Adsorption of Taxol into Ordered Mesoporous Silicas with Various Pore Diameters

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Taxol, an anticancer substance, was adsorbed into FSM-type mesoporous silicas with the pore sizes larger than 1.8 nm, while it was not adsorbed into the channels with the pore size less than 1.6 nm, indicating that mesoporous silicas have a molecular sieving property for relatively large molecules. The adsorption behavior of Taxol depended on the solvents; Taxol was not adsorbed into mesoporous silicas when methanol or acetone was used but was adsorbed when dichloromethane or toluene was used. The adsorption behavior of Taxol is discussed on the basis of the solubility parameters of the solvents. The proton-acceptor solubility parameter (δa) is a predominant factor in this liquid−solid partition system. The adsorption−desorption of an extract from yew needles was performed using the mesoporous silica and several elution solvents. In the adsorption, many components including Taxol were adsorbed into mesoporous silica. When the desorption of the extract components adsorbed into the mesoporous silica was performed with a mixture of methanol and water with varied ratios, the targeted molecule, Taxol, with a few addition of components was separated from the adsorbed components. This adsorption−desorption behavior was elucidated by the solubility parameter (δ) as well as δa.

Introduction

Ordered mesoporous silicas have attracted keen interests since the first successful synthesis through the reaction of a layered polysilicate kanemite with surfactants was reported by Yanagisawa et al.[1] After the discovery, various mesoporous silicas such as FSM,[2] M41S,[3] HMS,[4] and SBA[5] have been reported. These materials have high surface areas above 1000 m^2/g and ordered mesopores ranging from 1.5 nm to several tens of nanometers are created by changing the alkyl chain lengths of surfactants, solubilizing auxiliary substances into micelles,[3b] or employing polymeric micelles.[5b] Utilizing these mesopores has led to several reports on the applications to adsorbents, reaction vessels, and catalysts for relatively larger molecules.[6−11] However, as far as we know, there have been no reports on an effective use of variable pore sizes.

One of the characteristic features of this class of materials is the presence of silanol groups in the mesopores which can be silylated[3b,12,13,14] and esterified[15,16] to control the pore size and the surface property. Recently, it has been reported that a mesoporous silica modified with mercaptopropylsilyl groups exhibits a high adsorption property for heavy metals such as mercury.[17,18] The control of inner surface properties induced by such a surface modification is very effective for the design of adsorption media. On the basis of these

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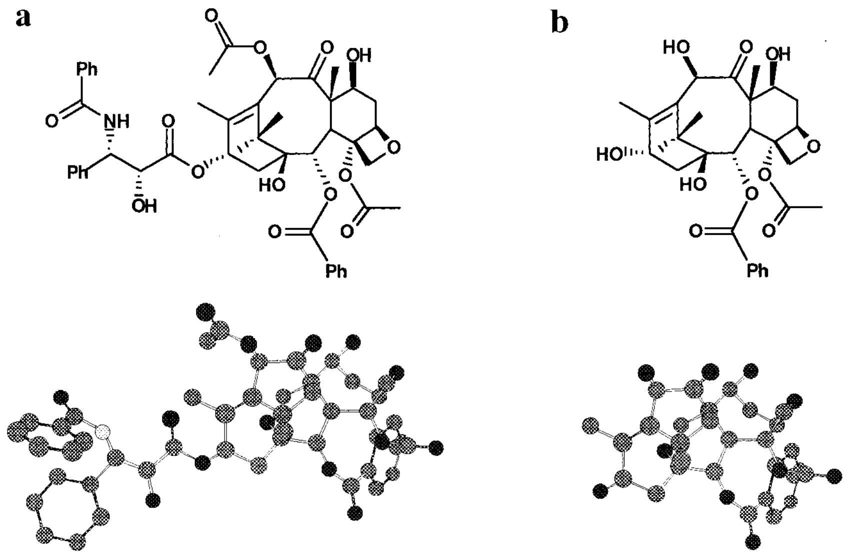

Figure 1. Structures of (a) Taxol and (b) 10-deacetyl baccatin III.

studies, it is very promising that we can develop a novel molecular sieving property and shape selective feature by the use of mesoporous materials whose pore sizes can be tuned to the size of targeted molecules.

Biologically active organic substances are generally difficult to synthesize in simple steps and naturally occurring substances are purified in various ways. These organic substances are normally very large in size and we can anticipate that mesoporous silica would be a potential adsorbent to isolate significant bioactive molecules by utilizing ordered mesopores. Among numerous bioactive molecules studied to date, Taxol has been focused on as one of the most important bioactive molecules in a few decades.

Taxol[19] (Figure 1a, $C_{47}H_{51}NO_{14}$, FW 853.9) was reported to be isolated from the stem bark of the western yew, *Taxus brevifolia* by Wani et al.[20] Horwitz et al. have shown that Taxol can act as an antitumor agent with a different mechanism from those of other known antitumor agents.[21] They have shown that Taxol binds to the microtubules which construct a part of the cytoskeleton. Microtubules are crucial for the dynamic process of cell division. Once Taxol is bound to microtubules, they become stable and static, which results in the prohibition of cell division. Because cancer cells divide more frequently than healthy cells, Taxol primarily attacks the tumors. Therefore, Taxol has attracted much attention as an excellent antitumor agent. The effectiveness of Taxol has been proved specifically for breast cancer and ovarian cancer. Although total syntheses of Taxol have been accomplished,[22-26] the processes are so complex that they do not afford a substantial amount for practical applications at present. Taxol has been found in the needles and twigs of the yew in addition to the stem bark.[27] The yield of Taxol in the bark is very low (1 g of Taxol/10 kg of bark)[28] and the supply was short after the discovery of the effectiveness of Taxol. The separation process employed currently requires many steps, including solvent partition, several column chromatographic separations, and recrystallization.[29] Considering that the process needs a large amount of solvents and takes a long time, the separation of Taxol in a higher yield by a simpler process is tremendously important for future medical development. The method described here would also be profitable for the separation of complex organic molecules and natural products.

In the first part of this study, pure Taxol as the model adsorbate with a relatively large molecular size was adsorbed into FSM-type highly ordered mesoporous silica with various pore sizes. In the second part, the effect of the solvent on the adsorption of Taxol was investigated, because the interactions among adsorbents, adsorbates, and solvents are very important for

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chromatographic separations of Taxol. Finally, adsorption−desorption experiments of an extract from yew needles into the mesopores were performed, and the possibility of the separation of Taxol is discussed.

