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STRUCTURE OF KORSEVINE

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From the bulb of Korolkowia sewerzowii Rgl. [1, 2] a new alkaloid korsevine with the composition $C_{28}H_{45}O_2N$, forming a crystalline perchlorate, hydrobromide, thiocyanate, methiodide, oxime, and semicarbazone, has been isolated [3].

In the base, one oxygen atom is present in the form of a carbonyl group and the second in the form of a hydroxyl. Acetylation of the alkaloids gives mono-O-acetylkorsevine, isolated as a crystalline perchlorate. The korsevine molecule contains four C-methyl groups and one N-methyl group. The reduction of korsevine in ethanolic solution and in acetic and hydrochloric acids in the presence of platinum black and in ethereal solution with lithium aluminum hydride forms dihydrokorsevine. Under these conditions, only the carbonyl group is reduced and the double bond remains unaffected. Dihydrokorsevine forms diacetyldihydrokorsevine, which gives a hydrobromide.

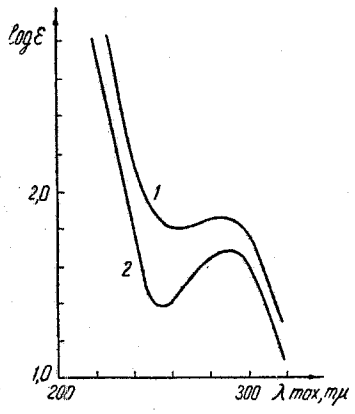


Fig. 1. UV spectra. 1) Korsevine; 2) imperialine and edpetiline.

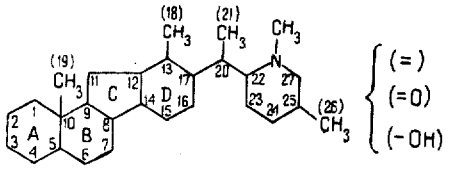
The oxidation of korsevine has given a diketone, korsevinone (perchlorate), and the oxidation of dihydrokorsevine has given korsevine and korsevinone. The reduction of korsevine by the Huang-Minlon method has given deoxodihydrokorsevine. In the UV region of the spectrum, korsevine has an absorption curve with λ_{max} 289 mμ (log ε 1.86), identical with respect to the position of the maximum and very similar in general nature to the UV spectra of imperialine and edpetiline (Fig. 1 [4-6]).

The four C-methyl groups, the composition with 28 carbon atoms in the molecule, and the nature of its absorption curve in the UV region of the spectrum enable us to regard korsevine as a steroid alkaloid. The fragmentation of korsevine under the conditions of mass spectrometry also takes place in the same way as that of the steroid alkaloid, i.e., with the formation of characteristic ion peaks with m/e 96; 98; 111; 112; 113; 114; 139; 287; 314; 316; $(M-29)^+$, $(M-18)^+$, $(M-15)^+$, $(M-1)^+$ and M^+ (Fig. 2) [7]. In the mass spectra of korsevine and dihydrokorsevine, the peak of the ion with m/e 112 has the maximum intensity. This peak is very intense and in the spectrum where, as can be seen, the molecular ion is situated right off the scale of the instrument. Thus, in korsevine α-cleavage of the bond relative to the nitrogen atom takes place with the formation of a high concentration of ions with m/e 112. Steroid alkaloids must behave in this way if the heterocyclic moiety is connected with the remainder of the molecule by only one σ-bond. We confirmed this assumption by recording the mass spectrum of tetrahydrosolasodine [8]. In its spectrum, the ion with m/e 98 has the maximum intensity. Tetrahydrosolasodine has a tendency to α-cleavage with retention of the piperidine nucleus. A similar bond rupture takes place in verarine [9] and veratramine [7].

The NMR spectrum of korsevine has resonance signals from methyl protons—a singlet at 7.78τ (3H, N-CH_3) [10-13], a singlet at 8.45τ (3H, C-18 CH_3) [14-15], a doublet at 9.07τ (3H, C-26 CH_3), a doublet at 9.32τ (3H, C-21 CH_3), and a singlet at 9.4τ (3H, C-19 CH_3) [16]. The conformity of the resonance signals at 9.07τ and at 9.32τ is confirmed by the results of a study of the NMR spectrum of solasodine. The spectrum of solasodine has a singlet at 9.04τ (3H, C-19 CH_3), a singlet at 9.22τ (3H, C-18 CH_3), a doublet at 9.07τ (3H, C-26 CH_3), a doublet at 9.2τ (3H, C-21 CH_3) and a multiplet at 4.7τ (H, olefinic proton at C-6). Consequently, in korsevine the doublet at 9.07τ is the signal from the protons of the C-26 CH_3. Hence, the doublets at 9.32τ (in korsevine) and at 9.2τ (in solasodine) are the signals of the protons of the C-21 CH_3. This attribution is confirmed by the presence in the NMR spectrum of tetrahydrosolasodine [8] of a doublet at 9.05τ (3H, C-26 CH_3).

