ALKALOIDS OF *Nitraria komarovii.*

X. STRUCTURES OF TETRAHYDRONITRAMARINE, TETRAHYDROKOMAROVININE, DIHYDROISOKOMAROVINE, AND TETRAHYDROISOKOMAROVINE

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The alkaloid nitraraine and four new bases have been isolated for the first time from the epigeal part of Nitraria komarovii. *Their structures have been established with the aid of chemical transformations and spectral characteristics: tetrahydronitramarine, tetrahydrokomarovinine, dihydroisokomarovine, and tetrahydroisokomarovine. Passages have been effected to nitramarine, komarovinine, isokomarovinine, and komarovidinine. Tetrahydroisokomarovine is a new alkaloid, while this is the first time that tetrahydronitroamarine, tetrahydrokomarovinine, and dihydroisokomarovine have been found in nature. Nitrarine, isonitrarine, and schoberidine have been isolated from the seeds with fruit.*

We have studied the plant *Nitraria komarovii* Iljin et Lava, growing in the environs of Krasnovodsk, Turkmen SSR [1]. To choose the optimum conditions, we performed extraction by three methods:

Method of extraction	Amount of plant, kg	Yield, g	Total, %
1. Methanol	0,8	1,12	0,14
2. a) 2% solution of CH_3COOH in chloroform	0,8	0,87	0,11 } 0,20
b) methanol		0,71	0,09 }
3. Chloroform (the plant was wetted with 8% NH_4OH)	0,8	1,60	0,20

The best results were obtained with the ordinary chloroform extraction of the plant that had been wetted with 8% ammonia. Subsequently, unless specially mentioned otherwise, only this method was used for the extraction of the mixture of alkaloids.

In the case of methanolic extraction, apparently, the majority of substances both of alkaloid and of nonalkaloid nature, including emulsifying agents, passes into the extract, which complicates the isolation of the total alkaloid material and lowers its yield.

The alkaloid content of *N. komarovii* was studied for different organs of the plant and different vegetation periods. It is generally accepted that the dynamics of the accumulation of alkaloids in various organs should be studied in the course of a single year but, unfortunately, we were unable to do this and, therefore, compared the results obtained in different years:

Plant organ	Date of collection	Vegetation period	Weight of plant, kg	Yield g	Yield %
Leaves	V.76	Flowering	7,8	39,0	0,50
"	VIII.79	Fruit-bearing	0.5	1,55	0,31
"	VIII.80	"	2,6	7,2	0,30
"	V.87	Flowering	0,7	3,86	0,55
Fruit with seeds	VIII.79	Fruit-bearing	2,25	3,25	0,14
Seeds	"	"	0,40	0,72	0,18
Fruit	"	"	0,36	0,44	0,12
Stems	V.87	Flowering	1,0	2,03	0,20
Roots	"	"	2,16	0,90	0,04
Buds	"	"	0,137	0,78	0,57
Epigeal part	"	"	2,10	5,67	0,27
One-year stems	"	"	1,35	4,87	0,36

The largest amount of alkaloids was observed in the flowering period in the leaves. By the end of the vegetation period the amount in the leaves and stems had decreased.

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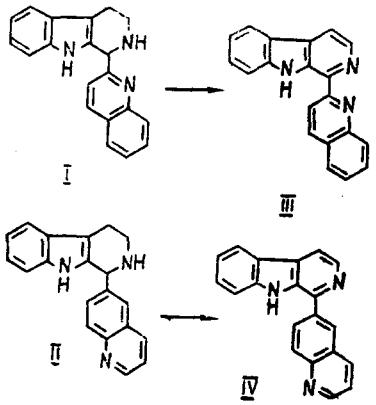
At the present time, in addition to alkaloids isolated previously [1], bases characteristic only for the species have been isolated from the epigeal part of *N. komarovii*, such as komarovine [2], komarovidine [3], komarovicine [4], isokomarovine and komarividinine [5], komarovinine [6], komaroine [7], nitramarine [8], and nitraraine, which was isolated from this species for the first time, and also two bases, the structures of which are discussed below.

