Phytochemical Studies on *Perovskia atriplicifolia*

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Summary: Ten compounds have been isolated for the first time from *Perovskia atriplicifolia* namely, lupeol (**1**), β-sitosterol (**2**), methyl 4-hydroxybenzoate (**3**), 4-hydroxy-3,5-dimethoxybenzaldehyde (**4**), 7-methoxy-5,4′-dihydroxyflavone (**5**), 4′,5-dihydroxy-6,7-dimethoxyflavone (**6**), (+)-pinoresinol (**7**), (+)-syringaresinol (**8**), (+)-lariciresinol (**9**) and (+)-taxiresinol (**10**). Their structures have been elucidated by EIMS, HREIMS, HRFABMS, ^{1}H- and ^{13}C- NMR techniques.

Introduction

The genus *Perovskia* belonging to the family Labiatae comprises seven species. One of these is *Perovskia atriplicifolia* Benth, commonly known as Russian sage, which is a shrubby plant found in central Asia, Pakistan, Afghanistan and Iran [1]. The plant has antibacterial activity and is also used as cooling medicine in the treatment of fever [2]. A number of new phenolic and flavonoidal constituents have previously been reported from the ethyl acetate soluble fraction of *P. atriplicifolia* [3, 4]. Studies on chloroform soluble fraction have now resulted in the isolation and structural characterization of lupeol (**1**), β-sitosterol (**2**), methyl 4-hydroxybenzoate (**3**), 4-hydroxy-3,5-dimethoxybenzaldehyde (**4**), 7-methoxy-5,4′-dihydr-oxyflavone (**5**), 4′,5-dihydroxy-6,7-dimethoxyflavone (**6**), (+)-pinoresinol (**7**), (+)-syringaresinol (**8**), (+)-lariciresinol (**9**) and (+)-taxiresinol (**10**), respectively, reported for the first time from this species.

Results and Discussion

The chloroform soluble fraction of the methanolic extract of *Perovskia atriplicifolia* was subjected to column chromatography over silica gel eluting with different mobile phases which resulted in the isolation and characterization of ten compounds namely, lupeol (**1**), β-sitosterol (**2**), methyl 4-hydroxybenzoate (**3**), 4-hydroxy-3,5-dimethoxy-benzaldehyde (**4**), 7-methoxy-5,4′-dihydroxyflavone (**5**), 4′,5-dihydroxy-6,7-dimethoxyflavone (**6**), (+)-pinoresinol (**7**), (+)-syringaresinol (**8**), (+)-lariciresinol (**9**) and (+)-taxiresinol (**10**). All of them have been reported for the first time from *P. atriplicifolia.* Compounds **1-10** were screened for antimicrobial activity but none of these showed significant activity.

Experimental

General

Column chromatography (CC): Silica gel 70-230 mesh; TLC: pre-coated silica gel 60 F_{254} (20 x 20 cm, 0.2 mm thick; E-Merck) plates; UV: detection at 254 nm and using ceric sulphate reagent. Optical rotations: Jasco-DIP-360 digital polarimeter. UV and IR spectra: Hitachi-UV-3200 and Shimadzu IR-460 spectrophotometer, respectively. ^{1}H-NMR spectra: Bruker spectrometers operating at 300 MHz, 400 MHz or 500 MHz. Chemical shift δ in ppm relative to $SiMe_4$ as internal standard and coupling constants J in Hz. EIMS, HREIMS: JEOL JMS–HX–110 and JMS–DA-500 mass spectrometers, *m/z*: (rel. int). The purity of the isolated compounds was checked on pre-coated high performance thin layer chromatography (HPTLC) plates of E. Merck.

Plant Material

The whole plant of *Perovskia atriplicifolia* Benth (Labiatae) was collected from Quetta (Pakistan) and identified by Prof. Rasool Bakhsh Tareen, Department of Botany, University of Balochistan, where a voucher specimen has been deposited (BU-68).

