THE POLYBUFFER SEPARATION OF THE ALKALOIDS OF Vinca erecta

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The total alkaloids of V. erecta consist of a complex mixture of bases [1, 2]. We have separated this mixture according to basicity on an apparatus for polybuffer separation (APS) [3].

The mixture of alkaloids in an organic solvent was passed successively through a series of phosphate buffer solutions with various pH values arranged in order of increasing pH. The initial buffer solutions were prepared by mixing a 25% solution of caustic potash and orthophosphoric acid. Each column of the APS was previously provided with 700 ml of chloroform to fill the outlet tubes, and then each pair of columns was charged with 1500 ml of buffer solution with a definite pH value (from 8 to 1). The solution of the mixture of bases (200 g) in 4000 ml of chloroform was passed through the apparatus. After the solution had passed through all the columns, washing with chloroform was carried out. Both the solution of the alkaloids and the chloroform were passed at the rate of 1000 ml/h. After the end of the distribution, the buffer and chloroform solutions from each column were poured off separately. The buffer solutions were made alkaline with 25% ammonia and extracted exhaustively with ether and then with chloroform. The solutions were distilled to dryness. The results of the distribution of the alkaloids are given below.

Fraction	pH of the buffer	Weight of the fraction, g	Fraction	pH of the buffer	Weight of the fraction, g
1	8.0	0.7	21	3.0	2.6
2	7.0	1.2	22	2.9	3.9
3	6.8	1.6	23	2.8	5.1
4	6.6	1.7	24	2.7	3.3
5	6.2	2.0	25	2.6	4.2
6	6.0	1.5	26	2.5	3.8
7	5.8	2.5	27	2.4	2.6
8	5.6	2.1	28	2.3	3.0
9	5.4	2.3	29	2.2	3.3
10	5.2	1.8	30	2.1	5.7
11	5.0	1.4	31	2.0	4.2
12	4.8	1.0	32	1.9	5.1
13	4.6	2.1	33	1.8	6.2
14	4.4	2.3	34	1.7	6.8
15	4.2	3.2	35	1.6	7.7
16	4.0	2.1	36	1.5	8.9
17	3.8	1.8	37	1.4	6.7
18	3.6	2.6	38	1.3	8.5
19	3.4	2.8	39	1.2	7.0
20	3.2	3.0	40	1.1	23.7
			41	1.0	

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The chloroform solutions that had passed through the APS were combined and distilled to dryness. The residue contained ervamine, ervine, kopsinilam, and kopsanone. The composition of the fractions obtained was checked by thin-layer chromatography [4].

The combined ethereal alkaloids (0.7 g) obtained from the first fraction were separated according to their solubility in organic solvents: benzene, acetone, and methanol. Concentration of the methanolic extract led to the deposition of 95 mg of a base with mp 279-280°C (methanol), $[\alpha]_D$ + 29.5° (c 0.6); methanol), R_f 0.38 (1), which we have called ervincidine. Its UV spectrum [λ_{max} 227, 282, 292 nm (log ε 4.8, 4.11, 4.0)] coincides with that of akummidine [5]. A broad absorption band (3000-3330 cm^{-1}) in the IR spectrum is due to a hydrogen bond between OH and NH groups. The mass spectrum of ervincidine has peaks with m/e 310 (M^+), 229, 279, 249, 182, 169, 168. Ervincidine is apparently a new alkaloid with the structure of 16- or 21-hydroxytombozine.

The second to sixth fractions were chromatographed in system 2. Bases with R_f 0.15, 0.2, 0.3, and 0.4 were detected.

On treatment with acetone, the seventh and eighth fractions deposited 2.1 g of tombozine [6].

The 9th to 13th fractions contained bases with R_f 0.5 and 0.7 (2).

The 14th to 16th fractions were dissolved in benzene, and on standing 1.4 g of akuammidine crystallized out.

The 17th fraction yielded 0.5 g of pseudokopsinine hydrobromide.

