Enantiomer Separation of the Four Diastereomers of Guaiacyl Glycerol from *Hydnocarpus annamensis* by Capillary Electrophoresis with HP-β-CD as a Chiral Selector

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Abstract

A capillary electrophoresis method with HP-β-CD as the chiral selector is established for the enantioseparation of two pairs of phenylpropanoids, which are isolated from *Hydnocarpus annamensis*. The effects of buffer pH, HP-β-CD and buffer concentration, applied voltage, and cartridge temperature on the enantioseparation are optimized. A baseline separation of the four diastereomers of guaiacyl glycerol is achieved in less than 10 min under these optimized conditions: 25 mmol/L Borax–NaOH buffer (pH 10.01) in the presence of 30 mmol/L HP-β-CD at 15°C and 30 kV. The experimental results show that the reported method by capillary electrophoresis for the separation of the four diastereomers of guaiacyl glycerol is powerful, sensitive, and fast, requires smaller amounts of reagents, and can be employed as a reliable alternative to other methods.

Introduction

Hydnocarpus annamensis (Gagnep.) M. Lescot et Sleum. (Flacourtiaceae) is an evergreen tree, mainly distributed in the Guangxi province of China, and it is used in folk medicine for the treatment of rheumatoid arthritis and syphilis (1). In the present study, two pairs of phenylpropanoids from this plant were isolated for the first time and identified as *erythro*-guaiacyl glycerol and *threo*-guaiacyl glycerol by comparing spectroscopic data (^{1}H, ^{13}C, and MS data) with those of corresponding authentic samples or values noted in the literature (2). This research work has indicated that these two pairs of phenylpropanoids only showed one peak each in high-performance liquid chromatography (HPLC) analysis, and their specific rotations were zero, suggesting that they possess a racemic nature. This paper deals with the enantiomer separation of two pairs of phenylpropanoids by capillary electrophoresis (CE), to further identify them to be four diastereomers. The chemical structures of these four diastereomers are shown in Figure 1. To the authors' knowledge, this is the first report about the separation of four diastereomers of guaiacyl glycerol by CE.

CE is increasingly employed in the enantiomeric separation of various kinds of racemic mixtures (3–10). This is because CE is a simple, rapid, and practical method providing high separation efficiency and requiring small amounts of samples and reagents. Generally, enantioseparation can be conveniently performed by adding chiral selectors to the background electrolyte. In the present study, the separation of (7*S*, 8*R*)-guaiacyl glycerol, (7*R*, 8*S*)-guaiacyl glycerol, (7*R*, 8*R*)-guaiacyl glycerol, and (7*S*, 8*S*)-guaiacyl glycerol in the presence of hydroxypropyl-beta-cyclodextrin (HP-β-CD) as a chiral selector was first investigated by CE. Many experimental conditions, including chiral selector types, buffer composition and pH, chiral selector and buffer concentration, applied voltage, and cartridge temperature, were tested for separating effects. Baseline separation for the four diastereomers of guaiacyl glycerol was achieved.

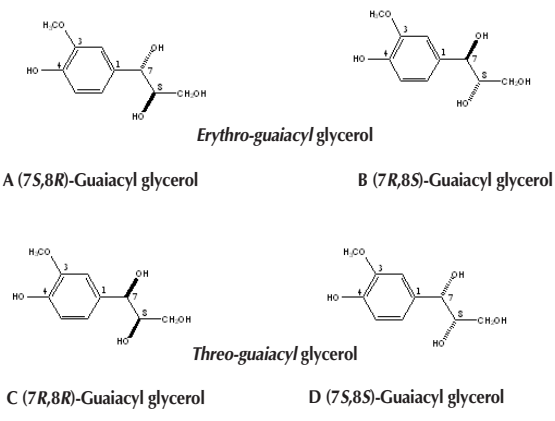

Figure 1. Chemical structures of four diastereomers of guaiacyl glycerol.

