ALKALOIDS OF Nitraria schoberi.

STRUCTURE OF DIHYDRONITRARAINE

A. A. Ibragimov and S. Yu. Yunusov

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The structure of dihydronitraraine (I) has been established on the basis of the results of a study of its chemical properties and spectral characteristics and also of a correlation with nitraraine. An internal quaternary salt of (I) has been obtained and, by means of a directed change in the configuration at $C_3$ in (I), the complete stereochemistry of the nitraraine and dihydronitraraine molecules have been determined: (±)-16-hydroxymethyl-3α,15α,20α-yohimb-16-ene and (±)-16β-hydroxymethyl-3α,15α,20α-yohimbane, respectively.

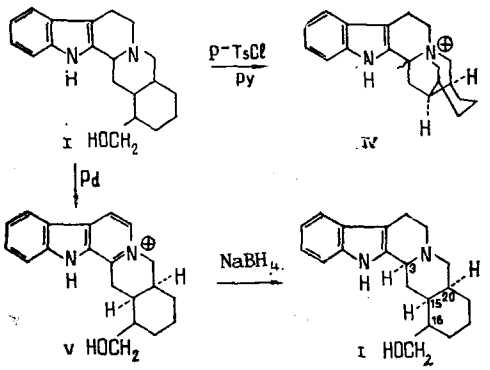
From the fractions following after the separation of the nitraraine [1] we have isolated an alkaloid with the composiiton $C_{20}H_{26}N_2O$, mp 286-287°C, $[\alpha]_D \pm 0°$ (Py, *c* 0,6), which we have called dihydronitraraine (I). The UV spectrum of (I) was typical for bases of the yohimbine group. Its mass-spectrometric fragmentation confirmed that (I) belonged to this series – m/z: 310 ($M^+$), 309, 293, 292, 279, 197, 184, 170, 169, 156, 144 – and showed the possible presence of a primary alcohol group in the molecule. The PMR spectrum of (I) taken in trifluoroacetic acid contained signals from the armomatic protons of an indole nucleus with their center at 6.93 ppm, of the protons of a hydroxymethyl group and of the $C_3$–H proton in the 3.86 ppm region, while a methylene hump appeared at 2.70-0.99 ppm.

The acetylation of (I) with acetic anhydride led to a mono-O-acetyl derivative (II) ($M^+$ 352) the IR spectrum of which contained the absorption band of an ester carbonyl group (1740 $cm^{-1}$).

The facts presented above permitted the assumption that (I) was a dihydro derivative of the alkaloid nitraraine (III), and this was confirmed by a direct comparison of it with one of the reduction products of (III) [1].

Compound (I) reacted with p-toluenesulfonyl chloride in pyridine with the formation of the internal quaternary salt (IV). The crystalline compound (IV) obtained had a high melting point, did not exhibit either acidic or basic properties, and did not dissolve in the usual organic solvents. The possibility of the internal quaternization of (I) permitted an idea to be obtained of the stereochemistry of the asymmetric $C_{15}$, $C_{16}$, and $C_{20}$ atoms: the protons attached to them are cis-oriented with respect to one another.

The dehydrogenation of (I) with palladium black in maleic acid led to the formation of the tetrahydro derivative (V). When the chloride of (V) was reduced with sodium tetrahydro-

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borate a compound was obtained (scheme) identical with dihydronitraraine. Consequently, the proton at $C_3$ was syn-oriented with respect to $C_{15}$–H, having the α configuration [2].

It must be mentioned that nitraraine, just like the analogous derivatives of yohimbine with a double bond at $C_{16}$ [3], undergoes practically no conversion into the corresponding 3,4,5,6-tetrahydro derivative on being heated with palladium in maleic acid. The formation of the tetrahydro derivative from the alkaloid nitraraine containing a nitrogen atom in position 16 of ring E also takes place with extreme difficulty and with insignificant yield [4]. Apparently, in these cases the selectivity of the reaction is lost.

Thus, the alkaloids dihydronitraraine and nitraraine have the structure and stereochemistry of (±)-16β-hydroxymethyl-3α,15α,20α- yohimban and (±)-16-hydroxymethyl-3α,15α,20α-yohimb-16-ene, respectively, and belong to the all series.

## EXPERIMENTAL

The UV spectrum was obtained on a Hitachi instrument in methanol and the PMR spectra of a JNM-4H 100/100 MHz spectrometer with HMDS as internal standard (δ scale). Mass spectra were recorded on a MKh-1303 instrument. For TLC we used type L 5/40 silica gel and the following solvent systems: 1) benzene–methanol (8:3); 2) chloroform–methanol (2:1); 3) chloroform–acetone–methanol (5:4:1); 4) chloroform–acetone–ethanol–conc. ammonia (6:2:1:0.1), and others.

Isolation of Dihydronitraraine (I). The mother liquor after the separation of the nitraraine and the subsequent fractions from the column of alumina [1] were combined and chromatographed on a column of silica gel (100/160) with elution by chloroform. The fractions enriched with (I) were rechromatographed in system 3. This gave 103 mg of (I), mp 286-287°C (sublimation).