The 17th-21st fractions consisted of vinervinine, eburnamine, quebrachamine, and a base with R_f 0.55 (3). From the 20th fraction was obtained 0.7 g of kopsinine nitrate contaminated with pseudokopsinine.

The 22nd fraction was dissolved in benzene and chromatographed on a column of Al_2O_3 (activity grade II) in a ratio of 1 : 10.

On treatment with acetone, the benzene eluates gave a base with mp 213-214°C (acetone) $[\alpha]_D^{25}$ ± 0° (c 0.8; methanol). UV spectrum: λ_{max} 230, 285, 292 nm (log ε 4.89, 3.87, 3.79). IR spectrum, cm^{-1}: 740, 3000-3300 (OH). The mass spectrum of the base had the peaks of ions with m/e 296 (M^+), 278, 267, 249, 208, 206, 193. The NMR spectrum of the alkaloid had the signals of the protons of the following groups: 0.87 δ (5H, triplet, C_2H_5), 5.39 δ (1H, quartet, OH), 7.05-7.61 δ (4H, aromatic).

The composition, developed formula, and the nature of the UV and IR spectral absorption curves showed that the base is similar to the alkaloid eburnamine [7] with the exception of the specific rotation. The mass and NMR spectra of the base and of eburnamine are identical. Thus, the base is dl-eburnamine.

The 23rd fraction was distributed between 85% ethanol and petroleum ether (40-70°C). The alcoholic fraction yielded 0.7 g of an oily base with $[\alpha]_D^{29}$ – 236°, 0 (c 1.0; methanol), R_f 0.5 (4), the IR spectrum of which lacked the band of the stretching vibrations of an NH bond and a carboxy group, while the UV spectrum had maxima at 216, 223, and 267 nm (log ε 4.21, 4.23, and 3.70) characterizing it as a 3H-indole derivative The mass spectrum of the base had peaks with m/e 280 M^+), 251, and 210. The NMR spectrum had the signals of three protons of a methyl group in the form of a triplet (δ 0.45 ppm) and the signals of aromatic protons (δ 7.15-7.65). From its physical and spectral properties, the base was identified as (–)– 1,2-dehydroaspidospermidine [8].

The petroleum ether fraction was chromatographed on a column [silica gel – Al_2O_3 (1 : 1)] in petroleum ether. The elution was performed with mixtures of ether and petroleum ether (2 : 1 and 3 : 1). On concentration, crystals were formed with mp 143-144°C, $[\alpha]_D^{28}$ + 142° (c 1.0; methanol). The IR spectrum had the absorption bands for a secondary nitrogen atom (3400 cm^{-1}), and the UV spectrum had maxima at 230, 287, and 294 nm (log ε 4.68, 3.92, and 3.90) which are characteristic of indole bases. The mass spectrum of the base had peaks of ions with m/e 282 (M^+), 267, 253, 157, 124, 110, 96.

The properties of the base mentioned above coincide with those of (+)-quebrachamine, isolated previously from the plant Stemmenia donnell-Smith [9, 10].

The 24th to 29th fractions contained vincarine, apovincamine, vincamine, and a base with R_f 0.3 and 0.75 (3).

When a methanolic solution of the 25th fraction was allowed to stand, ervincine [11] crystallized out. A study of its NMR and mass spectra showed that the base is not an individual compound. By preparative

separation of this mixture in a thin layer of silica gel in system 4, two alkaloids with R_f 0.5 and 0.7 were isolated. The first of them proved to be vincamine and the second a base with mp 160-162°C (methanol). IR spectrum, cm^{-1}: 750, 1610, 1630, 1725. Mass spectrum: M^+ 336 (100%), 307 (83%), 266 (75%), 251 (10%). From its physicochemical constants and spectroscopic properties, the base was identified as apovincamine [7].

