Quantitation of Aryltetralin Lignans in Plant Parts and among Different Populations of *Podophyllum peltatum* by Reversed-Phase High-Performance Liquid Chromatography

Jairo K. Bastos,[†,‡] Charles L. Burandt, Jr.,[‡] N. P. Dhammika Nanayakkara,[‡] Laura Bryant,[‡] and James D. McChesney*[,§]

Research Institute of Pharmaceutical Sciences and Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, Mississippi 38677

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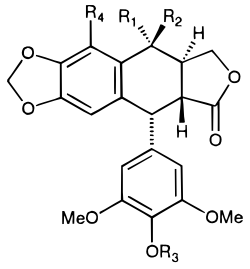
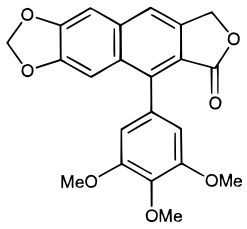
A simple procedure for the extraction of aryltetralin lignans from *Podophyllum peltatum* with addition of an internal standard (acetylpodophyllotoxin) was developed. Plant material was separated into rhizomes and roots, petioles, and blades, and the lignan content of the ground tissue of these different parts was analyzed by reversed-phase HPLC. Podophyllotoxin and seven of its congeners were quantified. It was observed that the lignan content of the different plant parts may differ by orders of magnitude. More significantly, this degree of variation found among tissues of plant parts was not consistent from one population to another. The recovery of podophyllotoxin and acetylpodophyllotoxin in these different tissues is discussed.

Podophyllotoxin and related compounds found in *Podophyllum* spp. are the commercial precursors of semisynthetic etoposide, etoposide phosphate, and teniposide,[1] important drugs in the treatment of testicular and small-cell lung cancer.[2] The demand for plant material, however, has endangered the more common source of podophyllotoxin, *Podophyllum emodii* Wall. ex Royale (Berberidaceae), a native of India.[3] The closely related American species, *Podophyllum peltatum* L., is widespread and abundant but has not been systematically evaluated as a potential alternative source of podophyllotoxin.[4] A prerequisite to an appraisal of the species as a drug source is the development of a reliable procedure to determine rapidly the content of podophyllotoxin and other aryltetralin lignans in a variety of plant tissues. In this study the reversed-phase HPLC method of Bastos et al.[5] has been enhanced by the incorporation of an internal standard and recovery study for each plant tissue. Reported are the analytical results for different plant parts from five populations of *P. peltatum*.

The aryltetralin lignan content in plant parts gathered from five populations of *P. peltatum* L. are recorded in Table 1. Representative lignan profiles for the different tissues are shown in the chromatograms of rhizomes/roots, petioles, and blades (Figure 1a, b, and c, respectively).

Epipodophyllotoxin 4-*O*-β-D-glucopyranoside (**1**), α-peltatin (**4**), podophyllotoxin (**6**), and β-peltatin (**7**) appear to be common to all parts studied. Their concentrations, however, varied by orders of magnitude among populations. More significantly, this degree of variation found among tissues of plant parts was not consistent from one popupation to another. Most notable was the presence of podophyllotoxin at concentrations higher in blades than in the rhizomes of the Hickman County material. Although rhizomes of *P. peltatum* are a well-established source of podophyllotoxin[6] (**6**) there are few reports on the content of podophyllotoxin in blades. Kusnetsova and Bogdanova have detected significant amounts of podophyllotoxin in blades by TLC,[7] while Yoo and Porter found only trace amounts using an enzyme immunoassay method.[8] This diversity of results suggests that the presence of podophyllotoxin and related compounds in the various parts of *P. peltatum* is influenced by complex biological, geographical, and environmental variables as yet unstudied. Probable sources of variation are the subject of an ongoing study wherein we are evaluating material of *P. peltatum* from throughout its range.