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Isolation

The shade-dried plant material (20 kg) was extracted with methanol (3×50 lit.) at room temperature. The extract was evaporated to yield the residue (780 g) which was divided into *n*-hexane (65 g), chloroform (60 g), ethyl acetate (48 g), *n*-butanol (70 g) and water (41 g) soluble sub-fractions. The chloroform soluble sub-fraction was subjected to column chromatography eluting with *n*-hexane, *n*-hexane-EtOAc and EtOAc-MeOH in increasing order of polarity to obtain seven fractions F_1-F_7. The fraction F_1 which eluted from *n*-hexane-ethyl acetate (9:1) was again chromatographed over silica gel using *n*-hexane-ethyl acetate (8:2) as eluent to afford lupeol (**1**) and *β*-sitosterol (**2**) from the top and tail fractions, respectively. The fraction F_3 obtained from *n*-hexane-EtOAc (6:4) gave two spots on TLC, and its preparative TLC in the same solvent system provided methyl 4-hydroxybenzoate (**3**) and 4-hydroxy-3,5-dimethoxybenzaldehyde (**4**) from the top and tail fractions, respectively. The fraction F_5 obtained from *n*-hexane-EtOAc (1:1) was subjected to column chromatography using solvent system *n*-hexane-EtOAc (1:1) to afford 7-methoxy-5,4′-dihydroxyflavone (**5**) and 4′,5-dihydroxy-6,7-dimethoxyflavone (**6**), respectively. The fraction F_6 obtained from *n*-hexane-EtOAc (4:6) was subjected to column chromatography eluting with *n*-hexane-EtOAc (3:7) to furnish (+)-pinoresinol (**7**) and (+)-syringaresinol (**8**) from the top and tail fractions, respectively. The fraction F_7 obtained from *n*-hexane-EtOAc (3:7) was subjected to column chromatography eluting with *n*-hexane-EtOAc (2.5:7.5) to furnish (+)-lariciresinol (**9**) and (+)-taxiresinol (**10**) from the top and tail fractions, respectively.

Lupeol (**1**)

Compound **1** (25 mg) was obtained as colorless needles from CH_3OH, M.p. 215-216 °C; $[\alpha]_D^{25}$ + 27 (*c* = 0.01, $CHCl_3$); UV λ_{max} (CH_3OH) nm: 205; IR (KBr) ν_{max} cm^{-1}: 3455 (OH), 3075, 1645 and 880 (C=CH_2); ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 4.62, 4.75 (1H, each, br.s. CH_2-29), 3.21 (1H, dd, *J* = 9.9, 4.5 Hz, H-3), 2.36 (1H, ddd, *J* = 10.5, 10.5, 5.4 Hz, H-19), 1.65 (3H, br.s. CH_3-30), 1.05 (3H, s, CH_3-26), 0.97 (6H, s, CH_3-25, CH_3-27), 0.94 (3H, s, CH_3-24), 0.85 (3H, s, CH_3-28), 0.79 (3H, s, CH_3-23); HREIMS showed $[M]^+$ at *m/z* 426.3827 (calcd. for $C_{30}H_{50}O$, 426.3861). The physical and spectral data showed complete resemblance with the reported values [5, 6].

β-Sitosterol (**2**)

Compound **2** (50 mg) was obtained as colorless needles; M.p. 135-137 °C; $[\alpha]_D^{25}$ -34 (c = 0.04, $CHCl_3$); IR ($CHCl_3$) ν_{max} cm^{-1}: 3445 and 1655; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 5.32 (1H, m, H-6), 3.36 (1H, m, H-3), 0.92 (3H, s, CH_3-19), 0.88 (3H, d, *J* = 6.5 Hz, CH_3-21), 0.83 (3H, d, *J* = 6.5 Hz, CH_3-26), 0.81 (3H, d, *J* = 6.5 Hz, CH_3-27), 0.77 (3H, t, *J* = 7.0 Hz, CH_3-29), 0.63 (3H, s, CH_3-18); HREIMS showed $[M]^+$ at *m/z* 414.3857 (calcd. for $C_{29}H_{50}O$, 414.3861). The physical and spectral data coincided with the reported values [7, 8].

