ALKALOIDS FORMED BY SOMATIC HYBRIDS OF \textit{Rauwolfia serpentina} + \textit{Rhazya stricta}


Somatic hybridization of higher plants is known to result in the creation of new "synthetic" cells whose genomes contain inherited determinants from both parents; the genomes of the hybrid cells and plants regenerated from them often show significant reconstructions, whose extent and direction can only poorly, if at all, be predicted. There are also significant changes in the secondary metabolism of the reconstructed cells and tissues.

We have studied producer plants of the Apocynaceae family: \textit{Rauwolfia serpentina} Benth. and \textit{Rhazya stricta} Decaisne. \textit{R. serpentina} is a valuable raw material for the preparation of a number of antiarrhythmic, hypotensive, and cardiostimulatory drugs, whose major active agents are indole alkaloids — ajmaline, ajmalicine, reserpine, etc. \textit{Rhazya stricta} is a small bush which grows in a number of areas of India and Pakistan; it is used in the folk medicine of these countries for the treatment of a number of diseases [1]. Previous studies have produced, selected, and analyzed hybrid cell lines using \textit{Rauwolfia} and \textit{Rhazya} [2], which were maintained as suspension and callus strains with quite high genetic stability over several years in culture [3].

EXPERIMENTAL

Hybrid cell line RR17 was cultured on production growth medium APII [4]. Biomass was collected from several passages and different subcultures and was frozen in liquid nitrogen and lyophilized. Alkaloids were extracted by our modification of the method described by A. Parr et al. [5]. Lyophilized biomass (62.5 g) was ground and extracted with 2 liters of 0.005 M orthophosphoric acid (pH 2.0) in Erlenmeyer flasks on a rotary shaker (100 rpm) for 12 h. The aqueous extract was filtered and the dry residue was re-extracted with 200 ml of 0.005 M orthophosphoric acid (pH 2.0); the filtrates were pooled. The pH of the filtrate was adjusted to 2.0 with concentrated orthophosphoric acid (COA), and 0.5 liter of toluene was added. The mixture was shaken vigorously for 2-3 min. The phases were separated in a separating funnel and the toluene was removed. The aqueous phase was treated with two further 0.5-liter volumes of fresh toluene. The pH of the aqueous extract was adjusted to 8.5 using 25% ammonia solution, and again extracted three times with 0.6-liter aliquots of toluene. The toluene — aqueous emulsion was separated by centrifugation (Beckman SW-40 rotor, 7500 rpm, 15 min); the organic layers were pooled and evaporated to dryness. A total of 81 mg of extract was obtained. The extract was separated by thin-layer chromatography (TLC) on 0.20-mm Silicagel F254 plates (Merck) in the following systems:

- **FM1**: chloroform:methanol:ammonia (9:1:0.1),
- **FM2**: chloroform:hexane:diethylamine (6:3:1),
- **FM3**: chloroform:methanol:ammonia (4:1:0.05), and
- **FM4**: acetone:petroleum ether:diethylamine (2:7:1).

The separated components of the extract were detected by the absorption of plate fluorescence on exposure to ultraviolet light at 254 nm.
After two-dimensional TLC separation of the extract, plates were dried in a hot air flow and sprinkled with a 5% solution of cerium (IV) ammonium sulfate in COA(CAS). After treatment with CAS, colored compounds were detected in daylight and by fluorescence on exposure to ultraviolet light at 366 nm.

Use of systems FM1 and FM2 resulted in separation of the total alkaloid extract of tissues produced from hybrid cell line RR17 into 50 fractions.

Fractions 1-10 were scraped from 35 TLC plates and eluted with a mixture of dichloromethane and methanol (8:1), loaded onto TLC plates and again separated, this time using systems FM3 and FM4; silica gel was again scraped from the plates and eluted with dichloromethane/methanol (8:1), and evaporated to dryness. The dry residue was dissolved in 50 μl of dichloromethane, and 15 μl was placed in a mass spectroscopy sample vessel, evaporated to dryness and finally dried in vacuo. Mass spectra were recorded using a Finnigan Mat-44S mass spectrometer (T 45-300°C, 60°C/min, electron ionization energy 70 eV).

Indole alkaloids were identified using the COA reaction, fluorescence in UV light after treatment with CAS, Rf values in various systems, and by mass spectrometric data.

1. Tubotaiwine (fraction 1-1).

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\text{CAS reaction: dark blue (turning brown after 30-40 min); fluorescence (366 nm): none.}
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\text{Rf values: 0.43, 0.49, 0.63 and 0.33 in systems FM1, FM2, FM3 and FM4 respectively.}
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\text{Mass spectrum, m/z (relative intensity, %): 324 (M^+, 43), 295 (2), 293 (2.4), 267 (35), 240 (9.5), 229 (100), 182 (60), 181 (78), 180 (92), 168 (52), 167 (84).}
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\text{These data are in agreement with results presented in [6].}
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2. Vallesiachotamine (fraction 6).

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\text{CAS reaction: yellow color; fluorescence (366 nm): yellow.}
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\text{Rf values: 0.65, 0.44, 0.72, and 0.29 in systems FM1, FM2, FM3 and FM4 respectively.}
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\text{Mass spectrum, m/z (relative intensity, %): 350 (M^+, 50), 335 (10), 322 (46), 321 (16), 319 (16), 318 (20), 307 (30), 291 (50), 279 (97), 265 (42), 264 (60), 263 (100), 249 (23), 247 (17), 223 (22), 222 (23), 221 (77), 219 (16), 209 (38), 208 (20), 184 (21), 178 (1), 170 (28), 169 (33), 168 (27), 156 (21), 144 (28).}
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\text{These data are in agreement with results presented in [7].}
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3. Vomilenine (fraction 8).

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\text{CAS reaction: colorless; fluorescence (366 nm): light blue.}
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\text{Rf values: 0.46, 0.12, 0.67, and 0.14 in systems FM1, FM2, FM3 and FM4 respectively.}
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\text{Mass spectrum, m/z (relative intensity, %): 350 (M^+, 22), 307 (8), 291 (8), 183 (13), 169 (100), 156 (8).}
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