ALKALOIDS OF *Nitraria sibirica*. STRUCTURE AND ABSOLUTE CONFIGURATION OF SIBIRININE

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The chemical properties of sibirinine have been studied, its synthesis has been performed, and its ^{1}H and ^{13}C NMR spectra have been analyzed. From the results obtained its structure and absolute configuration have been deduced.

In the present paper we give the results of an investigation of the structure of a new alkaloid from the epigeal part of *Nitraria sibirica*.

Sibirinine (I) consists of a crystalline low-melting base which, on standing under normal conditions, changes into an oil; it is optically active $[\alpha]_D^{20}$ −9.4° (c 0.53; chloroform). Its composition, $C_{12}H_{21}NO_2$, and its molecular weight (211.1572) were determined by high-resolution mass spectrometry. The mass spectra of (I) revealed the following fragmentation, m/z (%): 211 (M^+; 24), 195 (M − 16, 67), 180 (M − 31; 81), 167 (M − C_2H_4O; 57), 166(27), 152(46), 150(80), 138(29), 124(98), 122(32), 111(43), 110(100), 99(62), and others. Figure 1 shows the presumed structures of some fragmentary ions, of which the composition was determined. The low intensity of the molecular ions, and also the ready detachment of 60 mass units was evidence in favor of an N-oxide structure for (I). Following the loss of oxygen (ion *a*, m/z 195.1619: $C_{12}H_{21}NO$, in Fig. 1) the ejection of CH_3 and CH—CH_3 groups (the ions b (180.1396, $C_{11}H_{18}NO$) and c (167.1315, $C_{10}H_{17}NO$, in Fig. 1) apparently take place in parallel. The further fragmentation is comparable with that of the alkaloids nitramine and isonitramine [1].

Under the conditions of Adams hydrogenation, sibirinine was transformed into a single end-product (after 19 h) the composition of which corresponded to a dihydrodeoxysibirinine (II), M^+ 197.1772, $C_{12}H_{23}NO$). A comparison of (II) with the product of the ethylation of isonitramine (III), see scheme 1) showed their identity. In the mass spectra of (II) the peaks of an ion with m/z 182, corresponding to the detachment of a methyl group $(M - CH_3)^+$ was the maximum ion. Chromatographic monitoring of the hydrogenation of (I) showed that two substances were formed in the reduction process, and with an increase in its duration the intermediate product was converted completely into the final N-ethylisonitramine. The mass spectrum of a mixture of the two products isolated from the initial substance after hydrogenation for 3 h contained the peak of a molecular ion (m/z 213) and the peak of an ion with m/z 197 due to the ejection of oxygen, which was the molecular ion for component (II), and also the intense peak of an ion with m/z 182. No peak with m/z 195 was observed in the spectrum. These facts permit the conclusion that the hydrogenolysis of the C_{12}—O bond took place first with the formation of N-ethylisonitramine, and this was followed by the reduction of the N-oxide bond. The reduction of sibirinine with zinc in acid led to a similar result.

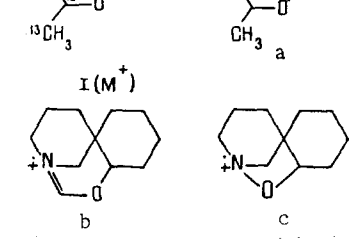

Fig. 1. Probable structures of ions in the mass spectrum of sibirinine.

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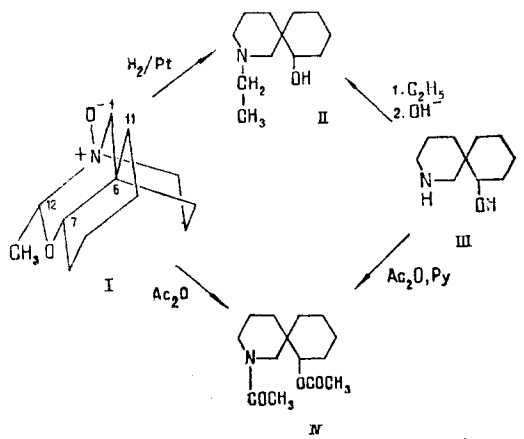
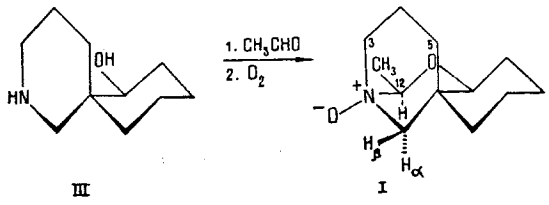


