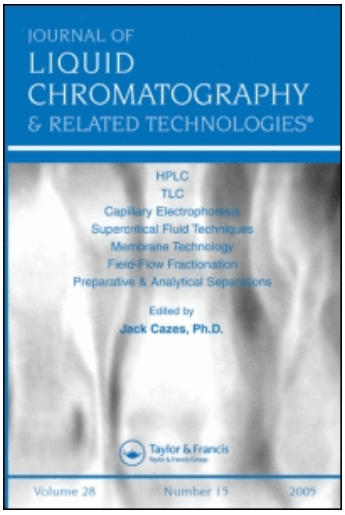

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Preparation of Solanesol from a Tobacco Leaf Extract Using High Speed Countercurrent Chromatography

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Abstract: Separation of solanesol from the tobacco leaves extract was performed by high speed countercurrent chromatography using a solvent system composed of petroleum ether-ethanol-methanol (200 : 1 : 100, v/v). In each separation, one gram of the tobacco leaves extract was injected to the multilayer coil separation column to yield 121 mg of solanesol with a purity of 90.7%. The obtained solanesol was identified by ESI-MS, ^{1}H, and ^{13}C-NMR.

Keywords: Solanesol, Tobacco leaves, Separation, High speed countercurrent chromatography

INTRODUCTION

Solanesol is an unsaturated polyisoprenol with low polarity. It is an intermediate compound for the synthesis of ubiquinone medicines such as coenzyme Q10 and vitamin K2.[1–3] Solanesol also possesses bioactivities of anti-bacteria, anti-inflammation and anti-ulcer.[4,5] The content of solanesol is 0.3–3% of dried tobacco leaves, which provides a natural resource to

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prepare solanesol.[6,7] High speed countercurrent chromatography (HSCCC) permits the use of a non-aqueous two-phase solvent system[8,9] to separate non-polar components. The present paper describes the preparative separation of solanesol in tobacco leaves extract using a non-aqueous two-phases solvent system composed of petroleum ether-ethanol-methanol (200 : 1 : 100, v/v).

EXPERIMENTAL

Materials

The tobacco leaves extract containing about 15% of solanesol was provided by Three Power Group Co. Ltd., Weifang, China.

TLC Analysis

TLC analysis was performed on a 4 plate (Merck, Germany) developed with petroleum ether-ethyl acetate (4 : 1), and colorized with sulfuric acid-anisaldehyde-glacial acetic acid (5 : 5 : 90, v/v) at 110°C.

Selection of HSCCC Solvent System

The partition effect in a two-phase solvent system was tested by TLC analysis. First, a small amount of tobacco leaves extract was added into a test tube containing equal volumes of each phase of a given solvent system, and the tube was shaken to thoroughly mix the contents. Finally, the solanesol concentration in each phase was analyzed by TLC to estimate the partition coefficient.

Isolation of Solanesol with HSCCC

A J-type HSCCC instrument (Institute of Food and Biological Engineering, Zhejiang Gongshang University, Hangzhou, China) was used for the separation of tobacco leaves extract. 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. The column holder hub was 15 cm in length and 6 cm in OD. The multilayer coils were prepared by winging 60 m of 3.0 mm I.D. PTFE (polytetrafluoroethylene) tubing (Zeus Industrial Products, Raritan, NJ) onto the holder hub. The capacity of the column was 450 mL. The mobile phase was delivered using a Waters 510 HPLC pump (Millipore Corporation, Milford, MA). An injection loop was

used for sample loading. A two-phase solvent system composed of petroleum ether-ethanol-methanol (200 : 1 : 100, v/v) was applied for the HSCCC separation with the upper phase as the stationary phase and the lower phase as mobile phase. In each separation, the HSCCC column was first filled with stationary phase. Then, the apparatus was started at 750 rpm, and the sample solution, 1 g of the tobacco leaves extract in 30 mL of the lower phase, was injected into the column through the injection loop. Afterward, the mobile phase was delivered into the column to elute the components. The effluent was collected with a BS-160 mode fraction collector, collecting 5 mL of each fraction. The fractions were analyzed by TLC to find the solanesol fractions.

HPLC Analysis

The HPLC system was composed of a Waters 510 pump, a manual injector, a Lichrospher C18 column (5 μm, 250 × 4.6 i.d. mm, Merck, Germany), a Waters 480 UV variable wavelength monitor, and a data processing system. The separation of solanesol was performed at 30°C, eluting with acetonitrile-isopropanol (60 : 40). The detection wavelength was 213 nm.[10]

ESI-MS and NMR

ESI-MS experiments were performed on a Bruker Esquire LC–MS ion trap multiple mass spectrometer (Bremen, Germany) in positive and negative ionization mode analyzing ions up to m/z 2200. ^{1}H-, ^{13}C-, and DEPT 90/135-NMR spectra were recorded in $CDCl_3$ on a Bruker Avance 500 (Karlsruhe, Germany) with 500 MHz for ^{1}H- and 100.5 MHz for ^{13}C-measurements, respectively.

RESULTS AND DISCUSSION

Solvent Systems

Solvent system selection is a key step of countercurrent chromatographic separation. The partition coefficients of solanesol in five different non-aqueous two-phase solvent systems, including methanol-petroleum ether (b.p. 60–90°C) (1 : 2, v/v), acetonitrile-petroleum ether (1 : 2, v/v), petroleum ether-ethanol-methanol (200 : 5 : 100, v/v), petroleum ether-ethanol-methanol (200 : 1 : 100, v/v), and petroleum ether-ethanol-methanol (200 : 0.5 : 100, v/v) were investigated with TLC. Finally, petroleum ether-ethanol-methanol (200 : 1 : 100, v/v) was selected as the solvent system in which the partition coefficient of solanesol was about 1, which was significantly different from the partition coefficients of the other components.