Experimental Section

Materials. High-purity water glass ($SiO_2 = 21.12\%$, $Na_2O = 6.57\%$, Nippon Chemical Ind. Co.) was used as a silica source. Quaternary ammonium surfactant compounds [$C_nH_{2n+1}(CH_3)_3NCl$, $n = 10-18$ and $C_8H_{17}(CH_3)_3NBr$] were obtained from Tokyo Kasei Co. As the silylating reagent, chlorotrimethylsilane was obtained from Chisso Co. Toluene and pyridine (GR grade) were obtained from Junsei Chemical Co. and distilled before use. Taxol (paclitaxel) is commercially available from Sigma Co. **(Warning: Taxol has a potential cytotoxicity in small amount, so that special care should be taken when handling it.)** The liquids extracted from yew needles were kindly supplied by Osaka Organic Chemicals Co. The solvents used in the adsorption and desorption experiments were dichloromethane, methanol, acetone, and toluene (GR grade), purchased from Junsei Chemical Co. and Kokusan Chemical Co. Acetonitrile (HPLC grade, Tokyo Kasei Co.), ultrapure water, and ammonium acetate (Kanto Kagaku Co.) were used for the elution solvents for high performance liquid chromatography (HPLC) analysis. All chemicals except for toluene and pyridine were used as received.

Synthesis of Mesoporous Silica and Surface Modification. FSM-type mesoporous silicas were typically prepared by the following method. Kanemite was prepared by dispersing 5 g of δ-$Na_2Si_2O_5$ in 100 mL of water, followed by stirring for 30 min. The wet kanemite was dispersed in 100 mL of a 0.1 M quaternary ammonium surfactant solution. After the suspension was stirred at 343 K for 3 h, the pH was adjusted to 8.5 by adding 2 M HCl and the stirring was continued at 343 K for 3 h. The obtained solid product was recovered, washed, air-dried, and calcined at 823 K for 6 h in air. FSM-type mesoporous silicas were designated as C*n*FSM where *n* means the carbon number of the alkyl chains ($n = 8$, 10, 12, 16 and 18). The preparation conditions of C8FSM were slightly different: 70 mL of a 0.14 M surfactant solution was used without varying the Si/N ratio of 0.2 and the reaction time was 3 days.

Trimethylsilylation of C18FSM[14] was conducted as follows: C18FSM was pretreated at 150 °C for 3 h in vacuo to remove adsorbed water. Dried C18FSM (1.0 g) was dispersed in distilled toluene (50 mL), and then chlorotrimethylsilane (10 mL) and distilled pyridine (10 mL) were added to the mixture, and the mixture was refluxed for 24 h with stirring in an N_2 atmosphere. After the reaction, the product (C18FSM-TMS) was washed with chloroform (50 mL), toluene (50 mL), and acetone (50 mL) to remove pyridine hydrochloride, excess pyridine, and residual chlorotrimethylsilane and air-dried at room temperature.

Adsorption of Taxol. The adsorption experiments were performed as follows. C*n*FSM or C18FSM-TMS (0.2 g) was added into 200 mL of a dichloromethane solution of Taxol (Taxol contents of 5 or 12.5 mg), and the mixture was stirred for 3 h in the dark at room temperature. Then, the mixture was centrifuged and the solvent of the supernatant (dichloromethane) was evaporated and was changed to methanol in order to stabilize the Taxol dissolved. The resultant adsorbents were air-dried in the dark. The contents of Taxol that remained in the supernatants were determined by HPLC analysis. In the case of C18FSM, the same adsorption experiments were performed using methanol, acetone, or toluene as the solvent instead of dichloromethane.

Adsorption and Desorption of an Extract Obtained from Yew Needles. A dichloromethane solution containing an extract from yew needles contained 0.0016 wt % of Taxol. C18FSM (0.15 g) was dispersed in the dichloromethane solution (25 mL) and the suspension was stirred for 3 h in the dark at room temperature. Then the suspension was centrifuged and the solvent of the supernatant was changed from dichloromethane to methanol. The resultant C18FSM was air-dried in the dark.

The desorption experiment with methanol or hexane as the elution solvent was performed as follows: C18FSM (0.05 g) which adsorbed a part of the extract components including Taxol was dispersed in the elution solvent (60 mL) and stirred for 3 h in the dark at room temperature. The suspension was centrifuged and the solvent in the supernatant was changed to methanol.

When a water−methanol mixed solvent was used as the elution solvent, repeated desorption procedures were employed. At first, C18FSM (0.05 g) which adsorbed a part of the extract components was dispersed in water (60 mL) and the suspension was stirred for 3 h in the dark at room temperature and centrifuged. The resultant C18FSM was dispersed in 60 mL of a mixture of methanol and water (methanol/$H_2O = 0.54$) and the process described above was repeated. Finally, the resultant C18FSM was again dispersed in 60 mL of methanol and the same process was performed. The supernatant solutions were quantitatively analyzed by HPLC.

Instrumentation. X-ray powder diffraction (XRD) patterns were obtained with a Mac Science MXP[3] diffractometer (monochromated Cu Kα radiation). The nitrogen adsorption−desorption isotherms were measured at 77 K in the relative pressure range from about 10^{-5} to 0.99 on a Belsorp 28SA instrument (Japan Bell Inc.). The pore size distributions were obtained by means of the Horváth−Kawazoe method[30] from the adsorption data. The BET surface areas were determined by using the data under the relative pressures between 0.05 and 0.35 just before the capillary condensation. The pore volumes and the external surface areas were obtained by the *t*-plot method.[31] The water vapor adsorption−desorption isotherms were obtained on a BELSORP 18 instrument (Japan Bell Inc.) at 298 K in the relative pressure range from about 10^{-2} to 0.90. For the characterization of porous materials, they were preheated at 393 K for 3 h under 10^{-2} Torr before the nitrogen and water vapor adsorption measurements. The pretreatment conditions of the samples, which adsorbed Taxol, were changed to 313 K for 6 h under 10^{-2} Torr because Taxol is thermally unstable. These two different pretreatment conditions did not produce any substantial differences in the adsorption isotherms. Infrared spectra were recorded on a Perkin-Elmer FTIR 1640 spectrometer. Solid-state ^{29}Si MAS NMR measurement was performed on a JEOL GSX-400 spectrometer at 79.3 MHz using 45° pulses at 60 s intervals. The HPLC system consisted of HP-LC1900C (Hewlett-Packard Co.), and the column was packed with Pegasil C-8 (Sensyuu Kagaku Co.) with the length and the diameter of the column being 250 and 4.6 mm, respectively. The peak at 225 nm in a UV detector was used for the quantitative analysis of Taxol. The mobile phase for elution was $CH_3CN/10\ mM\ CH_3COONH_4(aq) = 45/55$ at a flow rate of 0.6 mL/min. The column temperature was set at 313 K and the injection volume of samples was 20 μL throughout the analysis.

