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Note

Thermospray liquid chromatographic–mass spectrometric analysis of *Catharanthus* alkaloids

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Catharanthus roseus (L.) G. Don is a source of the medicinally important bis-indole alkaloids vinblastine and vincristine (Fig. 1)[1]. The biosynthesis of these compounds is studied actively in order to produce them in cell or tissue cultures[2,3]. In this connection, specific and reliable analytical methods are necessary when the levels of metabolic intermediates and products are determined. High-performance liquid chromatography (HPLC) in combination with UV or fluorescence detection is widely used for this purpose[4,5], although the specificity of these techniques is often questionable. The purity of separated peaks may be evaluated by scanning their UV spectra[6,7] and mass spectral analysis of collected peaks using chemical[8] or electronic[9] ionization has been used for structure elucidation and identification.

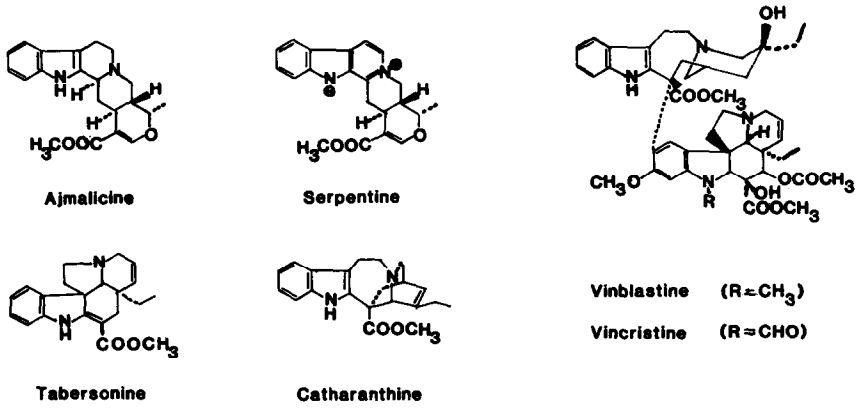

Fig. 1. Chemical structures of four monomeric and two dimeric indole alkaloids from *C. roseus*.

Thermospray liquid chromatography–mass spectrometry (LC–MS) offers a possibility to analyze non-volatile and polar compounds directly without derivatization[10–12]. In this study, this technique was utilized to check the specificity of an HPLC method in which indole alkaloids of *C. roseus* were analyzed using electrochemical and UV detection. Monitoring the MH^+ ions of tryptamine, ajmalicine, serpentine, catharanthine and tabersonine was used to reveal their presence in cell suspension samples. Thermospray mass spectral fragmentation of a bis-indole alkaloid, vinblastine, was also studied.

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EXPERIMENTAL

Chemicals

Catharanthine hydrochloride and tabersonine were generously provided by Professor W. G. W. Kurz (Plant Biotecnology Institute, National Research Council, Saskatoon, Canada). Serpentine tartrate was obtained from ICN, K&K Labs. (New York, NY, U.S.A.), ajmalicine hydrochloride and vinblastine sulphate from Sigma (St. Louis, MO, U.S.A.) and tryptamine from Aldrich Chemie (Steinheim, F.R.G.).

HPLC conditions

A Beckman 342 gradient liquid chromatograph equipped with two Beckman solvent delivery modules, a Beckman 420 controller a Beckman 165 variable-wavelength detector (at 280 nm) and an Altex 210 A injector (20-μl loop) were used. The results were recorded with a Goerz Se 120 recorder. A μBondapak C_{18} (30 cm × 3.9 mm I.D., 10 μm) reversed-phase column (Waters Assoc., Milford, MA, U.S.A.) was used. The isocratic solvent system was 0.1 *M* ammonium acetate (pH 7.2)–acetonitrile (51:49). The flow-rate was set to 1.0 ml/min.

Chromatographic conditions for thermospray LC–MS were as described above, but a Kontron 420 HPLC pump was used for solvent delivery and the samples were injected with a Rheodyne 7125 injector (loop volume 20 μl).

Mass spectrometry

The thermospray system used was a VG thermospray-plasmaspray probe coupled to a VG Trio-2 quadrupole mass spectrometer. The instrument was operated in the thermospray ionization mode. The thermospray probe temperature was 220°C, the ion source temperature was 230°C, the repeller voltage was 50 V and other ion source conditions were optimized daily.

Sample preparation

Samples from freeze-dried cell suspensions of *C. roseus* were extracted and purified as described before[7,13].

RESULTS AND DISCUSSION

A good chromatographic separation for the alkaloids of interest was achieved in 18 min with isocratic elution (Fig. 2) when 0.1 *M* ammonium acetate was used as a buffer in the mobile phase. Ammonium acetate offers the best sensitivity in acetonitrile–water and methanol–water solutions in thermospray analysis[11], and the NH_4^+ decreases polar interactions by masking free silanol groups on the bonded stationary phase[14].

