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Determination of coumarins from *Chrysanthemum segetum* L. by capillary electrophoresis

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Abstract

The separation of seven closely related coumarins, i.e., herniarin, coumarin, umbelliferone, aesculetin, dihydrocoumarin, coumarinic acid and 4-hydroxycoumarin, by capillary electrophoresis was studied using different buffer systems. The best conditions chosen were applied for the determination of coumarins in extracts from roots and aerial parts from the plant *Chrysanthemum segetum*.

1. Introduction

The chemical composition of *Chrysanthemum* species is not yet fully elucidated. It was reported that these species contain sesquiterpene lactones [1], flavonoids [2] and coumarins [3]. Coumarins, which are derivatives of benzopyran, are widely distributed in plants and essential oils. They are used as fragrance components in perfumes, toothpastes and tobacco products [4]. Moreover, coumarins are pharmacologically active and have been used in the treatment of a diverse range of diseases, such as brucellosis, burns, rheumatic disease and even cancer [5,6].

A number of techniques have been applied to the determination of coumarins. Most of the previous papers have described results of semiquantitative TLC determinations [3,7–9], but gas chromatography [10], high-performance liquid chromatography [6,9,11] and centrifugal partition chromatography [12] have also been applied for the separation and determination of coumarins in various natural products.

The great diversity of coumarin structures and their wide range of polarities present special problems for their simultaneous determination. This work represents an attempt to determine coumarins using a modern and fully quantitative capillary electrophoresis (CE) method. The structural formulae of the coumarins under investigation are presented in Fig. 1.

2. Experimental

2.1. Chemicals

Herniarin (7-methoxycoumarin), coumarin, 4-hydroxycoumarin and dihydrocoumarin were obtained from Aldrich-Europe and umbelliferone (7-hydroxycoumarin), aesculetin (6,7-dihydroxycoumarin) and coumarinic acid were purchased from Merck (Darmstadt, Germany).

Methanol, chloroform, lead acetate, boric acid and borax (POCh, Gliwice, Poland) were of

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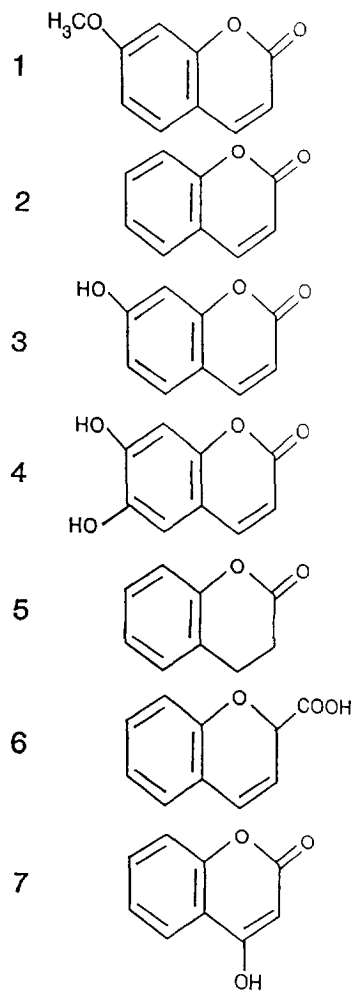

Fig. 1. Structural formulae of investigated coumarins. 1 = Herniarin (7-methoxycoumarin); 2 = coumarin; 3 = umbelliferone (7-hydroxycoumarin); 4 = aesculetin (6,7-dihydroxycoumarin); 5 = dihydrocoumarin; 6 = coumarinic acid; 7 = 4-hydroxycoumarin.

analytical-reagent grade. Water was purified by double distillation.

2.2. Plant material

The plant material was collected from the Gdańsk district (Poland) and identified in the Department of Botany. A voucher specimen is deposited in the herbarium of the Faculty of Pharmacy (Medical Academy, Gdańsk, Poland), International Herbarium Index GDMA.

