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Stereoisomeric separation of some flavanones using highly succinate-substituted α-cyclosophoro-octadecaoses as chiral additives in capillary electrophoresis

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ABSTRACT

α-Cyclosophoro-octadecaoses (α-C18), produced by *Rhodobacter sphaeroides*, are mostly homogeneous in size with 18 glucose units per ring as the predominant form. α-C18s are linked by β-(1→4)-linkages and one α-(1→6)-linkage and are also known to be highly substituted by acetyl (0–2 per mol) and/or succinoyl groups (1–7 per mol). We isolated and purified α-C18 and successfully used it in capillary electrophoresis (CE) as a chiral additive for the separation of five flavanones and flavanone-7-*O*-glycosides, including naringenin, hesperetin, eriodictyol, homoeriodictyol, isosakuranetin, and hesperidin. Throughout the CE experiment with unsubstituted α-C18 (uα-C18) obtained after alkaline treatment of the isolated α-C18, we found that successful chiral separation critically depends on the presence of succinate substituents attached to α-C18 in CE, suggesting that succinoylation of α-C18 is decisive for effective stereoisomeric separation.

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1. Introduction

Many pharmaceutical compounds have one or more chiral centers that are responsible for their optical activity. It is well known that the pharmacological effect is, in general, restricted to one of the enantiomers.[1] At present, liquid chromatography (LC) still dominates chromatographic enantiomeric analyses in industry.[2] However, over the last two decades, capillary electrophoresis (CE) has become a powerful separation technique for enantiomeric separations of various kinds of racemic mixtures. The advantages of CE, compared to other chromatographic methods, are that it is a simple, rapid, and practical method for providing high separation efficiency that requires small amounts of samples and reagents. CE offers a tremendous flexibility for enantiomeric separations because a wide variety of chiral additives are available. Cyclodextrins,[3,4] crown ethers,[5,6] proteins,[7,8] and various oligo- or polysaccharides[9,10] have been successfully applied as chiral additives in CE for chiral separations.[11]

Flavonoids are a group of polyphenolic compounds of low molecular weight[12] that present a common benzo-γ-pyrone structure.[13] They have beneficial health-related properties and are commonly distributed in fruits and vegetables. In addition, among the large family of flavonoids, flavanones possess chirality, which distinguishes them from all other classes of flavonoids. Flavanones exist in two enantiomeric forms due to the stereogenic center in position C2 (Fig. 1A). Flavanone-7-*O*-glycosides also have an additional D-configured mono- or disaccharide moiety and accordingly exist as pairs of epimers (Fig. 1B). Separation of enantiomeric flavanones and epimeric flavanone-7-*O*-glycosides has been performed by high-performance liquid chromatography (HPLC)[14,15] and more recently by CE[16–19] with native or modified cyclodextrins as chiral selectors.[17–21] Recently, chiral separation of some flavonoids was performed with microbial cyclic or noncylic oligosaccharides produced by proteobacteria in CE.[22–26]

α-Cyclosophoro-octadecaoses (α-C18), produced by *Rhodobacter sphaeroides*, are mostly homogeneous in size, and have 18 glucose units per ring as the predominant form. α-C18 is linked by β-(1→4)-linkages and one α-(1→6)-linkage and is also known to be highly substituted by acetyl (0–2 per mol) and/or succinoyl groups (1–7 per mol).[27] *R. sphaeroides* is a member of the α-proteobacteria, and as the most extensively studied photosynthetic organism in relation to renewable energy sources, it has been genetically analyzed in detail.[28] Although α-C18 of *R. sphaeroides* is believed to play roles for osmotic adaptation,[27] the exact biological functions and roles of α-C18 are not yet clear.

In the present study, we purified α-C18s from *R. sphaeroides* and used them as novel chiral additives for the stereoisomeric separation of five flavanones and three flavanone-7-*O*-glycosides in CE. Various parameters were investigated for optimal chiral separation of flavanones under background electrolytes (BGEs) with varied concentrations of additives, buffer pH values, buffer concentrations, and temperatures. Moreover, unsubstituted α-C18 (uα-C18), obtained by the removal of succinoyl and acetyl substituents by

