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Short communication

Enantioseparation of 2-*O*-β-D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one and its 7-chloro-derivative by capillary zone electrophoresis using native and substituted β-cyclodextrins as chiral additives

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Abstract

Efficient, rapid and inexpensive methods were established for the chiral separation of two glucopyranosyl compounds from plant extracts, by capillary zone electrophoresis (CZE). Baseline separation was achieved for both compounds. Several native cyclodextrins and their derivatives were tried as chiral selectors. CM-β-CD and HP-β-CD (with addition of acetonitrile in the buffer) gave rise to optimal chiral *separation* for the two compounds, respectively, each within a few minutes. The effects of several parameters on the chiral separation were studied.

Keywords: Capillary zone electrophoresis; Enantiomers; Chiral separation; Cyclodextrins

1. Introduction

Acanthus ilicifolius L. (Acanthaceae) is a plant widely distributed in southeastern Asia. Traditionally, the plant is used as an anti-inflammatory and anti-hepatitis drug [1]. Two chiral ingredients were isolated from the plant, 2-*O*-β-D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one (I) [2] and 7-chloro-2-*O*-β-D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one (II) [3] were identified by comparison of physical data with literature values and from spectroscopic evidence [4]. The chemical structures of these two compounds are shown in Fig. 1. As verified by TLC and HPLC analyses, samples of both compounds were pure, however, the C^{13} NMR spectral data of either compounds showed split peaks for both of the C-1 of the glucose moiety and the α-carbon of the aglycone, respectively. These results would indicate that each of the compounds was epimers at the α-carbon of the aglycone. To the authors' knowledge, there is no report up to now about the *S*-configuration of these enantiomers obtained from natural plants, moreover, there is no report at all about the *S*-configuration of compound II. The above-mentioned discovery called for a chiral separation means to investigate the compounds involved. All our previous attempts to separate the two enantiomer pairs without using a chiral HPLC column, however, were to no avail, This paper deals with the chiral separation of these two ingredients.

The enantioseparation methods have been described based on different techniques [5–12]. HPCE is an effective tool for the separation of enantiomers. In many cases, this was accomplished by adding chiral selectors into the background electrolyte to discriminate between the enantiomers concerned. A large number of chiral selectors have been reported, currently available and were comprehensively reviewed in references

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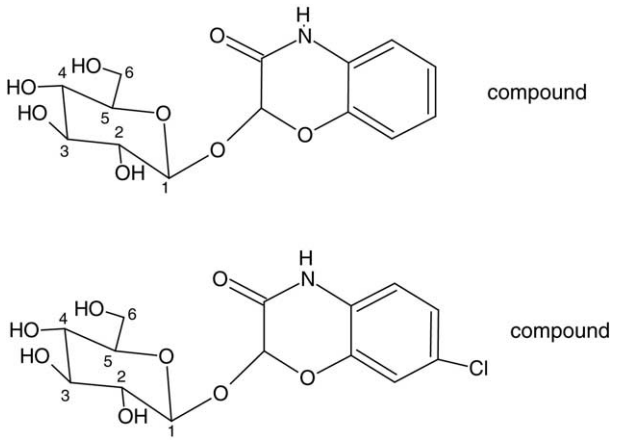

Fig. 1. Structures of 2-*O*-β-D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one (compound I) and 7-chloro-2-*O*-β-D-glucopyranosyl-2H-1,4-benzoxazin-3(4H)-one (compound II).

[10–12]. Up to now, the most suitable chiral selector for each specific purpose is usually chosen by trial and error [9].

In this paper, the chiral separation of the two glucopyranosyl compounds were tested using HPCE, with native and several substituted β-CDs as the chiral selector. Baseline separation for both compounds was achieved.

2. Experimental

2.1. Capillary electrophoresis system

All experiments were performed on an HP 3D CE apparatus (Agilent, Waldbronn, Germany) equipped with a diode array detector as well as the HPCE ChemStation software (Agilent). Non-coated polyimide-clad fused silica capillaries (I.D. 50 μm, O.D. 360 μm), used for separation, were purchased from Yongnian Optical Fiber Factory (Hebei, China). Compounds I and II were separated on columns with total lengths of 38.5 and 68.5 cm and effective lengths of 30 and 60 cm, respectively. Before use, the capillaries were rinsed with 0.1 mol/L NaOH solution for 20 min, and subsequently with double-distilled water and the running buffer, each for 5 min.