O-Acetyldihydronitraraine (II). A well-mixed combination of 35 mg of (I), 0.5 ml of freshly distilled pyridine, and 1 ml of freshly distilled acetic anhydride was left at room temperature for a day. The pyridine and the excess of the reagent were evaporated off in vacuum, the residue was treated with 1.5 ml of water, and the resulting solution was neutralized to pH 7 with 25% $NH_4OH$; the precipitate that deposited was filtered off and was washed with water and with methanol and was then dried. This gave 19 mg (47%) of (II) with mp 94-95°C (methanol). Mass spectrum, m/z: 352 ($M^+$), 351, 309, 293, 279, 223, 184, 170, 169, 156, 144.

Quaternary Salt of Dihydronitraraine (IV). With stirring by means of a magnetic stirrer, 70 mg of p-toluenesulfonyl chloride in 1.5 ml of pyridine was added to 40 mg of (I) in 5 ml of dry purified pyridine. The mixture was left for 5 days in the refrigerator, and then the pyridine was distilled off in a rotary evaporator. The reaction product was crystallized from methanol. This gave colorless prisms with mp 334-337°C, Yield 92%. The mixed tosylate-chloride (1:1) salt crystallized with three molecules of water.

Reduction of the Tetrahydro Derivative (V). A mixture of 15 mg of (I), 40 mg of maleic acid, 40 mg of palladium black, and 2 ml of water was stirred at 80°C for 26 h. The catalyst was filtered off and was washed with 10% sulfuric acid solution and with water; chloroform was added to the filtrate and, after this had been made alkaline with caustic potash solution, the reaction product was exhaustively extracted with chloroform. On a Silufol plate in the benzene–methanol (3.5:1) system the dehydro base obtained clearly differed in its $R_f$ value from the initial (I). A chloroform solution of the substance was acidified with an ethanolic solution of hydrogen chloride, whereupon the solution became yellow. The solvents were evaporated off to dryness, the residue was treated with 2 ml of methanol, and 30 ml of sodium tetrahydroborate was added in portions over 15 min. Then the reaction was continued for another 25 min. The product of the reduction of tetrahydrodihydronitraraine so obtained was identical with (I) according to TLC in systems 1 and 4 and IR spectroscopy.

## SUMMARY

The structure of the alkaloid dihydronitraraine from the epigeal part of Nitraria schoberi has been established. The configurations of all the symmetric centers in the nitraraine and dihydronitraraine molecules have been determined.

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# PURIFICATION OF NERVE GROWTH FACTOR FROM THE VENOM OF THE CENTRAL ASIAN COBRA *Naja oxiana*

D. Kh. Khamidov, R. S. Salikhov
M. G. Khafizova, and L. Ya. Yukel'son

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A highly purified electrophoreticaly homogeneous protein with a NGF activity of $10 \cdot 10^5$ BU*/mg of protein have been isolated from the venom of the Central Asian cobra by gel-filtration and ion-exchange chromatography followed by preparative isolectric focusing in a thin layer of Sephadex. It has been shown that the NGF isolated is characterized by a molecular weight in the range of 20-30 kD and a pI value of about 7.0.

Nerve growth factor (NGF) is a protein which is known for its capacity for stimulating the growth of nerve fibers with the formation of a so-called halo of axons in cultivated spinal ganglia of chick embryos. Various effects of NGF have been described in the literature and an analysis of these shows its importance in the development of the nervous system, especially the sympathetic innervation [1, 2]. As a molecular factor of development, NGF presents considerable interest, particularly for neurobiology and this explains the numerous attempts to obtain in the purified state from various tissues and biological fluids [2-4]. Snake venom is considered to be a rich source of NGF and, in the relevant literature [3], detailed information is given which characterizes methods of isolating snake venon NGF and its molecular properties. This information indicates the existence of NGFs differing in structure and properties from different sources. In this connection, attention must be directed to the absence of sufficient information relative to NGF in the venoms of Central Asian snakes. Only recently has there been publication of investigations characterizing the level of activity of NGF in these venoms and attempts to obtain it in the purified state [5-7]. The present paper gives the results of work on the purification of NGF from the venom of the Central Asian cobra *Naja oxiana* Eichw.

In the first stage of purification of the venon of the Central Asian cobra was fractionated by gel-filtration on Sephadex under the conditions developed previously in connection with the isolation of pure phospholipases $A_2$, and also neuro- and cytotoxins from this venom [8-12]. A standard separation of the venom into three fractions [I-III, Fig. 1) was achieved, but small changes in the parameters of the gel-containing column and the eluent, and also in the basic technique of recording the optical density of the eluate, which was performed continuously with the aid of a Uvicord II instrument (LKB, Sweden) permitted the identification of six protein fractions 1-6. Biological testing showed that the main zone of NGF activity corresponded to the area of peak 4, which contained components with molecular weights in the range of 20-30 kD. Slight NGF activity was also detected in fractions 3 and 5. The results of isolectric focusing on PAAG plates with Ampholines in the pH range of 3.5-9.5 showed the presence in fractions 4 to 20 of protein components with pI values between 6.0 and 9.5. In the light of the predominant presence in these fractions of basic components, the further purification of the NGF was performed by chromatography on CM-cellulose.

*BU signifies biological units – Publisher.

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