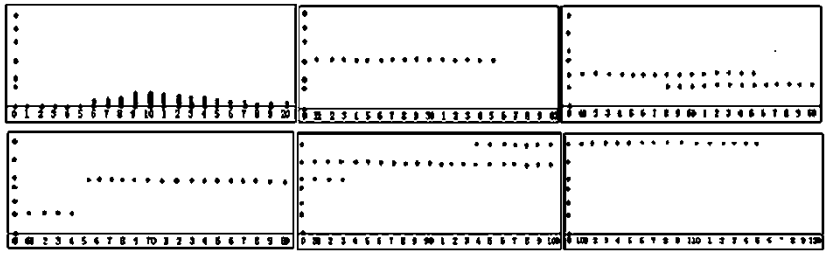

Figure 1. HSCCC separation result of 1 g of tobacco leaves extract. Solvent system: petroleum ether-ethanol-methanol (200 : 1 : 100, v/v); Stationary phase: upper phase; flow rate: 1.0 mL/min; Solution volume in each tube: 5 mL. TLC analysis was performed on a 4 plate (Merck, Germany) developed with petroleum ether-ethyl acetate (4 : 1), and colorized with sulfuric acid-anisaldehyde-glacial acetic acid (5 : 5 : 90, v/v) at 110°.

HSCCC Separation

Figure 1 shows the HSCCC separation result of 1 g of tobacco leaves extract with the two-phase solvent system petroleum ether-ethanol-methanol (200 : 1 : 100, v/v) obtained at a flow rate of 1 mL/min. The separation yielded 120 tubes of fractions in which tubes 22–35, tubes 41–47, tubes 56–64, tubes 65–80, tubes 84–93, and tubes 101–115 gave single spots in TLC analysis. The Rf value of the component in tubes 22–35 was identical to that of solanesol in reference [11]. These fractions were combined, decolorized with active carbon, and then evaporated in vacuum to dryness yielding 121 mg of light yellow powder that gave a purity of 90.7% determined by HPLC (Fig. 2).

The separation efficiency in unit time depends on the flow rate of elution. The preliminary runs were performed at two flow rates of 1.5 and 1.2 mL/min.

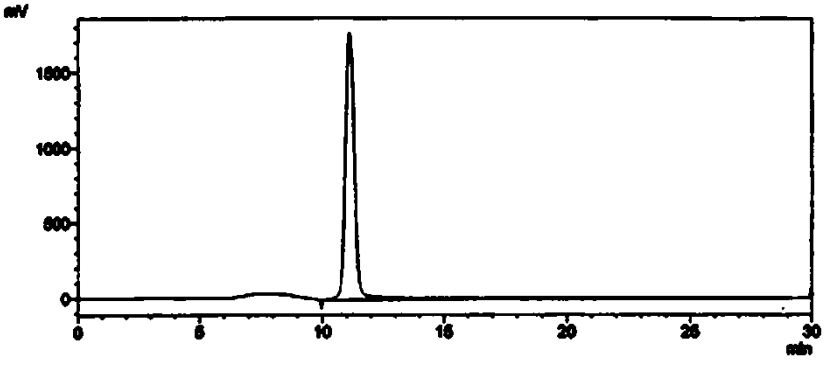

Figure 2. HPLC analysis of solanesol prepared by HSCCC separation.

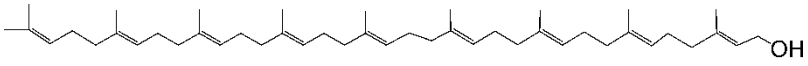

Figure 3. The chemical structure of solanesol.

At the flow rate of 1.2 mL/min, 2 fractions containing solanesol intercrossed with the following component, while at the flow rate of 1.5 mL/min 6 fractions containing solanesol overlapped with the following component. Therefore, the optimum flow rate was set at 1.0 mL/min. The stationary phase retention at flow rates of 1.0, 1.2, and 1.5 mL/min was 65%, 57%, and 48%, respectively, which agreed with the loss of resolution caused the increased flow rates.

Identification of Solanesol from CCC Separation

ESI-MS: m/z 653.2 $[M - H + Na]^+$, suggested the possible molecular formula $C_{45}H_{74}O$. ^{1}H-NMR (δppm, in $CDCl_3$) can be assigned as below: 1.60 (s, 24H, $8CH_3$), 1.68 (s, 2 6H, CH_3), 1.99 (t, 16H, $8CH_2$), 2.02 (s, OH), 2.09 (m, 16H, $8CH_2$), 4.15, (d, 2H,CH_2), 5.11 (8H, m, CH), and ^{13}C-NMR (δppm, in $CDCl_3$) can be assigned as below: 139.9 (1C, q), 136.5 (1C, q), 136.2 (3C, q), 135.1 (3C, q) 131.6 (91C, q), 124.6 (1C, CH), 124.3 (6C, CH), 124.2 (1C, CH), 123.9 (1C, CH), 69.6 (1C, CH_2), 39.9 (7C, CH_2), 39.7 (2C, CH_2), 26.8 (5C, CH_2), 26.4 (1C, CH_2), 25.8 (CH_2), 17.8 (1C, CH_3), 16.4 (1C, CH_3), 16.2 (8C, CH_3). ESI-MS, ^{1}H- and ^{13}C- NMR data[12] are identical to the chemical structure of solanesol (Fig. 3).

In conclusion, the present study demonstrates that HSCCC is a useful method to separate solanesol from tobacco leaves extract.

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