Scheme 1. Correlation of sibirinine (I) with isonitramine (III).

The PMR spectrum of sibirinine was fairly complex because of the mutual overlapping of the multiplets of a group of methylene protons. It was nevertheless possible to identify the most imformative signals. A doublet at 1.65 ppm with ^3J = 5.7 Hz and a quartet (1:3:3:1) with the lines split additionally (^4J = 1.2 Hz) at 4.58 ppm corresponded to the protons of a CH_3—CH— group. The protons of an isolated methylene group at C-1 appeared in the form of two doublets at 3.17 and 3.31 ppm with ^2J = —12.0 Hz. The lines of the first of them were appreciably broadened, while the second were narrow. When the signals were recorded with the use of a procedure for increasing resolution, it was found that the lines of the first doublet were split additionally into a doublet of doublets with ^4J 2.3 Hz each.

It was concluded from these results that H-12 had one long-range (W) interaction, one of the protons at C-1 two such interactions, and the other not even one. Analysis of the structure of sibirinine on Dreiding models showed that only in the case of the axial orientation of H-12 is agreement with the experimental results observed. Under these conditions, H_{ax} —12 (4.58 ppm) interacts with H_{ax} —3; H_α —1 (3.17 ppm) experiences two long-range spin-spin interactions with H_{eq} —3 and H_{eq} —5, while H_β —1 (3.31 ppm) can have no W-like interactions. In the alternative structure with equatorial H-12 a splitting of the signal from H_β —1 should have been expected, which was not in fact observed (see (I) in scheme 2).



Scheme 2. Synthesis of sibirinine.

Thus, the analysis of the PMR spectrum given above permits the conclusion that the methyl group in sibirinine is oriented equatorially, It must be mentioned that in the case of isonitramine (see (III) in schemes 1 and 2), the difference between the chemical shifts (CSs) of the protons at C-1 is 0.44 ppm [1], which is appreciably greater than that for sibirinine (0.14 ppm). This is apparently explained by the presence in the sibirinine molecule of a new oxazine ring, which is included in the oxazabicyclononane system A-C (see (i) in Fig. 1) with a common C-1 methylene group, and also by the presence of an N-oxide function.

The parameters of the ^{13}C spectrum of sibirinine in two solvents were given in Table 1. For comparison, the table includes information for isonitramine [1]. A comparison of the CS values for the carbon atoms in the α-position to the nitrogen (C-1 and C-3) in (I) and (III) shows that on sibirinine they are considerably descreened ($\Delta\delta \sim 16$ ppm) as compared with isonitramine. The magnitude of the downfield shift is of the same order as has been found in other alkaloid N-oxides (see, for example, [2]). Apparently, this may indirectly indicate a C-12 methine carbon, since in the spectrum of the related alkaloid nitramine the signal of the analogous carbon atom in the oxazine ring appears at 82.2 ppm [3]. A methyl group resonates in the form of a quartet at 14.4 ppm. The signals of the other carbon atoms in the spectrum of sibirinine are comparable with those of isonitramine (see Table 1).