2.2. Reagents and materials

Native β-CD was from Nankai Fine Chemical Laboratories (Tianjin, China), derivatives of β-cyclodextrin, apart from carboxymethyl-β-cyclodextrin (CM-β-CD), which was synthesized in our laboratory, were from Acros Organics (New Jersey, USA). Acetonitrile was acquired from Fisher Co. (USA); 20 mmol/L borate buffer (pH 9.3) was obtained from Agilent (USA). Tris and phosphoric acid used in this study were Analytical Reagent Grade, both from Beijing Chemical Reagent Factory (Beijing, China). Double-distilled water was produced in this laboratory. All buffers and solutions used in the study were filtered through 0.45 μm membranes (Agilent, Germany) before use. Compounds I and II were isolated from the natural plant by two of the authors, Huo and Zhao.

2.3. Procedures

For the separation of (I), the running buffer was composed of 40 mmol/L Tris, its pH adjusted to 8.5 with phosphoric acid. For (II), 20 mmol/L borate buffer (pH 9.3, Agilent) was used. Different amounts of various CDs were dissolved in the buffer solutions to test the effect of chiral selectors on the separation. For (II), buffer solutions containing various amounts of acetonitrile were also tested. The capillary cassette was kept at a constant temperature of $20 \pm 0.1\,^{\circ}C$; the applied voltage was 20 kV, unless stated otherwise. The sample was hydrodynamically injected at 50 mbar × 5 s. After every two runs, the capillaries were rinsed with running buffer for 5 min.

3. Results and discussion

3.1. Chiral selector CD used

Among the native and derivative CDs tested, only with CM-β-CD and HP-β-CD could (I) and (II) be baseline separated, in each case within a few minutes. For (II), the separation could be realized only with the addition of organic additives in the mobile phase.

3.2. Optimization of the enantioseparation of compound I

Fig. 2 shows the effects of CM-β-CD concentrations on the separation efficiency of compound I. It can be seen that the apparent mobility difference increased with the concentration of CM-β-CD, and baseline separation could be achieved at a selector concentration above 1.2% (Fig. 2f). Fig. 2 shows the distinct increase of the resolution with CM-β-CD concentra-

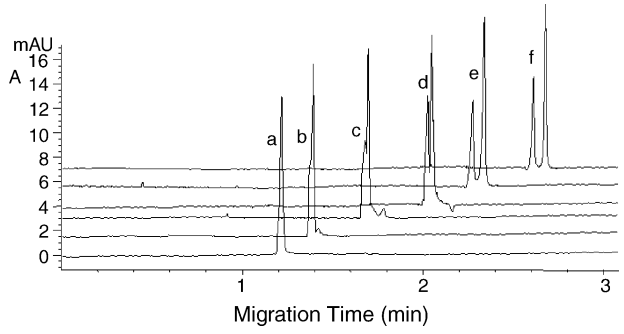

Fig. 2. Electropherograms showing the separation of enantiomers of compound I as function of CM-β-CD concentration. Conditions: column effective length, 30 cm; applied voltage, 20 kV; column temperature, 20 °C; BGE, 40 mmol/L Tris–H_3PO_4 buffer at pH 8.5 containing following concentration of CM-β-CD: (a) 0%, (b) 0.2%, (c) 0.5%, (d) 0.8%, (e) 1.0% and (f) 1.2%.

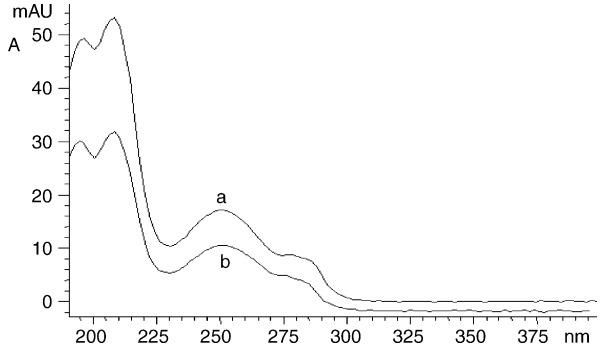

Fig. 3. The UV spectra of each of the enantiomers of compound I, extracted from DAD plot at apex of each peak. The upper spectrum is from that of the latter peak in Fig. 2, the lower from the former.

tion. On account of both the enantioseparation efficiency and saving of the expensive chiral selector, a CM-β-CD concentration of 1.2% was selected for CE separation.

Fig. 3 shows the UV spectra, extracted from the DAD 3-D graph, of the enantiomers concerned.

3.3. Optimization of the enantioseparation of compound II

Compound II could be partly separated using HP-β-CD as the selector, reaching a maximal apparent mobility difference at a HP-β-CD concentration of about 50–70 mmol/L (see Fig. 4), resulted in minute but discernable separation when the abscissa of electropherogram is elongated. As the viscosity of the aqueous BGE increased with HP-β-CD concentration, it was not possible to increase separation without sacrificing analysis speed. The dissatisfactory separation in the aqueous system could be improved when acetonitrile was added into the BGE [13,14]. At an acetonitrile concentration up to 20%, the analytes can be baseline separated (resolution, $R > 1.5$), as is shown in Fig. 5.