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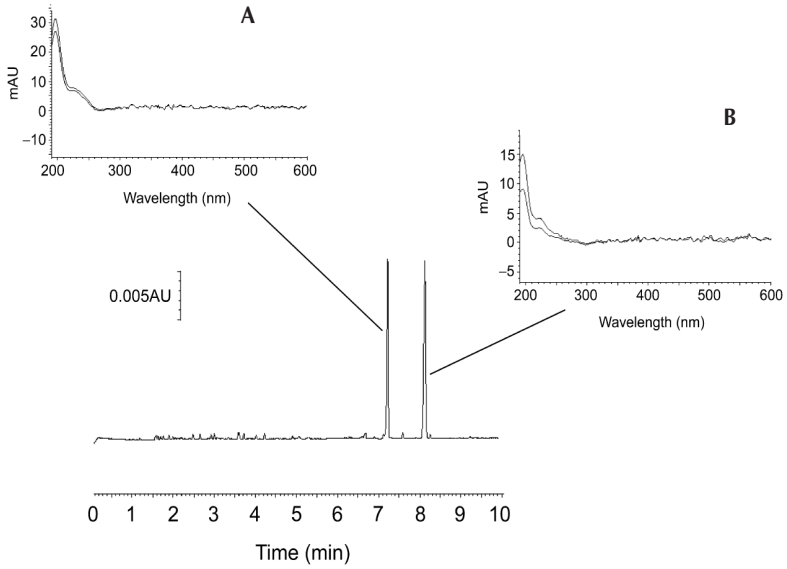

Figure 2. Electropherograms of compounds I and II. The conditions used were as follows: injection, 0.5 psi for 10 s; applied voltage, 30 kV; capillary, total length 60.2 cm (effective length 50 cm) × 50-µm i.d.

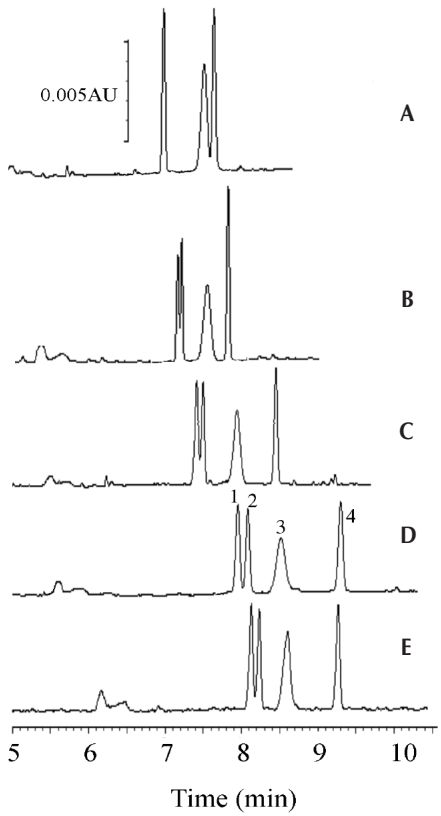

Figure 3. Electropherograms of compounds I and II in the running buffer system with the different concentrations of HP-β-CD in mmol/L (5, A; 10, B; 20, C; 30, D; and 40, E). The conditions used were as follows: injection, 0.5 psi for 10 s; applied voltage, 30 kV; capillary, total length 60.2 cm (effective length 50 cm) × 50-µm i.d.

Experimental

Apparatus and CE conditions

All experiments were performed on a Beckman P/ACE™ MDQ system (Beckman Coulter, Inc., Fullerton, CA) equipped with a photodiode array detector as well as the 32 Karat™ software, version 5.0 (Beckman). A capillary tube (Yongnian Optical Fibre Corporation, Hebei, China) with an internal diameter of 50 µm was used. The total and effective lengths of the capillary were 60.2 cm and 50 cm, respectively. Before use, the new capillaries were rinsed with 0.1 mol/L NaOH solution for 20 min, and subsequently with deionized water for 5 min.

The semi-preparative HPLC was equipped with Waters 600 controller, Waters column (Prep Nova-Pak HR C18 7.8 × 300 mm), and Waters 2487 dual × absorbance detector (detection wavelength 210, 254 nm) (Waters, Milford, MA).

Chemicals and materials

Native beta-cyclodextrin (β-CD) was from Nankai Fine Chemical Laboratories (Tianjin, China), HP-β-CD was obtained from Shijiazhuang Biotechnological Company (Shijiazhuang, China), and sodium dodecyl sulfate (SDS) was purchased from Sigma (St. Louis, MO). All chemicals were of analytical grade unless otherwise indicated. Boric acid and borax used in this study were from Beijing Chemical Reagent Factory (Beijing, China). Deionized water was prepared using a Millipore Milli-Q-Plus system (Millipore, Bedford, MA). All buffers and solutions used in the study were filtered through 0.45-µm membranes (Agilent, Santa Clara, CA) before using.

The barks of *Hydnocarpus annamensis* (Gagnep.) M. Lescot et Sleum. were collected from Pingxiang, Guangxi Province, China, in December 2004, and were identified by Mr. Chao-Liang Zhang. A voucher specimen (No. 041212) was deposited in the herbarium of the Modern Research Center for TCM, Peking University, Beijing, China.

