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Phytochemical Studies on the Underground Parts of *Asperula taurina* subsp. *caucasica*

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One naphthohydroquinone (mollugin) (**1**), 3 anthraquinones (1-hydroxy-2-methyl-9,10-anthraqinone (**2**), 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone (**4**) and 1,3-dihydroxy-2-carboxy-9,10-anthraquinone (**7,** munjistin)), β-sitosterol (**3**), 1 naphthalene glycoside (2-carbomethoxy-3-prenyl-1,4-naphthohydroquinone, 1,4-di-O-β-glucoside (**5**)) and 1 anthraquinone glycoside (lucidin-3-O-β-primeveroside (**6**)) were isolated from the underground parts of *A. taurina* subsp. *caucasica*. The structures of the isolates were established by MS, ^{1}H-NMR and ^{13}C-NMR analysis.

Key Words: *Asperula taurina* subsp. *caucasica,* Rubiaceae, anthraquinone, anthraquinone glycoside, naphthohydroquinone, naphthalene glycoside.

Introduction

The family Rubiaceae is represented by about 500 genera and 6000 species, most of them tropical trees and shrubs[1]. Some species belonging to this family contain quinonic compounds (anthraquinones, naphthoquinones, naphthohydroquinones and their glycosides)[2−5], iridoids[6], coumarins[7], triterpenes[8] and flavonoids[9]. The subterranean parts of some genera belonging to Rubiaceae are rich in quinonic compounds. *Rubia, Galium, Asperula* and *Morinda* species contain quinonic compounds[2−5]. Some 9,10-anthraquinones and their glycosides were isolated from the underground parts of *Asperula odorata* and *A. besseriana*[2,10].

The genus *Asperula* has about 200 known species[1]. This genus has 39 species in Turkey, and 26 taxa belonging to these species are endemic. *Asperula taurina* subsp. *caucasica* grows in northeast Turkey[11]. A survey of the literature revealed that there have been no phytochemical studies dealing with *A. taurina*. We herein report the isolation and characterization of some different structural compounds from *A. taurina* subsp. *caucasica*.

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Experimental

General: NMR spectra were recorded on a Varian Mercury 400 MHz NMR spectrometer and 270.05 (67.80) JEOL NMR spectrometer. EI-MS spectra were recorded on a Thermo-Finnigan and JEOL JMS D300 mass spectrometer. Column chromatography was performed on silica gel 60 (0.063-0.200 μ, Merck), RP-18 (LiChroprep®, 25-40 μ, Merck) and Sephadex LH-20 (Sigma-Aldrich). Preparative TLC was performed with silica gel F_{254} plates (20 x 20 cm, 0.5 mm, Merck).

Plant Material: The underground parts (roots and rhizomes) of *A. taurina* L. subsp. *caucasica* (Pobed.) Ehrend. (Syn.: *A. caucasica* Pobed.) were collected from Ormanüstü village (625 m) (Maçka district, Trabzon province, Turkey) in August 2000. It was identified by Dr. Ufuk Özgen. A voucher specimen (AEF 19791) is deposited at the Ankara Üniversitesi Eczacılık Fakültesi Herbaryumu (AEF).

Extraction and Isolation: The air-dried and powdered underground parts (roots and rhizomes) (700 g) of *A. taurina* subsp. *caucasica* were extracted with methanol (3000 mL x 3) under reflux for 3 h for each extraction at 40 °C. The combined methanolic extracts were evaporated to dryness (73 g, yield 10.4%) under reduced pressure at 40 °C. Methanol extract was suspended with 300 mL of water:methanol (9:1). This mixture was partitioned against chloroform (300 mL x 3). Chloroform fractions were combined and evaporated at reduced pressure at 40 °C. The chloroform extract was 14 g. The aqueous fraction was evaporated to give a residue (59 g).

The chloroform fraction (12 g) was subjected to silica gel column chromatography. Elution was performed with an n-hexane-ethyl acetate mixture with gradient elution. Similar fractions determined by TLC were combined. Mollugin (**1**, 300 mg), 1-hydroxy-2-methyl-9,10-anthraquinone (**2**, 10 mg), β-Sitosterol (**3**, 50 mg), and 1,3-dihydroxy-2-methoxymethyl-9,10-anthraquinone (**4**, 15 mg) were obtained. Column chromatography, preparative TLC and recrystallization were used to obtain pure compounds.

