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Capillary electrophoretic analysis of oxindole alkaloids from *Uncaria tomentosa*

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ABSTRACT

The main oxindole alkaloids from the root bark of *Uncaria tomentosa* were separated by capillary electrophoresis. The electrophoretic parameters for the separation of the six alkaloids were optimized by studying the impact of the buffer composition, the ionic strength and the pH and the applied electric field on the separation efficiency of the analytical system. The best separation was achieved with a fused-silica capillary tube and 20 m*M* phosphate running buffer (pH 5.6) at a constant voltage of 10 kV. The determination of oxindole alkaloids in a crude methanolic extract of *Uncaria tomentosa* demonstrates the applicability of this method.

INTRODUCTION

Capillary electrophoresis (CE) is a powerful separation technique that has become an important analytical and micropreparative tool in peptide and protein chemistry [1]. In addition to its special selectivity, the CE method provides short separation times, small sample requirements and virtually no waste production. Therefore, it can be considered as an ideal complement to high-performance liquid chromatography (HPLC). In spite of these advantages, few papers have been published so far describing the application of CE to the separation of natural plant constituents [1,2]. In this paper, the separation and determination of oxindole alkaloids in *Uncaria tomentosa* with CE is reported.

Uncaria tomentosa (Willd.) DC. (Rubiaceae), commonly known as "una de gato", is a woody liana growing in South America. Decoctions of the root bark are used in traditional Peruvian medicine for the treatment of arthritis, gastritis, cancer and certain epidermic diseases [3]. Phytochemical studies of the root bark have shown the presence of triterpenes, some minor constituents and six main oxindole alkaloids, which previously have been identified as pteropodine, isopteropodine, mitraphylline, isomitraphylline, speciophylline and uncarine F (Fig. 1) [4–7]. Because of the wide range of pharmacological and biological activities of these compounds, their separation and determination are of considerable interest.

So far, analyses for oxindole alkaloids have been accomplished by thin-layer and gas–liquid chromatography and HPLC [5,8,9]. The application of these methods to the separation of the stereoisomeric oxindole alkaloids from *Uncaria tomentosa* however, led to unsatisfactory resolution of adjacent pairs of alkaloids. In this work, a CE method for the rapid separation and determination of oxindole alkaloids in *Uncaria tomentosa* extracts was

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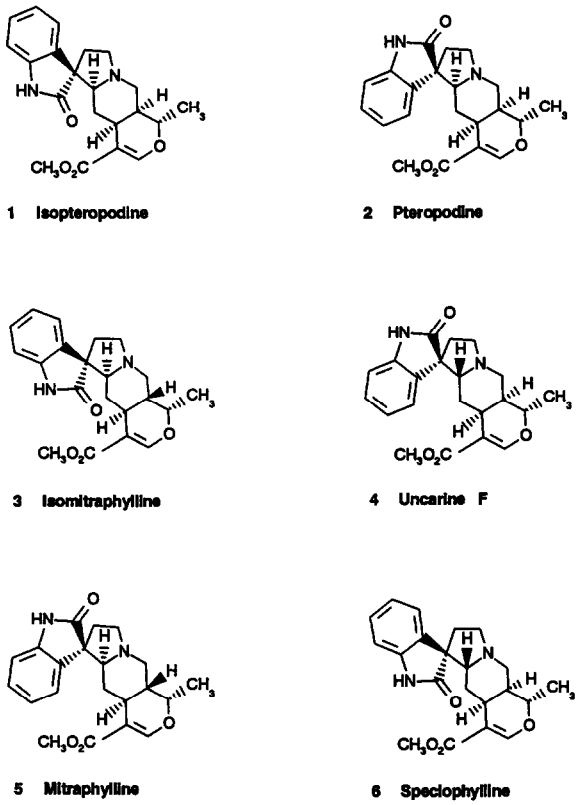

Fig. 1. Structures of the investigated oxindole alkaloids.

developed. The influence of pH, the ionic strength of the buffer and the applied voltage on the resolution of oxindole alkaloids from *Uncaria tomentosa* is discussed.

EXPERIMENTAL

Materials

Methanol, mesityl oxide, Na_2HPO_4 and KH_2PO_4 were purchased from Merck (Darmstadt, Germany). The reference alkaloids **1–6** (Fig. 1) were a gift from Professor J. D. Phillipson (University of London). Plant material of *Uncaria tomentosa* (root bark) was obtained from Mr. K. Kepplinger (Innsbruck, Austria). A voucher specimen is deposited in the Institute of Pharmacognosy, University of Innsbruck.