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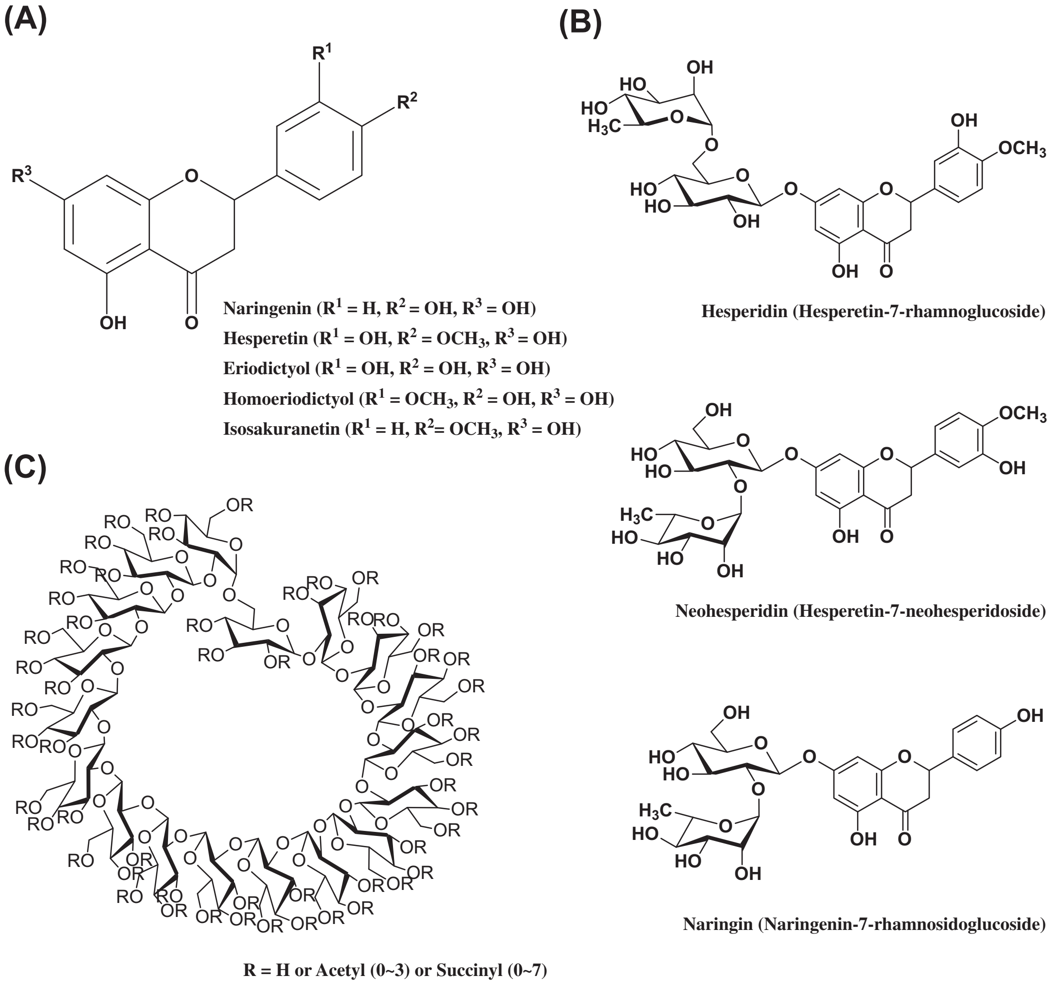

Figure 1. Chemical structures (A) of five flavanones, (B) three flavanone-7-*O*-glycosides and (C) α-cyclosophoro-octadecaoses (α-C18s) involved in this work.

alkaline treatment, was prepared for clarification of the role of substituents in the chiral separation of flavanones.

2. Experimental

2.1. Chemicals and reagents

All chemicals were of analytical grade purity. Naringenin, naringin, hesperetin, hesperidin, and neohesperidin were purchased from Sigma–Aldrich Chemical Co. (St. Louis, Mo, USA). Eriodictyol, homoeriodictyol, and isosakuranetin were purchased from Carl Roth (Karlsruhe, Germany) (Fig. 1A and B).

2.2. Preparation of α-cyclosophoro-octadecaoses

R. sphaeroides (ATCC 17023) was grown at 30 °C in sistrom's succinic acid minimal (SIS) medium under anaerobic conditions illuminated with light.[29] For the production of OPGs, aerobic chemoheterotrophic cultures were grown in a Luria–Bertani (LB) broth with shaking.[24] The bacterial cells were collected after 2 days by centrifugation at 8000 rpm for 10 min. The cell pellets were extracted with 5% trichloroacetic acid, and the extract was neutralized with ammonia water and desalted on a Sephadex G-15 column. The desalted materials were concentrated and applied to a Bio-Gel P-4 column. The fractions of the putative glucans detected by the phenol–sulfuric acid method[30] were pooled and concentrated by rotary evaporation. Fractions containing oligosaccharides were pooled and lyophilized. The structural analysis of α-cyclosophoro-octadecaoses (α-C18) was carried out by NMR spectroscopic and MALDI-TOF mass spectrometric analyses. For NMR spectroscopic analyses, α-C18s were dissolved in D_2O at room temperature. A Bruker Avance 500 spectrometer was used to record the ^1H NMR spectrum. For MALDI-TOF mass spectrometric analyses, α-C18s were dissolved in water and mixed with the matrix (2,5-dihydroxybenzoic acid). Mass spectra were recorded on a mass spectrometer (Voyager-DETM STR BioSpectrometry, PerSeptive Biosystems, Framingham, MA, USA) in positive-ion mode.

2.3. Deesterification of α-cyclosophoro-octadecaoses

For removal of the succinoyl and acetyl substituents, α-cyclosophoro-octadecaoses (α-C18) were treated in 0.1 M KOH at 37 °C for 1 h. After neutralization with AG 50W-X8 (H^+) (Bio-Rad), the samples were desalted on a Bio-Gel P-2 column.[27]

2.4. Apparatus and procedure

All capillary electrophoretic experiments were performed on an Agilent 3D CE System (Wilmington, DE, USA) equipped with a diode array detector. Separations were carried out on an uncoated 50-μm ID fused-silica capillary with a total length of 50.5 cm and an effective length of 42 cm to the detector window. The capillary

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