THE STRUCTURE OF VINCARINE

P. Kh. Yuldashev and S. Yu. Yunusov

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Continuing the separation of the mixture of alkaloids of Vinca erecta on alumina, we have isolated a crystalline base with mp 263-264°; $[\alpha]_D$ +13.98° (methanol; 0.0032% in the epigeal part and 0.012% in the roots) [1]. Elementary analysis of the base and a determination of its molecular weight by mass spectroscopy has established its composition as $C_{21}H_{24}O_3N_2$. The alkaloid proved to be new and has been named vincarine.

Vincarine contains one methoxyl group and two active hydrogen atoms. The IR spectra show the presence of an OH or an NH group (3335 cm^{-1}) and also an ester group (1733, 1250 cm^{-1}). The oxidation of vincarine by the Kuhn-Roth method showed the presence of one C-alkyl group [2], which has the form of an ethylidene chain, as is shown by the presence in the NMR spectrum of a doublet of the three protons of a methyl group (δ = 1.55 ppm) and a quartet of the vinyl proton (δ = 5.10 ppm).

Vincarine is a derivative of an o-disubstituted benzene (770 cm^{-1}). This is confirmed by the presence in the NMR spectrum of a signal of four aromatic protons (δ = 6.56-7.25 ppm).

The acetylation of vincarine with acetic anhydride gave an O,N-diacetyl derivative with mp 170-171°; $[\alpha]_D$ – – 63.3° (methanol), $C_{25}H_{28}O_5N_2$, M 436 (by mass spectroscopy). The IR spectrum of O,N-diacetylvincarine has bands characteristic for an amide carbonyl group (1680 cm^{-1}) and an O-acetyl group (1765 cm^{-1}). The NMR spectrum of O,N-diacetylvincarine shows the signals of the six protons of the methyl groups of an O-acetate (δ = 1.78 ppm) and an N-acetate (δ = 2.35 ppm).

The hydroxyl group in vincarine must be secondary, since the NMR spectrum exhibits the signal of the single proton of the –CH–O group (δ = 5.5 ppm). Consequently, the developed formula of vincarine has the form

$$C_{19}H_{19}(=N-H)(=N-)(COOCH_3)(OH).$$

UV spectrum: λ, mμ: 242 and 292.

The nature of the absorption curve shows that the base is an indoline derivative [3].

The UV spectrum of O,N-diacetylvincarine has four maxima – λ, mμ: 210, 250, 280, and 290, which is characteristic for derivatives of N-acylindoline derivatives [4].

The reduction of O,N-diacetylvincarine with sodium borohydride leads to acetolysis with the formation of O-acetylvincarine with mp 56-57°, M 394 (by mass spectroscopy). The UV spectrum of O-acetylvincarine has two maxima – λ, mμ: 244 and 293 – and the IR spectrum of O-acetylvincarine has a band at 1740 cm^{-1}, the integral intensity of which corresponds to two ester carbonyl groups.

On heating in a sealed tube with 15% hydrochloric acid and 20% alcoholic alkali, vincarine forms an amino acid with mp 289-290° and composition $C_{20}H_{22}O_3N_2$.

The mass spectrum of vincarine and O,N-diacetylvincarine has two intense peaks of the molecular ions with 352 and 436 m/e. Besides the intense peak of the molecular ion at 352 m/e, there are medium-intensity peaks at 130 and 143 m/e formed from the indole part of the molecule and at 190 and 222 m/e formed by the non-indole part.

The decomposition of the 352 m/e molecular ion into the 130 m/e and 222 m/e ions is shown by the presence in the mass spectrum of a metastable peak at 139 m/e (calculated: 140.0 m/e).

The 190 m/e ion is formed from the 222 m/e ion by the splitting off of a hydroxymethyl group with one proton. The correctness of this transition $(222 \xrightarrow{-32} 190)$ is confirmed by the presence in the spectrum of vincarine of a metastable peak at 163 m/e (calculated 162.6 m/e).

The mass spectrum of O,N-diacetylvincarine has peaks at 130, 143, 190, 222, 264, 377, and 436 m/e. The intense peak at 377 m/e is formed by the splitting off of an acetoxy group from O,N-diacetylvincarine.

