Isolation studies on *Cotoneaster racemiflora*

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Summary: Seven compounds have been isolated for the first time from *Cotoneaster racemiflora* namely, β-sitosterol (**1**), scopoletin (**2**), 7,8-dimethoxy-6-hydroxycoumarin (**3**), ursolic acid (**4**), cereotagaloperoxide (**5**), 3,3′,4′-tri-*O*-methylellagic acid (**6**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**7**), respectively. Their structures have been elucidated by spectroscopic techniques.

Introduction

The genus *Cotoneaster* belongs to family Roseaceae which comprises 85 species, occurring mainly in Iran, Syria, Turkey and Europe. It is represented in Pakistan by 9 species [1]. *Cotoneaster racemiflora* Desf. occurs in Western Himalayas from Kashmir to Kuman. It is widely distributed from Siberia and Cabul to France. In Pakistan it is very common in Baluchistan, Landi Kotal, Chitral, Swat, Kaghan and Kashmir. The plant has a bitter and sharp taste and used medicinally in Indo-China. Various species of the genus *Cotoneaster* are used as cooling, laxative, aperient, astringent, expectorant and stomachic effect and are also used in the diseases of the eye, bronchitis, abdominal pain, stangury, thirst, itch, leucoderma, fevers, wounds, piles and in urinary calculi [2-4]. Literature survey revealed that no phytochemical work has so for been carried out on *C. racemiflora*. The diverse medicinal applications attributed to genus *Cotoneaster* prompted us to carry out phytochemical investigations on this plant. Herein we report the isolation and structure elucidation of β-sitosterol (**1**), scopoletin (**2**), 7,8-dimethoxy-6-hydroxycoumarin (**3**), ursolic acid (**4**), cereotagaloperoxide (**5**), 3,3′,4′-tri-*O*-methylellagic acid (**6**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**7**), respectively, from the ethylacetate fraction.

Results and Discussion

The methanolic extract of the whole plant showed strong toxicity in brine shrimp lethality test. It was subsequently divided into *n*-hexane, ethylacetate, *n*-butanol and water-soluble fractions. Out of these the ethylacetate soluble fraction showed strong toxicity in brine shrimp lethality test. This fraction was, therefore, subjected to a series of column and flash chromatographic techniques as described in the experimental to obtain seven compounds reported for the first time from this species. These could be identified as β-sitosterol (**1**), scopoletin (**2**), 7,8-dimethoxy-6-hydroxy-coumarin (**3**), ursolic acid (**4**), cereotagaloperoxide (**5**), 3,3′,4′-tri-*O*-methylellagic acid (**6**) and β-sitosterol 3-*O*-β-D-glucopyranoside (**7**) on the basis of their respective spectral data. All these compounds individually showed lower level of toxicity compared to the crude ethylacetate extract thereby indicating that the strong toxicity of the latter is due to combined effect of various constituents present therein.

Experimental

General

Column chromatography was carried out using silica gel of (70-230) and flash chromatography on silica gel (230-400) mesh. Aluminium sheets precoated with silica gel 60 F_{254} (20×20 cm, 0.2 mm thick; E-Merck) were used for TLC to check the purity of the compounds and were visualized under UV light (254 and 366 nm) followed by ceric sulfate as spraying reagent. Optical rotations were measured on a Jasco DIP-360 digital polarimeter. The UV spectra were recorded on a Hitachi UV-3200 spectrometer (λ_{max} in nm). IR spectra were recorded on Shimadzu IR-460 spectrophotometer (ν in cm^{-1}).

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EI-MS, HR-EI-MS, FAB-MS and HR-FAB-MS spectra were recorded on Jeol JMS-HX 110 spectrometer with data system. The ^{1}H-NMR spectra were recorded on Bruker AMX-400 MHz instruments using TMS as an internal reference. The chemical shift values are reported in ppm (δ) units and the scalar coupling constants (J) are in Hz.

