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SEPARATION AND PURIFICATION OF TAXOL AND CEPHALOMANNINE FROM *Taxus Cuspidada* BY NORMAL PHASE CHROMATOGRAPHY AND TWICE-REVERSED-PHASE CHROMATOGRAPHY

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Taxol is a kind of terpene compounds, which was separated from *Taxus brevifolia* by Wani et al. [1] originally. Because of the special anticancer mechanics and special curative effect for some kinds of tumor, taxol has attracted much attention recently. At present, an effective method used to obtain taxol is extraction and separation frorm taxus. It is very difficult to separate and purify taxol from taxus, because the content of the taxol in taxus is very low, and there are also some analogues of taxol in taxus, such as cephalomannine, etc. The structures of taxol and cephalomannine are very similar, the difference between taxol and cephalomannine being the functional group at C-13 only, so the separation and purification of taxol and cephalomannine are very difficult. Although there are some references on separating and purifying taxol and cephalomannine from taxus, most of them are complicated and expensive. Normal phase chromatography is a common method, but it has some disadvantages, such as low separation efficiency and low recovery of taxol. Preparative high performance liquid chromatography has been used to separate taxol and cephalomannine from taxus, but the throughput is very little and the cost is too high to use on a large scale. Ozone [2] was used to treat cephalomannine, which can be separated from taxol, but this method is complicated.

In this paper, a novel high-molecular resin (PRP-6) developed by Kailu Liu et al. [3] was applied to reversed- phase preparative chromatography for extracting taxol and its analogues from taxus. This method can avoid the break and wastage of taxol and cephalomannine using an oxidation process, and improve the recovery. Because the retention values of taxol and 7-epi-taxol are very close in reversed-phase chromatography, the elimination of 7-epi-taxol from the digestion products of taxus is very difficult. In this work, normal phase chromatography was used to eliminate the pigments, 7-epi-taxol, grease impurities etc. from the digestion products of taxus first, then the selectivity of PRP-6 resin was utilized to separate and purify taxol and cephalomannine by twice-reversed-phase chromatography. Under the selected experimental conditions, the taxol and cephalomannine separated and purified from *Taxus cuspidada*, which only contained 0.003% of taxol, were determined by HPLC, ^{1}H-NMR, and ^{13}C-NMR, and the determination results were satisfactory.

Procedure of Separation and Purification. The dry leaves of *Taxus cuspidada* from China were smashed and sifted; then 7.5 kg of them was digested three times with methanol at room temperature, and the digestion solutions were concentrated using reduced pressure distillation. The concentrated solutions were extracted by methylene chloride–H_2O (1:1, v/v) solution, and the crude products were obtained after the methylene chloride in the soak liquor was evaporated to dry. The crude products were added to a silica gel column (5.0×100 cm, 65 cm high), the acetone-hexane solutions with different acetone volume percentage (0, 25, 30, 40, 50 and 60%) were eluted in turn, and the eluates collected were passed through a Shimadzu LC-10AT high-performance liquid chromatograph. The eluates containing taxol and cephalomannine were merged and concentrated. Then the concentrates were added to a reversed-phase chromatographic column (2.86×150 cm) packed with a novel high-molecular resin PRP-6 (polystyrene-diethenoid benzene porous particulates synthesized in our laboratory, degree of cross-linking 9%, grain size in the range of 50–80 mm, and specific surface area in the range of 450–500 m^2/g) 116 cm high, and the acetone–H_2O solutions with different acetone volume percentages (20, 42, 46, 50, and 54%) were eluted in turn.

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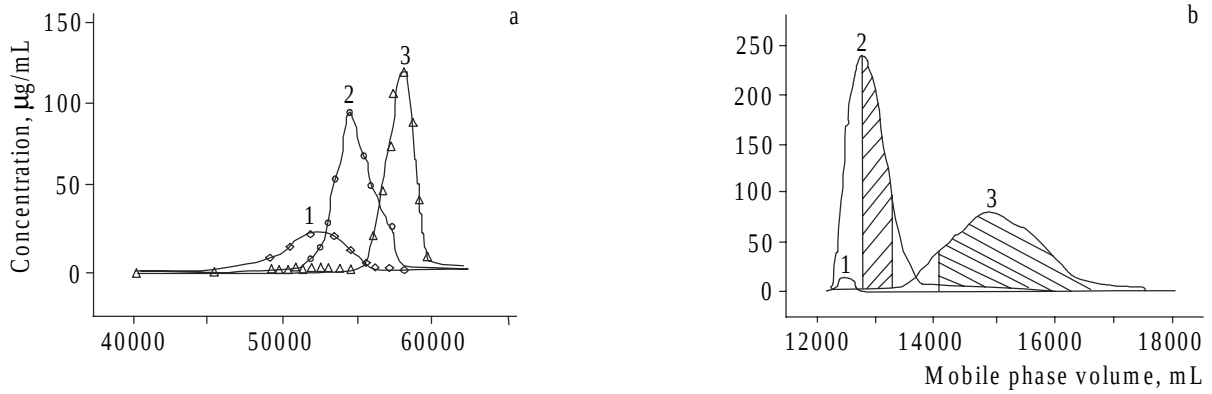

Fig. 1. Elution curves of the three compounds in normal phase chromatography (a) and in one-time reversed phase chromatography (b); 1 – 7-epi-taxol, 2 – cephalomannine, 3 – taxol.

