STRUCTURES OF ERVINCEINE, ERVAMICINE, AND ERVINCININE

D. A. Rakhimov, V. M. Malikov, M. R. Yagudaev, and S. Yu. Yunusov

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By the further separation of the mixture of alkaloids from the epigeal part of Vinca erecta we have isolated another four bases: ervinceine, ervamicine, ervincinine and (-)-eburnamonine [1-4].

Ervinceine (I), $C_{22}H_{28}N_2O_3$, contains two methoxyl and one C-ethyl groups. Its UV spectrum is characteristic for α-methyleneindoline alkaloids.

Ervamicine (II), $C_{22}H_{26}N_2O_3$, is an amorphous base. It forms a crystalline hydriodide and hydrochloride. Its UV spectrum is similar to those of ervinceine and ervincinine. The IR spectra of I and II have the absorption bands of a secondary nitrogen atom, an ester carbonyl group conjugated with a double band, and a 1, 2, 4-trisubstituted benzene ring. On the basis of these data, ervinceine and ervamicine have the following developed formulas:

$C_{19}H_{21}(>N-H)(>N-)(OCH_3)(COOCH_3)(=)$ (I)

$C_{19}H_{19}(>N\ \ H)(>N-)(OCH_3)(COOCH_3)(=)_2$ (II)

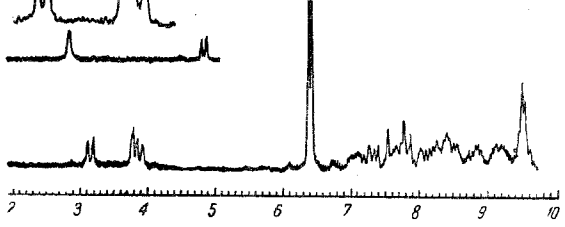
The reduction of I with zinc in methanolic sulfuric acid gave an indole base, dihydroervinceine $C_{22}H_{30}N_2O_3$ (IV).

The mass spectrum of IV (table) has the molecular peak M^+ with m/e 370, and also the peaks of ions with m/e 284 and 124. The formation of the fragment with m/e 284 in IV is confirmed by a metastable peak with m/e 218 (calculated 218.3) for 370 → 284. The mass spectra of ervincinine and of 16-methoxyvincadifformine are similar [5], but differ in the intensities of the peaks. Since, according to the IR spectrum, ervinceine is a 1, 2, 4-trisubstituted benzene derivative, its methoxyl group may be present in position 15 or 16.

Relative Intensities, m/e, %

Substance	M^+	Fragments	
		indole	nonindole
Ervinceine (I)	368 (45)	—	124 (100). 125 (16)
Ervamicine (II)	366 (60)	—	135 (100). 107 (47)
Ervincinine (III)	382 (100)	351 (10). 244 (78)	138 (9). 108(16)
Dihydroervinceine (IV)	370 (15)	284 (26). 174 (4)	124 (100). 125 (11)

In the NMR spectrum of ervinceine (figure), the three protons of the aromatic ring (14, 15, and 17) form a three-spin system of the ABX type which can be subjected to first-order analysis. Directly from the spectrum we find the chemical shifts (CSs) and spin-spin coupling constants (J) of the A (17), B (15), and X (14) protons, namely: τ_X 3.12, τ_A 3.72, and τ_B 3.85 ppm; $J_{BX} = 8.0$ Hz, $J_{AB} = 2.0$ Hz, and $J_{AX} = 0$.



NMR spectrum of ervinceine.

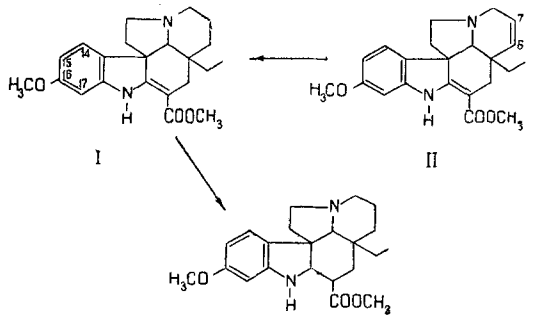
The appearance of the CS of the X (14) proton in the weaker field and also the displacement of its signal by 0.37 ppm in the strong-field direction in the spectrum of dihydroervinceine as compared with ervinceine shows that

there is a OCH_3 at C_{16} in the benzene ring. In actual fact, if the OCH_3 group were present in position 15, the CSs of all three protons could be similar and would be shifted in the strong-field direction because of the electron-donating influence of the methoxyl on the 14- and 16-protons [6].

The signals of the ethyl protons of the methoxyl and ester groups of ervinceine give two three-proton singlets at τ 6.34 and 6.37 ppm, respectively. The CH_3 protons of the ethyl group appear in the form of a triplet at τ 9.47 ppm (3H), J = 4.5 Hz, and the signals of the CH_2 group are in the 8.0 ppm region. The quartet at τ 7.20 ppm corresponds to two nonequivalent protons of a CH_2—N group, and the singlet at τ 7.48 ppm (1H) to a CH—N group. The signal of an NH proton is found in the weak field at τ 1.12 ppm.