Sample preparation

Alkaloids **1–6** (0.75–3.15 mg) were dissolved in 1 ml of methanol and diluted with the eluent buffer (1:5) in order to give final concentrations of 15–63 μg/ml. Exhaustive extraction of 1.0 g of *Uncaria tomentosa* root bark material was carried out with a Soxhlet apparatus using methanol as solvent. For qualitative and quantitative analyses the extract obtained was evaporated to dryness and the residue dissolved in 1.00 ml methanol and diluted with phosphate buffer (20 m*M*, pH 5.6) in a ratio of 1:5. Mesityl oxide (diluted 1:1250 with water) was added to all sample solutions as a neutral marker (0.1 ml/ml).

Calibration

The calibration graph was obtained from standard solutions containing the alkaloid **1** in concentrations between 0.005 and 0.300 mg/ml in methanol–eluent buffer (1:5).

Analytical method

A Beckman CZE System 2000 (Model P/ACE) equipped with a UV detector, an automatic injector, a temperature-controlled column cartridge (57 cm x 75 μm I.D.) (Beckman No. 338427), an autosampler and a printer was used. The detection wavelength was 254 nm. All experiments were carried out at 25°C at a constant voltage of 10 kV. When studying the effect of electric field on electrophoretic mobilities, voltages between 5 and 20 kV were used. Injections were made using the pressure mode for 1 s each. When investigating the influence of the sample volume on the resolution, sampling times of 1, 3 and 5 s were used.

Phosphate buffer solutions (10–25 m*M*) were prepared by dissolving appropriate amounts of $Na_2HPO_4$and KH_2PO_4 in doubly distilled water. The pH of each buffer solution was checked with a pH meter. All sample and buffer solutions were filtered through 0.45-μm filters (Sartorius, Göttingen, Germany). Between runs, the capillary was washed with 0.1 *M* NaOH for 3 min, followed by equilibration with running buffer (3 min).

RESULTS AND DISCUSSION

The electropherogram of a model mixture of the alkaloids isopteropodine (**1**), pteropodine (**2**), iso-

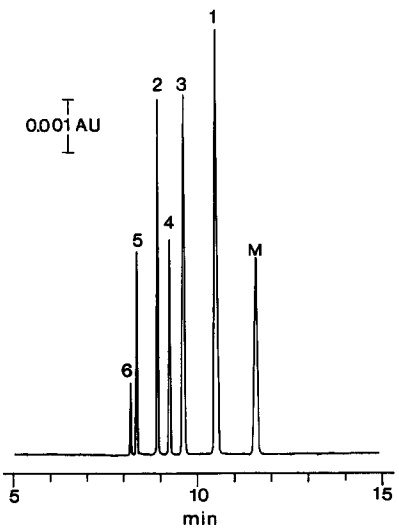

Fig. 2. Electropherogram of a model mixture of the alkaloids isopteropodine (**1**), pteropodine (**2**), isomitraphylline (**3**), uncarine F (**4**), mitraphylline (**5**) and speciophylline (**6**). M is the neutral marker mesityl oxide. Buffer, 20 m*M* phosphate buffer (pH 5.6); column, fused silica (570 × 0.075 mm I.D.); injection, pressure mode, 1 s (4.8 nl); injected amounts (ng), (**1**) 0.32, (**2,3**) 0.30, (**4**) 0.16, (**5**) 0.15, (**6**) 0.07, (M) 0.30; voltage, 10 kV; current, 17 μA; detection, UV at 254 nm; temperature, 25°C.

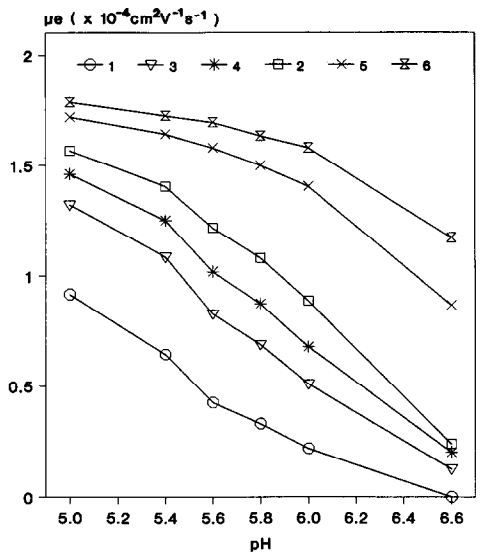

Fig. 3. Effect of pH on electrophoretic mobilities (μ_e) of alkaloids **1**–**6**. pH, 5.0–6.6; other conditions same as in Fig.2.

mitraphylline (**3**), uncarine F (**4**), mitraphylline (**5**) and speciophylline (**6**) is shown in Fig. 2. Baseline separation of the six compounds could be achieved in less than 15 min using a fused-silica capillary tube with phosphate buffer (20 m*M*, pH 5.6) as running electrolyte at a constant voltage of 10 kV. UV detection was performed at 254 nm. In this system the alkaloids are positively charged and migrate faster to the cathode than the neutral marker mesityl oxide.

