ALKALOIDS OF Vinca major

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Four bases have previously been isolated from the epigeal part of Vinca Major: reserpinine, majdine, akuammine, and majoridine [1]. Continuing an investigation of the alkaloids of this plant collected in April, 1974, in Tashkent, by the usual ethereal extracting we obtained 0.7% of combined alkaloids. These were separated into six fractions by treatment with citrate-phosphate buffer solution. The ethereal fractions were chromatographed on columns of alumina. In this way, in addition to the bases mentioned, we isolated akuammicine [2], ervine [3], vincamajine, and vincamajoreine [4] and a new base with the composition $C_{22}H_{24}N_2O_4$ (mass spectrometrically), mp 195-196° (acetone), which we have called majorinine (I). The UV spectrum of (I) shows absorption maxima at $\lambda_{max}^{C_2H_5OH}$ 213, 222, and 280 nm (log ε 4.31, 4.32, and 3.92), which are characteristic for 2H-indole derivatives with substituents at C_{10} (10-methoxyvinorine, (+)-10-methoxyakuammiline) [5, 6], and in the IR spectra (KBr) there are bands at 830 and 880 cm^{-1} (1,2,4-trisubstituted benzene ring), 1748 cm^{-1} ($\nu_{C=O}$ of an acetyl group), 1635 cm^{-1} ($\nu_{C=N}$ of 2H-indole), and 1595 cm^{-1} (skeletal vibrations of an aromatic ring) and a broad band with its center at 3200 cm^{-1} (OH group). The mass spectrum of the base is characterized by the peaks of ions with m/e 380 M^+, 362 $(M-18)^+$, 352 $(M-28)^+$, 351 $(M-29)^+$, 350 $(M-30)^+$, 337 $(M-43)^+$, 321 $(M-59)^+$ and peaks of β-carboline ions with m/e 213 and 199. The facts given permit the hypothesis that (I) belongs to the 2H-indole bases and is structurally close to 10-methoxyvinorine [5].

The molecular ions of these alkaloids differ by 16 mass units, and in the IR spectrum of (I) there is the band of an OH group, which gives grounds for assigning majorinine to hydroxy derivatives of 10-methoxyvinorine. The presence of an OH group is also confirmed by the formation of a O-acetyl derivative of majorinine (II).

The reduction of (I) with sodium tetrahydroborate gives a mixture of substances with molecular weights of 342, 340, and 324 (mass spectrum). The substance with mol. wt. 324 had the same R_f values and gave the same color with cerium sulfate as lochnerine.

Treatment of the base with sodium tetrahydroborate led to the reduction of the OAc and the O groups and to a desmotropic transformation of the 2H-indole base into the 1H-indole base. As a result, lochnerine was formed. The substances with mol. wt. 342 and 340 are products of incomplete reduction of (I). On this basis, it was assumed that in majorinine there is an OH or an OAc group at the C_{21} atom.

To establish the structure and stereochemistry of majorinine we studied the PMR spectra of the base itself and of its acetyl derivative (II).

The assignment of the signals of the protons, the analysis of their interaction, and the determination of the values of the CSs and spin-spin coupling constants (SSCCs) of (I) and (II) was made by using the double-resonance collapse, and the INDOR methods. The spectral results obtained on spectrometers with working frequences ν_0 of 100 and 360 MHz (for the O-acetyl derivatives) are given in Table 1 and Fig. 1.

In the PMR spectra of compounds (I) and (II), the three protons of the aromatic ring give a pattern corresponding to a three-spin system of the AMX type, the CS and SSCC values of which can be determined directly from the spectrum.