TABLE 1. Chemical Shifts of the Carbon Atoms in the Spectra of (I) and (III), ppm, O —TMS*

Substance	Carbon atom							Solvent
	C-1 (t)	C-3 (t)	C-6 (s)	C-7 (d)	C-12 (d)	C-13 (q)	C-4, 5, 8–11 (triplets)	
Sibirinine	77,1	62,0	38,1	84,3	101,9	14,4	34,5; 26,8; 26,1; 24,6; 21,0; 19,0	$CDCl_3$
Sibirinine	77,5	62,6	38,2	84,0	102,1	14,6	34,5; 26,8 26,4; 24,8 21,4; 19,7	$Py-d_5$
Isonitramine	60,3	47,3	36,2	79,8	—	—	36,3; 29,8; 28,7; 24,3; 23,1; 20,4	$CDCl_3$

*The latters in parentheses signify multiplicity in the "off-resonance" spectrum; S — singlet, d — doublet, t — triplet, q — quartet.

Under the action of acetic anhydride, sibirinine underwent a Polonovski transformation [4]. The only product of the reaction was diacetylisonitramine (IV, see scheme 1), which was identified by a direct comparison with an authentic sample obtained from isonitramine.

With the aim of demonstrating the structure of sibirinine unambiguously, we performed the synthesis of compound (I) from isonitramine and acetaldehyde followed by oxidation with atmospheric oxygen (see scheme 2). The synthetic product (I) was identical in TLC, specific rotation and PMR and mass spectra with the natural alkaloid sibirinine. This shows the stereoselectivity of the synthesis performed: of the two possibilities in the formation of the new center at C-12, the one with the equatorial methyl group is formed.

Starting from the known absolute configuration of isonitramine [5], we analyzed the structure of (I) assembled from Dreiding models and found that sibirinine has the following absolute configuration: C-6(S), C-7(S), N-2(S), C-12(R).

EXPERIMENTAL

IR spectra were taken on a UR-20 instrument with the substances in the form of films. Mass spectra were obtained on a MKh-1310 spectrometer. ^1H and ^{13}C NMR spectra were recorded on Tesla BS-567A (100 MHz) and XL-200 Varian spectrometers in $CDCl_3$ and C_5D_5N using TMS as internal standard. The chromatographic conditions are given in the description of the experiments. Specific rotations were determined on a Lippich-Landolt polarimeter at 20°C.

Isolation of Sibirinine. The material from the mother solution of pH 7 and 8 fractions after the separation of isonitramine [1] (1.1 g) was chromatographed on a column of silica gel (KSK) with elution by ether. Fractions with a volume of 200 ml were collected, and fractions 18-25 were combined (0.56 g) and rechromatographed on a column on alumina also with elution by ether (100-ml fractions). Fractions 4-9 yielded 0.18 g (0.00045% on the weight of the dry plant) of sibirinine, consisting of crystals in a colorless oil of identical composition. After repurification, mp 40°C, M^+ 211 [6].

Hydrogenation of Sibirinine. a) A solution of 0.05 g of sibirinine in 3 ml of ethanol was treated with 0.05 g of platinum oxide and was saturated with hydrogen under constant shaking. The course of the reaction was monitored by TLC in the chloroform—methanol (1:1) system; after 0.5 h a spot with an impurity nature having R_f ∿0.1 had formed, but the bulk (∿90%) of the initial material remained unchanged; after 1 h a second product arose with R_f ∿0.2; its amount increased with time (5 and 10 h) and that of the starting material and the first product decreased. After 19 h, the second product was the only remaining component of the reaction.

The catalyst was separated off and the solvent was evaporated, and the residue was treated with 2 ml of water and, after alkalinization with a concentrated solution of ammonia, the product was extracted with ether. The extract was dried over anhydrous sodium sulfate and the ether was distilled off. This gave 0.035 g (yield 75%) of an oily product. Mass spectrum m/z (%): 197 (M^+,28), 196(21), 182(100), 169(12), 168(15), 154(12), 152(8), 150(3), 138(6), 137(20), 126(7), 124(23), 110(6), 98(4), 96(4), 84(5). Main bands in the IR spectrum, ν_{max}: 3420-3280, 2935, 2860, 2810, 1455, 1145, 1100 cm^{-1}.

