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TAXOL CONTENT IN BARK, WOOD, ROOT, LEAF, TWIG, AND SEEDLING FROM SEVERAL *TAXUS* SPECIES

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ABSTRACT.—Taxol content in various parts of several *Taxus* species have been determined. The weight percent ranged from 0.00003 to 0.069.

Taxol, a diterpene with exceptional anticancer activity (1), occurs as a very minor component in several species of *Taxus*. Although a partial synthesis from a congener has been achieved (2) and a biosynthesis employing tissue culture has been reported (3), the currently available source of the chemical is the bark of *Taxus brevifolia* Nutt. (Taxaceae). To obtain 1 kg of taxol requires about 10,000 kg of bark; several thousand trees must be cut to procure this quantity of bark. Compounding this problem is the relative scarcity and the slow growth of *T. brevifolia*. The obvious need for additional sources prompted this search for taxol in all parts of the tree as well as in other *Taxus* spp.

In this note we report taxol content in various parts (separated bark, roots, wood, branches, leaves, twigs, and whole seedlings) of several *Taxus* spp. including *T. brevifolia, Taxus baccata* L., *Taxus media* Rehder, and *Taxus cuspidata* Sieb. et Zucc., although specimens of the last three species constitute only 10% of the total samples examined. The bark gave the highest average yield of taxol, although not every bark sample was worthy of consideration. The next highest average yield came from the seedlings. The lower average yields were found in the twigs and the leaves; the lowest average yield was associated with the wood.

The weight percentages of taxol in various parts of *T. brevifolia* are tabulated in Table 1; those in various parts of *T. baccata, T. media,* and *T. cuspidata* are listed in Table 2.

EXPERIMENTAL

PLANT MATERIAL, EXTRACTION, AND TAXOL DETERMINATION.—The *T. brevifolia* specimens were collected from national forests in Northern California, Oregon, and British Columbia; the samples of *T. baccata, T. media,* and *T. cuspidata* were obtained from private property in Virginia. The air-dried plant materials were milled to irregular-shaped particles ranging from 1 to 40 mm and kept at room temperature in capped, opaque plastic containers. Although some leaf-containing specimens felt moist, moisture content of the samples was not determined. Most samples weighed hundreds of grams, some tens of grams, and two were less than 10 g. If material

TABLE 1. Weight Percent of Taxol in Various Parts of *Taxus brevifolia*.

Plant Material	No. of Samples	Samplings Analyzed	Avg. % Taxol	Range of %
Bark	15	51	0.015, s = 0.018[a]	0.0001–0.069
Roots	8	17	0.004, s = 0.004	0.0008–0.010
Wood	7	13	0.0006, s = 0.0004	0.0001–0.0012
Wood with bark	5	6	0.0003, s = 0.0002	0.00003–0.004
Branches	8	22	0.0017, s = 0.0020	0.0001–0.005
Leaves/needles	6	12	0.0015, s = 0.0012	0.00003–0.0030
Twigs	6	12	0.0012, s = 0.0013	0.0002–0.0034
Seedlings	14	23	0.0058, s = 0.0040	0.0007–0.015

[a]s=standard deviation.

TABLE 2. Weight Percent of Taxol in Various Parts of Other *Taxus* Species.

Species	Plant Material	No. of Samples	Samplings Analyzed	Avg. % Taxol	Range of %
Taxus baccata	stem	1	2	0.001	0.0009–0.001
	twig	2	4	0.0006	0.0004–0.0009
	leaf	1	2	0.003	0.003
Taxus media	stem	1	2	0.002	0.002
	twig	2	4	0.009	0.009
	leaf	1	2	0.002	0.002
Taxus cuspidata	twig	1	2	0.0006	0.0002–0.0009

was available, at least duplicate samplings were taken.

Anhydrous MeOH (100–140 ml) was added to weighed samplings of 10 g to 30 g in 500-ml, screw-capped polyethylene bottles, and the mixtures were mechanically agitated for 16 h at room temperature. The MeOH was decanted through Whatman No. 1 filter paper, and the filtrate was evaporated to near dryness at 40° and ca. 25 mm pressure. A second extraction of the plant material was similarly carried out, and the second extract was added to the first. The combined extract was partitioned between CH_2Cl_2 and H_2O. For mixtures that separated with difficulty, centrifugation was required. The combined CH_2Cl_2 layer was evaporated to dryness at 35° and under reduced pressure.

The residue was dissolved in a known volume of a CH_2Cl_2-MeOH (1:1) and filtered; aliquots were streaked onto 0.25 × 20 × 20 cm silica GF plates, on which reference taxol was spotted. The chromatoplates were developed with a CH_2Cl_2-MeOH (95:5) solvent system. The taxol-containing band was located under uv light, marked, scraped off the plate, packed into a small column, and eluted with 3 to 4 volumes of MeOH. The eluate was gently evaporated to dryness. If the resulting residue was highly colored, it was redissolved in a minimal amount of MeOH, streaked onto a second plate, and developed in a CH_2Cl_2-MeOH (98:2) solvent system after reference taxol spots had been applied. The taxol-containing band was scraped from the plate, packed into a small column, and eluted with 3 volumes of MeOH. The eluate was gently evaporated to dryness, and the residue was dissolved in a known volume of MeOH for taxol determination by hplc.

An improved method to enrich the taxol was to use liquid-solid phase extraction in place of tlc. A C-18 disposable cartridge, prepared by washing with MeOH followed by H_2O, was charged with the CH_2Cl_2 extract dissolved in a minimal amount of MeOH. The short column was developed stepwise with 10-ml volumes of 30%, 50%, and 70% MeOH in H_2O, and finally with neat MeOH. The enriched taxol, eluting exclusively during the 70% MeOH development, was collected, made to volume with more MeOH, and chromatographed under the following analytical conditions: column, C_{18}, 5 μm, 4.6 × 150 mm, preceded by a C_{18} guard column; mobile phase, MeOH-H_2O (68:32) at 1 ml/min; detection, 230 nm at 0.1 AUFS; references, (a) authentic taxol, ≥98% purity, (b) cephalomannine. The external standard method of quantitation was employed.

CONDITIONS AND CALCULATIONS.—The hplc conditions were adjusted such that cephalomannine was at least 50% resolved from taxol and little or no cephalomannine was included in the taxol measurements.

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