The aqueous extract (25 g) was subjected to a Sephadex LH-20 column. Elution was performed with methanol. Six fractions were collected. A white powder was obtained from the third fraction (800 mg). It was subjected to a silica gel column ($CHCl_3$:MeOH:water 70:30:3, v/v/v) and then an RP-18 silica gel column (MeOH:H_2O, 1:1, v/v). 2-Carbomethoxy-3-prenyl-1,4-naphtho-hydroquinone, 1,4-di-O-β-glucoside (**5**, 20 mg) was obtained. Fraction 4 (600 mg) gave a yellow powder. It was purified using water on a Sephadex column and lucidin-3-O-β-primeveroside was obtained (**6**, 20 mg). Fraction 5 (100 mg) was subjected to a Sephadex column using MeOH and 1,3-dihydroxy-2-carboxy-9,10-anthraquinone (**7,** munjistin) (8 mg) was obtained.

Mollugin (6-hydroxy-2,2-dimethyl-2H-naphtho[1,2-b]pyran-5-carboxylic acid methyl ester) (1): Yellow crystal; **EI-MS (m/e)** 284 [M^+] (33%), 269 (21%), 252 (39%), 237 (100%); **^{1}H-NMR** (270 MHz, $CDCl_3$): δ 12.16 (s, 1H, OH), 8.38 (bd, 1H, H-7 or H-10, J = 8.3 Hz), 8.18 (bd, 1H, H-7 or H-10, J = 8.3 Hz), 7.61 (ddd, 1H, H-8 or H-9, J = 8.3, J = 6.9, J = 1.3 Hz), 7.51 (ddd, 1H, H-8 or H-9, J = 8.3, J = 6.9, J = 1.3 Hz), 7.12 (d, 1H, H-4, J = 9.9 Hz), 5.68 (d, 1H, H-3, J = 9.9 Hz), 4.01 (s, 3H, OCH_3), 1.48 (s, 6H, 2xCH_3); **^{13}C-NMR** (67.8 MHz, $CDCl_3$): δ 172.5 (s), 156.5 (s), 141.6 (s), 129.4 (d), 129.1 (s), 128.8 (d), 126.5 (d), 125.1 (s), 124.0 (d), 122.3 (d), 121.9 (d), 112.6 (s), 102.2 (s), 74.6 (s), 52.3 (q), 26.8 (q). EI-MS, ^{1}H-NMR and ^{13}C-NMR data agree with the literature[12–14].

1-Hydroxy-2-methyl-9,10-anthraquinone (2): Yellow crystal; **EI-MS (m/e)** 238 [M^+] (100%), 209 (14%), 181 (22%), 152 (23%), 76 (12%); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 8.34-8.29 (m, 2H, H-5 and

H-8), 7.82-7.80 (m, 2H, H-6 and H-7), 7.77 (d, 1H, H-3 or H-4, $J = 7.7$ Hz), 7.55 (d, 1H, H-3 or H-4, $J = 7.7$ Hz), 2.39 (s, 3H, CH_3). EI-MS and ^{1}H-NMR are in good agreement with the data given in the literature[15].

β-Sitosterol (5-Stigmasten-3β-ol) (3): White crystal; **EI-MS (m/e)** 414 [M^+] (100%), 396 (54%), 381 (21%); **^{1}H-NMR** (270 MHz, $CDCl_3$) (selected data): δ 5.34 (m, 1H, H-6), 3.51 (m, 1H, H-3), 0.98 (s, 3H, Me-19), 0.90 (d, 3H, Me-21, $J = 6.0$ Hz), 0.87 (t, 3H, Me-29, $J = 5.6$ Hz), 0.86 (d, 3H, Me-26, $J = 5.6$ Hz), 0.84 (d, 3H, Me-27, $J = 6.6$ Hz), 0.65 (s, 3H, Me-18); **^{13}C-NMR** (67.8 MHz, $CDCl_3$): δ 140.8 (s), 121.7 (d), 71.8 (d), 56.8 (d), 56.0 (d), 50.1 (d), 45.8 (d), 42.3 (t), 42.3 (s), 39.8 (t), 37.2 (t), 36.5 (s), 36.1 (d), 33.9 (t), 31.9 (t), 31.9 (d), 31.7 (t), 29.1 (d), 28.2 (t), 26.1 (t), 24.3 (t), 23.0 (t), 21.1 (t), 19.8 (q), 19.4 (q), 19.0 (q), 18.8 (q), 12.0 (q), 11.9 (q). EI-MS, ^{1}H-NMR and ^{13}C-NMR data agree with the literature[16].

