Journal of Natural Products
Vol. 58, No. 4, pp. 577-582, April 1995

ANALYSIS OF RHUBARB BY MICELLAR ELECTROKINETIC CAPILLARY CHROMATOGRAPHY

YU-YING ZONG[1] and CHUN-TAO CHE*

Department of Chemistry, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong

ABSTRACT.—A micellar electrokinetic capillary chromatographic method for the separation and quantitative determination of aloe-emodin, emodin, and rhein in rhubarb samples was established. The running electrolyte was a mixture of 0.025 M 3-(cyclohexylamino)-1-propane sulfonic acid (CAPS) buffer [containing 0.025 M sodium dodecyl sulfate (SDS)] and acetonitrile (100:10), pH 10.96, with an applied voltage of 12 kV. This method resolved the three components as well as chrysophanol and physcion from the other constituents present. The technique is simple, rapid, and reproducible.

Originating from northwestern China and Tibet, rhubarb has been used in medicine for thousands of years. Prepared from the dried roots and rhizomes of *Rheum palmatum* L., *R. tanguticum* Maxim. ex Balf., or *R. officinale* Baill. (Polygonaceae), rhubarb (known as "Da-Huang" in the Chinese language) is among the oldest and best-known herbal medicines officially listed in the pharmacopoeias of many countries. The drug is commonly used as a laxative, stomachic, and liver cleanser in the treatment of indigestion and jaundice (1,2). Decoctions of rhubarb can also be applied topically to treat thermal burns and skin diseases (1,2). The plants are known to contain biologically active compounds such as anthraquinone derivatives (e.g., aloe-emodin, chrysophanol, emodin, physcion, rhein, and their glucosides), bianthrones (e.g., palmidins, rheidins, and sennosides), and tannins (3).

In addition to the species listed in the pharmacopoeia, more than 30 other species of *Rheum* grow in China, and several of these have been found in use as rhubarb substitutes (4). At present, the method of choice for evaluation of the quality of rhubarb is to determine the composition of the non-polar solvent solubles as well as the H_2O solubles by hplc or tlc scanning (5,6). It is desirable to establish alternative analytical approaches that may offer advantages in convenience and efficiency over existing techniques. Capillary electrophoresis is a microanalytical technique which offers rapid separation of charged and neutral compounds and has many attractive features such as simplicity, efficiency, and sensitivity (for recent reviews, see 7–9). The technique has proven suitable not only for analysis of macromolecules (10–12), drugs (13–15), and natural compounds (16–18), but also for separation of herbal preparations (19,20). In the present study, the micellar mode of capillary electrophoresis, known as micellar electrokinetic capillary chromatography (MECC or MEKC), has been used to analyze rhubarb samples (including *Rheum palmatum, R. tanguticum,* and *R. emodi*) for their anthraquinone content. The technique offers satisfactory results and can be developed into an important and useful analytical tool for herbal drug analysis.

In a preliminary study, both the capillary zone electrophoresis (CZE) and micellar electrokinetic capillary chromatography (MECC) methods were used to separate anthraquinones from extracts of *Rheum* spp. In general, the MECC method gave better resolution and was therefore studied at length. Due to the phenolic (and acidic) nature of anthraquinones, alkaline conditions were preferred dur-

[1]On leave from the Qinghai Institute for Drug Control, Xining, Qinghai, People's Republic of China.

ing electrophoresis. After examining a series of buffer solutions differing in pH, ionic strength and composition, it was found that a mixture of 0.025 M CAPS buffer (containing 0.025 M SDS) and MeCN (100:10), at pH 10.96, could best resolve the anthraquinone mixture within 14 min. An increase in the concentration of CAPS, SDS, or MeCN gave little improvement in resolution but greatly increased the migration time. The effect of pH was also critical. Thus, at pH values over 11, the resolution was poor; while at pH values lower than 10.7, the migration time was significantly increased and the samples became only partially soluble. An electrolyte consisting of 0.025 M CAPS, 0.025 M SDS, and MeCN (buffer-organic solvent, 100:10), adjusted to pH 10.96 with NaOH, was subsequently chosen as the buffer solution used in this study.