In the NMR spectrum of dihydroervinceine, the CSs of the signals of the protons, apart from the X (14) proton, practically coincide with those of the protons of ervinceine, with the exception of the NH proton which is markedly displaced in the strong-field direction at τ 5.81 ppm, which is in harmony with the characteristic feature found previously for these alkaloids [7].

On the basis of what has been said above, structure I, isomeric with 16-methoxyvincadifformine is proposed for ervinceine.



A comparison of the developed formulas I and II shows that ervamicine differs from ervinceine only by the presence of a double bond. When ervamicine was subjected to Adams hydrogenation in ethanol, 1 mole of hydrogen was absorbed and 6,7-dihydroervamicine, $C_{22}H_{28}N_2O_3$, was formed. The latter was shown to be identical (R_f, UV, IR, and mass spectra), with ervinceine.

The NMR spectrum of ervamicine has the signals of three aromatic protons A (17), B (15), and X (14): τ_X 3.06, τ_A 3.71, and τ_B 3.82 ppm, J_{BX} = 8.0 Hz, J_{AB} = 2.0 Hz, and J_{AX} = 0, it also has the signals of an Ar—OCH_3 group at τ 6.34 ppm, of a $COOCH_3$ group at τ 6.37 ppm (singlet, 3H each), and of a N—CH_2—C=C group at τ 6.70 ppm (poorly resolved quartet, 2H), i.e., the two CH_2 protons are almost equivalent.

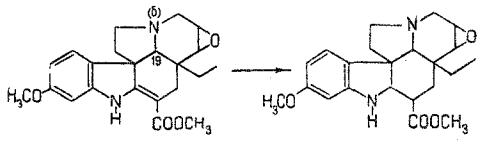
The two olefinic protons of ring D form a AB system and give a quartet with τ_A 4.39 and τ_B 4.47 ppm, J_{AB} = 10 Hz, which shows the cis arrangement of these hydrogen atoms relative to the double bond. The CH_3 group of the ethyl appears at τ 9.42 ppm (triplet, 3H) with J = 6.0 Hz and the CS of the signal of the NH proton is 1.05 ppm. The mass spectrum of ervamicine is similar to that of 16-methoxytabersonine [8], isolated from V. minor. However, the melting points of the hydrochlorides of these alkaloids and the CS of the protons in the NMR spectra are different. Consequently ervamicine is perhaps a stereoisomer of methoxytabersonine and has the structure II.

Ervincinine (III), $C_{22}H_{26}O_4N_2$, contains two methoxyl groups and an active hydrogen. The IR absorption spectrum of III shows the presence of an ester carbonyl group conjugated with a double bond, a secondary nitrogen atom, and a 1,2,4-trisubstituted benzene ring. The UV spectrum of ervincinine is similar to the spectra of I and II.

The oxidation of ervincine by a modified Kuhn-Roth method yielded acetic and propionic acids, which shows the presence of a C-ethyl group in it. The reduction of the base with zinc in methanolic H_2SO_4 gave an indoline base, dihydroervincinine (V), $C_{22}H_{28}O_4N_2$. Its IR spectrum contained the bands of an unconjugated carbonyl group (1720 cm^{-1}).

The saponification of ervincinine in HCl (15%) gave desmethoxycarbonylervincinine, the UV spectrum of which had the maximum characteristic for the indolenine bases.

The mass spectrum of ervincinine proved to be similar to that of lochnerinine (differing only by the low intensity of the peaks with m/e 138 and 108). The peak with m/e 138 shows the presence of an aspidospermine skeleton containing oxygen attached to the piperidine part of the molecule. The position of attachment of the ethereal oxygen was established from a comparison of the properties of ervincinine with those of other alkaloids of similar structure [9].



III V

The signals of the AB part of the aromatic protons X (14), A (15), and B (17) in ervincine were identical with the signals of dihydroervinceine: τ_X 3.02, τ_A 3.77, and τ_B 3.66 ppm, and $J_{AX} = 8.0$ Hz, $J_{AB} = 2.0$ Hz, and $J_{BX} = 0$. The two OCH_3 groups give a six-proton singlet at τ 6.29 ppm, the signal of the methine proton in the N—CH_2CH—O grouping appears in the form of a quartet at τ 5.72 ppm with $J_1 = 12.0$ Hz and $J_2 = 5.0$ Hz, and the second CH—O methine forms a broadened singlet at τ 6.44 ppm. The CH_2—N protons appear at τ 7.36 ppm, those of the CH_3—CH_2 group at τ 9.38 ppm (triplet), and that of the NH group at τ 1.10 ppm.

The IR spectra of ervinceine, ervamicine, and ervincinine lack absorption bands in the 2700–2800 cm^{-1} region, which shows the cis-orientation of the hydrogen with respect to the unshared pair of electrons of the N^{δ} nitrogen atom.

In all its properties (mp, $[\alpha]_D$, UV, IR, and mass spectra), the quaternary base resembles (–)-eburnamonine, isolated from V. minor.

A comparison of the structures of ervinceine, ervincinine, and ervamicine shows that they are very similar. It is extremely likely that mutual transitions take place in the plant between these alkaloids [10].

