ALKALOIDS OF *Nitraria sibirica*.

STRUCTURE OF NITRABIRINE

A. A. Ibragimov, Z. Osmanov,
M. R. Yagudaev, and S. Yu. Yunusov

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From the epigeal part of *Nitraria sibirica* has been isolated a new alkaloid with mp 184-185°C (methanol), $[\alpha]_D \pm 0°$, which has been called nitrabirine. On the basis of chemical transformations and an analysis of the spectral characteristics (mass, IR, UV, ^1H and ^{13}C NMR spectra), its structure has been established as spiro{cyclohexane-1,3'-(3',4',5',6'-tetrahydropyrido[1',2':1",2"]imidazole)}-2-ol, and a conformation has been suggested.

Continuing a study of the alkaloids of the epigeal part of *Nitraria sibirica* Pall., we have isolated a crystalline optically inactive base with the composition $C_{12}H_{18}N_2O$ (high-resolution mass spectrum), mp 184-185°C. The alkaloid has proved to be new, and we have called it nitrabirine (I).

The mass spectrum of (I) contains (with others), the peak of the molecular ion with m/z 206 (M^+, 70%), and also fragments with m/z 189 (M − 17; 15%) and 188 (M − 18, 9%), indicating the ejection of a hydroxy group and of water: The peak with the maximum intensity has m/z 135.

The IR spectrum of the alkaloid shows the absorption bands characteristic for the vibrations of active hydrogen (3350-3180 cm^{-1}), of saturated C—H bonds (2945, 2875), of aromatic >C=C< and —C=N— bonds (1670, 1595, 1530, 1490), of C—OH in alcohols (1250, 1090, 935), and others. The acetylation of nitrabirine led to the formation of a mono-O-acetyl derivative (II) (M^+ 248) in the spectrum of which the absorption band of active hydrogen had disappeared and a strong band had appeared at 1740 cm^{-1} region due to an ester carbonyl group.

In the UV spectrum of (I) there is a single absorption band at 212 nm (log ε 3.84) showing the presence of an alkyl-substituted imidazole or pyrazole ring in the molecule of the base [1, 2]. The most informative for elucidating the skeleton of (I) proved to be the ^{13}C NMR spectrum, in which the following signals (off-resonance) were observed (ppm): 150.9 (singlet, C_{8a}); 127.7 (doublet, C_2); 117.9 (d, C_3); 74.7 (d, C_9); 44.7 (triplet, C_5); 42.7 (s, C_8); 35.4 (t); 29.1 (t); 24.6 (t); 21.6 (t); 20.8 (t); and 19.7 (t). It follows from these facts that the molecule of (I), just like those of the alkaloids nitramine (III) and isonitramine (IV) [3] is based on an azaspirobicyclic system. Since, as mentioned above, the UV spectrum of (I) does not permit preference to be given to one of two heterocycles — pyrazole or imidazole — the assignment of the signal with chemical shifts (CSs) greater than 100 ppm was carried out by comparison with literature information. A comparison of the ^{13}C NMR spectrum of the heteroaromatic part of (I) with the CSs of the analogous carbon atoms of a number of azoles permitted the conclusion that an imidazole ring was condensed with nitramine in the 1—2 position. Below we give the CSs of the heteroaromatic carbon atoms of nitrabirine and of a number of azoles (ppm, $CDCl_3$, 0 — TMS):

Substance	C_2	C_3	C_4	C_5	Literature
Nitrabirine	150,9(C_{8a})	—	127,7	117,9(C_3)	—
1-Methylimidazole	137.6	—	129.3	119.7	[4]
1-Amino-2-ethyl-4-phenylimidazole	150.5	—	138.0	116.7	[5]
1-Methylpyrazole	—	138.7	105.1	129.3	[4]
1-5-Dimethylpyrazole	—	138.7	105.0	139.1	[3]

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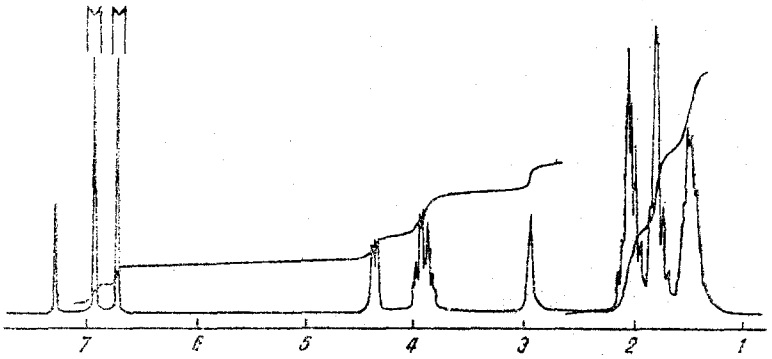


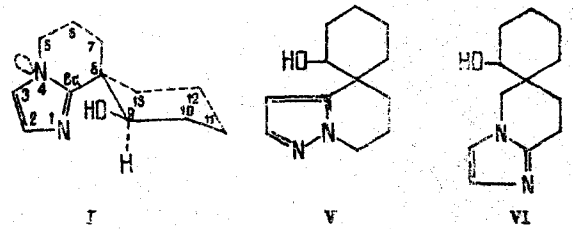
Fig. 1. PMR spectrum of nitrabirine.