Methyl 4-hydroxybenzoate (**3**)

Compound **3** (15 mg) was obtained as a colorless needles from CH_3OH, M.p. 129-130 °C; UV λ_{max} (CH_3OH) nm: 287, 326; IR ($CHCl_3$) ν_{max} cm^{-1}: 3430, 3045, 1600, 815; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 7.95 (2H, d, *J* = 8.7 Hz, H-2, 6), 6.84 (2H, d, *J* = 8.7 Hz, H-3, 5), 3.86 (3H, s, OCH_3); HREIMS showed $[M]^+$ at *m/z* 152.0446 (calcd. for $C_8H_8O_3$, 152.0450). The physical and spectral data coincided with the literature values [9, 10].

4-Hydroxy-3,5-dimethoxybenzaldehyde (**4**)

Compound **4** (12 mg) was obtained as colorless needles; M.p. 115-116 °C; UV λ_{max} (CH_3OH) nm : 215, 227 and 232; IR (KBr) ν_{max} cm^{-1}: 3580, 3430 and 1610 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 9.80 (1H, s, aldehyde-H), 7.13 (2H, s, H-2, 6), 3.95 (6H, s, 2×OCH_3); HREIMS showed $[M]^+$ at *m/z* 182.0571 (calcd. for $C_9H_{10}O_4$, 182.0579). The physical and spectral data corresponded to the reported values [11].

7-Methoxy-5,4′-dihydroxyflavone (**5**)

Compound **5** (10 mg) was obtained as yellow crystalline solid; M.p. 200 °C; UV λ_{max} (CH_3OH) nm : 205, 290, 325, 370; IR (KBr) ν_{max} cm^{-1}: 3300, 1730, 1630: ^{1}H-NMR (CD_3OD, 400 MHz) δ 13.65 (1H, s, 5-OH), 7.86 (2H, d, *J*= 1.9 Hz, H-2′, 6′), 6.92 (2H, d, *J*= 1.9 Hz, H-3′, 5′), 6.63 (1H, s, H-3), 3.89 (3H, s, OCH_3); HREIMS showed $[M]^+$

