Isolation and Identification of Stilbenes in Two Varieties of *Polygonum cuspidatum*

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The roots of two varieties of *Polygonum cuspidatum* (Hu Zhang and Mexican Bamboo) were analyzed for resveratrol and analogues. The roots of each variety were dried and ground into a powder. The powdered roots were then extracted with methanol and ethyl acetate. The ethyl acetate fraction of the Mexican Bamboo was then subjected to fractionation and purification using silica gel column chromatography and semipreparative HPLC. In addition to resveratrol (3,5,4′-trihydroxystilbene), three stilbene glucosides were identified by ^{1}H NMR, ^{13}C NMR, and MS. The stilbene glucosides were shown to be a piceatannol glucoside (3,5,3′,4′-tetrahydroxystilbene 4′-*O*-*β*-D-glucopyranoside), resveratroloside (3,5,4′-trihydroxystilbene 4′-*O*-*β*-D-glucopyranoside), and piceid (3,5,4′-trihydroxystilbene 3-*O*-*β*-D-glucopyranoside). The levels of the piceatannol glucoside and piceid were twice as high in the Mexican Bamboo as compared to the Hu Zhang.

Keywords: *Polygonum cuspidatum; stilbene glucoside; resveratrol; piceatannol; resveratroloside; piceid; Hu Zhang; Mexican Bamboo; Japanese Knotweed*

INTRODUCTION

The roots of *Polygonum cuspidatum* (PC) have been used in ancient Chinese and Japanese herbal medicines for a variety of therapeutic purposes. Ancient folk medicines have used PC as laxatives and occasionally as foods (Spainhour, 1997). In addition, the powder of dried PC roots has been used in Asia to treat atherosclerosis (Kimura et al., 1983, 1985; Shan et al., 1990) as well as other medical ailments including cough, asthma, hypertension, and cancer (Su et al., 1995). Originally PC is from China where it is commonly referred to as Hu Zhang (HZ) or Hu Chang. It then migrated to Japan where it is known as Kojo Kon. In addition to Asia, PC can also be found growing throughout North America. The variety that grows in North America may be referred to as Mexican Bamboo (MB), Japanese Bamboo, or Japanese Knotweed. MB has gained much notoriety mainly due to its virtually indestructible growing characteristics and as a pernicious weed here in the Northeast.

Stilbenes are a class of biologically active components found in PC roots that have been shown to possess various medicinal properties (Goldberg et al., 1996). Resveratrol (3,5,4′-trihydroxystilbene) has been most extensively investigated. It has been proposed that resveratrol protects human low-density lipoprotein (LDL) against copper-catalyzed oxidation (Frankel et al., 1993), has cancer chemopreventive activity in the three major stages of carcinogenesis (Jang et al., 1997), and could modulate hepatic synthesis of triglyceride and cholesterol in the rat (Arichi et al., 1982). In addition, resveratrol inhibits platelet clotting (Waterhouse et al., 1994) and reduces injury to the liver as a result of peroxidized oil (Kimura et al., 1983). The antioxidant properties of stilbene analogues have also been investigated. The most potent of these analogues against oxidation of the human LDL is piceatannol (3,5,3′,4′-tetrahydroxystilbene) (Teguo et al., 1998).

Much research has been done on the identification of stilbenes in wines cultivated throughout the world (Goldberg et al., 1996; Lamuela-Raventos et al., 1995; Sato et al., 1997). However, the amount of stilbenes that occurs in wine is far less than the amount found in the roots of PC. The objective of this study was to determine the structures of the naturally occurring stilbenes present in two varieties of PC roots and compare the amount of stilbenes in MB to that in the HZ in hopes that this pernicious weed may be used as an alternative crop in the nutraceutical industry.