It is known that in the formation of tetrahydrosolasodine the double bond is hydrogenated and an -O-bond is cleaved [8]. These structural changes are remote from the C-26 CH_3, in consequence of which practically no screening and descreening of the C-26 CH_3 takes place. Alkaloids containing four C-methyl groups in the molecule belong to the C-nor-D-homosteroid alkaloids of the veratramine group and to the steroid alkaloids of the solasodine and solanidine group [17]. The latter groups of alkaloids have two tertiary methyl groups in their molecule. The absence of a C-18 tertiary methyl group from korsevine enables us to assign it to the C-nor-D-homosteroid alkaloids.

On the basis of the data given above, we propose the following structural formula for korsevine:



In the NMR spectrum of korsevine, the resonance signal from chemically equivalent protons at 8.45τ shows that the C–18 CH_3 is located on an unsaturated carbon atom [14, 15] and, consequently, the double bond may be between C–12 and C–13 or between C–13 and C–17. The other two secondary methyl groups cannot be attached to an unsaturated carbon atom since the ion with m/e 112 has the maximum peak in the mass spectrum of korsevine. The presence in the mass spectrum of korsevine of peaks of ions with m/e 287, 314, and 316 excludes the location of the double bond between C–13 and C–17. The results given show that the double bond in korsevine is located between C–12 and C–13.

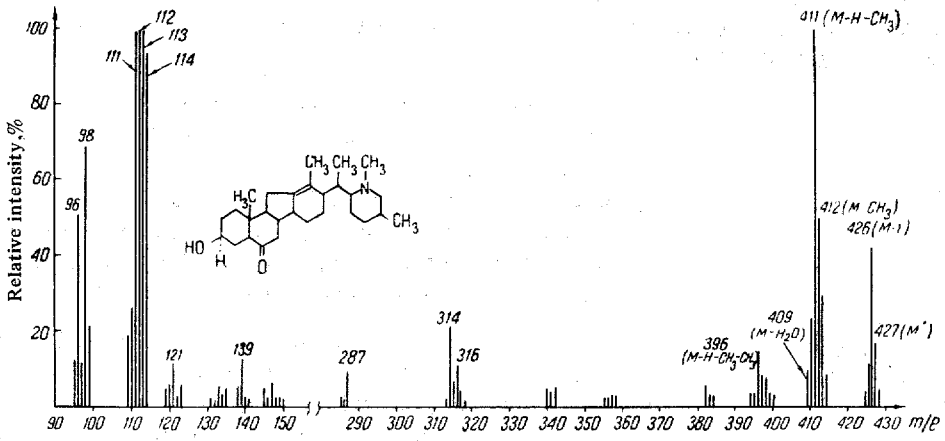


Fig. 2. Mass spectrum of korsevine (intensity of the peaks with m/e 111, 112, and 113 greater than 100%).

The carbonyl group in korsevine must occupy one of positions 1, 2, 3, 4, 6, or 7; the secondary hydroxy group must also be in one of positions 1, 2, 3, 4, 6, or 7. The carbonyl and hydroxy groups cannot be in position 11, 15, or 16 for the following reasons. If the carbonyl group were present in position 11, korsevine should have a maximum in the UV region characteristic for α, β-unsaturated ketones. There is no such absorption in the UV spectrum of korsevine. If the carbonyl group is located in position 15 or 16, displacement of the double bond would take place with the formation of an α, β-unsaturated ketone, which is not the case. The main features of the NMR spectrum of korsevinone are a singlet at 7.76τ (3H, N–CH_3), a singlet at 8.45τ (3H, C–19 CH_3), a singlet at 9.2τ (3H, C–19 CH_3), a doublet at 9.07τ (3H, C–26 CH_3), and a doublet at 9.32τ (3H, C–21 CH_3), and in the NMR spectrum of diacetyldihydrokorsevine there is a singlet at 7.76τ (3H, N–CH_3), a singlet at 8.05τ (6H, 2 $OCOCH_3$), a singlet at 8.45τ (3H, C–18 CH_3), a doublet at 9.07τ (3H, C–26 CH_3), a singlet at 9.15τ (3H, C–19 CH_3), and a doublet at 9.23τ (3H, C–21 CH_3). As can be seen, in the NMR spectra of korsevinone and diacetyldihydrokorsevine the signals from the methyl protons, other than the singlet from the chemically equivalent protons of the C–19 CH_3, do not change their positions; no screening or descreening of the methyl groups at C–18, C–21, and C–26 has been observed. If hydroxy and carbonyl groups were present in rings C and D there should be a change in the chemical shifts of the C–18 CH_3 and the C–21 CH_3, but this did not occur.

