Accelerated Solvent Extraction of Paclitaxel and Related Compounds from the Bark of *Taxus cuspidata*

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Accelerated solvent extraction (ASE) of paclitaxel and related compounds from *Taxus cuspidata* (Japanese yew) bark has been investigated under various conditions. In ASE, pressure is applied to the sample extraction cell to maintain the heated solvent in a liquid state during the extraction. This method is able to shorten the extraction time and to increase the recovery of target compounds. In this study, ASE of paclitaxel, baccatin III, and 10-deacetylbaccatin III produced amounts of these compounds that were higher than those from ordinary solvent extraction at room temperature. The conditions providing the highest recovery of paclitaxel were as follows: solvent, MeOH−H_2O (90:10); temperature, 150 °C; and pressure, 10.13 MPa (0.128% w/w recovery based on oven-dried sample powder). ASE does not require chlorinated solvents and can reduce solvent consumption because of its strong dissolving power. Moreover, with water alone, the recovery of paclitaxel and related compounds using ASE is much higher than with other extraction methods.

Paclitaxel (generally known in the scientific literature as Taxol), a complex nitrogen-containing diterpenoid, was first isolated from the bark of the Pacific yew tree, *Taxus brevifolia*, and its structure determined.[1] It has significant anticancer activity and is clinically effective both alone and when employed with other cancer therapy treatments.[2−4] Until now, the total synthesis and the in vivo production of paclitaxel in plant tissue and cell culture have not provided economically feasible solutions to the paclitaxel supply problem.[5−9] Although paclitaxel can now be prepared from 10-deacetylbaccatin III and/or baccatin III using a semisynthetic procedure[10−12] (this is the route used for its commercial production), the direct isolation of paclitaxel is still a matter of considerable interest.

Various surveys have placed the paclitaxel content of *Taxus* spp. in the range of 0.001−0.08% w/w.[13−16] The recovery of paclitaxel extracted from the various parts of mature *T. cuspidata* trees was reported to be in the following order: bark > needles > roots > branches > seeds > wood.[17] Because paclitaxel occurs in small amounts in *Taxus* spp. and the tree grows slowly, it is important that any extraction procedure effectively remove all this compound from the bark. Taxanes such as paclitaxel, baccatin III, and 10-deacetylbaccatin III (10-DAB) have been extracted from the bark and needles of various yew species by ordinary solvent extraction (OSE). The majority of OSE processes reported in the literature have made use of MeOH as the extraction solvent at room temperature. However, there are also reports of extraction using other solvents, for example, MeOH−$CHCl_3$ (1:1),[18] MeOH−CH_2Cl_2 (1:1),[16,19] and 95% EtOH.[20] Supercritical fluid extraction methods exhibit high selectivity for taxane compounds, although organic cosolvents (e.g., EtOH, MeOH, and CH_2Cl_2) are required to obtain high taxane recovery.[21−24] Recently, microwave-assisted extraction has been reported as an extraction method for taxanes and found to reduce considerably both the extraction time and solvent consumption.[25]

Accelerated solvent extraction (ASE) is a new extraction method and enhances the traditional extraction process using solvent at elevated temperatures. Pressure is applied to the sample extraction cell to maintain the heated solvent in a liquid state during the extraction procedure and hence augments its dissolving power.[26−28] This paper reports on the ASE of paclitaxel, baccatin III, and 10-DAB from the bark of *Taxus cuspidata* Sieb. et Zucc. (Japanese yew, Taxaceae), and compares its efficiency with that of OSE at room temperature. Various temperatures, pressures, times, and solvents for the ASE procedure were investigated to optimize the efficiency of the extraction. Particular attention has been paid to ASE with water, from which paclitaxel was isolated and identified by means of NMR spectroscopy.