Results

1. Characterization of Mesoporous Silicas. The powder XRD analysis of the C*n*FSMs showed diffraction peaks in lower 2θ angles due to a hexagonal structure after calcination. The lattice constants ($a = 2d_{100}/\sqrt{3}$) are given in Table 1. The surface areas, the pore volumes, and the pore diameters are also shown in Table 1. The pore diameters of C*n*FSM varied from <1.6

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Table 1. Lattice Constant, Surface Area, Pore Volume and Pore Diameter for CnFSMs and C18FSM-TMS

sample	a_0 (nm)[a]	S_{BET} (m^2/g)[b]	S_{out} (m^2/g)[c]	S_p/S_{BET}[d]	V_p (mL/g)[e]	W (nm)[f]
C18FSM	4.8	978	206	0.79	0.68	3.6
C16FSM	4.3	1015	82	0.92	0.68	3.1
C12FSM	3.4	1127	133	0.88	0.52	2.0
C10FSM	3.3	985	84	0.91	0.38	1.8
C8FSM	3.1	661	100	0.85	0.22	<1.6
C18FSM-TMS	4.8	697	159	0.77	0.40	3.0

[a] a_0 = lattice constant (distance between pore centers). [b] S_{BET} = BET surface area. [c] S_{out} = outer surface area. [d] S_p = pore surface area ($S_{BET} - S_{out}$). [e] V_p = pore volume. [f] W = pore diameter determined by the Horváth–Kawazoe method.

to 3.6 nm with the chain lengths of surfactants used. Trimethylsilylation of the surface of C18FSM was confirmed by ^{29}Si MAS NMR and FT-IR. The surface area, the pore volume, and the pore diameter for C18FSM-TMS are also shown in Table 1. The water vapor adsorption measurement showed that the adsorbed amount of water was drastically decreased after trimethylsilylation, so that the enhancement of the hydrophobicity of the surface was verified. On the basis of elemental (C, H, N) analysis, the number of trimethylsilyl groups per unit surface area was 1.86 groups/nm^2. It is reported that the cross sectional area of a trimethylsilyl group is 0.43 nm^2.[32] Therefore, the surface coverage by trimethylsilyl groups was estimated as ca. 80%. CnFSMs and C18FSM-TMS were used as the adsorbents in the Taxol adsorption experiments.

2. Adsorption of Taxol into Mesoporous Silicas with Various Pore Sizes. Two Taxol solutions with different concentrations were used: 5 mg of Taxol/200 mL of CH_2Cl_2 for 0.2 g of FSM (that is 25 mg/L for 1 g of FSM) and 12.5 mg of Taxol/200 mL of CH_2Cl_2 for 0.2 g of FSM (62.5 mg/L for 1 g of FSM). Figures 2 and 3 show the chromatograms of Taxol solutions (in methanol) and the supernatant solutions after adsorption in two different concentrations of Taxol. The chromatograms of the Taxol solutions clearly show the peak due to Taxol at a retention time of 38 min (Figures 2a and 3a). Table 2 lists the pore volumes of CnFSMs after adsorption and the amounts of adsorbed Taxol which were calculated by the difference in the peak areas of Taxol before and after adsorption.

C18FSM as the Adsorbent. When C18FSM was used as the adsorbent, the peak at the retention time of 38 min due to Taxol disappeared completely after the adsorption in the lower concentration system (5.0 mg of Taxol/200 mL of CH_2Cl_2 for 0.2 g of FSM, Figure 2b). Even when the higher concentration system (12.5 mg of Taxol/200 mL of CH_2Cl_2 for 0.2 g of FSM) was used, only a very small peak was observed (Figure 3b). The amounts of adsorbed Taxol into 1 g of C18FSM increased from 25.0 to 60.7 mg with the concentration of Taxol (Table 2). The adsorption isotherms of the C18FSM also changed after the adsorption (Figure 4a). The amount of nitrogen adsorbed in the range of capillary condensation decreased with the increase in the amount of adsorbed Taxol. The pore volume decreased from 0.68 to 0.61 mL/g and further decreased to 0.54 mL/g, depending on the adsorbed amount of Taxol (Table 2).

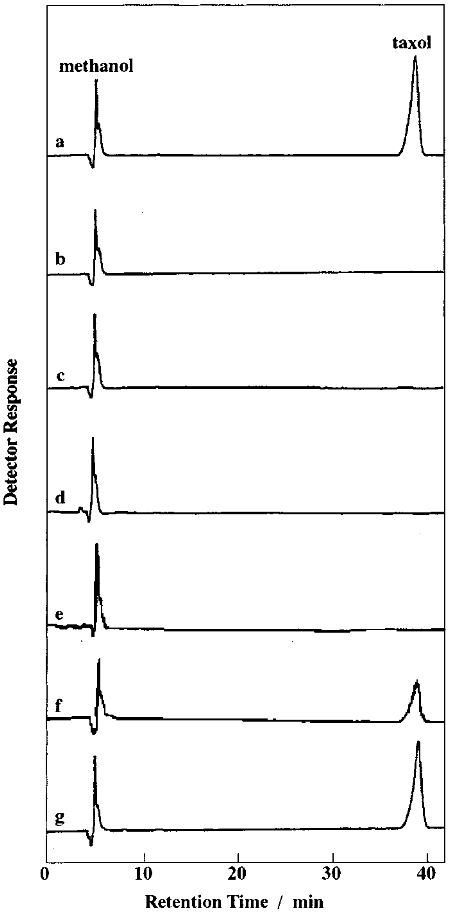

Figure 2. Chromatograms of (a) Taxol in methanol (5.0 mg/200 mL) and supernatants after the adsorption with (b) C18FSM, (c) C16FSM, (d) C12FSM, (e) C10FSM, (f) C8FSM, and (g) C18FSM-TMS.

This finding strongly suggests that Taxol was successfully introduced into the mesopores of C18FSM. However, there were little changes in the pore diameter of the two C18FSMs with different Taxol loadings, being slightly decreased from 3.6 to 3.4 nm, which will be discussed later.

C16FSM and C12FSM as the Adsorbents. When C16FSM and C12FSM were used as the adsorbents, Taxol was not detected in the supernatant solutions in the lower concentration system (Figures 2c and 2d). The pore volumes of C16FSM and C12FSM also decreased from 0.68 to 0.59 mL/g and from 0.52 to 0.44 mL/g after the Taxol adsorption, respectively (Table 2). Accordingly, Taxol was adsorbed into the mesopores of C16FSM and C12FSM as in the case of C18FSM.