b) Sibirinine (0.005 g) was dissolved in 0.5 ml of 15% hydrochloric acid, and zinc dust was added in 0.01-g portions. Hydrogen was slowly evolved. The reaction lasted 1.5 h, after which the mixture was made alkaline with concentrated ammonia solution and the products were extracted with ether and then, exhaustively, with chloroform. The result was similar to that obtained in the preceding experiment: the mixture consisted of the starting material and two reaction products (TLC).

c) Sibirinine (0.01 g) was subjected to hydrogenation under the conditions of experiment a). The reaction ceased after 3 h; the mixture isolated was chromatographed on a column of silica gel in the system mentioned above. This gave 0.004 g of the initial sibirinine and 0.003 g of a mixture of two reduction products. Mass spectrum, m/z (%): 213 (M^+, 16), 197(17), 182(39), 169(12), 168(11), 151(16), 150(12), 149(40), 137(26), 125(32), 124(23), 123(30), 111 (62), 110(30), 109(45), 97(90), 96(50), 95(66), 85(82), 84(48), 83(100), 82(44), 81(76).

N-Ethylisonitramine. A solution of 0.011 g of isonitromanine in 1 ml of ethanol was treated with 0.5 ml of freshly purified ethyl iodide, and the mixture was boiled under reflux for 2 h. The solvent and excess of the reagent were evaporated off to dryness. The residue was treated with 1 ml of water, the mixture was made alkaline with dilute caustic potash solution, and the reaction product was extracted with ether. After drying over anhydrous sodium sulfate, filtration, and elimination of the solvent, 0.007 g (55%) of N-ethylisonitramine was obtained. Mass spectrum, m/z (%): 197 (M^+, 27), 197(20), 182(100), 169(8), 168(13), 154(9), 152(4), 150(3), 138(4), 137(12), 126(7), 124(12), 110(8), 98(13), 96(13), 84(16). According to TLC and IR spectroscopy, this was identical with the product of the hydrogenation of sibirinine.

Reaction of Sibirinine with Acetic Anhydride. A mixture of 0.017 g of sibirinine and 2.5 ml of freshly distilled acetic anhydride was boiled for 1 h for 20 min. The excess of anhydride was eliminated in vacuum, and the residue was fractionated between ether and saturated aqueous $NaHCO_3$ solution and was then exhaustively extracted with chloroform. The ethereal and chloroform extracts were combined, dried over anhydrous sodium sulfate, filtered from the desiccant, and the extractants were evaporated off. This gave 0.016 g of an oily product. Yield 78%. Mass spectrum, m/z (%): 253 (M^+, 55), 210(20), 219(100), 194(28), 193 (68), 168(28), 164(28), 152(30), 151(32), 150(39), 138(33), 124(32), 122(63), 110(15), 109 (16), 96(35), 83(15), 82(23), 81(25), 80(20), 79(24). IR spectrum, ν_{max}: 2940, 2870, 1740, 1650, 1450, 1375, 1250, 1145 (w), 1040, 1000 (w), 965 (w), 940 (w), 870 (w), 800 (w), cm^{-1} (w — weak bands, the others being strong).

N,O-Diacetylisonitramine. A mixture of 0.02 g of isonitramine and 1 ml of acetic anhydride was left at room temperature for two days. Practically no reaction took place (TLC); 0.4 ml of pyridine was added to the reaction mixture and acetylation was continued for another two days under the same conditions. The pyridine and the excess of anhydride were eliminated in a rotary evaporator, and the residue was fractionated between ether and saturated $NaHCO_3$ solution and then the product was isolated by the procedure described above. This gave 0.017 g of oily N,O-diacetylisonitramine. Yield 56%, mass spectrum, m/z (%): 253 (M^+, 48), 211(21), 210(100), 194(21), 193(75), 168(40), 164(39), 152(40), 151(51), 150(55), 138(55), 124(14), 122(60), 110(15), 109(18), 96(40), 83(25), 82(30), 81 (36), 80(18), 79(44).

IR spectrum, ν_{max}: 2942, 2870, 1740, 1650, 1450, 1377, 1250, 1145 (w), 1040, 1000 (w), 970 (w), 938 (w), 870 (w), 800 (w) cm^{-1}.

