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# Screening of the needles of different yew species and cultivars for paclitaxel and related taxoids

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**Abstract**

The needles of several yew species and cultivars were analysed by high-pressure liquid chromatography for paclitaxel, 10-deacetylpaclitaxel, cephalomannine, baccatin III, 10-deacetylbaccatin III and brevifoliol. About 750 samples were collected from five different locations in the Netherlands and the UK. The results of this screening show a large variation in taxane content between the different species and cultivars. The content of paclitaxel and 10-deacetylbaccatin III varied from 0 to 500 μg/g and 0 to 4800 μg/g dried needles, respectively. Brevifoliol was found in a very high concentration in *Taxus brevifolia*. 10-Deacetylpaclitaxel, cephalomannine and baccatin III were found in concentrations ranging from 0 to 500 μg/g dried needles. 

*Keywords:* *Taxus* cultivars; Taxaceae; High-pressure liquid chromatography; Taxanes; Paclitaxel

## 1. Introduction

Paclitaxel (Taxol®, Yewtaxan®) (**1**) is an important anticancer drug which was first isolated from the bark of the Pacific yew (*Taxus brevifolia* Nutt.) (Wani, Taylor, Wall, Coggon, & McPhail, 1971), a slow growing evergreen from western US and Canada. Because of the lack of a sustainable supply and a growing demand for the drug, various means to increase the supply of paclitaxel have been investigated. Some promising possibilities are semisynthesis from a readily available paclitaxel precursor such as 10-deacetylbaccatin III (**5**), or isolation of paclitaxel from the needles of more common *Taxus* species (Denis et al., 1988; Witherup et al., 1990; Wiegerinck et al., 1996). Furthermore, a lot of effort is invested in producing or isolating paclitaxel analogues with less toxicity, improved water solubility and/or improved biological activity (Deutsch et al., 1989; Kingston, Samanarayake, & Ivey, 1990; Guéritte-Voegelein et al., 1991; Amato, 1993). For these reasons, many *Taxus* species and cultivars have been screened for their taxane content (Witherup et al., 1990; Vidensek, Lim, Campbell, & Carlson, 1990; Mattina & Paiva, 1992; ElSohly et al., 1995). However, most yew samples have only been analysed for paclitaxel. Furthermore, it is known that there is a significant seasonal influence on the taxane content of yew needles (Vance, Kelsey, & Sabin, 1994; ElSohly, Croom, Kopycki, Joshi, & McChesney, 1997). Therefore, it is not correct to compare analytical results from yew samples that were collected during different times of the year. We report here the analysis of yew species and cultivars collected during the same time period.

## 2. Results and discussion

For the large-scale screening of yew needles for their content of taxanes a labour-efficient and selective clean-up method in combination with an HPLC quantitation on standard $C_{18}$ columns was developed (van Rozendaal, Lelyveld, & van Beek, 1997). The needles

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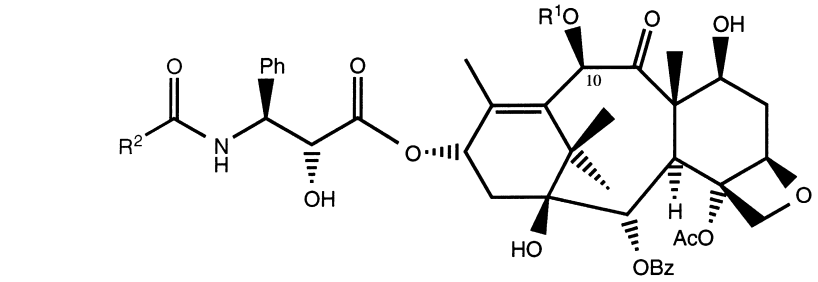
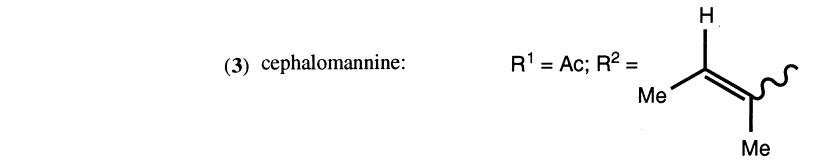
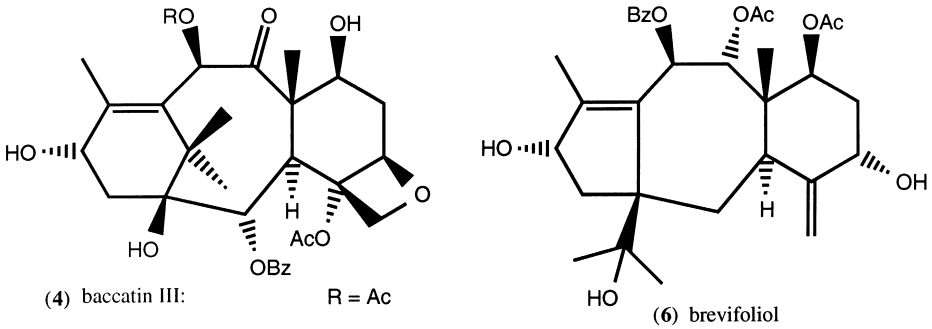