Sample extraction and isolation

The dried barks (21.4 kg) were milled and extracted three times with 95% EtOH for 2 h each time, with the solvent removed under reduced pressure. The 95% ethanolic extract was suspended in water, then partitioned with $CHCl_3$ and *n*-BuOH successively. The *n*-BuOH-soluble fraction (198.2 g) was concentrated and subjected to silica gel column eluting with a $CHCl_3$–CH_3OH (100:1, 100:2, 100:4, 100:8, 100:16, 100:32, 100:50, 100:100) gradient system to yield 1–8. Fr. 5 (20 g) was chromatographed on a silica gel column eluting with $CHCl_3$-CH_3OH (15:1) to afford frs. 5-1, 5-2, 5-3, and 5-4. Fr. 5-3 (2.8 g) was purified by semi-preparative HPLC (CH_3OH:H_2O=5:95) to afford sample erythro-guaiacyl glycerol (36 mg, t_R = 8.6 min) and sample threo-guaiacyl glycerol (40 mg, t_R = 9.2 min). The structures were identified by mass spectrometry (MS) and nuclear magnetic resonance (NMR).

The conditions were as follows: (7*S*, 8*R*)-guaiacyl glycerol and (7*S*, 8*R*)-guaiacyl glycerol ($C_{10}H_{14}O_5$), electrospray ionization (ESI)–MS: 237 $[M+Na]^+$; NMR: H–NMR(CD_3OD, 300MHz): 7.00 (1H, d, *J* = 1.8 Hz, H-2), 6.81(1H, dd, *J* = 1.8, 8.1 Hz, H-5), 6.75 (1H, d, *J*= 8.1 Hz, H-6), 4.52 (1H, d, *J* = 6.3 Hz, H-7), 3.85 (3H, s, 3-OCH_3), 3.73 (1H, m, H-8), 3.66 (1H, dd, *J* = 3.9, 11.1 Hz, H-9), 3.57 (1H, dd, *J* = 6.6, 11.1Hz, H-9). ^{13}C-NMR(CD_3OD, 75 MHz): 148.7(C-3), 146.9 (C-4), 134.7 (C-1), 121.0(C-6), 115.7 (C-5), 111.8 (C-2), 76.6 (C-8), 76.0 (C-7), 64.5 (C-9), 56.3 (3-OCH_3).

(7*S*, 8*S*)-guaiacyl glycerol and (7*R*, 8*R*)-guaiacyl glycerol ($C_{10}H_{14}O_5$), ESI–MS $[M+Na]^+$: 237; NMR: ^{1}H–NMR (CD_3OD, 300 MHz): 6.92(1H, d, *J* = 1.5 Hz, H-2), 6.76(1H, dd, *J* = 1.5, 8.1 Hz, H-5), 6.72(1H, d, *J*= 8.1Hz, H-6), 4.48(1H, d, *J* = 6.3 Hz, H-7), 3.80(3H, s, 3-OCH_3), 3.63 (1H, m, H-8), 3.43 (1H, dd, *J* = 3.9, 11.4 Hz, H-9), 3.31 (1H, dd, *J* = 6.3, 11.4Hz, H-9). ^{13}C-NMR (CD_3OD, 75 MHz): 148.8 (C-3), 147.0 (C-4), 134.7 (C-1), 120.6 (C-6), 115.8 (C-5), 111.4(C-2), 77.5 (C-8), 75.4 (C-7), 64.2 (C-9), 56.3 (3-OCH_3).

CE of the separation of the four diastereomers of guaiacyl glycerol

For the separation of the two pairs of phenylpropanoids, the running buffer was composed of different borax concentrations, and its pH was adjusted with NaOH. Different amounts of various CDs were dissolved in the buffer solutions; the different running temperatures and the applied voltage were also tested. Stock solutions of the samples (1 mg/mL) were prepared in methanol and were diluted to the desired concentration with the running buffer. The samples were injected using the pressure injection mode at 0.5 psi for 10 s (1 psi = 6894.76 Pa). The capillary was washed between runs with the running buffer for 5 min at 20 psi. Each condition was run in duplicate.

