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Short communication

Preparative separation of gambogic acid and its C-2 epimer using recycling high-speed counter-current chromatography

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Abstract

A recycling counter-current chromatographic system was first set up with a high-speed counter-current chromatography instrument coupled with a column switching valve. This system was first successfully applied to the preparative separation of epimers, gambogic acid and epigambogic acid from *Garcinia hanburyi* using *n*-hexane–methanol–water (5:4:1, v/v/v) as the two-phase solvent system. As a result, 28.2 mg gambogic acid and 18.4 mg epigambogic acid were separated from 50 mg of mixture. Their purities were both above 97% as determined by HPLC. The chemical structures were then identified by their ^{1}H NMR and ^{13}C NMR spectra.

Keywords: Recycling counter-current chromatography; Preparative separation; *Garcinia hanburyi*; Epimer; Gambogic acid; Epigambogic acid

1. Introduction

Gambogic acid (GA, CAS No. 2752-65-0) is the principal active component of gamboge, the resin from various *Garcinia* species including *Garcinia Morella* and *Garcinia hanburyi*. Many modern pharmaceutical studies are focused on its extensive and potent anti-tumor activities [1,2]. In 1970s, it had been developed as an anti-tumor drug for clinical testing via intravenous injection in China. However GA had been believed to be an inseparable C-2 epimeric mixture [3]. The stereochemistry had not been determined until the single crystal of pyridine salt of *R*-epimer was obtained and then analyzed by X-ray diffraction [4]. In our previous report, this epimer pair was separated first by semi-preparative HPLC. The *S*-epimer was found more potent against CYP 2C9 than *R*-epimer [5]. The chemical structures of gambogic acid epimers are very similar, with only one stereochemical difference at C-2 (Fig. 1). This made their ^{1}H and ^{13}C NMR spectra extremely similar to each other (Fig. 2), and also made this epimer pair difficult to separate by common chromatography methods. Moreover, the reverse-phase HPLC separation of these epimers with semi-preparative column was time-consuming [5]. Therefore, a simpler isolation is necessary to achieve a better separation.

High-speed counter-current chromatography (HSCCC), a special liquid–liquid partition chromatography without solid support matrix, prevents peak tailing and sample loss due to irreversible adsorption [6]. This method has been successfully applied in the preparative separation of natural products and chiral compounds [7–10]. In recent years, multidimensional CCC which effectively extends the separation journey has been successfully applied to natural products separation [11–13]. Accordingly, it is also theoretically feasible to separate any compounds including epimers if the CCC separation course is long enough. This paper describes a successful separation of gambogic acid and its C-2 epimer using a newly built recycling HSCCC system.

2. Experimental

2.1. Apparatus

The preparative HSCCC instrument used in this study was TBE-300A high-speed counter-current chromatography (Shenzhen, Tauto Biotech, China) with three polytetrafluoroethylene preparative coils (diameter of tube, 2.6 mm, total volume, 300 ml). The revolution radius or the distance between the holder

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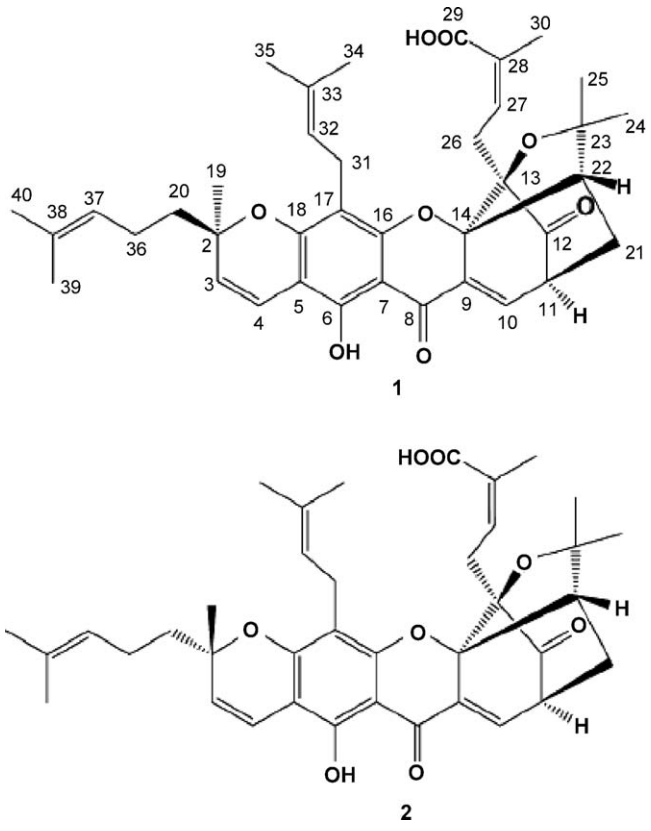