C10FSM as the Adsorbent. When C10FSM was used as the adsorbent, there was no peak due to Taxol in the chromatogram of the supernatant in the lower concentration system of Taxol/CH_2Cl_2 = 5.0 mg/200 mL (Figure 2e). In the higher concentration system of Taxol/CH_2Cl_2 = 12.5 mg/200 mL, a small peak due to Taxol was observed (Figure 3c). The amount of Taxol adsorbed per 1 g of C10FSM increased from 25.0 to 57.8 mg with the concentration of Taxol (Table 2), although the amount in the higher concentration system is somewhat lower in this case than in the case of C18FSM (60.7 mg). The nitrogen adsorption isotherms of the samples before and after the adsorption of Taxol are shown in Figure 4b. With the increase in the amount of adsorbed Taxol,

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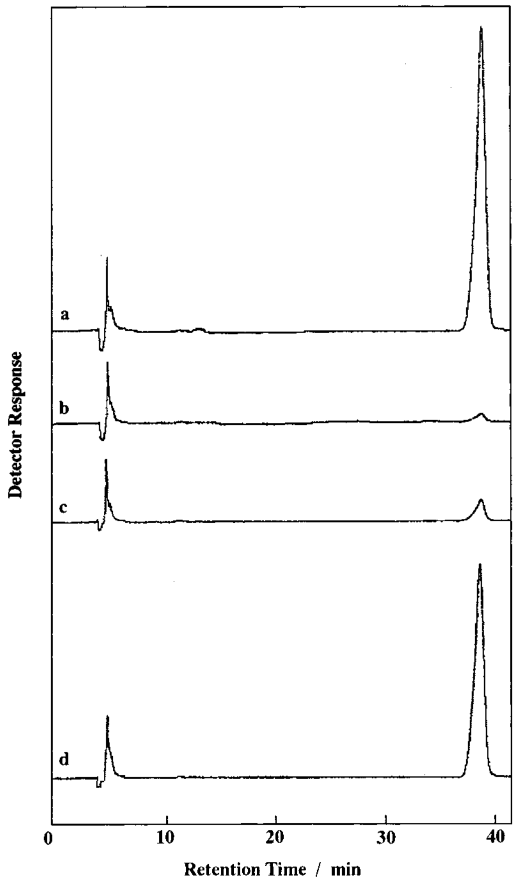

Figure 3. Chromatograms of (a) Taxol in methanol (12.5 mg/200 mL) and supernatants after the adsorption with (b) C18FSM, (c) C10FSM, and (d) C8FSM.

Table 2. Amount of Taxol Adsorbed onto CnFSMs and Pore Volume of C*n*FSMs after Adsorption

	adsorbed amount per 1 g of FSM (mg) for different Taxol/CH_2Cl_2		pore volume (mL/g) for different Taxol/CH_2Cl_2	
adsorbent	25.0 mg/L	62.5 mg/L	25.0 mg/L	62.5 mg/L
C18FSM	25.0	60.7	0.61	0.54
C16FSM	25.0		0.59	
C12FSM	25.0		0.44	
C10FSM	25.0	57.8	0.34	0.28
C8FSM	16.2	21.1	0.16	0.16

the amount of nitrogen adsorbed into the pores decreased. The pore volume decreased from 0.38 to 0.34 mL/g and further decreased to 0.28 mL/g (Table 2). These findings tell us that Taxol was adsorbed in the pores.

C8FSM as the Adsorbent. On the other hand, when C8FSM was used as the adsorbent, a part of Taxol remained in the supernatant in the lower concentration system of Taxol/CH_2Cl_2 = 5.0 mg/200 mL (Figure 2f). In the higher concentration system, the amount of adsorbed Taxol did not change so much irrespective of the concentration of Taxol (Figure 3d and Table 2). The nitrogen adsorption isotherms also displayed that the isotherms hardly changed between the two samples after the adsorption at different concentrations (Figure 4c). The pore volume decreased from 0.22 to 0.16 mL/g for the lower concentration system but did not decrease further (Table 2). These results indicate that there is a large distinction between C10FSM and C8FSM in the adsorption of Taxol.

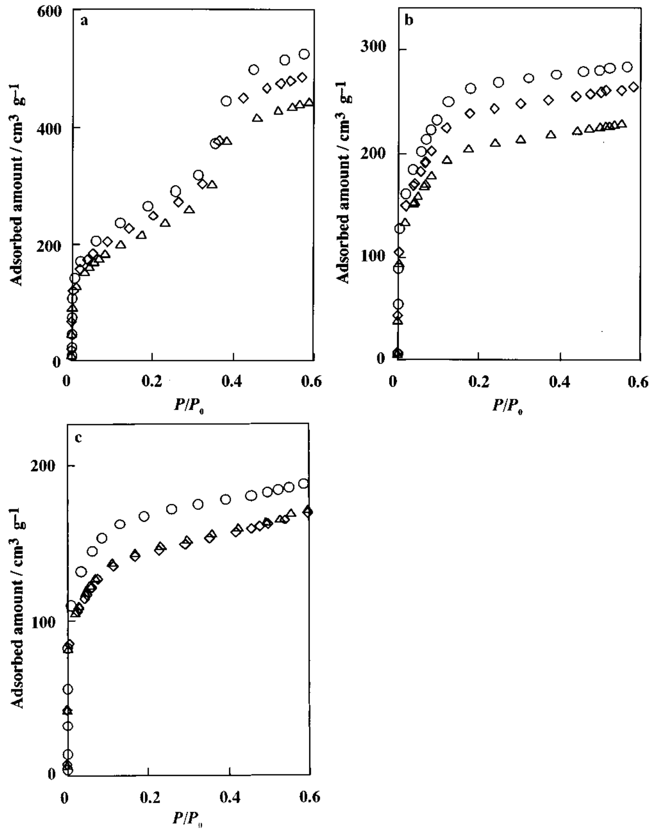

Figure 4. Nitrogen adsorption isotherms for (a) C18FSM, (b) C10FSM, and (c) C8FSM before and after adsorption (Taxol/CH_2Cl_2 = 5.0 mg/200 mL and 12.5 mg/200 mL). (○, before adsorption experiment; ◇, Taxol/CH_2Cl_2 = 5.0 mg/200 mL; △, Taxol/CH_2Cl_2 = 12.5 mg/200 mL).