Results and Discussion

Influence of the chiral selector types and HP-β-CD concentration on separations

The effect of chiral selector types on enantioseparation is very important in CE (11). If there is no chiral selector in this media, there are two peaks in the electropherogram of Figure 2. The first peak contains (7*R*, 8*R*)-guaiacyl glycerol and (7*S*, 8*S*)-guaiacyl glycerol, and their UV spectra extracted from diode array detector (DAD) 3-D graph is given in Figure 2A. The second peak contains (7*S*, 8*R*)-guaiacyl glycerol and (7*R*, 8*S*)-guaiacyl glycerol, and their UV spectra extracted from DAD 3-D graph is shown in Figure 2B. However, no enantiomeric separation for each pair of phenylpropanoids was observed. In order to choose a chiral selector for the enantiomeric separation of the four stereoisomers, the enantioseparations of the four diastereomers of guaiacyl glycerol were carried out using HP-β-CD, β-CD, and SDS in the Borax–NaOH buffer solution systems. The other experiment conditions (25 mmol/L Borax–NaOH, pH 10.01 of background electrolyte, 15°C of cartridge temperature, and 30 kV of running voltage) were the same in all the systems. Among

Table I. μ_{app} and Enantiomeric Resolution (R_s) for the Four Stereoisomers with Different Running Conditions

	μ_{app} (cm^2/V/s)				R_s		
	$\mu_{app}1$	$\mu_{app}2$	$\mu_{app}3$	$\mu_{app}4$	$R_{s\,1,2}$	$R_{s\,2,3}$	$R_{s\,3,4}$
Borate–NaOH concentration (mmol/L)							
15	–	–	2.43×10^{-4}	2.31×10^{-4}	–	–	2.88
20	2.04×10^{-4}	2.01×10^{-4}	1.93×10^{-4}	1.77×10^{-4}	1.07	1.11	2.48
25	1.92×10^{-4}	1.89×10^{-4}	1.80×10^{-4}	1.66×10^{-4}	1.51	2.65	4.56
30	1.64×10^{-4}	1.59×10^{-4}	1.53×10^{-4}	1.38×10^{-4}	1.69	1.14	4.36
35	1.51×10^{-4}	1.46×10^{-4}	1.42×10^{-4}	1.26×10^{-4}	2.00	0.88	3.41
pH							
9.42	2.22×10^{-4}	2.20×10^{-4}	2.09×10^{-4}	1.95×10^{-4}	0.89	2.89	3.77
9.78	1.98×10^{-4}	1.94×10^{-4}	1.87×10^{-4}	1.71×10^{-4}	1.41	1.35	3.24
10.01	1.92×10^{-4}	1.89×10^{-4}	1.80×10^{-4}	1.66×10^{-4}	1.51	2.65	4.56
10.65	1.45×10^{-4}	1.41×10^{-4}	1.32×10^{-4}	1.19×10^{-4}	0.98	3.77	1.83
Voltage (kV)							
30	1.92×10^{-4}	1.89×10^{-4}	1.80×10^{-4}	1.66×10^{-4}	1.51	2.65	4.56
25	1.46×10^{-4}	1.44×10^{-4}	1.38×10^{-4}	1.26×10^{-4}	1.60	2.72	5.56
20	1.14×10^{-4}	1.12×10^{-4}	1.07×10^{-4}	9.82×10^{-4}	1.86	2.91	6.04
15	8.39×10^{-5}	8.24×10^{-5}	7.89×10^{-5}	7.20×10^{-5}	1.80	3.16	6.61
Temperature (°C)							
15	1.92×10^{-4}	1.89×10^{-4}	1.80×10^{-4}	1.66 × 10–4	1.51	2.65	4.56
20	2.39×10^{-4}	2.36×10^{-4}	2.24×10^{-4}	2.10×10^{-4}	0.97	3.62	4.95
25	2.73×10^{-4}	2.70×10^{-4}	2.56×10^{-4}	2.41×10^{-4}	0.875	3.40	4.20
30	3.06×10^{-4}	3.04×10^{-4}	2.87×10^{-4}	2.71×10^{-4}	0.59	3.64	4.12

these tested chiral selectors, only HP-β-CD displayed the enantioselectivity to the studied diastereomers.

The HP-β-CD concentration is an essential parameter for optimizing chiral separations. The influence of HP-β-CD concentration on the resolution of enantioseparations was studied in the range from 20 to 40 mmol/L in the system with 25 mmol/L Borate-NaOH, pH 10.01 of background electrolyte, 15°C of cartridge temperature, and 30 kV of running voltage. As seen in Figure 3, no baseline separation was observed at 20, 25, 35, and 40 mmol/L HP-β-CD. However, the enantiomer resolution increases when HP-β-CD concentration is increased from 20 to 30 mmol/L, and decreases when HP-β-CD concentration is increased from 30 to 40 mmol/L. The optimal baseline separation was obtained at 30 mmol/L of HP-β-CD concentration, which is the optimal concentration of HP-β-CD for separating the four diastereomers of guaiacyl glycerol.