As can be seen from the figures given in Table 1, the CS value of the signal of the X proton – a doublet with its center at 7.53 ppm, $J_{ortho} = 8.5$ Hz – shows the position of the OCH_3 group at C_{10}, since it is precisely in the 10-methoxy-substituted 2H-indole that the signal of the H_{12} proton is observed in the weakest field at

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TABLE 1. Characteristics of the PMR Spectra of Majorinine and its Acetate

Assignment	Chemical shift, ppm		SSCC, Hz
	majorinine	21-O-acetylmajorinine	
9—H	7,02 d	7,01 d	$J_{9,11}=2,3$
11—H	6,90 q	6,90 q	$J_{11,12}=8,5$; $J_{11,9}=2,3$
12—H	7,53 d	7,53 d	$J_{12,11}=2,3$
17—H_α	4,98 m	4,97 br.s	$J_{17,16}<0,5$; $^4J_{17,6}\approx 1,5$
16—H_α	2,37 t	2,46 t	$J_{16,15}=6$; $J_{16,5}=6$; $J_{16,17}<0,5$
5-H_α	3,85 m	3,70 q	$J_{5,6}=5$; $J_{5,6\alpha}<0,5$; $J_{5,16}=6$
6—H_β^{eq}	2,71 q	2,70 q	$J_{6\beta,6\alpha}=12$; $J_{6\beta 5}=5$
6—H_α^{ax}	1,67 q	1,70 q	$^2J_{6\alpha,6\beta}=12$; $^4J_{6\alpha,17}\approx 1,5$; $J_{6\alpha,5}<0,5$
3—H_α^{eq}	4,30 q	4,38 q	$J_{3,14}=7$; $J_{3,14\beta}=3$
14—H_α, H_β	1,95 m	1,93 m	$W_{1/2}=11$
15—H_α	3,28 m	3,32 t	$J_{15,16}=6$; $J_{15,14}=6$; $^4J_{15,19}<0,5$
21—H_α	4,98 m	6,03 t	$^4J_{21\alpha,19}\approx 2$; $^5J_{21\alpha,18}\approx 2$
19—H	5,70 v	5,48 v	$J_{19,18}=6,5$; $^4J_{19,21\alpha}\approx 2$
18—CH_3	1,67 q	1,71 q	$J_{19,18}=6,5$; $^5J_{18,21\alpha}\approx 2$
17—$OCOCH_3$	2,19 s	2,19 s	—
21—$OCOCH_3$	—	2,16 s	—
10—OCH_3	3,82 s	3,83 s	—

Abbreviations: s) singlet; d) doublet; t) triplet; q) quartet; o) octet; m) multiplet; br. s) broadened singlet.

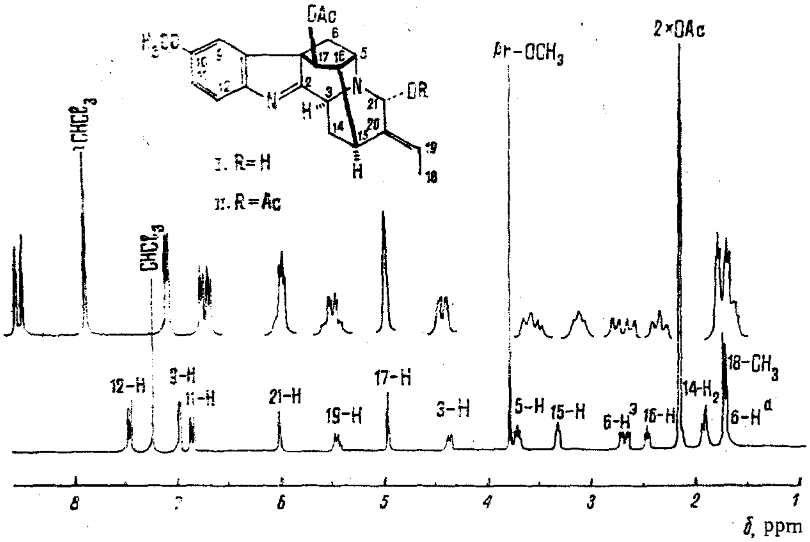


Fig. 1. PMR spectrum of majorinine acetate (II) at 360 MHz in $CDCl_3$.

