Chemistry of Natural Compounds, Vol. 46, No. 2, 2010

CHEMICAL STUDY OF *Lophanthus chinensis*

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UDC 582.949.2:677.46

The genus *Lophanthus* Adanson (Lamiaceae) contains 20 species [1]. The aerial part of *L. chinensis* Benth. (*pri yang ku*) is used in Tibetan medicine for diseases of the cardiovascular and central nervous systems that are comprised by the name "planetary diseases" [2]. Water-soluble polysaccharides from the aerial part were investigated earlier during a chemical study of *L. chinensis* [3]. Information on other classes of compounds in *L. chinensis* has not appeared. The goal of our work was to study the composition of extracted substances from the aerial part of *L. chinensis*.

The aerial part of *L. chinensis* was collected during flowering in August 2008 near Atsagat (Republic of Buryatia, Russia; sample No. L/L1589-08, located in the Herbarium of the Department of Biologically Active Compounds, IGEB, SB, RAS). The species was verified by Doctor of Pharmaceutical Sciences Professor T. A. Aseeva (IGEB, SB, RAS).

Dried and ground raw material (0.6 kg) was extracted with EtOH (70%, 8×, 1:20) at 80°C. The alcohol extract was concentrated to an aqueous residue that was extracted with hexane, $CHCl_3$, and EtOAc. This produced four fractions: hexane (10.05 g, 1.68% of air-dried mass); $CHCl_3$ (17.81 g, 2.97%); EtOAc (8.72 g, 1.45%); and H_2O (157.65 g, 26.17%). The hexane fraction (8 g) was chromatographed over a SiO_2 column (3.5 × 40 cm) using gradients of hexane:$CHCl_3$ (100:0→75:25) and $CHCl_3$:EtOH (100:0→80:20) to isolate **1** {35 mg, mp 138–140°C, $[\alpha]_D^{20}$ –50° (*c* 0.3, $CHCl_3$)} and **2** {12 mg, mp 309–310°C, $[\alpha]_D^{20}$ +79° (*c* 0.32, $CHCl_3$)}, which were identified as *β*-sitosterol and oleanolic acid, respectively [4]. Subfractions containing carotenoids afforded after HPTLC (petroleum ether:Me_2CO 7:3) **3** [3 mg; UV spectrum (hexane, λ_{max}, nm): 420, 446, 474], **4** [15 mg; UV spectrum (hexane, λ_{max}, nm): 424, 449, 476], **5** [27 mg; UV spectrum (hexane, λ_{max}, nm): 421, 445, 474], and **6** [2 mg; UV spectrum (EtOH, λ_{max}, nm): 425, 450, 475], which were characterized as *α*- and *β*-carotenes, lutein, and zeaxanthin [5].

The $CHCl_3$ fraction (15 g) was dissolved in MeOH (1:10) and left at 4°C for 30 h. An amorphous precipitate separated and was repeatedly recrystallized from $CHCl_3$:EtOH to afford **7** [157 mg, mp 290–292°C, $[\alpha]_D^{20}$ +62° (*c* 1.0, MeOH)], which was identified as ursolic acid [6]. Next the $CHCl_3$ fraction was chromatographed over a SiO_2 column (3 × 50 cm) using hexane:$CHCl_3$ (100:0→75:25) and $CHCl_3$:Me_2CO (100:0→0:100). Subfractions were also rechromatographed using TLC (petroleum ether:Et_2O:HCOOH 62:31:7) to afford **2** (22 mg), **7** (92 mg), **8** [11 mg, mp 202°C. UV spectrum (MeOH, λ_{max}, nm): 228, 255, 295, 343], **9** [13 mg, mp ~350°C. UV spectrum (MeOH, λ_{max}, nm): 265, 333; +$AlCl_3$ 278, 300, 368, 381; +$AlCl_3$/HCl 278, 300, 343, 380; +NaOMe 275, 325, 393], and **10** [19 mg, mp ~330°C. UV spectrum (MeOH, λ_{max}, nm): 252, 266, 290, 350; +$AlCl_3$ 272, 300sh, 326, 420; +$AlCl_3$/HCl 260, 275, 300, 355, 385; +NaOMe 266, 329, 400]. According to the results, **8**, **9**, and **10** were identified as scopoletin [7], apigenin, and luteolin [8].

