BERBERIS ALKALOIDS.
XXIX. AN INVESTIGATION OF THE ALKALOIDS OF *Berberis sibirica*

A. Karimov, M. G. Levkovich, N. D. Abdullaev, and R. Shakirov

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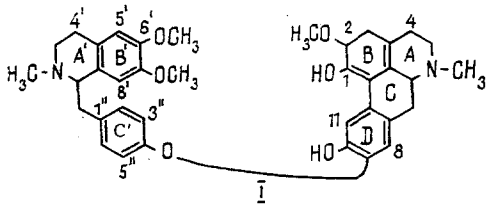
The alkaloid compositions of the roots, young shoots, and leaves of Berberis sibirica have been studied, and berberine, palmatine, columbamine, berberrubine, oxyacanthine, berbamine, 8-oxoberberine, 8-oxoberrberubine, pakistanine, and pronunciferine, and also the new base N-acetylhomoveratrylamine have been isolated. This is the first time that any of these alkaloids, except berberine, have been isolated from this plant.

Berberis sibirica Pall., a ramose prickly shrub with a height of about 1 m grows in Western and Eastern Siberia, in Central Asia and Kazakhstan, and in China and Mongolia.

The roots are used in folk medicine for the treatment of tuberculosis, gastrointestinal and catarrhal diseases and as a hemostatic agent, the epigeal part for jaundice, and the leaves in cases of merorrhagia [1]. Berberine (0.36%) has been isolated from the roots of this plant previously [2]. Flavonoids have been isolated from the leaves, and organic acids and vitamin C from the fruit [3].

We have studied the alkaloid composition of various organs of *Berberis sibirica* Pall. gathered in the fruit-bearing phase in the Kent Mountains, Karaganda province (Kazakhstan). By extracting the roots, young shoots, and leaves by published procedures [4-6] we isolated 2.74, 0.62, and 0.26% of alkaloids, respectively. The amount of berberine in the roots was 0.98% and in the young shoots 0.14%, while only trace amounts of it were detected in the leaves. By chromatography on a column of silica gel the total alkaloids of the roots yielded palmatine, columbamine, berberrubine, oxyacanthine, and berbamine, and the total alkaloids of young shoots yielded berberrubine, 8-oxoberberine, 8-oxoberberrubine, and the crystalline base (I) with mp 155-156°C, base (II), with mp 185-186°C, and base (III), with mp 104-105°C. From the total alkaloids of the leaves, by chromatography on a column of silica gel, we isolated oxyacanthine, trace amounts of berberine, and base (V). More than 80% of the total alkaloids of the leaves consisted of base (V).

Base (I), optically active, had the composition $C_{37}H_{40}N_2O_6$. According to its mass and UV spectra it belonged to the aporphine-benzylisoquinoline alkaloids [7]. Its PMR spectrum showed 3H singlets from two N—CH_3 groups at 2.47 and 2.50 ppm, and three methoxy groups at 3.44, 3.71, and 3.85 ppm, assigned to OCH_3 groups at C-7, -2, and -6′, respectively. Signals from aliphatic protons appeared in the interval of 2.1-3.5 ppm in the form of multiplets. In the region of aromatic protons there were three one-proton singlets at 5.85, 6.74, and 8.08 ppm, which were assigned to the H-8′, H-8, and H-11 protons, respectively. A two-proton singlet at 6.62 ppm was assigned to the H-3 and H-5′ protons, and two 2H doublets at 6.83 and 6.96 ppm to two pairs of *ortho*-protons in ring *C*′. From the spectral characteristics given, (I) was identified as pakistanine, which has been isolated previously from *B. baluchistanica* [8].



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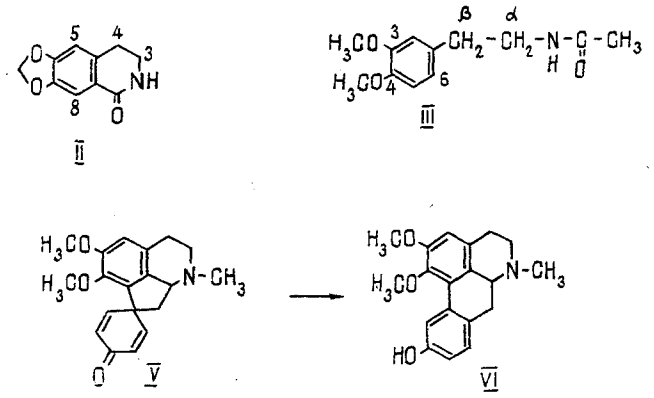