2.3. Sample preparation

Roots and aerial parts of the plants were dried, powdered and then extracted with methanol. The extract was concentrated in vacuo and the concentrate was diluted with an equal volume of 4% lead acetate solution in water. After standing overnight, the precipitate that formed was removed by filtration. The filtrate was concentrated in vacuo and extracted with chloroform. The chloroform was removed from the extracts and the residue was dissolved in buffer.

2.4. Capillary electrophoresis

Analyses were performed with a Beckman P/ACE 2100 System Gold electrophoresis apparatus with UV detection at 280 nm. The column was a 58 cm × 50 μm I.D. uncoated silica capillary, with a length to the detector of 51 cm. The best overall separation was obtained with 7-s pneumatic injection using a buffer solution of 0.2 *M* boric acid–0.05 *M* of borax in water (11:9, v/v) (pH 8.5). The voltage was maintained at 25 kV, which gave a current of 32.6 mA, and the temperature was set at 25°C. Under these conditions the analysis time was 10 min and a baseline separation of herniarin, umbelliferone, esculetin, dihydrocoumarin, coumarinic acid and 4-hydroxycoumarin was achieved.

Stock solutions of standards were prepared in methanol at concentrations of 1 mg/ml. From these solutions, appropriate injection solutions were prepared by mixing the required volume with water.

3. Results and discussion

3.1. Separation of standard mixture

Fig. 2 shows electrophoretic separation of a standard mixture under the conditions described under Experimental. As can be seen, herniarin is not separated from coumarin. Our aim was to develop a method to separate as many compounds as possible. Hence the final conditions

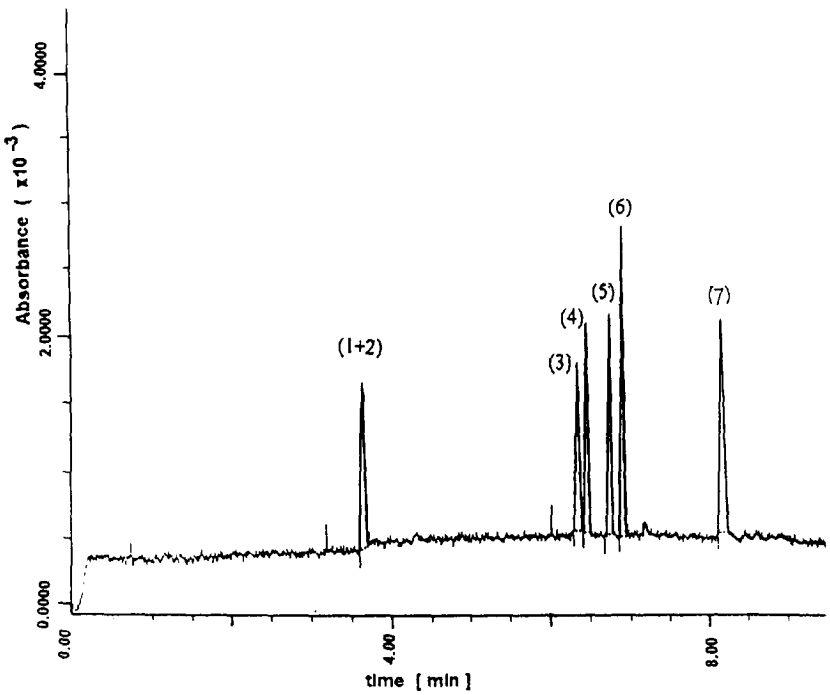

Fig. 2. CE separation of standard coumarin mixture. Conditions as under Experimental. Peaks: 1 + 2 = herniarin and coumarin; 3 = umbelliferone; 4 = aesculetin; 5 = dihydrocoumarin; 6 = coumarinic acid; 7 = 4-hydroxycoumarin.

were not necessarily the best for every type of coumarin mixture, but were selected to provide the best overall separation of the extracts considered. For example, at pH 9.33 the migration time of the last compound (4-hydroxycoumarin) is shorter (6 min), but a separation between umbelliferone and coumarinic acid is not achieved. One of the major advantages of this CE method is that the entire electrophoresis process is relatively rapid, comprising a 7-min analysis time followed by a 3-min wash. In HPLC, for comparison, the retention time of herniarin (7-methoxycoumarin) is 25.27 min [11].

