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Research Article

TWO NEW PHENOLIC GLYCOSIDES FROM THE FLOWERS OF *SALIX CAPREA* L.

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ABSTRACT
Two new phenolic glycosides characterized as o-methoxycatechol-O-β-D- glucopyranoside and o-cresol-O-α–D-glucopyranoside have been isolated from the flowers of *Salix caprea* L. (Salicaceae) along with the known compounds n- tritetracontane, 4'–methoxyapigenin-7-glucoside and 4-methoxyluteolin-7- glucoside. The structures of all the phytoconstituents have been elucidated on the basis of spectral data analysis and chemical reactions.

KEYWORDS: *Salix caprea,* Salicaceae, floweres, o-methoxycatechol glucoside, o-cresol glucoside.

INTRODUCTION

Salix caprea L. (Salicaceae), commonly known as Bed mushk, Sallow or Willow-bark, is a large shrub or small tree planted for ornamental purpose in the north-western Himalayas, Iran and Afghanistan. It has been reported to possess antioxidant, antifungal and antimicrobial[1] activities. The flowers of the plant have antioxidant activity[2] and utilized for hemolysis[3]. The bark is useful as astringent application to treat piles. This drug is beneficial as stimulant, aromatic and to cure influenza[4]. The plant is prescribed in Swedish traditional medicine to subside inflammatory diseases and wounds. Luteolin-7-β-D-glucopyranoside, (+)-epigallocatechin[5], (+)-gallocatechin, salicin[6], saligenin, (+)-gallocatechin, rutin, cymaroside, quercetin and luteolin[7] are reported from the leaves. Luteolin- 7- glucoside[8] and a flavone bioside are present in the plant. Aromadendrin, (+)- catechin, dihydrokaempferide, naringenin, prunin, taxifolin and 4, 2'-dihydroxy-3,5-dimethoxybiphenyl were isolated from the wood[9]. The present manuscript describes the isolation and characterization of the phytoconstituents from the flowers of *S.caprea*.

MATERIAL & METHODS

General experimental procedures

Melting points were measured on a Perfit apparatus and are uncorrected. IR spectra were recorded in KBr pellet on Win IR FTS 135 instrument (Biorad, USA). ^{1}H NMR (300 MHz), ^{13}C NMR (75 MHz), and 2D NMR spectra were recorded by Bruker spectrospin NMR instrument in $CDCl_3$, using TMS as internal standard. FAB ionization at 70 eV was scanned on a Jeol D-300 instrument (Jeol, USA). For Column chromatography, silica gel (60-120 mesh, Merck, Mumbai, India) was used and thin-layer chromatography was performed on silica gel G coated TLC plates (Merck, Mumbai, India).

Plant material

The dried flowers of *Salix caprea* were purchased from Khari Baoli market of Delhi. The authenticity of the material was established by Prof. M.P.Sharma, Department of Botany, Faculty of Science, Jamia Hamdard, New Delhi. A voucher specimen No-PRL/ JH/08/37 in deposited in the herbarium of the Department of Pharmacognosy and Phytochemistry, Jamia Hamdard, New Delhi.

Extraction and fractionation

The air dried flowers (1.32 kg) were exhaustively extracted with ethyl alcohol in a Soxhlet apparatus. The solvent was removed by distillation under reduced pressure to obtain a dark brown mass (250 g). The alcoholic extract was treated with petroleum ether (60-80^{o} C) at room temperature to obtain petroleum ether soluble and petroleum ether insoluble fractions.

Isolation

The petroleum ether insoluble fraction (100 g) of the ethyl alcohol extract was chromatographed over silica gel column (60-120 mesh, 1.5 kg). The column was eluted with petroleum ether, petroleum ether–chloroform (9:1, 3:1, 1:1, 1:3) mixtures, chloroform, chloroform-methanol (99:1, 95:5, 9:1, 4:2, 3:1, 1:1) and finally with methanol to isolate the following compounds:

n-Tritetracontane (1)

Elution of the column with petroleum ether-chloroform (9:1) gave colourless amorphous powder of **1**, recrystallized from chloroform-methanol (1:1), 21 mg (0.0015% yield), R_f 0.83 ($CHCl_3$: acetone, 1:1), 20 mg, m.p. 110-112 °C. ^{1}H NMR ($CDCl_3$): δ 1.53 (4H, brs, 2 × CH_2), 1.25 (78H, brs, 39 × CH_2), 0.81 (3H, t, J = 6.1 Hz, Me-1), 0.79 (3H, t, J = 6.2 Hz, Me-43); +ve ESI MS m/z (rel. int) : 604 $[M]^+$ ($C_{43}H_{88}$) (1.9).