The thermospray mass spectrum of ajmalicine (Fig. 3) shows MH^+ (*m/z* 353) as the base peak and no important fragment or ammonium adduct ions are present. Thermospray mass spectra of the other monomeric indole alkaloids examined were of the same type. The mass spectrum of a dimeric alkaloid, vinblastine (found only in intact plants), was more informative, containing a protonated molecular ion (*m/z* 811) and abundant fragment ions (Fig. 3). The loss of water from the parent ion gave the peak at *m/z* 793 ($MH^+ - H_2O$). Other peaks were observed at *m/z* 779 (MH^+ –

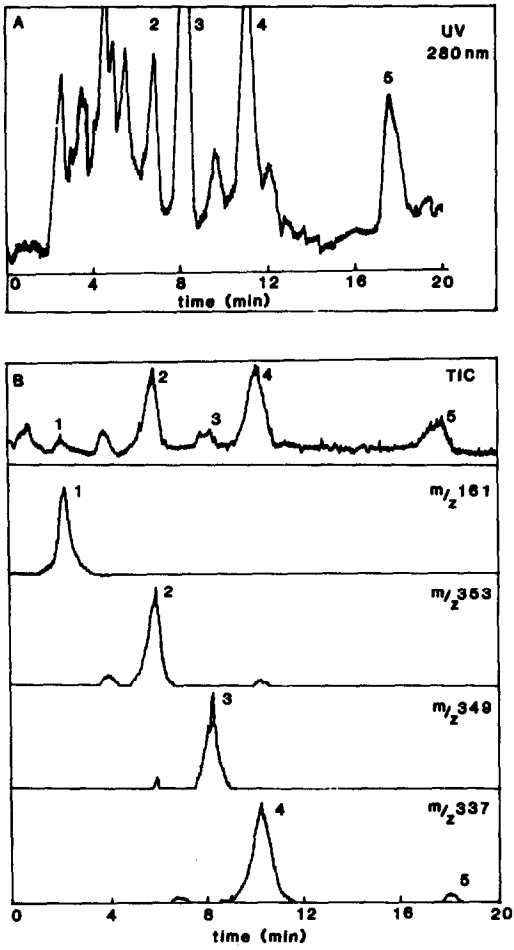

Fig. 2. HPLC UV chromatogram (A) and thermospray mass chromatograms (B) of a cell suspension sample of *C. roseus*. Compounds monitored: 1 = tryptamine; 2 = ajmalicine; 3 = serpentine; 4 = catharanthine; 5 = tabersonine. Column: 30 cm × 3.9 mm μBondapak C_{18}. Eluent: 51% acetonitrile in 0.1 *M* ammonium acetate (pH 7.2); flow-rate, 1.0 ml/min.

CH_3OH) and 753 ($MH^+ - COOCH_2$). These three fragment ions were also present in the ammonia desorption chemical ionization (DCI) spectrum published by Kuntebommanahalli *et al.*[8]. In the thermospray spectrum there also exist various other ions absent in the ammonia DCI spectrum. Of these peaks, that at *m/z* 457 might originate from the vindoline moiety (MW 456).

The peak observed at the retention time of tabersonine (18 min) with UV and electrochemical detection proved to be a mixture of two or more compounds when analyzed with thermospray LC–MS. Tabersonine was only a minor component in that peak (Fig. 2). Serpentine was not detected with electrochemical oxidation, but was easily monitored with the thermospray technique (Fig. 2).

Because of the absence of fragment ions in the thermospray mass spectra of

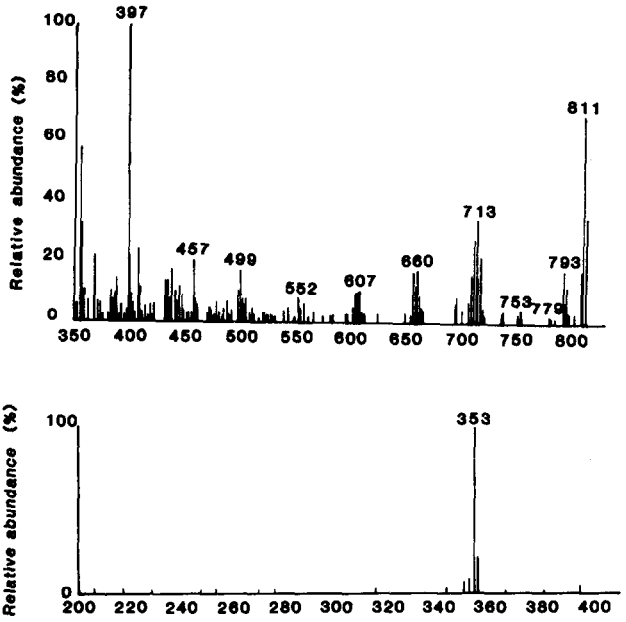

Fig. 3. Direct flow injection thermospray mass spectra of vinblastine (MH^+ 811) and ajmalicine (MH^+ 353). Eluent as in Fig. 2.

monomeric indole alkaloids, more informative ionization techniques are needed when structures of unknown compounds are elucidated. These include techniques described by McFadden and Lammert[15], *i.e.*, filament on or discharge ionization and collision-induced dissociation (CID) with the thermospray repeller or CID with MS–MS mass spectrometer.

However, single-ion monitoring of the prominent MH^+ of monomeric alkaloids is a very suitable method for screening these secondary metabolites from cultured cells of *C. roseus*. The thermospray method was more sensitive than our HPLC assay with UV detection[7], the limit of detection being 4 ng per injection for each alkaloid.

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