MATERIALS AND METHODS

General Procedures. ^{1}H and ^{13}C NMR spectra were obtained on a VXR-200 instrument, and MS analysis was performed on a Micromass Platform II system (Micromass Co., MA) equipped with a Digital DECPc XL560 computer for analysis of data. Mass spectra were obtained using atmospheric pressure chemical ionization (APCI) in the negative-ion mode. The ion source temperature was set at 150 °C, and the probe temperature was set at 450 °C. The sample cone voltage was 10 V, and the corona discharge was 3.2 kV. HPLC analysis was performed on a Varian Vista 5500 liquid chromatograph pump coupled to a Varian 9065 Polychrom diode array detector. Fractionation of purified compounds was obtained on a Waters 600E HPLC pump coupled to a Waters Lambda-Max model 481 LC spectrophotometer. Selecto Scientific silica gel (100−200 mesh particle size) was used for column chromatography. All fractions were screened on Whatman silica gel thin-layer chromatography (TLC) plates (250 μm thickness, 60 A silica gel medium) with compounds revealed under fluorescent light. The column packing and TLC plates were both purchased from Fisher Scientific (Springfield, NJ). *trans*-Resveratrol was purchased from Sigma Chemical Co. (St. Louis, MO) for HPLC quantitation. All solvents used for extraction and isolation were of HPLC grade and purchased from Fisher Scientific.

Plant Material. The dried roots of HZ were imported from China and purchased from Sam Luen Co. (48 KO Shing St., Hong Kong). The roots of MB were obtained from a wild batch growing along the Raritan River in Piscataway, NJ.

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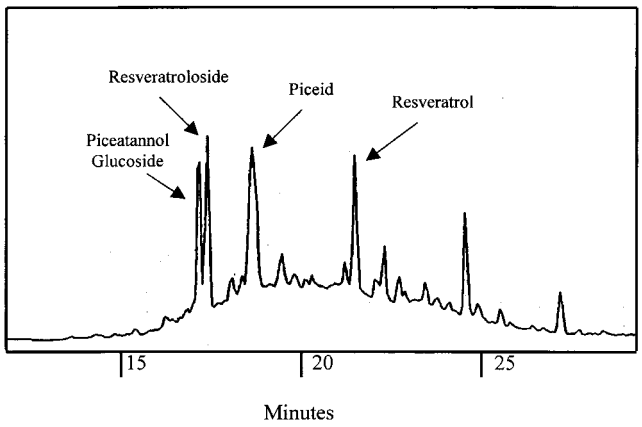


Figure 1. Reversed-phase HPLC chromatogram of MB ethyl acetate extract at 254 nm. The piceatannol glucoside eluted at 17.2 min, resveratroloside eluted at 17.4 min, piceid eluted at 18.6 min, and resveratrol eluted at 21.5 min.

Extraction and Isolation Procedures. The roots were washed and dried prior to being ground into a powder. The powdered roots of HZ (2 × 35 g) and MB (4 × 45 g) were continuously extracted with methanol in a Soxhlet extraction apparatus. The methanol extracts were concentrated under vacuum using rotary evaporation. The remaining concentrate was then partitioned with acidified ethyl acetate (3% HCl). The ethyl acetate extracts of HZ and MB were quantitatively analyzed for stilbene analogues using HPLC and external standards of *trans*-resveratrol (see Figure 1).

Separation was performed on a Discovery C18 reversed-phase column (250 mm × 4.6 mm, 5 μm) with a column guard purchased from Supelco (Bellefonte, PA). The solvent program was a gradient system: A, water with 0.15% triethylamine (TEA) purchased from Sigma Chemical Co. and 0.18% formic acid (FA) purchased from Fisher Scientific; B, acetonitrile. The elution program at 1 mL min^{-1} was as follows: 100% A (0−5 min); 100% A to 100% B (5−35 min); 100% B (35−55 min). The wavelengths monitored were 220−320 nm with a Varian 9065 diode array detector.

