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Journal of Chromatography A, 813 (1998) 397–401

JOURNAL OF CHROMATOGRAPHY A

Short communication

Separation and purification of 10-deacetylbaccatin III by high-speed counter-current chromatography

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Received 16 February 1998; received in revised form 24 April 1998; accepted 27 April 1998

Abstract

High-speed counter-current chromatography (HSCCC) was used for the separation and purification of 10-deacetylbaccatin III from the needle extract of Chinese yew (*Taxus chinensis*). The crude needle extract (500 mg/5 ml) was first separated with a two-phase solvent system composed of *n*-hexane–ethyl acetate–ethanol–water (2:5:2:5, v/v) and the partially purified fraction was again purified with a different solvent system composed of *n*-hexane–chloroform–methanol–water (5:25:34:20, v/v). HPLC analysis of the final fraction showed that the purity of 10-deacetylbaccatin (20 mg) was over 98%. The chemical structure was confirmed by fast atom bombardment MS and ^{1}H NMR. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: Counter-current chromatography; *Taxus chinesis*; Deacetylbaccatin III

1. Introduction

The anticancer drug taxol is a natural product obtained from the bark of the Pacific yew, *Taxus brevifolia*. The quantity of taxol in the bark, however, is so low that a large amount of bark is required to meet the increasing demand for taxol. Since the removal of the bark kills the whole tree, intensive efforts have been devoted to search for the alternative resources such as leaves and branches. At present the most promising approach is the semi-synthesis of paclitaxel or its analogs from 10-deacetylbaccatin III, a compound available in a relatively high quantity from the foliage of several yew species [1,2]. 10-Deacetylbaccatin III is also used for synthesizing taxotere (analog of taxol) that possesses an improved pharmaceutical property [3]. Consequently, considerable interest exists in the isolation of this compound and its analogs [4,5].

In the present study, we report a method for the isolation and purification of 10-deacetylbaccatin III (10-DAB) from the needles of Chinese yew (*T. chinensis*) directly from the crude water extract by high-speed counter-current chromatography (HSCCC). Since HSCCC is support-free liquid–liquid partition chromatography, it eliminates sample loss caused by its irreversible adsorption to the solid support used in the conventional chromatographic column and has been successfully used for analysis

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PII: S0021-9673(98)00360-4

and separation of various kinds of natural products [6–8].

2. Experimental

2.1. Apparatus

HSCCC was performed with a Model GS10A2 multilayer coil planet centrifuge produced by Beijing Institute of New Technology Application, Beijing, China. The multilayer coil separation column was prepared by winding a 1.6 mm I.D. PTFE (polytetrafluoroethylene) tube (total capacity: 260 ml) coaxially onto the column holder hub at β=0.5–0.75 ($\beta = r/R$ where r is the distance from the holder axis to the coil and R, the distance from the holder axis to the centrifuge axis). The revolution speed is adjustable with a speed controller in a range of 0–1000 rpm with an 8 cm radius. The system was also equipped with an NS-1007 constant-flow pump, a Model 8823A-UV monitor operating at 254 nm, a Yokogawa Model 3057 recorder, and a sample injection valve with a 10-ml loop.

2.2. Reagents

All solvents used for HSCCC were of analytical grade and purchased from Beijing Chemical Factory, Beijing, China. Acetonitrile used for HPLC analysis was of chromatographic grade and purchased from Tianjin Huaxi Special Reagent Factory, Tianjin, China.

2.3. Sample preparation

A 500 mg amount of ground needles of *T. chinensis* with an average diameter of about 1 mm was extracted twice with 2.5 l of demineralized water at 50°C for 1 h under ultrasonication. The filtered aqueous solution was then extracted three times with 3 l of ethyl acetate. The organic phases containing the target compound were combined and washed with 2 l of 0.1 *M* sodium carbonate solution and then twice with 0.9 l of demineralized water. The extract was finally concentrated to dryness. The above procedure was based on Margraff and Chatillon's patent [5].

2.4. HSCCC separation procedure

In each separation the coiled column was first entirely filled with the upper organic stationary phase. Then the apparatus was rotated at 800 rpm, while the lower aqueous mobile phase was pumped into the column at a flow-rate of 2.0 ml/min. After the mobile phase front was emerged and the system established steady state hydrodynamic equilibrium, the sample solution was injected through the injection valve. The effluent from the outlet of the column was continuously monitored with a UV detector at 254 nm. Peak fractions were collected according to the chromatogram.

Because the extract was a complex mixture, a two-step operation was employed in the present separation. In the first separation, a quaternary solvent system composed of *n*-hexane–ethyl acetate–ethanol–water (2:5:2:5, v/v) was used. The fraction containing 10-deacetylbaccatin III was collected and concentrated, and then subjected to the second separation by HSCCC using another solvent system composed of *n*-hexane–chloroform–methanol–water (5:25:34:20, v/v).

