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ALKALOIDS OF *Eminium lehmannii*

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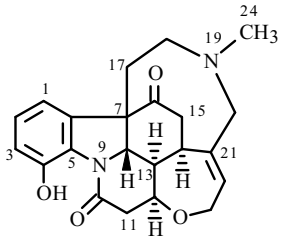
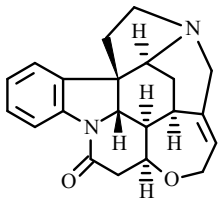
Eminium lehmannii (Bunge) O. Kuntze is a rare relict species of the ancient family Araceae [1]. The genus *Eminium* includes eight species that are distributed primarily in the Mediterrean and Western Asian countries. Two species grow in Kazakhstan, *E. lehmannii* and *E. alberty* (Rgl.) Engl. [1]. Both are endemic and cited in the Red Book of Kazakhstan [2]. Tubers of this plant are used in folk medicine [1, 3]. According to the literature [3, 4], tubers contain alkaloids, saponins, and starch. The quantitative composition of leaf lipids and the composition of glycolipids and pigments from leaves and skin and core of tubers have been studied [5, 6].

Herein we communicate results from the first study of the alkaloid composition of *E. lehmannii* tubers.

Alkaloids were isolated from the thick alcohol extract of the subterrean part of *E. lehmannii* (14% yield of extracted substances) by partitioning extraction using petroleum ether:water. This separated a fraction of nonpolar components (20% yield). The remaining aqueous alcohol solution was extracted with $CHCl_3$. Column chromatography of the $CHCl_3$ extract over silica gel isolated successively the alkaloids vomicine (4-hydroxy-19-methyl-16,19-*seco*-strychnidin-10,16-dione) (**1**) (0.015% yield of dry wt.) and strychnine (**2**) (0.025% yield). The molecular structures of these alkaloids were established by x-ray structure analyses (XSA). Figure 1 shows the molecular structure of **1** and intramolecular interactions from the XSA. Figure 2 shows the crystal packing of **1** with intermolecular interactions.



1



2

The geometry, conformations of rings, and intermolecular interactions of **1** were analyzed using the programs Platon [7] and Mercury [8]. The indole fragment of **1** was practically planar. The mean-square deviation from the plane passing through all nonhydrogen atoms of this fragment was 0.036 Å. The tetrahydropyridone group adopted a twist-boat conformation; the seven-membered oxazine ring, a twist-chair; the cyclohexane ring, a half-chair. The bond lengths and angles in **1** agreed with the mean-statistical values within 3σ [9] and with those in icajine [10]. The presence in **1** of an OH group in the C4-position led to the formation of a strong intramolecular H-bond O1–H...O2 with O1–H1OH 0.88(2), 02...H1OH 1.69(2), and 01...O2 2.532(2) Å and O1–H...O2 159(2)°. Molecules of **1** in the crystal were bonded to each other into chains along the crystallographic *a* axis through weak intermolecular O...H and C...H interactions (Fig. 2), among which were nonbonding O2...H17B contact shortened to 2.48 Å (sum of van der Waals radii 2.72 Å). Compound **1** was isolated previously in a pure form from *Strychnos icaja* Baill [11]. Methods for its preparation from strychnine were reported [12].

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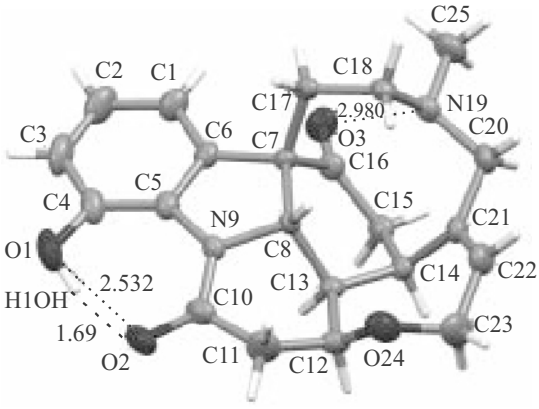

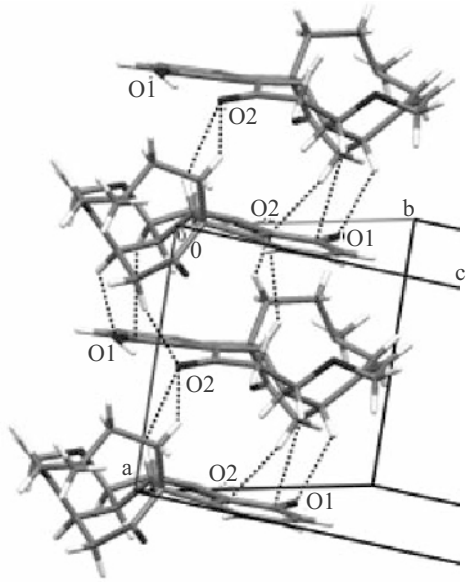

Fig. 1 Fig. 2

Fig. 1. Molecular structure of vomicine (**1**) and intramolecular interactions according to XSA.
Fig. 2. Crystal packing fragment of vomicine (**1**) with intermolecular interactions.