Results and Discussion

ASE of the taxanes from *T. cuspidata* bark was conducted with MeOH, and the taxanes in the extracted solids were quantitatively determined by HPLC. The influence of temperature in ASE at 10.13 MPa for 15 min was investigated, and the recoveries of extracted solids and taxanes from oven-dried bark were calculated (Figure 1). The higher the temperature, the greater the recovery of the MeOH-extracted solids from the bark. However, the content of taxanes in the MeOH extracts decreased with an increase of temperature. Moreover, at temperatures over 160 °C, degradation was likely to occur. The recoveries of paclitaxel and 10-DAB from the bark were highest in the temperature range of 100−150 °C and 120−150 °C, respectively.

The influence of pressure on the recoveries of taxanes by ASE at 150 °C for 15 min was investigated. The recovery of taxanes from the bark (paclitaxel, 0.083−0.093%; baccatin III, 0.013−0.021%; 10-DAB, 0.049−0.058%) hardly differed in the pressure range of 1.01−20.27 MPa (detailed data not shown). In the ASE at 150 °C with MeOH, the extraction occurred with high recovery at the very low pressure of 1.01 MPa.

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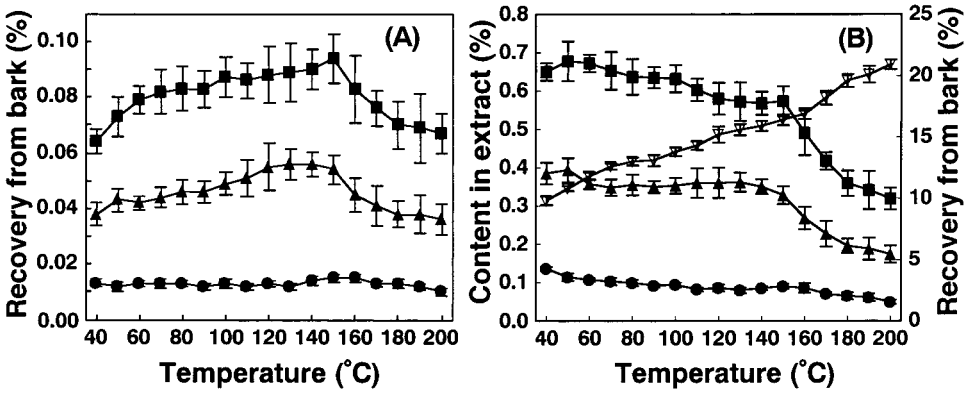

Figure 1. Influence of temperature on the recovery of taxanes from *T. cuspidata* bark with MeOH. (**A**) Taxane recovery from bark. (**B**) Taxane recovery as a percentage of the extract (left scale) and total extract recovery (right scale). Conditions for ASE: pressure, 10.13 MPa; extraction time, 15 min; solvent, MeOH. ■, Paclitaxel; ▲, 10-deacetylbaccatin III; ●, baccatin III; ▽, total extracts.

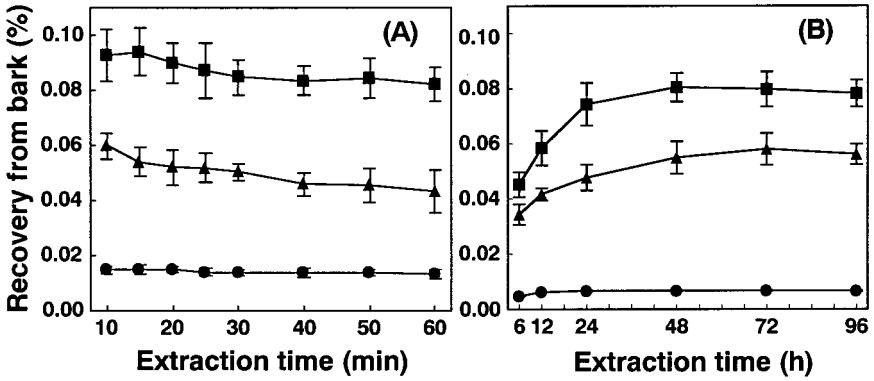

Figure 2. Changes in recoveries of taxanes with extraction time in ASE (**A**) and OSE (**B**). Conditions for ASE: temperature, 150 °C; pressure, 10.13 MPa; solvent, MeOH. ■, Paclitaxel; ▲, 10-deacetylbaccatin III; ●, baccatin III.

