STRUCTURE AND CONFIGURATION OF SEVERTZIDINE

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We have continued an investigation of the alkaloids of the epigeal part of Korolkovia sewertzovii Rgl. [1-3] collected at Saryagach near Tashkent in the budding and incipient flowering period. A mixture of bases was extracted from the plant with chloroform. Separation of the combined alkaloids yielded sevkorine, korseveriline, korseveridine, korseverinine [4, 5], a base with mp 212-214°C, and a new alkaloid – severtzidine with mp 244-245°C $[\alpha]_D$ – 46.4°, $C_{27}H_{45}NO_3$ (I). Severtzidine forms a hydrochloride with mp 224-226°C.

Severtzidine is a tertiary saturated base containing two secondary and one tertiary hydroxyls. The IR spectrum of the base shows absorption bands at (cm^{-1}) 3450 (hydroxy groups), 2960-2860 ($-CH_3$, $-CH_2-$), and 2785 (trans-quinolizidine); the fingerprint region of the spectrum of (I) is similar to that of the C-nor-D-homosteroid alkaloid korseveramine [6].

The mass spectrum of (I) shows peaks of ions with m/e 98, 111 (100%), 112, 124, 125, 126, 128, 138, 139, 140, 150, 159, 162, 164, 178, 180, 234, 258, 272, 274, (M–71), (M–56), (M–55), (M–33), (M–29), (M–18), (M–15), and 431 M^+, which are characteristic for the C-nor-D-homosteroid alkaloids of the cevine group [7, 8].

The acetylation of severtzidine with acetic anhydride in pyridine yielded diacetylsevertzidine (II). The IR spectrum of (II) showed absorption bands at (cm^{-1}) 3500 (hydroxy group) 1742 and 1245 (ester C=O).

The oxidation of severtzidine with chromium dioxide formed a diketone – severtzidinedione, $C_{27}H_{41}NO_3$ (III). The UV spectrum of the diketone, with λ_{max} 250, 305 mμ (log ε 2.60, 2.07), is characteristic for diketones. The IR spectrum of (III) shows absorption frequencies at 3570 cm^{-1} (hydroxy group) and 1715 cm^{-1} (carbonyl in a six-membered ring). The characteristics of the NMR spectra of compound (I)-(III) are given in Table 1.

Thus, in severtzidine and its conversion products there are two secondary and one tertiary methyl group, and it contains the sevanine skeleton [9-15].

The chemical shifts (CSs) of the 19-CH_3 protons vary by 2-6 Hz in the NMR spectra of (II) and (III) with a change in the substituents, but no appreciable changes were observed in the CSs of the 21-CH_3 and 27-CH_3 protons in the presence of acetyl and carbonyl groups. Consequently, in severtzidine the secondary hydroxy groups can be present only in rings A, B, and C [15, 16]. By comparing the signal from the 19-CH_3 and 21-CH_3 protons of compounds (I-III) with those of imperialine (korseveramine [5, 6, 15], and korsinine [17]) it may be concluded that rings A/B, B/C, and D/E are trans-linked and C/D cis-linked, as in cevine [7, 8].

In the IR spectrum of severtzidine the trans-quinolizidine band shows that the E and F rings are trans-linked. Ring C is excluded for a hydroxy group because of the absence of the absorption of a carbonyl in a five-membered ring in the spectrum of (III). In the IR spectra of severtzidine and diacetyl severtzidine there are absorption bands at 1060 and 1037 cm^{-1}, respectively, showing the presence of a β-oriented hydroxy group at C_3. This is confirmed by the presence of a multiplet at 4.65 ppm for C-3αH in the NMR spectrum of diacetylsevertzidine [18, 19].

On comparing the CSs of the protons of the C-19 methyl groups in compounds (I-III) with those of korsinine [17], a position at C_6 and the β orientation may be proposed for the secondary hydroxy group, as is also shown by the presence in the NMR spectra of diacetylsevertzidine of a multiplet at 4.98 ppm from 6αH [15]. According to the NMR spectra of (I-III), the tertiary hydroxy group does not affect the CSs of the 19-CH_3, 21-CH_3, and 27-CH_3 protons. Consequently, the tertiary hydroxy group may be present at C_{14} as in the known alkaloids korseveriline and korseveramine [5, 6] and it has the α orientation.