Crystallographic data and unit-cell constants of **2** were obtained by XSA and searched for in the Cambridge Crystallographic Database [13]. The search was successful; a crystal of strychnine with similar constants, a = 11.267(2), b = 11.892(11), c = 12.105(4) Å, V = 1621.9 Å^3, space group $P2_12_12_1$, was reported [14]. The identity of compound **2** was verified by measuring the intensities of 110 independent reflections using ω-scanning. Atomic coordinates from the previous study [14] and identical isotropic thermal parameters (U = 0.05 Å^2) for all atoms were used. Only the overall scaling factor was refined to obtain R = 0.0744 for 101 reflections with F > 4σ(F). According to these data, **2** was strychnine.

Thus, alkaloids of the strychnine type were isolated from the subterrean organs of *E. lehmannii*. In addition to valuable the therapeutic properties of strychnane-type alkaloids due to the excitatory action on the CNS [15], vomicine (**1**) and other alkaloids of this type are viewed as promising antimalarial agents [16, 17].

The purity of the isolated components was monitored by TLC on Silufol UV-254 plates ($CHCl_3$:EtOH, 1:1). PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solutions on Bruker AV 600 and DRX-500 instruments at operating frequencies 600.13 MHz (1H) and 125.76 MHz (^{13}C). Resonances in PMR spectra were assigned using two-dimensional correlation ^1H–^1H (COSY) and ^1H–^{13}C (COLOC) spectra. Mass spectra were obtained on a Finnigan MAT 8200 spectrometer with electron-impact ionization. IR spectra were recorded in KBr disks on an Avatar 360 instrument. Optical rotation $[\alpha]_D^{20}$ was measured in EtOH on a Pol AAr3005 polarimeter. Melting points were determined on a Boetius heating stage. Column chromatography over silica gel (KSK) was used to isolate the alkaloids. Raw material of *E. lehmannii* was collected in southern Kazakhstan in 2006 during fruiting.

The XSA of **1** was performed on a Kappa Apex II (Bruker) diffractometer with a two-coordinate CCD detector (MoK_α-radiation, graphite monochromator, ω-ψ-scanning for 2θ < 55°) at –54°C. The XSA of **2** was performed on a Bruker P4 diffractometer (MoK_α-radiation, graphite monochromator, 2θ/θ-scanning for 2θ < 50°).

Isolation of Alkaloids. Tubers of *E. lehmannii* were ground in a blender. The ground raw material was extracted exhaustively with EtOH (96%) for 2 h at 80°C at a raw material:EtOH ratio of 1:6. The extraction was carried out five times. The resulting extracts were combined and evaporated to afford total thick extract (14%).

The resulting thick EtOH extract was dissolved in aqueous EtOH (800 mL) at a 1:2 ratio. Extraction with petroleum ether (PE) (5 × 700 mL) isolated a fraction of nonpolar components. The PE extracts were evaporated and combined to afford a nonpolar fraction (20%, oily yellow mass).

Components of medium polarity were extracted from the remainder of the aqueous EtOH solution by $CHCl_3$ until the $CHCl_3$ part was no longer colored (5 × 600 mL). The $CHCl_3$ extracts were combined and evaporated to afford a thick $CHCl_3$ extract (16%). Qualitative reactions of the $CHCl_3$ extract for alkaloids (Dragendorff's, Sonnenschein's, and Mayer's) gave a positive result. Column chromatography of the $CHCl_3$ extract over silica gel (KSK, PE, PE:EtOAc with gradient of increasing

polarity) with elution by PE:EtOAc (100:15) isolated fractions containing crystalline **1** (0.015% yield). Elution by PE:EtOAc (2:1) isolated fractions containing crystalline **2** (0.025% yield).

Vomicine [4-hydroxy-19-methyl-16,19-*seco*-strychnidin-10,16-dione] (1); colorless needle-like crystals; very soluble in $CHCl_3$, EtOAc, EtOH; poorly soluble in hexane and PE; mp 284–285°C (EtOAc) (lit. mp [11, 18] 281–282°C], $[\alpha]_D^{20}$ –98.1° (*c* 3.2, $CHCl_3$).