Methoxycatechol glucoside (2)

Elution of the column with chloroform-methanol (19:5) furnished colourless crystals of **2**, recrystallized from $CHCl_3$-MeOH (1:1), 15 mg (0.0011% yield), R_f 0.50 ($CHCl_3$- MeOH, 4:1), m.p. 162-164 °C; UV λ_{max} (MeOH): 261 nm (log ε 2.1); IR υ_{max} (KBr): 3360, 2950, 2845, 1550, 1455, 1418, 1241, 1191, 1117, 1084, 1041,1014, cm^{-1}. ^{1}H NMR ($CDCl_3$): δ 7.89 (2H, m, H–5), 7.67 (1H, m, H–2), 7.13 (2H, m, H–3, H–4), 5.13 (1H, d, J=7.1 Hz, H–1′), 4.16 (1H, m, H–5′), 3.89 (1H, m, H–2′), 3.63 (2H, m, H–3′, H–4′), 3.17 (1H, brs, H_2–6′a), 3.11 (1H, brs, H_2–6′b), 3.35 (3H, brs, OMe); ^{13}C NMR ($CDCl_3$) : δ 154.67 (C-1), 127.61 (C-2), 114.77(C-3), 121.65 (C-4), 127.16 (C-5), 161.47 (C-6), 101.44 (C-1'), 76.46 (C-2'), 73.35 (C-3'), 69.70 (C-4'), 77.04 (C-5'), 60.73 (C-6'), 58.23 (OMe); +ve ESI MS m/z (rel. int): 286 $[M]^+$ ($C_{13}H_{18}O_7$) (1.8).

o-Cresol glucoside (3)

Further elution of the column with chloroform-methanol (9:1) afforded colourless crystals of **3 ,** recrystallized from with $CHCl_3$: MeOH (1:1), 45 mg (0.0034% yield), R_f 0.82 ($CHCl_3$: MeOH, 6:3), m.p. 173 -175 °C; UV λ_{max} (MeOH): 269 nm (log ε 4.3).IR υ_{max} (KBr) : 3510, 3450, 3371, 2934, 2855, 1544, 1450, 1420, 1241, 1191, 1117, 1085, 1042, 1015 cm^{-1}. ^{1}H NMR ($CDCl_3$) :δ 7.16 (1H, dd, J=7.11, 2.3 Hz, H–6), 7.09 (1H, m, H-5), 6.94 (1H, dd, J = 7.61, 2.31 Hz, H–3), 6.88 (1H, m, H–4), 5.12 (1H, d, J = 3.9 Hz, H–1′ α), 4.78 (1H, m, H–5′), 4.48 (1H, m, H–2′), 4.27 (1H, m, H–4′), 3.52 (1H, d, J= 9.3 Hz, H_2–6′a), 3.34 (1H, d, J = 9.3Hz, H_2–6′b), 2.59 (3H, brs, Me-7). ^{13}CNMR ($CDCl_3$) : δ 154.73 (C-1), 131.54 (C-2), 127.25(C-3), 121.79 (C-4), 115.86 (C-5), 127.74 (C-6), 28.30 (C-7), 101.48 (C-1'), 116.54 (C-2'), 73.60 (C-3'), 69.86 (C-4'), 77.10 (C-5'), 60.82 (C-6') (OMe); +ve ESI MS m/z (rel. int): 270 $[M]^+$ ($C_{13}H_{18}O_6$) (1.5).

4'- Methoxyapigenin glucoside (4)

Elution of the column with chloroform-methanol (9:1) gave a yellow amorphous powder of **4,** recrystallized from MeOH, 25.2 mg (0.018% yield), R_f. 2.5 (EtOAc: MeOH, 7:3), m.p. 280 -282 °C, UV λ_{max} (MeOH): 273, 322 nm (log ε 3.6, 3.9); IR υ_{max} (KBr): 3490, 3360, 1709, 1586, 1460, 1210, 1085, 1035, 1020, 895 cm^{-1}; ^{1}H NMR (DMSO-d6): δ 7.41 (2H, m, H–2′, H–6′), 7.13 (2H, m, H–3′, H–5′), 6.78 (1H, d, J = 2.9 Hz, H–8), 6.29 (2H, m, H–6, H–3), 5.11 (1H, d, J = 7.2 Hz, H–1′), 3.96 (1H, m, H–5′), 3.50 (1H, m, H–2′), 3.46 (2H, m, H–3′,H-4′), 3.31 (3H, brs, OMe), 3.23 (2H, brs, H_2–6′). ^{13}C NMR ($CDCl_3$) : δ 163.01 (C-2), 101.42 (C-3), 181.75(C-4), 161.10 (C-5), 99.54 (C-6), 164.11 (C-7), 94.79 (C-8), 156.95 (C-9), 103.81 (C-10), 121.70 (C-1'), 114.78 (C-2'), 127.65 (C-3'), 151.79 (C-4'), 112.13 (C-5'), 127.15 (C-6'), 99.93 (C-1"), 76.48 (C-2"), 73.39 (C-3"), 69.75 (C-4'), 77.09 (C-5"), 60.75 (C-6"), 58.21 (OMe); +ve ESI MS m/z (rel. int): 446 $[M]^+$ ($C_{22}H_{22}O_{10}$) (2.1).

