Phytochemical Studies of *Salix Acmophylla* Boiss.

[1]K. IQBAL, [1]A. MALIK,* [1]A. MEHMOOD, [1]N. MUKHTAR AND [2]R. B. TAREEN
[1]*International Centre for Chemical Sciences*
H.E.J. Research Institute of Chemistry
University of Karachi, Karachi-75270, Pakistan
[2] *Department of Botany, Baluchistan University, Quetta, Pakistan*

(Received 20th February, 2004, revised 22nd May, 2004)

Summary: Eight compounds have been isolated for the first time from *Salix acmophylla* namely, benzyl β-D-glucopyranoside (**1**), 2-(hydroxymethyl)phenyl β-D-glucopyranoside (salicin) (**2**), 2-hydroxybenzyl β-D-glucopyranoside (isosilicin) (**3**), 4-hydroxyphenyl β-D-glucopyranoside (arbutin) (**4**), 2′-*O*-acetyl salicin (**5**), phenethyl β-D-glucopyranoside (**6**), 4-hydroxycinnamoyl alcohol-1-β-D-glucopyranoside (triandrin) (**7**) and quercetin-3-*O*-β-D-glucopyranoside (**8**). Their structure have been elucidated by EIMS, HREIMS, FAB, HRFABMS, ^{1}H and ^{13}C NMR spectroscopic methods.

Introduction

The genus *Salix* belonging to the family Salicaceae comprises of 520 species, represented in Pakistan by 26 species [1]. It has a wide range of activities against different diseases. The bark and leaves are used for the treatment of various ailments such as ulcerated nose, abscesses, rheumatism, chronic dysentery, small pox, jaundice, fever, gonorrhea and skin diseases [2]. The literature survey revealed that a large number of different classes of compounds have so far been reported from the genus *Salix*. The diverse medicinal uses attributed to this genus prompted us to carry out phytochemical studies on one of its species *Salix acmophylla* Boiss. on which no work has so far been reported. It is a large shrub or small tree widely distributed in Turkey, Syria, Palestine, Iraq, Iran, Afghanistan, Pakistan, Kashmir, India and Central Asia [1]. The methanolic extract of this plant showed strong phytotoxicity. On further fractionation the major phytotoxicity was detected in ethylacetate and *n*-butanol fractions. Studies on ethylacetate fraction have now resulted in the isolation and structural characterization of benzyl β-D-glucopyranoside (**1**), 2-(hydroxymethyl)phenyl β-D-glucopyranoside (salicin) (**2**), 2-hydroxybenzyl β-D-glucopyranoside (isosilicin) (**3**), 4-hydroxy-phenyl β-D-glucopyranoside (arbutin) (**4**), 2′-*O*-acetyl salicin (**5**), phenethyl β-D-glucopyranoside (**6**), 4-hydroxy-cinnamoyl alcohol-1-β-D-glucopyranoside (triandrin) (**7**) and quercetin-3-*O*-β-D-glucopyra-noside (**8**), respectively.

Results and Discussion

Column chromatography of the ethylacetate fraction of *Salix acmophylla* resulted in the isolation and characterization of phenolic and flavonol glycosides, namely benzyl β-D-glucopyranoside (**1**), 2-(hydroxymethyl)phenyl β-D-glucopyranoside (Salicin) (**2**), 2-hydroxybenzyl β-D-glucopyranoside (isosilicin) (**3**), 4-hydroxyphenyl β-D-glucopyranoside (arbutin) (**4**), 2′-*O*-acetyl salicin (**5**), phenethyl β-D-glucopyranoside (**6**), 4-hydroxy-cinnamoyl alcohol-1-β-D-glucopyranoside (triandrin) (**7**) and quercetin-3-*O*-β-D-glucopyranoside (**8**). All of them have been reported for the first time from *Salix acmophylla* following earlier reports from other sources [3-9]. The compounds **1-8** were respectively screened for phytotoxicity but none of these was found to be active. Therefore it can be concluded that the strong phytotoxicity of the ethylacetate fraction is a combined effect of various constituents present therein.

