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PHENOLIC GLYCOSIDES FROM *SALIX BABYLONICA*

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Key Word Index—*Salix babylonica*; Salicaceae; 2′-*O*-acetyltrichocarpin; trichocarpin; salicin; flavonoid glycosides.

Abstract—From the leaves of *Salix babylonica*, the new naturally occurring benzyl ester of gentisic acid 2′-*O*-acetyl β-D-glucoside has been isolated along with the known compounds-trichocarpin, salicin, kaempferol 7-*O*-glucoside, apigenin 7-*O*-galactoside, luteolin 4′-*O*-glucoside and an ester of terephthalic acid.

INTRODUCTION

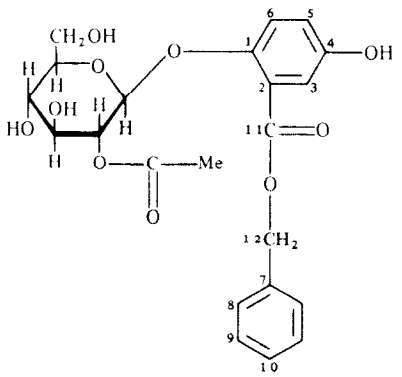
Previous investigations on various species of the Salicaceae have led to the isolation and structure elucidation of a number of phenolic glycosides [1–4]. The present paper describes the isolation of a new glycoside, the benzyl ester of gentisic acid 2′-*O*-acetyl β-D-glucoside (**1**) together with trichocarpin (**2**) and salicin (**3**) in addition to kaempferol 7-*O*-glucoside, apigenin 7-*O*-galactoside, luteolin 4′-*O*-glucoside and ester of terephthalic acid from the leaves of *Salix babylonica* L.

RESULTS AND DISCUSSION

Methanol extract of the dried and powdered leaves after purification by solvent treatment was separated into ethyl acetate soluble and insoluble fractions. Ethyl acetate insoluble material on repeated column chromatography on silica gel yielded compounds, **1**–**3**. Benzyl ester of gentisic acid 2′-*O*-acetyl β-D-glucoside (**1**) analysed for $C_{22}H_{24}O_{10}$, showed in the IR spectrum, a carbonyl group at 1730 cm^{-1}, an aromatic conjugated carbonyl group at 1678 cm^{-1} and hydroxyl groups at 3500 cm^{-1} (non-hydrogen bonded) and 3250 cm^{-1} (hydrogen bonded). The peak at 1730 cm^{-1} attributed to the acetyl carbonyl in (**1**) was missing in (**2**). The mass spectrum of (**1**) showed no molecular ion peak, but the peak at *m/z* 244 and 205 were observed, probably due to the primary rupture of glycosidic linkage. The fragment *m/z* 244 supported the presence of acetyl group in the glucose unit of the glycoside. There were several other expectable fragments (see Experimental). Enzymic hydrolysis of (**1**) yielded the benzyl ester of gentisic acid (mp, mmp, co-TLC and spectral data) and D-glucose in the aqueous layer (PC). The ^{1}H NMR (80 MHz) spectrum of (**1**) showed a singlet at δ 1.96 integrating for 3H was due to an aliphatic acetate methyl group. A multiplet at δ 3.29–3.57 (5H) was assigned to C_3'-H, C_4'-H, C_5'-H and C_6'-H. The anomeric proton (C_1'-H) showed a doublet at δ 4.52 with a coupling constant of 7 Hz. The large coupling constant ($J = 7$ Hz) due to *trans* diaxial coupling with C_2'-H proton, indicated the sugar linkage with β-configuration. The β-linkage was also evident from the release of D-glucose by the enzymic hydrolysis (β-glucosidase) of the glycoside. The signals between δ 4.63 and 5.34 integrated for six protons were attributed to C_2'-H, the hydroxyl protons of the glucose residue and of the C_{12}-proton [5]. The position of acetyl group in glucose unit of the molecule was ascertained by the fact that acetylation of a ring hydroxyl causes deshielding effect on the neighbouring protons. The anomeric proton (C_1'-H) was shifted downfield from δ 4.49 (trichocarpin) to δ 4.52, indicating the acetoxyl group at C_2' in the glucose unit. The acetoxyl signal (δ 1.96) at C_2' was also supported by the observation that the acetate signal at C_2' appears at highest field (δ 1.95–1.97), while the other acetate signals appear at relatively low field (δ 2.07–2.09) [6]. Trichocarpin (**2**) and salicin (**3**) were identified from their spectroscopic properties and hydrolysis products. The ethyl acetate soluble fraction on successive CC (silica gel) and PC (Whatman No. 3) afforded an ester of terephthalic acid, kaempferol 7-*O*-glucoside, apigenin 7-*O*-galactoside and luteolin 4′-*O*-glucoside. These were identified by standard procedures.

