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Short communication

Purification of long-chain fatty acid ester of epigallocatechin-3-*O*-gallate by high-speed counter-current chromatography

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Abstract

Epigallocatechin-3-*O*-gallate (EGCG) was modified by catalytic esterification with hexadecanoyl chloride. A long-chain fatty acid ester derivative was obtained from the purification of this reaction product by high-speed counter-current chromatography using a solvent system composed of *n*-hexane–ethyl acetate–methanol–water (1:1:1:1, v/v). The structure of the derivative, epigallocatechin-3-*O*-gallate-4′-hexadecanate, was elucidated by IR, MS and ^{1}H NMR.
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Keywords: Counter-current chromatography; Epigallocatechin-3-*O*-gallate; Epigallocatechin-3-*O*-gallate-4′-hexadecanate; Fatty acids; Catechins

1. Introduction

Green tea extracts are used as food antioxidants and medicines since epigallocatechin gallate (EGCG), the major catechin in tea leaves, possesses several bioavailability such as anti-arteriosclerosis, resistant to oxidation and anticancer [1–4]. However, the use of EGCG is greatly limited because of its low solubility in lipid-soluble medium. Therefore, it is important to modify the molecule structure of EGCG to prepare an EGCG derivative for use in lipid-soluble medium. The present paper describes a purification of long-chain fatty acid ester derivative of EGCG from the reaction product of EGCG and C_{16}-fatty acid by high-speed counter-current chromatography (HSCCC) and the elucidation of the chemical structure of the derivative.

2. Experimental

2.1. Apparatus

A J-type HSCCC instrument was used in the present study. It holds a separation column at a distance of 10 cm from the center of the centrifuge. The column revolves around the central axis of the centrifuge and simultaneously rotates about its own axis at the same angular velocity in the same direction [5]. The column holder hub was 25 cm in

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length and 6 cm O.D. The multilayer coils was prepared by winging 48 m of 5.5 mm I.D. PTFE (polytetrafluoroethylene) tubing (Zeus Industrial Products, Raritan, NJ, USA) onto the holder hub. The capacity of the column was 1100 ml. The experiment was performed at a revolution speed of 750 rpm. The mobile phase was delivered using a Waters 510 HPLC pump (Millipore, Milford, MA, USA). An injection loop was used for sample loading and a UV–Vis detector (Model UV-752, Shanghai Instrument Factory, Shanghai, China) was used for monitoring the effluent.

2.2. Reagents

Epigallocatechin gallate (EGCG) used for the preparation of EGCG derivative was purchased from Sigma, and hexadecanoyl chloride was freshly prepared by collecting 152–156 °C/267 Pa fractions of distillation of reaction product between hexadecanoic acid and thionyl chloride. *n*-Hexane, methanol and ethyl acetate used for high-speed counter-current chromatography were of analytical grade solvents. Methanol used for HPLC analysis was of HPLC grade reagent.

2.3. Decision and preparation of two-phase solvent system

The preparative HSCCC purification was performed with a two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water (1:1:1:1, v/v). After thoroughly equilibrating the mixture of the solvents in a separatory funnel at room temperature, two phases were separated shortly before use. The upper organic phase was used as mobile phase, and the lower aqueous phase as stationary phase.

2.4. Preparation of lipid-soluble derivative of EGCG

The catalytic esterification was carried out according to a method previously described [6]. The lipid-soluble derivative of EGCG was prepared using 0.01 mol of EGCG and 0.01 mol of hexadecanoyl chloride in 50 ml of ethyl acetate at 40 °C. The reaction was kept for 3 h under stirring. Then the reaction solution obtained by filtration was washed with deionized water (30 ml, 3 times), and the upper organic layer was evaporated and dried in vacuum at 40 °C to yield 6.7 g of the white powdery product.

The HSCCC sample solution was prepared by dissolving 5.0 g of the derivative in 50 ml of upper phase.

2.5. Separation procedure

The multilayer coiled column was first entirely filled with the lower aquatic phase as the stationary phase. Then, the columns were rotated at 200 rpm. The sample solution was injected through the injection loop, and the mobile phase was pumped into the column at a flow-rate of 3.2 ml/min while the column rotation was increased to 750 rpm. The effluent was monitored at 280 nm and collected with a fraction collector.

2.6. HPLC analysis

An Agilent 1100 HPLC system composed of a quaternary pump with a degasser, a thermostatted column compartment, a variable wavelength detector, a manual injector, and 1100 ChemStation software. The HPLC separation was performed on a Zorbax-ODS column (5 μm, 4.6 mm I.D.×25 cm) eluted with 85% of methanol in water from 0 to 30 min at a flow-rate of 1.0 ml/min by monitoring at 280 nm.