3.2. Chemical composition of the extracts

Fig. 3A shows a typical electrophoretic separation of an extract obtained from aerial parts of *Chrysanthemum segetum* and Fig. 3B shows the electropherogram of the same extract spiked with standards. Fig. 4 illustrates a typical electrophoretic separation of coumarins from roots extract. With our method, coumarin cannot be separated from herniarin (peaks 1 and 2, Fig. 2); however, most of the plants containing herniarin do not contain coumarin. Using semi-preparative CE and by comparison of UV spectra we found that peak 2 from *Chrysanthemum segetum* extracts represents almost pure herniarin.

The mean concentrations (peak areas) of coumarins found in the aerial part and root samples together with standard deviations (S.D.) and relative standard deviations (R.S.D.) are given in Table 1. The mean concentration values were obtained from six independent extraction runs both for aerial parts and roots of the plant material. The relative standard deviation in each instance is <6%. Therefore, it can be concluded that both the extraction procedure and CE determination are highly reproducible.

As it can be seen in Table 1, 4-hydroxycoumarin was not found in extracts from *Chrysanthemum segetum*. Nevertheless, 4-hydroxycoumarin is an important intermediate product in the biochemical transformation of coumarins to dicoumarol [13]. This transformation is typical of

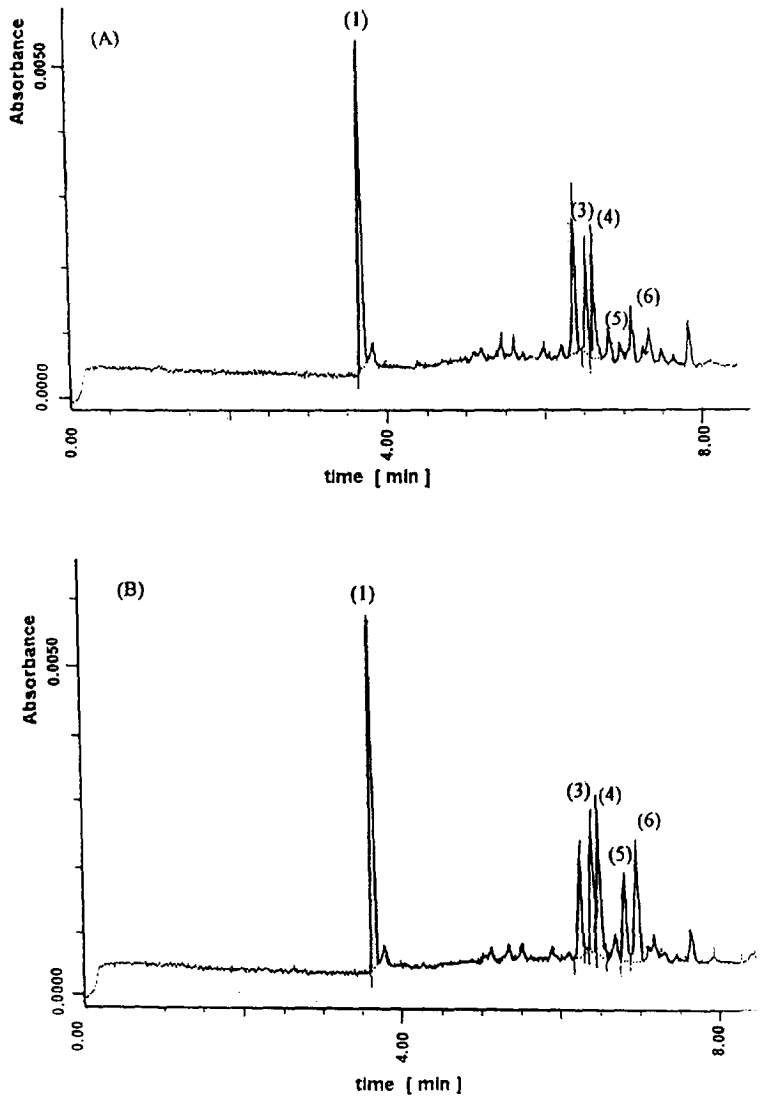

Fig. 3. (A) CE separation of an extract obtained from aerial parts of *Chrysanthemum segetum* L. Peaks: 1 = herniarin; 3 = umbelliferone; 4 = aesculetin; 5 = dihydrocoumarin; 6 = coumarinic acid. (B) The same extract spiked with standards. Conditions as under Experimental.

putrefactive processes. The absence of this compound in the extracts investigated confirms the freshness of the plant material.

