STRUCTURE AND CONFIGURATION OF KORSEVERININE

R. N. Nuriddinov and S. Yu. Yunusov

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From the epigeal part of Korolkowia sewerzowii Rgl. we have isolated an optically active tertiary base – korseverinine, $C_{27}H_{43}O_2N$, which forms a hydrochloride, hydrobromide, hydriodide, and methiodide as crystalline compounds [1].

The IR spectrum of the alkaloid shows characteristic absorption bands at (cm^{-1}) 3420, 3180, 1085, 1060, 1030, and 990 (hydroxy groups), 2980-2830, 1470, 1430 (C-methyl groups), 2790 (trans-quinolizidine), and 1620 (double bond) (Fig. 1).

Korseverinine is an unsaturated compound which in weakly acid solution instantaneously decolorizes a dilute solution of potassium permanganate. In the alkaloid the oxygen atoms are present in the form of secondary hydroxy groups, as is confirmed by the preparation of a diacetyl derivative. The IR spectrum of the latter shows absorption at (cm^{-1}) 1735, 1725, and 1270-1230 (acetyl carbonyl groups), 2960-2835 and 1465-1490 (C-methyl groups), and 2750 (trans-quinolizidine) and lacks the absorption band of a hydroxy group, while in the NMR spectrum there are the signals of protons of secondary carbon atoms to which acetyl groups are attached at 5.04 and 4.62 ppm [2].

In addition, the oxidation of korseverinine with chromium trioxide in acetic acid forms korseverininone and korseverininedione. The IR spectrum of korseverininone shows absorption at (cm^{-1}) 3390 and 1060 (hydroxy group), 2980-2860 and 1460-1420 (C-methyl group), 1710 (carbonyl group), and 2750 (trans-quinolizidine), and the IR spectrum of korseverininedione absorption at 2960-2860 and 1460-1415 (C-methyl groups), 1695-1710 (carbonyl groups), and 2755 (trans-quinolizidine), and lacks the absorption of a hydroxy group.

Under the conditions of mass spectrometry, korseverinine gives fragments with m/e 98, 111, 112, 125, 139, 162, 164 $(M-29)^+$, $(M-15)^+$ and 413 (M^+), which are characteristic for the C-nor-D-homosteroid alkaloids of the cevine group [3, 4] (Fig. 2).

The fragmentation of korseverininone and korseverininedione under mass-spectrometry conditions takes place in the same way as that of korseverinine. Their mass spectra differ only with respect to the molecular ions.

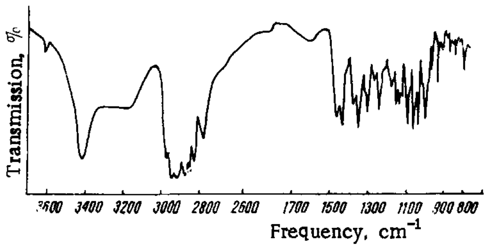

Fig. 1. IR spectrum of korseverinine.

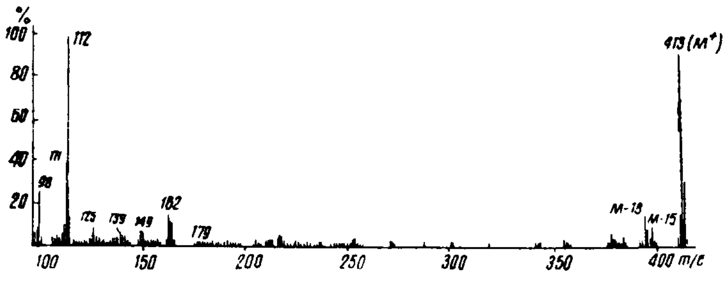

Fig. 2. Mass spectrum of korseverinine.

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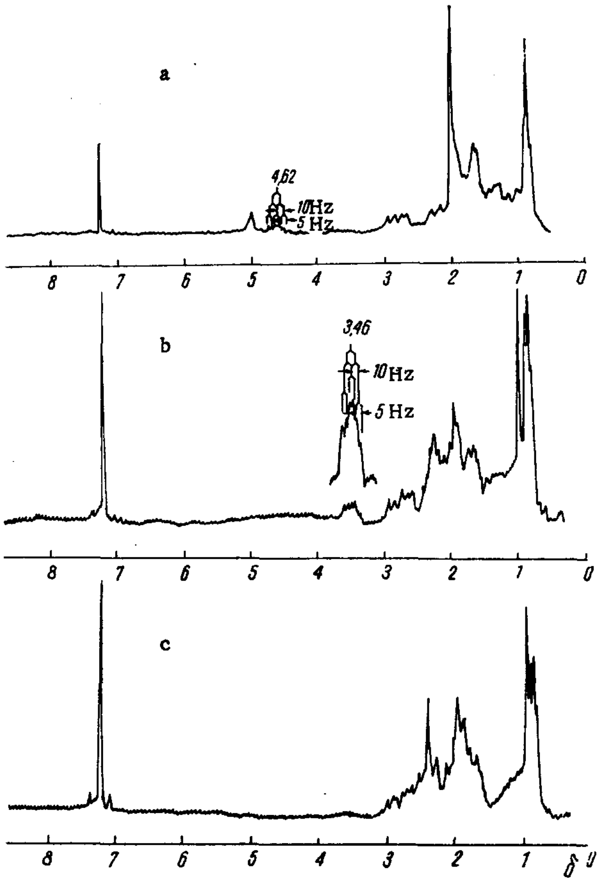