Methoxyluteolin glucoside (5)

Further elution of the column with chloroform-methanol (9:1) afforded pale yellow crystals of **5**, recrystallized from chloroform – methanol (1:1), 25 mg (0.0019% yield), R_f 0.70 ($CHCl_3$ – MeOH (1:1), m.p 240-242 °C; UV λ_{max} (MeOH): 260, 321 nm (log ε 3.8,3.1); IRυ_{max} (KBr) : 3495, 3345, 2945. 2850, 1709, 1590, 1460, 1260, 1035, 890 cm^{-1}; ^{1}H NMR (DMSO–d6): δ 7.29 (1H,d,J=8.1 Hz,H-5′), 7.21(2H,m, H-2′ H-5′), 6.75 (1H, d, J = 2.7 Hz, H–8), 6.30 (2H, m, H–6, H–3), 5.17 (1H, α, J = 7.3 Hz, H–1′), 4.36 (1H, m, H–5′), 3.99 (1H,m, H-2′) 3.45 (2H, m, H–2′, H–3′, H–4′), 3.32 (3H, brs, OMe), 3.21 (2H, brs, H_2–6′); ^{13}CNMR ($CDCl_3$) : δ 163.03 (C-2), 103.84 (C-3), 181.77 (C-4), 161.13 (C-5), 99.59 (C-6), 164.11 (C-7), 94.82 (C-8), 156.98 (C-9), 103.89 (C-10), 122.90 (C-1'), 113.14 (C-2'), 146.81 (C-3'), 151.81(C-4') 112.16 (C-5'), 118.92 (C-6'), 99.89 (C-1"), 76.41 (C-2"), 73.12 (C-3"), 69.55(C-4") 77.18 (C-5"), 60.75 (C-6"), 58.21 (OMe); +ve ESI MS m/z (rel. int): 462$[M]^+$ ($C_{22}H_{22}O_{11}$) (2.5)

RESULTS AND DISCUSSION

Compounds **1, 4** and **5** were the known phytoconstituents characterized as n- tritetracontane, 5,7-dihydroxy-4'-methoxyflavone-7-O-β–D-glucopyranoside and 5,7,3'–trihydroxy–4'–methoxyflavone–7–O–β–D-glucopyranoside, respectively, on the basis of the spectral data analysis.

Chloroform–methanol (19:5) eluant on crystallization with methanol gave colourless crystals of **2.** Its UV spectrum displayed absorption maximum at 261 nm. Its IR spectrum displayed characteristic absorption bands for hydroxyl groups (3360 cm^{-1}) and aromaticity (1550, 1014 cm^{-1}). Its +ve ESI mass spectrum displayed a molecular ion peak at m/z 286 consistent with the molecular formula of a phenolic glucoside $C_{13}H_{18}O_7$. The ^{1}H NMR of **2** displayed two one-proton multiples at δ 7.89 and δ 7.67 assigned correspondingly to H-5 and

H-2 aromatic protons. A two-proton multiplet at δ 7.13 was ascribed to H-3 and H-4 aromatic protons. A one-proton doublet at δ 5.13 (J= 7.1 Hz) appeared due to H-1′ anomeric proton. Two one-proton broad signals at δ 3.17 and δ 3.11 were attributed to H_2-6′a and H_2-6′b oxygenated methylene protons. The remaining sugar protons resonated as multiples at δ 4.16 (1H), 3.89 (1H) and 3.63 (2H). The absence of any signals after δ 3.11 ruled out the presence of any aliphatic moiety in the molecule. The ^{13}C NMR spectrum of **2** displayed important signals for phenolic carbons at δ 154.67 (C-1) and 161.47 (C-6), remaining aromatic carbons between δ 127.61 – 114.77, anomeric C-1′ at δ 101.44, other sugar carbons between 77.04-60.73 and methoxy carbon at δ 58.23. Acid hydrolysis of **2** yielded D-glucose (TLC comparable). On the basis of spectral data analysis and chemical reaction the structure of **2** has been elucidated as o-methoxyocatechol-O-β-D-glucopyronoside. This is a new catechol glycoside isolated from a natural or synthetic source for the first time.