By chromatography on a column of silica gel, the ethereal sum of the seeds with fruit yielded — together with nitrarine [10], isonitrarine [11] and schoberidine [12], which were isolated for the first time from the epigeal part of *N. schoberi* — a base with a molecular mass of 580, mp 71-72°C (benzene) $[\alpha]_D$ −30.5° (c 1.25; ethanol). This base had earlier been obtained from the phenolic sum from the epigeal part of *N. komarovii* and, according to preliminary results, it belongs to the glucoalkaloids with a D-glucose sugar residue.

By chromatographic separation of the mother solutions from the total bases of *N. komarovii* (1978, 1979, and 1980 harvests) we isolated two bases (I and II). Base (I): $C_{20}H_{17}N_3$, M^+ 299, mp 193-194°, $[\alpha]_D$ ±0. Base (II): $C_{20}H_{17}N_3$, mp 252-253°, $[\alpha]_D$ ±0.

Scheme 1



The alkaloid komarovicine with an identical composition and comparable spectral characteristics had been isolated from the material under investigation previously [4]. Starting from this fact, we dehydrogenated bases (I) and (II) with Pd black (Scheme 1). This gave products identical in all their parameters with nitramarine (III) and komarovinine (IV), respectively. A direct comparison of bases (I) and (II) with the tetrahydro derivatives of these alkaloids showed their identity.

Thus, for alkaloids (I) and (II) the structures of 1-(quinolin-2-yl)-1,2,3,4-tetrahydro-β-carboline and 1-(quinolin-6-yl)-1,2,3,4-tetrahydro-β-carboline have been established, and they have been called tetrahydronitramarine and tetrahydrokomarovinine, respectively.

From the epigeal part of the plant collected in 1987, two other bases (V) and (VI) were isolated by column chromatography. Base (V) with mp 252-253°C had the composition $C_{20}H_{15}N_3$, molecular mass 297 (mass-spectrometrically).

PMR, mass, UV, and IR spectra permitted the assumption that base (V) was a substituted 1-(quinolinyl)-dihydro-β-carboline.

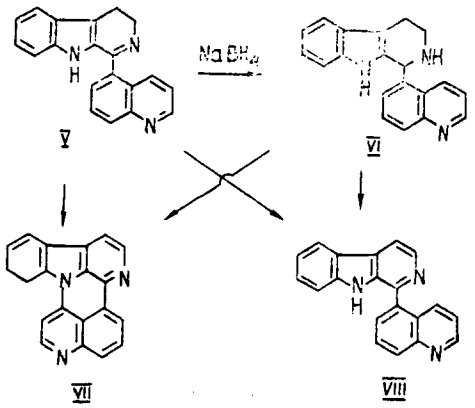
Base (VI) was optically inactive, with mp 274-275°C and the composition $C_{20}H_{17}N_3$, M^+ 299.

The PMR spectrum of (VI) contained, in addition to a complex group of signals in the aromatic region at 7.1-8.7 ppm, two two-proton multiplets at 2.10 and 2.95 ppm ($Ar—\underline{CH_2}—\underline{CH_2}—N<^{H}$) and two one-proton signals at 3.19 and 5.70 ppm ($>N—\underline{H}$, $Ar_2CH—\underline{H}$). On the basis of these facts, it is possible to suggest for compound (VI) the probable structure of a 1-(quinolinyl)-tetrahydro-β-carboline.

The dehydrogenation of (V) and (VI) with Pd black led to the formation of the known alkaloid komarovidinine (VII) [5]. On the mild oxidation of both bases with nitrobenzene, isokomarovine (VIII) [5] was obtained. The reduction of (V) with sodium tetrahydroborate formed the base (VI) (Scheme 2).

Chemical transformations and spectral characteristics enabled us to suggest the following most probable structures: 1-(quinolin-5-yl)-3,4-dihydro-β-carboline for base (V) and 1-(quinolin-5-yl)-1,2,3,4-tetrahydro-β-carboline for base (VI). In actual fact, a comparison of base (V) with the dihydro derivative of isokomarovine, an intermediate in the synthesis of isokomarovine, showed their complete identity. Bases (V) and (VI) have been called dihydroisokomarovine and tetrahydroisokomarovine,

Scheme 2



respectively. This is the first time that dihyroisokomarovine has been found in nature, while tetrahydroisokomarovine is a new alkaloid.

EXPERIMENTAL

UV spectra were recorded on an EPS-3T spectrophotometer (Hitachi), IR spectra on UR-20 instrument (KBr tablets), mass spectra on an MKh 1310 spectrometer, and PMR spectra on a JNM-4H 100/100 MHz instrument with HMDS as internal standard in a mixed chloroform–methanol solvent.