Experimental

General

Column chromatography (CC): silica gel 70-230 mesh. Flash chromatography (FC): silica gel 230-400 mesh. TLC: pre-coated silica gel 60 F_{254} plates. UV: 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-302-A spectrophotometer, respectively. ^{1}H- and ^{13}C-NMR, spectra: Bruker spectrometers operating at 500, 400, 100 MHz; chemical shifts δ in ppm relative to $SiMe_4$ as internal standard and coupling constants J in Hz. EI-, FAB-, HR-EI-, HR-FAB-MS: JEOL JMS-HX-110 and JMS-DA-500

*To whom all correspondence should be addressed.

mass spectrometers, m/z (rel. int). The purity of the isolated compounds was check by high performance liquid chromatography (HPLC) over RP-18 column as well as thin layer chromatography over pre-coated RP-18 TLC cards of E-Merck.

Plant Material

The whole plant Salix acmophylla was collected from Quetta district of Baluchistan province in June, 2000. The identity of the plant was verified by Prof. Rasool Bukhsh Tareen, Department of Botany, University of Baluchistan, where a voucher specimen has been deposited.

Isolation

The whole plant (5 kg) was extracted thrice with MeOH at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy mass. It was suspended in water and successively extracted with *n*-hexane, chloroform, ethylacetate and *n*-butanol. All these fractions were submitted to the plant screening section for location of phytotoxicity. The ethylacetate and *n*-butanol fractions revealed pronounced phytotoxicity while *n*-hexane and chloroform fractions were weakly active. The ethyl acetate fraction was subjected to column chromatography over silica gel eluting with *n*-hexane, ethylacetate, ethylacetate-methanol and methanol in increasing order of polarity. The fractions obtained from *n*-hexane-ethylacetate (1:1-1:9) were combined and again column chromatographed over silica gel using chloroform and chloroform-methanol (9:1-8:2) to obtain benzyl β-D-glucopyranoside (**1**), 2-(hydroxymethyl)phenyl β-D-glucopyranoside (salicin) (**2**), 2-hydroxybenyl β-D-glucopyranoside (isosilicin) (**3**), 4-hydroxyphenyl β-D-glucopyranoside (arbutin) (**4**), 2′-*O*-acetyl salicin (**5**), phenethyl β-D-glucopyranoside (**6**), 4-hydroxycinnamoyl alcohol-1-β-D-glucopyranoside (triandrin) (**7**) The fractions which were obtained from ethylacetate were also combined and column chromatographed over flash silica eluting with chloroform-methanol (8:2-7:3) to furnish quercetin-3-*O*-β-D-glucopyranoside (**8**).The *n*-butanol extract which was the other active fraction was subjected to column chromatography over Sephadex LH-20 but failed to provide any uniform constituents.

Benzyl β-D-glucopyranoside (1):

The fraction which eluted with *n*-hexane-ethylacetate (1:1) was chromatographed over silica gel and eluted with chloroform-methanol (9.6:0.4) to give compound 1 which crystallized from methanol, m.p 123°C, $[\alpha]_D^{24}$ –52.9° (MeOH); HRFABMS (+): *m/z* 271.1175 $[M+H]^+$ (calcd for $C_{13}H_{19}O_6$, 271.1181). The physical and spectral data showed complete agreement with the literature [3].

2-(Hydroxymethyl)phenyl β-D-glucopyranoside (salicin) (2):

The fraction which eluted with *n*-hexane-ethylacetate (3:7) was rechromatographed over flash silica and eluted with chloroform-methanol (9.5:0.5) to give compound **2**, crystallized from methanol, m.p 204°C, $[\alpha]_D^{24}$ –62.56°; HRFABMS (+) showed $[M+H]^+$ at *m/z* 287.1125 (calcd for $C_{13}H_{19}O_7$, 287.1130). The physical and spectral data coincided with literature values [4].

2-Hydroxybenzyl β-D-glucopyranoside (isosilicin) (3):

The fraction which eluted with *n*-hexane-ethylacetate (2:8) was subjected to flash chromatography using chloroform-methanol (9.5:0.5) as eluent to give compound **3**, m.p 65°C, $[\alpha]_D^{24}$ –43° (MeOH); HRFABMS (+) showed $[M+H]^+$ at *m/z* 287.1121 (calcd for $C_{13}H_{19}O_7$, 287.1130). The physical and spectral data resembled to reported values [5].