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	R_1	R_2	R_3	R_4
1	Oglc	H	Me	H
2	H	Oglc	Me	H
3	OH	H	H	H
4	H	H	H	OH
5	H	OH	Me	H
6	OH	H	Me	H
7	H	H	Me	OH
8	OAc	H	Me	H

Experimental Section

General Experimental Procedures. Melting points were determined on a Thomas-Hoover Uni-melt capillary apparatus or a Fisher-Johns digital melting point analyzer Model 355 and were not corrected. Optical

[†] Present address: Pharmacy School, University of São Paulo, Faculdade de Ciências Farmacêuticas de Ribeirão Preto−USP, Via do Café S/N, Ribeirão Preto−São Paulo, 14040-903, Brazil.
[‡] Research Institute of Pharmaceutical Sciences.
[§] Department of Pharmacognosy.
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Table 1. Aryltetralin Lignan Content of Plant Parts from Five Populations of *P. peltatum*

source	compound (mg/100 g of dried plant material)[a]							
	1	2	3	4	5	6	7	9
Desoto County, MS								
rhizomes/roots	trace[b]	103.6	1.9	115.0	0[c]	76.4	15.2	0
petioles	0	17.1	trace	21.6	0	6.3	1.1	0
blades	trace	0	0	185.3	trace	2.6	7.7	0
Drew County, AR								
rhizomes/roots	trace	459.6	2.8	72.9	0	260.4	9.2	0
petioles	0	47.6	0	14.7	0	13.0	1.8	0
blades	1.0	11.3	trace	32.5	0	0	4.9	0
HickmanCounty, TN								
rhizomes/roots	0	34.2	11.4	108.0	0	128.4	7.1	trace
petioles	trace	14.2	0	9.2	trace	31.3	trace	0
blades	trace	274.2	1.4	1.9	trace	542.6	trace	0
Lafayette County, MS								
rhizomes/roots	trace	191.7	3.0	144.0	0	190.9	28.8	trace
petioles	0	37.2	trace	20.1	0	12.4	4.8	0
blades	trace	0	42.2	trace	1.3	2.8	8.9	0
Putnam County, TN								
rhizomes/roots	trace	242.7	3.1	63.2	0	122.5	12.9	trace
petioles	trace	4.3	0	24.3	0	6.9	3.9	0
blades	7.4	1.7	trace	108.0	1.1	trace	53.0	0

[a] Key to compounds: podophyllotoxin 4-*O*-*β*-D-glucopyranoside (**1**), epipodophyllotoxin 4-*O*-*β*-D-glucopyranoside (**2**), 4′-demethylpodophyllotoxin (**3**), α-peltatin (**4**), epipodophyllotoxin (**5**), podophyllotoxin (**6**), *β*-peltatin (**7**), 1,2,3,4-dehydrodesoxypodophyllotoxin (**9**). [b] Trace means peak was present at retention volume of compound but below limits of quantification. [c] 0 means no detectable peak was present at retention volume of compound.

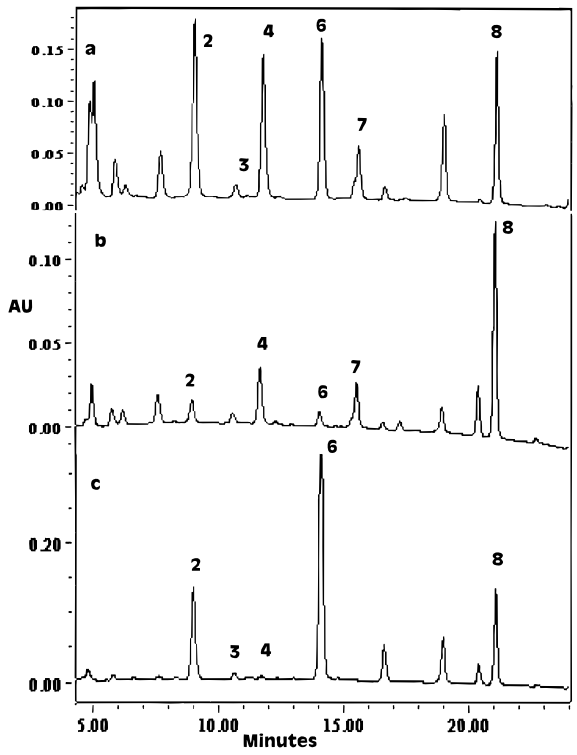

Figure 1. (a) Chromatogram obtained for the aryltetralin lignans extracted from rhizomes/roots of *P. peltatum* L. collected in Drew County, AR. (b) Chromatogram obtained for the aryltetralin lignans extracted from petioles of *P. peltatum* L. collected in Lafayette County, MS. (c) Chromatogram obtained for the aryltetralin lignans extracted from blades of *P. peltatum* L. collected in Hickman County, TN.