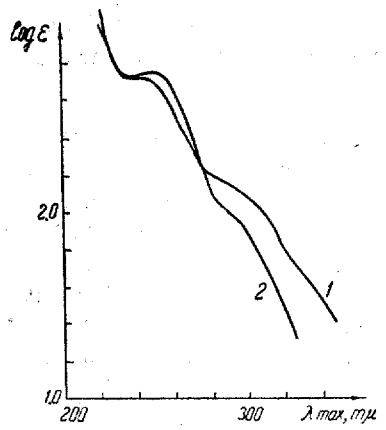
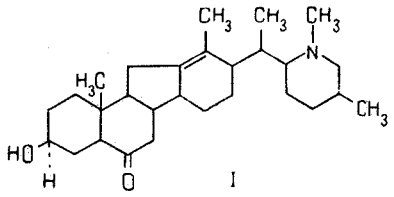


Fig. 3. UV spectra. 1) Korsevinone; 2) imperialone.

Thus, the introduction of acetyl and carbonyl groups into the molecule of korsevine has almost no effect on the chemical shifts of the chemically equivalent protons of the C–19, C–21, and C–26 methyl groups. All this convincingly shows that the carbonyl and hydroxy groups are located in rings A and B. On considering literature data concerning the NMR spectra of steroid compounds, we came to the conclusion that when a carbonyl group was present in ring A or B a C–29 CH_3 had different chemical shifts according to its position, i.e., the resonance signals from the C–19 CH_3 protons were dissimilar. Signals are observed in a strong field when the carbonyl group is present in position 2, 4, or 6 [18].

In korsevine, the signal from C–19 CH_3 protons is found at 9.4τ and in imperialine the singlet from the C–19 CH_3 protons is found at 9.325τ. These two figures are very close and make it possible to assume that the carbonyl group is in position 6. Furthermore, positions 2 and 4 are excluded for the carbonyl group since in the UV region of the spectrum korsevinone, like imperialone, has λ_{max} 250 and 295 mμ (log ε 2.74 and 2.16), which is characteristic for diketones (Fig. 3). In imperialone the carbonyl groups are also located in position 3 and 6 [19, 22]. In korsevinone, the signal from the C–19 CH_3 is located at 9.2τ and is displaced by 0.2 ppm in the direction of weaker fields in comparison with the signals from the C–19 CH_3 of korsevine. Similar descreening takes place in the passage from 3β-hydroxy-5α,14β-androstane to 3-oxo-5α,14β-androstane. Similar examples are frequently encountered [17]. Consequently, the hydroxy group in korsevine is located at C-3 and has the β-configuration. In accordance with this, in the NMR spectrum of diacetyldihydrokorsevine there is a multiplet at 5.35τ (H, C-3α H) [20, 21], and a multiplet at 5.05τ (H, C-6 H). Finally, the carbonyl and hydroxy groups cannot be present in the same ring, A or B. If they were arranged in this way, the singlet from the the C-19 CH_3 in korsevine should be between 8.9 and 9τ, which was not found to be the case.