Fig. 3. NMR spectra of diacetylkorseverinine (I) (a), korseverininone (II)(b), and korseverininedione (III)(c).

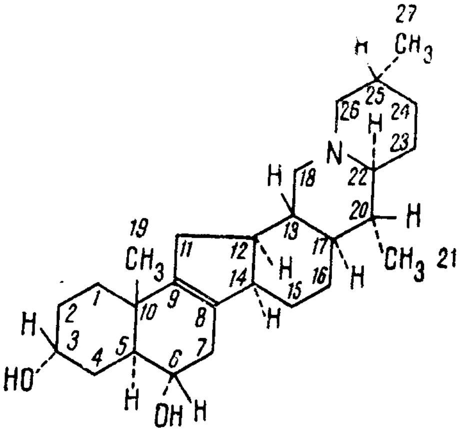
Consequently, korseverinine has the heterocyclic skeleton of cevine. This is also confirmed by the IR spectrum, which shows that its molecule contains a trans-quinolizidine system, and by the characteristic values of the chemical shifts of the equivalent 19-CH_3, 21-CH_3, and 27-CH_3 protons in the NMR spectra of diacetylkorseverinine, korseverininone, and korseverininedione [6-10] (Fig. 3).

The NMR spectrum of korseverinine was not recorded because of the poor solubility of this substance in benzene, deuterochloroform, and pyridine.

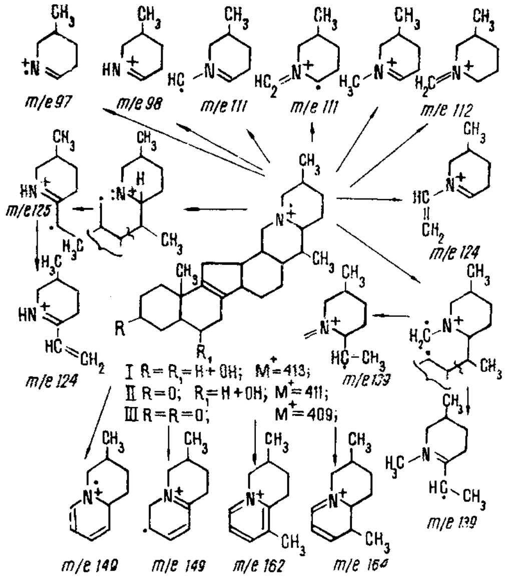
The NMR spectrum of diacetylkorseverinine shows singlets at (ppm) 0.85 (3H, 19-CH_3), 2.0 (3H, $OCOCH_3$), and 1.98 (3H, $OCOCH_3$), doublets at 0.85 (3H, 21-CH_3), and 0.80 (3H, 27-CH_3), a multiplet at 5.04 (H_e, $H-COOCCH_3$), and a signal split into six lines at 4.62 (H_a, $HCOOCCH_3$). The NMR spectrum of korseverininone has a singlet at (ppm) 0.99 (3H, 19-CH_3), doublets at 0.85 (3H, 21-CH_3), and 0.8 (3H, 27-CH_3), and a one-proton signal split into six lines at 3.46 ppm (H, $H-\overset{|}{\underset{|}{C}}-OH$), while in the NMR spectrum of korseverininedione there are a singlet at (ppm) 0.93 (3H, 19-CH_3), and doublets at 0.85 (3H, 21-CH_3), and 0.8 (3H, 27-CH_3) [2, 6-10]. It can be seen from what has been said above that with a change in the substituents in the korseverinine molecule the chemical shifts of the 19-CH_3 protons change while the 21-CH_3 and 27-CH_3 protons undergo absolutely no similar influences. This shows that in korseverinine both hydroxy groups are located on carbon atoms of rings A, B, and C. Korseverinine and the products of its transformations undergo under mass-spectrometry conditions fragmentation characteristic for the saturated quinolizidine systems of the C-nor-D-homosteroid alkaloids [3, 4]. This pattern of decomposition, and also the absence from the NMR spectra of diacetylkorseverinine and the products of the oxidation of korseverinine of the signal of an olefinic proton, permit the assumption that in korseverinine the double bond is located in ring C. According to the IR spectra, in korseverininone and korseverininedione the carbonyl groups have the absorption frequencies characteristic of such groups in a six-membered ring. Consequently, position 11 in ring C is excluded for the hydroxy group.