Figure 2 shows the variations in recovery of taxanes in terms of extraction time by ASE at 150 °C and 10.13 MPa (**A**) and those of OSE (**B**). In the OSE method, the recoveries of taxanes leveled off at 48 h. On the other hand, the recoveries of paclitaxel and 10-DAB with ASE from bark were the highest at 10−15 min, indicating that taxane degradation could be minimized by the use of short extraction times even if the temperature was as high as 150 °C. In general, it is recognized that paclitaxel is unstable at high temperatures, and it should be extracted usually at room temperature with MeOH only or MeOH-chlorinated methane.[16,19,25] The ASE method made possible the shortening of the extraction time, allowing the possibility of extracting large amounts of paclitaxel at a high temperature and a high pressure. The optimal conditions for ASE with MeOH were as follows: temperature, 150 °C; pressure, 1.01−20.27 MPa; extraction time, 10−15 min.

A comparison of ASE (10.13 MPa, 100 °C) and ordinary solvent extraction OSE at room temperature (0.10 MPa, 22 °C) with various solvents was conducted. The recovery of paclitaxel from oven-dried bark was calculated (Figure 3). In the ASE method, the recovery of paclitaxel was highest with MeOH. Except for MeOH−CH_2Cl_2 (1:1), the recoveries by ASE were higher than those of OSE, particularly when water was used for extraction. Similar results were found for baccatin III and 10-DAB. It was estimated that ASE has a high recovery with MeOH (not containing chlorinated solvents), H_2O, and EtOH and can reduce solvent consumption because of its strong dissolving power. Figure 4 shows the content of taxanes in extracts of ASE (**A**) and OSE (**B**) with various solvents. The content of paclitaxel in water extract produced by OSE was very low; however, it was improved by ASE.

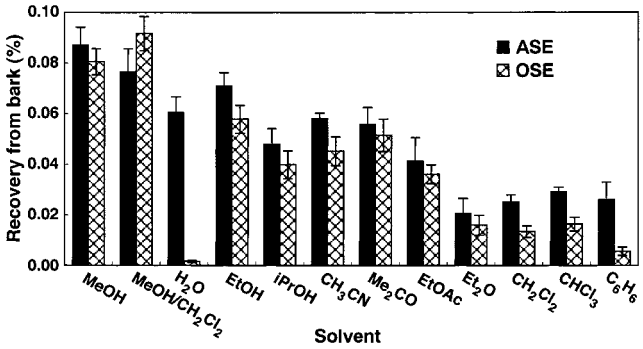

Figure 3. Influence of solvent on recovery of paclitaxel from the bark of *T. cuspidata*. Conditions for ASE: temperature, 100 °C; pressure, 10.13 MPa; extraction time, 15 min. Conditions for OSE: temperature, 22 °C; pressure, 0.10 MPa; extraction time, 48 h. MeOH/CH_2Cl_2 (1:1, v/v).

Although paclitaxel has been reported to have poor solubility in water,[3,29] in the ASE method extraction with water showed high recovery. The influence of temperature on taxane recovery in ASE with water (Figure 5) at 10.13 MPa for 15 min was examined. The recovery of paclitaxel and 10-DAB from bark was high and in the ranges of 130−140 °C and 120−130 °C, respectively. The recovery of paclitaxel with ASE at 140 °C was the same as that of OSE with MeOH−CH_2Cl_2 (1:1). Water extraction was performed with various procedures (Figure 6). In a comparison of ASE (140 °C, 10.13 MPa, 15 min) with OSE (22 °C, 0.10 MPa, 48 h) and hot-water extraction (100 °C, 0.10 MPa, 1 h), the recoveries of paclitaxel by ASE were *ca.* 50 times and 5 times those by OSE and hot-water extraction, respectively. It was confirmed that the yield of paclitaxel by water