7.45-7.55 ppm as compared with the CS of H_9 [5, 6]. Furthermore, although the PMR spectrum of the derivative reduced at the C=N double bond shows the formation of a mixture of products, there is nevertheless an appreciable upfield shift of the doublet with δ 7.53 ppm, J=8.5 Hz, to δ 6.90 ppm, which unambiguously shows the position of OCH_3 group in (I) at C_{10}.

The signal of the 19-H olefinic proton of the ethylidene group in the spectra of (I) and (II) appears in the form of an octet with $J_1=6.5$ Hz and $J_2=2.0$ Hz. It has been found by the double-resonance collapse and INDOR methods that the high SSCC of 6.5 Hz is due to interaction of the olefinic proton with the 18-CH_3 proton and the low SSCC (2.0 Hz) to its allyl interaction with the 21-H gem-hydroxyl proton in (I) and gem-acyl proton in (II), since when the signal of the olefinic proton is saturated, a contraction and an increase take place in the intensity of the 21-H signal at 4.98 ppm in (I) which is superposed on the signal of the gem-acyl proton (17-H) in (II), and conversely, on irradiation with ν_2=498 Hz in (I), the 21-H signal, the 19-H octet at 5.70 ppm is transformed into a quartet with J=6.5 Hz.

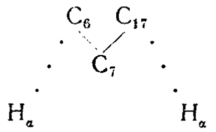
When (I) was acetylated to give (II), in the spectrum of the latter (see Fig. 1) the signal of the 21-H gem-acyl proton had shifted downfield by $\Delta\delta$ =1.05 ppm as compared with (I) and had a triplet structure with $J_1=J_2=$ 2.0 Hz.

As already mentioned, one of the SSCCs of this proton is due to allyl interaction with the 19-H olefinic protons, and the second is caused by the homoallyl interaction of 21-H with the 18-CH_3 protons, as has been shown by the double-resonance method.

The one-proton quartets at 4.30 and 4.38 ppm with J_1=7 and J_2=3 Hz relate to 3-H in (I) and (II) respectively, and shows its α-orientation [6, 7]. By double irradiation we found that 3-H_α interacts with the 14-CH_2 protons – multiplet (2H) at 1.95 ppm – and the latter is connected with a broad signal at 3.28 ppm in the spectrum of (I) (in the spectrum of (II) on an instrument with ν_0=360 MHz, this signal appears in the form of a poorly resolved triplet, see Fig. 1). Consequently, the signal at 3.28 ppm relates to 15-H_α. We then established that when the 15-H_α signal is saturated (ν_2=328 Hz), the triplet at 2.37 ppm, $J_1=J_2$=6 Hz in the spectrum of (I) is converted into a doublet, i.e., the latter relates to the 16-H proton.

By the application of the INDOR method to the lines of the 16-H triplet it was possible to detect a connection with the signal of the proton at 3.85 ppm, which, in its turn, interacted with the quartet at 2.71 ppm, J_1=12 Hz and J_2=5 Hz. It is obvious that the signal at 3.85 ppm relates to 5-H and the quartet at 2.71 ppm, which has a geminal SSCC of 12 Hz, belongs to the equatorial proton of the 6-CH_2 group. INDOR signals on the lines of the quartet at 2.71 ppm enabled us to find the signal of the axial proton 6-H_α^{ax} at 1.67 and 1.70 ppm in the spectra of (I) and (II), respectively, this normally being masked by the 18-CH_3 signal.

It must be observed that the results of double-resonance experiments show the absence of coupling between the 17-H_α and 16-H_α protons in the spectrum of (II) (see Fig. 1) and, therefore, the $H_\alpha-C_{17}-C_{16}-H_\alpha$ dihedral angle must be close to 90°. In actual fact, it can be seen from a Dreiding model of (I) that the size of this angle is approximately 100°. At the same time, we found a long-range stereospecific interaction by the M or the W route between the 6-H_α^{ax} and the 17-H_α protons with a value of $^4J \approx 1.5$ Hz.