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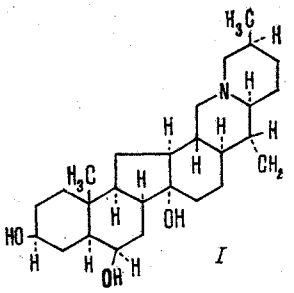
TABLE 1. Chemical Shifts (δ, ppm)

Substance	19-CH_3, s	21-CH_3, d	27-CH_3, d	H, H-C-OH, m	3H, OCO-CH_3, s	3H, -OCO-CH_3, s	H, CH-O-$COCH_3$, m	H, CH-$OCOCH_3$, m
I	0,97	0,76	1,05	3,77	1,99	2,01	4,65	4,98
II	0,99	0,76 J=7 Hz	1,04 J=7 Hz					
III	0,91	0,78	1,04					

Note. s) singlet; d) doublet; m) multiplet; the spectrum of substance (I) was recorded in $CDCl_3$ + CD_3OD and those of (II) and (III) in $CDCl_3$.

It follows from the CSs that the 21-CH_3 group is oriented α-equatorially and the 27-CH_3 group β-axially [11]. Hence it follows that severtzidine differs from korseveramine [6] by the orientation of the hydroxy groups at C_3 and C_6 and of the methyl group at C_{25}, and from korseveriline [5] by the orientation of the hydroxy group at C_3 and the methyl group at C_{25}. A confirmation of this is the fact that severtzidinedione is not identical with korseverilinedione. According to these facts, severtzidine has the most probable structure and configuration of 3β, 6β,14α-trihydroxycevanine (I).



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EXPERIMENTAL

Thin-layer chromatography (TLC) was performed with KSK silica gel (100 mμ) alumina (activity grade II) and the ethyl acetate–chloroform–methanol (15 : 10 : 3) solvent system, the chromogenic agent being Dragendorff's solution. The UV spectrum was taken on a Hitachi spectrophotometer, the IR spectra on a UR-20 double-beam spectrophotometer (molded tablets with KBr), the mass spectra on an MKh-1303 mass spectrometer, and the NMR spectra on a JNM-4H-100-MHz instrument with HMDS as internal standard (δ scale).

Isolation of the Alkaloids. The epigeal part of K. sewertzovii (30 kg) was moistened with a 10% solution of ammonia and extracted with chloroform until the bases had been completely removed. The concentrated chloroform extract was repeatedly treated with 10% sulfuric acid. The acid solution was shaken out with ether and was then made alkaline with ammonia, and the alkaloids were extracted with benzene (149 g) and with chloroform (258 g).

The chloroform extract of combined alkaloids deposited sevkorine (0.4 g), mp 236-238°C (methanol). The total yield of combined alkaloids was 407.4 g (1.35% of the weight of the dried plant).

From the benzene solution of combined alkaloids, on concentration, 10.7 g of a mixture of crystals with mp 240-270°C deposited. After the separation of the mixture of crystals, 138 g of the residue from the evaporation of the mother solution was dissolved in benzene and was separated by means of citrate-sulfate buffer solutions with pH 8-3 (intervals of 1 unit). In the pH 8 extract an oil formed, which was taken up in chloroform (53 g). Then it was dissolved in 250 ml of 5% sulfuric acid and the said solution was washed with petroleum ether (70-100°C) and was then made alkaline with ammonia and extracted with ether (35 g) and with chloroform. The ether-soluble fraction was dissolved in 100 ml of chloroform and passed through a column of alumina (*l* 60 cm, d 2.6 cm) and eluted with chloroform in 30-ml portions, 29 fractions being collected. Then elution was continued with mixtures of chloroform and methanol in ratios of 40 : 1, 20 : 1, and 4 : 1 in 100-ml portions.

Korseveridine. Chloroform fractions 1-9 were combined and evaporated, and when the residue was treated with acetone a mixture of two substances was obtained with R_f 0.47 and 0.60 (alumina), mp 271-273°C. The mixture of two substances was separated by fractional recrystallization, and korseveridine with mp 290-292°C (methanol), R_f 0.47 was isolated.