(1) paclitaxel: $R^1$ = Ac; $R^2$ = Ph

(2) 10-deacetyl paclitaxel: $R^1$ = H; $R^2$ = Ph



(3) cephalomannine: $R^1$ = Ac; $R^2$ =



(4) baccatin III: R = Ac

(5) 10-deacetyl baccatin III: R = H

(6) brevifoliol

Table 1
Taxane content (**1**–**6**) in needles of several Taxaceae

| Taxaceae species (No. of samples) | Average taxane concentration (μg/g dried needles) | | | | | | |
|---|---|---|---|---|---|---|---|
| | (**1**) | (**2**) | (**3**) | (**4**) | (**5**) | (**6**) | Total |
| *T. baccata* (49) | 41 | 198 | 22 | 14 | 762 | 3 | 1039 |
| *T. baccata* cv. (473) | 63 | 179 | 42 | 10 | 468 | 6 | 768 |
| *T. brevifolia* (1) | 130 | 0 | 0 | 296 | 41 | 9132 | 9599 |
| *T. canadensis* (2) | 285 | 253 | 289 | 224 | 2665 | 77 | 3793 |
| *T. celebica* (6) | 26 | 81 | 0 | 0 | 70 | 46 | 223 |
| *T. cuspidata* (10) | 105 | 113 | 40 | 15 | 120 | 6 | 399 |
| *T. cuspidata* cv. (60) | 136 | 198 | 93 | 18 | 116 | 1 | 562 |
| *T. floridana* (1) | 516 | 515 | 0 | 0 | 1689 | 0 | 2720 |
| *T. globosa* (1) | 433 | 229 | 480 | 168 | 1395 | 0 | 2705 |
| *T.* × *hunnewelliana* (9) | 41 | 100 | 0 | 0 | 63 | 0 | 204 |
| *T.* × *media* cv. (108) | 211 | 205 | 131 | 36 | 230 | 6 | 819 |
| *T. wallichiana* (1) | 272 | 420 | 0 | 0 | 1092 | 0 | 1784 |
| *Amentotaxus formosana* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cephalotaxus fortunei* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Cephalotaxus harringtonia* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Pseudotaxus chienii* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Torreya grandis* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| *Torreya californica* (1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

were screened for paclitaxel (**1**), 10-deacetylpaclitaxel (**2**), cephalomannine (**3**), baccatin III (**4**), 10-deacetylbaccatin III (**5**) and brevifoliol (**6**).

About 750 samples of yew needles were collected, belonging to the following species: *T. baccata* L. (78 cultivars), *T. brevifolia* Nutt., *T. canadensis* Marshall, *T. celebica* (Warb.) Li, *T. cuspidata* Sieb.&Zucc. (10 cultivars), *T. floridana* Chapm., *T. globosa* Schltdl., *T.* × *hunnewelliana* Rehder, *T.* × *media* Rehder (15 cultivars) and *T. wallichiana* Zucc. Furthermore, the following other Taxaceae have been collected: *Amentotaxus formosana* Li, *Cephalotaxus fortunei* Hook, *Cephalotaxus harringtonia* (Forbes) K. Koch, *Pseudotaxus chienii* (Cheng) Cheng, *Torreya grandis* Fort. and *Torreya californica* Torr. Table 1 gives the average in taxane content (**1**–**6**) measured in needles of the different *Taxus* genera and the other Taxaceae. Figs. 1–3 give the taxane content of the different *T. baccata*, *T. cuspidata* and *T.* × *media* cultivars. The highest paclitaxel concentrations (400–500 μg/g dried needles) were found in *T. floridana* and *T. globosa*. Unfortunately, these species are not widespread. The best candidates for the isolation of paclitaxel are