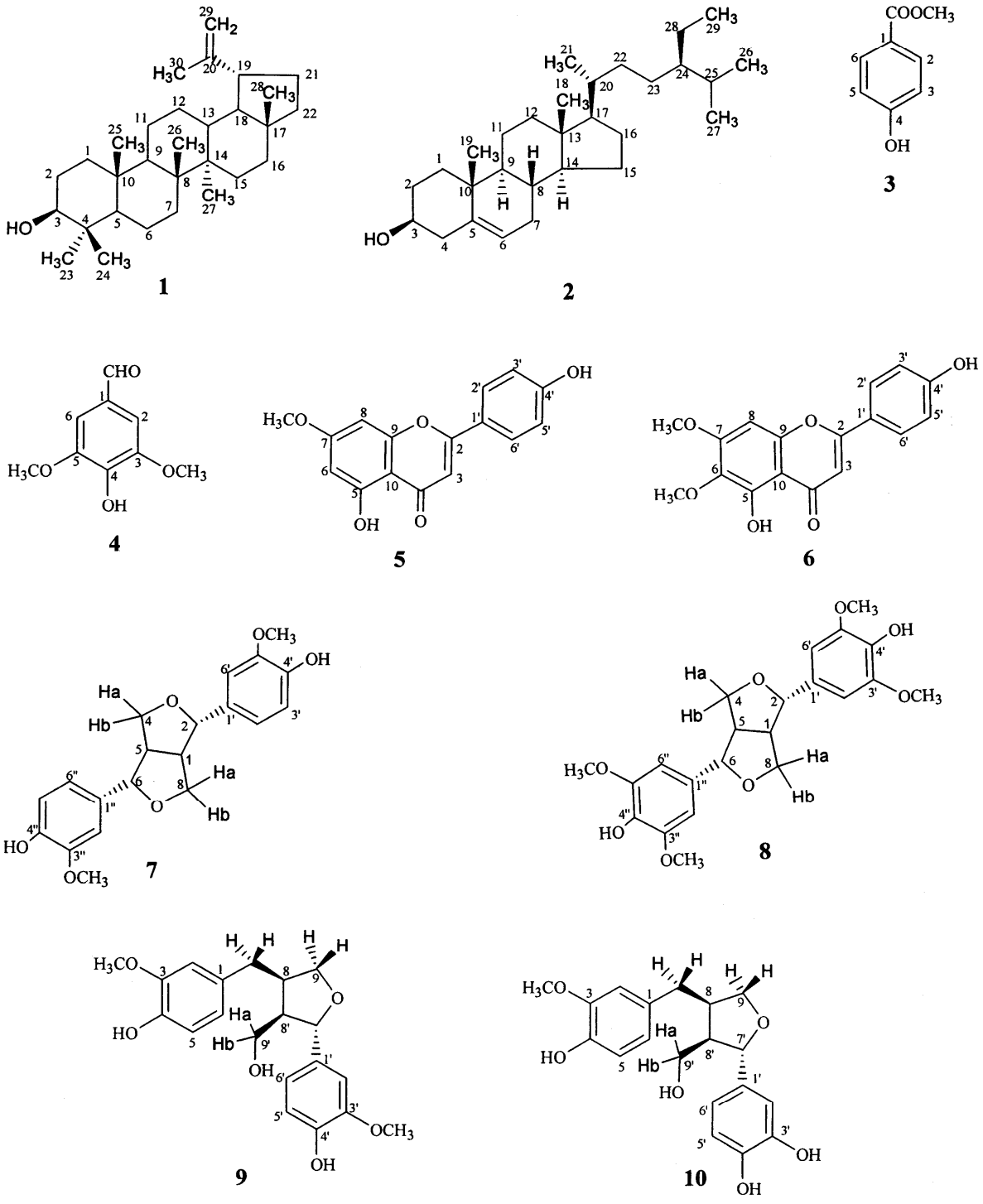

Fig. 1: Structures of compounds **1-10**.

at *m/z* 284.0667 (calcd. for $C_{16}H_{12}O_5$, 284.0684). The physical and spectral data were similar to those reported previously in literature [12, 13].

4′,5-Dihydroxy-6,7-dimethoxyflavone (**6**)

Compound **6** (12 mg) was obtained as yellow needles from CH_3OH; M.p. 193-194 °C; UV λ_{max} (CH_3OH) nm : 212, 271, 341; IR ($CHCl_3$) ν_{max} cm^{-1}: 3457, 1670, 1650, 1503, 1460 and 1310-1180; ^{1}H-NMR (CD_3OD, 400 MHz) δ 12.90 (1H, s, 5-OH), 7.90 (2H, d, J= 8.6 Hz, H-2′, 6′), 6.93 (2H, d, J= 8.6 Hz, H-3′, 5′), 6.91 (1H, s, H-8), 6.82 (1H, s, H-3), 3.91 (3H, s, OCH_3), 3.72 (3H, s, OCH_3); HREIMS showed $[M]^+$ at *m/z* 314.0782 (calcd. for $C_{17}H_{14}O_6$, 314.0791). The physical and spectral data showed complete agreement with the reported values [14].

(+)-Pinoresinol (**7**)

Compound **7** (26 mg) was obtained as colorless crystals, M.p. 124-126 °C; $[\alpha]_D^{25}$ + 85 (c = 0.01, $CHCl_3$); UV λ_{max} (CH_3OH) nm: 233 and 280; IR (KBr) ν_{max} cm^{-1}: 3450, 1615 and 1510; ^{1}H-NMR (CD_3OD, 400 MHz) δ 6.95 (2H, d, J = 1.6 Hz, H-2′, 2″), 6.81 (2H, dd, J = 8.0, 1.6 Hz, H-6′, 6″), 6.78 (2H, d, J = 8.0 Hz, H-5′, 5″), 4.70 (2H, d, J = 4.5 Hz, H-2, 6), 4.24 (2H, dd, J = 8.8, 6.7 Hz, H-4b, 8b), 3.85 (2H, dd, J = 8.8, 3.6 Hz, H-4a, 8a), 3.84 (6H, s, 2×OCH_3), 3.12 (2H, m, H-1, 5); HREIMS showed $[M]^+$ at *m/z* 358.1396 (calcd. for $C_{20}H_{22}O_6$, 358.1416). The physical and spectral data coincided with the literature values [15].

