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High-performance liquid chromatography and micellar electrokinetic chromatography of taxol and related taxanes[☆] from bark and needle extracts of *Taxus* species

King C. Chan[a], A. Belinda Alvarado[a], Mary T. McGuire[a], Gary M. Muschik[a], Haleem J. Issaq*[,a], Kenneth M. Snader[b]

[a]*Program Resources, Inc./DynCorp, NCI-Frederick Cancer Research and Development Center, Frederick, MD 21702, USA*
[b]*Natural Products Branch, DTP, DCT, National Cancer Institute, Frederick, MD 21702, USA*

Abstract

High-performance liquid chromatography (HPLC) and micellar electrokinetic chromatography (MEKC) were applied for the separation of taxol, cephalomannine, and baccatin III in crude extracts from the needle and bark of *Taxus* species. The chromatogram of the bark extract was cleaner than that of the needle allowing a more reliable detection of taxol and cephalomannine in the bark extract. However, HPLC quantitation of taxol in the needle extract would be difficult due to coeluting taxinines. Nevertheless, this was not a problem in the MEKC experiment. In comparison to HPLC, MEKC offered baseline resolution of taxol from taxinines in the needle extract, less solvent waste, a smaller sample requirement, and the simultaneous detection of taxol, cephalomannine and baccatin III in a relatively simpler electrophoretic run.

1. Introduction

Taxol is a diterpene amide that was initially isolated from the bark of the Pacific yew (*Taxus brevifolia*) which shows unique antitumor and antileukemic activities [1,2]. It has been shown to produce responses in patients with different types of cancer, such as ovarian [3], breast [4], lung [5], head and neck region [6] and malignant melanoma [7]. To date, total synthesis of taxol has not been successful [8]. However, due to environmental concerns, procedures other than extraction of taxol from the tree bark are needed to maintain the natural source. One of these procedures involves the extraction of taxol and baccatin III from the needles rather than the bark [9]. Baccatin III is a very useful compound in this case because it can be converted to taxol via a semi-synthetic route [10]. This is not only environmentally sound but it is also an important economical and time-saving step. In addition, extraction of taxol from the plant tissue culture could also be a potential long-term source of taxol [11]. The resolution of taxol from other closely related taxanes, especially cephalomannine, in the bark and needle extracts has been the focus of numerous separation studies involving high-performance liquid chromatography (HPLC) [12–20], thin-layer chromatography [21] and counter-current chromatography

* Corresponding author.

☆ Taxol refers to the compound that now has the generic name paclitaxel and the registered name Taxol (Bristol-Myers Squibb, New York, NY).

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[22]. Although HPLC separations of standard mixtures of taxol, cephalomannine, baccatin III and other related compounds have been reported, with HPLC it is difficult to baseline resolve taxol, cephalomannine, and baccatin III especially from needle extracts in a single chromatographic run due to interfering compounds such as taxinines and other natural products [23] that coelute with the compounds of interest. It is important to be able to quantitate for baccatin III and taxol in tree parts because taxonomical studies have shown that the amounts of these compounds in needles and bark are dependent on the age of the tree, the time that the needles are harvested, geographical area, and environmental conditions [1]. In addition, the method and duration for drying *Taxus* needles appear to be critical for achieving optimum yields of these compounds [24].

Since the time of harvesting, maturity of the needles, and other environmental conditions are critical in determining the amounts of taxol and baccatin III in the needles, it is important to develop an analytical procedure that can resolve taxol, cephalomannine and baccatin III in a single experiment that is simple, fast, economical, requires small amounts of extract and does not produce relatively large amounts of organic solvent waste. Capillary electrophoresis is a very attractive candidate in this case because it is a highly efficient and fast separation technique that requires only a few nl of sample and a few μl of electrolyte [25]. In a recent study performed by this laboratory [26], we demonstrated that micellar electrokinetic chromatography (MEKC) [27] is a very efficient tool for the separation of a standard mixture of taxol, cephalomannine, baccatin III and their deacetylated derivatives. The objective of this study is to test the applicability of the MEKC procedure for the separation of taxol, cephalomannine and baccatin III from needle and bark crude extracts in a single, fast and simple experimental run that may be adopted as a test to determine the optimum needle and bark harvesting time, geographical location and environmental conditions.