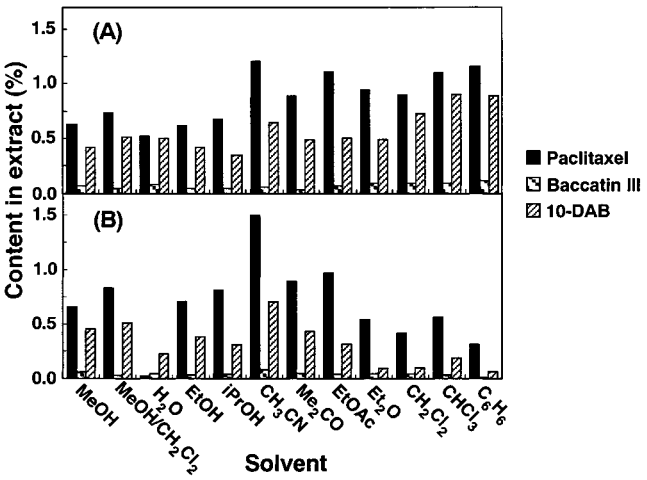

Figure 4. Content of taxanes in extracts produced by ASE (**A**) and OSE (**B**) with various solvents. Conditions for ASE are the same as those in Figure 3.

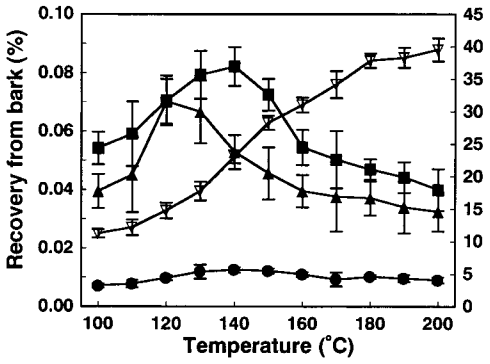

Figure 5. Relationship between temperature and recovery of taxanes by ASE with water. Conditions for ASE: pressure, 10.13 MPa; extraction time, 15 min; solvent, MeOH. Left scale: ■, paclitaxel; ▲, 10-deacetylbaccatin III; ●, baccatin III. Right scale: ▽, total extracts.

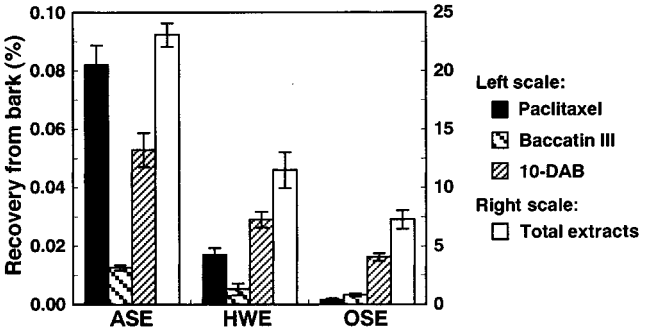

Figure 6. Recovery changes with various H_2O extraction methods. Extraction method (temperature, pressure, extraction time): ASE (140 °C, 10.13 MPa, 15 min); hot-water extraction (HWE) (100 °C, 0.10 MPa, 1 h); OSE (22 °C, 0.10 MPa, 48 h).

extraction with ASE was higher than that of the other extraction methods.