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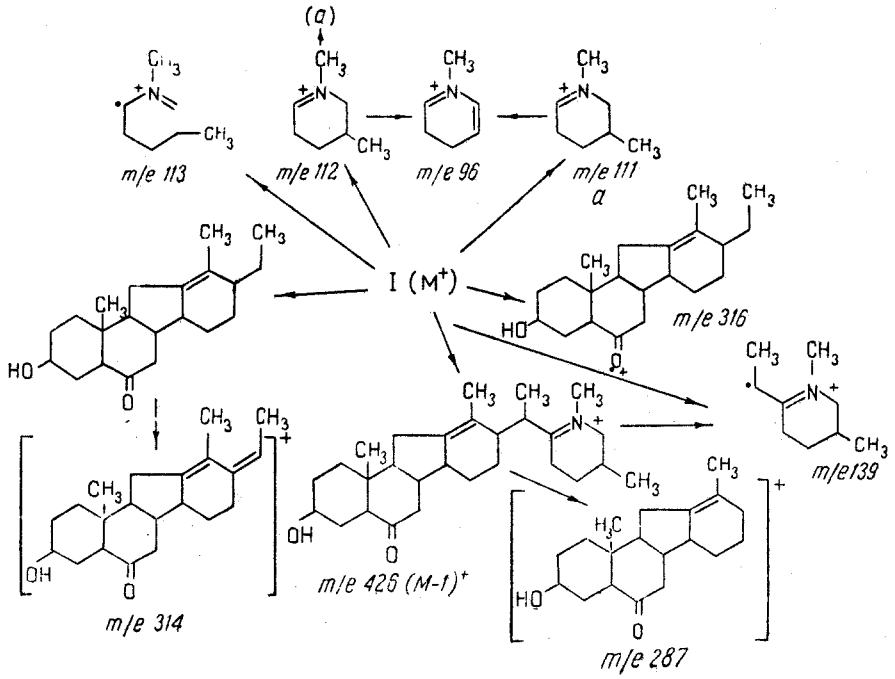
The fragmentation of korsevine confirms the correctness of the structural formula proposed.

As a result of the α-cleavage of the korsevine bond, the peaks of ions with m/e 111, 112, 314, and 316 are formed. The ion with m/e 111 can also be obtained after the elimination of a hydrogen atom from the ion with m/e 112. The ion with m/e 96 can be formed from the ion with m/e 112 after the elimination of hydrogen and a methyl group and also from the ion with m/e 111 after the splitting off of a methyl group.



The fragment with m/e 98 is obtained from the molecular ion after the splitting off of a methyl group (C-26, CH_3), the migration of hydrogen from C-27 to C-25, and α-cleavage between C-20 and C-22, with the simultaneous migration of hydrogen from C-17 to C-22. The fragment with m/e 113 appears as the result of the α-cleavage of a bond with the simultaneous cleavage of the bond between C-20 and C-22 and the migration of hydrogen from C-17 to C-25. The splitting off of an α-atom of hydrogen and subsequent β-cleavage leads to ions with m/e 139 and 287. The ion $(M-29)^+$

is formed after the splitting off of an ethyl radical as a consequence of cleavage of the bond between C-22 and C-23 and the migration of hydrogen from C-27 to C-23 and also by the subsequent cleavage of the bond between C-24 and C-25. The appearance of the ion $(M-29)^+$ is possible in part after the splitting off of a formyl radical from ring B. The ion $(M-18)^+$ is formed by the splitting out of water, and the ion $(M-15)^+$ by the elimination of one of the methyl groups.

Experimental

The substances were chromatographed on a fixed thin layer of alumina in the petroleum ether–toluene–methanol (5:5:1.5) system, and the spots were revealed with Dragendorff's reagent.

The bulbs of K. sewerzowii (3 kg) collected in the stage of the dying-off of the epigeal part in August 1960 in the Chatkal valley were comminuted, moistened with 8% ammonia, and extracted with chloroform. The acid solution of total alkaloids obtained by the usual treatment of the chloroform extracts was made alkaline and extracted with petroleum ether (0.22%), ether (0.48%), and chloroform (0.083%).

Korsevine. On treatment with benzene, the mixture of alkaloids dissolved in petroleum ether gave crystals (10% of the combined alkaloids) with mp 169–170° C (from methanol), $[\alpha]_D$ −84° (c 0.9; methanol), R_f 0.25. UV spectrum: λ_{max} 298 mμ (log ε 1.86). IR spectrum: ν_{max} 3250, 1080 cm^{-1} (OH); 1715 (C=O); 2830–2940, 1460 ($C-CH_3$) 1630 cm^{-1} (>C=C<).

Found, %: C 78.4; 78.4; H 10.6, 10.65, N 3.24, 3.28, $N-CH_3$ 3.8, 3.54, mol. wt 427. Calculated for $C_{28}H_{45}O_2N$, %: C 78.6, H 10.6, N 3.27, $N-CH_3$ 3.5, mol. wt. 427.57.

The hydrobromide was obtained by mixing an acetone solution of korsevine with hydrobromic acid; mp 289–290° C (from acetone).