We detected a similar long-range interaction of protons in the spectrum of vincamajine [8] in which, on saturation of the 6-H_α signal with ν_2=165 Hz (v_0=100 MHz, $CDCl_3+CD_3OD$, TMS), the half-width of the 17-H signal at 4.21 ppm decreased considerably, and its intensity increased. Apparently, the long-range spin–spin coupling between the 6-H_α and 17-H protons is characteristic for alkaloids of the ajmaline and sarpagine type and can serve as a reliable criterion in determining the orientation of substituents at C_{17} in them. The orientation of the OH group at C_{21} was chosen on the basis of the fact that the PMR spectra of majorinine and vomilenine [9] coincide, with the exception of the signals of the aromatic protons.

Thus, on the basis of the results of an analysis of NMR, mass, IR, and UV spectra, and also of chemical transformations, the structure of the new alkaloid majorinine has been established as 21-hydroxy-10-methoxyvinorine, and its stereochemistry as shown by formula (I).

EXPERIMENTAL

The UV spectra were taken on a Hitachi EPS-3T spectrophotometer, the IR spectra on a UR-20 instrument (KBr), the mass spectra on a MKh-1303 mass spectrometer fitted with a system for direct introduction into the ion source, and the PMR spectra on JEOL JNM-4H-100/100 MHz and Varian XL-100 spectrometers in $CDCl_3$ with TMS as internal standard, δ scale. The double resonance collapse experiments were performed on the JNM-4H-100 instrument and the INDOR experiments on the XL-100. The spectrum of majorinine acetate was obtained on a Brüker WH-360 NMR spectrometer with a working frequency for ^1H nuclei of 360 MHz in $CDCl_3$, 0–TMS.

Isolation and Separation of the Combined Alkaloids. The ground epigeal part of the plant (9 kg) was wetted with a 6% solution of ammonia, and the alkaloids were exhaustively extracted with ether. By the usual treatment, 63.58 g (0.7%) of combined alkaloids was obtained from the concentrated ethereal extract. The whole of the material was dissolved in one liter of benzene and was separated by means of citrate-phosphate buffer solutions into six fractions: pH 6.5 – 3.60 g; pH 5.64 – 2.90 g; pH 3.87 – 5.58 g; pH 3.54 – 7.00 g; pH 2.85 – 5.50 g; and pH 2.38 – 5.00 g. The remainder of the combined material (26 g) remained in the benzene solution.

By separating each fraction on a column of alumina, the following bases were obtained: from the pH 6.5 fraction – akuammine and reserpinine; pH 5.64 – akuammine, vincamajoreine, and ervine; pH 3.87 – akuammicine; pH 3.54 – vincamajine and majoridine; pH 2.85 – majdine and majorinine; 2.38 – majdine, ervine, and reserpinine. From the material remaining in the benzene, reserpinine, a mixture of reserpinine and ervine, and a mixture of reserpinine, majoridine, and ervine were obtained.

O-Acetylmajorinine (II). A solution of 74 mg of majorinine in 2 ml of acetic anhydride was treated with two drops of pyridine and left at room temperature for a day. After the appropriate working up, compound (II) was obtained with mol. wt. 422.

Reduction of Majorinine with Sodium Tetrahydroborate. With stirring, 100 mg of sodium tetrahydroborate was added over 30 min to a solution of 40 mg of majorinine in 30 ml of methanol. A mixture of crystalline substances with mol. wt. 342, 340, and 324 was isolated from the reaction products.

SUMMARY

1. From the epigeal part of *Vinca major* have been isolated the known bases akuammicine, vincamajine, vincamajoridine, and ervine, and the new alkaloid majorinine with the composition $C_{22}H_{24}N_2O_4$, mp 195-196°C.

2. On the basis of chemical transformations and IR, UV, mass, and PMR spectra using the methods of double resonance collapse and INDOR the structure of 21-hydroxy-10-methoxyvinorine and the stereochemistry (I) have been established for majorinine.

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