Base (II) had the composition $C_{10}H_9NO_3$. Its IR spectrum contained absorption bands from an amide carbonyl (1660 cm^{-1}) and from active hydrogen (3200 cm^{-1}). According to the characteristics of its mass and UV spectra, (II) belonged to the isoquinolone series [9]. In the PMR spectrum of (II), the signals of methylene protons at C-4 appeared in the form of a two-proton triplet at 2.84 ppm with ^3J = 7.0 Hz, and the signals of methylene protons at C-3 in the form of two triplets at 3.44 and 3.48 ppm. The difference of 0.04 ppm between the signals of these two protons was due to hindered inversion of the unshared pair of electrons on the nitrogen, although it is reported in the literature that the signals of methylenes at C-3 give the same triplet as methylenes at C-4 [10, 11]. A 2H singlet of the protons of a methylenedioxy group appeared at 5.96 ppm and the singlets of aromatic at H-5 and H-8, respectively, at 6.61 and 7.47 ppm.

On the basis of the facts given above, base (II) was identified as noroxyhydrastinine [12].]

In the IR spectrum of base (III) there were absorption bands at 3280 and 1640 cm^{-1} (—NH—CO—). In the mass spectrum there were peaks of ions with *m/z* 223 (M^+), 164 (100%), 151, 149, 121, and 107. In the PMR spectrum of (III) a 3H singlet at 1.86 ppm was due to a N-acetyl group ($NHCOCH_3$). The signals of methylene protons at C_β appeared at 2.68 ppm in the form of a two-proton doublet with ^3J = 6.5 Hz, while the methylene protons at C_α appeared in the form of two triplets at 3.37 and 3.44 ppm with ^3J = 6.5 Hz and a total integral intensity of two proton units. The appearance of the signals of these protons in the form of two triplets can be explained, as in the case of (II) by hindered inversion of the unshared pair of electrons on the nitrogen. A six-proton singlet signal from two OCH_3 groups appeared at 3.77 ppm. In the region of aromatic protons, a multiplet of the AVC type of signal splitting was observed, i.e., a 1,3,4-substituted benzene system was present: the ortho-located protons H-5 and H-6 gave signals at 6.72 ppm (1H, d, ^3J = 8.8 Hz), and 6.66 ppm (1H, dd, ^3J = 8.8 Hz, ^4J = 2.0 Hz), while the signal of the H-2 proton appeared at 6.64 ppm (1H, d, ^4J = 2.0 Hz) and a NH proton gave a broad singlet at 5.4 ppm. On the basis of the facts given above, compound (III) should correspond to the structure of N-acetylhomoveratrylamine or N-acetyl-β-(2,4-dimethoxy)phenylethylamine (IV).

According to its TLC behavior, IR spectrum, and the absence of a depression of a mixed melting point, compound (III) and synthetic N-acetylhomoveratrylamine, obtained from homoveratrylamine, were identical. Thus, (III) was the natural N-acetyl derivative of homoveratrylamine, and this is the first time that it has been isolated from a plant. No β-phenylethylamine derivatives, which are biogenetic precursors of the isoquinoline alkaloids, have been isolated previously from plants of the genus *Berberis*.

According to its UV, mass, and IR spectra, base (V) was a proaporphine alkaloid [13, 14]. Its PMR spectrum revealed signals from N—CH_3 at (ppm) 2.29 (3H, s), from two OCH_3 groups at 3.49 (3H, s), and 3.70 (3H, s), and from seven aliphatic protons in the 2.00-2.50 ppm region in the form of multiplets. In the weak field there were the signals of five aromatic protons at (ppm) 6.18 (1H, dd, ^3J = 10.2, ^4J = 2.0 Hz), 6.29 (1H, dd, ^3J = 10.2, ^4J = 2.0 Hz), 6.53 (1H, s), 6.78 (1H, dd, ^3J = 9.5, ^4J = 3.0 Hz), 6.93 (1H, dd, ^3J = 9.5, ^4J = 3.0 Hz).

By a dienone—phenol rearrangement, (V) yielded nuciferoline (VI), which was identified by its spectral characteristics [16]. Thus, (V) was pronuciferine which has been isolated previously from *Nelumbo nucifera* [15].