The medium-intensity peak at 264 m/e must be formed from that part of the molecule which contains the acetoxy group, since the acetyl group present on the indole nitrogen atom is readily split off in the mass spectroscopy of the N-acylindoline alkaloids [5]. Consequently, the masses 42, 130, and 264 m/e make up the 436 m/e molecular ion of O,N-diacetylvincarine.

O-Acetylvincarine decomposes similarly to O, N-diacetylvincarine and has peaks at 130, 143, 190, 222, 264, 335, and 394 m/e. The 130 and 264 m/e ions make up the mass of the molecular ion.

A comparison of the mass spectrum of vincarine with the spectrum of the alkaloid quebrachidine [5] established their identity. However, the properties of quebrachidine and vincarine differ markedly. Consequently, vincarine is perhaps a stereoisomer of quebrachidine.

The oxidation of vincarine (I) with lead tetraacetate gave a base with mp 159-160° (II).

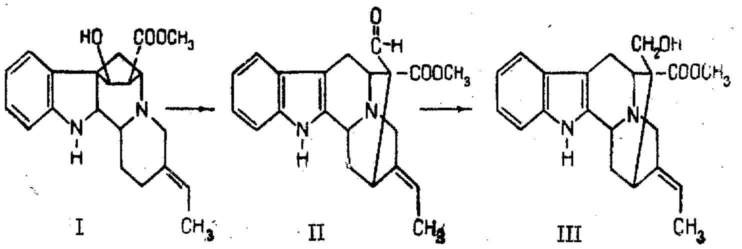
Bands of the UV spectrum (λ, mμ) at 223 and 274 are characteristic for alkaloids consisting of indole derivatives. The IR spectrum of compound (II) has bands of an ester carbonyl group (1755 cm^{-1}), an aldehyde group (1710 cm^{-1}), and a secondary nitrogen atom (3400 cm^{-1}).

Reduction of the indole base (II) with sodium borohydride gave the base (III) with mp 241-242°, which forms an O-acetyl derivative with mp 272-274°. Its UV and IR spectra and other properties coincide with the properties of the alkaloid polyneuridine [7], and the transition to this showed the structure of vincarine.

In quebrachidine the hydroxyl and ester groups occupy the cis position, and in vincarine they must occupy the trans position, since the IR spectrum of vincarinic acid has an absorption band of the carboxyl ion at 1600 cm^{-1} and deformation vibrations of the NH_2 group at 1660 cm^{-1} because of the formation of an internal salt.

The frequencies mentioned are characteristic for amino acids with secondary nitrogen atoms [6], and therefore the methoxycarbonyl group in vincarine is located close to the secondary atom and lies in the same plane as this.

Thus, vincarine differs from quebrachidine by the configuration of the C_{16} carbon atom and is its diastereoisomer:



EXPERIMENTAL

Vincarine was recrystallized from methanol, mp 263-264°. In a thin-layer chromatogram, it gave R_f = 0.55 in the ethyl acetate-methanol (8:2) system and R_f = 0.60 in the ethyl acetate-methanol (6:4) system. $[\alpha]_D$ + 13.98° (c 0.785; methanol).

Found %: C 71.65; 71.38; H 6.59; 6.60; N 8.01; 7.93; OCH_3 9.2; 8.50. H_{act} 0.55, 0.64; M 352.0 (by mass spectroscopy). $C_{21}H_{24}O_3N_2$. Calculated %: C 71.56; H 6.86; N 7.96 OCH_3 8.73; $2H_{act}$ 0.56; M 352.4.

UV spectrum λ, mμ: 242, 292 (log ε 3.84, 3.50).

Vincarinic acid. A solution of 270 mg of vincarine in 10 ml of 15% hydrochloric acid was heated for 4 hr at 115° in an evacuated and sealed tube. After the tube had been opened, the contents were made alkaline with ammonia solution and were extracted with ether. This gave 185 mg of vincarine. The mother liquor was evaporated to dryness and washed with hot water. The residue consisted of 48 mg of vincarinic acid with mp 289-290° (decomp., from alcohol). The acid is readily soluble in ammonia solution and precipitates on evaporation. It is very sparingly soluble in water.

Found %: N 7.92; 7.70. $C_{20}H_{22}O_3N_2$. Calculated %: N 8.27.

