THE STRUCTURE OF VINERINE AND VINERIDINE

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We have previously reported that the main alkaloids in the epigeal part of Vinca erecta Rgl. et. Schmalh., collected in the Fergana Oblast are kopsinine and ervamine [1, 2]; in the Oshsk Oblast they are kopsinine and pseudokopsinine [3]; and in the Surkhan Dar'ya Oblast the main alkaloid is vinervine [4].

The present paper gives the results of an investigation of the alkaloids from the epigeal part of V. erecta growing in the Tashkent Oblast, and of the structure of vinerine and vineridine. The maximum accumulation of total alkaloids in the epigeal part of the plant is found in the early vegetation period (2.7%).

Ethereal extraction of the plant with subsequent separation of the total bases according to their basicities and by chromatography on alumina yielded vincanine, vincanidine, vinervine, akuammine, kopsinine, pseudokopsinine, and the new alkaloids vinerine and vineridine [5]. Vinerine, with the composition $C_{22}H_{26}O_5N_2$, contains two methoxy groups. The characteristic curve of its UV spectrum is close to that of the hydroxyindole bases rincophylline and majdine [6, 7]; there is an absorption maximum at 220 mμ (log ε 4.54). The following vibrational bands are found in the IR spectrum:

3295 cm^{-1} (N–H bond), 1605, 1730 (the groupings $H_3COOC-\underset{|}{C}=\underset{|}{C}-O-$), 1665 (amide carbonyl group) and 800 and 830 cm^{-1} (1, 2, 4-trisubstituted benzene). The NMR spectrum of vinerine has the signals of the three protons of a methyl group in the form of a double t at 1.23 ppm, one of olefinic proton in the form of a singlet at 7.45 ppm, and the six protons of the methyl groups of an ester methoxyl at 3.65 ppm and an aromatic methoxyl at 3.80 ppm. The saponification of vinerine with alcoholic caustic potash gave an amorphous amino acid whose methylation gave the initial base. These facts enable the formula of vinerine to be developed in the following way:

$$C_{18}H_{19}(=NH)\ (=N-)\ (COOCH_3)\ (-OCH_3)\ (=CO)\ (-O-).$$

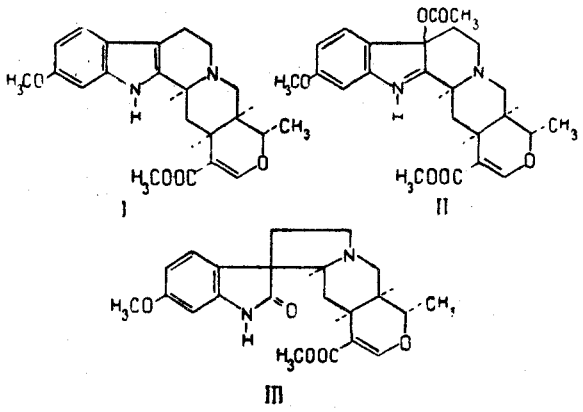
On acetylation with acetic anhydride, vinerine forms a N-acetyl derivative $C_{24}H_{28}O_6N_2$. The UV spectrum of the latter has three absorption maxima: 212, 240, 280 mμ (log ε 4.64, 4.40, 3.58). The IR spectrum of N-acetylvinerine lacks the absorption band of the N–H bond and has absorption bands at 1765, 1700, and 1640 cm^{-1} showing the presence of $H_3COOC-\underset{|}{C}=\underset{|}{C}-O-$ and $H_3COC-\underset{|}{N}-\underset{|}{CO}-$ groups. The NMR spectrum of this product has the signals of three aromatic protons. As a result of spin-spin interaction with neighbouring proton 10, the signals of the proton in position 9 are split into two peaks (doublet at 7.15 ppm), and the signals of proton 10 are split into four peaks (quartet at 6.55 ppm) under the action of protons 9 and 12. The proton in position 12 gives a doublet at 7.70 ppm. Consequently, only position 11 remains for the methoxy group.

A comparison of the mass spectra of vinerine that we have recorded with the spectrum of the known hydroxyindole base carapanaubine [8] showed that they differ with respect to the fragments of the hydroxyindole part of the molecule by 30 m/e. Consequently, vinerine has one methoxy group in the benzene ring.

