Supercritical CO₂ extraction of velutinol \( A \) from \textit{Mandevilla velutina} (\textit{Apocynaceae}) cultured cells and MALDI-TOF MS analysis

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**Abstract**

MALDI-TOF MS analysis of supercritical CO₂ extracted samples obtained from \textit{Mandevilla velutina} cell cultures allowed the detection of the anti-bradykinin pregnanic steroid, velutinol \( A \), using low amount of sample (1 g lyophilized cells), with minimum analyte isolation.

**Introduction**

Much attention has been paid in obtaining bradykinin (BK) – Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg – antagonists, a nonapeptide released from plasma globulin by snake venom and also by trypsin, that participates in several physiological and pathological processes. After the demonstration of a BK-potentiating effect by \textit{Bothrops jararaca} venom, there was an increasing interest in kinin action but its physiological and pathological role in many biological systems still remains unknown, due to the lack of a selective and competitive kinin antagonist (Calixto \textit{et al.} 1991). Aqueous/alcoholic extracts and some pure pregnane (velutinol \( A \), e.g., Figure 1) compounds isolated from tubers of the Brazilian plant \textit{Mandevilla velutina} selectively antagonize, in a concentration-dependent manner, functional responses to BK and related kinins in several smooth muscle preparations (Calixto \textit{et al.} 1995). Based on these findings, the compounds mentioned are of interest for development of new antiinflammatory medicines. These anti-BK compounds are secondary metabolites, chemically characterized as pregnanic steroids. However, only small amounts are found in crude plant extracts [tuber, 0.001\% to 0.0001\%, w/w] (Calixto \textit{et al.} 1989) and large-scale biomass production by conventional methods seems not be economically feasible. \textit{M. velutina} cell cultures have been thought to be a suitable production system, because both callus (Calixto \textit{et al.} 1989) and cell suspension cultures (Maraschin 1998) produce such pregnane compounds in higher amounts than the plant \([0.0032\%, \text{ w/w}]\) and revealed an anti-BK action about 31- to 79-fold greater than that obtained from crude tuber (Calixto \textit{et al.} 1989). These metabolites still represent only minor constituents of the cell biomass, so that sensitive detection methods are needed for further studies aiming at increasing the \textit{in vitro} production of the active compounds.

Over the last years, the application of supercritical fluid extraction (SFE) has continuously grown in several areas, supported by the development of new...
automated and low-cost systems. The characterization and analysis of food, drugs, pharmaceuticals, and natural products have been performed with interesting results (Overmeyer et al. 1999). For example, the analysis of polyprenols in Ginkgo biloba leaves by SSCO₂ revealed the existence of a C₁₂₀ isoprenolog, which was not detected by previous chromatographic methods (Huh et al. 1992). Similarly, progresses in mass spectrometry techniques and matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) have also been reported. The latter has proved suitable for the determination of molecular weight of biomolecules as proteins, peptides, oligonucleotides, oligosaccharides and synthetic polymers. The highly efficient TOF mass detection method coupled with the relatively gentle MALDI ionization method allow the routine analyses of biomolecules, using in some cases as little as femtomole amounts of material (Pfenninger et al. 1999). As depicted, this highly sensitive and fast technique might be especially important when one is interested in screening any plant secondary metabolite (taxol, e.g., Gimon et al. 1994) or new compounds in cell culture systems with pharmaceutical potential.

This study was carried out in order to evaluate the feasibility of the application of SFE and MALDI-TOF MS techniques in detecting velutinol A in small amounts of Mandevilla velutina cultured cell biomass.

Material and methods

Cell cultures

Callus cultures of Mandevilla velutina were initiated using nodal segments (6.5–8.0 mg), from a single 4-month-old platelet native to Cerrado ecosystem (Coromandel, Minas Gerais State/Brazil), on MS medium (Murashige & Skoog 1962) supplemented with 2 mg 2,4-dichlorophenoxyacetic acid ₁⁻¹, 2 mg 6-benzylaminopurine ₁⁻¹, and 3 mg 6-furfurylaminopurine ₁⁻¹. From these cultures, 0.5 g of 21-day-old cells were subcultured in liquid medium to obtain cell suspension cultures as previously described (Maraschin 1998). Cell suspensions were maintained in 250-ml Erlenmeyer flasks under continuous light (1100 lux) and shaking (110 rpm), at 24 ± 1 °C. Subculturing was performed every three weeks.

Supercritical fluid extraction

The following experiments were carried out after the collection of 15 g cells (fresh wt) from 21-day-old cell cultures (inoculum density = 3 g cells/50 ml culture medium) and centrifugation (10 000 × ɡ/5 min). The cell biomass was lyophilized and stored at −20 °C. The compound of interest was extracted from 1 g lyophilized cells, using a laboratory home-made device unit previously described (Coelho et al. 1997). Acetone was selected as SFE modifier of polarity as 10% (v/v) of the CO₂ supercritical streaming. Fifty ml was introduced into a high-pressure vessel (500 ml) in the screw cap with a T-connection to permit introduction of CO₂ (99.9% purity) and an inlet for the

Fig. 1. Velutinol A (15R, 16R, 20S)₁₄,₁₆:₁₅,₂₀₁₆:₁₅,₂₁-triepoxy-1₄,₁₆-seco-1₄β,₁₇α-pregn-5-ene-3β,₁₅-diol — a steroidal pregnane isolated from tubers and cell cultures of Mandevilla velutina (Apocynaceae).