The 30th fraction was dissolved in a mixture of acetone and methanol (1 : 1), and from the solution 0.26 g of vincanine was obtained. The material from the mother liquor (2.1 g) was separated chromatographically on a column of Al_2O_3. On treatment with acetone, the benzene eluates gave 0.17 g of a mixture of crystals which, on TLC, showed two spots with R_f 0.35 (dl-vincanine) and 0.26 (3) (vincanicine).

The 32nd fraction was dissolved in ethanol and, with cooling, the solution was acidified with nitric acid. A mixture of nitrates amounting to 1.9 g deposited. Their separation according to their solubility in methanol gave ervine and reserpinine. The mother liquor after the isolation of the nitrates was made alkaline and extracted with ether. This gave 7.5 g of mixed alkaloids. The latter contained picrinine and bases with R_f 0.1 and 0.2 (4).

The 33rd fraction yielded eburnamonine, and the chromatography of the 33rd-40th fractions showed the presence of the following alkaloids: ervine, isoreserpiline, ervancinine, ervinidinine, and bases with R_f 0.2 and 0.79 (4).

The 41st fraction was subjected to chromatographic separation on a column. Benzene eluates gave 2 g of a mixture of crystals consisting of kopsinilam, ervinidine, and a base with mp 284-285°C, $[\alpha]_D^{20}$ + 98.6° (c 0.18; chloroform), R_f 0.4 (5). Its UV spectrum [λ_{max} 243, 298 nm (log ε 3.94, 3.61)] is characteristic for indoline bases. The IR spectrum had absorption bands at 3400 cm^{-1} (NH), 1750 cm^{-1} (CO), and 1670 cm^{-1} (five-membered lactam); mol. wt. 320 (mass spectrum). From its constants and UV, IR, and mass spectroscopic features, this base corresponds to kopsanone [12].

To identify the base, by heating it in ethanolic alkali with subsequent esterification by means of gaseous hydrochloric in methanol, a product was obtained from it which was identified by its mass spectrum and R_f value as kopsinilam [11, 13]. Consequently, the base that we isolated is indeed kopsanone.

Thus, by using the APS it is possible to separate the combined alkaloids into fractions each containing from 3 to 6 bases.

EXPERIMENTAL

The KSK silica gel used for thin-layer chromatography was previously ground in a ball mill, washed with conc. hydrochloric acid until the reaction for iron was negative and then with water to neutrality, and finally with methanol. The silica gel was dried in the air and was sieved, a fraction with grains of 120-200 nm being selected.

The sorbent consisted of a mixture of silica gel and gypsum (9 : 1). The layer was deposited by means of a device made as described by Stahl. The plates with the deposited layer of silica gel (without additional activation) were kept at room temperature for 12 h.

The spots were revealed with iodine vapor, Dragendorff's solution, and a 1% solution of cerium sulfate with conc. orthophosphoric acid.

The following solvent systems were used for TLC: 1) methanol, 2) ethyl acetate–methanol (3 : 1), 3) benzene–methanol (9 : 1), 4) chloroform–methanol (9 : 1), and 5) chloroform–methanol (96 : 4).

The UV spectra were taken on an SF-4 instrument (ethanolic solutions), the IR spectra on a UR-10 instrument (molded tablets with KBr and solutions in dry chloroform), the mass spectra on an MKh-1303 mass spectrometer with a glass inlet device at 40 eV and 150 mA, and the NMR spectra on a JNM-4H-100/100 MHz instrument with HMDS as the internal standard (δ scale). The pH values of the buffer solutions were determined on a LPU-1 potentiometer.

SUMMARY

By separating the total alkaloids of V. erecta according to their basicities in an APS, in addition to bases known previously we have isolated ervincidine, apovincamine, (+)-quebrachamine, dl-eburnamine,

(−)-1,2-dehydroaspidospermidine, and kopsanone. This is the first time that these alkaloids have been obtained from this plant. Thin-layer chromatography showed the presence of another 10-12 uncharacterized alkaloids.

The region of the passage into the buffer solutions of some alkaloids has also been established.

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