The dry ethyl acetate extracts were then chromatographed on a silica gel column. Elution was performed using a solvent mixture of chloroform/methanol with an increasing amount of methanol (30:1, 25:1, 20:1, 15:1, 10:1, 7.5:1, 5:1, 4:1, 3:1, 2:1, 1:1, 0:1; each 500 mL) (Chen et al., 1999). Fractions were screened for the presence of stilbenes using silica gel TLC plates, reversed-phase HPLC, and APCI LC−MS in the negative-ion mode. The fraction eluted with 7.5:1 chloroform/methanol was determined to contain three stilbene glucosides. This fraction was then rechromatographed on a silica gel column and was eluted with ethyl acetate/methanol/water at a ratio of 15:1:1 (Chen et al., 1999). Successive fractions were collected and screened for stilbenes using silica gel TLC plates. Final separation of pure compounds was obtained using semipreparative HPLC on a Zorbax Rx-C18 reversed-phase column (9.4 mm × 240 mm, 5 μm) purchased from Mac-Mod Analytical (Chadds Ford, PA). Compounds were eluted by an isocratic solvent system containing 85% water with 0.15% TEA and 0.18% FA; 15% acetonitrile. The solvent program was at 3 mL min^{-1}, and the wavelength monitored was 254 nm.

RESULTS AND DISCUSSION

The ethyl acetate extracts of HZ and MB were both analyzed versus external standards of *trans*-resveratrol using reversed-phase HPLC and APCI LC−MS in the negative-ion mode. The HPLC conditions are given in the Materials and Methods section. The HPLC chromatogram of the MB ethyl acetate extract at 254 nm is presented in Figure 1. Quantitation for the amounts of resveratrol and the three stilbene glucosides is shown in Table 1 and assumes that all compounds have the same response factor as *trans*-resveratrol. It was evident that the levels of both the piceatannol glucoside and piceid are approximately 2 times greater in the roots of MB than in the roots of HZ. The level of resveratroloside was determined to be twice as high in the roots of HZ as compared to the roots of MB, and the level of free resveratrol was slightly higher in the roots of HZ than in MB. However, material from only one source was tested for each variety.

Table 1. Quantitation of Stilbene Analogues in Two Varieties of PC Roots[a]

PC variety	piceatannol glucoside (mg/g dry root)	resveratroloside (mg/g dry root)	piceid (mg/g dry root)	resveratrol (mg/g dry root)
HZ	1.22	5.31	2.32	3.77
MB	2.76	2.77	5.31	2.96

[a] The coefficient of variation for these determinations was 0.23.

The fraction eluted with 7.5:1 chloroform/methanol was evaporated to dryness under nitrogen at room temperature. The fraction was then reconstituted in methanol and analyzed by APCI LC−MS in the negative-ion mode. It was determined that the peak at 17.2 min on the HPLC chromatogram had a mass of 406 (M^- ion) and a fragment at *m/z* 405 ([M − H]$^-$ ion) and was tentatively identified as a piceatannol glucoside. Its corresponding aglycon (A) was observed at mass 243 ([A − H]$^-$ ion), as shown in Figure 2. The peak at 17.4 min had a mass of 389 ([M − H]$^-$ ion) and was tentatively identified as resveratroloside. Its corresponding aglycon was observed at mass 227. The peak at 18.6 min on the HPLC chromatogram also had a mass of 389 ([M − H]$^-$ ion) and was ultimately determined to be piceid. Its corresponding aglycon was observed at mass 227. The peak which eluted at 21.5 min was identified as resveratrol and had a mass of 227 ([M − H]$^-$ ion).

For ultimate proof of structure, isolation and purification of these three stilbene glucosides were necessary for NMR studies. The isolation of the total stilbene glucoside content from the 7.5:1 chloroform/methanol fraction was performed by rechromatographing this fraction on a silica gel column, eluting with ethyl acetate/methanol/water at a ratio of 15:1:1 (Chen et al., 1999). Eluted fractions were collected and screened by TLC. Final purification of the three stilbene glucosides was performed using reversed-phase semipreparative HPLC. The HPLC parameters for purification are given in the Materials and Methods section. The three stilbene glucosides were determined to be a piceatannol glucoside (3,5,3′,4′-tetrahydroxystilbene 4′-*O*-β-D-glucopyranoside), resveratroloside (3,5,4′-trihydroxystilbene 4′-*O*-β-D-glucopyranoside), and piceid (3,5,4′-trihydroxystilbene 3-*O*-β-D-glucopyranoside). The structures of the three stilbene glucosides, as shown in Figure 3, were determined by NMR studies.

