Phytochemical Studies on the Underground Parts of

*Asperula taurina* subsp. *caucasica*

Ufuk ÖZGEN1*, Cavit KAZAZ2, Hasan SEÇEN2,  
Maksut COŞKUN3  

1Department of Pharmacognosy, Faculty of Pharmacy, Atatürk University,  
25240 Erzurum- TURKEY  
e-mail: uozgen@atauni.edu.tr  
2Department of Chemistry, Faculty of Arts and Science, Atatürk University,  
25240 Erzurum- TURKEY  
3Department of Pharmaceutical Botany, Faculty of Pharmacy, Ankara University,  
06100 Tandojian, Ankara-TURKEY

Received 10.03.2005

One naphthohydroquinone (mollugin) (1), 3 anthraquinones (1-hydroxy-2-methyl-9,10-anthraquinone (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-primeveroside (6)) were isolated from the underground parts of *A. taurina* subsp. *caucasica*. The structures of the isolates were established by MS, 1H-NMR and 13C-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. Some species belonging to this family contain quinonic compounds (anthraquinones, naphthohydroquinones, naphthalene glycosides and their glycosides), iridoids, coumarins, triterpenes and flavonoids. The subterranean parts of some genera belonging to Rubiaceae are rich in quinonic compounds. *Rubia, Galium, Asperula* and *Morinda* species contain quinonic compounds. Some 9,10-anthraquinones and their glycosides were isolated from the underground parts of *Asperula odorata* and *A. besserianna*.

The genus *Asperula* has about 200 known species. This genus has 39 species in Turkey, and 26 taxa belonging to these species are endemic. *Asperula taurina* subsp. *caucasica* grows in northeast Turkey. 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*.  

*Corresponding author
**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 Ormanü Stü 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 methanic 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 (CHCl₃:MeOH:water 70:30:3, v/v/v) and then an RP-18 silica gel column (MeOH:H₂O, 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-naphto[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%); ¹H-NMR (270 MHz, CDCl₃): 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, OCH₃), 1.48 (s, 6H, 2xCH₃); ¹³C-NMR (67.8 MHz, CDCl₃): δ 172.5 (s), 165.6 (s), 151.4 (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, ¹H-NMR and ¹³C-NMR data agree with the literature²⁻⁴.

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%); ¹H-NMR (400 MHz, CDCl₃): δ 8.34-8.29 (m, 2H, H-5 and
Phytochemical Studies on the Underground Parts of..., U. ÖZGEN, et al.,

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, CH₃). EI-MS and ¹H-NMR are in good agreement with the data given in the literature.

β-Sitosterol (5-Stigmaster-3β-ol) (3): White crystal; EI-MS (m/e) 414 [M⁺] (100%), 396 (54%), 381 (21%); ¹H-NMR (270 MHz, CDCl₃) (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); ¹³C-NMR (67.8 MHz, CDCl₃): 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 (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, ¹H-NMR and ¹³C-NMR data agree with the literature.

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%); ¹H-NMR (400 MHz, CDCl₃): δ 12.30 (s, 1H, OH), 9.40 (s, 1H, OH), 8.26-8.30 (m, 2H, H-5 and H-6), 7.77-7.81 (m, 2H, H-6 and H-7), 7.30 (s, 1H, H-4), 4.94 (s, 2H, CH₂), 3.58 (s, 3H, OCH₃); ¹³C-NMR (100 MHz, CDCl₃): 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, CH₂-O), 59.6 (q, OCH₃). EI-MS data agree with the literature, ¹H-NMR and ¹³C-NMR agree with the literature.

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%); ¹H-NMR (400 MHz, CD₃OD): 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 = CMe₂), 4.84 (m, 9H, overlapped 8xOH and an anomeric proton), 4.65 (d, 1H, anomeric H, J = 7.7 Hz), 3.85 (s, 3H, OCH₃), 3.58-3.82 (m, 6H, protons of sugars and CH₂CH = ), 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 = CMe₂), 1.68 (s, 3H, one of C = CMe₂); ¹³C-NMR (100 MHz, CD₃OD): 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), 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 (OCH₃), 25.6 (CH₂), 23.9 (CH₃), 16.4 (CH₃).

EI-MS fragmentation is good agreement with the data given the literature and ¹H-NMR is agreement with the data given in the literature.

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%); ¹H-NMR (400 MHz, DMSO-d₆): 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 CH₂OH, J = 11.0 Hz), 4.56 (B part of AB system, d, 1H, one of CH₂OH, 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); ¹³C-NMR (100 MHz, DMSO-d₆): 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). ¹H-NMR and ¹³C-NMR data agree with the literature.
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%); **¹H-NMR** (400 MHz, D₂O): 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 **¹H-NMR** is in agreement with the data given in the literature¹⁵.

**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.

![Chemical structures](image)

1; Mollugin

2 ; R¹ = CH₃, R² = H

4 ; R¹ = CH₂OH CH₃, R² = OH

6 ; R¹ = CH₂OH H R² = O·β-primeveroside

7 ; R¹ = COOH R² = OH

The EI-MS spectrum of mollugin 1 showed an M⁺ ion peak (284) in regard to its structure. In the
1H-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 1H-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 13C-NMR spectra of 6 belonging to the sugar moiety and chemical shifts and coupling constants measured in 1H-NMR showed that the sugar moiety is primeveroside.

The 1H-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.

β–Sitosterol 3 was primarily characterized by comparing its EI-MS spectrum with the data given in the literature. Its 1H and 13C-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 1H and 13C-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 1H and 13C-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.

Acknowledgment

We thank Professor İhsan Çalış for his sending the original NMR spectrum of lucidin-3-O-β-primeveroside for comparison. We especially thank Dr. Hamdullah Kilic for recording the EI-MS spectra of the compounds.

References

Phytochemical Studies on the Underground Parts of..., U. ÖZGEN, et al.,


