H), 1704 (C=O, ester),1510, 1430, 1210 cm^{-1}; ^1H and ^{13}C NMR: Table 1; FAB-HR-MS [M-H]: 567.8250; EI-MS *m/z* (rel. int.): 244 [M – (2 × glucose)]$^+$ (65), 140 [$C_6H_3(OH)_2CH_2OH$, gentisyl alcohol]$^+$ (78), 123 [$C_6H_3(OH)_2CH_2$]$^+$ (49), 122 [gentisyl alcohol - H_3O]$^+$ (80), 122 [$C_6H_5CO_2H$]$^+$ (80), 105 [$C_6H_5CO_2$]$^+$ (100), 77 [C_6H_5]$^+$ (55).

[1] S. G. Joshi, Medicinal Plants, p. 389, Oxford and IGH Publishing Co. Pvt. Ltd., New Delhi (2000).

[2] V. U. Ahmad, M. A. Abbasi, H. Hussain, M. N. Akhtar, U. Farooq, N. Fatima, M. I. Choudhary, Phytochemistry **68**, 217 (2003).

[3] V. U Ahmad, M. A. Abbasi, M. Zubair, U. Farooq, N. Fatima, M. I. Choudhary, Helv. Chim. Acta **87**, 57 (2004).

[4] B. Mathieu, G. Rik, C. Hugo, S. Willy, and S. Cristiana, Biochem. Mol. **35**, 393 (2000).

[5] W. E. Razzell, Phosphodiesterases Methods in Enzymology, Vol **VI**, p. 236 – 42, Academic Press, New York (1963).

[6] J. Kristen, H. Sanshiro, L. Martin, P. Kenneth, G. James, T. Robet, Arthritis Rheum **44**, 1071 (2001).

[7] N. Jahan, W. Ahmed, A. Malik, J. Nat. Prod. **58**, **8**, 1244 (1995).

[8] Q. Ye, W. Zhao, Planta Med. **68**, 723 (2002).

[9] J. J. Harding, P. Hassett, K. C. Rixon, A. J. Bron, D. J. Harvey, Curr. Eye Res. **19**, **2**, 131 (1999).