The perchlorate was formed by treating an aqueous solution of korsevine hydrochloride with a saturated solution of sodium perchlorate; mp 244–245° C (from water).

The thiocyanate was obtained by mixing a 1% sulfuric and solution of korsevine with a saturated solution of potassium thiocyanate; mp 264–265° C (from water).

Methiodide. 0.2 g of korsevine in 12 ml of methanol was mixed with 1 ml of methyl iodide and the mixture was boiled for 3 hr. The residue after the elimination of the solvent melted at 244–245° C [from acetone–methanol (10:1)].

Oxime. 200 mg of korsevine, 200 mg of hydroxylamine hydrochloride, and 200 mg of sodium acetate were boiled in 10 ml of 65% ethanol for 6 hr. The reaction mixture was evaporated in vacuum, the residue was dissolved in 5% hydrochloric acid, and the solution was washed with chloroform, made alkaline with 5% caustic soda, and extracted with chloroform. After the chloroform solution had been washed with water, the solvent was distilled off and the residue was recrystallized from acetone; mp 200–201° C.

Found, %: N 6.06, 6.12. Calculated for $C_{28}H_{46}O_2N_2$, %: N 6.33.

Semicarbazone. 200 mg of korsevine, 200 mg of sodium acetate, and 200 mg of semicarbazide hydrochloride were boiled in 10 ml of 85% ethanol for 6 hr. The semicarbazone was isolated under the conditions used for the isolation of the oxime. It softened at 160° C and melted at 190° C.

Found, %: N 10.85, 11.09. Calculated for $C_{29}H_{48}O_2N_4$, %: N 11.58.

O-Acetylkorsevine perchlorate. A mixture of 0.4 g of korsevine, 6 ml of pyridine, and 2 ml of acetic anhydride was left at room temperature for 2 days. The solvent was evaporated off and the residue was treated with sodium acetate solution and extracted with chloroform. After being washed with water, the chloroform was distilled off and then the residue was dried in vacuum and dissolved in 10% hydrochloric acid, and the solution was mixed with a saturated solution of sodium perchlorate. The perchlorate precipitated, with mp 178–179° C (from water). IR spectrum: ν_{max} 1715 cm^{-1} (−C=O); 1735 (O=C−OCH_3); 1630 cm^{-1} (C=C).

Found, %: N 2.45; 2.56. Calculated for $C_{30}H_{47}O_3N \cdot HClO_4$, %: N 2.45.

Dihydrokorsevine. A solution of 100 mg of korsevine in 10 ml of ethanol was shaken in an atmosphere of hydrogen in the presence of platinum black (from 50 mg of PtO_2). About 5 ml of hydrogen was absorbed. After the catalyst had been separated off, dihydrokorsevine separated from the concentrated solution with mp 218–219° C (from methanol), $[\alpha]_D$ −87° (c 0.16; methanol); IR spectrum: ν_{max} 3420 cm^{-1}, 1080, 1050 (OH), 1640 (C=C), no. 1715 cm^{-1} (C=O).

Korsevine (100 mg) was hydrogenated in 10 ml of hydrochloric acid in the presence of platinum black (from 50 mg of PtO_2). The dihydrokorsevine was isolated by the usual method, mp 218–219° C (from methanol).

100 mg korsevine was hydrogenated in 10 ml of glacial acetic acid in the presence of platinum black. Dihydrokorsevine with mp 218–219° C (from methanol) was isolated.

A mixture of 100 mg of korsevine in 50 ml of absolute ether and 100 mg of $LiAlH_4$ was heated for 8 hr. After treatment with water, the ethereal layer was separated off and the aqueous layer containing the residue was extracted with chloroform. The ethereal and chloroform solutions were extracted with 5% sulfuric acid. The acid solution was made alkaline with ammonia and extracted with chloroform. This gave dihydrokorsevine with mp 218–219° C (from methanol).

Diacetyldihydrokorsevine. A mixture of 460 mg of dihydrokorsevine, 5 ml of pyridine, and 2 ml of acetic anhydride was left at room temperature for 5 days. The solvent was evaporated off, and the oily residue was treated with water, made alkaline with sodium carbonate, and extracted with chloroform. The chloroform solution was washed with water. The residue after the chloroform had been distilled off was dried in vacuum at room temperature and recrystallized from acetone; mp 133–134° C (from acetone). The NMR spectrum had a singlet at 8.05 (6H, 2 $COCH_3$).