Korseverinine. A solution of the material of fraction 10-15 in acetone deposited a mixture of substances with mp 316-318°C which was passed through a column of alumina. A chloroform–methanol (15 : 1) eluate yielded korseverinine with mp 231-323°C (methanol).

Korseveriline. Fractions 25-29 of the chloroform eluate were also combined and evaporated to dryness. On treatment with methanol, korseveriline deposited with mp 240-242°C (methanol).

Severtzidine. After the separation of the oily fraction, the buffer solution with pH 8 was made alkaline with ammonia and extracted with chloroform. The residue from the distillation of the chloroform (10 g) was dissolved in 100 ml of 5% sulfuric acid. The acid solution was washed with ether. Then it was made alkaline with ammonia and was extracted with benzene (6 g), ether (2.3 g), and chloroform (1.3 g). The benzene-soluble fraction was chromatographed on a column of silica gel, and eluted with ether–methanol (1 : 1). The first 100 ml of eluate yielded a base with mp 212-214°C (acetone). The last 100 ml of ether–methanol elutates yielded severtzidine with the composition $C_{27}H_{45}NO_3$ mp 244-245°C (acetone), $[\alpha]_D$ –46.4° (c 0.69; chloroform), M^+ 431.

Severtzidine Hydrochloride. When 0.03 g of the base was treated with an ethanolic solution of hydrogen chloride it yielded severtzidine hydrochloride with mp 224-226°C (methanol–acetone; 1 : 5).

Acetylation of Severtizdine. A mixture of 0.06 g of severtzidine, 1 ml of pyridine, and 2 ml of acetic anhydride was kept at room temperature for 3 days and was then evaporated in vacuum. The residue was dissolved in 5% sulfuric acid and this solution was made alkaline with ammonia and extracted with chloroform, and the chloroform extract was washed with water and evaporated to dryness. Amorphous diacetylsevertzidine was obtained with R_f 0.24 (silica gel). M^+ 515.

Oxidization of Severtzidine. A solution of 0.12 g of chromium trioxide in 3 ml of 80% acetic acid was added to a solution of 0.17 g of severtzidine in 3 ml of acetic acid. The mixture was heated on the water bath for 30 min and was then evaporated in vacuum, the residue was dissolved in water, and the solution was made alkaline and was extracted with chloroform. The oxidation product was chromatographed on a column of alumina and was diluted with chloroform–methanol (20 : 1). This gave the diketone severtzidinedione with mp 137-139°C (with foaming) (acetone–petroleum ether; 1 : 5); M^+ 427.

SUMMARY

1. From the combined alkaloids of the epigeal part of Korolkowia sewertsovii Rgl. collected at Saryagach we have isolated sevkorine, korseveriline, korseveridine, korseverinine, a base with mp 212-214°C, and the new alkaloid severtzidine.

2. On the basis of a study of the chemical and physical characteristics of the alkaloids itself and the products of its transformations, the most probable structure and configuration of severtzidine has been established as 3β,6β,14α-trihydroxycevanine.

LITERATURE CITED

1. S. Yu. Yunusov, R. A. Konovalova, and A. P. Orekhov, Zh. Obshch. Khim., 1911 (1939).
2. G. Kittél and A. S. Sadykov, Dokl. Akad. Nauk UzSSR, 11 (1948).
3. R. N. Nuriddinov and S. Yu. Yunusov, Dokl. Akad. Nauk UzSSR, 47 (1962).
4. R. N. Nuriddinov and S. Yu. Yunusov, Khim. Prirodn. Soedin., 60 (1968).
5. R. N. Nuriddinov and S. Yu. Yunusov, Khim. Prirodn. Soedin., 101 (1968); 258 (1968); 767 (1971).
6. R. N. Nuriddinov and S. Yu. Yunusov, Khim. Prirodn. Soedin., 773 (1971).
7. R. N. Nuriddinov, R. Shakirov, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 316 (1967).
8. H. Budzikiewicz, Tetrahedron, 20, 2264 (1964).
9. IUPAC-IUB Revised Tentative Rules for the Nomenclature of Steroids, J. Org. Chem., 34, 1517 (1969).
10. F. Bohlman, Ber., 91, 2157 (1958).
11. T. M. Moynehan, K. Schofield, R. A. V. Jones, and A. R. Katritzky, J. Chem. Soc., 2637 (1962).
12. H. Mohrle, C. Karl, and U. Schedegger, Tetrahedron, 24, 6813 (1968).
13. S. Ito, J. B. Stothers, and S. M. Kupchan, Tetrahedron, 20, 913 (1964).
14. F. Bohlmann, D. Schumann, and C. Arndt, Tetrahedron Lett., 31, 2705 (1965).