2.5. High-performance liquid chromatographic analysis

The crude extract of taxus needles, partially purified fraction by the first-step CCC separation and the final fraction after the second-step CCC separation were analyzed by high-performance liquid chromatography (HPLC). The analysis was performed with a Rainin (Woburn, MA, USA) HPX HPLC system. It equipped with a Microsob-MV C_8 column (150×4.6 mm I.D., 5 μm diameter). The mobile phase composed of acetonitrile–water (25:75, v/v) was isocratically eluted at a flow-rate of 1.0 ml/min, and the effluent monitored by a UV detector at 230 nm.

2.6. Fast atom bombardment (FAB) MS and ^1H NMR identification

The purified fraction of 10-deacetylbaccatin III obtained from the HSCCC separation was analyzed by FAB mass spectrometry (MS) and ^1H nuclear magnetic resonance (NMR). FAB-MS was taken on

a Finnigan MAT711, Tabspec instrument, and ^{1}H NMR spectra on a Bruker AM-500 spectrometer (in $^{2}HO^{2}H$).

3. Results and discussion

It has been reported that 10-deacetylbaccatin III was separated from a crude extract of European yew (*T. baccata*) by centrifugal partition chromatography [9]. In the present study, the crude needle extract of *T. chinensis* contained much greater number of components, some of which elute close to the target component of 10-deacetylbaccatin III as shown by HPLC analysis (Fig. 1).

In order to obtain a suitable two-phase solvent system which provides the partition coefficient for the aimed compound, we have examined several solvent systems including ethyl acetate–ethanol–water (5:1:5), hexane–ethyl acetate–ethanol–water (1:5:1:5) and (2:5:2:5). Enhancing the hydrophobicity of the solvent system by increasing the relative concentration of hexane in the solvent system optimized the retention time of the analytes, but it failed to resolve the target compound from the surrounding impurities. Although the last solvent system yielded the improved separation shown in Fig. 2, we found that it was difficult to obtain relatively pure 10-deacetylbaccatin III through a one step separation. Therefore, two-step HSCCC separation was conducted. Finally we found that the second solvent system composed of hexane–chloroform–methanol–water (5:25:24:20) could eliminate impurities present in the fraction obtained by the first solvent system. This may be due to a large dipole moment of chloroform which can selectively influence the parti-

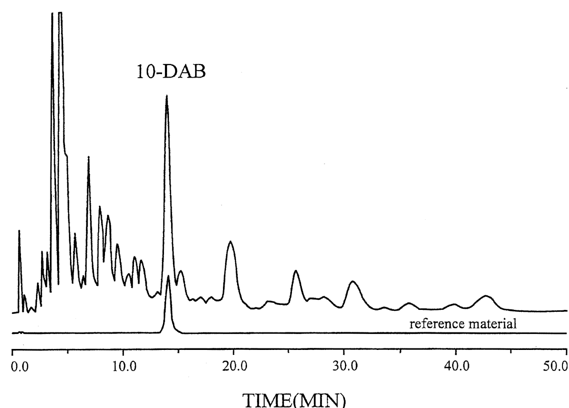
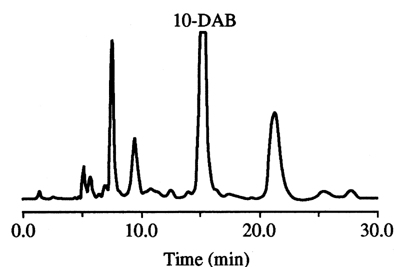


Fig. 1. HPLC analysis of the crude needle extract of *Taxus chinensis*. 10-DAB = 10-deacetylbaccatin III.



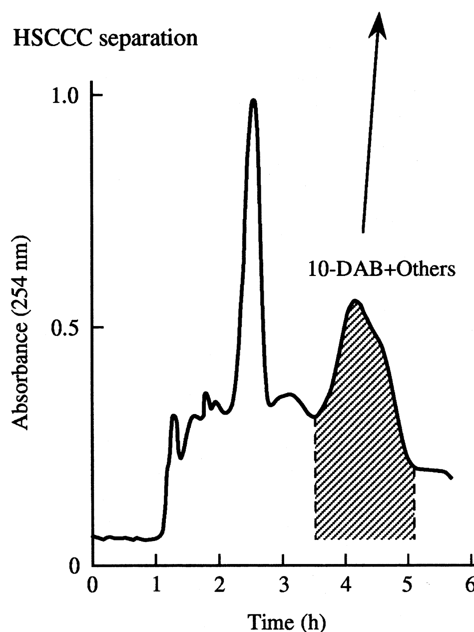


Fig. 2. The first-step HSCCC separation of the crude needle extract of *Taxus chinensis* and HPLC analysis of the 10-DAB fraction. Experimental conditions: apparatus: a Model GS10A2 multilayer coil planet centrifuge with 8 cm revolution radius; column: multilayer coil of 1.6 mm I.D. and 260 ml capacity (β=0.5–0.75); solvent system: *n*-hexane–ethyl acetate–ethanol–water (2:5:2:5, v/v); mobile phase: lower phase; sample: 500 mg of crude needle extract of *Taxus chinensis* in 5 ml solvent; flow-rate: 2 ml/min; detection: 254 nm. HPLC conditions: column: Microsob-MV C_8 (150×4.6 mm I.D., 5 μm, Rainin); mobile phase: acetonitrile–water (25:75, v/v); flow-rate: 1 ml/min (isocratic elution); detection: 230 nm. 10-DAB = 10-deacetylbaccatin III.

tion behavior of solutes in the solvent system. The similar strategy has been reported for separating anti-HIV-1 lignans from a crude extract of *Larrea tridentata* by HSCCC [10].