Structure Determination of Isolated Compounds. *Piceatannol glucoside (3,5,3′,4′-tetrahydroxystilbene 4′-O-β-D-glucopyranoside):* APCI *m/z* 405 [M − H]$^-$; ^{1}H NMR δ 7.17 (1H, br d, J = 8.4 Hz, H-6′), 7.05 (1H, d, J = 1.9 Hz, H-2′), 6.94 (1H, d, J = 16.0 Hz, H-8), 6.92 (1H, d, J = 8.4 Hz, H-5′), 6.84 (1H, d, J = 16.0 Hz, H-7), 6.47 (2H, br s, H-2, 6), 6.19 (1H, br s, H-4), glucose 4.80 (1H, d, J = 7.2 Hz, H-1″), 3.90 (1H, br d, J = 12.0 Hz, H-6″a), 3.72 (1H, dd, J = 12.0, 5.6 Hz, H-6″b), 3.36−3.56 (4H, m, H-2″, 3″,4″,5″); ^{13}C NMR δ 160.1 (C-3,5), 148.8 (C-4′), 146.9 (C-3′), 141.2 (C-1), 134.9 (C-1′), 129.3

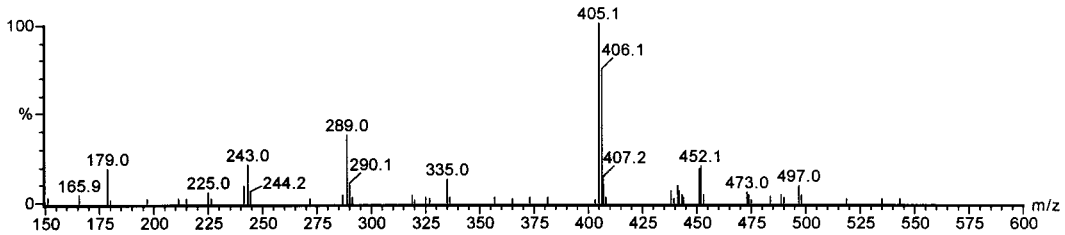
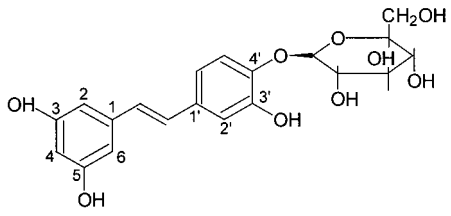
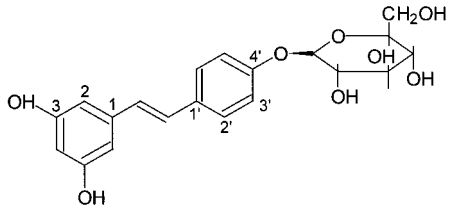


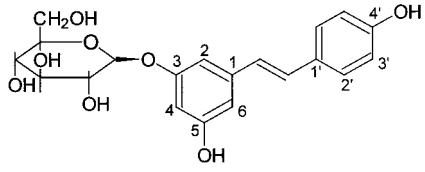
Figure 2. APCI negative-ion mass spectrum of a piceatannol glucoside (3,5,3′,4′-tetrahydroxystilbene 4′-*O*-*β*-D-glucopyranoside): *m/z* 406 = M^-, 405 = $[M - H]^-$, 243 = aglycon − H^-, 179 = glucose − H^-; other major peaks are due to formic acid adducts from the mobile phase.



Piceatannol Glucoside (3,5,3′,4′-tetrahydroxystilbene-4′-*O*-β-D-glucopyranoside)



Resveratroloside (3,5,4′-trihydroxystilbene-4′-*O*-β-D-glucopyranoside)



Piceid (3,5,4′-trihydroxystilbene-3-*O*-β-D-glucopyranoside)

Figure 3. Structures of stilbene glucosides isolated from MB.

(C-8), 129.2 (C-7), 120.1 (C-6′), 118.9 (C-5′), 114.8 (C-2′), 106.3 (C-2,6), 104.5 (C-4), glucose 103.3 (C-1″), 78.7 (C-3″), 77.9 (C-5″), 75.2 (C-2″), 71.6 (C-4″), 62.7 (C-6″).