C18FSM-TMS as the Adsorbent. Trimethylsilylated C18FSM (C18FSM-TMS) was also used as the adsorbent for further investigation of the adsorption property of FSM-type mesoporous silica. As shown in Table 1, the pore size of C18FSM-TMS was 3.0 nm, being large enough to adsorb Taxol molecules into the pores. By using a dichloromethane solution, however, almost all Taxol remained in the supernatant and Taxol was not adsorbed on C18FSM-TMS, indicating that the modification by trimethylsilylation largely affects the adsorption behavior of Taxol.

3. Adsorption of Taxol into C18FSM in Various Solvents. The adsorption into C18FSM was conducted in other solvents with different affinities for Taxol in place of dichloromethane. The adsorption was carried out by using methanol, toluene, or acetone in a manner similar to the case of the lower concentration system in dichloromethane. When toluene was used (Figure 5c), Taxol was adsorbed on C18FSM at the same level as in a dichloromethane solution (Figure 5b). On the other hand, when a methanol or acetone solution was used, Taxol was not adsorbed on C18FSM (Figure 5d,e). The adsorption behavior differed with the kind of solvent.

4. Adsorption and Desorption of an Extract from Yew Needles into Mesoporous Silica. Adsorption of the Extract into C18FSM. We tried to separate Taxol from an extract containing many components besides Taxol by utilizing adsorption−desorption processes of C18FSM. In the chromatogram of the extract, very many peaks appeared in addition to Taxol at 38

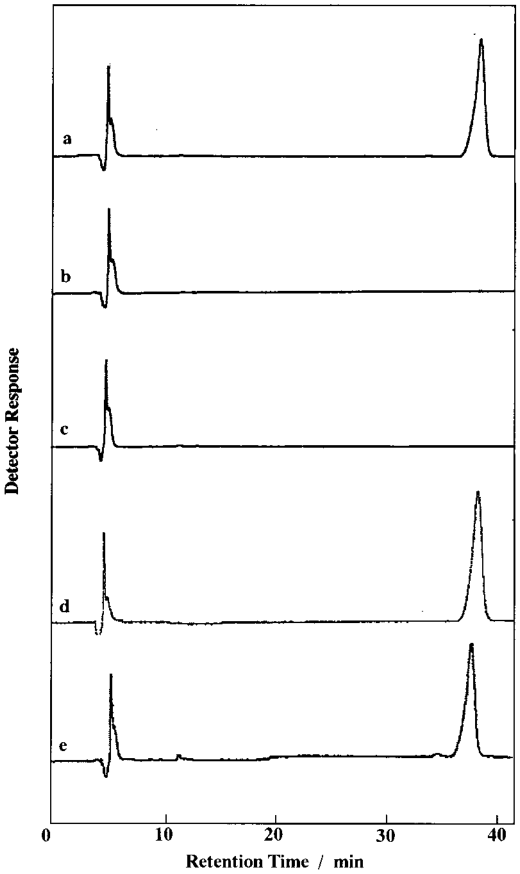

Figure 5. Chromatograms of (a) Taxol in methanol (5.0 mg/200 mL) and supernatants after the adsorption experiments with (b) dichloromethane, (c) toluene, (d) methanol, and (e) acetone.

min (Figure 6a). The peak due to Taxol was confirmed by a comparison with that of pure Taxol.

The chromatogram of the supernatant after the adsorption is shown in Figure 6b. The peaks due to components in the extract appearing longer than 13 min largely diminished, although the peaks before 13 min also seem to have diminished. The nitrogen adsorption of C18FSM containing the components from the extract exhibited that the pore volume decreased from 0.68 to 0.36 mL/g and that the pore diameter decreased from 3.6 to 2.8 nm. Consequently, many components including Taxol were adsorbed into C18FSM, and the adsorbed components are supposed to be located in the mesopores. Therefore, many components were adsorbed into C18FSM simultaneously, and no molecular sieving effect was confirmed. When C8FSM was used instead of C18FSM, almost all the extract components remained in the supernatant after adsorption (Figure 6c), so few components were adsorbed into C8FSM, and a molecular sieving effect was not observed.

Desorption of the Extract Components Adsorbed into Mesoporous Silica C18FSM. On the basis of the fact that many components including Taxol were simultaneously adsorbed into C18FSM, desorption using various solvents and the separation of those components were conducted. The supernatants after the desorption were analyzed by HPLC; the chromatograms are shown in Figure 7. A wide range of components including Taxol were desorbed when methanol was used as the solvent (Figure 7a). On the other hand, no peaks in the chromatogram were observed when hexane was used as the solvent. Accordingly, this procedure is not so effective for the selective desorption of Taxol or selective binding in the mesopores.

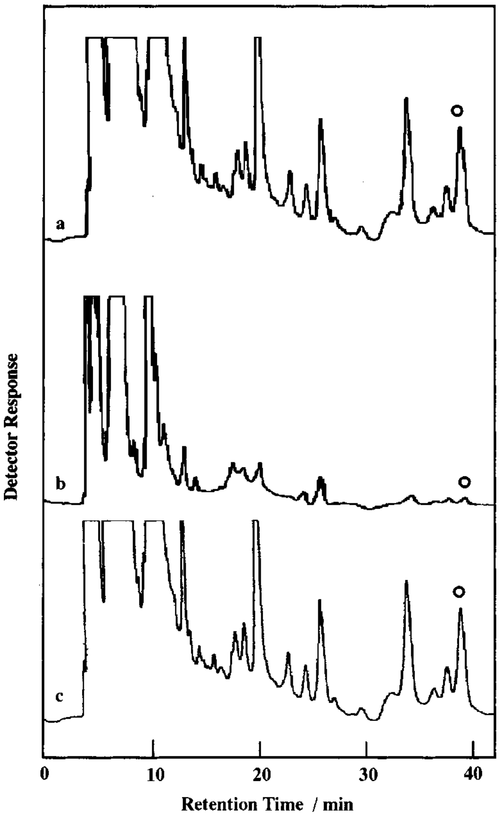

Figure 6. Chromatograms of (a) the extract from the needles of yews in methanol (Taxol content, 0.0016 wt %), (b) supernatant after adsorption with C18FSM, and (c) supernatant after adsorption with C8FSM. (○, Taxol).