EXPERIMENTAL

Ervinceine (I). Seventy grams of the combined ethereal alkaloids was chromatographed on alumina (activity grade II, 1:30). Then, 5 g of the combined alkaloids eluted by benzene (fractions 1–3) was distributed between 85% ethanol and petroleum ether (40–70° C). The petroleum ether fraction yielded 3 g of a mixture of hydriodides of bases. After four recrystallizations from methanol, a hydriodide with mp 214–215° C was obtained. This hydriodide yielded base with mp 99–100° C (methanol), $[\alpha]_D^{12}$ –448° (c 0.9; chloroform). IR spectrum, cm^{-1}: 3335, 1622, 1682, 805, 868. UV spectrum, $\lambda_{max}^{C_2H_5OH}$, mμ: 248, 328 (log ε 4.12, 4.26). Mol wt 368.

Dihydroervinceine (IV). A solution of 100 mg of the base in 50 ml of 10% methanolic sulfuric acid was treated with 2 g of zinc dust, and the mixture was boiled in the water bath for 40 min. The filtered solution was evaporated in vacuum to small volume, and the residue was dissolved in 30 ml of water, made alkaline, and extracted with ether. Dihydroervinceine was obtained with mp 118–119° C (methanol), $[\alpha]_D^{20}$ +33.0 (c 0.9; chloroform), R_f 0.78 [TLC on silica gel, ether–petroleum ether (1:2)]. IR spectrum: 1720 cm^{-1}. UV spectrum, $\lambda_{max}^{C_2H_5OH}$, 249, 305 (log ε 3.78, 3.73). Mol wt 370.

Ervamicine (II). The material (11 g) obtained from the mother liquor after the isolation of the ervinceine was dissolved in benzene, and the benzene solution was extracted successively with citrate-phosphate buffer solutions at pH 4.5 and 2.8, and with 1 N HCl. The latter fraction deposited crystals of a hydrochloride with mp 213–214° C (methanol) from which an amorphous base was obtained (0.4 g), $[\alpha]_D^{20}$ –264.4° (c 1.4; chloroform), R_f 0.8 [TLC, ether–chloroform (1:1)] and R_f 0.87 [TLC, benzene–ether (3:1)]. IR spectrum (in KBr), cm^{-1}: 1670, 3390. Hydriodide, mp 207–208° C (methanol); IR spectrum, cm^{-1}: 795, 860; mol wt 366.

6,7-Dihydroervamicine. Ervamicine (70 mg dissolved in 30 ml of ethanol) was reduced in an atmosphere of hydrogen in the presence of a platinum catalyst for 3 hr. The ethanolic solution was separated from the catalyst and was evaporated to dryness under vacuum. Yield 60 mg, R_f 0.36 [ether–petroleum ether (1:2)]. UV spectrum, $\lambda_{max}^{C_2H_5OH}$, mμ: 248, 329 (log ε 4.12, 4.26). Mol wt 368.

Ervincinine (II). On treatment with acetone, fractions IV–VII, eluted with benzene, deposited a base with mp 247–248° C [from acetone–methanol (1:3)], $[\alpha]_D^{22}$ –80.5° (c 0.39; chloroform), R_f 0.84 [TLC on silica gel,

benzene–methanol (9 : 1)], IR spectrum, cm^{-1}: 3265, 1615–1685, 800, 840; UV spectrum, $\lambda_{max}^{C_2H_5OH}$, mμ: 250, 330 (log ε 4.08, 4.18); mol wt 382.

Dihydroervincinine (V). The reduction of 25 mg of ervincinine by the method described above yielded 20 mg of dihydroervincinine with mp 206.5–207.5° C (from ether), mol wt 384, R_f 0.7 [TLC on silica gel, benzene–methanol (9 : 1)]. IR spectrum: 1720 cm^{-1}. UV spectrum: $\lambda_{max}^{C_2H_5OH}$, mμ: 245, 303, (log ε 4.06, 4.02).

(–)-Eburnamonine. The combined alkaloids were separated according to their basicities by means of a phosphate buffer solution. The pH 1.8 fraction, on treatment with acetone, gave a base with mp 167–168° C. IR spectrum, cm^{-1}: 760, 1700; mass spectrum: 294 M^+, 265, 237, 224; mass spectrum, $\lambda_{max}^{C_2H_5OH}$, mμ: 243, 268, 297–305 (log ε 4.55, 4.16, 3.36).

The mass spectra were taken on a MKh-1303 instrument at 110° C with an energy of the ionizing electrons of 40 eV; the NMR spectra of all the alkaloids were recorded on a JNM-4H-100/100 MHz instrument with HMDS as internal standard (τ scale). The spectra of ervinceine, dihydroervinceine, and ervamicine were obtained in CCl_4, and that of ervincinine in $CDCl_3$.

CONCLUSIONS

1. The epigeal part of Vinca erecta has yielded (–)-eburnamonine and the new bases ervinceine, ervamicine, and ervincinine.

2. The structures of these bases have been established by means of spectroscopic data and some chemical reactions.

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