EXPERIMENTAL

Isolation. Defatted leaves of *Salix babylonica* (4 kg) (procured from Royal Botanical Garden, Godawari, Lalitpur, Nepal) were



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exhaustively extracted with MeOH and the extracts were concd under red. pres. The residue was treated successively with petrol (bp 60–80°), C_6H_6 and $CHCl_3$.The gummy residue (20 g) left was treated with EtOAc. The EtOAc insoluble material (10 g) was chromatographed over silica gel column. Elution with $CHCl_3$–MeOH (4:1) gave a mixture of **1**, **2** and **3** (2 g) (TLC, silica gel, EtOAc–MeOH–H_2O, 8:1:1) which on fractionation over a silica gel column using EtOAc–MeOH–H_2O (8:1:1) as the eluant, afforded compounds (**1**) (R_f 0.62, 200 mg), (**2**) (R_f 0.53, 300 mg) and (**3**) (R_f 0.41, 350 mg).

2′-*O*-Acetyltrichocarpin (**1**). Needle shaped crystals, mp 170–171°. (Found: C 58.77; H 5.26; $C_{22}H_{24}O_{10}$ requires C 58.93; H 5.39%.) MS *m/z* (rel. int.): 244 (23.07), 205 (33.3), 187 (17.94), 145 (15.38), 127 (25.6), 137 (10.25), 107 (100), 91 (2.56), 43 (66.7). ^{1}H NMR (DMSO-d_6): δ3.29–3.57 (5H, *m*, C_3, -H, $C_{4'}$-H, $C_{5'}$-H and $C_{6'}$-H), 4.52 (1H, *d*, $J = 7$ Hz, $C_{1'}$-H), 4.63–5.34 (unresolved peaks for $C_{2'}$-H, $C_{3'}$-OH, $C_{4'}$-OH, $C_{6'}$-OH and C_{12}-H), 6.81–7.74 (8H, *m*, aromatic protons), 10.46 (1H, *s*, phenolic-H), 1.96 (3H, *s*, $C_{2'}$–OAc). Hydrolysis of **1** (20 mg) with β-glucosidase in citrate phosphate buffer (pH ~ 6) at 37° for 30 hr provided an aglycone, trichocarpigenin mp 103–105°. The aq. layer was acidified with HCl, heated for 3 hr on a water bath and the neutralized aq. soln [amberlite IR-45(OH)] was analysed for glucose by PC (R_f, co-PC and colour developed by aniline hydrogen phthalate, identical with authentic sugar.

Trichocarpin (**2**). Needle shaped crystals, mp 137° (lit. [7] mp 134°–136°). (Found: C, 59.23; H 5.31; $C_{20}H_{22}O_9$; C 59.11; H 5.45%). MS *m/z* (rel. int.): 244 (12.82), 163 (0.66), 145 (1.92), 137 (12.82), 127 (1.92), 107 (100), 91 (7.69). ^{1}H NMR (DMSO-d_6): δ3.28–3.71 (6H, *m*, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H, $C_{5'}$-H and $C_{6'}$-H) 4.49 (1H, *d*, $J = 7$ Hz, $C_{1'}$-H anomeric proton), 4.77, 4.88, 4.93, 5.23 prominent peaks from complex resonances, $C_{2'}$–OH, $C_{3'}$–OH, $C_{4'}$–OH and $C_{6'}$–OH), 5.45 (2H, *s*, C_{12}-H), 6.90–7.84 (8H, *m*, aromatic protons), 10.5 (1H, *s*, phenolic proton). Hydrolysis of (**2**) as described for (**1**) yielded trichocarpigenin and D-glucose identified by comparison with authentic specimens.

Salicin (**3**). Colourless needles, mp 195–196°; pentaacetate mp, 128–129°, ^{1}H NMR ($CDCl_3$): δ2.05 (15H, *s*, 5 × OAc), 3.88 (1H, *m*, $C_{5'}$-H), 4.24 (2H, *m*, $C_{6'}$-H), 5.02–5.33 (6H, *m*, $C_{1'}$-H, $C_{2'}$-H, $C_{3'}$-H, $C_{4'}$-H and C_7-H), 6.92–7.42 (4H, *m*, aromatic protons).

EtOAc-soluble fraction (4 g) was chromatographed over a silica gel column. C_6H_6–EtOAc eluates yielded ester of terephathalic acid (80 mg) and a yellow mixture. The mixture by prep. PC (Whatman No. 3, 15% HOAc) yielded kaempferol 7-*O*-glucoside (R_f 0.13, 200 mg), apigenin 7-*O*-galactoside (R_f 0.23, 150 mg) and luteolin 4′-*O*-glucoside (R_f 0.34, 100 mg). These were identified by spectral and chromatographic studies of the glycosides as well as the hydrolysed products.

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