1,3-Dihydroxy-2-methoxymethyl-9,10-anthraquinone (4): Yellow crystal; **EI-MS (m/e)** 284 [M^+] (9%), 252 (100%), 196 (55%), 168 (45%), 139 (30%); **^{1}H-NMR** (400 MHz, $CDCl_3$): δ 13.30 (s, 1H, OH), 9.40 (s, 1H, OH), 8.26-8.30 (m, 2H, H-5 and H-8), 7.77-7.81 (m, 2H, H-6 and H-7), 7.30 (s, 1H, H-4), 4.94 (s, 2H, CH_2), 3.58 (s, 3H, OCH_3); **^{13}C-NMR** (100 MHz, $CDCl_3$): δ 187.2 (s) (C = O), 182.5 (s) (C = O), 164.3 (s), 162.1 (s), 134.4 (d, 2C), 134.3 (s), 133.8 (s), 133.7 (s), 127.6 (d), 127.0 (d), 114.4 (s), 110.0 (d), 110.0 (s), 69.2 (t, CH_2-O), 59.6 (q, OCH_3). EI-MS[17] data agree with the literature, ^{1}H-NMR and ^{13}C-NMR agree with the literature[18].

2-Carbomethoxy-3-prenyl-1,4-naphtho-hydroquinone, 1,4-di-O-β-glucoside (5): Colorless needles; **EI-MS (m/e)** 286.1 ([M^+] of aglycone +2) (50%), 254 (100%), 239 (14%), 198 (18%), 165 (6%), 105 (6%), 85 (7%), 73 (17%); **^{1}H-NMR** (400 MHz, CD_3OD): δ 8.55 (bd, 1H, H-5 or H-8, $J = 7.4$ Hz), 8.53 (bd, 1H, H-5 or H-8, $J = 7.7$ Hz), 7.57 (dt, 1H, H-6 or H-7, $J = 7.7$ Hz, $J = 1.1$ Hz), 7.52 (dt, 1H, H-6 or H-7, $J = 7.4$ Hz, $J = 1.1$ Hz), 5.14 (m, 1H, $CH = CMe_2$), 4.84 (m, 9H, overlapped 8xOH and an anomeric proton), 4.65 (d, 1H, anomeric H, $J = 7.7$ Hz), 3.85 (s, 3H, OCH_3), 3.58-3.82 (m, 6H, protons of sugars and $CH_2CH =$), 3.38-3.48 (m, 2H, protons of sugars), 3.26-3.34 (m, 2H, protons of sugars, 3.07-3.13 (m, 2H, protons of sugars), 1.73 (s, 3H, one of C = CMe_2), 1.68 (s, 3H, one of C = CMe_2); **^{13}C-NMR** (100 MHz, CD_3OD): δ 169.2 (C = O), 146.3 (s), 146.0 (s), 131.1 (s), 129.1 (s), 128.7 (s), 126.7 (s), 126.5 (d), 125.8 (s), 125.4 (d), 122.7 (d), 122.5 (d), 122.5 (d), 104.5 (d), 104.3 (d), 76.1 (d), 76.0 (d), 75.9 (d), 75.8 (d), 73.9 (d), 73.6 (d), 70.0 (d), 69.5 (d), 61.3 (t), 60.6 (t), 51.0 (OCH_3), 25.6 (CH_2), 23.9 (CH_3), 16.4 (CH_3) .

EI-MS fragmentation is good agreement with the data given the literature and ^{1}H-NMR is agreement with the data given in the literature[14].