Compound **3** was obtained as colourless crystals from chloroform–methanol (9:1) eluants. It gave positive tests of glycosides. Its IR spectrum exhibited characteristic absorption bands for hydroxyl groups (3510, 3450, 3371 cm^{-1}) and aromatic ring (1544, 1015 cm^{-1}). Its +ve ESI mass spectrum displayed a molecular ion peak at m/z 270 corresponding to the molecular formula of toludine glucoside, $C_{13}H_{18}O_6$. The ^{1}H NMR spectrum of **3** displayed two one- proton double doubles at δ 7.16 (J = 7.11, 2.3 Hz) and δ 6.94 (J = 7.61, 2.3 Hz) assigned correspondingly to ortho-, meta - coupled H-6 and H-3 aromatic protons. Two one–proton multiplets at δ 7.09 and 6.88 were attributed to aromatic H-5 and H-4 protons, respectively. A one-proton doublet at δ 5.12 (J = 3.9 Hz) was due to anomeric H-1′α-protons. Three one-proton multiplets at δ 4.78, 4.48, and 4.27 were ascribed to the sugar carbinol H-5′, H-2′ and H-4′ protons, respectively. Two one-proton doublets at δ 3.52 (J = 9.3 Hz) and δ 3.34 (J = 9.3 Hz) were associated with H_2-6′a and H_2-6′b oxygenated methylene protons. A three-proton broad signal at δ 2.59 was assigned to C-7 methyl protons attached to the aromatic ring. The ^{13}C NMR spectrum of **3** displayed important signals for phenolic carbon C-1 at δ 154.73, anomeric carbon C-1′ at δ 101.48 and other sugar carbons appeared between 77.10-60.72. The methyl carbons C-7 resonated at δ 28.30. On the basis of above spectral data analysis the structure of **3** has been elucidated as *o*-cresol-O–α-D-glucopyranoside. This is a new glycoside isolated from a natural or synthetic source for the first time.

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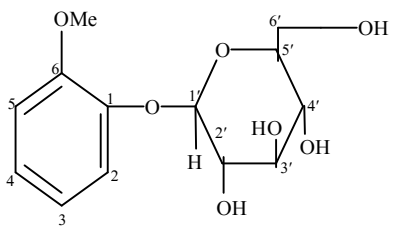
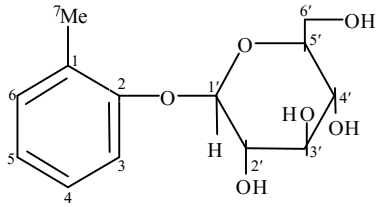
$$\overset{43}{CH_3}\ (CH_2)_{41} - \overset{1}{CH_3}$$

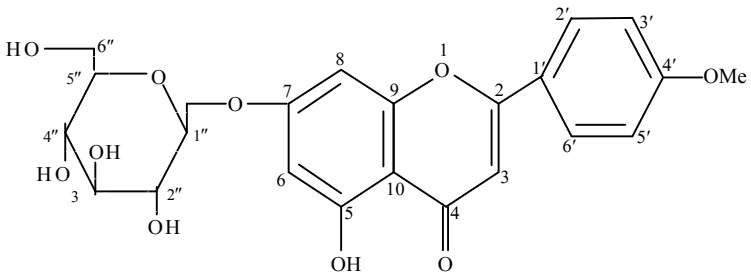
n-Tritetracontane **(1)**



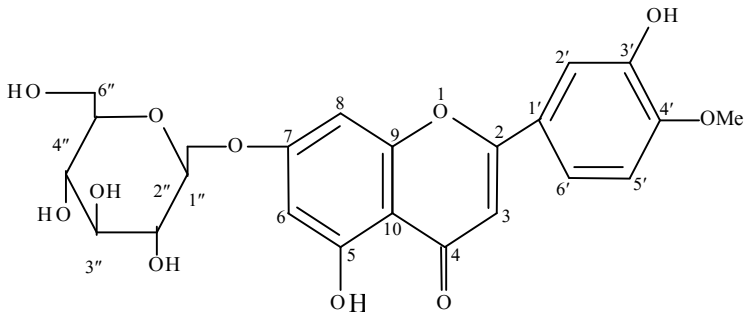
o-Methoxyocatechol-O-β-D-glucopyronoside **(2)**



o-Cresol -O –**α**-D-glucopyranoside **(3)**



5,7-Dihydroxy-4′-methoxy-flavone-7-O-β-D- glucopyranoside **(4)**



5, 7, 3′- Trihydroxy-4′-methoxyflavone-7-O- β-D- glucopyranoside (**5)**

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