rotations were determined on a Perkin-Elmer 141 automatic polarimeter using MeOH solutions. UV spectra were obtained on a Perkin-Elmer Lambda 3B UV/vis spectrophotometer in MeOH solutions. IR spectra were obtained as KBr pellets on a Mattson Genesis Series FT-IR spectrometer. NMR spectra were recorded at 300 MHz for proton and 75 MHz for carbon on a Varian VXR-300 instrument, and the solvent signal was used as reference. LREIMS and HRMS were obtained in the Department of Chemistry, University of Kansas, at Lawrence. Si gel (230−400 mesh, Merck) was used for column chromatography. All solvents used for chromatographic purposes were AR grade.

Plant Material. *P. peltatum* L. (mayapple) was harvested from five populations: Drew County, AR; Desoto and Lafayette Counties, MS; and Hickman and Putnam Counties, TN. Plants were separated (while fresh) into petioles, blades, and rhizomes with their roots attached. These were immediately dried at 40 °C and ground to powders. Sufficient material was collected to yield a minimum of 25 g of dried biomass for each plant part. Ethanol (10 mL) containing 400 *μ*g/mL of the internal standard was added to 2 g of dried plant material in a 125-mL Erlenmeyer flask. Flasks were sealed and shaken for 2 h at 40 °C. The EtOH extract was filtered through filter paper, and 5 mL was transferred to a 50-mL round-bottomed flask and evaporated to dryness. The resulting resin was dissolved and partitioned between 20 mL of EtOAc and 20 mL of H_2O. After partition, 10 mL of the organic phase was evaporated to dryness under reduced pressure and then dissolved in 10 mL of EtOH. A 10-*μ*L sample of the final EtOH solution was analyzed on HPLC using a Taxsil (MetaChem Technologies Inc., Torrance, CA) column following the work of Bastos et al.[5]

Standard Compounds. The isolation and structural identification of the standard compounds podophyllotoxin 4-*O*-*β*-D-glucopyranoside (**1**), 4′-demethylpodophyllotoxin (**3**), α-peltatin (**4**), epipodophyllotoxin (**5**), podophyllotoxin (**6**), *β*-peltatin (**7**), and 1,2,3,4-dehydrodesoxypodophyllotoxin (**9**) are described in a previous paper.[5] In addition to these seven standards, epipodophyllotoxin 4-*O*-*β*-D-glucopyranoside (**2**) was isolated from rhizomes of a Lafayette County, MS, collection by a similar procedure using column chromatography on Si gel. The identity of compound **2** was confirmed by comparison of its physical and spectroscopic data (mp, UV, ^{1}H and ^{13}C NMR) with the literature values.[6,9,10]

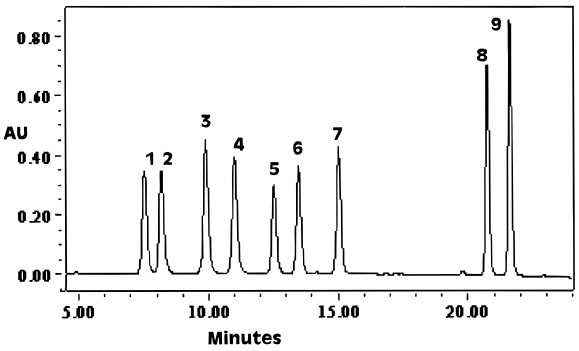

Figure 2. Chromatogram of aryltetralin lignan standards from *Podophyllum* spp. and the internal standard: podophyllotoxin 4-*O*-β-D-glucopyranoside (**1**), epipodophyllotoxin 4-*O*-β-D-glucopyranoside (**2**), 4′-demethylpodophyllotoxin (**3**), α-peltatin (**4**), epipodophyllotoxin (**5**), podophyllotoxin (**6**), β-peltatin (**7**), acetylpodophyllotoxin (**8**), 1,2,3,4-dehydrodesoxypodophyllotoxin (**9**).

Acetylpodophyllotoxin (**8**) was chosen as an internal standard because of its structural similarity to the lignans of interest and the ease of synthesis from podophyllotoxin by acetylation of its hydroxyl group using the procedure described by Aiyar and Chang[11] and its elution in a region of the chromatogram devoid of other peaks using the cited methodology[5] (Figure 2).