Figure 1 shows a standard capillary electropherogram of a mixture of reference compounds (aloe-emodin, chrysophanol, emodin, physcion, and rhein), with migration times of 7.89 min for aloe-emodin, 8.58 min for an internal standard (1,8-dihydroxyanthraquinone), 9.30 min for chrysophanol, 9.91 min for emodin, 10.53 min for physcion, and 12.17 min for rhein. All components were well separated. Figure 2 shows a typical electropherogram of a $CHCl_3$ extract of *Rheum tanguticum* following hydrolysis. All anthraquinones could be separated without any interference from other constituents; the resolution was as

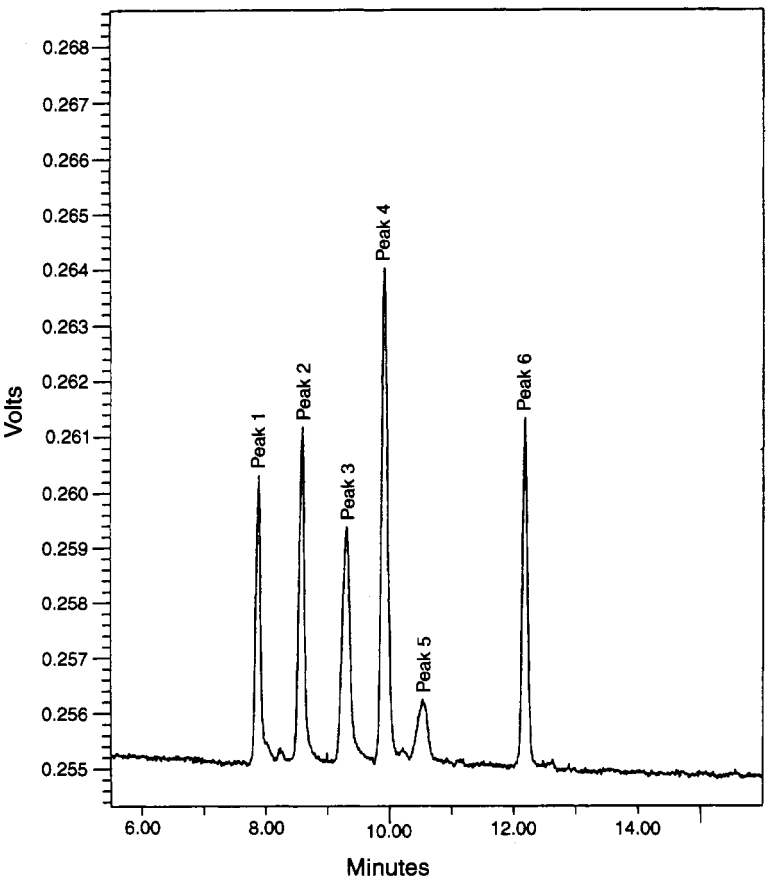

FIGURE 1. Capillary electropherogram of reference compounds and internal standard. [peak 1, aloe-emodin; peak 2, 1,8-dihydroxyanthraquinone; peak 3, chrysophanol; peak 4, emodin; peak 5, physcion; peak 6, rhein].