Synthesis of Sibirinine. To 0.03 g of isonitramine was added 1 ml of cooled acetyaldehyde; the reaction solution was left in the refrigerator overnight. After 16 h, the excess of the aldehyde was distilled off and the residue was dissolved in a small volume of chloroform—methanol (1:1) and was chromatographed in this system on a column (12 × 140 mm) of type KSK silica gel with a particle size of 50-90 mμ. Fractions with a volume of 8-10 ml were collected; fractions 4-6 were combined, the solvents were distilled off, and the residue was dried in the vacuum of water pump. This gave 0.008 g of pure condensation product. After it had been kept at room teperature for a month, a substance had formed which, according to TLC, specific rotation ($[\alpha]_D$ −9.8°; c 0.31; chloroform) and PMR and mass spectroscopy was identical with natural sibirinine.

CONCLUSIONS

The structure and absolute configuration of the alkaloid sibirinine have been established on the basis of the results of a study of its chemical properties and its ^1H and

^{13}C NMR spectra of a correlation with isonitramine, and also by direct comparison with a synthesized sample.

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ALKALOIDS OF *Aconitum coreanum*. I. STRUCTURE OF ACORINE

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Two C_{20}-diterpene alkaloids have been isolated from the epigeal part of *Aconitum coreanum* (Levl.) Rapaics: the known 14-hydroxy-2-isobutyrylhetesine (I) (Guan-Fu base Z) and a new one — acorine (II), $C_{22}H_{29}NO_5$, mp 214-215°C (from acetone), α_D^{20} + 9° (c 0.01; methanol), for which the structure of 2-acetyl-14-hydroxyhetesine has been established. Details of the IR, mass, and ^{13}C NMR spectrum of (I) and (II) and of the PMR spectrum of (II) are given.

Korean monkswood (*Aconitum coreanum* (Levl.) Rapaics (A. Komarovii Steinb.) is occasionally found in low oak woods and thickets of Siberian filbert of the southern part of the Maritime Territory [1, 2], in north-eastern China, and on the Korean peninsula. A crystalline hydrobromide of a base with the composition $C_{35}H_{41}NO_{10}$ has been isolated previously from the epigeal part of this plant [3]. The presence of hypoconitine and a number of new alkaloids that are acyl derivatives of 14-hydroxyhetesine in it has been established [4].

We have investigated the epigeal part of the plant collected in the environs of the village of Chernyatino, Maritime Territory, in the flowering period. The amount of combined alkaloids was 0.8% on the weight of the air-dry raw material. By chromatography, from the mixture of alkaloids we obtained two individual crystalline bases with mp 229-230°C (I) and 214-215°C (II). Base (I) had the composition $C_{24}H_{33}NO_5$ (M^+ 415.2358 (HRMS)). Its IR spectrum contained absorption bands of hydroxy and carbonyl groups (3400 and 1745 cm^{-1}). Its PMR spectrum showed signals the chemical shifts and multiplicities of which agreed completely with those published for Guan-Fu base Z, mp 230-231°C, isolated from Korean monkswood growing in China [5]. For the latter the structure of 14-hydroxy-2-isobutyrylhetesine has been established on the basis of the results of an analysis of its 1-D and 2-D NMR spectra. The strongest peaks in the mass spectrum of (I) were the following (m/z, %): 415(82), 398(88), 387(89), 370(100), and 328(70). The results obtained indicate that the base obtained was identical with the Guan-Fu base Z.

Base (II), with the composition $C_{22}H_{29}NO_5$ (M^+ 387.2038 (HRMS)) was not optically active; it had the same absorption bands in the IR spectrum as (I): 3400 cm^{-1} (OH) and 1745 cm^{-1} (C=O). A comparison of the PMR spectra of (I) and (II) showed that they were close, differing by the fact that in the spectrum of (II), in place of the signals of the isobutyryl substituent (δ 2.47 and 1.12 ppm), there was a singlet from the protons of an acetoxy group at 1.99 ppm.

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