Plant Material

The whole plant of *Cotoneaster racemiflora* Desf. was collected from Swat (Pakistan) in July 2004 and identified by Prof. Dr. Jahndar Shah, Plant Taxonomist, Islamia College Peshawar, University of Peshawar, where a voucher specimen (CR-119-04) is deposited.

Isolation

The shade dried whole plant of *C. racemiflora* (15 kg) was extracted with MeOH (3 × 50L) at room temperature. The combined methanolic extract was evaporated under reduced pressure to obtain a thick gummy residue (500 g). It was suspended in water and successively extracted with *n*-hexane, ethylacetate and *n*-butanol. The ethylacetate soluble fraction (40 g) was subjected to column chromatography over silica gel eluting with *n*-hexane, *n*-hexane-ethylacetate, ethylacetate, ethylacetate-methanol and methanol in increasing order of polarity. The fractions obtained from *n*-hexane-ethylacetate (8.5:1.5) were combined and chromatographed over silica gel using *n*-hexane-ethylacetate (8.4: 1.6) to obtain β-sitosterol (**1**). The fractions obtained from *n*-hexane-ethylacetate (8: 2) gave two major spots on TLC, which on final purification by flash chromatography with the solvent system *n*-hexane-ethylacetate (7.8: 2.2) afforded scopoletin (**2**) from the top fractions and 7, 8-dimethoxy-6-hydroxycoumarin (**3**) from the tail fractions, respectively. The fractions obtained from *n*-hexane-ethylacetate (7: 3) were combined and rechromatographed over silica gel using the same solvent system to obtain ursolic acid (**4**). The fractions obtained from *n*-hexane-ethylacetate (6: 4) gave two spots on TLC, which on final purification by flash chromatography with the solvent system *n*-hexane-ethylacetate (5.7:4.3) afforded cereotagaloperoxide (**5**) from the top fractions and 3,3′,4′-tri-*O*-methylellagic acid (**6**) from the tail fractions, respectively. The fractions obtained from *n*-hexane-ethylacetate (2: 8) were combined and rechromatographed over silica gel using *n*-hexane-ethylacetate (1.7: 8.3) as eluent to obtain β-sitosterol 3-*O*-β-D-glucopyranoside (**7**).

β-Sitosterol (1)

Crystallized from acetone (24 mg), m.p. 136–137°C; $[\alpha]_D^{28}$ – 35° (*c* 0.002, $CHCl_3$); 1R (KBr) ν_{max} cm^{-1}: 3446, 3050, 1650; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.23 (1H, m, H-6), 3.32 (lH, m, H-3), 1.01 (3H, s, Me-19), 0.92 (3H, d, J = 6.2 Hz, Me-21), 0.84 (3H, t, J = 7.0 Hz, Me-29), 0.83 (3H, d, J = 6.5 Hz, Me-26), 0.81 (3H, d, J = 6.5 Hz, Me-27), 0.68 (3H, s, Me-18); HR-EI-MS, *m/z:* 414.3861 (calcd. for $C_{29}H_{50}O$, 414.3849). The physical and spectral data showed complete agreement with those reported in the literature [5-7].

Scopoletin (2)

Crystalline solid (21 mg), m.p. 202–204 °C; UV (MeOH): λ_{max} (log ε) nm = 227 (3.6), 252 (4.0), 298 (2.6), 341 (4.3); IR ($CHCl_3$) ν_{max} cm^{-1}: 3333, 1701, 1625, 1584, 1100; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.57 (1H, d, J = 9.6 Hz, H-4), 6.89 (1H, s, H-8), 6.82 (1H, s, H-5), 6.24 (1H, d, J = 9.6 Hz, H-3), 3.92 (3H, s, OCH_3-6); HR-EI-MS showed $[M]^+$ at *m/z:* 192.0416 (calcd. for $C_{10}H_8O_4$, 192.0422). The physical and spectral data showed complete agreement with the literature [8].