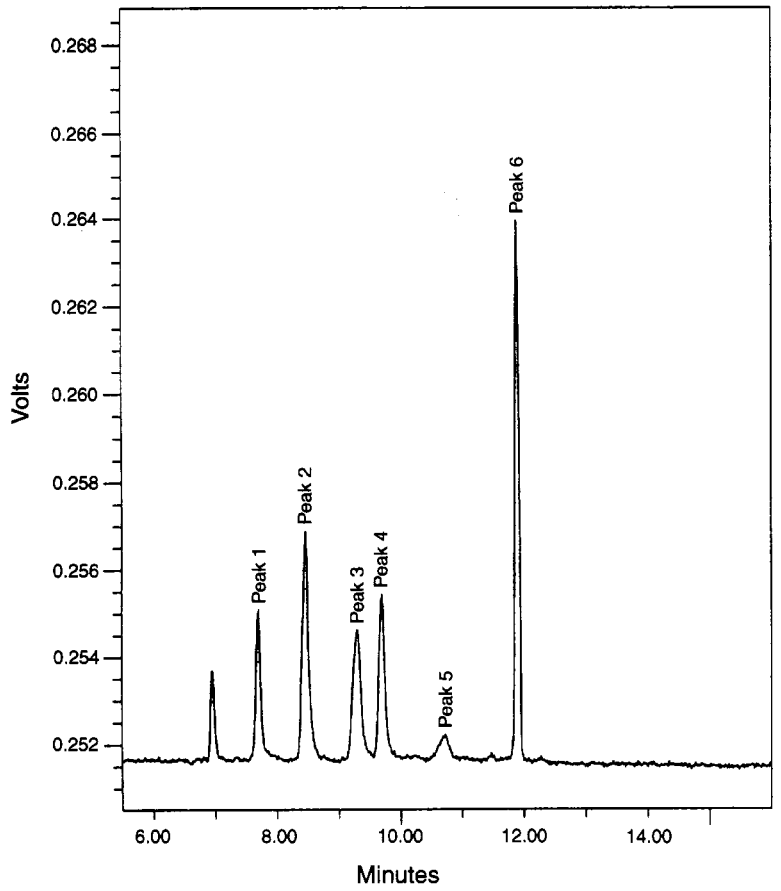

FIGURE 2. Capillary electropherogram of a $CHCl_3$ extract of hydrolyzed *R. tanguticum* sample. [Unlabelled peak, unknown component; peak 1, aloe-emodin; peak 2, 1,8-dihydroxyanthraquinone; peak 3, chrysophanol; peak 4, emodin; peak 5, physcion; peak 6, rhein].

good as that obtained with the reference compounds.

In order to establish a procedure for quantitative measurement, calibration graphs for each reference compound were constructed, using 1,8-dihydroxyanthraquinone as an internal standard. The curves (peak-area ratio vs. concentration ratio) were constructed in the range of 0.0025–0.0175 mg/ml for aloe-emodin, 0.005–0.05 mg/ml for emodin, and 0.005–0.035 mg/ml for rhein. The regression equations of these curves and their correlation coefficients were calculated as follows: aloe-emodin, $Y=1.0801x+0.0021$ ($r=0.9999$); emodin, $Y=0.8825x-0.0096$ ($r=0.9999$); rhein, $Y=1.2037x-0.0044$ ($r=0.9996$).

For chrysophanol and physcion, the linear correlation coefficients were found to be unacceptably low and were therefore considered unsuitable for quantitative measurement. Such poor responses might arise from the fact that these compounds were incompletely dissolved in the buffer solution.

The reproducibility (expressed as relative standard deviation, R.S.D.) of this proposed method, on the basis of peak-area for seven replicate injections, was 1.27% (intraday) and 2.46% (interday) for aloe-emodin, 0.89% (intraday) and 2.05% (interday) for emodin, and 1.36% (intraday) and 3.15% (interday) for rhein. For a series of ten consecutive injections, the migration time reproducibility for

TABLE 1. Reproducibility of Migration Time.

Peak No.	Compound[a]	Migration Time	
		Mean (min) (n=10)	R.S.D.[b] (%)
1	Aloe-emodin	7.86	1.91
2	1,8-Dihydroxyanthraquinone	8.58	1.93
3	Chrysophanol	9.34	2.03
4	Emodin	9.90	2.06
5	Physcion	10.56	2.20
6	Rhein	12.17	1.92

[a]Concentration of individual component was 0.01–0.05 mg/ml.
[b]Relative standard deviation.

individual compounds was between 1.91% and 2.20% R.S.D. (Table 1).

Five samples of *R. palmatum*, *R. tanguticum*, and *R. emodi* were then extracted, with and without prior hydrolysis, and analyzed by MECC. All five anthraquinones were detected in the test samples, but their quantities varied. Both the total amount and the free form of aloe-emodin, emodin, and rhein could be estimated with reference to the calibration curves of individual compounds. The results (Table 2) indicated that the majority of the anthraquinones were present in the glycosidic form, and the concentrations of aloe-emodin and rhein derivatives were higher in the official drug species (i.e., *R. palmatum* and *R. tanguticum*) than in *R. emodi*. The latter sample contained mainly emodin and chrysophanol, with small amounts of aloe-emodin and rhein.

The results of a standard addition-recovery study of aloe-emodin, emodin, and rhein were also calculated. When added to hydrolysate samples, the recovery was 98.2–101.8% for aloe-emodin, 99.9–101.9% for emodin, and 99.2–101.2% for rhein. In $CHCl_3$ extracts of *R. tanguticum*, the recovery was 100.5–102.9% for aloe-emodin, 94.5–102.7% for emodin, and 100.3–102.5% for rhein.