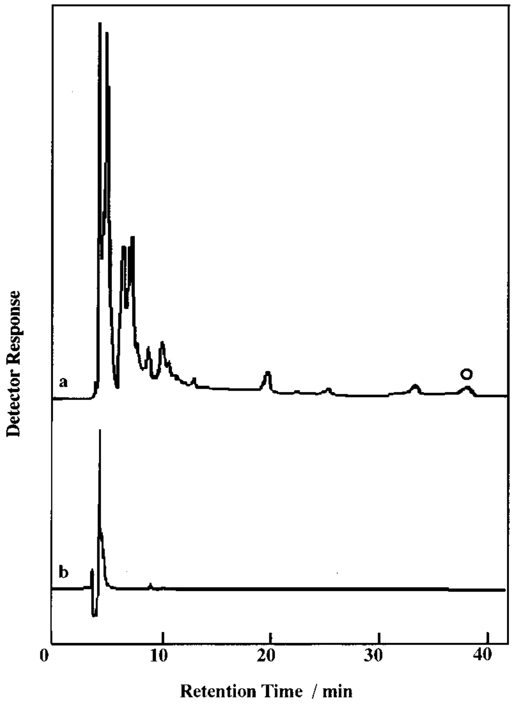

Figure 7. Chromatograms of supernatants after desorption with (a) methanol and (b) hexane. (○, Taxol).

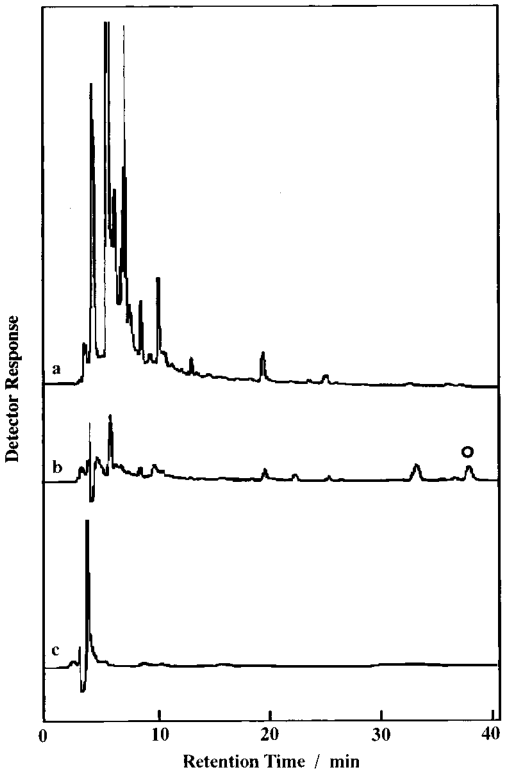

Figure 8. Chromatograms of supernatants after desorption with (a) water, (b) methanol/H_2O = 0.54, and (c) methanol. (○, Taxol).

Because Taxol is not soluble in water, mixed solvents of water−methanol with varied proportions were used; the chromatograms of the supernatants after desorption are shown in Figure 8. When water was used as the first solvent, relatively polar components up to the retention time of 13 min were mainly desorbed, and the peak due to Taxol was not observed (Figure 8a). When a mixed solvent (methanol/H_2O = 0.54) was used as the second step, the components up to 13 min were detected in a much lesser degree, and the peak due to Taxol appeared at 38 min, indicating desorption of Taxol (Figure 8b). Thus, almost all the components up to 13 min were desorbed in the first treatment with water. When methanol was used as the solvent as the final step, there were few peaks except the solvent. Consequently, Taxol was able to be separated to some extent by gradual desorption of the extract adsorbed into the mesopores, although the separation of Taxol as a single component was not successful.

Discussion

1. Adsorption of Taxol into Mesoporous Silicas with Different Pore Sizes. The results of HPLC analysis and all the nitrogen adsorption data clearly indicate that Taxol was favorably adsorbed into pores of C18-, C16-, C12- and C10FSMs. On the other hand, the adsorption behavior of Taxol drastically changed between C10FSM and C8FSM. The variation in the adsorption behavior of Taxol arising from the difference of the pore sizes (<1.6−3.6 nm) of FSM-type mesoporous silica is discussed here.

The mesoporous silica with largest pore size used in this study is C18FSM, whose pore size is 3.6 nm. Considering that the molecular size of Taxol is estimated to be ca. 1.0 × 1.5 × 2.0 nm on the basis of a space filling model,[33] the pore size of C18FSM is large enough for Taxol molecules to enter into the pores. However, the decrease in the pore size (from 3.6 to 3.4 nm) in both of the experiments with different concentrations was low. It seems that such a decrease would be too small if the size of Taxol is taken into consideration. The occupied density of Taxol in mesopores is estimated to be at most very low, 0.023 molecules/nm^2 in the lower concentration system and 0.055 molecules/nm^2 in the higher concentration system. The pore volume decreased by 0.07 and 0.14 mL/g in both of the adsorption systems. Therefore, Taxol molecules are not distributed so densely, which affords ample space where Taxol molecules are not located, corresponding to the lower degree of decrease in the pore diameter of C18FSM after adsorption of Taxol.

The adsorption of an extract from yew needles into C18FSM resulted in the adsorption of many components, which induces a larger decrease in the pore volume from 0.68 to 0.36 mL/g and the pore diameter decreased from 3.6 to 2.8 nm. This finding clearly indicates that many components were introduced into the mesopores. On top of that, Taxol adsorption does not reach to the saturation state when the higher concentration of a Taxol solution was used, and it should be noted that more Taxol molecules should be adsorbed into the pores of C18FSM.

The possibility of adsorption of Taxol onto the outer surface should be considered. The contribution of inner pores and outer surface to the adsorption in the lower concentration system (5.0 mg of Taxol/200 mL CH_2Cl_2) is discussed. The ratio of Taxol adsorbed into the inner pores is estimated by eq 1

$$\mathrm{Tax}_{\mathrm{inner}}/\mathrm{Tax}_{\mathrm{total}} = \Delta V_{\mathrm{p}}/V_{\mathrm{taxtotal}} \tag{1}$$

where $\mathrm{Tax}_{\mathrm{inner}}$ is the amount of Taxol adsorbed into inner pores, $\mathrm{Tax}_{\mathrm{total}}$ is the total amount of adsorbed Taxol, ΔV_{p} is the amount of the decrease of the pore volume after adsorption, and V_{taxtotal} is the total volume of adsorbed Taxol.[34] The equation is derived from the assumption that the decrease in the pore volume comes from the occupancy of Taxol molecules in the inner pores. Because the inner surface areas can be calculated by the nitrogen adsorption data, the ratio of outer surface to inner pores is calculated.