The results of a comparison of the chemical shifts of the 19-CH_3 protons of diacetylkorseverinine and korseverininedione with those of diacetylisoimperialine and imperialine, respectively, show that the hydroxy groups in korseverinine are located at C_3 and C_6. The downfield shift of the signals in the korseverinine derivatives by 6 Hz takes place because of the presence of a double bond between C_8 and C_9 [9-11]. In the NMR spectrum of korseverininone, the signal from the 19-CH_3 group shifts downfield by 6 Hz as compared with the corresponding signal in the spectrum of korseverininedione.

Such a shift is observed in the oxidation of an α-oriented equatorial hydroxy group of steroid compounds in position 6. This is confirmed by a one-proton signal at 3.46 ppm (in the NMR spectrum of korseverininone) split into six lines from the axial hydrogen. The latter is located on a carbon atom to which a hydroxy group is attached. This splitting off of the signal from the C_6 proton takes place through the interaction of the two axial protons at C_5 and C_7 and the equatorial proton at C_7. The spin–spin coupling constants of 5 and 10 Hz so arising show this interaction. In diacetylkorseverinine the signal from this proton is considerably shifted downfield, being found at 4.62 ppm, and it has the same order of splitting [2, 12, 13].



As already mentioned, the NMR spectrum of diacetylkorseverinine has two one-proton signals from equatorial and axial protons attached to the carbons bearing the acetyl groups [2]. If the axially oriented proton is located at C_6, the equatorial proton must be at C_3. Then the hydroxy group at C_3 will have the axial orientation.



The values of the signals of the protons of the C_3-methyl groups in the NMR spectra of diacetylkorseverinine, korseverininone, and korseverininedione show that in korseverinine the linkage of the rings is the same as in the alkaloids of the cevine group [6-10]. The presence of a trans band in the IR spectra of the alkaloid and its transformation products shows the trans linkage of rings E/F [5]. From the values of their signals in the NMR spectra, the 21-CH_3 and 27-CH_3 groups have the equatorial orientation.

The facts given above enable us to put forward for korseverinine the most probable structure and configuration shown above.

A scheme of the fragmentation of korseverinine (I), korseverininone (II), and korseverininedione (III), is given on the preceding page.

EXPERIMENTAL

The IR spectra were taken on a UR-20 spectrometer (potassium bromide), the mass spectra on an MKh-1303 instrument, and the NMR spectra on a JNM-4H-100 MHz instrument with HMDS as internal standard (δ scale).

The fresh epigeal part of Korolkowia sewerzowii (22 kg) collected in the flowering stage at Saryagach, near Tashkent, was comminuted. The resulting porridge-like green mass was repeatedly treated with water. The aqueous extract was separated from the plant material, made alkaline with ammonia, and extracted with chloroform. The chloroform solution, after concentration, was treated with 5% sulfuric acid. The acid solution was made alkaline with ammonia and was exhaustively extracted with petroleum ether (2.1 g), petroleum ether—diethyl ether (6 : 4) (11.6 g), diethyl ether (12.8 g), and chloroform [18].

After the petroleum ether—diethyl ether fraction of the mixture of bases had been freed from petroleum ether, a white amorphous product was obtained which, on trituration in acetone, gave 0.5 g of a crystalline substance. Fractional recrystallization of the latter yielded 0.4 g of korseverine (from acetone) [14] and 0.02 g of korsinine (from methanol) [15]. The mixture of alkaloids from the petroleum ether—diethyl ether fraction, on standing in acetone, yielded 0.52 g of crystals with mp 250-260°C (from methanol). The combined bases from the ethereal fraction were treated with acetone. After prolonged standing, 0.68 g of crystalline mixtures with mp 250-260°C deposited. The mother solution from the 0.68 g of crystals was evaporated and the amorphous mixture of bases obtained was dissolved in benzene. On standing, the concentrated benzene solution formed a sticky voluminous mixture of crystals (8.47 g). The mother liquors from the crystals were combined and freed from solvent, and the residue was again dissolved in chloroform. The mixture of bases in chloroform was separated into ten fractions by extraction with 0.01 N sulfuric acid.

The first two fractions of the mixture of bases (5.4 g) gave sticky crystals from benzene [4, 5]. The other fractions did not crystallize from benzene.

By separating into fractions the mixtures of crystals isolated from benzene solution according to their solubilities in acetone and methanol, we obtained 5.7 g of korseverilline (from acetone) and 4.1 g of korsine (from methanol) [16, 17].

On fractional crystallization of the mixture of crystalline bases with mp 250-260°C (1.19 g) from methanol, korseverinine with mp 320-322°C, korseveridine with mp 290-292°C, and korseveramine with mp 304-305°C were isolated [1, 18].