The increasing resolution of the enantiomers of compound II with the concentration of acetonitrile is probably as follows: the addition of acetonitrile could be expected to reduce the affinity of the enantiomer pair for HP-β-CD, leading to

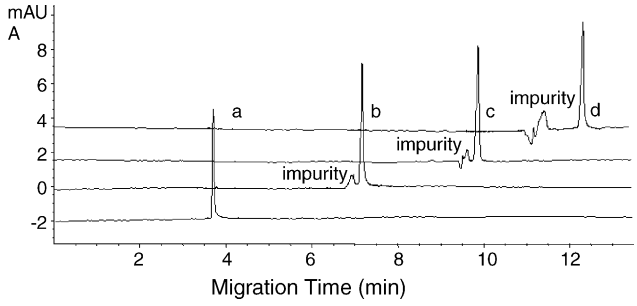

Fig. 4. The effect of HP-β-CD concentration on the chiral separation of compound II. Conditions: column effective length, 60 cm; applied voltage, 30 kV; column temperature, 20 °C; BGE, 20 mmol/L borate buffer at pH 9.3 containing following concentration of HP-β-CD: (a) 0 mM, (b) 50 mM, (c) 70 mM and (d) 90 mM.

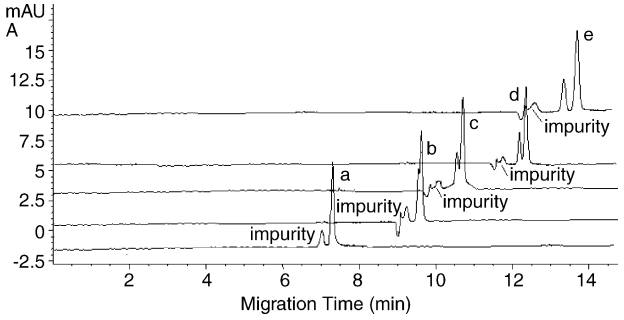

Fig. 5. The effect of acetonitrile concentration on the chiral separation of compound II. Conditions: column effective length, 60 cm; applied voltage, 30 kV; column temperature, 20 °C; BGE, 20 mmol/L borate buffer at pH 9.3 with 50 mM HP-β-CD containing following concentration of acetonitrile (a) 0%, (b) 5%, (c) 10%, (d) 15% and (e) 20%.

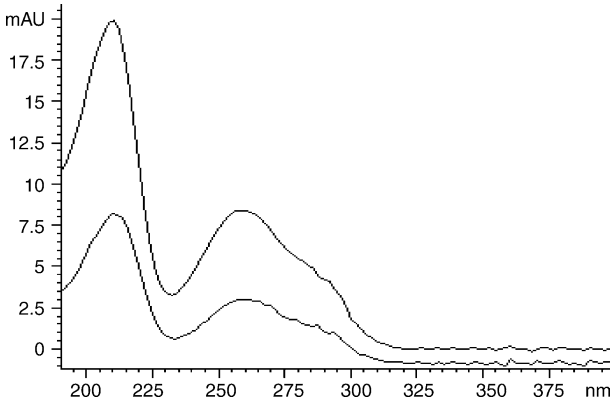

Fig. 6. The UV spectra of each of the enantiomers of compound II extracted from DAD plot at apex of each peak. The upper spectrum is from that of the latter peak in Fig. 5, and the lower from the former.

the decrease of the association equilibrium constants [14], thereby increasing the apparent mobility difference between the two enantiomers. The result was also in accordance with the conclusions proposed by Stephen et al. [15].

That the migration time tended to increase with acetonitrile concentration was also shown in Fig. 5. This indicated that the addition of acetonitrile led to a decrease of the actual mobility. The effect can be attributed to the medium effect and the smaller change in viscosity of the binary water–ACN mixture [14,16].

Take both the analysis speed and the resolution of the enantiomers into consideration, an acetonitrile concentration of 20% was chosen as optimal.

Fig. 6 shows the UV spectra, extracted from the DAD 3-D graph, of the enantiomers concerned.

4. Conclusions

Rapid and effective methods for the chiral separation of compounds I and II are reported. The optimum conditions were: 40 mmol/L Tris–H_3PO_4 buffer at pH 8.5 containing 1.2% CM-β-CD for (I), and 20 mmol/L borate buffer at pH

9.3 containing 50 mmol/L HP-β-CD for (II). Under these conditions the two enantiomer pairs could be baseline separated, respectively. Most interestingly, the ratio of the heights and/or the areas of the electrophoretic peaks of the enantiomer pairs, obtained from two natural products, were not equal. The ratio herein is consistent with that obtained from C^{13} NMR. This deserves a further study.

Acknowledgement

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