In the case of C18FSM, $\mathrm{Tax}_{\mathrm{inner}}/\mathrm{Tax}_{\mathrm{total}}$ is calculated to be 0.78, which nearly agreed with the ratio of inner surface area to total BET surface area ($S_{\mathrm{p}}/S_{\mathrm{BET}} = 0.79$ shown in Table 1). Therefore, the adsorption of Taxol into inner mesopores and onto external surfaces uniformly and spontaneously occurred. Accordingly, in the adsorption process of Taxol into C18FSM, the influence of diffusion of Taxol, which is possibly restricted by the pore structure, seems to be negligible. The coincidence of the values of $\mathrm{Tax}_{\mathrm{inner}}/\mathrm{Tax}_{\mathrm{total}}$ and $S_{\mathrm{p}}/S_{\mathrm{BET}}$ is also observed in both C16FSM and C12FSM, indicating that the diffusion of Taxol is not restricted into the pores of C16- and C12FSMs.

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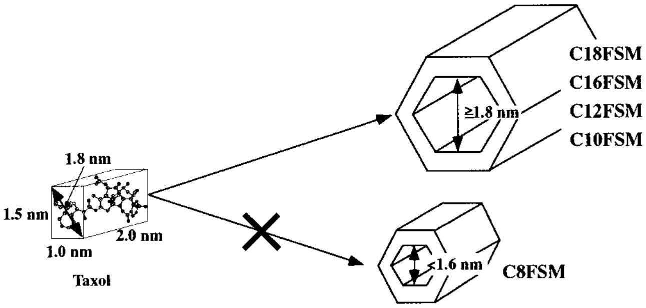
(33) The size of Taxol (including hydrogen atoms) was estimated by considering the van der Waals radii, and the model was energy-minimized.

(34) V_{taxtotal} was estimated to be 5.1 nm^3 by regarding the Taxol molecule as a cylinder (radius, 0.9 nm; length, 2.0 nm).

Scheme 1. Image of the Adsorption with C18-, C16-, C12-, C10- and C8FSMs



When C10FSM was used, the amount of Taxol in the higher concentration system was somewhat lowered as compared with C18FSM. Additionally, the value of $\mathrm{Tax_{inner}}/\mathrm{Tax_{total}}$ (0.44) is not consistent with the value of S_p/S_{BET} (0.91), which is fairly different than those of C18-, C16- and C12FSMs. This result indicates that the further decrease in the pore size tends to restrain the diffusion of Taxol molecules into the pores; that is, the direction of Taxol molecules on adsorption into the narrower pores (1.8 nm) of C10FSM is largely restricted.

In the case of C8FSM, Taxol molecules were not adsorbed into the pores. Because the size of Taxol is larger than the pore size of C8FSM, Taxol molecules cannot enter into the pores, suggesting that Taxol molecules were partly adsorbed onto the external surface. The pore volume of C8FSM decreased further than that of C10FSM, suggesting that the molecules covered the entrance of the pores, judging from the lesser amount of Taxol and the preference of adsorption onto the outer surface in the case of C8FSM.

All these results on adsorption of Taxol utilizing mesoporous silicas of C18-, C16-, C12- and C10FSM with various pore sizes confirmed that Taxol molecules were able to be adsorbed into the pores. On the contrary, Taxol was not adsorbed into the pores of C8FSM because the pore size is too small for Taxol molecules to enter (Scheme 1). Consequently, the adsorption behavior of Taxol changed drastically between the two slightly different pore sizes of C10FSM and C8FSM, verifying that mesoporous silica has a sort of molecular sieving property due to its various pore diameters.

The adsorption of 10-deacetyl baccatin III (Figure 1b, $C_{29}H_{36}O_{10}$, FW 544.6) molecules into C10FSM and C8FSM was also conducted in a manner similar to the lower concentration system of Taxol adsorption. 10-Deacetyl baccatin III, which is smaller in size than Taxol, has a taxane ring, a main structural component of Taxol, and one of the key substances for semisynthesis of Taxol.[35] The HPLC analysis of the supernatant solution after the adsorption by C10FSM showed a complete adsorption of 10-deacetyl baccatin III into C10FSM. The attempt by C8FSM resulted in partial adsorption by 45%. As in the case of Taxol, 10-deacetyl baccatin III could not be adsorbed into the pores of C8FSM. If compared with the cases of Taxol, the number of 10-deacetyl baccatin III molecules adsorbed onto C8FSM are roughly equal to that of Taxol molecules, supporting strongly the adsorption of Taxol onto the outer surface. These facts also imply that mesoporous silica with well-regulated pores are quite effective as a reaction vessel with confined space.

Table 3. Solvent Properties[36]

solvent	δ^a	δd^b	δo^c	δa^d	δh^e
hexane	7.3	7.3	0	0	0
toluene	8.9	8.9	0	0.5	0
acetone	9.4	6.8	5	2.5	0
dichloromethane	9.6	6.4	5.5	0.5	0
methanol	12.9	6.2	5	7.5	7.5
water	21	6.3	large	large	large

[a] δ = Solubility parameter (calculated from the boiling point). [b] δd = Dispersion solubility parameter. [c] δo = Orientation solubility parameter. [d] δa = Proton-acceptor solubility parameter. [e] δh = Proton-donor solubility parameter.

2. The Effect of Solvents on the Adsorption of Taxol. The kind of solvents for the adsorption of Taxol has affected remarkably, as described in the Results; Taxol was adsorbed into mesopores in dichloromethane or toluene solution, whereas Taxol was not adsorbed in methanol or acetone solution. To understand the different behavior, the solubility parameter (δ) of the solvents,[36] which denotes the polarities of solvents, can be introduced in this discussion. Generally, higher solubility parameters mean higher polarity of solvents. On the basis of the intermolecular interactions, the δ parameters are grouped into several parameters such as δd for dispersion interactions, δo for dipole interactions, and δa and δh for hydrogen-bonding interactions. These parameters are good indices to reveal the solubility of solvents for specific substances. The parameters of the solvents used in this study are listed in Table 3.

The proton-acceptor solubility parameter (δa) values of dichloromethane and toluene are 0.5, which are much lower than those of other solvents ($\delta a = 7.5$ for methanol and $\delta a = 2.5$ for acetone), which is the most critical point to distinguish the different adsorption behavior. The value of δa indicates the degree of dissolving alcohol, phenol, and carboxylic acid. Taxol has both hydroxyl groups for possible hydrogen bonding and carbonyl groups as possible acceptors for hydrogen bonding. Therefore it has a good affinity with solvents having high solubility parameters of proton acceptors (δa). In other words, the affinity of Taxol and the solvents with lower δa values is low and the interaction (hydrogen bonding) between Taxol with silanol groups in FSM-type mesoporous silicas is relatively stronger, which results in adsorption of Taxol into the pores of the mesoporous silicas. When Taxol-containing mesoporous silica was dispersed into methanol, Taxol was fully eluted, indicating that full desorption of Taxol from the mesopores is facile by using a solvent with high δa value. Mutual affinities among Taxol, solvent, and FSM-type mesoporous silica substantially influence the present adsorption system.

