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ALKALOIDS OF *Phellodendrone lavallei* INTRODUCED TO WESTERN GEORGIA

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Medicinal species of cork trees *Phellodendron amurense* Rupr., *P. chinease* Sohneid, *P. wilsonii* Hayate et Kemachica, and *P. amurense* Rupr. var. *sachalinense* Fr. (Rutaceae) are distributed in the Far East, China, and Korea. They all are productive sources of the protoberberine alkaloids berberine, palmatine, jatrorrhizine, and phellodendrine. Cork phloem, leaves, and fruit are used for medicinal purposes in Eastern medicine. Decoctions and tinctures of cork trees exhibit antimicrobial, analgesic, anti-inflammatory, antidiabetic, and other activities [1–6].

The Lavalle cork species that was introduced to Western Georgia is insufficiently studied. The content of berberine in it has been reported [7].

Pharmacological *in vitro* tests of the dry aqueous extract of bark from this plant for anti-inflammatory, antimicrobial, and cytotoxic activity at the Basic Research Department of Chicoutimi University (Canada) showed pronounced anti-inflammatory activity.

Pharmacological *in vivo* tests at the Pharmacology Department of Tbilisi State Medical University found hypoglycemic activity for the dry aqueous extract of Lavalle cork. This prompted our studies of the alkaloid composition in Lavalle cork bark collected in 2008-2009 on the shores of the Adzhari Black Sea.

The aqueous extract of cork bark was studied. Purification of the extract from polypeptides, resins, and water-soluble polysaccharides; distribution according to solubility between butanol and the aqueous phase; and separation over a column of silica gel isolated the hydrochlorides and iodide of four constituents (**1**–**4**), of which **1** and **4** dominated and **2** and **3** were minor compounds.

Analysis of DEPT, PMR, and ^{13}C NMR spectral data of **1** and **2** (Table 1) in addition to the TLC mobility, the lack of melting point depression of samples of these alkaloids and standard berberine and jatrorrhizine samples, and a comparison with the literature enabled **1** and **2** to be identified as berberine and jatrorrhizine, respectively [5, 8, 9, 10].

Mass spectrum of **3** (EI, 12 eV, *m*/*z*): 387.46, $C_{21}H_{22}O_4N^+Cl^-$, 351 $[M - HCl]^+$, 337 (100), 353, 339, 365, 367; (70 eV, *m*/*z*): 351 $[M - HCl]^+$, 337 (100), 322, 320, 308, 294, 282.5, 278, 270, 268, 265, 250, 236, 220, 206, 191, 168.5. ^{13}C NMR spectrum (75 MHz, CCl_4, DMSO-d_6): C-1 (108.5), C-2 (147.1), C-3 (147.1), C-4 (111.3), C-4a (132.6), C-5 (28), C-6 (57.7), C-8 (145.6), C-8a (120.0), C-9 (152.6), C-10 (145.0), C-11 (122.0), C-12 (123.5), C-12a (128.4), C-13 (119.8), C-13a (137.9), C-13b (123.0).

Compound **3** was identified as palmatine [5, 8].

Mass spectrum of **4** (EI, 70 eV, *m*/*z*): 468.9, $C_{20}H_{24}O_4N^+ I^-$, 341 $[M - HI]^+$, 327, 270, 268, 254, 186.5, 173, 142, 138, 57.4 (100), 54.3, 42, 30, 18, 15; (12 eV, *m*/*z*): 341 $[M - HI]^+$, 327, 270, 268, 252 (27%), 242, 240, 238, 128, 58 (100%).

PMR spectrum (300 MHz, CCl_4, DMSO-d_6, δ, ppm): 3.0 and 3.50 [3H each, s, $N(CH_3)_2$], 3.88 (3H, s, 10-OCH_3), 3.90 (3H, s, 2-OCH_3), 6.84 (1H, s, 3-H), 6.91 (2H, s, 8-H, 9-H), 9.74 (2H, br.s). ^{13}C NMR spectrum (75 MHz, CCl_4, DMSO-d_6): C-1 (140.7), C-1a (119.5), C-2 (145.8), C-3 (108.7), C-3a (123.9), C-4 (29), C-5 (53.5), C-6a (62.7), C-1b (120.0), C-7 (35.5), C-7a (129.6), C-8 (118.9), C-9 (110.7), C-10 (149), C-11 (143.6), C-11a (119).

Compound **4** was identified as magnoflorine [5, 8, 9, 10, 11].

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Table 1. PMR, ^{13}C NMR, DEPT, and HSQC Spectral Data for Berberine Hydrochloride and Jatrorrhizine Hydrochloride

C atom	Berberine hydrochloride			Jatrorrhizine hydrochloride		
	δ_C	DEPT	HQSC	δ_C	DEPT	HQSC
1	105.46	CH	7.78 (1H, s, H-1)	109.60	CH	7.68 (1H, s, H-1)
2	147.80	C		149.70	C	
3	149.10	C		147.80	C	
4	107.98	CH	6.96 (1H, s, H-4)	114.80	CH	6.87 (1H, s, H-4)
4a	130.00	C		133.30	C	
5	26.50	CH_2	3.25 (2H, t, J = 6.0, H-5)	25.90	CH_2	3.16 (2H, t, J = 6.0, H-5)
6	54.97	CH_2	5.03 (2H, t, J = 6.0, H-6)	55.30	CH_2	4.96 (2H, t, J = 6.0, H-6)
8	145.50	CH	10.05 (1H, s, H-8)	145.00	CH	9.86 (1H, s, H-8)
8a	121.30	C		117.20	C	
9	143.60	C		150.30	C	
10	150.00	C		143.50	C	
11	126.20	CH	8.04 (1H, d, J = 9.1, H-11)	126.50	CH	8.14 (1H, d, J = 9.2, H-11)
12	123.40	CH	8.10 (1H, d, J = 9.1, H-12)	123.10	CH	8.02 (1H, d, J = 9.2, H-12)
12a	133.10	C		128.30	C	
13	120.20	CH	8.63 (1H, s, H-13)	119.40	CH	8.99 (1H, s, H-13)
13a	137.20	C		138.10	C	
13b	120.30	C		130.20	C	
Δ2,3	101.60		6.14 (2H, s)			
2-O$\underline{C}H_3$				56.8		3.96 (3H, s)
9-O$\underline{C}H_3$	56.50		4.17 (3H, s)	61.50		4.14 (3H, s)
10-O$\underline{C}H_3$	61.51		4.11 (3H, s)	56.1		4.07 (3H, s)
C_3-O$\underline{H}$						9.93 (1H, br.s)