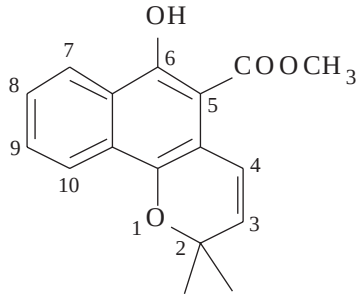
Lucidin-3-O-β-primeveroside (6): Yellow powder; **EI-MS (m/e)** 254 [M^+] (100%), 239 (28%), 207 (14%), 197 (8%), 152 (22%), 129 (28%), 115 (30%); **^{1}H-NMR** (400 MHz, DMSO-d_6): δ 8.25-8.23 (m, 1H, H-5 or H-8), 8.19-8.17 (m, 1H, H-5 or H-8), 7.94-7.92 (m, 2H, H-6 and H-7), 7.47 (s, 1H, H-4), 5.10 (d, 1H, H-1 gluc, $J = 6.6$ Hz), 4.64 (A part of AB system, d, 1H, one of CH_2OH, $J = 11.0$ Hz), 4.56 (B part of AB system, d, 1H, one of CH_2OH, $J = 11.0$ Hz), 4.13 (d, 1H, H-1 xylose, $J = 7.3$ Hz), 3.94 (d, 1H, sugar proton, $J = 9.5$ Hz), 3.72-3.58 (m, 3H, sugar protons), 3.40-3.25 (m, 4H, sugar protons), 3.01 (bt, 1H, sugar proton, $J = 7.0$ Hz), 2.99 (bt, 2H, sugar protons, $J = 10.6$ Hz); **^{13}C-NMR** (100 MHz, DMSO-d_6): δ 187.8 (s) (C = O), 182.2 (s) (C = O), 162.7 (s), 162.6 (s), 135.6 (d), 135.4 (d), 134.5 (s), 133.7 (s), 133.6 (s), 127.7 (d), 127.3 (d), 124.4 (s), 112.1 (s), 107.1 (d), 104.8 (d), 101.5 (d), 77.1 (d), 76.6 (d), 76.4 (d), 74.0 (d, 2C), 70.2 (d), 69.9 (d), 68.7 (t), 66.3 (t), 51.7 (t). ^{1}H-NMR and ^{13}C-NMR agree with the literature[19,20].

1,3-Dihydroxy-2-carboxy-9,10-anthraquinone (Munjistin) (7): Orange substance; **EI-MS (m/e)** 284 [M^+] (0.5%), 240 (44%), 239 (100%), 212 (15%), 184 (18%), 128 (16%), 77 (9%), 69 (12%); ^{1}**H-NMR** (400 MHz, D_2O): δ 7.68 (d, 1H, H-5 or H-8, J = 7.4 Hz), 7.60 (d, 1H, H-5 or H-8, J = 6.7 Hz), 7.49 (t, 1H, H-6 or H-7, J = 7.5 Hz), 7.40 (t, 1H, H-6 or H-7, J = 7.4 Hz), 6.44 (s, 1H, H-4). EI-MS fragmentation is in good agreement with the data given in the literature and ^{1}H-NMR is in agreement with the data given in the literature[15].

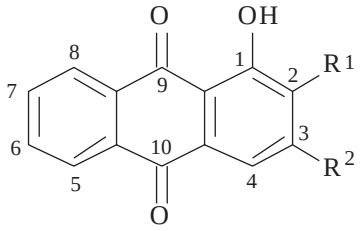
Results and Discussion

The underground parts of *A. taurina* subsp. *caucasica* were extracted with methanol. The extract was fractionated between chloroform and water. The chloroform fraction was subjected to a silica gel column, eluting with n-hexane-ethyl acetate by gradient elution. Similar fractions were collected and combined. As a result of repeated column chromatography and preparative TLC, 4 compounds (**1-4**) were purified. Using Sephadex LH-20, RP-18 and silica gel column chromatography, **5-7** were obtained from the aqueous fraction (Figure).

Characterization of compounds **1-7** was performed by extensive NMR studies plus EI-MS.