*T.* × *media* 'Hillii' and 'Hicksii' (300–500 μg/g dried needles) because these are available and relatively fast growing cultivars. Another potentially interesting paclitaxel precursor, 10-deacetylpaclitaxel, occurred in high concentrations (400–500 μg/g dried needles) in *T. floridana*, *T. wallichiana* and in several *T. baccata* cultivars. *T. globosa* had the highest cephalomannine content (480 μg/g dried needles). Baccatin III showed a relatively high content (200–300 μg/g dried needles) in *T. brevifolia* and *T. canadensis*. All the other yew

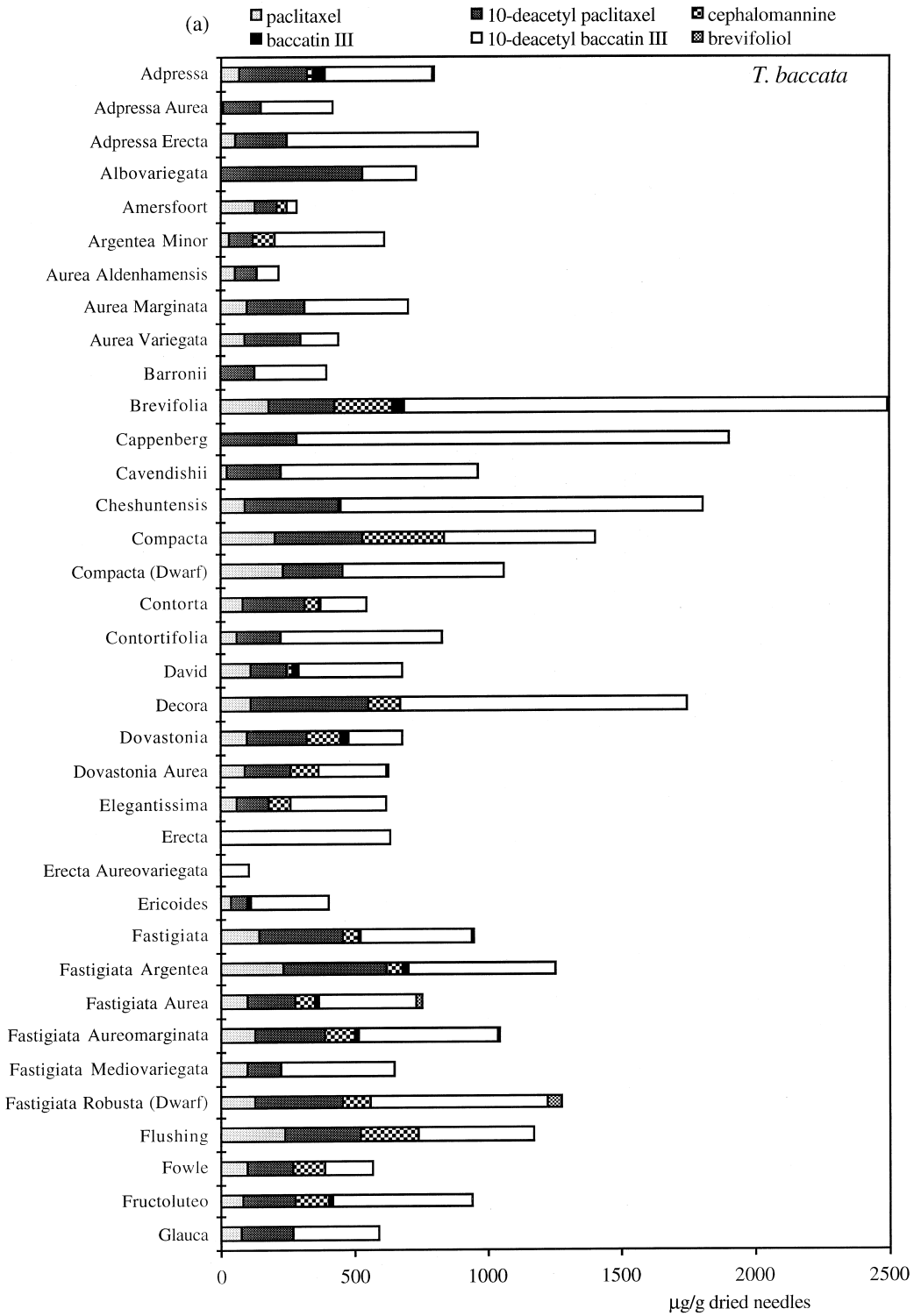



Fig. 1. (a) Taxane content of *T. baccata* cultivars.

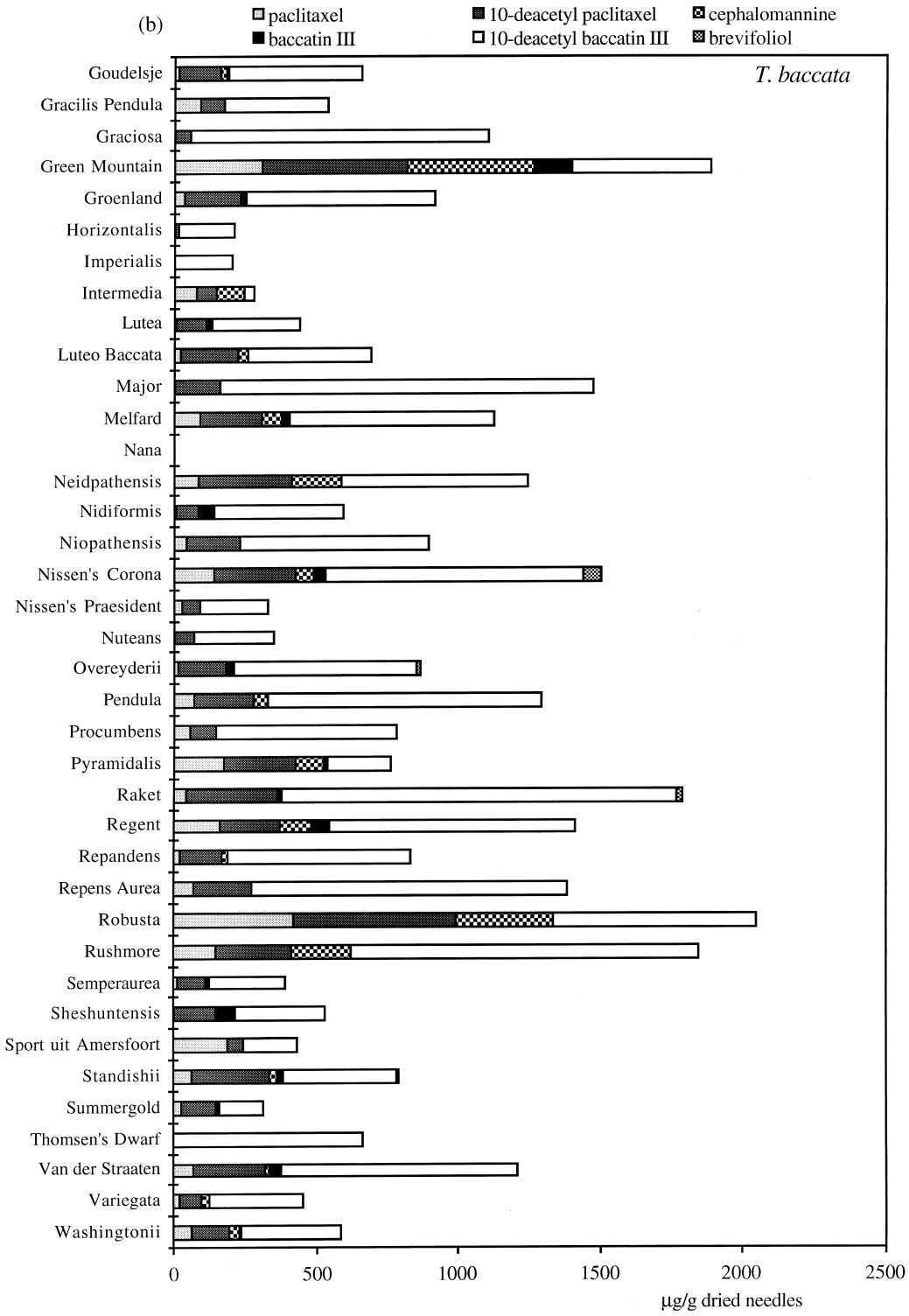



Fig. 1. (b) Taxane content of *T. baccata* cultivars.