The present findings demonstrate the applicability of MECC to the determination of the aloe-emodin, emodin, and rhein constituents of rhubarb samples. The proposed method can be used not only to quantify chemical components, but also to aid in identification and quality control of these plant drugs. In addi-

TABLE 2. Content of Aloe-emodin, Emodin, and Rhein in *Rheum* spp. Samples.

Sample	Aloe-emodin		Emodin		Rhein	
	Total[a]	Free[a]	Total[a]	Free[a]	Total[a]	Free[a]
R. palmatum (Zhangye, Gansu)	1.84	0.23	4.12	0.42	2.98	<0.05
R. tanguticum (Guoluo, Qinghai)	2.42	0.07	3.29	0.21	7.89	0.14
R. tanguticum (Yushu, Qinghai)	1.91	0.07	2.52	0.11	10.04	0.09
R. tanguticum (Huangnan, Qinghai)	4.56	0.10	5.62	0.17	10.41	0.10
R. emodi (Tibet)	<0.01	<0.01	7.71	1.38	<0.05	<0.05

[a]Expressed as mg/g dried plant material.

tion to its rapid and accurate performance, the method allows subsequent injections to be made after 14 min with a thoroughly cleaned capillary. Therefore, it should be especially useful for routine analysis of pharmaceutical plant products for quality control purposes. The present study extends the utility of the capillary electrophoretic technique from the analysis of plant constituents such as alkaloids (17–20) and flavonoids (16, 21–23) to anthraquinones.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Analyses were carried out on a Bio-Rad HPE 100 capillary electrophoresis system equipped with a uv detector set at 500 nm and a microsampler 100 cartridge (uncoated capillary, 50 cm×50 μm i.d.). Aloe-emodin, chrysophanol, emodin, physcion, and rhein were purchased from the National Institute for the Control of Pharmaceutical and Biological Products, Beijing, People's Republic of China. 1,8-Dihydroxyanthraquinone and 3-(cyclohexylamino)-1-propane sulfonic acid (CAPS) were obtained from Fluka, and sodium dodecyl sulfate (SDS) from Bio-Rad Laboratories. Deionized H_2O (Milli-Q) was used to prepare buffer solutions. MeCN was hplc grade, and $CHCl_3$ and EtOAc were A.R. grade (Fisher).

PLANT MATERIAL.—The roots and rhizomes of *Rheum palmatum, R. tanguticum,* and *R. emodi* were collected from Gansu Province, Qinghai Province (Guoluo, Huangnan, and Yushu areas) and Tibet, respectively, in September 1993. They were authenticated at the Qinghai Institute for Drug Control, where voucher specimens have been deposited. Plant materials were air-dried, milled, and stored in enclosed containers until used.

PREPARATION OF PLANT EXTRACTS.—To prepare extracts of hydrolyzed samples, dried plant powder (100 mesh, 0.04 g) was added to 0.5 ml of 20% H_2SO_4 and stirred for 5 min. $CHCl_3$ (50 ml) was then added and refluxed for 1 h. After cooling to room temperature, anhydrous Na_2SO_4 (2.5 g) was added, and the solution was filtered through a No. 1 filter paper. The residue was further washed by $CHCl_3$. The $CHCl_3$ extract and washings were then combined and excess solvent was removed *in vacuo*. The residue was dissolved in EtOAc and diluted to 5 ml. An aliquot (0.1 ml) was then removed and, after addition of 6 μl of internal standard solution (1 mg of 1,8-dihydroxyanthraquinone in 1 ml EtOAc), dried *in vacuo*. Finally, the residue was redissolved in 0.2 ml of running buffer and filtered through a 0.5 μm filter. The filtrate was loaded (4 sec) into the capillary electrophoresis system directly.

To prepare extracts of non-hydrolyzed samples, dried plant powders (100 mesh, 0.1–0.3 g) were added to $CHCl_3$ (50 ml) and refluxed for 20 min. After cooling to room temperature, the extract was filtered, and the extraction procedure was repeated several times until the $CHCl_3$ extract became colorless. The $CHCl_3$ solutions were combined, dried, and redissolved in 2 ml of EtOAc. An aliquot (0.1 ml) of the EtOAc solution was treated as described for the hydrolyzed samples.