2.7. IR, MS and NMR analysis

A Bruker Vector 22 IR spectrophotometer, a Bruker Esquire-LC mass spectrograph and a Bruker Advance 400 MHz NMR spectrometer were used for the IR, MS and NMR analysis of the derivative, respectively.

3. Results and discussion

3.1. Purification of lipid-soluble derivative of EGCG

Since eight hydroxyl groups in the structure of EGCG may react with acyl chloride, the reaction product was complicated though the reaction con-

dition was strictly controlled (Fig. 1A). However, it is invigorating that the main product was obtained by the separation of HSCCC (Fig. 1B). The fraction corresponding to the major peak was collected and evaporated the solvent to yield 3.1 g of white powder. The HPLC analysis (Fig. 1B) of the powder showed that it is a pure EGCG derivative.

3.2. Elucidation of the structure of the derivative

The IR spectra of EGCG and the EGCG derivative (KBr) showed that there were two strong absorption peaks at 2924 cm^{-1} (ν_{as}) and 2853 cm^{-1} (ν_{s}) that were the characteristic absorption peaks of long chain saturated aliphatic hydrocarbon while the other parts were similar to that of the EGCG [7]. There was another characteristic absorption peak at 1735 ($\nu_{C=O}$) cm^{-1} which indicated the saturated fatty acid ester linkage between the long fatty acid chain and EGCG.

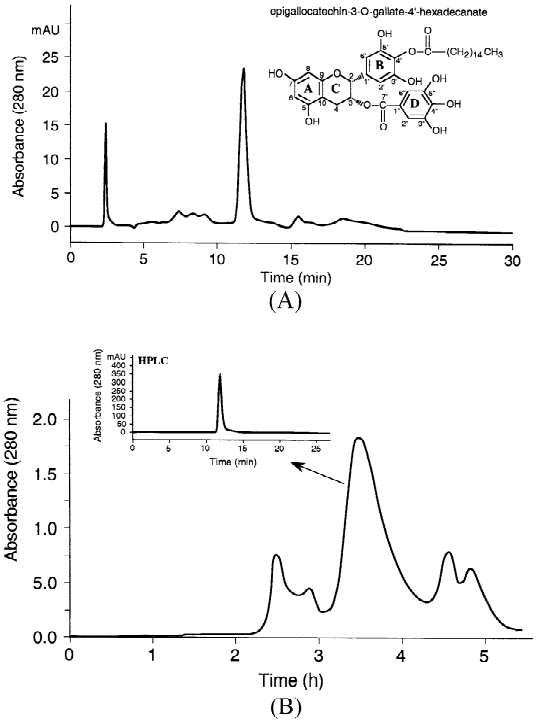

Fig. 1. (A) HPLC analysis of the reaction product between EGCG and hexadecanoyl chloride. Zorbax-ODS column (5 μm, 4.6 mm I.D.×25 cm); mobile phase: 85% of methanol in water; flow-rate: 1.0 ml/min; wavelength: 280 nm. (B) HSCCC separation of the reaction product. Solvent system: *n*-hexane–ethyl acetate–methanol–water (1:1:1:1, v/v); mobile phase: upper organic phase; flow-rate: 3.2 ml/min; rotating speed of the column: 750 rpm.

The first- and second-class electrospray ionization (ESI) MS spectrogram of the EGCG derivative produced a negative ion of the EGCG derivative at m/z 695.4 that is the EGCG esterified with C_{16} fatty acid chain. The main fragment of the negative ion of EGCG was found at m/z 457.9. Therefore, it can be inferred from the MS analysis that the derivative should be EGCG–$COOC_{15}H_{31}$.

The ^{1}H NMR studies on chemical shifts (δ) of the fatty acid derivative of EGCG and EGCG in DMSO-d_6 revealed that the δ values of the derivative were near to the corresponding protons in EGCG while one proton (δ 8.01) missed and three kinds of protons {δ 0.85 (3H,t, J=6.72 Hz), δ 1.24 (26H,m), δ 1.58 (2H,m)} increased. Proton corresponding to δ 8.01 is the proton at 4′-OH on the B-ring of EGCG structure [7], and protons corresponding to δ 0.85 (3H,t, J=6.72 Hz), δ 1.24 (26H,m) and δ 1.58 (2H,m) are those in the groups of $-CH_3-(CH_2)_{13}-$ and $-CO-CH_2-$, respectively.

On the basis of the above analyses, it is clear that the derivative is epigallocatechin-3-*O*-gallate-4′-hexadecanate, a single-substitution derivative at 4′ on the B-ring of EGCG structure (Fig. 1A).

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