The PMR spectrum of (I) (Fig. 1) shows signals at 6.94 ppm (H_2) and 6.73 ppm (H_3) due to the neighboring protons of an imidazole ring (1 H, each a doublet with J ≈ 1 Hz); a quartet at 4.37 ppm (1 H, J_1 ≈ 10, J_2 ≈ 4.5 Hz) is due to a proton geminal to a hydroxy group. The values of the spin–spin coupling constants of the signals of this proton with the neighboring ones and its quartet nature unambiguously show (as in (III) and (IV)) the equatorial orientation of the hydroxy group in the chair conformation of the ring and the fact that there are two protons adjacent to the gem-hydroxylic proton. The latter fact gives grounds for considering that the OH group is located in the α position to the spiro center. A two-proton multiplet in the 3.91 ppm region is due to the protons of a methylene group directly bound to the nitrogen of the imidazole ring (see below). The hydrogen of the hydroxy group gives a signal in the form of a broadened singlet at 2.93 ppm (assignment made with the aid of deuterium exchange with CD_3OD and on heating). Three four-proton multiplets in the 2.00, 1.77, and 1.47 ppm regions are due to the remaining twelve protons of six methylene groups (Fig. 1).

We give a comparison of the CSs of the protons of nitrabirine and of a number of azoles ($CDCl_3$, TMS):

	Heteroaromatic moiety				
Substance	H_1	H_5	H_2	$N—CH_2$ ($—CH_3$)	*Literature*
Nitrabirine	6,94	6,73	—	3,91	—
1-Ethylimidazole	6,98	6,88	7,41	3,91	[7]
1,2-Dimethylimidazole	6,87	6,77	—	—	[8]
1,5-Dimethylimidazole	6,68	—	7,27	3,42	[7]
1,5-Dimethylpyrazole	5,81	$—H_3$	7,10	3 57	[9]

An analysis of the PMR characteristics of (I) and a comparison of them with those given above (together with the ^{13}C NMR results) permits us to exclude the alternative formula (V) with a pyrazole ring. The nature of the linkage of the imidazole ring with the nitramine system was determined on the basis of two factors. In the first place, the complex multiplicity of the signal of the $N—CH_2—$ protons permits us to give our preference to structure (I) over (VI). This is also indicated by the absence of the signals of the protons of a methylene group bound directly to the imidazole ring, which could be expected in the ∿3 ppm region.



I V VI

Experiments using a double-resonance method (total and in the INDOR regime) show that the protons at C_5 and C_9 interact with different protons (the protons of the methylene group in the α position to N_4 at 3.91 ppm in the INDOR spectrum interact only with the multiplet at 2.00 ppm, while the gem-hydroxylic proton giving a signal at 4.37 ppm interacts with those giving two other signals, in the 1.77 and 1.47 ppm regions). This confirms the presence of a hydroxy group in the carbocyclic ring.

It must be mentioned that the CS of the signal the gem-hydroxylic proton in (I) is appreciably shifted downfield ($\Delta\delta \sim 0.80$ ppm) in comparison with those of nitramine and isonitramine [3]. This fact can obviously be explained by the descreening influence of the imidazole ring on the CS of the H_9 proton in (I), which is present in almost the same plane as it, and also by the action of the unshared pairs of electrons of the N_1 nitrogen on H_9, which enables us to select the most probable conformation as (I) or its anitpode.

The IR spectrum of (I), taken in chloroform solution with a concentration of 70 mg/ml has the band of a free OH group of 3600 cm^{-1} and a broad band in the 3400-3200 cm^{-1} region due to association through intermolecular hydrogen bonds of the O–H...O or O–H...N type. On 20-fold dilution, the broad low-frequency band underwent a marked decrease in intensity and shifted in the direction of higher frequencies.

In conclusion, it must be mentioned that imidazole is widely represented in both natural and synthetic organic compounds. However, the alkaloid nitrabirine is the first in this series and has no analogs in the literature. In the IUPAC nomenclature (I) may be called spiro{cyclohexane-1,3'-(3',4',5',6'-tetrahydropyrido[1',2':1",2"]imidazole)}-2-ol.

EXPERIMENTAL

Mass spectra were obtained on MKh-1303 and MKh-1310 instruments with systems for the direct introduction of the substance in the ion source, and IR spectra on a UR-20 spectrophotometer with the substances in the form of tablets with KBr, unless stated otherwise. UV spectra were taken in a Hitachi instrument in ethanol. ^1H and ^3C NMR spectra were obtained on a WM-250 superconducting spectrometer (Bruker) in $CDCl_3$, 0 – TMS. The ^{13}C NMR spectra were recorded in the pulsed regime with Fourier transformation under conditions of complete and partial decoupling of C–H interactions (off-resonance). PMR spectra in the INDOR regime were obtained on a Varian XL-100-15 instrument in $CDCl_3$, 0 – TMS.