According to HPLC analysis, the active constituents in the studied pharmacologically active aqueous extract were the protoberberine derivatives berberine (7.6%), jatrorrhizine (0.9), and palmatine (0.77). The aporphine base magnoflorine was dominant. Its content in the extract was 57%.

Thus, the alkaloids palmatine, jatrorrhizine, and magnoflorine were isolated and identified for the first time from Lavalle cork.

The anti-inflammatory and hypoglycemic activity of the dry aqueous extract of bark from a cork tree introduced into Georgia enabled this species to be considered a potential medicinal plant.

PMR, ^{13}C NMR, DEPT, HSQC, HMBC, and NOESY spectra were recorded in DMSO-d_6/CCl_4 on a Varian Mercury-300 Vx instrument at 300.077 MHz for ^1H and 75.462 MHz for ^{13}C. Mass spectra were taken on an LKB spectrometer (Sweden) at 12 and 70 eV energy.

Total alkaloids were separated over a column of silica gel. TLC was performed on Silicagel Merck 60 F_{254} plates. The mobile phase was *n*-BuOH:EtOAc:HCO_2H:H_2O (30:50:10:10). Detection used Dragendorff's reagent.

HPLC was carried out in an Agilent-1200 Series chromatograph using a column (250 × 4.6 mm), SB-C18 sorbent, UV detector at 235 nm, mobile phase MeOH/20 mL/mol KH_2PO_4 48/52, pH 2.6, flow rate 0.7 mL/min. The volume of injected sample was 20 μL. The analysis time was 10 min.

The standards were berberine, palmatine, jatrorrhizine, and magnoflorine hydrochlorides (Phytomarker LTD, China).

Alkaloids were precipitated as hydrochlorides from aqueous solutions of HCl (1 N) at pH 4–5; iodides, by saturated KI solution (pH 3–4). The resulting salts were dried in vacuo over P_2O_5.

Pharmacological tests were performed at the Pharmacology Department of Tbilisi Medical University and in the Basic Science Department of Chicoutimi University (Quebec, Canada).

Extraction and Isolation of Alkaloids. Air-dried ground bark of Lavalle cork (200 g) was extracted with H_2O (2 × 2 L) by standing at room temperature for 50 h. The aqueous extracts were combined, filtered, and condensed to 1.5 L. Coagulated proteins that formed on cooling were separated by centrifugation. The supernatant liquid was washed with hexane (2 × 500 mL) and $CHCl_3$ (2 × 500 mL). The aqueous phase was condensed to dryness, dissolved again in H_2O (500 mL), and filtered. Water-soluble polysaccharides were precipitated by a five-fold excess of EtOH. The EtOH was distilled. The aqueous-alcohol solution was condensed to dryness to afford a brownish amorphous powder (A, 17 g).

The extract (5 g) was separated over a column of silica gel by a dry method. The mobile phase was $CHCl_3$:MeOH:NH_4OH (10%) (15:4:1). The yellow effluents were combined. The solvent was distilled. The solid was taken to dryness, dissolved in MeOH, and precipitated by EtOH to afford a greenish-yellow amorphous fraction, dissolution of which in H_2O (15 mL) and addition of HCl (1 N) to pH 5 isolated on standing **1** (360 mg), mp 196–197°C, R_f 0.62.

The MeOH:Et_2O mother liquor was taken to dryness. The solid was purified over a column of silica gel. The mobile phase was $CHCl_3$:MeOH:H_2O (7:13:8). The yellow effluents were combined. The solvent was removed. The solid was taken to dryness, dissolved in H_2O, and precipitated by HCl (1 N) to pH 4 to isolate on standing a reddish crystalline compound **2** (40 mg), mp 205–207°C, R_f 0.57.

The aqueous extract (A, 4.0 g) was dissolved in H_2O (120 mL), filtered, and extracted with *n*-BuOH (3 × 50 mL). The combined n-BuOH extracts were condensed, dried in a vacuum chamber, and separated over a column of silica gel. The mobile phase was C_6H_6:EtOAc:*n*-PrOH:MeOH:$EtNH_2$ (8:4:2:1:1). Two fractions, A and B, were obtained. Compound **1** dominated in fraction A. Compound **3** (28 mg) was isolated as the hydrochloride from fraction B, mp 238–241°C, R_f 0.65.

The aqueous phase after work up with *n*-BuOH was made basic with NH_4OH (25%) to pH 9–10 and extracted with $CHCl_3$ (50, 30, 20 mL). The alkaline solution was made acidic with H_2SO_4 (10%) to pH 3 and treated with saturated KI solution. Extraction by $CHCl_3$ separated a fraction of iodides that was separated after removal of solvent over a column of silica gel. The column was eluted with $CHCl_3$ and $CHCl_3$:MeOH (1–20%). The effluents with 5–10% MeOH afforded **4** (1.16 g), mp 248–250°C (dec.), R_f 0.79.

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