2. Experimental

2.1. Chemicals

Taxol, cephalomannine and baccatin III were obtained from the National Cancer Institute Repository, and were dissolved in methanol as stock solutions. Other chemicals were reagent grade and purchased from Fisher (Fairlawn, NJ, USA). The crude methanolic extracts prepared from the bark and needle were provided by the Developmental Therapeutics Program, National Cancer Institute.

2.2. Apparatus

The HPLC system used in this study was similar to that described previously [9,12]. It consisted of a Waters 600E LC pump, a Waters 700 WISP autoinjector and a Waters 990 photodiode array detector. Separation was performed with a Dynamax-60 A phenyl column (5 μm particle size, 150 mm $\times$ 4.6 mm) with guard modules. The mobile phase consisted of methanol–acetonitrile–50 m*M* ammonium acetate (20:32:48) and was adjusted to pH 4.4 with acetic acid. Isocratic elution at a flow-rate of 1 ml/min were used. All samples were prepared in methanol and filtered (0.2 μm) before injection. Chromatograms were plotted at 230 nm.

MEKC separations were performed with Beckman P/ACE 2000 or 5500 capillary electrophoresis units. The Model 5500 was equipped with a diode array detector. Uncoated fused-silica capillaries were used for all experiments. The electrophoresis buffer contained 25 m*M* Tris–phosphate (pH 8.5), 50 m*M* sodium dodecyl sulfate and 25% acetonitrile. The capillary was flushed with the buffer after each run. The needle and the bark crude extracts were first dissolved in methanol and then diluted with the running buffer and filtered. Sample injections were performed by applying pressure for 3 s. Normalization of spectra was performed by dividing each data point in a scan by the absorbance at 200 nm using the Beckman System

Gold 8.0 software. Electropherograms were plotted at 230 nm.

3. Results and discussion

The HPLC procedure selected for this study has been used in our laboratory for the determination of taxol and cephalomannine in hundreds of bark and needle extracts. We were able to baseline resolve a standard mixture of taxol, cephalomannine and baccatin III using the same HPLC run. However, the separation of these three compounds from other impurities, especially in the needle extracts in a single HPLC experiment could be difficult. This is due to the presence of many interfering compounds that coelute with or close to the baccatin III elution time at the beginning of the chromatogram (Fig. 1). In general, a different mobile phase composition can be used to improve the detection of baccatin III whereby the methanol–acetonitrile–ammonium acetate ratio is changed to 20:25:55. This change in the mobile phase results in the resolution of baccatin III from other coeluting compounds; however, cephalomannine and taxol would not elute off the HPLC column (data not shown). With this new mobile phase, we were still unable to detect baccatin III in this sample. In Fig. 1, the presence of taxol and cephalomannine in the crude needle extract was determined by spiking the extract solution with standards and also by comparison of the peaks' UV spectra (using photodiode array detection) with that of standard solutions. Also, note that other compounds (taxinines) coeluted with taxol, which made its quantitation unreliable.

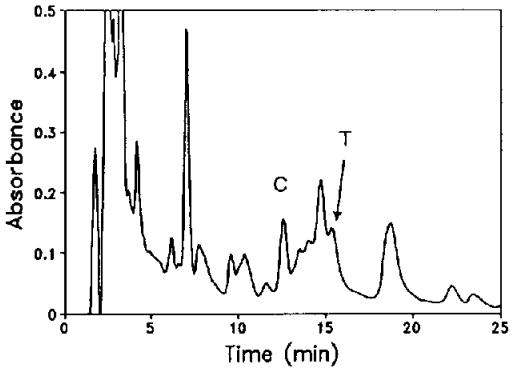

Fig. 1. HPLC chromatogram of a crude needle extract from the *Taxus* species. T = Taxol; C = cephalomannine. For detailed running conditions, see Experimental section.