species had very low baccatin III concentrations. The currently most useful paclitaxel precursor, 10-deacetylbaccatin III, was found in high concentrations (1000–1700 µg/g dried needles) in *T. floridana*, *T. globosa* and *T. wallichiana*. Even higher concentrations (>2500 µg/g dried needles) were measured in *T. canadensis* and several *T. baccata* cultivars. The 10-deacetylbaccatin III concentration of *T. baccata* L. samples showed a large variation (100–4800 µg/g dried needles). Nevertheless, it seems the most prom-

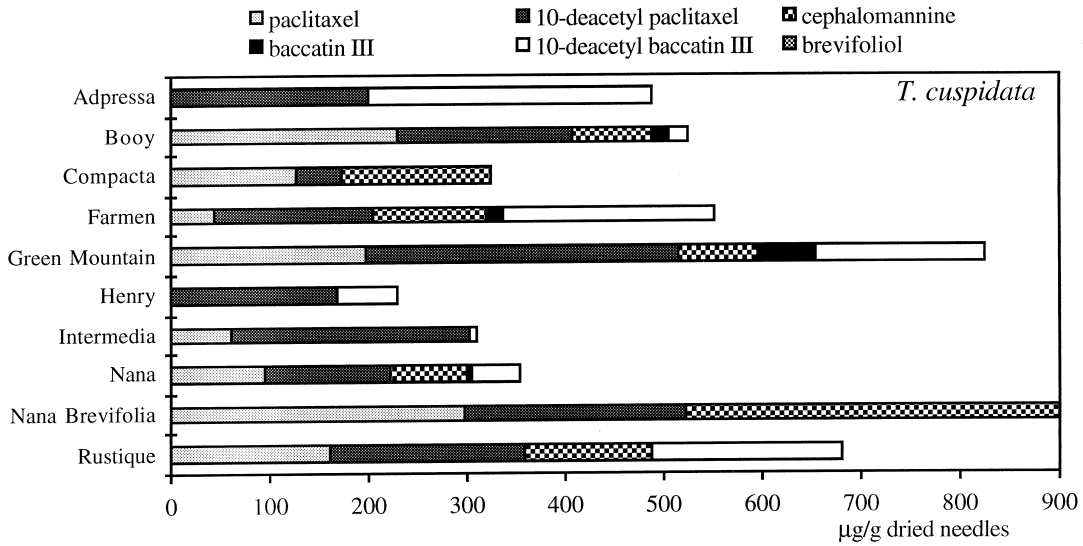



Fig. 2. Taxane content of *T. cuspidata* cultivars.

ising yew for the isolation of 10-deacetylbaccatin III because this species is very common. An extremely high brevifoliol concentration (>9000 µg/g dried needles) was measured in the needles of *T. brevifolia*. All the other species had only very small brevifoliol concentrations. None of the taxanes (**1**–**6**) could be