PMR spectrum ($CDCl_3$, δ, ppm, J/Hz): 1.61 (1H, d, J = 12.8, 3.6, H-17), 1.71 (1H, ddd, $J_{8,13}$ = 11.2, $J_{13,14}$ = 2.8, $J_{12,13}$ = 3.2, H-13), 2.04 (3H, s, CH_3), 2.13–2.25 (2H, m, H-15, H-18), 2.60–2.77 (3H, m, H-15, H-17, H-20), 2.94 (1H, td, J = 13.0, 4.2, H-18), 3.08–3.28 (3H, m, H-11, H-11, H-20), 3.48 (1H, m, H-14), 4.03 (1H, dd, J = 14.8, 5.6, H-23), 4.21 (1H, m, $J_{12,13}$ = 3.2, H-12), 4.27 (1H, t, J = 14.8, 7.4, H-23), 4.34 (1H, d, J = 11.2, H-8), 6.00 (1H, m, H-22), 6.79 (1H, d, J = 7.6, H-3), 7.04 (1H, t, J = 7.6, H-2), 7.33 (1H, dd, J = 7.6, 1.8, H-1), 11.65 (1H, s, OH).

^{13}C NMR spectrum (δ, ppm): 35.12 (d, C-14), 39.48 (q, CH_3), 40.57 (t, C-20), 42.67 (t, C-15), 45.67 (C-18), 46.89 (d, C-13), 47.89 (t, C-17), 54.75 (s, C-7), 59.83 (d, C-8), 62.32 (t, C-11), 65.11 (t, C-23), 78.92 (d, C-12), 117.29 (d, C-1), 117.74 (d, C-3), 126.32 (s, C-21), 127.53 (d, C-2), 130.35 (d, C-22), 136.17 (s, C-6), 141.11 (s, C-5), 145.26 (s, C-4), 168.00 (s, C-10), 192.58 (s, C-16).

Mass spectrum (EI, 70 eV, *m*/*z*, I_{rel}, %): 380.2 (80) $[M]^+$, 352.2 (10), 351.2 (22), 337.2 (6), 323.2 (18), 322.2 (24), 321.2 (100), 320.1 (14), 307.1 (64), 306.1 (28), 304.1 (17), 292.1 (7), 279.1 (8), 267.1 (11), 264.2 (5), 252.1 (8), 251.1 (20), 250.1 (12), 212.1 (6), 199.1 (11), 184.1 (8), 161.1 (6), 160.1 (7), 159.1 (11), 146.1 (9), 126.1 (11), 77.0 (7), 58.0 (7), 57.0 (7). $C_{22}H_{24}O_4N_2$, MW 380.17.

IR spectrum (KBr, ν, cm^{-1}): 2854 (>N–CH_3), 2750 (C–C), 1650 (C=O), 1473, 1445, 1113, 845.

XSA of 1. A crystal of **1** (0.20 × 0.10 × 0.05 mm) was selected. The crystals were orthorhombic, *a* = 8.1166(7), *b* = 14.174(1), *c* = 15.698(2) Å, *V* = 1806.0(4) $Å^3$, space group $P2_12_12_1$, *Z* = 4, $C_{22}H_{24}N_2O_4$, d_{calc} = 1.399 g/cm^3, μ = 0.097 mm^{-1}. Intensities of 11,995 reflections were measured. Of these, 4,147 were independent (R_{int} = 0.046). Absorption corrections were applied using the SADABS program [19] (transmission 0.62–0.75). The structure was solved by direct methods using the SIR2002 program [20]. Parameters of H atoms were calculated in each refinement cycle from the coordinates of the corresponding C atoms (riding model). The H atom of the OH group (H1OH) was located using a difference synthesis. The structure was refined using an anisotropic-isotropic (for H1OH) full-matrix least-squares methods in the SHELXL-97 program [21]. Final refinement of the structure over all F^2 gave wR_2 = 0.0947 and *S* = 1.04 for 257 parameters (*R* = 0.0349 for 3836 $F > 4\sigma$). Coordinates and thermal factors of the atoms and geometric parameters of **1** were deposited in the Cambridge Crystallographic Data Centre CCDC 729046. Data for the XSA are available at www.ccdc.cam.ac.uk/data_request/cif.

Strychnine (2), mp 286–288°C (EtOH) (lit. mp [15] 286–290°C).

XSA of 2. Crystallographic data: orthorhombic system, space group $P2_12_12_1$, *a* = 11.325(1), *b* = 11.905(1), *c* = 12.115(1) Å, *V* = 1633.2(3) $Å^3$.

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