4-Hydroxyphenyl β-D-glucopyranoside (arbutin) (4):

The fraction which eluted with *n*-hexane-ethylacetate (2:8) was subjected to flash chromatography using chloroform-methanol (9.5:0.5) to afford compound **4**, m.p 141°C, $[\alpha]_D^{24}$ –61.3° (MeOH); HRFABMS (+) showed $[M+H]^+$ at *m/z* 273.0899 (calcd for $C_{12}H_{17}O_7$, 273.0974). The physical and spectral data showed complete agreement with the literature [4].

2′-O-Acetyl salicin (5):

The fraction which eluted with *n*-hexane-ethylacetate (1:9) was subjected to flash chromatography using chloroform-methanol (9.2:0.8) as eluent to provide compound **5**, m.p 190°C (MeOH); HRFABMS (+) showed $[M+H]^+$ at *m/z* 329.1225 (calcd for $C_{15}H_{21}O_8$, 329.1236). The physical and spectral data coincided with the literature [6].

Phenethyl β-D-glucopyranoside (6)

The fraction which eluted with *n*-hexane-ethylacetate (0.5:9.5) was chromatographed over

flash silica gel and eluted with chloroform-methanol (9:1) to obtain compound **6** as an amorphous powder, $[\alpha]_D^{24}$ –35.9° (MeOH); HRFABMS (+): *m/z* 285.1329 $[M+H]^+$ (calcd for $C_{14}H_{21}O_6$, 285.1338). The physical and spectral data showed complete agreement with reported values [7].

4-Hydroxycinnamoyl alcohol-1-β-D-glucopyranoside (triandrin) (7):

The fraction which eluted with *n*-hexane-ethylacetate (0.5:9.5) was chromatographed over flash silica gel eluting with chloroform-methanol (9.8:1.2) to furnish compound **7** as colourless crystals from methanol, m.p 159°C, $[\alpha]_D^{24}$ –50° (MeOH); HRFABMS (+): *m/z* 313.1285 $[M+H]^+$ (calcd for $C_{15}H_{21}O_7$, 313.1287). The physical and spectral data showed complete resemblence with reported values [8]

Quercetin-3-O-β-D-glucopyranoside (8):

The fraction which eluted with ethylacetate-methanol (9.5:0.5) was chromatographed over flash silica gel using chloroform-methanol (8:2) as eluent to afford compound **9**, a yellow amorphous powder, $[\alpha]_D^{24}$ –20° (MeOH); HRFABMS (+): *m/z* 465.1025 $[M+H]^+$ (calcd for $C_{21}H_{21}O_{12}$, 465.1033). The physical and spectral data showed complete agreement with reported values [9].

References

1. S. I. Ali and M. Qaiser, Flora of Pakistan, No.203. Department of Botany, University of Karachi, Karachi. p 1 (2001)
2. L. M. Perry and J. Metzger, Medicinal plants of East and South East Asia. Attributed properties and uses. The MIT press, Cambridge, England, pp 372 (1980).
3. T. Miyase, A. Ueno, N. Takizawa, H. Kobayashi, H. Karasawa, *Chem. Pharm. Bull.*, **35**(3), 1109 (1987).
4. R. A. Dommisse, L. Hoof, A. J. Vlietinck, *Phytochemistry*, **25**, 1201 (1986).
5. J. Kitajima, T. Ishikawa, Y. Tanaka, M. Ono, *Chem. Pharm. Bull.*, **46**(10), 1587 (1998).
6. P. B. Reichardt., H. M. Merken, T. P. Clausen, *J. Nat. Prod.*, **55**(7), 970 (1992).
7. T. Miyase, A. Ueno, N. Takizawa, H. Kobayashi, H. Oguchi, *Chem. Pharm. Bull.*, **36**(7), 2475 (1988).
8. L. A. C. Naidoo, S. E. Drewes, J. V. Staden, A. Hutchings, *Phytochemistry*, **31**(11), 3929-3931 (1992).
9. S. Canigueral, J. Iglesias, M. Hamburger, K. Hostettmann, *Planta Medica,* **55**, 92 (1989).