7,8-Dimethoxy-6-hydroxycoumarin (3)

Pale yellow needles (18 mg), m.p. 145–146°C; UV (MeOH) λ_{max} (log ε) nm: 290 (4.5), 330 (4.04), 383 (3.45); IR (KBr) ν_{max} cm^{-1}: 3348, 1690, 1641, 1546; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.93 (1H, dd, J = 9.6, 1.4 Hz, H-4), 6.71 (1H, d, J = 1.4 Hz, H-5), 6.24 (1H, d, J = 9.6 Hz, H-3), 4.00 (3H, s, OCH_3-8), 3.94 (3H, s, OCH_3-7), HR-EI-MS showed $[M]^+$ at *m/z:* 222.0535 (calcd. for $C_{11}H_{10}O_5$, 222.0514). The physical and spectral data corresponded to the reported values [9].

Ursolic acid (4)

Amorphous powder (25 mg), m.p. 291–292°C, $[\alpha]_D^{28}$ + 66.2° (*c* 0.01, $CHCl_3$); IR (KBr) ν_{max} cm^{-1}: 3510, 3050, 1697, 1635, 820; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.11 (1H, m, H-12), 3.19 (1H, dd, $J_{ax,ax}$ = 10.0 Hz, $J_{ax,eq}$ = 4.5 Hz, H-3α), 1.20 (3H, s, Me-27), 1.07 (3H, s, Me-23), 0.94 (3H, s, Me-25), 0.91 (3H, d, J = 6.6 Hz Me-30), 0.86 (3H, s, Me-26), 0.81 (3H, s, Me-24), 0.80 (3H, d, J = 6.8 Hz, Me-29);