ASE (10.13 MPa, 150 °C, 15 min) was conducted with various ratios of MeOH−H_2O (Figure 7). The higher the proportion of water, the greater was the recovery of overall extracts from the bark. The content of taxanes in the extracts was the highest for a MeOH−H_2O ratio of 90:10. Greater proportions of water resulted in decreased taxane content. It is considered that the increase of extract recovery when the water content increased over 20% was caused by an increase in the amount of carbohydrates extracted. The yield of taxanes from bark was also highest for the MeOH−H_2O (90:10) solution. It has been reported

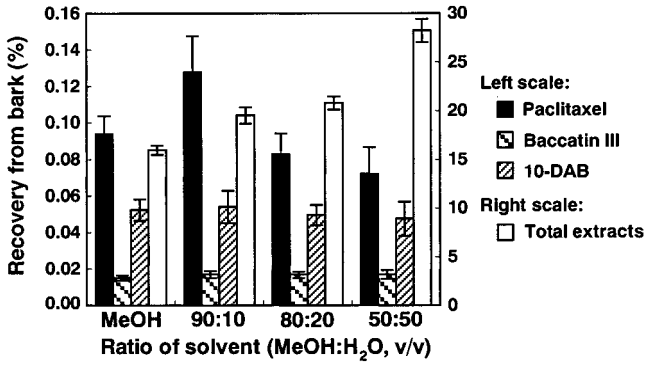

Figure 7. Recovery of taxanes by ASE using various ratios of MeOH−H_2O. Conditions for ASE: temperature, 150 °C; pressure, 10.13 MPa; extraction time, 15 min.

that the use of CH_2Cl_2 as a cosolvent in the extraction of paclitaxel by SFE with CO_2 from the needles of *T. cuspidata* largely improved the paclitaxel extraction capability.[23] In another report, the optimized SFE with NO_2 + EtOH (cosolvent) gave 0.014% recovery of paclitaxel from the bark of *T. brevifolia*, and this value was a 99% against the recovery by exhaustive OSE with EtOH.[24] In the present investigation of ASE (MeOH−H_2O, 90:10; temperature, 150 °C; and pressure, 10.13 MPa), the recoveries of paclitaxel vs the mass paclitaxel based on OSE with both EtOH and MeOH−CH_2Cl_2 (1:1) were >100% (Figures 3 and 7). Accordingly, ASE can rapidly extract taxanes from *T. cuspidata* bark in high recovery without chlorinated solvents.

A preparative ASE (10.13 MPa, 140 °C) with water was conducted, and the paclitaxel was isolated from the water extract by chromatographic methods. The final recovery of paclitaxel from bark was 0.022%, and the isolated material had NMR spectroscopic data that matched those of an authentic sample. HPLC analysis of the water extract produced by ASE proved the high extractivity of paclitaxel using this procedure (0.082% w/w recovery from the bark, Figure 5). Extraction with water can be useful in the treatment of effluent and reduction of solvent costs. These results using ASE expand the potential industrial applications for the direct extraction of paclitaxel from *Taxus* species.

Experimental Section

General Experimental Procedures. Optical rotations were determined with a Horiba SEPA-300 polarimeter. ^1H and ^{13}C NMR spectra were obtained with JEOL JNM-LA400 (400 MHz) NMR spectrometer, using TMS as an internal standard. HPLC was performed with Shimadzu LC-10AD (analytical) and LC-6AD (preparative) pumps, SPD-M10A (analytical) and SPD-10AV (preparative) UV detector, and OTO-10A column oven, using columns prepacked with Senshu Kagaku PEGASIL C_8 (250 × 4.6 mm i.d., analytical; 250 × 20.0 mm i.d., preparative). Column chromatography was carried out with Si gel (Merck, Kieselgel 60, 70−230 mesh).

Plant Material. The bark of a 16-year-old *T. cuspidata* tree cultivated in Monbetsu, Hokkaido, Japan, was sampled in June 1996. The cut ends of the samples were sealed and kept at −30 °C. The sample powder was prepared with a Wiley mill (Retsh, cutting mill SM1) and was sieved to obtain a meal (40−60 mesh). The sample powder was air-dried to give a moisture content of 14%.