For TLC we used KSK and L 5/40 silica gels. The following solvent systems were used for chromatography: 1) benzene–chloroform (8:1), 2) chloroform–acetone–methanol–ammonia (5:4:1:0.1), 3) chloroform–acetone (4:1), 4) benzene–methanol (4:1), 5) benzene–ethyl acetate–diethylamine (7:2:1), 6) chloroform–acetone–methanol (5:4:1), and 7) chloroform–acetone–ethanol (5:4:1). Revealing agents were the Dragendorff reagent and iodine vapor.

Choice of Solvent for Extraction. For this purpose we used 24 kg of the stems of *N. komarovii* collected in the second half of May, 1976.

a) The finely comminuted stems (0.8 kg) were wetted with 8% ammonia, left for 2 h, and then extracted with chloroform ten times. The bases were extracted from the chloroform solution with 10% sulfuric acid, the acid extract was decomposed with 10% caustic soda, and the bases were reextracted first with ether and then with chloroform. The phenolic fraction was obtained after the addition of NH_4Cl. The total yield of mixed alkaloids was 1.6 g, which corresponds to 0.2% on the weight of the air-dry plant.

b) The finely comminuted stems (0.8 kg) were extracted ten times with methanol, the methanol was distilled off to dryness, and the residue was dissolved in chloroform. The alkaloids were extracted from the chloroform with 10% sulfuric acid and the extract was worked up by the method described above. The total yield of alkaloids was 1.12 g, 0.14% on the weight of the air-dry plant.

c) The finely comminuted stems (0.8 kg) were extracted with 2% acetic acid in chloroform ten times. Then the products were worked up in a similar way to method a). The total yield of alkaloids was 0.87 g (0.108%). The meal was extracted three times with methanol. The methanol was distilled off completely and the residue was dissolved in 10% sulfuric acid and worked up by the method described above. This gave 0.71 g (0.09%) of alkaloids. The total yield was 1.58 g, which corresponds to 0.2% on the weight of the air-dry material. The extraction and separation of the mixed alkaloids has been described in detail in [2, 5].

Extraction of the Stems. The finely comminuted stems (1.0 kg) were wetted with 8% ammonia, left for 2 h, and extracted with chloroform 13 times. The bases were extracted from the chloroform solution with 10% sulfuric acid. The acid extract was decomposed with 10% sulfuric acid. The acid extract was decomposed with 10% caustic soda and the bases were extracted with ether and then with chloroform. The phenolic fraction was obtained after the addition of NH_4Cl. The total yield was 2.03 g, which corresponds to 0.2% on the weight of the air-dry plant.

Extraction of the Roots. The finely comminuted roots (2.16 kg) were extracted in the way described for the stems. This gave a total of 0.9 g (0.04%) of the mixed bases.

Extraction of the Buds. The comminuted buds (0.137 kg) were extracted by the method described above. This gave a total of 0.78 g (0.57%) of mixed bases.

Extraction of the Leaves. The leaves (0.7 kg) were extracted by the method described for the stems, giving a total of 3.86 g (0.55%) of mixed alkaloids.

Extraction of the Epigeal Part. The epigeal part (2.1 kg) was extracted in the usual way. This gave a total of 5.67 g of the mixed bases. Yield 0.27% on the weight of the air-dry plant.

Extraction of One-Year Stems. The finely comminuted stems (1.36 kg) were extracted by the method described above, giving a total of 4.87 g (0.36%) of mixed bases.

Nitraraine. The combined fractions with pH 4, 5, and 6 from the polybuffer separation of the total ether-extracted material from *N. komarovii* were chromatographed on a column of alumina. Elution was performed with ether, chloroform, and chloroform—methanol. The chloroform—methanol (10:1) eluates yielded 0.06 g (0.00076% on the weight of the air-dry plant) of a base with mp 280-281°C. M^+ 308.

