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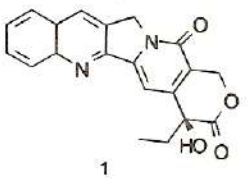
Over a 12-week period, new growth was collected at different intervals from *Camptotheca acuminata* trees to determine whether a leaf harvest strategy would be an efficient means for the production of the alkaloid camptothecin. Because camptothecin accumulates in young leaves and because the harvesting of young tissue stimulates axillary bud outgrowth, this strategy increased the harvestable amount of camptothecin from trees in a nondestructive manner.

Camptothecin (CPT, **1**), a monoterpene-derived indole alkaloid produced by the Chinese tree *Camptotheca acuminata* Decaisne (Nyssaceae), was first isolated and structurally characterized in the 1960's.[1] CPT is a valuable compound as a chemical precursor for the semisynthetic derivatives topotecan and irinotecan, which are used clinically as anticancer agents.[2,3] Its antitumor activity is due to an ability to inhibit DNA topoisomerase I.[4] CPT also inhibits retroviruses such as the human immunodeficiency virus and the equine infectious anemia virus.[5,6] The anti-HIV activity of CPT is due to the inhibition of Tat-mediated transcription from the viral promoter.[7] Recently, CPT has also shown promising results against parasitic trypanosomes and *Leishmania*.[8]



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Young leaves contain the highest concentration of CPT in the tree.[9] Knowing this, our goal was to determine if young leaves could be used as a repeatedly harvestable source of CPT. This study was designed to determine the effect of repeated harvest of new growth on biomass production, CPT yield, and CPT concentration in the young leaves of *C. acuminata* grown under greenhouse conditions.

Samples from 24 trees were lyophilized, and alkaloids were extracted for reversed-phase HPLC analysis. CPT concentration in new growth was measured for each tree at several time points. Total CPT was calculated using the total dry weight of each sample and the concentration of CPT present in that sample as measured using HPLC. Because a small amount of tissue was collected, only one extraction was done per tree at each time point. These data were combined into four groups based upon the time interval between collections (Figure 1). New growth was clipped from each group of six trees at 2-,3-, 4-, or 6-week intervals. At the time of first harvest, the four groups yielded relatively equal amounts of total CPT. Total CPT was calculated by summing the total CPT present in each of the samples from the six trees. Throughout the experiment, groups harvested at 2-, or

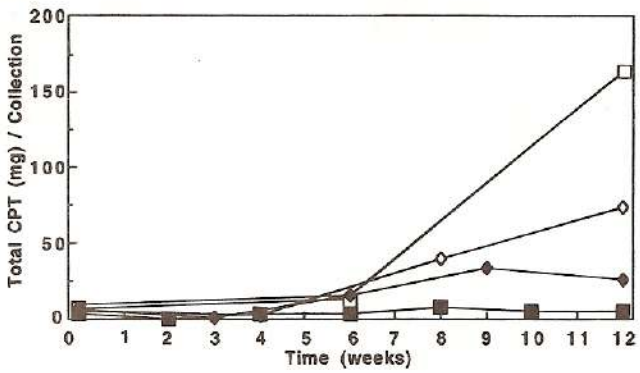

Figure 1. Total amount of CPT collected at each time point for four groups of six trees. Points represent the sum of the CPT collected from six plants expressed in mg CPT/collection at a given week: ■, 2-week harvest interval; ◆ 3-week harvest interval; ◇, 4-week harvest interval; □, 6-week harvest interval.

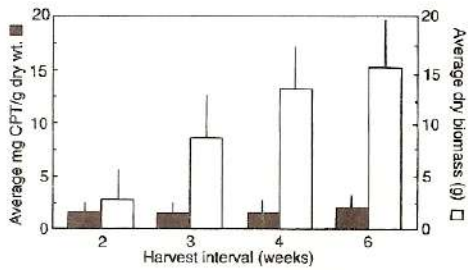

Figure 2. Comparison of average CPT concentration in young tissue and average biomass of young tissue produced by each group of 6 17 month old, 1.5 m tall trees. Vertical lines represent standard deviations.

3-week intervals showed little increase in CPT production. In groups harvested at 4-, and 6-week intervals, the total amount of CPT increased with time. By week 12, the total amount of CPT collected from trees harvested at 6-week intervals was 6.5 times greater than from trees harvested at 2-week intervals (175 mg vs 26.9 mg).

The amount of CPT produced per g of tissue was not significantly different (Figure 2). However, the mass of young leaves produced by the plants in each group rose with an increase in the number of weeks between collection (Figure 2). Initial CPT concentrations ranged from 0.45 mg/g dry weight to 3.49 mg/g dry weight. Even though the variation between individuals was high, the plants within each group showed the same trends in biomass production.

During each harvest, the apical bud was removed from these trees which in turn induced growth from

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lateral buds. This release from apical dominance then accounts for the increase in biomass production. Thus, although the CPT concentration in these leaves stayed constant, the increased biomass produced by the young trees led to an overall increase in yield.