The eluates containing taxol and cephalomannine were collected and determined by HPLC. The eluates containing taxol and cephalomannine were concentrated and frozen, and then the crude products of taxol and cephalomannine were obtained. The PRP-6 reversed-phase chromatography was used once again to separate and purify the crude products of taxol and cephalomannine, the eluates containing taxol and cephalomannine were collected and concentrated, and crystals of them were obtained after freezing. The crystals of taxol and cephalomannine obtained were dissolved with methanol–H_2O and then frozen, filtered, and vacuum dried. Finally, 0.17 g of taxol and 0.14 g of cephalomannine were obtained.

Qualitative and Quantitative Procedures. The qualitative and quantitative analysis of taxol and cephalomannine was carried out by HPLC. Twenty microliters each of the two kinds of crystals obtained and the standard solutions were injected into a Shimadzu LC-10AT high-performance liquid chromatograph. The conditions of HPLC are as follows: Dikma Diamonsil C_{18} column (5 μm, 250 mm × 4.6 mm), CH_3CN–H_2O solution (45:55, v/v) as the mobile phase, 1.0 mL/min flow rate, 30°C and detection wavelength 227 nm.

After digestion and extraction, the crude products obtained were separated by normal phase chromatography first. The separation results are shown in Fig. 1, *a*. It can be seen that 7-epi-taxol, cephalomannine, and taxol were separated preliminarily by normal phase chromatography; the taxol was enriched to 4% (determined by HPLC), and a great deal of 7-epi-taxol was eliminated.

The eluates containing taxol and cephalomannine obtained by normal phase chromatography were concentrated and dissolved in acetone, then separated by PRP-6 reversed- phase chromatography. The separation results are shown in Fig. 1,*b*.

As shown in Fig. 2, the separation of taxol and cephalomannine was good, the resolution was 0.99, and the peaks of each compound are basically symmetrical. Then, the eluates of the two shadow parts collected were separated by reversed-twice phase chromatography once again, and all of the other parts (no shadow) were separated by twice-reversed- phase chromatography again. Finally, all of the eluates containing taxol and cephalomannine separated by twice- reversed phase chromatography were collected together, then evaporated to dryness, and re-crystallized, and white acicular crystals of taxol and cephalomannine were obtained.

The qualitative analysis of the two kinds of white acicular crystals was determined by HPLC. The experimental results showed that the retention time of the acicular crystal (**1**) agrees with the retention time of the cephalomannine standard, and the retention time of the acicular crystal (**2**) agrees with the retention time of the taxol standard, proving that the acicular crystal (**1**) is cephalomannine, and the acicular crystal (**2**) is taxol.

The purities of the two kinds of white acicular crystals of cephalomannine and taxol were determined by an external standard method. The standard solutions (1–50 μg/mL) were determined by HPLC under the selected chromatographic conditions. The linear regression equation and correlation coefficients for taxol are $Y = 9.022 \times 10^7 X - 0.009 \times 10^7$ (0.9989); for cephalomannine, $Y = 3.98 \times 10^7 X - 0.012 \times 10^7$ (0.9985).

According to the above procedure, the purities of taxol and cephalomannine were 98.5 and 98.3%, respectively.

Dry leaves of *Taxus cuspidada* (7.5 kg) were smashed, sifted, digested, and extracted, then separated and purified by normal phase chromatography and twice PRP-6 reversed- phase chromatography. The contents of taxol and cephalomannine in *Taxus cuspidada* determined by HPLC were 0.0030 and 0.0025%, respectively. The yields and recoveries are shown in Table 1.

TABLE 1. The Yields and Recoveries

Component	Output, g	Yield, %	Recoveries, %
Taxol	0.17	0.0022	70.8
Cephalomannine	0.14	0.0018	74.7

The taxol and cephalomannine separated and purified from *Taxus cuspidada* by the proposed method were determined by an AV-600MHz NMR spectrometer to determine their structures. The experimental results show that all the NMR data correspond to the ones published in early reports [4], proving that the two kinds of white acicular crystals obtained are taxol and cephalomannine.

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