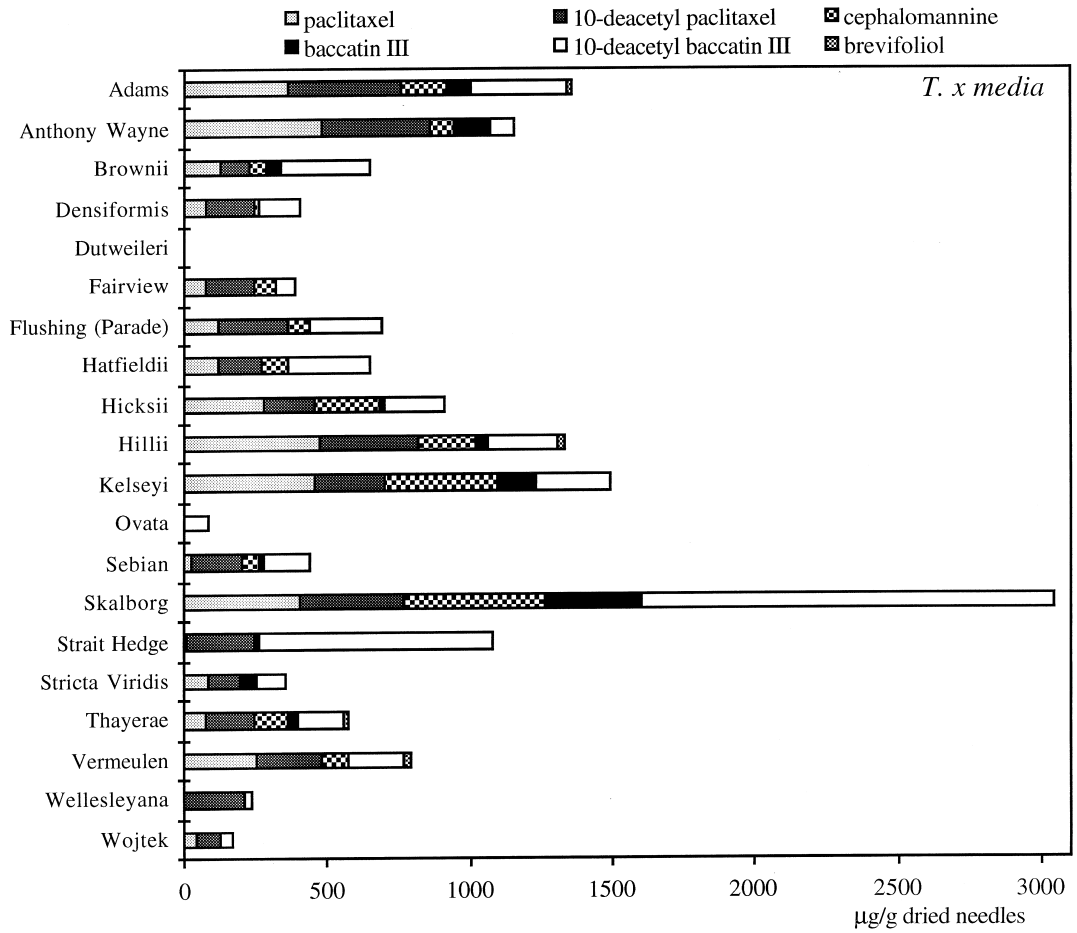



Fig. 3. Taxane content of *T.* × *media* cultivars.

Table 2
Average taxane content for the yew species at the five locations

| Location (No. of samples) | Average taxane concentration (μg/g dried needles) | | | | | | |
|---|---|---|---|---|---|---|---|
| | (1) | (2) | (3) | (4) | (5) | (6) | Total |
| Wageningen (69) | 29 | 156 | 8 | 14 | 489 | 134 | 830 |
| Boskoop (217) | 89 | 191 | 60 | 8 | 373 | 11 | 732 |
| Horst (272) | 98 | 176 | 48 | 25 | 392 | 4 | 743 |
| Hilversum (25) | 119 | 247 | 52 | 7 | 532 | 0 | 957 |
| Bedgebury (138) | 113 | 195 | 102 | 11 | 443 | 4 | 868 |
| Total (721) | 92 | 185 | 58 | 16 | 417 | 19 | 787 |

detected in Taxaceae genera other than those of *Taxus* itself. The relative standard deviation (RSD) of the taxane content of a group of the same species or cultivars varied from 4 to 420%.

Plant samples have been collected from five different locations. Table 2 gives the average taxane content for the yew species on these five places. There is no significant difference in taxane content between these locations. The relatively high concentration of brevifoliol in Wageningen was caused by the *T. brevifolia* on this site. The relatively low paclitaxel content in Wageningen can be explained by the fact that most of the samples from this location were *T. baccata* cultivars.