Found, %: C 74.70, 74.55, H 9.94, 10.1. Calculated for $C_{32}H_{51}O_4N$, %: C 74.82, H 10.00.

Hydrobromide. An ethanolic solution of diacetyldihydrokorsevine was mixed with hydrobromic acid and evaporated in vacuum. The melting point of the residue was 266–268° C (from acetone).

Found, %: N 2.29, 2.31, Br 12.9, 12.9. Calculated for $C_{32}H_{51}O_4N \cdot HBr$, %: N 2.35, Br 13.43.

Deoxodihydrokorsevine. A solution of 100 mg of korsevine in 6 ml of diethylene glycol was mixed with 120 mg of caustic potash and 0.6 ml of hydrazine hydrate. Then the mixture was heated in the water bath for 80 min and at 200 and 205° C (temperature of the reaction solution) for 4 hr. The reaction product was cooled, mixed with 100 ml of water, and extracted with chloroform. The chloroform solution, after being washed with water, was extracted with acid, and the acid solution was washed with ether, made alkaline with ammonia, and extracted with chloroform. The residue after the chloroform had been driven off was crystallized from benzene, mp 165–167° C (from acetone). A mixture with korsevine melted at 150–154° C.

Found, %: C 81.8, 82.1, H 11.3, 11.6. Calculated for $C_{28}H_{47}ON$, %: C 81.31, H 11.45.

Korsevinone. A mixture of 350 mg of korsevine and 200 mg of chromic anhydride (in a few drops of water) in 7 ml of acetic acid was heated at 80° C for 30 min. The acetic acid was partially distilled off, the residue was dissolved in chloroform, and the solution was extracted with a saturated solution of sodium carbonate. The chloroform solution was separated off and washed with water and was then extracted with 5% sulfuric acid. The acid solution was made alkaline and again extracted with chloroform. The product obtained gave two spots. One of them was the initial korsevine, with R_f 0.25. The oxidation product was dissolved in 20 ml of benzene and passed through alumina of activity grade II (l = = 3 cm, d = 2 cm). The base was eluted from the adsorbent with benzene. This gave 15 eluate fractions, the first 10 of which were 25 ml each and the others 100 ml. The first seven eluates gave 200 mg of korsevinone with R_f 0.63. IR spectrum: ν_{max} 1722 cm^{-1} (C = O), no 3250, 1080 cm^{-1} (OH).

Perchlorate. A solution of 100 mg of korsevinone in ethanol was mixed with an ethanolic solution of hydrochloric acid and the mixture was evaporated in vacuum. The residue was dissolved in water and mixed with 20% sodium perchlorate solution. Korsevinone perchlorate precipitated with mp 240° C (from water). A mixture with korsevine perchlorate melted at 225° C.

Reduction of korsevinone. A solution of 30 mg of korsevinone in 10 ml of ethanol was hydrogenated in the presence of platinum black (from 50 mg of PtO_2). This gave dihydrokorsevine with mp 218–219° C. A misture with dihydrokorsevine melted at 218–219° C.

Oxidation of dihydrokorsevine. A mixture of 100 mg of dihydrokorsevine, 100 mg of chromic anhydride (in a drop of water), and 3 ml of acetic acid was heated at 80° C for 30 min. The acetic acid was evaporated off at 20° C, and the residue was dissolved in chloroform and treated with a solution of sodium carbonate. The chloroform solution was separated off, washed with water, and evaporated in vacuum. The residue was crystallized from benzene, mp 169–170° C (from acetone). A mixture with korsevine melted at 169–170° C, R_f 0.25. From the korsevine mother liquor, korsevinone with R_f 0.63 was isolated by the method described above.

The solasodine was isolated from Solanum aviculare. The tetrahydrosolasodine was obtained by the method of Briggs and Locker [8], and the imperialone from imperialine [19].

The IR spectra were recorded in a UR-10 double-beam spectrophotometer (compressed tablets with KBr), the UV spectra on a SF-4 spectrophotometer (ethanolic solutions), the mass spectra on a MKh-1303 mass spectrometer with a glass inlet system at 30 eV and 50 ma, and the NMR spectra in deuterochloroform on a JHM-4H-100 instrument. Tetramethylsilane was used as an internal standard.

Summary

Structure I has been established for the alkaloid korsevine on the basis of the results of a study of its chemical properties and its IR, UV, NMR, and mass spectra.

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