Optimization of the parameters was carried out by investigating the influence of electrolyte composition, pH, electric field and ionic strength on the mobilities of **1**–**6**. The parameter with the strongest influence on the mobilities of the alkaloids is pH. As alkaloids are weak bases, an acidic buffer system was chosen in order to ensure an adequate degree of dissociation. Fig. 3 shows the mobilities of the analytes at different pH values of the phosphate buffer. The true electrophoretic mobility (μ_e) was calculated using the equation

$$\mu_e = (Ll/V)\,[(1/t_m) - (1/t_{eo})] \quad (1)$$

where L is the total capillary length (cm), l the distance from injection to the detector (cm), V the applied voltage (V), t_m the solute migration time (s) and t_{eo} the hold-up time of the electroosmotic flow marker mesityl oxide (s) [10]. The reproducibility of the solute mobilities was good. Six analyses of the standard mixture gave a relative standard deviation of 0.66%.

It is evident from Fig. 3, that the alkaloids with higher pK_a values are eluted faster than those with lower values [11,12]. The lower mobilities of the alkaloids at higher pH values are due to the increased electroosmotic flow. Excellent resolution (R_s) of adjacent pairs of alkaloids was observed at pH of 5.6 (Fig. 4).

The influence of the electric field strength on resolution is shown in Fig. 5. As the number of theoretical plates is proportional to the applied voltage, resolution was enhanced up to an electric field strength of 175 V/cm [13]. Beyond this point, the resolution decreased as the voltage was increased further. This is due to Joule heating effects [13,14].

Fig. 6 shows the effect of ionic buffer strength on the separation efficiency of the system at a constant voltage of 10 kV and a pH value of 5.6. In addition to the higher current, increasing the buffer concen-

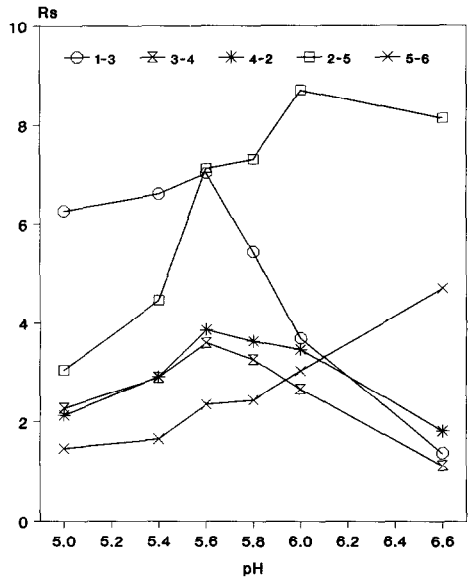

Fig. 4. Effect of pH (5.0–6.6) on resolution (R_s) of the alkaloid pairs **1–3**, **3–4**, **4–2**, **2–5** and **5–6**. Conditions as in Fig. 3.

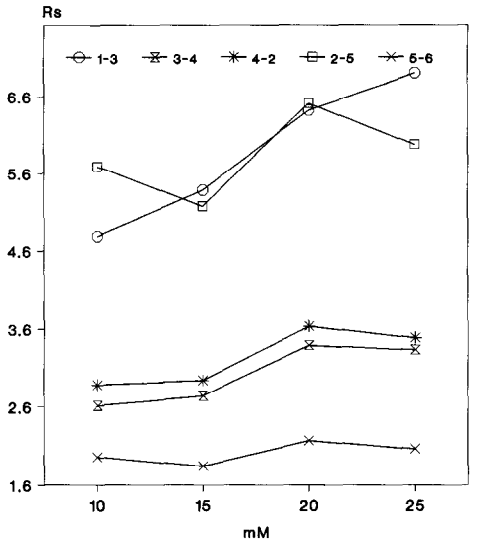

Fig. 6. Effect of buffer concentration on resolution (R_s) of the alkaloid pairs **1–3**, **3–4**, **4–2**, **2–5** and **5–6**. Buffer, 10–25 m*M* phosphate buffer; other conditions as in Fig. 2.

tration resulted in an increased spread of the electrophoretic mobilities between the investigated alkaloids. The alkaloids **1–4** exhibited faster and alkaloids **5–6** slower migration. In addition, peak broadening was significantly reduced. The best separation of all compounds was achieved with a buffer concentration of 20 m*M*. In contrast to the other alkaloid pairs, the resolution of the pair **2–5** decreased on increasing the buffer concentration from 10 to 15 m*M* and from 20 to 25 m*M*.