(+)-Syringaresinol (**8**)

Compound **8** (20 mg) was obtained as colorless crystals, M.p. 170-172 °C; $[\alpha]_D^{25}$ + 64 (c = 0.01, $CHCl_3$); UV λ_{max} (CH_3OH) nm: 215, 240, 275; IR (KBr) ν_{max} cm^{-1}: 3400, 1600 and 1500; ^{1}H-NMR (CD_3OD, 400 MHz) δ 6.67 (4H, s, H-2′, 6′, 2″, 6″), 4.65 (2H, d, J = 4.1 Hz, H-2, 6), 4.21 (2H, dd, J = 8.9, 6.9 Hz, H-4a, 8a), 3.86 (12H, s, 4×OCH_3), 3.48 (2H, m, H-4b, 8b), 3.07 (2H, m, H-1, 5); HREIMS showed $[M]^+$ at *m/z* 418.1586 (calcd. for $C_{22}H_{26}O_8$, 418.1627). The physical and spectral data coincided with the literature values [16, 17].

(+)-Lariciresinol (**9**)

Compound **9** (20 mg) was obtained as white crystals, M.p. 167-168 °C; $[\alpha]_D^{25}$ +18 (c = 0.01, CH_3OH); UV λ_{max} (CH_3OH) nm: 233 and 280; IR (KBr) ν_{max} cm^{-1}: 3375, 2940, 1607, 1450; ^{1}H-NMR (CD_3OD, 400 MHz) δ 6.76 (1H, br.s. H-2′), 6.68 (1H, br.s. H-2), 6.63 (1H, br.s. H-5′), 6.62 (1H, d, 4.89 Hz, H-6′), 6.60 (1H, br.s. H-5), 6.51 (1H, dd, J = 1.85, 7.96 Hz, H-6), 4.55 (1H, d, J = 6.3 Hz, H-7′), 3.80 (1H, t, J = 6.5 Hz, H-9α), 3.62 (6H, s, 2×OCH_3), 3.60 (1H, m, H-9′a), 3.48 (1H, t, J = 6.7 Hz, H-9β), 3.40 (1H, m, H-9′b), 2.75 (1H, dd, J = 4.8, 13.4 Hz, H-7α), 2.49 (1H, m, H-8), 2.35 (1H, t, J = 12.4 Hz, H-7β), 2.15 (1H, dd, J = 7.0, 13.7 Hz, H-8′); HREIMS showed $[M]^+$ at *m/z* 360.1562 (calcd. for $C_{20}H_{24}O_6$, 360.1573). The physical and spectral data coincided with the literature values [18].

(+)-Taxiresinol (**10**)

Compound **10** (20 mg) was obtained as white crystals, M.p. 158-160 °C; $[\alpha]_D^{25}$ +32 (c = 0.01, CH_3OH); UV λ_{max} (CH_3OH) nm: 225 and 280; IR (KBr) ν_{max} cm^{-1}: 3372, 2935, 1605, 1450; ^{1}H-NMR (CD_3OD, 400 MHz) δ 6.84 (1H, br.s. H-2), 6.80 (1H, br.s. H-2′), 6.77 (1H, d, J = 8.3 Hz, H-6), 6.75 (1H, d, 8.3 Hz, H-6′), 6.70 (1H, m, H-5′), 6.68 (1H, br.s. H-5), 4.73 (1H, d, J = 7.0 Hz, H-7′), 3.99 (1H, m, H-9α), 3.86 (3H, s, OCH_3), 3.84 (1H, m, H-9′a), 3.75 (1H, m, H-9β), 3.68 (1H, m, H-9′b), 2.95 (1H, dd, J = 13.5, 4.6 Hz, H-7α), 2.73 (1H, d, 6.1 Hz, H-8), 2.50 (1H, t, J = 12.4 Hz, H-7*β*), 2.41 (1H, d, J = 6.8 Hz, H-8′); HREIMS showed $[M]^+$ at *m/z* 346.1367 (calcd. for $C_{19}H_{22}O_6$, 346.1416). The physical and spectral data coincided with the literature values [19].

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