It was found that coumarins are distributed differently, both quantitatively and qualitatively, in roots and aerial parts of the plants. The extracts from roots showed abundant herniarin (40%), dihydrocoumarin (30%) and umbelliferone (5%). Herniarin (26%) is also the major component of extracts from aerial parts of the plant, but the second highest is umbelliferone (18%) and then esculetin (12%), dihydrocoumarin (11%) and coumarinic acid (4%). Hence it can be concluded that *Chrysanthemum segetum* L. is a herniarin-type species. In the literature there, no quantitative data concerning

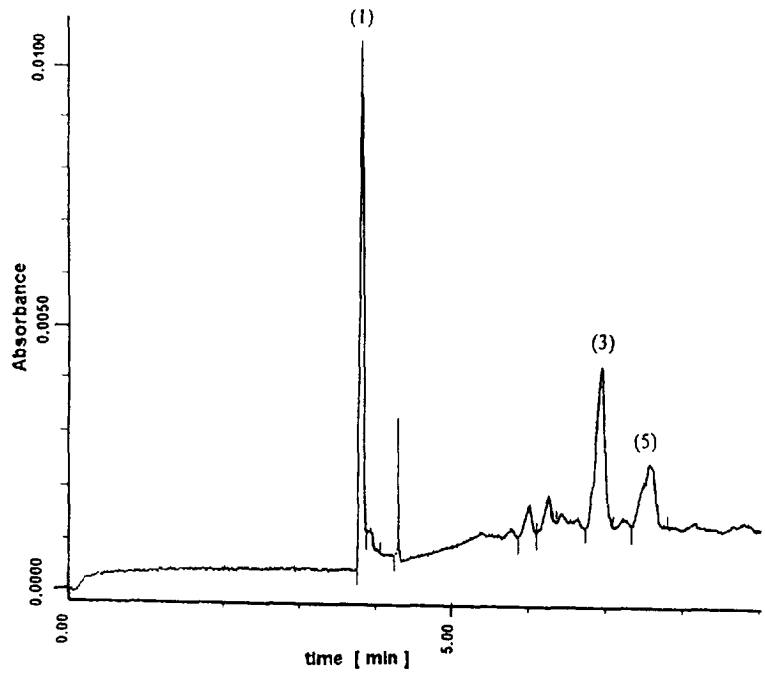

Fig. 4. CE separation of an extract obtained from roots of *Chrysanthemum segetum* L. Conditions as under Experimental. Peaks: 1 = herniarin; 3 = umbelliferone; 5 = dihydrocoumarin.

coumarin contents in *Chrysanthemum segetum* are available. In only one study [3] it was mentioned that herniarin and umbelliferone are the major components of extracts from whole plant material. From this point of view, our conclusions are similar to the results published previously.

The main advantages in the application of CE for the determination of coumarins were the short time of analysis, baseline separation and the low cost of the reagents used in the mobile phase.

Table 1
Contents of coumarins in extracts obtained from aerial parts and roots of *Chrysanthemum segetum* L. ($n = 6$)

Compound	Mean content ±S.D. (%)	R.S.D. (%)
Aerial parts		
Herniarin	26.5 ± 0.42	1.6
Umbelliferone	19.0 ± 0.36	1.9
Aesculetin	12.1 ± 0.13	1.1
Dihydrocoumarin	11.3 ± 0.08	0.7
Coumaric acid	4.1 ± 0.0001	0.002
Roots		
Herniarin	39.0 ± 2.2	5.6
Umbelliferone	4.6 ± 0.02	0.4
Dihydrocoumarin	29.9 ± 1.2	4.0

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