UV spectrum: λ, mμ: 242, 292 (log ε 3.98, 3.65).

Vincarinic acid was obtained in good yield by the saponification of vincarine with 20% alcoholic alkali for 3 hr.

O, N-Diacetylvincarine. A solution of 580 mg of vincarine in 5 ml of acetic anhydride was heated for 4 hr under reflux in a sand bath. The excess of acetic anhydride was evaporated off under vacuum, the residue was dissolved in water, and the solution was made alkaline with 10% caustic potash solution and was extracted with ether. The residue after the distillation of the ether amounted to 0.61 g, mp 170-171° (from acetone). $[\alpha]_D^{19}$ – 63.3° (c 2.719; methanol).

Found %: C 68.90; 69.10; H 6.63; 6.53; N 6.26; 6.16. $C_{25}H_{28}O_5N_2$. Calculated %: C 68.78; H 6.47; N 6.41; M 436 (by mass spectroscopy).

UV spectrum: λ, mμ: 210, 250, 280, 290 (log ε: 4.30, 4.09, 3.45, 3.30).

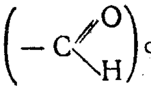
O-Acetylvincarine. A mixture of a solution of 94 mg of O, N-diacetylvincarine in 5 ml of aqueous methanol and 100 mg of sodium borohydride was left for 4 hr and was then diluted with water and extracted with ether. The residue after the distillation of the ether was dissolved in benzene and the solution was passed through alumina. On elution with a mixture of benzene and ether (1:1), the 3rd-5th fractions gave 35 mg of O-acetylvincarine with mp 56-57° (from petroleum ether).

On a thin-layer chromatogram in the ethyl acetate-methanol (9:1) system, R_f 0.54. M 394.0 (by mass spectroscopy).

UV spectrum λ, mμ: 244, 293 (log ε: 3.82, 3.38).

Oxidation of vincarine with lead tetraacetate and isolation of the aldehyde (II). A solution of 500 mg of vincarine (1 mole) in 10 ml of glacial acetic acid was treated with 630 mg (1 mole) of lead tetraacetate and the mixture was left for 24 hr at room temperature. It was then diluted with water and made alkaline with ammonia, and the base was extracted with ether. After drying, the ethereal solution was concentrated; 180 mg of crystals with mp 159-160° (from methanol) deposited. In the butanol-acetic acid-water (100:5:100) system, R_f 0.80.

UV spectrum λ, mμ: 223, 274 (log ε: 3.72, 3.14).

IR spectrum in KBr: 3400 (=N−H), 1755 ($COOCH_3$), 1710  $\left(-C\begin{smallmatrix}\nearrow O\\ \searrow H\end{smallmatrix}\right)$ cm^{-1}.

Reduction of the aldehyde (II) with sodium borohydride and isolation of polyneuridine (III). With stirring, 200 mg of sodium borohydride was added over 1.5 hr to a solution of 142 mg of the aldehyde in 10 ml of aqueous methanol (9:1). The mixture was diluted with water and extracted with ether. From the ether, 126 mg of polyneuridine with mp 241-243° was isolated.

UV spectrum λ, mμ: 228, 283, 290 (log ε 3.88, 4.22, 4.11).

IR spectrum in KBr: 3150 (=N−H), 3470, 3570 (OH), 1730, 1220 ($COOCH_3$) cm^{-1}.

O-Acetylpolyneuridine. A solution of 97 mg of polyneuredine in 4 ml of a mixture of pyridine and acetic anhydride (1:1) was left at room temperature for 2 days. The excess of acetic anhydride and pyridine was evaporated off in vacuum. The residue was recrystallized from methanol: mp 272-274°.

IR spectrum in KBr: 3150 (=N−H), 1752 ($COOCH_3 \cdot OCOCH_3$) cm^{-1}.

SUMMARY

A new base, vincarine, has been isolated from the roots and epigeal part of *V. erecta*. Its formula is $C_{21}H_{24}O_3N_2$.

The structure of vincarine has been established by analysis of the UV, IR, NMR, and mass spectra and also by its conversion into polyneuridine; it is a diastereoisomer of quebrachidine.

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Institute of the Chemistry of Plant Substances AS Uzbek SSR