Molecular ion	Peaks of the fragments of the hydroxyindole part	Peaks of the fragments of the alicyclic part
Vinerine M^+ 398	160, 174, 176, 189	223, 208, 69
Carapanaubine M^+ 428	190, 204, 206, 219	223, 208, 69

The UV spectrum of vineridine $C_{22}H_{26}O_5N_2$ is similar to that of vinerine. In the IR spectrum, the stretching vibrations of the N–H bond give a broad band at 3240 cm^{-1} which is probably due to an intermolecular hydrogen bond. This was confirmed by a study of the IR spectrum in chloroform solution. Absorption bands at 1630 and 1720 cm^{-1} show the vibrations of the grouping $H_3COOC-\underset{|}{C}=\underset{|}{C}-O$ and an amide carbonyl group. On acetylation with acetic anhydride,

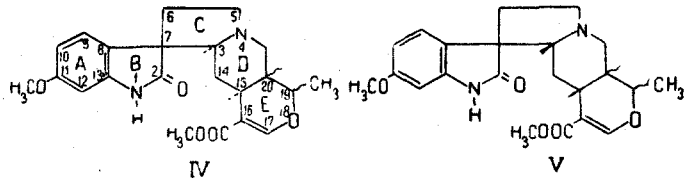
vineridine forms a N-acetyl derivative which is identical with N-acetylvinerine. Reduction of the latter with sodium borohydride leads to saponification with the formation of vinerine. Vineridine also isomerizes into vinerine on heating in pyridine. This behavior is characteristic for twin hydroxyindole alkaloids, i.e., on isomerization the less stable hydroxyindole base changes its configuration at C_7 and C_3 [9], the base having a cis-C/D linkage having a higher basicity and lower stability than the base with the trans-C/D linkage. Consequently, vinerine and vineridine are isomeric bases differing by the configuration of the substituents at C_3 and they are possibly spiroisomers about C_7. If the α-orientation of the substituent at C_{15} in the indole [10] and the hydroxyindole alkaloids is taken into account, only the configuration of the substituents at C_{19} and C_{20} in vinerine and vineridine remains uncertain. To elucidate this point, we effected the synthesis of hydroxyindole-reserpinine by Taylor's reaction [11]. Acetoxyresperinine $C_{24}H_{28}O_5N_2$ (II) was obtained; its IR spectrum has two maxima, at 236 and 284 mμ (log ε 4.50 and 3.42). The IR spectrum lacks the band of an N–H bond and exhibits a band at 1760 cm^{-1} showing the vibrations of a $-OCOCH_3$ group. To confirm the formation of acetoxyresperinine, the latter was reduced with sodium borohydride to the initial resperinine (I). The isomerization of acetoxyreserpinine in an acid medium gives hydroxyindole reserpinine, which proved to be identical with vinerine and vineridine.



I II III

A comparison of the UV, IR, and NMR spectra of hydroxyindole-reserpinine and vinerine shows that these substances are isomers.

Thus, in vinerine (IV) and vineridine (V) the configurations of the substituents at C_{19} and C_{20} remain uncertain.



IV V

Experimental

Isolation and separation of the alkaloids. The ethereal extraction of 300 kg of the plant collected on 20–28 June 1962 in the Tashkent Oblast gave 2950 g (0.98%) of total alkaloids. On standing, the concentrated ethereal extracts of the mixture of alkaloids deposited crystals of vineridine – 122 g (0.04%). After the separation of the vineridine, the total mixture was separated into a phenolic fraction, 151 g (0.05%), and a nonphenolic fraction, 2670 g (0.89%). The concentration of an ethereal extract of the phenolic bases gave 42 g (0.014%) of a mixture of crystals, the purification of which from ether yielded vincanidine (0.7 g), akuammine (13.0 g), and vinervine (0.37 g). The nonphenolic fraction was dissolved in 14 *l* of benzene and treated with an equal volume of citrate-phosphate buffer (pH 2.6). The buffer fraction of the mixture of alkaloids (980 g) gave kopsinine nitrate –610 g (0.15%). The mother liquors (365 g) were chromatographed on alumina (4500 g). The benzene eluates gave pseudokopsinine (3.5 g) and vincanine (11.2 g). The benzene fraction of the mixture of alkaloids – 1659 g (0.55%) –was dissolved in methanol (500 ml). On standing, vinerine crystallized out –110 g (0.036%).