Influence of running buffer composition, concentration, and pH on separations

The composition and the concentration of the buffer can affect the baseline stability, the peak shape, and the separation selectivity (12). In the experiment, the four stereoisomers were separated using Tris–H_3PO_4 (pH 7.0–8.0), boric acid–borax (pH 8.0-9.0), and borax–NaOH (pH 9.0–10.5) as buffer solution systems to optimize the composition of the buffer, for better separation. The experimental results suggested that the composition of the buffer had a great effect on chiral separations in CE. Borax–NaOH (pH 9.0–10.5) provided better resolution for the four analytes, but boric acid–borax (pH 8.0–9.0) and Tris–H_3PO_4 (pH 7.0–8.0) did not yield enantioresolution. The combination of HP-β-CD and borate was shown to be an effective tool for the chiral separation. A dual chiral recognition mechanism based on both inclusion into the chiral cavity of the CD and borate complexation with the four stereoisomers has been proposed (13).

In order to determine the appropriate concentration of borax–NaOH for the enantiomeric separation of four diastereomers of guaiacyl glycerol, the concentration of borax–NaOH was changed from 15 to 35 mmol/L, and the other experiment conditions in all systems were as follows: 30 mmol/L HP-β-CD, pH 10.01 of background electrolyte, 15°C of cartridge temperature, and 30 kV of running voltage. The effects of various concentrations of borax–NaOH (15, 20, 30, and 35 mmol/L) on the resolution (Rs) of the four diastereomers is shown in Table I. The enantiomer resolution increases when borax-NaOH concentration rises from 15 to 25 mmol/L, but decreases when borax–NaOH concentration rises from 25 to 35 mmol/L. With an increasing buffer concentration, the migration time of the four diastereomers increases. And when borax–NaOH concentration is 25 mmol/L, the optimal baseline separation is achieved.

The pH of the buffer produced some influence upon both resolution and separation. Keeping 30 mmol/L HP-β-CD, 25 mmol/L borax–NaOH, 15°C of cartridge temperature, and 30 kV of running voltage the same in all systems, the change of buffer pH obviously affects the Rs of the four stereoisomers, as shown in Table I. It can be seen that there is no baseline separation at pH 9.42, 9.77, and 10.65 except at pH 10.01. When the buffer pH increases from 9.42 to 10.01, the chiral separation of the four compounds is optimal. When the buffer pH is 10.65, the enantiomer resolution worsens. In addition, the migration time increases when the buffer pH increases, and is the longest at pH 10.65; at the same time, the width of peak increases in the case of pH 10.65 as well. Therefore, the optimal buffer pH is 10.01 for separating the four diastereomers of guaiacyl glycerol.

Influence of the applied voltage and cartridge temperature on separations

The effect of applied voltage (30, 25, 20, and 15 kV) on the Rs of the four diastereomers is also shown in Table I. The baseline separation is always obtained at four different voltages for the four diastereomers of Guaiacyl glycerol. Therefore, taking both the analysis speed and the resolution of the enantiomers into consideration, 30kV was chosen as optimal.

The effect of cartridge temperature was studied in our experiments: using 30°C, 25°C, 20°C, and 15°C, the electrophoretic system was equilibrated at each temperature for at least 10 min prior to each experiment. The best chiral resolutions were achieved for all studied analytes when the cartridge temperature is kept at 15°C (Table I).

Conclusions

Rapid and effective methods for the chiral separation of the two pairs of phenylpropanoids were reported. The HP-β-CD was proven to be a powerful selector to achieve the enantioseparation of four chiral natural products, as well as its concentrations, and was clearly identified as a factor influencing enantiomeric selectivity. Under the optimal conditions [30 mmol/L borax–NaOH buffer (pH 10.01) containing 25 mmol/L HP-β-CD, an applied voltage of 15 kV, and a cartridge temperature of 15°C], the four compounds (7*S*, 8*R*)-guaiacyl glycerol, (7*R*, 8*S*)-guaiacyl glycerol (7*R*, 8*R*)-guaiacyl glycerol, and (7*S*, 8*S*)-guaiacyl glycerol) could be baseline separated.

Acknowledgments

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