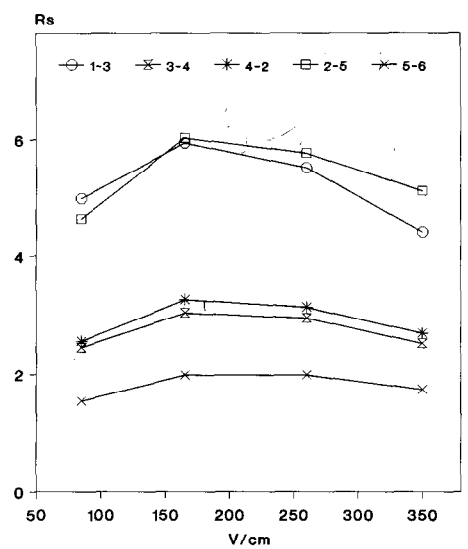

Fig. 5. Effect of electric field strength (V/cm) on resolution (R_s) of the alkaloid pairs **1–3**, **3–4**, **4–2**, **2–5** and **5–6**. Voltage, 5–20 kV; other conditions as in Fig. 2.

The effect of injection volume on CE performance has already been reported [15]. Accordingly, zone distortion and band broadening are only avoided if the sample volumes are kept as small as possible (< 1% of the total capillary volume). Table I shows the number of theoretical plates (N) and the resolution (R_s) obtained on increasing the sample volume, the actual amount of sample introduced being kept constant (sampling times of 1, 3 and 5 s). Both N and R_s decreased with increasing injection time.

To confirm the applicability of the proposed separation method, a crude methanolic extract of *Uncaria tomentosa* was investigated. CE yielded a baseline separation within 15 min. Peaks **1–6** were identified by comparison with standards. Quantitative information was obtained by using the external standard method. As the investigated oxindole alkaloids are stereoisomers with very similar UV molar absorptivities (ε) [8], calibration was performed only with compound **1**. The graph obtained was lin-

TABLE I

EFFECT OF INJECTION TIME ON NUMBER OF THEORETICAL PLATES AND RESOLUTION

Sampling times: 1, 3 and 5 s. Other conditions as in Fig. 2.

Injection time (s)	Compound	Concentration (μg/ml)	Plates per metre, N ($\times 10^5$)	R_s
1	**1**	66.09	1.260	
	3	61.95	2.194	6.42
	4	39.13	2.858	3.39
	2	61.95	3.149	3,62
	5	32.17	3.541	6.50
	6	14.78	4.680	2.17
3	**1**	22.03	0.800	
	3	20.65	1.037	4.70
	4	13.04	1.136	2.19
	2	20.65	1.321	2.29
	5	10.72	1.452	4.14
	6	4.93	1.748	1.36
5	**1**	13.22	0.316	
	3	12.39	0.379	3.10
	4	7.83	0.456	1.37
	2	12.39	0.471	1.30
	5	6.43	0.671	2.78
	6	2.96	0.920	1.09

ear in the range of 5–300 μg/ml ($y = 0.14298x + 0.00204$; $r^2 = 0.998$). The detection limit was *ca.* 1.2 μg/ml. The results of the quantification are shown in Table II. The relative standard deviation (six experiments) was between 1.2 and 5.3% for all compounds studied.

In conclusion, the described CE technique represents an excellent method for the qualitative and quantitative analysis of *Uncaria tomentosa* for stereoisomeric oxindole alkaloids.

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TABLE II

RESULTS OF THE DETERMINATION OF ALKALOIDS IN A CRUDE METHANOLIC EXTRACT OF *Uncaria tomentosa*

Average results (g per 100 g) ± S.D. for six analyses.

Compound	Calibration	Concentration (g per 100 g)
1	$y = 0.14298x + 0.00204$ ($r^5 = 0.998$)	0.0285 ± 0.0012
2	Calculated with eqn. 1	0.0551 ± 0.0028
3	Calculated with eqn. 1	0.0186 ± 0.0006
4	Calculated with eqn. 1	0.0104 ± 0.0001
5	Calculated with eqn. 1	0.0276 ± 0.0007
6	Calculated with eqn. 1	0.0142 ± 0.0003
Total		0.1544 ± 0.0036

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