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ALKALOIDS FROM *Crambe kotschyana* ENDEMIC TO UZBEKISTAN

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Plants of the genus *Crambe* (Cruciferae, mustard) are perennial herbs and number 12 species [1], four of which are encountered in Uzbekistan: *C. kotschyana* Boiss., *C. edentula* F., *C. schugnana* Korsh., and *C. gordjadinii* Spryg. et Pol. [2], mainly in Namangan (Kasan-Sai) and Fergan (Fedchenko-Gorchakovo) Oblasts [2].

C. kotschyaa is a perennial endemic feed plant that reaches 2.5 m in height and inhabits rocky and humus-rich slopes of the lower and middle mountain belt of Central Asia [2].

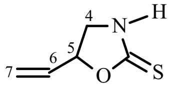
Fruit contained up to 17.5% plant oil; seeds, from 30.0 to 38.0 % [3–5]. The aerial part was reported to contain coumarins, vitamin C, and β-carotene [6]. Seeds are used in fold medicine for upper respiratory tract congestion. Roots and stems of this plant are edible and are used by the local population as food and in animal feed. Carbohydrates such as starch, disaccharides, and monosaccharides were found in roots [7, 8]. *C. kotschyana* is cultivated in Uzbekistan. The harvest reaches 16–20 cwt/ha of dry mass [9].

Alkaloids from this plant species were not previously studied. Only brief mention of the alkaloid content of *C. kotschyana* has appeared [10, 11]. However, S-containing alkaloids are characteristic of plants from the family Cruciferae [12].

We studied the aerial part of *C. kotschyana* collected in April 2009 in Dzhizak Oblast. According to preliminary data, total alkaloids from the aerial part make up 0.2% and contain (TLC) 5–6 alkaloids [13].

The $CHCl_3$ extract of the aerial part afforded after the appropriate work up the total bases. The basic solution remaining after separation of total bases continued to give a reaction with silicotungstic acid for alkaloids, which were extracted by *n*-BuOH. This afforded an additional amount (1.6 g) of bases.

The mother liquor from the $CHCl_3$ fraction of alkaloids was placed on a silica-gel column and separated by elution with $CHCl_3$ and then a gradient of hydrocarbons and $CHCl_3$. A liquid base **1** (goitrin) was isolated from individual hydrocarbon:$CHCl_3$ fractions, liquid alkaloid **2**, from succeeding fractions.



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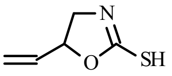

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The structures of both alkaloids were established using IR, mass, PMR, and ^{13}C NMR spectral data and comparative analyses. The IR spectrum of **1** had absorption bands for active H at 3225 cm^{-1} (>NH) and stretching (3047, 2895, 2885) and bending (1416, 988, 921) CH_2 and CH vibrations. An ether (C–O) band was observed at 1032; an >N–C–S band, at 1163.

The mass spectrum of **1** showed peaks for a molecular ion with *m*/*z* 129 (100%) and for fragment ions with *m*/*z* 102, 85, 68, and 51.

The PMR spectrum of **1** in DMSO contained seven separate well resolved resonances from all protons of the molecule. Two doublets of triplets characteristic of two exo-methylene protons on C-7 appeared at 5.20–5.35 ppm. The doublet splitting was due to spin–spin coupling with the third olefinic proton H-6 with SSCC 17.1 and 10.4 Hz. Small SSCC 1.2–1.4 Hz resulted from coupling of the H-7 protons with each other and with methine H-5. Olefinic H-6 resonated at 5.88 as a ddd resonance with well resolved lines. Resonances of H-5 and H-6 were easily identified from the chemical shifts and their SSCC (Table 1).

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TABLE 1. NMR Spectra of **1** and **2** in DMSO (chemical shifts, ppm; SSCC, Hz; proton partner by SSCC, in parentheses)

C atom	**1**			**2**		
	δ_C	δ_H	SSCC	δ_C	δ_H	SSCC
2	159.44			118.62		
4c	45.58	3.14	8.9 (4t), 6.9 (5), 0.9 (6), ~0.2	25.85	2.51	16.8 (41), 5.9 (5), ~ 0.2 (6)
4t		3.59	8.9 (4c), 7.8 (5), ~0.2 (6)		2.60	16.8 (4c), 4.8 (5), ~0.2 (6)
5	76.51	4.95	7.8 (4t), 6.9 (4c), 6.5 (6), 1.3 (7c), 1.2 (7t)	67.42	4.25	5.9 (4c), 4.8 (4t), 4.8 (6), 1.6 (7c), 1.4 (7t)
6	135.76	5.88	17.1 (7c), 10.4 (7t), 6.5 (5), 0.9 (4c), 0.2 (4t)	139.25	5.81	17.2 (7c), 10.5 (7t), 4.8 (5), ~ 0.2 (4c), ~0.2 (4t)
7c	118.20	5.31	17.1 (6), 1.4 (7t), 1.3 (5)	115.89	5.26	17.2 (6), 1.7 (7t), 1.6 (5)
7t		5.21	10.4 (6), 1.4 (7c), 1.2 (5)		5.11	10.5 (6), 1.7 (7c), 1.4 (5)
NH(SH)		7.23			5.41	

4c, 4t: *cis*- and *trans*-orientation relative to proton H-5; 7c, 7t: *cis*- and *trans*-orientation relative to proton H-6.

