Chemistry of Natural Compounds, Vol. 46, No. 3, 2010

ALKALOIDS FROM *Haplophyllum leptomerum*

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UDC 547.944/945

The plant *Haplophyllum leptomerum* Lincz. et Vved. (Rutaceae) is indigenous to Uzbekistan and produces alkaloids [1a], flavonoids, and coumarins [2].

The air-dried aerial part (1 kg) of this plant that was collected in Babataga Mountains on May 20, 2008, during budding was extracted with MeOH. The dry MeOH extract was distributed between aqueous H_2SO_4 (10%) and $CHCl_3$. A mixture of bases (1 g, 0.1% of the dry raw material mass) was obtained as usual from the acidic solution and was separated over a column of silica gel (KSK, 1:60) using gradient elution by hydrocarbons:EtOAc. The hydrocarbon:EtOAc effluents (1:4) afforded successively the known alkaloids skimmianine (**1**, 55 mg) [1b], γ-fagarine (**2**, 17 mg) [1c], *N*-methyl-2-phenylquinolin-4-one (**3**, 23 mg) [1d]; the EtOAc ones, leptomerine (**4**, 15 mg) [1e], acutine (**5**, 12 mg) [1f], and 2-heptylquinolin-4-one (**6**, 13 mg).

Alkaloids **1**–**5** were identified using TLC and mixed samples with authentic compounds that were obtained previously from this species (**1**–**4**) [1a] and *Haplophyllum acutifolium* (**5**) [1f].

Compound **6** was identified from its PMR spectrum (400 MHz, $CDCl_3$, δ, ppm, J/Hz, 0 = HMDSO): 0.72 (3H, t, ^3J = 7.02, CH_3), 1.14 (8H, m, 4 × CH_2), 1.72 (2H, m, CH_2), 2.90 (2H, t, ^3J = 7.78, Ar-CH_2), 6.74 (1H, s, H-3), 7.43 and 7.64 (1H each, m, H-6, 7), 8.25 (1H, d, ^3J = 9.63, H-8), 8.33 (1H, dd, ^3J = 8.28, ^4J = 1.50, H-5), which indicated that **6** was 2-heptylquinolin-4-one, which was isolated previously from *Pseudomonas* microorganisms [3, 4] and synthesized from acutine [4]. Direct comparison of **6** with a synthetic sample (TLC, mixed sample) showed that they were identical.

In addition to the aerial part, we collected roots of *H. leptomerum*, which had not been previously studied. The total alkaloid fraction from the roots (67 g) was obtained by the aforementioned method (0.17 g, 0.25% of dry raw material mass) and separated over a column of silica gel 60 (0.063–0.100 mm, Merck) at a mixture:adsorbent ratio of 1:100 using $CHCl_3$ elution. The known alkaloids dictamnine (**7**, 7 mg) [1g] and **2** (10 mg) were isolated successively from the effluents and were identified using TLC and PMR spectra.

PMR spectrum of **2** (400 MHz, $CDCl_3$, δ, ppm, J/Hz, 0 = HMDSO): 4.02 (3H, s, OCH_3), 4.39 (3H, s, OCH_3), 6.88 (1H, dd, ^3J = 7.68, ^4J = 1.16, H-7), 7.02 (1H, d, ^3J = 2.84, H-3), 7.30 (1H, dd, $^3J_1$ = 8.63, $^3J_2$ = 7.68, H-6), 7.58 (1H, d, ^3J = 2.84, H-2), 7.78 (1H, dd, ^3J = 8.63, ^4J = 1.16, H-5).

PMR spectrum of **7** (400 MHz, DMSO-d_6, δ, ppm, J/Hz, 0 = HMDSO): 4.41 (3H, s, CH_3O-4), 7.45 (1H, d, ^3J = 3.75, H-3), 7.46 and 7.64 (1H each, ddd, $^3J_1$ = 8.48, $^3J_2$ = 6.74, ^4J = 1.50, H-6, 7), 7.84 (1H, dm, ^3J = 8.48, H-8), 8.01 (1H, d, ^3J = 3.75, H-2), 8.18 (1H, ddd, ^3J = 8.48, ^4J = 1.50, ^5J = 0.62, H-5).

Thus, the aerial part of *H. leptomerum* afforded six (**1**-**6**) alkaloids; the roots, two (**2**, **7**). Acutine (**5**), 2-heptylquinolin-4-one (**6**), and dictamnine (**7**) were observed for the first time in this plant.

Herein we report also results from a study of the anticancer properties of dictamnine (**7**) on two human cancer-cell lines HeLa and HCT-116 that was conducted according to an *in vitro* method [5]. The compound was dissolved in DMSO. Six different concentrations (1–100 μM) were used for the test. Cytotoxicity was evaluated using WST-1 reagent and ELISA (450 nm). Dictamnine exhibited moderate cytotoxicity against both cancer-cell lines (Table 1).

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 0009-3130/10/4603-0502

TABLE 1. Anticancer Activity* of Dictamnine (**7**), γ-Fagarine (**2**)**, and Skimmianine (**1**)**

Compound	IC_{50}, μM	
	HeLa cells	HCT-116 cells
7	65.0	85.0
2	34.9	>100
1	11.55	>100
Colchicine	1.1	1.3

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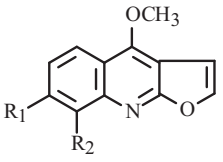
**in vitro* experiments on human HeLa and HCT-116 cancer cells. **Published data [5] are given for comparison.



1, 2, 7

1: $R_1 = R_2 = OCH_3$
2: $R_1 = H$, $R_2 = OCH_3$
7: $R_1 = R_2 = H$

A comparison of the results with those obtained by us earlier [5] led to conclusions about the structure–anticancer activity relationship of furanoquinoline alkaloids **1**, **2**, and **7** (Table 1). Increasing the number of methoxyls in the molecule (**7** → **2** → **1**) increased the cytotoxicity of the compound against HeLa cancer cells whereas the inverse relationship was observed for HCT-116 cells. The least methoxylated dictamnine (**7**) exhibited moderate cytotoxicity whereas **1** and **2** were weakly toxic.

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