1; Mollugin

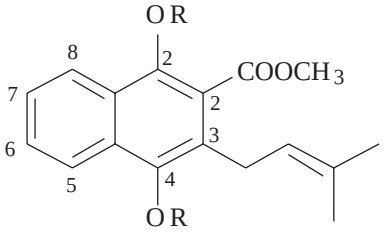


2 ; R^1 = CH_3, R^2 = H

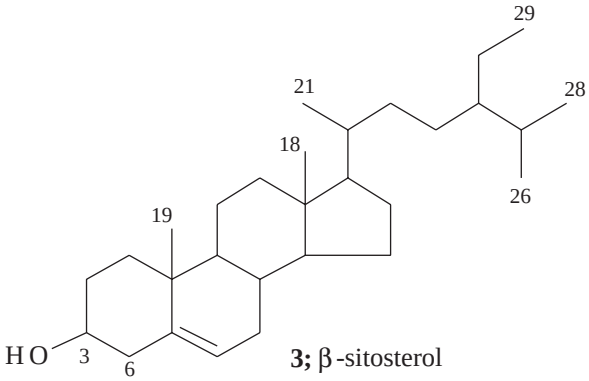
4 ; R^1 = CH_2OCH_3, R^2 = OH

6 ; R^1 = CH_2OH R^2 = O -β-primeveroside

7 ; R^1 = COOH R^2 = OH



5; R = β–D -glucoside



3; β -sitosterol

Figure

The EI-MS spectrum of mollugin **1** showed an M^+ ion peak (284) in regard to its structure. In the

^{1}H-NMR spectrum of mollugin **1**, signals of 2 methyls at C-2 arose at δ 1.48 as 1 singlet, and methoxymethyl at δ 4.01. Olefinic hydrogens were seen as a doublet of doublets at δ 5.68 and 7.12 (J = 9.9 Hz). The signals of 4 protons in the benzene ring were also in accordance with the structure. While H-7 and H-10 resonated as a doublet of doublets, H-8 and H-9 were seen as ddd. All data were in agreement with the data given in the literature[12,14].

As expected, a similarity was seen between the ^{1}H-NMR spectra of the aromatic hydrogens of compounds **4, 6** and **7**. The H-4's of these compounds were shown as singlets. While 4 protons (H-5, H-6, H-7, H-8) of **4** and **6** showed multiplicity in the aromatic area, the same protons of **7** were uncomplicated (H-5 and H-8 as doublets; H-6 and H-7 as triplets). This differentiation probably arises from the diversity of the functional group at C-2 of compound **7**. The signals observed at δ 4.94 and δ 3.58, with 2 and 3 proton intensities, respectively, were assigned to methylene and methyl protons of the methoxymethyl group. Characterization of the sugar moiety in molecule **6** was achieved by comparing with the literature[19]. Eleven carbon signals in the ^{13}C-NMR spectra of **6** belonging to the sugar moiety and chemical shifts and coupling constants measured in ^{1}H-NMR showed that the sugar moiety is primeveroside.

The ^{1}H-NMR spectrum of the aromatic hydrogens of compound **2** differs from those of compounds **4**, **6** and **7**, owing to an AB system made of H-3 and H-4. A methyl singlet of **2** arose at δ 2.39.

$\beta-$ Sitosterol **3** was primarily characterized by comparing its EI-MS spectrum with the data given in the literature. Its ^{1}H and ^{13}C-NMR spectroscopic data were in agreement with the data given in the literature[16].

Two sugar moieties, 1 prenyl group and 1 carboxymethyl group of compound **5** were easily determined from the ^{1}H and ^{13}C-NMR spectra. Signals belonging to 4 protons in the aromatic ring of **5** were also in accordance with the structure. While H-5 and H-8 resonated as broad doublets, H-6 and H-7 were seen as a doublet of triplets in accordance with the structure. An evaluation of the ^{1}H and ^{13}C-NMR spectra of the sugar moiety in compound **5** in comparison with the literature showed that this part should be glucose[15].

In conclusion, in this work we showed the isolation and characterization of 7 compounds from *Asperula taurina* subsp. *caucasica* for the first time.

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