In the first step of HSCCC separation, 500 mg of the crude extract in 5 ml sample solution was injected and separated using a two-phase solvent system composed of *n*-hexane–ethyl acetate–ethanol–water (2:5:2:5, v/v) and 120 mg of the partially purified fraction was obtained (shaded portion of chromatogram in Fig. 2). HPLC analysis of this fraction showed that many interfering compounds, which was present in the original sample solution, have been removed (Fig. 2, HPLC analysis).

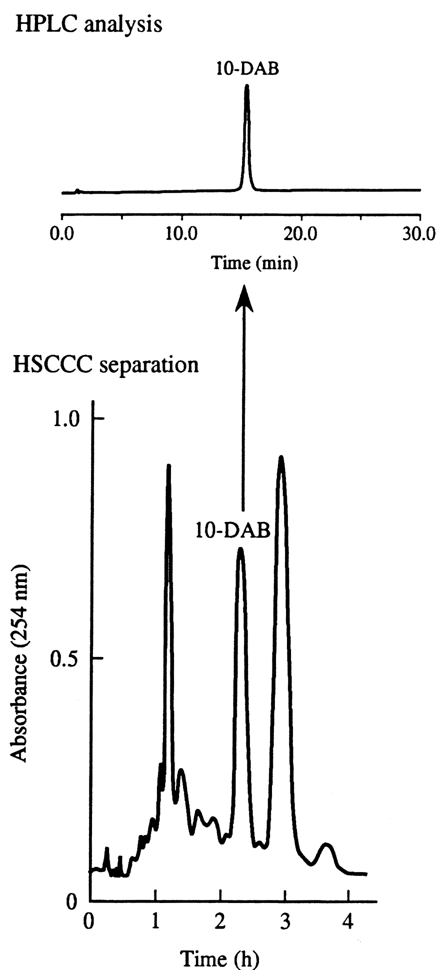


Fig. 3. The second-step HSCCC separation of the partially purified fraction and HPLC analysis of the 10-DAB fraction. Experimental conditions: apparatus: a Model GS10A2 multilayer coil planet centrifuge with 8 cm revolution radius; column: multilayer coil of 1.6 mm I.D. and 260 ml capacity (β=0.5–0.75); solvent system: *n*-hexane–chloroform–methanol–water (5:25:34:20, v/v); mobile phase: lower phase; sample: 120 mg of first-step HSCCC fraction (shaded portion of chromatogram in Fig. 2) in 5 ml solvent; flow-rate: 2 ml/min; detection: 254 nm. For HPLC conditions see Fig. 2. 10-DAB=10-deacetylbaccatin III.

Then, this semi-pure product (120 mg in 5 ml sample solution) was subjected to the HSCCC purification using a different two-phase solvent system composed of *n*-hexane–chloroform–methanol–water (5:25:34:20, v/v). This second step purification yielded 20 mg of the final product. HPLC analysis indicated that the purity of 10-deacetylbaccatin III in this fraction was over 98% (Fig. 3).

The final fraction was identified by both FAB-MS and ^{1}H NMR. FAB-MS showed three fragments of $[MH]^{+}$, i.e., 545, 527, 509 which are corresponding to $[M+1]^{+}$, $[M+1-H_2O]^{+}$, $[M+1-2H_2O]^{+}$, respectively.

This indicates that the compound has a molecular mass of 544 which is identical to that of 10-deacetylbaccatin III. ^{1}H NMR showed the spectra same as that of the 10-deacetylbaccatin III standard. These findings confirmed that our final product is 10-deacetylbaccatin III.

The overall results of the present studies led us to conclude that HSCCC is a suitable method for the semi-preparative separation and purification of 10-deacetylbaccatin III from a crude extract of *Taxus* needles and it may also be applied to other parts of *Taxus* plants. The present method can produce a pure fraction of 10-deacetylbaccatin III with a high recovery rate through a relatively simple separation procedure.

Acknowledgements

This work is supported by Beijing Commission of Science and Technology and Beijing Academy of Science and Technology. The authors wish to thank super engineer X.N. Li for his technical assistance in HSCCC instrumentation. Finally, we thank the Instrument Center, Academy of Military Medical Science (Beijing) and Institute of Materia Medica, Chinese Academy of Medical Sciences (Beijing) for

their help in FAB-MS and ^{1}H NMR analysis, respectively.

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