

Capillary Electrophoresis of Anthraquinones from *Cassia siamea*

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The separation and determination of two anthraquinones, emodin and chrysophanol, and two bianthraquinones, cassiamin A and cassiamin B, were achieved by capillary electrophoresis (CE). The running electrolyte used in this method was 0.05 M hydroxypropyl- γ -cyclodextrin in 0.1 M borate buffer (pH 9) containing 10% acetonitrile, with an applied voltage of 20 kV. Application of this technique in the determination of the main bianthraquinones, cassiamin A and cassiamin B, of *Cassia siamea* is demonstrated in this paper.

Key words capillary electrophoresis; anthraquinone; bianthraquinone; Leguminosae; *Cassia siamea*

In a continuation of our work on quinones that occur widely in the plant kingdom and that may have biological activities, we have developed a simple and rapid cyclodextrin electrokinetic chromatography (CD-EKC) to separate and determine anthraquinones and bianthraquinones. Some naturally occurring anthraquinones have already been examined by thin layer chromatography,¹⁾ HPLC methods,^{2–6)} and capillary electrophoresis (CE).^{7–10)} In this study, a simple and rapid CE method for separating and determining anthraquinones and bianthraquinones has been established. This separation method has also been applied to analyze the extract of *Cassia siamea* (Leguminosae) containing anti-tumor-promoting anthraquinones.¹¹⁾

The root and bark of *C. siamea*, a tree which is endemic to Central and East Africa, have been used in folklore medicine to treat stomach complaints and as a mild purgative. It was the first identification of a bianthraquinonyl occurrence in a plant.¹²⁾ In the course of our screening for anti-tumor-active anthraquinones, it was found that cassiamin B might be valuable as an anti-tumor-promoting and chemopreventive agent.¹³⁾

Experimental

Reagents and Materials Sodium tetraborate, sodium dodecyl sulphate (SDS), and hydroxypropyl- γ -CD (HP- γ -CD) were purchased from Wako (Osaka, Japan), and boric acid from Fluka (Buchs, Switzerland). Acetonitrile, methanol, and water were of HPLC grade. Chrysophanol (1), emodin (2), cassiamin A (3) and cassiamin B (4) were isolated from the root bark of *C. siamea*.¹⁴⁾ The extract of the root bark and leaf extract of *C. siamea* were procured from the University of Maiduguri Campus and Ahmadu Bello University Campus, Nigeria, during November 1998.

Procedure for CE CE analyses were carried out on a Beckman P/ACE System 5000 apparatus (Fullerton, CA, U.S.A.), equipped with a UV detector set at 254 nm and a Diode Array detector, and a Beckman untreated fused-silica capillary (570 mm \times 75 mm i.d.; 500 mm effective length). The analytical conditions were as follows: sampling time, 5 s (hydrodynamic mode; 0.5 p.s.i.); applied constant voltage, 20 kV; column temperature, 20 °C. The electrolyte of cyclodextrin modified micellar electrokinetic chromatography (CD-MEKC) was a buffer solution prepared by mixing 0.03 M boric acid solution with appropriate volumes of 0.03 M sodium tetraborate solution (pH 9), followed by the addition of 0.015 M SDS, 0.005 M HP- γ -CD and 10% methanol; and the electrolyte of CD-EKC was a buffer solution prepared by mixing 0.1 M boric acid solution with appropriate volumes of 0.1 M sodium tetraborate solution (pH 9), followed by the addition of 0.05 M HP- γ -CD and 10% acetonitrile. Standard solutions for each anthraquinone were prepared by dissolving compounds in the electrolyte at a concentration of ca. 10 ppm.

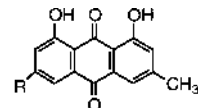
Sample Preparation of *C. siamea* Extracts Powdered leaves (10 g) of *C. siamea* were exhaustively extracted in a Soxhlet apparatus with methanol (100 ml) for 10 h. After concentration, the residue was diluted with methanol to 100 ml. This solution was passed through a 0.45 μ m filter. After centrifu-

gation, 1 ml of the methanol solution was diluted to 10 ml with borate buffer (pH 9), yielding the sample for CD-CZE analysis.