Fig. 2a and b are the electropherograms for the extract used in Fig. 1 and the same extract fortified with taxol, cephalomannine and baccatin III, respectively. These figures show that the three compounds are resolved both from each other and other interfering compounds in under 20 min. Taxol and cephalomannine present in the extract were identified by spiking with standards (Fig. 2b) and by UV spectra comparison of the standard components (Figs. 3 and 4). The excellent spectra matching (correlation coefficients ≈ 0.999) with the standards indicates that a pure peak for each of the taxol and cephalomannine was obtained with the MEKC separation, unlike the HPLC method where taxol was not fully resolved from other coeluting taxinines. In addition, the total analysis time in both methods is comparable; but the solvent waste produced by MEKC is negligible (μl *vs.* ml by HPLC).

Fig. 5 is the HPLC chromatogram for a crude

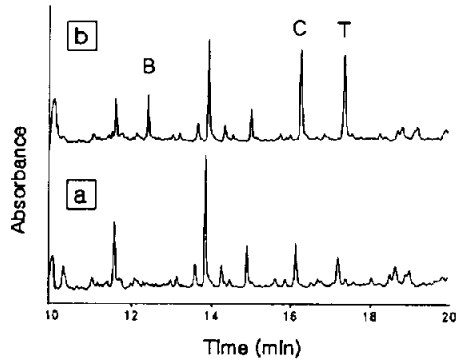

Fig. 2. MEKC electropherograms of the needle exctract used in Fig. 1 (a), and the spiked needle extract (b). T = Taxol; C = cephalomannine; B = baccatin III. Separation was performed at + 25 kV in a 87 cm × 50 μm uncoated fused-silica capillary. The electrophoresis buffer consisted of 25 m*M* Tris–phosphate (pH 8.5), 50 m*M* sodium dodecyl sulfate and 25% acetonitrile.

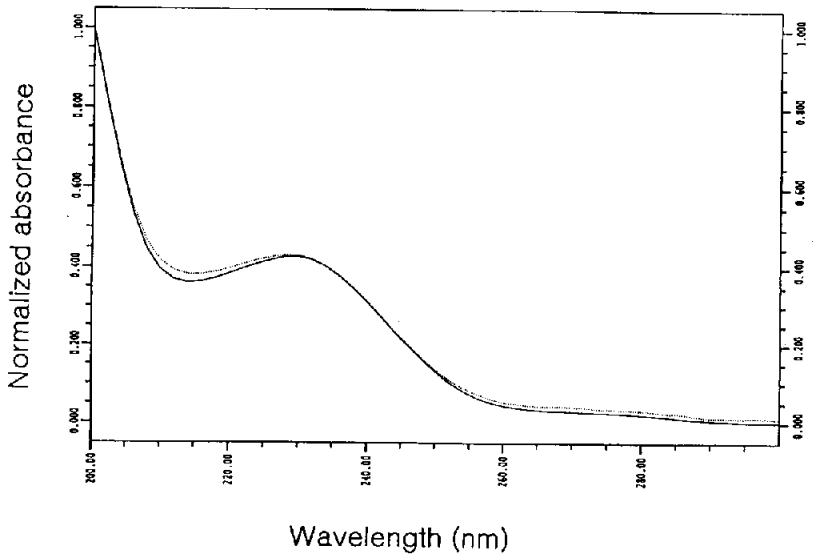

Fig. 3. Normalized UV spectra of a standard taxol peak (solid line), and the identified taxol peak (dotted line) in crude extracts separated by MEKC.

bark extract. A comparison of the chromatograms in Figs. 1 and 5 shows that the bark extract is cleaner than the needle extract, and taxol and cephalomannine are baseline resolved from other interfering peaks. Fig. 6a and b are the electropherograms for the crude bark extract used in Fig. 5 and the same extract fortified with taxol, cephalomannine and baccatin III, respectively. As in the case of the needle extract, taxol, cephalomannine and baccatin III are well resolved from other taxinines. The high purity of taxol and cephalomannine peaks in the original