The fact that Taxol was not adsorbed on C18FSM-TMS can be ascribed to the reduced affinity between Taxol and the trimethylsilyl groups in C18FSM-TMS. When hexane ($\delta a = 0$) was used as a desorbing solvent of Taxol adsorbed into C18FSM, Taxol was not eluted. This finding also indicates that there is no affinity between Taxol and hexane. The inner surface of C18FSM

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(35) Denis, J. N.; Greene, A. E.; Guénard, D.; Guéritte-Voegelein, F.; Mangatal, L.; Potier, P. *J. Am. Chem. Soc.* **1988**, *110*, 5917−5919.

(36) Kirkland, J. J. *Modern Practice of Liquid Chromatography*; Wiley-Interscience: New York, 1971; Chapter 4.

became more hydrophobic after trimethylsilylation, which resulted in a very low adsorption of Taxol into these modified mesopores.

The adsorption behavior of Taxol is qualitatively elucidated by considering the differences of the affinities between solvents and the adsorbents toward Taxol molecules, which is related to the solubility parameters of the solvents.

3. Adsorption and Desorption of an Extract from Yew Needles. The results of the adsorption and desorption of an extract from yew needles showed that separation of Taxol as a single component was not achieved. One of the reasons may come from the large pore diameter of C18FSM, which accommodates other components. The outer surface can also contribute to the adsorption, which is not related to the function as a molecular sieving property. When C8FSM was used as an adsorbent, few extract components were adsorbed, indicating that almost all the molecules present in a yew extract solution were relatively large. Therefore, the use of mesoporous silicas with narrower pore sizes is not suitable for the first step to remove a large fraction of the components of the yew needle extract.

In the desorption process of the extract components adsorbed into C18FSM, almost all components were eluted when methanol was used as the elution solvent and the components were hardly detected in the chromatogram when hexane was used as the solvent. This finding can be explained by the solubilities of the extract components to the solvents. The separation processes using mixed methanol−water solvents exhibit a promising result; the first step of the process using water and the following second step using a mixed solvent (methanol/H_2O = 0.54) resulted in favorable separation of Taxol with additional limited components. The solubility parameters should be efficient for the understanding of the elution behavior.

The HPLC column modified with C8 groups was employed as a reversed-phase chromatography in this study. Polar components are eluted at earlier retention times. Accordingly, the polarity of Taxol is not so high because it elutes at 38 min. Taxol does not dissolve in water with very high δ value (21.0). It has been known that the solubility parameters of mixed solvents vary linearly with the mixing ratio.[36] The δa values of mixed solvents of methanol and water should be high because the values of both of the solvents are very high, so the δa values are not so sufficient for the understanding of desorption behavior. In place of δa values, the δ values themselves are more suitable for the discussion. Because the δ values of methanol and water are 12.9 and 21.0, respectively, the variation of the mixed solvents should vary from 12.9 to 21.0, depending on the ratio. By changing the ratio of methanol/H_2O (from 0 to 0.54) the δ value can be decreased gradually from 21.0 to 18.2, and Taxol can be eluted by the increasing affinity with the mixed solvent. With such a variation upon changing the ratio of the mixed solvents, we can narrow down to very limited components including Taxol from a broad range of components in the extract. Therefore, by combining various solvents with different parameters, the separation of Taxol from an extract can be optimized.

The separation of Taxol from yew needles takes many steps until now: (1) extraction of soluble components in yew needles, (2) solvent partition using water and dichloromethane (or chloroform), (3) column chromatographic separation of Taxol from the substances dissolved in the organic phase, and (4) recrystallization of Taxol for higher purity.[29] In these consecutive processes, the solvent partition requires the organic solvent and water in large quantities (ca. 35 dm^3 each for 1 kg of the components extracted in methanol). Additionally, it takes several tens of hours to obtain clear phase separation from an emulsion state during solvent partition. Nevertheless, the substances dissolved in the organic phase, being charged to chromatographic separation, have many components including Taxol, in a pattern similar to Figure 6a. (The ratio of Taxol is only 0.78%, based on the peak areas of HPLC.) Consequently, the chromatographic separation is very complex, and the amounts of the solvents used in these processes are large.

On the other hand, the separation process presented here is superior to the above processes, though the first extraction process is the same. Compared with the former solvent partition processes, it takes only several hours to conduct the adsorption and desorption operations. The amounts of dichloromethane, for example, can be reduced to 10−20 dm^3 in the adsorption procedure for 1 kg of the components extracted in methanol, and the solvent is reusable. In the desorption process, the amount of methanol can also be reduced to 15 dm^3, which indicates that the total amount of the solvents is also reduced in comparison with that in the former processes, including solvent partition process.

The most noteworthy point is that Taxol is able to be concentrated with a limited number of the other components from the extract (from 0.78% in the above process to 10.8% in the present one), as shown in Figure 8b. This finding remarkably simplifies the process of subsequent column chromatographic separation and accordingly reduces the amount of the solvents used as a mobile phase substantially. Therefore, the process using ordered mesoporous silica and controlling the solubility parameters of solvents is considered to be a quite effective pretreatment for the purification of Taxol in place of the usual solvent partition.

Conclusions

Taxol was adsorbed into ordered mesoporous silica with different pore diameters, and a very effective molecular sieving effect was confirmed for the first time. The affinity of Taxol for solvents and/or the adsorbent is critical in the adsorption, which is elucidated by the solubility parameters of the solvents. The solubility parameters are a very efficient indication for designing practical processes (selection of solvent, mode of surface modification, and so on) for the adsorption of various organic substances with mesoporous silicas. The possible isolation of Taxol with a few additional components from yew was also done by utilizing these mesoporous silicas. In the present adsorption−desorption process of the yew extract, it would be interesting to use other mesoporous silicas with three-dimensional channel systems instead of two-dimensional hexagonal mesoporous silicas because the diffusion behavior of the extract should be changed.

The results obtained here also implicate the potential application of the mesoporous silica as a new synthetic vessel in the process of organic semisynthesis or total synthesis of Taxol. It has been pointed out that one of the severe difficulties for total synthesis of Taxol is to control both the positions of various substituents and stereochemistry. The present study clarifies that Taxol and its precursor 10-deacetyl baccatin III can reside in the mesopores, depending on the solvents. Since a regiospecific synthesis in mesoporous silicas has recently been reported,[11] the shape selectivity of the products can be expected when using mesoporous silica as a reaction vessel, which would lead higher yield and simpler process in the Taxol synthesis, and this method can be extended to natural products chemistry.

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