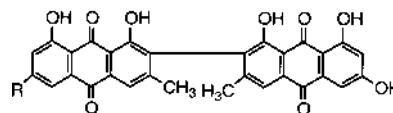
Results and Discussion

We have studied the application of CE to the isolation of anthraquinones, and found that owing to the phenolic (and hence weak acidic) or neutral nature of anthraquinones, the weak alkaline condition of borate buffer as an eluent could produce a good resolution. It is well known that when the buffer concentration increases, the electro-osmotic flow decreases, and therefore the migration times increase. In the case of SDS, as the concentration of SDS increased, the migration time increased. We first failed in the separation by MEKC, because the bianthraquinones and anthraquinones overlapped using various eluents. Changing the buffer solutions to those with different pH, composition, buffer concentration, organic solvent, and other modifiers, the separation of compounds (1–4) was achieved by adding HP- γ -CD to 0.03 M borate buffer (pH 9) containing 0.015 M SDS and 10% methanol. Figure 1 shows the electropherogram of the root bark extract of *C. siamea*, and 4 compounds (1–4) could be separated. Among the peaks, 1–4 were identified by spiking with those of standards.

In CD-MEKC, CD could not be solubilized in the SDS micelle and transported toward the negative electrode with the same velocity as that of the electro-osmotic flow. The solutes were distributed among three phases, an aqueous phase, the micelle and CD. Emodin and its dimer, cassiamin B, are faster than chrysophanol and emodin–chrysophanol dimer, cassiamin A. When a hydroxyl group is present at the



1 R = H chrysophanol
2 R = OH emodin



3 R = H cassiamin A
4 R = OH cassiamin B

Chart 1. Structures of Compounds 1–4

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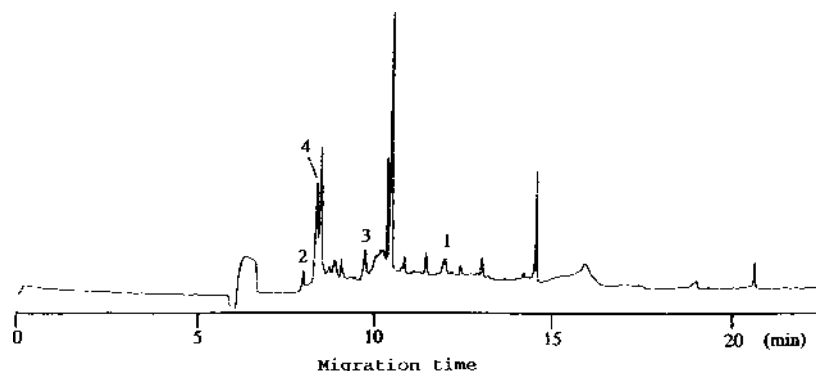


Fig. 1. CD-MEKC Electropherogram Showing the Separation of Anthraquinones from the Root Bark of *C. siamea*

Using 0.03 M borate buffer (pH 9) containing 0.015 M SDS, 0.005 M HP- γ -CD, and 10% methanol, peaks are identified by substance number as indicated in Chart 1.

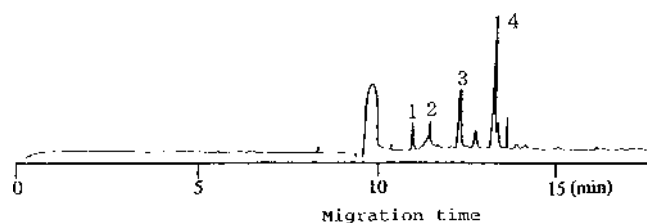


Fig. 2. CD-EKC Electropherogram Showing the Separation of Anthraquinones from the Root Bark of *C. siamea*

Using 0.1 M borate buffer (pH 9) containing 0.05 M HP- γ -CD and 10% acetonitrile, peaks are identified by substance number as indicated in Chart 1.

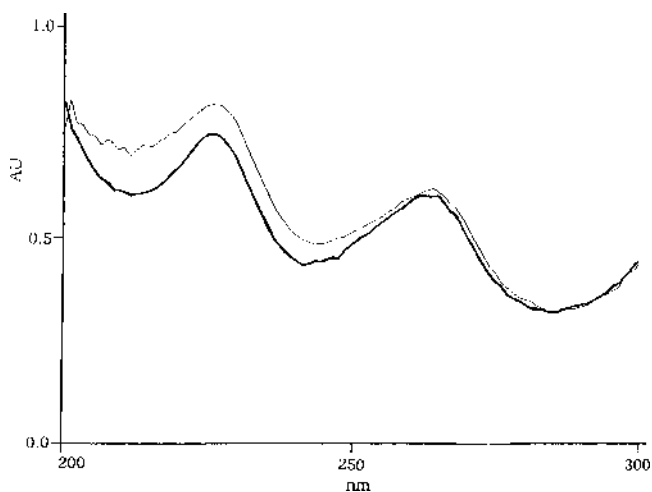


Fig. 3. Similarity Index with UV of Emodin (2)

—: standard emodin, - - -: emodin in the root bark of *C. siamea*.

peri position of the carbonyl group, a strong internal hydrogen bond is formed between the hydrogen of the hydroxyl and the carbonyl group, and therefore the carbonyl can no longer interact with the buffer.¹⁵ However, this method was not found to be entirely adequate for the separation of compound 4.