EXPERIMENTAL

General Observations. Melting points were determined on a Boëtius stage. Specific optical rotations were determined on a Jasco J-20 spectrometer. IR spectra were recorded on a UR-20 spectrometer by the method of molding tablets with potassium bromide. UV spectra were taken on a Hitachi EPS-3T instrument. Mass spectra of the alkaloids and their derivatives were obtained on a MKh-1310 double-focussing mass spectrometer with a system for the direct introduction of the sample into the ion source, at an energy of 60-70 eV and a temperature of 160-170°C. PMR spectra were taken on a Tesla BS 567 A/100 MHz instrument. HMDS was used as internal standard. The purity of the alkaloids was checked with the aid of TLC on plates coated with type LS 5/40 silica gel and 13% of gypsum in the following solvent systems: 1) chloroform—methanol (9:1 and 95:5); 2) benzene—ethanol (9:1 and 4:1); and 3) chloroform—ethanol (9:1 and 4:1). The revealing agents were Dragendorff's reagent and iodine vapor. Column chromatography was conducted on type KSK silica gel with a particle size of 125-160 μm as sorbent.

Isolation and Separation of the Alkaloids from *Berberis sibirica*. a) Roots. Dry comminuted roots (544 g) were extracted by the procedure of [4]. This yielded 5.34 g of berberine bisulfate, 5.1 g of a precipitate of tertiary bases, 1.64 g of ether fraction, 2.41 g of chloroform fraction, and 0.4 g of a quaternary fraction of alkaloids. When the total alkaloids were chromatographed on a column of silica gel by the method described in [5], palmatine (1.2 g), columbamine (0.62 g), berberrubine (0.54 g), oxyacanthine (4.61 g), and berbamine (0.82 g) were isolated.

b) Young Shoots. The isolation and separation of the alkaloids from young shoots were carried out as described in [6]. This yielded 5.0 g of ether fraction, 4.70 g of chloroform fraction, and 2.2 g of berberine bisulfate. Chromatography of the fractions obtained on a column of silica gel [5] led to the isolation of berberrubine, 8-oxoberberine, 8-oxoberberrubine, palistanine, noroxyhydrastinine, and base (III).

Pakistanine. mp 155-156°C (ethanol) (lit.: 154-156°C [7]), $[\alpha]_D$ +102° (*c* 0.1; CH_3OH), UV spectrum: 275, 305 nm (log ε 4.12, 2.02). Mass spectrum, *m/z* 608 (M^+), 402, 401, 206 (100%), 107. IR spectrum: 3400 cm^{-1}.

Noroxyhydrastinine. mp 185-186°C (ether). Mass spectrum: 191 (M^+, 86), 162 (80), 134 (100), 104 (20). UV: 225, 263, 306 (log ε 4.44, 3.88, 3.96). IR: 3200, 3040, 1660 cm^{-1}.

N-Acetylhomoveratrylamine (III). mp 104-105°C (benzene). IR spectrum: 3270, 3080, 3000, 2950, 1650, 1580, 1270, 1240 cm^{-1}.

Preparation of (III) from Homoveratrylamine. To 0.2 g of homoveratrylamine were added 2 ml of acetic anhydride and three drops of pyridine. The mixture was left at room temperature for two days. then it was evaporated in the water bath, and the residue was dissolved in 5 ml of benzene. On standing, the solution deposited crystals of (III), mp 101-102°C.

Leaves. The air-dried comminuted leaves (820 g) were moistened with 8% ammonia and were then extracted with chloroform (three times). This gave 1.53 g of ether fraction and 0.65 g of chloroform fraction. The ether fraction of the alkaloids was chromatographed on a column of silica gel (60 g). Elution with chloroform yielded 1.42 g of pronuciferine (V) and 0.05 g of oxyacanthine. Berberine was obtained in trace amounts.

Pronuciferine. mp 162-163°C (benzene). IR spectrum: 1610, 1670 cm^{-1}. UV spectrum: 236 (sh), 285 (log ε 4.87, 4.30). Mass spectrum: *m/z* (J, %): 311 (M^+, 100), 310 (45), 282 (65), 268 (50), 253, 237, 225.

Dienone-Phenol Rearrangement of Pronuciferine. A mixture of 200 mg of pronuciferine and 15 ml of 3 N hydrochloric acid was heated in the water bath under reflux for 15 h. Then the mixture was cooled to room temperature and it was washed (2 × 50 ml) with ether, made alkaline with conc. NH_4OH, and extracted with ether. The ethereal solution was dried over Na_2SO_4, filtered, and evaporated. The residue was chromatographed on a column of silica gel. Elution with a mixture of chloroform and methanol (98:2) gave 130 mg of nuciferoline (VI). mp 210-211°C (lit. 212-214°C [16]). Mass spectrum: 311 (M^+), 310, 296, 280, 268.

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