Extraction. Solvents for extraction were HPLC grade, except for Et_2O, which was special grade (Wako Chemical Co., Ltd., Osaka, Japan). ASE was conducted with a Dionex Accelerated Solvent Extractor ASE−200.[26−28] Conditions for ASE were as follows: pressure, 1.01−20.27 MPa; temperature,

40−200 °C; extraction time, 5−60 min; volume of cell, 22 mL; amount of sample bark, 5.0 g; and amount of solvent, 44 mL (including the amount for washing: flushing process, 22 mL). Conditions for OSE were as follows: pressure, 0.10 MPa; temperature, 22 °C; extraction time, 48 h (24 h × 2); amount of sample bark, 5.0 g; and amount of solvent, 120 mL. Conditions for boiling-water extraction were as follows: pressure, 0.10 MPa; temperature, 100 °C; extraction time, 60 min (30 min × 2); amount of sample bark, 5.0 g; amount of solvent, 240 mL (120 mL × 2). The extracted solids were obtained by removing solvent *in vacuo*. The H_2O extracts were freeze-dried for 24 h using an Eyela FD-81 freeze-dryer. The recoveries of extracted solids from bark were calculated as percentage values based on oven-dried sample meal. The extraction was repeated three times for each data point, and mean values of quantitative HPLC data were calculated.

Analytical HPLC Method. Solvents for HPLC were HPLC grade (Wako Chemical Co., Ltd., Osaka, Japan). Reference standards were paclitaxel (Taxol) (98% pure), baccatin III (95% pure), and 10-deacetylbaccatin III (10-DAB) (95% pure) (Sigma, St. Louis, MO). The specifications of peaks for paclitaxel, baccatin III, and 10-DAB were set by co-HPLC analysis, using these three compounds as reference standards. The quantitative determination by HPLC was based on the calibration curves prepared previously with reference standards. The recoveries of taxanes from bark were calculated as percentage values based on oven-dried sample meal. The eluent and conditions for analysis were as follows: eluent, CH_3CN−10 mM CH_3COONH_4 (45:55, v/v); flow rate, 1.0 mL/min; detection, UV at 228 nm; column oven temperature, 40 °C. The retention times (min) of different constituents were as follows: 10-DAB (4.7), baccatin III (7.1), and paclitaxel (20.6).

Paclitaxel Isolation with H_2O Extraction Using the Accelerated Solvent Extractor. Air-dried *T. cuspidata* bark (50.0 g) meal was extracted with H_2O by ASE. ASE conditions were as follows: pressure, 10.13 MPa; temperature, 140 °C; extraction time, 15 min. After extraction, the extracts were freeze-dried (9.94 g, 23.12% recovery, based on the oven-dried sample powder), followed by partition with H_2O−EtOAc (× 3). The EtOAc-soluble fraction was obtained by removing solvent *in vacuo* (1.36 g) and chromatographed using Si gel (C_6H_6−EtOAc, 60:40, v/v), and 45 fractions were collected in 50-mL portions. A mixture of eluates (nos. 4−17, 295.7 mg) was introduced into a Sep-Pak C_{18} cartridge and eluted with MeOH, and the eluates were chromatographed using C_8 reversed-phase preparative HPLC (MeOH−CH_3CN−H_2O, 20:35:45, v/v) to afford the crude paclitaxel (13.7 mg). The crude paclitaxel was chromatographed using C_8 reversed-phase preparative HPLC (CH_3CN−H_2O, 44:56, v/v) to afford colorless amorphous paclitaxel (9.3 mg, 0.022% recovery). The conditions for preparative HPLC were as follows: flow rate, 15.0 mL/min; detection, UV at 228 nm; column oven temperature, 40 °C. Paclitaxel: colorless amorphous powder, $[\alpha]^{25}_D$ − 45.2° (*c* 0.47, MeOH). The sample of paclitaxel isolated in this study was shown to be identical to a reference standard from its ^1H and ^{13}C NMR spectra.[30]

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