It can be concluded that there is a large variation in taxane content between the different yew species. The highest total taxane concentrations were found in the less common species. The most abundant taxanes were 10-deacetylbaccatin III (**5**) and 10-deacetylpaclitaxel (**2**). This is in contrast with the findings of ElSohly et al. (1995), who found 10-deacetylbaccatin III (**5**) and paclitaxel (**1**) to be the most abundant. They have collected their plant samples during the autumn and winter period and this could be the reason for the difference. When brevifoliol is not considered, the average total taxane content found by ElSohly is comparable with the results presented in this paper. For some of the more interesting plants the taxane analysis was repeated later in the season. It was found that the 10-deacetylbaccatin III content peaked at the end of summer. Very high concentrations of 8000 μg/g were detected in some *T. baccata* L. needles. This species is the most interesting for the isolation of 10-deacetylbaccatin III. Paclitaxel has its highest concentrations in the autumn. Some *T.* × *media* samples showed concentrations above 800 μg/g, which is considerably higher than the paclitaxel concentration found in the bark of *T. brevifolia*. For this reason, the widely occurring species *T.* × *media* ('Hillii' and 'Hicksii') is best suited for the isolation of paclitaxel.

## 3. Experimental

### 3.1. Plant material

Needles from several Taxaceae species and cultivars were collected between 16 February and 15 March 1996 from two sites of the Research Station for Nursery Stock (Boskoop and Horst, both in The Netherlands) and three botanical gardens (Wageningen Agricultural University Botanical Gardens, The Netherlands; Pinetum Blijdenstein, Hilversum, The Netherlands; Bedgebury National Pinetum, Kent, UK). Herbarium accession numbers are available on request. After being dried for 3 h at 60° in an oven with forced air ventilation, the needles were separated from the branches and stored in stoppered glass vials prior to the analysis.

### 3.2. Extraction procedure

Dried needles (≈1000 mg) were accurately weighed and placed in a 20-ml brown bottle. Then 10.0 ml EtOH–$H_2O$–HOAc (80:19:1) was added to the needles, after which the bottle was closed and left standing for 5 days at room temperature. After this period of time the bottle was shaken and a small quantity of the extract was pipetted off and used for further purification.

### 3.3. Purification procedure

A 4-ml polypropylene solid-phase extraction column (Varian, Bond Elut Reservoir, 4 ml, 20 μm frits) was filled with 750 mg Extrelut® (Merck). Aqueous sulphuric acid (0.5 %; 350 μl) was applied to the top of the column, followed by 200 ± 1.6 μl of the crude extract. The column with sample was allowed to stand for 30 min. The column was then eluted with 5.0 ml petroleum b.p. 40/60-*t*-butyl methyl ether (4:1), to remove interfering constituents. Next, the column was eluted with 4.0 ml 100% *t*-butyl methyl ether. This fraction was collected in a 4-ml vial and contained the taxanes of interest. The *t*-butyl methyl ether was removed under reduced pressure in a heated (40°) vacuum centrifuge. The residue was then dissolved in 200 ± 1.6 μl of the internal standard solution, which consisted of approximately 22 mg cinnamic acid, accurately weighed, in 1 l MeOH. After this, the sample was ready for HPLC analysis.

### 3.4. HPLC

Analysis was performed at room temperature on an Alltech Nucleosil $C_{18}$, 100-Å pores, 50-μm particle size, L 250 mm, ID 3.2 mm column. Chromatography was carried out using two solvents: A, acetonitrile and

Table 3
Gradient table for HPLC analysis

| Time (min) | Solvent A (%) | Solvent B (%) |
|---|---|---|
| 0 | 26 | 74 |
| 5 | 26 | 74 |
| 10 | 45 | 55 |
| 17 | 45 | 55 |
| 19 | 60 | 40 |
| 25 | 26 | 74 |
| 30 | 26 | 74 |

B, water adjusted to pH 2.5 with *ortho*-phosphoric acid, in a linear gradient programme detailed in Table 3. The flow-rate of the mobile phase was 0.8 ml/min. Injections were made by a sample injector equipped with a 10-μl loop. Peaks were detected over the 210–320 nm range of the absorption spectrum and all chromatograms were plotted at 227 and 280 nm. The $R_t$ for 10-deacetylbaccatin III (**5**), baccatin III (**4**), brevifoliol (**6**), 10-deacetylpaclitaxel (**2**), cephalomannine (**3**) and paclitaxel (**1**) was 10.30, 16.14, 18.97, 21.59, 24.40 and 25.38 min, respectively. The internal standard (cinnamic acid) had a $R_t$ of 13.60 min.

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