Resveratroloside (3,5,4′-trihydroxystilbene 4′-O-β-D-glucopyranoside): APCI *m/z* 389 $[M - H]^-$; ^{1}H NMR *δ* 7.46 (2H, d, *J* = 8.6 Hz, H-2′,6′), 7.09 (2H, d, *J* = 8.6 Hz, H-3′,5′), 7.03 (1H, d, *J* = 16.0 Hz, H-8), 6.89 (1H, d, *J* = 16.0 Hz, H-7), 6.49 (2H, br s, H-2,6), 6.20 (1H, br s, H-4), glucose 4.90 (1H, d, *J* = 7.6 Hz, H-1″), 3.92 (1H, dd, *J* = 12.0,1.6 Hz, H-6″a), 3.70 (1H, dd, *J* = 12.0, 5.6 Hz, H-6″b), 3.40−3.56 (4H, m, H-2″,3″,4″,5″); ^{13}C NMR *δ* 160.1 (C-3,5), 159.0 (C-4′), 141.0 (C-1), 133.4 (C-1′), 129.2 (C-8), 128.9 (C-2′,6′), 128.8 (C-7), 118.2 (C-3′,5′), 106.2 (C-2,6), 103.3 (C-4), glucose 102.5 (C-1″), 78.5 (C-3″), 78.3 (C-5″), 75.2 (C-2″), 71.7 (C-4″), 62.8 (C-6″) (Jayatilake et al., 1993).

Piceid (3,5,4′-trihydroxystilbene 3-O-β-D-glucopyranoside): APCI *m/z* 389 $[M - H]^-$; ^{1}H NMR *δ* 87.38 (2H, d, *J* = 8.5 Hz, H-2′,6′), 87.01 (1H, d, *J* = 16.0 Hz, H-8), 6.85 (1H, d, *J* = 16.0 Hz, H-7), 6.79 (1H, br s, H-2), 6.78 (2H, d, *J* = 8.4 Hz, H-3′,5′), 86.64 (1H, br s, H-6), 86.46 (1H, br s, H-4), glucose 4.90 (1H, d, *J* = 7.2 Hz, H-1″), 3.92 (1H, dd, *J* = 12.1,1.6 Hz, H-6″a), 3.74 (1H, dd, *J* = 12.1, 5.6 Hz, H-6″b), 3.38−3.50 (4H, m, H-2″,3″,4″,5″); ^{13}C NMR *δ* 160.8 (C-3), 160.0 (C-5), 141.7 (C-1), 130.5 (C-1′), 130.3 (C-8), 129.2 (C-2′,6′), 126.9 (C-7), 118.9 (C-4′), 116.9 (C-3′,5′), 108.6 (C-6), 107.2 (C-2), 104.4 (C-4), glucose 102.6 (C-1″), 78.6 (C-3″), 78.4 (C-5″), 75.3 (C-2″), 71.8 (C-4″), 62.8 (C-6″) (Jayatilake et al., 1993; Teguo et al., 1996).

Resveratrol, resveratroloside, and piceid have all previously been identified in roots of PC (Jayatilake et al., 1993) as well as in cell cultures of *Vitis vinifera* (Teguo et al., 1998). In addition, resveratrol and piceid have been identified in grape berries (Waterhouse et al., 1994) and in wine (Sato et al., 1997). Astringin, a piceatannol glucoside with its glucose in the 3 position, has been identified in cell cultures of *V. vinifera* (Teguo et al., 1998) and in wine (Ribeiro de Lima et al., 1999). In addition, astringin has been identified in the fruits of *Melaleuca leucadendron* (Tsuruga et al., 1991). A piceatannol glucoside has also been identified in Norway spruce needles; however, its exact structural isomer was not reported (Kettrup et al., 1991). The piceatannol glucoside which we have isolated from MB has its glucose in the 4′ position.

In summary, resveratrol and three naturally occurring stilbene glucosides were isolated from the roots of MB, and their precise chemical structures were elucidated. In addition, a quantitative analysis was performed on two different varieties of PC roots such that the amounts of the stilbene analogues could be compared. The potential use of MB in the nutraceutical industry as a medicinal crop may be an economical advantage due to its status as a pernicious weed growing throughout the Northeast and its high stilbene content.

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