The EtOAc fraction (8 g) was dissolved with heating in the minimum volume of EtOH (70%). Standing in the cold (4°C) produced a precipitate, HPLC analysis of which showed the presence of two dominant components in a 6.2:1 ratio. The precipitate was separated over polyamide (Woelm, 2 × 40 cm, eluent EtOH 0→95%) to afford **11** [125 mg, mp 239°C. UV spectrum (EtOH, λ_{max}, nm): 256, 267sh, 347; +$AlCl_3$ 275, 300sh, 330, 432; +$AlCl_3$/HCl 275, 297sh, 355, 389; +NaOMe 260, 301sh, 395; +NaOAc 260, 267sh, 365sh, 405; luteolin and glucose in the hydrolysate] and **12** [28 mg, mp 194°C. UV spectrum (EtOH, λ_{max}, nm): 255, 267, 348; +$AlCl_3$ 272, 295sh, 330sh, 429; +$AlCl_3$/HCl 275, 295, 360, 392; +NaOMe 267, 395; +NaOAc 260, 400; luteolin and glucose:rhamnose 1:1 in the hydrolysate]. The analyses characterized **11** and **12** as luteolin-7-glycoside [8] and luteolin-7-rutinoside (scolimoside) [9, 10]. The precipitate was removed. The EtOAc fraction was chromatographed over a SiO_2 column (3.5 × 30 cm) with elution by $CHCl_3$:Me_2CO (100:0→80:20) and Me_2CO:EtOH (95%) (100:0→70:30). The resulting fractions were rechromatographed over polyamide [2 × 40 cm, EtOH eluent (0→95%)]

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0009-3130/10/4602-0301

and by preparative PC [AcOH (15%) and BuOH:AcOH 3:1] to afford **11** (110 mg), **12** (52 mg), **13** [53 mg, mp 220°C. UV spectrum (EtOH, λ_{max}, nm): 247, 300, 328] (caffeic acid), **14** [22 mg, $[\alpha]_D^{20}$ +97° (*c* 0.5, EtOH). UV spectrum (EtOH, λ_{max}, nm): 210, 217, 330] (rosmaric acid methyl ester), and **15** [427 mg, $[\alpha]_D^{20}$ +76° (*c* 0.5, EtOH). UV spectrum (EtOH, λ_{max}, nm): 321, 330] (rosmaric acid) [11]. HPTLC (EtOAc:MeOH:H_2O 9.6:1.9:1, *p*-dimethylaminobenzaldehyde/HCl developer) of the EtOAc fraction detected iridoid compound, the chromatographic behavior of which coincided with that of 8-*O*-acetylharpagide.

Considering the pigmentation of *L. chinensis* flowers, we studied the anthocyans, for which the flowers (20 g) were extracted three times with HCl (1%) in EtOH (95%) (1:20 solid:liquid). The extract was concentrated in vacuo to dryness at 40°C. The dry solid was dissolved in MeOH (10 mL). The solution was treated with Et_2O (1:30) and held at 0°C for 1 h. The resulting precipitate was separated and washed with Et_2O. Total anthocyans were separated by preparative TLC (BuOH:AcOH:H_2O 5:1:2) to afford **16** [11 mg; UV spectrum (MeOH, λ_{max}, nm): 278, 525; +HCl 280, 542; +$AlCl_3$ 545. E_{440}/E_{max}: MeOH 28%; +HCl 18%]. Hydrolysis of **16** established that it contained delphinidine [UV spectrum (HCl/MeOH, λ_{max}, nm): 277, 547; +$AlCl_3$ 566. E_{440}/E_{max} (HCl/MeOH): 18%] and glucose in a 1:1 ratio. The spectral and chromatographic analyses showed that the isolated compound was delphinidine-3-glucoside [12]. The total anthocyan content in *L. chinensis* flowers was 0.14% [13].

HPTLC was performed on PTSKh-AF-V plates (Sorbfil, Imid Ltd.); preparative PC, on FN-8 paper (Filtrak). Flavonoids were hydrolyzed by placing a MeOH solution (2 μL, 1%) at the origin of an HPTLC plate, storing it in HCl vapor (6 M) at 60°C for 30 min, and chromatographing using *i*-PrOH:$CHCl_3$:H_2O (7:4:1, double elution to 4 and 8 cm, *p*-diphenylamine:H_3PO_4 developer, carbohydrates) and toluene:EtOAc:HCOOH (5:4:1, 2% H_3BO_3 developer; flavonoids). Spectrophotometric studies were performed on UV-Vis mini spectrophotometers (Shimadzu) in 10-mm quartz cuvettes. Optical rotation was determined on an SM-3 polarimeter (Zagorsk Optico-Mechanical Plant) in a 1-dm cuvette at 20°C. HPLC was carried out in a Summit (Dionex) liquid chromatograph with a Prodigy column (5 μm, ODS 3, 250 × 4.6 mm, Phenomenex) with elution by a gradient of A (0.1% aqueous TFA) and B (CH_3CN) with a UVD 170S UV detector at λ 254 and 280 nm.

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