Separation of the Mixed Alkaloids from the Seeds with Fruit. A solution of 4.15 g of the phenolic fraction of the total bases in chloroform was extracted with 10% sulfuric acid. The acid solution was washed with ether and then with chloroform. It was decomposed with 10% caustic soda solution, and the alkaloids were extracted with ether and then with chloroform. This gave a total of 3.0 g of ether-extracted bases and 1.0 g of chloroform-extracted bases. The 3.0-g mixture was separated on a column of silica gel with elution by chloroform and mixtures of chloroform and ethanol in various ratios. The eluates obtained at a ratio of (4:1) yielded bases with mp 255-256°C and 208-209°C. A direct comparison of these bases with authentic samples of nitrarine and isonitrarine, respectively, showed their identity.

On elution with chloroform—methanol—ammonia (4:1:0.05), a yellow technical base was obtained; this was dissolved in methanol and the solution was treated with a few drops of perchloric acid to give pH 3-4. On standing, yellow crystals deposited and these were filtered off and washed with acetone: mp 340-342°C. Decomposition of the salt yielded a base with mp 209-210°C. A direct comparison with an authentic sample showed its identity with schoberidine.

Tetrahydronitraramine (I). The products from the mother solutions obtained from the materials collected in 1978, 1979, and 1980 were combined and dissolved in 10% sulfuric acid. The acid solution was washed with ether and then with chloroform. Then it was decomposed with 10% caustic soda solution and extracted with chloroform. This gave 21 g of total chloroform-extracted material, which was chromatographed on a column of silica gel. Elution was performed with chloroform-acetone—ethanol (5:4:1), and 100- to 150-ml fractions were collected.

Fractions 3-8 were combined and rechromatographed on a column of silica gel. Elution was performed with chloroform—acetone (4:1) and, at the end, with chloroform—ethanol (4:1). Fractions 7-15 were combined, the solvent was evaporated off, and the residue was crystallized from benzene and then from methylene chloride. This gave 47 mg of base (I) with mp 193-194°C. Its IR spectrum contained absorption bands at 1430, 1505, 1570, 1700, 1620, 2860, 2930, 2960, and 3250-3350 cm^{-1}. The UV spectrum contained absorption maxima at $\lambda_{max}^{C_2H_5OH}$ 236, 269, 305, 309, and 316 nm (log ε 4.95, 4.08, 3.95, 3.84, 4.04). The PMR spectrum contained the signals of protons at 3.23, 3.38, 3.69, 5.33, and 7.23-8.16 ppm.

Tetrahydrokomarovinine (II). The chromatographically similar fractions 30-43 were combined, the solvent was distilled off, and the residue was separated on a column of silica gel with elution by chloroform—acetone—ethanol (5:4:1).

Fractions 17-25 were combined, the solvent was distilled off, and the residue was crystallized from benzene and then from methylene chloride. This gave 34 mg of a base with mp 252-253°C. M^+ 299. IR spectrum (ν_{max}, cm^{-1}): 750 (o-substituted benzene ring), 1455, 1505, 1580, 1625 (indole nucleus), 2850, 2970, 3290, 3320. UV spectrum, $\lambda_{max}^{C_2HOH}$: 226, 232 (sh), 275-283, 292, 318 nm (log ε 4.85, 4.80, 4.23, 4.17, 3.92). PMR spectrum (ppm): 2.83, 2.93, 3.25, 4.52, 5.22, 6.96-8.60.

1-(Quinolin-2-yl)-β-carboline (III). After careful mixing, 23 mg of base (I) and 15 mg of Pd black were immersed in a sand bath heated to 180°C. The temperature was maintained at 180-200°C for 1 h. After cooling, the reaction mixture was dissolved in a mixture of chloroform and methanol, the Pd was filtered off, and the solvent was evaporated off. The residue was dissolved in 10% sulfuric acid. The acid solution was washed with ether and, after the addition of 10% alkali, it was extracted with chloroform. Recrystallization of the extracted material from methylene chloride gave 9 mg of base (III) with mp 172-173°C.

1-(Quinolin-6-yl)-β-carboline (IV). A ground mixture of 17 mg of base (II) and 13 mg of Pd black was placed in a sand bath heated to 180°C. The temperature was maintained at 180-200°C. After cooling, the material was dissolved in a mixture of chloroform and methanol. The catalyst was filtered off, the filtrate was evaporated, and the residue was dissolved in 10% sulfuric acid. The acid solution was washed with ether and was then decomposed with 10% alkali and was extracted with ether and then with chloroform. After recrystallization from methylene chloride, 8 mg of a substance with mp 238-239°C was obtained.