This experiment was designed to study a possible harvest of CPT based on the fact that young leaf tissue contains the highest concentration of CPT in trees.[9] Leaves provide a readily renewable source of CPT, unlike plant parts such as bark or roots that can only be collected once. Leaves may also be harvested from trees prior to reproductive age, whereas seeds, another organ rich in CPT, must come from trees 5 years old or older. A leaf harvest system will save time, increase the number of harvests per year, and perhaps increase the yield when compared to current bark or seed collection methods. Trees harvested at 2- or 3-week intervals did not produce new tissue past the glossy stage of development. No new tissue was left on these trees after each harvest, and by the end of 12 weeks, these trees were losing all foliage. Most lateral buds grew and were harvested before they could be replaced during the course of the experiment. This would not be a suitable harvest strategy. For trees harvested at 4- and 6-week intervals, any growth beyond the glossy stage of leaf development was not collected. These trees responded by producing increasing amounts of new growth from lateral buds and retained older foliage. Trees collected at 6-week intervals in this experiment produced a total of 175 mg of CPT in 12 weeks using approximately 2 m^2 of greenhouse space. One of the trees in this group had a CPT concentration 10 times less than the others in the group. With careful selection and removal of low-level producers like the one mentioned, the yield of CPT could be even higher.

Due to the importance of CPT as a chemical precursor to medicinal compounds demand is sure to continue. An alternative source of CPT such as a leaf-harvesting strategy could be used to produce the alkaloid at a reduced cost. The results of this study show that a quickly renewed source of CPT is available in the form of young leaf tissue. Additional studies will be needed to determine the effects of increased harvest intervals and the long-term effect of repeated harvesting on plant growth and CPT yields.

Experimental Section

Plant material. *C. acuminata* trees were grown from seed in one gallon pots in a greenhouse. Seeds were obtained from the San Antonio Zoo in San Antonio, TX. Four groups of six trees were chosen at random from a larger population of 1.5 m tall trees 17 months old. New growth was clipped from the apex and tips of branches. New growth included buds, stems, and young leaves less than 14 cm in length that had a slightly shiny appearance on the upper surface of the leaf when compared to fully mature leaves. The fresh weight was measured for each sample, and the tissue was immediately frozen in liquid nitrogen.

Alkaloid Extraction. Frozen tissue was ground to a fine powder with a mortar and pestle. The powdered tissue was lyophilized and weighed, and alkaloids were extracted as described by van Hengel et al.[10] Briefly, 50 mg of lyophilized tissue from each sample was ground in 1 mL of methanol. After grinding, 5 mL of water was added and then extracted twice with dichloromethane. The extract was filtered through Whatman No. 1 paper and dried and the residue resuspended in HPLC-grade chloroform.

HPLC Analysis. Camptothecin standard was prepared by dissolving in chloroform. Camptothecin was quantified by reversed-phase HPLC on a C18 column (Waters No. WAT 025843) detected at 254 nm (Waters 996, Milford, MA). Although an internal standard was not used for each sample, CPT standards were run at the beginning and end of each HPLC run to verify retention times and to quantify CPT. The isocratic mobile phase was acetonitrile−water (3:7) at a flow rate of 2 mL/min. The retention time for camptothecin was 4.1 min, and peak area was used to calculate CPT concentration. All reagents were purchased from Sigma Chemical (St. Louis, MO).

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References and Notes

(1) Wall, M. E.; Wani, M. C.; Cook, C. C.; Palmer, K. H.; McPhail, A. T.; Sim, G. A. *J. Am. Chem. Soc.* **1966**, *88*, 3888−3890.
(2) Creemers, G. J.; Bolis, G.; Gore, M.; Scarfone, G.; Lacave, A. J.; Gustalla, J. P.; Despax, R.; Favalli, G.; Kreinberg, R.; Vanbelle, S.; Hudson, I.; Verweij, J.; Huinink, W. W. T. *J. Clin. Oncol.* **1996**, *14*, 3056−3061.
(3) Dancey, J.; Eisenhauer, E. A. *Brit. J. Cancer* **1996**, *74*, 327−338.
(4) Kjeldsen, E.; Svejstrup, J. Q.; Gromova, I. I.; Alsner, J.; Westergaard, O. *J. Mol. Biol.* **1992**, *228*, 1025−1030.
(5) Priel, E.; Showalter, S. D.; Blair, D. G. *AIDS Res. Hum. Retrovir.* **1991**, *7*, 65−72.
(6) Priel, E.; Showalter, S. D.; Roberts, M.; Oroszlan, S.; Blair, D. G. *J. Virol.* **1991**, *65*, 4137−4141.
(7) Li, C. J.; Wang, C.; Pardee, A. B. *J. Biol. Chem.* **1994**, *269*, 7051−7054.
(8) Bodley, A. L.; Shapiro, T. A. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3726−3730.
(9) López-Meyer, M.; Nessler, C. L.; McKnight, T. D. *Planta Med.* **1995**, *60*, 558−560.
(10) van Hengel, A. J.; Harkes, M. P.; Wichers, H. J.; Hesselink, G. M.; Buitelaar, R. M. *Plant Cell Tiss. Org. Cult.* **1992**, *28*, 11−18.

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