15. R. N. Nuriddinov, A. I. Saidkhodzhaev, M. R. Yagudaev, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 333 (1968).
16. R. F. Zürcher, Helv. Chim. Acta, 46, 2054 (1963).
17. R. N. Nuriddinov, A. I. Saidkhodzhaev, and S. Yu. Yunusov, Khim. Prirodn. Soedin., 61 (1969).
18. I. Fried, P. Grabowich, E. F. Sabo, and A. I. Cohen, Tetrahedron, 20, 2297 (1964).
19. M. L. Mihailovic, L. Lorens, M Rogic, A. Melera, and M. Stefanovic, Tetrahedron, 22, 2345 (1966).

AN INVESTIGATION OF THE CONFORMATIONAL STATES OF THE METHYLAMIDE OF N-ACETYL-L-HISTIDINE

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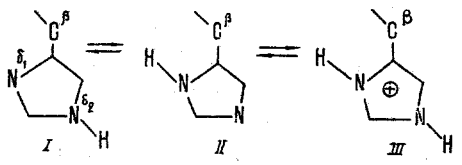
The present communication continues a series of communications devoted to the role of the short-range interactions in the formation of the structure of the peptide chain in proteins [1-7]. As had been reported, methylamides of N-acetylpeptides can serve as suitable objects for the investigation of the specific features of the interaction of the side chains of natural α-amino acids with adjacent peptide groups. In the present case, the conformational states of the methylamide of N-acetyl-L-histidine (AcL-His-NHMe) are considered. The increased interest in an investigation of the spatial structure of His is due to the essential participation of this residue in the acid–base stage of the enzymatic reactions of many proteins (chymotrypsin, elastase, carboxypeptidase, ribonuclease, etc.).

With respect to the nonvalent interactions of the atoms of the side and main chains, the methylamide of N-acetyl-L-histidine is a stereochemical analog of the methylamide of N-acetyl-L-phenylalanine, the calculation of which has been published previously [4]. However, the side chain of His has a number of specific features which may lead to conformational states of this residue substantially differing from the states of Phe and the other analogs of it–Trp and Tyr. Thus, the imidazole ring of histidine, unlike the benzene ring, may take part in considerable electrostatic interactions with the main chain, and also form hydrogen bonds. Under physiological conditions, the imidazole ring may exist in the protonated and in the nonprotonated states in equal measure. The capacity of His for undergoing protonation changes insignificantly if the residue is included in a branched peptide chain [8]. In addition to this, in the neutral form of the imidazole ring, the hydrogen atom can form covalent bonds with the N^{δ_1} and with the N^{ε_2} atoms with almost equal probability. Thus, in the His side chain an equilibrium is set up between the two neutral tautomeric forms (I and II) and the ionic form (III). It is quite realistic to expect stereochemical differences in the interactions of the main chain with the free forms of the side chain. In its turn, the conformational state of the His residue may have a definite influence on the stability of the above-mentioned forms of the imidazole ring. Consequently, we have performed a conformational analysis of the methylamide of N-acetyl-L-histidine with all the possible forms of the side chain.

Model of the Molecule and Potential Functions. A model of the molecule of the methylamide of N-acetyl-L-histidine is shown in Fig. 1. The null values for the listed parameters $\varphi(C^\alpha - N)$, $\psi(C^\alpha - C')$, $\chi_1(C^\alpha - C^\beta)$ and $\chi_2(C^\beta - C^\gamma)$ were selected in accordance with the accepted nomenclature [9]. The strength of the bonds in the main chain were taken as equal to the Pauling–Corey parameters [10], and the valence angles as the average parameters for a peptide group [3]. The geometry of the imidazole ring changed in dependence on its form. For



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