Vinerine. Mp 202°–203° C (from methanol). On paper chromatography it had R_f 0.9 in system 1 [benzene–cyclohexane (1:1), paper impregnated with a 15% alcoholic solution of formamide saturated with 5% ammonium formate]. On a thin-layer chromatogram the R_f value was 0.65 in system 2 [ethyl acetate– methanol (9:1)], $[\alpha]_D^{24}$ + 20.3° (c 1.23; pyridine), + 54.5° (c 1.10; acetone), + 36.6° (c 1.311; chloroform). UV spectrum: λ_{max}

(in alcohol) 220 mμ (log ε 4.54); IR spectrum (KBr): 3295, 1730, 1665, 1605, 830 and 800 cm^{-1}; IR spectrum ($CHCl_3$): 3430, 1720, and 1635 cm^{-1}.

Found, %: C 66.6, 66.5; H 7.00, 7.01; N 7.12, 7.10; OCH_3 15.5, 15.6; mol. wt. 398 (mass spectrometry). Calculated for $C_{22}H_{26}O_5N_2$, %: C 66.32; H 6.57; N 7.03; 2 OCH_3 15.58; mol. wt. 398.4.

Vinerine nitrate, mp 170° C (darkens). Found, %: C 57.1, 57.2; H 6.03, 6.32; N 8.98, 9.01. Calculated for $C_{22}H_{25}O_5N_2 \cdot HNO_3$, %: C 57.38: H 5.69; N 9.12.

Vinerine methiodide was isolated by heating an alcoholic solution of the base with methyl iodide for 6 hr, mp 194°-195° C.

Vineric acid was obtained in the amorphous state by heating the base with a 10% alcoholic solution of caustic potash.

Methylation of vineric acid. The amino acid (0.22 g) was dissolved in a mixture of absolute methanol and chloroform (1:1) and was methylated with an ethereal solution of diazomethane (24 hr) to form the initial vinerine (0.18 g).

N-Acetylvinerine was formed by heating vinerine (0.5 g) with acetic anhydride at 100° C for 2 hr, mp 159°-160° C, $[\alpha]_D^{20}$ −99.5° (c 1.90; acetone). On thin-layer chromatography in system 2, R_f 0.43. UV spectrum: λ_{max} (in alcohol) 212, 240, 286 mμ (log ε 4.68, 4.40, 3.58). IR spectrum (KBr), 1765, 1700, and 1640 cm^{-1}; the NMR spectrum was taken in CCl_4.

Found, %: C 64.4, 64.5; H 6.62, 6.84; N 6.65, 6.62; mol. wt. 440 (mass spectrometry). Calculated for $C_{24}H_{28}O_6N_2$, %: C 65.43; H 6.36; N 6.36; mol. wt. 440.4.

Vineridine. Mp 179°-180° C (from acetone), $[\alpha]_D^{22}$ +22.7° (c 2.32; pyridine), +42.4° (c 1.7; methanol), + 40.5° (c 2.395; chloroform). On paper chromatography in system 1, R_f 0.43, and on thin-layer chromatography in system 2, R_f 0.25. UV spectrum: λ_{max} (in alcohol) 220 mμ (log ε 4.54); IR spectrum (KBr): 3240, 1720, and 1630 cm^{-1}; IR spectrum ($CHCl_3$): 3440, 3200, 1720, and 1630 cm^{-1}.

Found, %: C 64.5, 64.5; H 6.83, 6.79; N 6.89, 7.10; OCH_3 14.8, 15.2; mol. wt. 398 (mass spectrometry). Calculated for $C_{22}H_{26}O_5N_2$, %: C 66.32; H 6.57; N 7.03; 2 OCH_3 15.58; mol. wt. 398.4.

Vineridine nitrate, mp 178° C (darkens). Found, %: C 57.00, 56.7, 46.03, 6.15; N 8.71, 8.84. Calculated for $C_{22}H_{26}O_5N_2 \cdot HNO_3$, %: C 57.38; H 5.69; N 9.12.

Vinerine methiodide was formed by heating an alcoholic solution of the base with methyl iodide for 1.5 hr, mp 209°-211° C.

N-Acetylvineridine was isolated in a similar manner to N-acetylvinerine and was identical with it.

Deacetylation of N-acetylvineridine. An alcoholic solution (1:9) of N-acetylvineridine (0.4 g) was reduced with sodium borohydride (0.3 g) for 1.5 hr, giving vinerine (0.29 g).

Isomerization of vineridine. A solution of 0.83 g of vineridine in 15 ml of pyridine was boiled for 16 hr. The solvent was evaporated off. The dry residue was dissolved in benzene (15 ml) and treated with an equal volume of citrate-phosphate buffer (pH 3.4). Vineridine (0.23 g) was isolated from the buffer fraction and vinerine (0.56 g) from the benzene fraction.