The homogeneity of the substances was determined in a thin layer of silica gel–gypsum with the solvent systems given previously [3].

Isolation of Nitrabirine (I). The chloroform extraction of 40 g of the air-dry comminuted epigeal part of the plant gave 66.9 g of ether fraction and 33.3 g of chloroform fraction of the total alkaloids [3]. The ether fraction was separated into water-soluble (24 g) and water-insoluble (42.5 g) parts. Isonitramine [3] was isolated from the latter. The water-soluble part was chromatographed on a column of silica gel with elution by chloroform and then by mixtures of chloroform and methanol with increasing concentrations of the latter. The chloroform eluates yielded (I) in the form of white crystals with mp 184-185°C (methanol), $[\alpha]_D \pm 0°$ (c 2,0 ; chloroform). Yield: 0.0002% on the weight of the dry raw material.

O-Acetylnitrabirine (II). A solution of 0.025 g of (I) in 2.5 ml of freshly purified acetic anhydride was treated with 0.07 g of p-toluenesulfonic acid, and the mixture was heated at 100°C for 4 h (chromatographic monitoring). After cooling, a solution of sodium carbonate was added and the product was extracted with chloroform. This gave 0.30 g of the mono-O-acetyl derivative; IR: 1740 cm^{-1} (film); M^+ 248.

Nitrabirine Hydrochloride. An ethanolic solution of hydrogen chloride was added dropwise to an acetonic solution of (I) to give a weakly acidic reaction, and the resulting salt precipitate was separated off. After crystallization from ethanol it had mp 224-225°C.

SUMMARY

The structure of the new alkaloid from the epigeal part of *Nitraria sibirica*, which has been called nitrabirine, has been established by chemical and spectral methods as spiro{cyclohexane-1,3'-(3',4',5',6'-tetrahydropyrido[1,2':1",2"]imidazole)}-2-ol, and its most probable conformation has been determined.

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PREPARATION OF TRITYL DERIVATIVES OF AMINO ACIDS WITH THE AID OF THE SILYLATION REACTION

A. K. Rabinovich, V. N. Karelskii,
E. P. Krysin, A. A. Antonov,
M. B. Smirnov, and G. I. Chipens

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A number of N-trityl-substituted amino acids (lysine, glutamine, tyrosine, valine, and proline) have been obtained with the aid of the silylation reaction. Bis(trimethylsilyl)acetamide was used as the silylating agent. The compounds were isolated with good yields and were distinguished by chromatographic homogeneity, and they were characterized by their angles of optical rotation, melting points, and elementary analyses. Their purity was checked by TLC and ^{13}C NMR.

One of the widely used methods of protecting amino groups in peptide chemistry is substitution by a triphenylmethyl (trityl) grouping. The usual method of obtaining trityl derivative of amino acids (AAs) is by the action of trityl chloride on hydrochlorides of the methyl (or ethyl) esters of the AAS in the presence of a tertiary base (triethylamine) followed by alkaline hydrolysis of the compounds obtained [1]. Disadvantages of this method include the necessity for a special stage of obtaining and isolating the corresponding AA ester which is associated in a number of cases with certain difficulties; for example, the methyl ester of N^{ε}-tert-butoxycarbonyl-L-lysine is obtained by using diazomethane. Furthermore, the process of hydrolyzing the esters is complicated by the steric hindrance connected with the large volume of the trityl radical. Consequently, to hydrolyze the esters of a number of tritylamino acids severe conditions are necessary (elevated temperature, excess of alkali), and this has an adverse effect on the quality of the desired product (partial racemization, degradation). The tritylation of free AAs in aqueous organic media considerably simplifies the process, but the hydrolysis of the trityl chloride, competing with the main reaction, leads to a large consumption of this reagent and, as a rule, the trityl derivatives are obtained in relatively low yield (40-50%). Recently, the trityl derivative of histidine has been obtained via the trimethylsilyl ester of the AA, which was subjected to tritylation with triphenylmethyl chloride without isolation [2].

Our task was to obtain trityl derivatives of a number of AAs (lysine, tyrosine, glutamine, valine, proline) with the aid of the silylation reaction in order to determine the possibilities of the method (to what extent this transformation has a general nature). The corresponding AA was first silylated in methylene chloride (or DMFA) solution and the trimethylsilyl ester so obtained, without isolation, was then treated with trityl chloride in the presence of trimethylamine:

$$H\text{--}A\text{--}OH \xrightarrow{BSA} [Me_3Si\text{--}A\text{--}OSiMe_3] \xrightarrow{(C_6H_5)_3CCl} Trit\text{--}A\text{--}OSiMe_3 \xrightarrow{H_2O} Trit\text{--}A\text{--}OH,$$

where A is an amino acid residue.

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