HPLC Analysis. Instrumentation consisted of a Waters LC Module I multisolvent delivery system, Waters 715 autoinjector, Waters pump 600, Waters detector 486 operating at 240 and 285 nm (Millipore Corp., Waters Chromatography Division, Milford, MA), and a computer (NEC power mate 386/33i, Millennium 2000) for control of the analytical system, data collection, and processing.

HPLC analysis was carried out using the methodology described earlier.[5] The linearity of the detector response was determined for each of nine lignan standards by a series of injections of solutions from 5 to 5000 μg/mL. The curve correlating concentration and area for podophyllotoxin, acetylpodophyllotoxin, and all other standards was linear through a concentration of 1250 μg/mL. All injected solutions were of concentrations less than this figure.

Recovery of Podophyllotoxin and Acetylpodophyllotoxin. The recovery of podophyllotoxin (**6**) and acetylpodophyllotoxin (**8**) from different plant tissues was determined on the mayapple population from Desoto County, MS. Initial screening of this collection showed low podophyllotoxin contents of 76.4, 6.3, and 2.6 mg/100 g of dry matter for rhizome/root, petiole, and blade tissue, respectively. For each of these plant materials, a 2-g sample was admixed with sufficient acetylpodophyllotoxin (**8**) to yield a concentration of 400 μg/mL in the extract, and podophyllotoxin (**6**) was added to the extraction solvent to yield an expected concentration of 2000 μg/mL. The extraction process was followed as described earlier, and in order to determine the actual amounts of acetylpodophyllotoxin (**8**) and podophyllotoxin (**6**) recovered, an external standard, 1,2,3,4-dehydrodesoxypodophyllotoxin (**9**) (Figure 2), not present in the plant material selected for the recovery study, was added to the final extract (80 μg/1.0 mL of solution) and then used to calculate recoveries. The procedure was repeated three times for each tissue. Using equations obtained from the plotted linearity of the detector response, the absolute amounts recovered of both podophyllotoxin and acetylpodophyllotoxin were calculated. The mean recoveries (% $\pm$ SE) of podophyllotoxin and the internal standard for rhizome/root, petiole, and blade tissues were 82.1 $\pm$ 1.2 and 80.3 $\pm$ 0.9, 108.2 $\pm$ 1.5 and 106.3 $\pm$ 2.2, and 97.9 $\pm$ 3.7 and 74.9 $\pm$ 1.5, respectively. Recoveries of other lignans are assumed to be equivalent to that of podophyllotoxin. Lignan contents of blades were adjusted to reflect the disparity between recovery of the internal standard and podophyllotoxin.

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References and Notes

(1) Stähelin, H. F.; von Wartburg, A. *Cancer Res.* **1991**, *51*, 5−15.
(2) Jardine, I. In *Anti Cancer Agents Based on Natural Products Models*; Cassady, J. M.; Douros, J. D., Eds.; Academic Press: New York, 1980; pp 319−351.
(3) Nautiyal, M. C.; Rawat, A. S.; Bhaduala, S. K.; Purohit, A. N. *Seed Res.* **1987**, *15*, 206−209.
(4) Meijer, W. *Econ. Bot.* **1974**, *28*, 68−72.
(5) Bastos, J. K.; Kopycki, W. J.; Burandt, C. L., Jr.; Nanayakkara, N. P. D.; McChesney, J. D. *Phytochem. Anal.* **1995**, *6*, 101−105.
(6) Broomhead, A. J.; Dewick, P. M. *Phytochemistry* **1990**, *29*, 3831−3844.
(7) Kuznetsova, G. A.; Bogdanova, V. P. *Rast. Resur.* **1970**, *6*, 240−243; *Chem. Abstr.* **1971**, *74*, 1085w.
(8) Yoo, K.-J.; Porter, J. R. *J. Nat. Prod.* **1993**, *56*, 715−721.
(9) Agrawal, P. K.; Thakur, R. S. *Mag. Reson. Chem.* **1985**, *23*, 389−418.
(10) San Feliciano, A.; Medarde, M.; Lopez, J. L.; Puebla, P.; Del Corral, M.; Barrero, A. F. *Phytochemistry* **1989**, *28*, 2863−2866.
(11) Aiyar, V. N.; Chang, F. C. *J. Org. Chem.* **1977**, *42*, 246−249.

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