Fig. 1. Chemical structures of gambogic acid (**1**) and its C-2 epimer (**2**).

axis and central axis of the centrifuge (R) was 5 cm, and the β value varied from 0.5 at the internal terminal to 0.8 at the external terminal ($\beta = r/R$ where r is the distance from the coil to the holder shaft). A HX 1050 constant-temperature circulating implement (Beijing Boyikang Lab Instrument Co. Ltd., Beijing, China) was used to control the separation temperature. The HSCCC system was equipped with a model S constant-flow pump, a 3725i-038 injector (Rheodyne, USA), the UV–vis G1365B photodiode array detector and Agilent HPLC workstation of a preparative Agilent 1100 HPLC Series. The recycling HSCCC separation was carried out on this system with a column switching valve.

HPLC analysis was carried out on an analytical Agilent 1100 series and Alltima-C_8 column (4.6 mm × 250 mm, 5 μm) at room temperature. The Agilent 1100 HPLC system included a G1311A solvent delivery unit, G1315B UV–vis photodiode array detector, Rheodyne 7725i injection valve with a 20 μl loop, G1332A degasser and Agilent HPLC workstation. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra were recorded on a Brucker DRX-400 spectrometer using TMS as internal standard.

2.2. Reagents

Methanol, acetonitrile, *n*-hexane, 1,4-dioxan anhydroscan, and acetic acid of HPLC grade were purchased from International Laboratory Ltd., USA. Distilled water was prepared using MILLI-Q SP reagent water system (Nihon Millipore Kogyo K.K., Japan), and was distilled twice before use. The mixture of gambogic acid epimers was isolated as previously reported, from the resin of *G. hanburyi* [5].

2.3. Preparation of two-phase solvent system and sample solution

Several two-phase solvent systems were tested for their partition abilities [7], including *n*-hexane–ethyl acetate–methanol–H_2O, *n*-hexane–methanol–H_2O, *n*-hexane–acetonitrile–H_2O in various ratios. As a result, *n*-hexane–methanol–water (5:4:1, v/v/v) was selected as the separation system, since the pigment GA could equally dissolve in both phases. The two-phase solvent system was prepared by adding the solvents to a separation funnel according to the volume ratios and fully equilibrated by shaking repeatedly at room temperature (20 °C). The upper and lower phases (1:1) were separated shortly before use and degassed by sonication for 30 min. Fifty milligrams of gambogic acid mixture was dissolved in 5 ml lower phase for HSCCC separation.

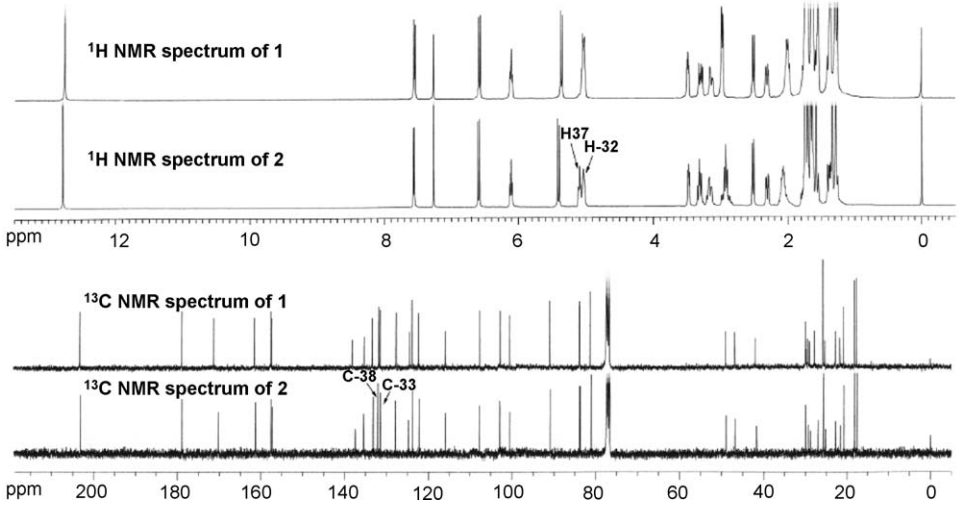

Fig. 2. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectra of **1** and **2** (in $CDCl_3$, TMS as internal standard).