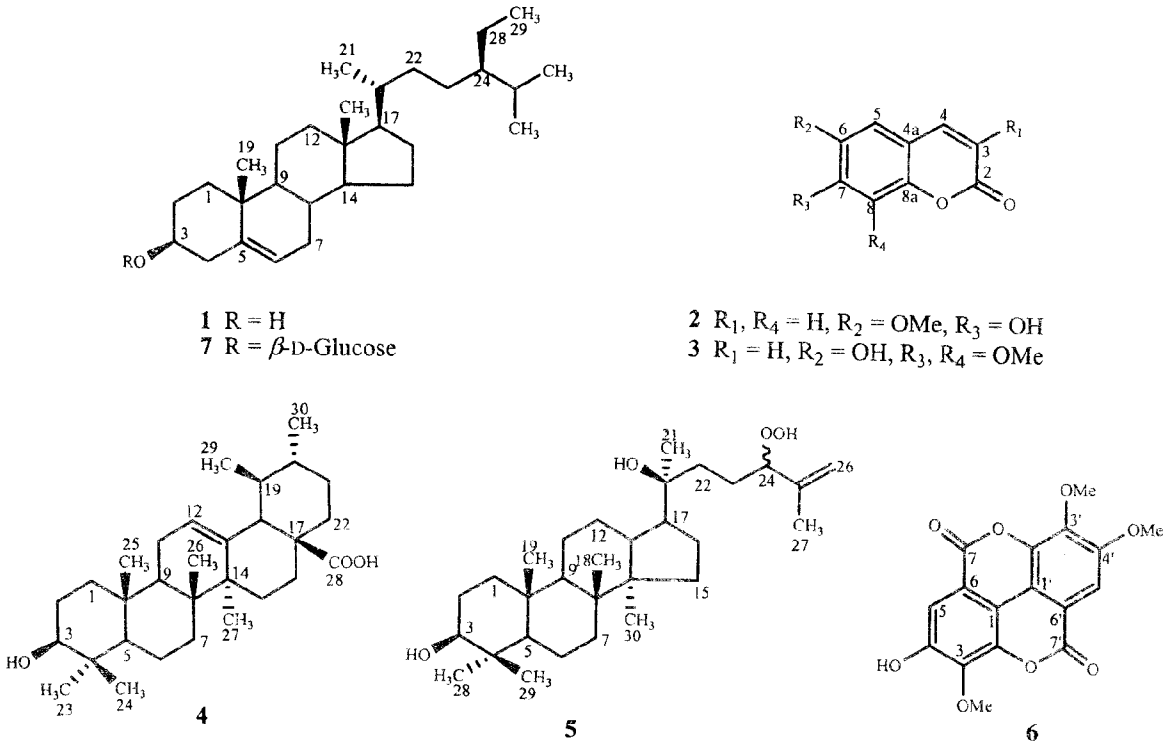

Fig. 1: Structure of compounds isolated from *C. racemiflora*

HR-EI-MS, *m/z:* 456.3603 (calcd. for $C_{30}H_{48}O_3$, 456.3595). The physical and spectral data showed complete resemblance with those reported in the literature [10, 11].

Cereotagaloperoxide (5)

Amorphous powder (14 mg), m.p. 183-185°C, $[\alpha]_D^{28}$ + 54.1° (c 0.04, MeOH); IR (KBr) ν_{max} cm^{-1}: 3450, 1655, 1460, 1375. ^{1}H-NMR ($CDCl_3$, 400 MHz)δ: 5.02 (2H, m, H-26), 4.31 (1H, t, J = 6.3 Hz, H-24), 3.20 (1H, dd, J = 11.1, 5.7 Hz, H-3), 1.75 (3H, s, Me-27), 1.72 (1H, m, H-17), 1.63 (1H, m, H-13), 1.33 (1H, m, H-9), 1.13 (3H, s, Me-21), 0.73 (1H, m, H-5), 0.97 (3H, s, Me-28), 0.96 (3H, s, Me-18), 0.87 (3H, s, Me-30), 0.85 (3H, s, Me-19), 0.78 (3H, s, Me-29), HR-EI-MS, *m/z*: 476.3860 $[M]^+$ (calcd. for $C_{30}H_{52}O_4$, 476.3865). The physical and spectral data showed complete agreement with the reported values [12].

3,3′,4′-Tri-O-methylellagic acid (6)

Crystallized in methanol (19 mg), m.p. 290°C, UV (MeOH) λ_{max} (log ε) nm: 246 (4.42), 373 (3.92); IR (KBr) ν_{max} cm^{-1}: 3500, 1755, 1730, 1610, 1350, 1320, 1285. ^{1}H-NMR (d_5-pyridine, 400 MHz) *δ*: 7.85 (1H, s, H-5′), 7.62 (1H, s, H-5), 5.45 (1H, s, OH-4), 3.95 (3H, s, OMe-3), 3.89 (3H, s, OMe-3′), 3.62 (3H, s, OMe-4′); HR-EI-MS, *m/z:* 344.2569 $[M]^+$ (calcd. for $C_{17}H_{12}O_8$, 344.2574). The physical and spectral data were in agreement with those reported in literature [13].

β-Sitosterol 3-O-β-D-glucopyranoside (7)

Obtained as a colorless amorphous powder (18 mg), m.p. 232-235°C, $[\alpha]_D^{28}$ – 45° (*c* 0.023, MeOH); IR (KBr) ν_{max} cm^{-1}: 3452, 3044, 1648. ^{1}H-NMR ($CDCl_3$+CD_3OD, 400 MHz) *δ*: 5.13 (1H, m, H-6), 4.57 (1H, d, *J* = 7.3 Hz, H-1'), 3.85 (1H, m, H-3), 3.24-3.80 (m, Glc-H), 1.01 (3H, s, Me-19), 0.92 (3H, d, *J* = 6.1 Hz, Me-21), 0.83 (3H, t, *J* = 7.0 Hz, Me-29), 0.83 (3H, d, *J* = 6.4 Hz, Me-26), 0.81 (3H, d, *J* = 6.4 Hz, Me-27) and 0.68 (3H, s, Me-18); HR-FAB-MS, *m/z:* 577.4444 $[M+H]^+$ (calcd. for $C_{35}H_{61}O_6$, 577.4438). The physical and spectral data were in agreement with those reported in the literature [5-7].

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