CDs have been successfully applied for the separation of several hydrophobic compounds. The CD's shape is similar to that of a truncated cone with a relatively hydrophobic cavity able to host analytes, as well as a hydrophilic outside region due to the presence of hydroxyl groups. In the inclusion-complexation mechanism, the compound fits the CD

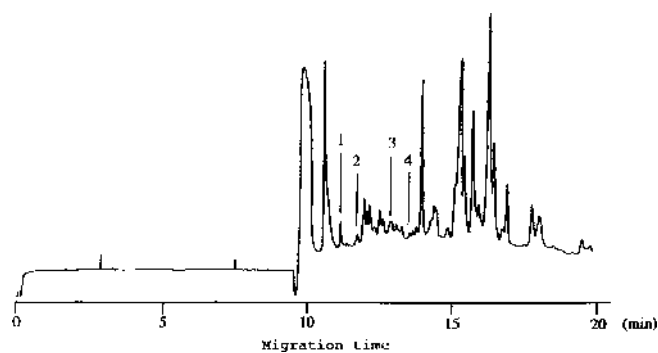


Fig. 4. CD-EKC Electropherogram Showing the Separation of Anthraquinones from the Leaf Extract of *C. siamea*

Using 0.1 M borate buffer (pH 9) containing 0.05 M HP- γ -CD and 10% acetonitrile.

cavity with the whole molecule or with its hydrophobic part and, thus, the CD has a very important role in the separation process. After examining a series of buffer solutions differing in pH, composition, concentration, and the nature and concentration of CD, it was found that 0.1 M borate buffer (pH 9) containing 0.05 M HP- γ -CD and 10% acetonitrile could resolve anthraquinones and bianthraquinones. An electropherogram of the root bark extract of *C. siamea* is shown in Fig. 2 (Similarity Index (with UV, Fig. 3); 1: 0.9681, 2: 0.9985, 3: 0.9941, 4: 0.9900). In the comparison of CD-EKC with CD-MEKC for analysis of the extract of *C. siamea*, CD-EKC has better resolution for 4. Figure 4 shows the electropherogram of the leaf extract of *C. siamea*.

For quantitative analysis, correlations between the peak area and the sample concentrations (3, 4) were studied. The curves (peak-area ratios, Y , vs. concentration, x , $\mu\text{g/ml}$) were constructed in the range of 5–50 $\mu\text{g/ml}$ for 3 and 5–100 $\mu\text{g/ml}$ for 4. The regression equations of these curves and their correlation coefficients were calculated as follows: 3, $Y=984.2x-196.8$ ($r=0.999$); 4, $Y=576.3x-1604.3$ ($r=0.996$). The extraction recovery was tested by adding known amounts of 3 and 4. The recovery of 3 was 101.0–103.3%, and that of 4 99.2–103.3% ($n=3$).

The reproducibilities, expressed as the relative standard deviation (RSD), of this method calculated on the basis of peak area over three replicate injections, are shown in Table 1. For a series of three consecutive injections, the migration time reproducibility for an individual compound was be-

Table 1. Reproducibility of Migration Times and Areas of Compounds 1–4

| Compound | Migration time (min) | RSD (%) (n=3) | RSD of areas (%) (n=3) |
|---------------------------------------|----------------------|---------------|------------------------|
| 1 | 10.89 | 0.09 | 3.09 |
| 2 | 11.39 | 0.09 | 2.88 |
| 3 | 12.22 | 0.08 | 1.02 |
| 4 | 13.22 | 0.13 | 2.90 |
| Root bark extract of <i>C. siamea</i> | | | |
| 1 | 10.89 | 0.11 | 2.39 |
| 2 | 11.45 | 0.10 | 0.87 |
| 3 | 12.25 | 0.13 | 3.93 |
| 4 | 13.26 | 0.16 | 1.50 |

tween 0.08 and 0.16% RSD. In conclusion, the CD-EKC described here has proven to be a useful technique for investigating mixtures of anthraquinones and bianthraquinones, as it is rapid, simple and reproducible.

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