Dihydroisokomarovine (V). The total bases (13.65 g) were separated chromatographically on a silica gel column. Elution was performed with chloroform–acetone–ethanol (8:2:1). Fractions with a volume of 100 ml were collected. Combined fractions 7-19 were rechromatographed on a silica gel column with elution by chloroform–acetone–ethanol (5:4:1). Fractions with a volume of 20-30 ml were collected. Fractions 9-17 were combined, the solvent was evaporated off, and the residue was crystallized from benzene and then from methylene chloride. This gave 87 mg of base (V) with mp 252-253°C.

In the UV spectrum of (V) there were absorption maxima, $\lambda_{max}^{C_2H_5OH}$, at 220, 246 sh, 293, 320 nm (log ε 4.60, 4.26, 4.10, 3.87); on acidification the spectrum changed: $\lambda_{max}^{C_2H_5OH+H^+}$ 216, 247-256, 305, 321 nm.

IR spectrum (ν_{max}, cm^{-1}): 770, 815, 1145, 1245, 1285, 1460, 1510, 1575, 1625, 2840, 2960, 3060, 3110-3170.

In the PMR spectrum of (I) signals of protons appeared at 3.20 and 4.70 ppm, and also in the 7.0-9.27 ppm region.

Tetrahydroisokomarovine (VI). Fractions 23-37 were combined, the solvent was evaporated off, and the residue was chromatographed on a column of silica gel with elution by chloroform–acetone–ethanol (5:4:1). Fractions with a volume of 30-35 ml were collected. Fractions 17-26 were combined. The solvent was distilled off, and the residue was recrystallized from benzene and then from methylene chloride. This gave 113 mg of base (VI) with mp 274-275°C. The IR spectrum contained the following absorption maxima: 760, 810, 1145, 1470, 1510, 1600, 1620, 2860, 2930, 3070, 3190, 3310 cm^{-1}.

UV spectrum: $\lambda_{max}^{C_2H_5OH}$ 232, 284-288, 295, 316 nm (log ε 4.65, 4.08, 4.04, 3.57), $\lambda_{max}^{C_2H_5OH+H^+}$ 222, 275-280, 285, 291, 316 nm.

Dehydrogenation of the Bases (V) and (VI). Komarovidinine (VII). A. A well-ground mixture of 23 mg of base (V) and 20 mg of Pd black in a round-bottomed flask was immersed in a sand bath previously heated to 180°C. The temperature was maintained at 180-200°C for 40 min. After cooling, the substance was dissolved in a mixture of chloroform and methanol. The catalyst was filtered off, and the filtrate was evaporated. The residue was dissolved in 10% sulfuric acid. The acid solution was washed with ether and was then decomposed with 10% caustic soda and was extracted with ether and then with chloroform. The product was crystallized from benzene and then from methylene chloride. This gave 11 mg of base (VII), mp 254-255°C.

B. The reaction of a mixture of 26 mg of base (VI) and 21 mg of Pd black was carried out and the products were worked up in the same way as described in experiment A. This gave 13 mg of base (VII) with mp 254-255°C.

Oxidation of Bases (V) and (VI). Isokomarovine (VIII). A. A mixture of 19 mg of base (I) and 5 ml of dry nitrobenzene was boiled for 35-40 min. After cooling, the solution was diluted with an equal volume of chloroform. The substance was extracted from the mixture with 10% sulfuric acid. The acid solution was washed with ether and was then decomposed with 10% caustic soda solution and extracted with ether and then with chloroform. After recrystallization from methylene chloride, 9 mg of base (VIII) was obtained with mp 321-323°C.

B. A mixture of 27 mg of base (VI) and 5 ml dry nitrobenzene was boiled for 35 min. The products were worked up as described in experiment A. This gave 14 mg of base (VIII) with mp 321-323°C.

Tetrahydroisokomarovine (VI). A solution of 26 mg of base (V) in 5 ml of methanol was treated with 132 mg of sodium tetrahydroborate in portions. The mixture was stirred at room temperature for 1 h and then another 84 mg of sodium tetrahydroborate was added and the resulting mixture was boiled for 1 h. The solvent was distilled off, the excess of reagent in the residue was decomposed with water, and the reaction product was extracted with chloroform and crystallized from methylene chloride. This gave 15 mg of base (VI) with mp 274-275°C. M^+ 299.

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