CAPILLARY ELECTROPHORETIC CONDITIONS.—Sampling time, 4 sec; run time, 15 min; applied voltage, 12 kV (constant voltage, positive to negative polarity). The electrolyte was a buffer solution consisting of 0.025 M CAPS and 0.025 M SDS mixed with MeCN (100:10). NaOH was added to a pH value of 10.96.

RECOVERY STUDY.—Known amounts of aloe-emodin, emodin, or rhein standards were added to $CHCl_3$ extracts of *R. tanguticum* having known anthraquinone contents; the mixtures were processed and analyzed as described above.

ACKNOWLEDGMENTS

We thank Drs. Penju Guo, Ziqiang Zhu, and Mantang Yu, Qinghai Institute for Drug Control, and Ms. Jing Lu, National Institute for the Control of Pharmaceutical and Biological Products, Beijing, for their interest in support of this work. Messrs. Hubert Tang, Timothy Tam, Kai-Lun Huen, and Rongbin Huang (at the Hong Kong University of Science and Technology) are acknowledged for their skillful help during the course of the study.

LITERATURE CITED

1. "Chinese Pharmacopoeia," People's Press of Public Health, Beijing, People's Republic of China, 1990, Part 1, pp. 15–16.
2. "Dictionary of Chinese Medicinal Materials," Shanghai Scientific and Technological Press, Shanghai, People's Republic of China, 1977, Vol. 1, pp. 102–108.
3. W. Tang and G. Eisenbrand, "Chinese Drugs of Plant Origin," Springer-Verlag, Berlin, 1992, pp. 855–876.
4. J.R. Cheng and Z.J. Kao, *J. Beijing Med. Univ.*, **25** Suppl., 9 (1993).
5. L.W. Shi and J.H. Zheng, *J. Beijing Med. Univ.*, **25** Suppl., 15 (1993).
6. J. Zhou, Z.Z. Fang, C.F. Li, and G. Zhao, *Chinese Traditional Patent Medicine*, **10**, 31 (1987).
7. R. Kuhn and S. Hoffstetter-Kuhn, "Capillary Electrophoresis: Principles and Practice," Springer-Verlag, Berlin, 1993.
8. G.M. Janini and H.I. Issaq, *J. Liq. Chromatogr.*, **15**, 927 (1992).

9. W.G. Kuhr and C.A. Monnig, *Anal. Chem.*, **64**, 389R (1992).
10. X.C. Huang, M.A. Quesada, and R.A. Mathies, *Anal. Chem.*, **64**, 2149 (1992).
11. F.T.A. Chen, *J. Chromatogr.*, **559**, 445 (1991).
12. W. Nashabeh and Z. El Rassi, *J. Chromatogr.*, **600**, 279 (1992).
13. G.M. McLaughlin, J.A. Nolan, J.L. Lindahl, R.H. Palmieri, K.W. Anderson, S.C. Morris, J.A. Morrison, and T.J. Bronzert, *J. Liq. Chromatogr.*, **15**, 961 (1992).
14. M.T. Ackermans, J.L. Beckers, F.M. Everaerts, H. Hoogland, and M.J.H. Tomassen, *J. Chromatogr.*, **596**, 101 (1992).
15. G.L. Chee and T.S.M. Wan, *J. Chromatogr.*, **612**, 172 (1993).
16. U. Seitz, P.J. Oefner, S. Nathakarnkitkool, M. Popp, and G.K. Bonn, *Electrophoresis*, **13**, 35 (1992).
17. K.J. Lee, G.S. Heo, N.J. Kim, and D.C. Moon, *J. Chromatogr.*, **577**, 135 (1992).
18. Y.M. Liu and S.J. Sheu, *J. Chromatogr.*, **600**, 370 (1992).
19. Y.M. Liu and S.J. Sheu, *J. Chromatogr.*, **639**, 323 (1993).
20. J.M. Betz, in: "Abstracts of the International Research Congress on Natural Products," Halifax, Nova Scotia, Canada, July 31 to August 4, 1994, Abstr. No. P:96, 1994.
21. C.L. Ng, C.P. Ong, H.K. Lee, and S.F.Y. Li, *Chromatographia*, **34**, 166 (1992).
22. P. Morin, F. Villard, and M. Dreux, *J. Chromatogr.*, **628**, 153 (1993).
23. P. Morin, F. Villard, and M. Dreux, *J. Chromatogr.*, **628**, 161 (1993).

Received 25 August 1994