Korseverinine, $C_{27}H_{43}O_2N$, has mp 320-322°C (from methanol), $[\alpha]_D$ + 23° (c 0.823; 10% acetic acid); mol. wt. 413 (mass spectrum), and is sparingly soluble in ether, benzene, chloroform, and pyridine.

Hydrochloride. This was prepared by dissolving 0.18 g of the base in ethanolic hydrogen chloride. The melting point of the residue after evaporation was 310-312°C (from acetone—ethanol).

Hydrobromide. By the action of 60% hydrobromic acid, 0.2 g of korseverinine was converted into the hydrobromide with mp 285-287°C (from ethanol—acetone).

Hydriodide. A solution of 0.19 g of the base in ethanol was mixed with 57% hydriodic acid to give the hydriodide with mp 219-220°C (from ethanol—acetone).

Methiodide. A mixture of 0.2 g of the base in 30 ml of methanol and 1.5 ml of methyl iodide was boiled for 2.5 h. The residue after the evaporation of the solvent had mp 295-296°C (from acetone).

Diacetylkorseverinine. A mixture of 0.25 g of korseverinine, 1 ml of acetic anhydride, and 2 ml of pyridine was heated in the water bath for 4 h. On cooling, the solution deposited 60 mg of crystals of the initial base.

The alkaline solution after the separation of the crystals was evaporated in vacuum, the residue was dissolved in chloroform, and the solution was washed with 5% sulfuric acid. The chloroform solution was treated repeatedly with a saturated solution of sodium acetate and with water. The residue after the chloroform had been distilled off was dried in vacuum. The resulting solid amorphous powder was dissolved in petroleum ether (bp 50-70°C) and chromatographed on alumina (activity grade II).

After evaporation of the solvent, the diacetylkorseverinine, which had been eluted with petroleum ether, gave a solid powder. From this, crystals with mp 138-140°C were isolated (from aqueous acetone).

The NMR spectrum of the substance showed three-proton singlets from two acetyl groups at 2.0 and 1.98 ppm.

Korseverininone and Korseverininedione. A solution of 0.5 g of korseverinine in 10 ml of acetic acid was mixed with 0.25 g of chromium trioxide in 6 ml of acetic acid and two drops of water. A flocculent precipitate was formed, which rapidly dissolved when the mixture was heated in the water bath. The acid solution was stirred continuously at 80°C for 20 min and was then evaporated in vacuum. The residue was treated with saturated sodium carbonate solution and extracted with chloroform. The solvent was distilled off, and the residue (0.45 g) was dried in vacuum. The resulting amorphous powder, on chromatography in a thin layer of Al_2O_3 and $CaSO_4$ (9 : 1) in the toluene–petroleum ether–methanol (5 : 5 : 0.5) system (1) gave four spots with R_f 0.15, 0.3, and 0.72, the initial base remaining at the starting line.

The oxidation products (0.45 g) were dissolved in benzene and chromatographed on alumina (activity grade II). The fractions of the benzene eluates were checked by GLC in system 1. The first eluates contained as the main component a base with R_f 0.72, and traces of a substance with R_f 0.3.

After the amount of product with R_f 0.72 in the eluates had decreased sharply, chloroform was used for elution. The first chloroform eluates contained almost equal amounts of substances with R_f 0.3 and 0.15. In the later eluates, the amount of the substance with R_f 0.15 became predominant, the substance with R_f 0.3 being present only in the form of an impurity. These fractions, after the elimination of the chloroform, gave korseverininone with R_f 0.15, mp 263-265°C (from acetone). Mol. wt. 411 (mass spectrum).

The fraction of substances obtained from the benzene eluates containing large amounts of the base with R_f 0.72 was dissolved in petroleum ether and crystallized. The mixture of crystals with R_f 0.72 and 0.3 that deposited was separated off, and the solution was chromatographed on Al_2O_3 (activity grade II). The first petroleum ether eluates contained only korseverininedione with R_f 0.72, while the final fractions contained a mixture of substances with R_f 0.72 and 0.3.

From the pure fractions of the base after the distillation of the solvent, korseverininedione was isolated with mp 227-229°C (from acetone). Mol. wt. 409 (mass spectrum).

CONCLUSIONS

1. By separating the mixture of bases obtained from the fresh epigeal part of Korolkowia sewerzowii Rgl. with respect to solubility in different solvents, the alkaloids korseverine, korsinine, korsine, korseveriline, korseveridine, korseveramine, and korseverinine have been been isolated.

2. On the basis of a study of chemical transformations and IR, NMR, and mass spectra, the configuration, 5α,12α,14α,13β,17α,20β,22α,25β-cev-8-ene-3α,6α-diol has been proposed for korseverinine.

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