The ^{13}C NMR spectrum of **1** showed at strong field a resonance for a C atom bonded to S at 159.44 ppm. A resonance at 135.76 was assigned to C-6; at 118.20, to the terminal vinyl C atom.

Table 1 presents the complete data for the PMR and ^{13}C NMR spectra of **1** in DMSO.

IR and mass spectra of **1** showed that the compound was the S-containing alkaloid goitrin, which has the structure 5-vinyloxazolidin-2-thione [14]. Goitrin was observed previously in several plant species of the family Cruciferae. This alkaloid has been reported to be present in many *Brassica* species (Cruciferae). Goitrin was isolated and characterized by spectral data from plants of the genus *Crambe* for the first time by us.

The structure of **2** was established by comparing its spectral data with those of goitrin. Thus, the mass spectrum of **2** was similar to that of goitrin and contained peaks for a molecular ion with *m/z* 129 and fragment ions differing only by intensity. A comparison of the PMR spectrum of **2** with that of goitrin showed that they were similar. Important changes in the resonances for skeletal protons were observed only for H-4 and H-5 (shifts to strong field by 0.6–1.0 ppm). This suggested that atoms 2 and 3 had different types of substitution that affected the chemical shifts of these protons and caused such a strong effect. The NH resonance was also shifted to strong field by greater than 1.8 ppm. This indicated that the resonance of the NH proton was shifted to that of an SH group, which would explain such a shift.

The ^{13}C NMR spectrum of **2** was analogous to that of goitrin. Table 1 presents complete PMR and ^{13}C NMR spectral data for **2** in DMSO. A comparison of the spectra for both alkaloids showed that important changes in the resonances for **2** were found only for C-2 and C-4, which shifted to weak field by ~41 and ~20 ppm, respectively (Table 1).

Thus, the previously unreported alkaloid **2**, which we called goitridin, was a geometric isomer of goitrin with the structure 5-vinyl-4,5-dihydrooxazol-2-thiol. Goitridin was a native alkaloid of *C. kotschyana*. This was confirmed by the detection of it in the alcohol extracts of the plant.

The purity of the compounds was monitored by TLC on silica gel with detection by silicotungstic acid (5%) or I_2 vapor. Column chromatography was performed over silica gel with elution by hydrocarbons:$CHCl_3$ (1:1, system 1; 1:2, system 2) and $CHCl_3$:MeOH (4:1, system 3). IR spectra were recorded in pressed KBr pellets on a Perkin–Elmer model 2000 Fourier-IR spectrometer. NMR spectra were recorded on a Varian Unity-400+ spectrometer at operating frequencies 400 MHz (^1H) and 100 MHz (^{13}C). The standards were HMDSO for protons (0 ppm) and DMSO for C (39.51 ppm). Spectra were recorded at room temperature.

Preparation of Total Alkaloids from *Crambe kotschyana*. The ground aerial part of *C. kotschyana* (6.0 kg) was wetted beforehand with NH_4OH solution (5%) and extracted exhaustively with $CHCl_3$. The combined $CHCl_3$ extracts were worked up with dilute H_2SO_4 (4 × 100 mL). The H_2SO_4 solutions were combined, washed with hydrocarbons, and made basic with conc. NH_4OH. Alkaloids were extracted by $CHCl_3$ and then *n*-BuOH. The eluates were condensed. The solids were dried *in vacuo* to afford $CHCl_3$ (2.2 g, 0.036%) and BuOH (1.6 g, 0.026%) total alkaloids.

The basic aqueous solution remaining after total alkaloids were separated was worked up with *n*-BuOH to afford an additional amount (1.6 g) of bases. TLC of the *n*-BuOH extract showed spots with R_f 0.3 and at the origin (system 3). The remaining basic aqueous solution continued to show a reaction for alkaloids so it was taken to dryness. The dry solid showed according to TLC at least three alkaloids.

Isolation of Alkaloids 1 and 2. The $CHCl_3$ alkaloids were chromatographed over a silica-gel column (1:20). Alkaloids were eluted by system 1. Fractions 1–10 contained oily **1** with R_f 0.5 (system 1) (goitrin). Further elution by system 2 produced oily **2** with R_f 0.3 (goitridin).

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