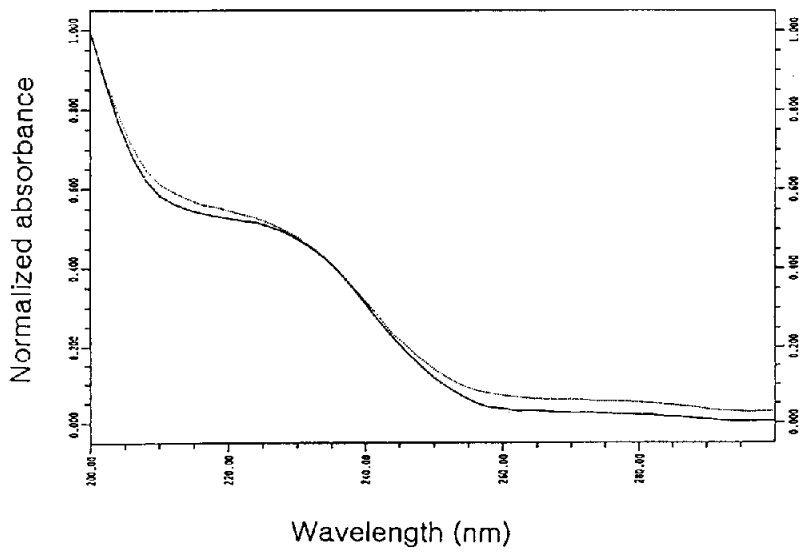

Fig. 4. Normalized UV spectra of a standard cephalomannine peak (solid line), and the identified cephalomannine peak (dotted line) in crude extracts separated by MEKC.

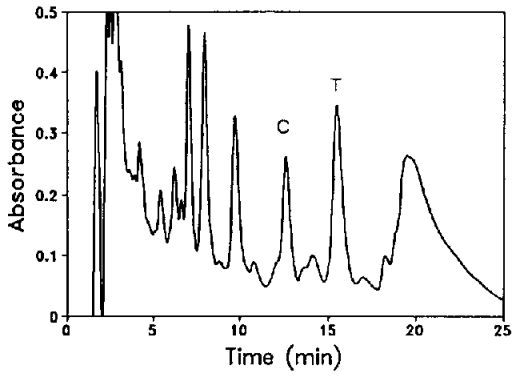

Fig. 5. HPLC chromatogram of a crude bark extract from the *Taxus* species.

extract were confirmed by the spectra matching technique as before. However, baccatin III was not detected in the original sample by both techniques (Figs. 5 and 6a).

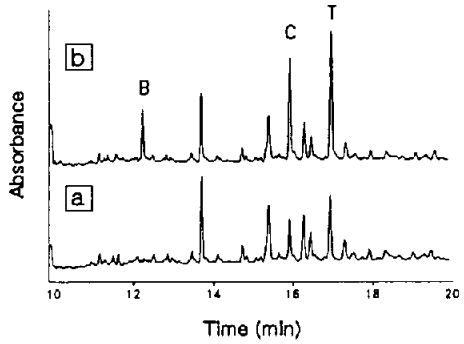

Fig. 6. MEKC electropherograms of the bark extract used in Fig. 5 (a), and the spiked extract (b). Other conditions as in Fig. 2.

4. Conclusions

This study demonstrates the feasibility of using MEKC for the separation of taxol, cephalomannine and baccatin III in the crude needle and bark extracts of *Taxus* species. MEKC allows for the separation of taxol, cephalomannine and baccatin III from each other and from other interfering compounds in the bark and needle samples in a single electrophoretic run. The method is simpler, more economical, and produces considerably less solvent waste, as compared to HPLC. This MEKC procedure should also be applicable to the determination of taxol in plant tissue culture, semi-organic synthesis, and the study of taxol metabolites in biological fluids.

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Note added in proof

After the submission of this manuscipt, Holton et al. [*J. Am. Chem. Soc.*, 116 (1994) 1597 and 1599], and Nicolaou et al. [*Nature*, 367 (1994) 630] reported the total synthesis of taxol by different routes.

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