Acetoxyreserpinine. A solution of 1.2 g of reserpinine in 30 ml of methylene chloride was treated with 1.8 g of lead tetraacetate dissolved in 20 ml of methylene chloride. The mixture was shaken at room temperature for 15 min. The precipitate of lead diacetate that deposited was separated off. The filtrate was distilled in vacuum. The dry residue (1.32 g) was dissolved in 5 ml of methylene chloride and chromatographed on alumina (activity grade II, 25 g). On treatment with ether, the 1st-3rd fractions (0.06 g) gave acetoxyreserpinine with mp 206°-207° C, $[\alpha]_D^{18}$ +125.2° (c 1.11; chloroform), UV spectrum: λ_{max} (in alcohol) 236, 285 mμ (log ε 4.50, 3.42). IR spectrum (KBr): 1750, 1730, 1640, 1605 cm^{-1}.

Found, %: C 65.7, 65.8; H 6.15, 6.41; N 6.15, 6.30. Calculated for $C_{24}H_{28}O_5N_2$, %: C 65.43; H 6.41; N 6.36.

Deacetylation of acetoxyreserpinine. Over 1 hr, 0.5 g of sodium borohydride was added to 0.41 g of acetoxyreserpinine in 20 ml of aqueous methanol (1:9). Extraction of the reaction mixture with ether yielded reserpinine (0.29 g).

Hydroxyindole reserpinine. A solution of 0.36 g of acetoxyreserpinine in aqueous methanol (1:9) containing five drops of glacial acetic acid was heated under reflux for 1.5 hr. The dry residue (0.34 g) obtained by the ethereal extraction of the reaction mixture was dissolved in benzene and chromatographed on alumina (1.0 g). Rechromatography of the ethereal eluate gave hydroxyindole-reserpinine (0.12 g) with mp 201°-202° C, $[\alpha]_D^{23}$ − 95.7° (c 1.182; acetone), UV

UV spectrum: λ_{max} (in alcohol) 220 mμ (log ε 4.54), IR spectrum ($CHCl_3$): 3435, 1720, and 1630 cm^{-1}; mol. wt. 298 (mass spectrometry).

Summary

1. The epigeal part of V. erecta gathered in the Tashkent Oblast in the early vegetation period contains a mixture of alkaloids (2.7%). Separation of the total alkaloids gave vincanine, vincanidine, vinervine, akuammine, kopsinine, pseudokopsinine, and the new bases vinerine and vineridine.

2. The structure of vinerine has been established by a study of the IR, NMR, and mass spectra. Vineridine is isomeric with vinerine at C_3 and C_7.

3. A new base, hydroxyindole reserpinine, has been obtained from reserpinine.

REFERENCES

1. P. Kh. Yuldashev, V. M. Malikov, and S. Yu. Yunusov, DAN UzSSR, no. 1, 25, 1960.
2. V. M. Malikov, P. Kh. Yuldashev, and S. Yu. Yunusov, DAN UzSSR, no. 4, 21, 1963.
3. N. Abdurakhimova, P. Kh. Yuldashev, and S. Yu. Yunusov, DAN UzSSR, no. 2, 29, 1964.
4. M. A. Kuchenkova, P. Kh. Yuldashev, and S. Yu. Yunusov, DAN UzSSR, no. 11, 42, 1964.
5. Sh. Z. Kasymov, P. Kh. Yuldashev, and S. Yu. Yunusov, DAN SSSR, 162, no. 1, 102, 1965; 163, no. 6, 1400, 1965.
6. N. Abdurakhimova, P. Kh. Yuldashev, and S. Yu. Yunusov, KhPS [Chemistry of Natural Compounds], 224, 1965.
7. M. Raymond-Hamet, Compts. rend., 235, 547, 1952.
8. B. Gilbert, J. A. Brissolese, N. Finch, W. J. Taylor, H. Budzikiewicz, J. M. Wilson, and C Djerassi, J. Amer. Chem. Soc., 85, 1523, 1963.
9. J. C. Seaton, M. D. Nair, O. E. Edwards, and L. Marion, Can. J. Chem. Soc., 38, no. 7, 1035, 1960.
10. M. Shamma and J. M. Richey, J. Amer. Chem. Soc., 85, 2505, 1963.
11. N. Finch, W. C. Gemenden, I. H. Hsu, and W. J. Taylor, J. Amer. Chem. Soc., 85, 1520, 1963.

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