Full Papers

Polyphenolic Constituents of *Actaea racemosa*[⊥]

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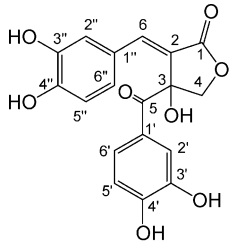
A new lignan, actaealactone (**1**), and a new phenylpropanoid ester derivative, cimicifugic acid G (**2**), together with 15 known polyphenols, protocatechuic acid, protocatechualdehyde, *p*-coumaric acid, caffeic acid, methyl caffeate, ferulic acid, ferulate-1-methyl ester, isoferulic acid, 1-isoferuloyl-β-D-glucopyranoside, fukinolic acid, and cimicifugic acids A, B, and D−F, were isolated from an extract of the rhizomes and roots of black cohosh (*Actaea racemosa*). The structures of the new compounds were determined on the basis of NMR spectroscopic analysis. Compounds **1** and **2** displayed antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical assay with IC_{50} values of 26 and 37 μM, respectively. Other antioxidants identified from *A. racemosa* include cimicifugic acid A (**3**), cimicifugic acid B (**4**), and fukinolic acid (**5**). Compounds **1** and **2** also exhibited a small stimulating effect on the growth of MCF-7 breast cancer cell proliferation 1.24-fold (14 μM) and 1.14-fold (10 μM), respectively, compared to untreated cells.

Black cohosh [*Actaea racemosa* L. (synonym: *Cimicifuga racemosa*)] is a native North American plant in the buttercup family (Ranunculaceae). Its rhizomes and roots have long been used by Native Americans to treat a variety of ailments, including malaise, gynecological disorder, diarrhea, sore throat, and rheumatism.[1] Black cohosh is now one of the most important botanical dietary supplements for the treatment of menopausal symptoms in the United States and Europe. Numerous studies have shown the beneficial effect of black cohosh extract on the treatment of menopausal symptoms, and these are summarized in recent review papers.[2,3] Black cohosh extracts have exhibited various biological activities including anticancer,[4−6] anti-inflammatory,[7] and antioxidant.[8] A mechanism of action of black cohosh for any of the aforementioned biological activities has not been determined, but it is possibly the result of complex synergistic action of its components.[9]

Previous chemical investigations on black cohosh reported the isolation of two principal groups of compounds, triterpenoid glycosides and polyphenolic derivatives.[10−18] To date, over 40 triterpenoid glycosides have been isolated from black cohosh, and they exhibited various biological activities including anticancer activity,[6,16,19] anti-HIV activity,[20] and inhibitory effect on catecholamine secretion.[21] With regard to polyphenolic derivatives, 13 compounds have been isolated from the rhizomes and roots of black cohosh, including hydroxycinnamic acid derivatives (e.g., caffeic acid, ferulic acid, and isoferulic acid), fukiic acid ester derivatives (e.g., fukinolic acid and cimicifugic acids A and B), and piscidic acid ester derivatives (e.g., cimicifugic acids E and F).[17,18] Certain black cohosh compounds have been reported to have estrogenic,[17] anti-inflammatory,[22] and antioxidant activities[8] and exhibit inhibitory effects on the enzymatic activities of α-amylase, carboxypeptidase A,[23] and collagenase.[24]

Because of the increasing use of black cohosh by menopausal women, it is important to better understand the phytochemical constituents of black cohosh. In a preliminary phytochemical study of black cohosh, we detected many minor polyphenols by an HPLC-PDA method. As part of a continuing phytochemical study of black cohosh, we have investigated in greater detail the polyphenolic constituents of black cohosh. A black cohosh alcoholic extract was fractionated, and two new polyphenolic compounds, actaealactone (**1**) and cimicifugic acid G (**2**), were isolated, along with 15 known



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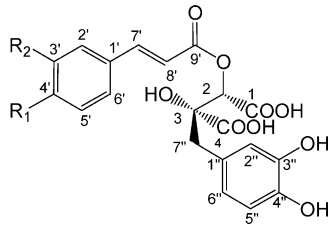

2	$R_1 = OCH_3$	$R_2 = OCH_3$
3	$R_1 = OH$	$R_2 = OCH_3$
4	$R_1 = OCH_3$	$R_2 = OH$
5	$R_1 = OH$	$R_2 = OH$

polyphenolic compounds. Compounds **1** and **2** were evaluated for their antioxidant activity in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radical assay and for their growth effects on the MCF-7 estrogen-sensitive breast cancer cell line, using a cell proliferation assay. In addition, six phenolic compounds, protocatechuic acid, protocatechualdehyde, *p*-coumaric acid, 1-isoferuloyl-β-D-glucopyranoside, ferulate-1-methyl ester, and cimicifugic acid D, are reported for the first time from black cohosh.

[⊥] Dedicated to Dr. Norman R. Farnsworth of the University of Illinois at Chicago for his pioneering work on bioactive natural products.

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Table 1. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) Spectroscopic Data of Compounds **1** and **2** in CD_3OD

	1			**2**			
C/H	δ_C	δ_H (int., mult., *J* in Hz)	HMBC (^{13}C no.)	δ_C	δ_H (int., mult., *J* in Hz)	HMBC (^{13}C no.)	NOSEY (^{1}H no.)
1	172.2 s			169.5 s			
2	123.8 s			76.5 d	5.66 (1H, s)	1, 3, 4, 9′, 7″	7″
3	80.3 s			78.7 s			
4	77.7 t	4.43 (1H, d, 10.5) 4.69 (1H, d, 10.5)	1, 3, 5	173.5 s			
5	195.8 s						
6	144.4 d	7.60 (1H, s)	2″, 6″, 1, 3				
1′	124.9 s			127.4 s			
2′	115.9 d	7.30 (1H, d, 2.1)	4′, 6′, 5	110.2 d	7.30 (1H, d, 1.8)	4′, 6′, 7′	7′, 8′
3′	144.5 s			149.5 s			
4′	151.5 s			151.7 s			
5′	114.2 d	6.71 (1H, d, 8.4)	1′, 3′	111.3 d	7.02 (1H, d, 8.4)	1′, 3′	6′
6′	122.7 d	7.24 (1H, dd, 8.4, 2.1)	2′, 4′, 5	122.9 d	7.25 (1H, dd, 8.4, 1.8)	2′, 4′	5′, 7′, 8′
7′				146.2 d	7.82 (1H, d, 15.9)	2′, 6′, 9′	2′, 6′
8′				114.3 d	6.58 (1H, d, 15.9)	1′, 9′	2′, 6′
9′				166.6 s			
1″	124.9 s			126.7 s			
2″	118.1 d	7.10 (1H, d, 2.4)	4″, 6″, 6	117.4 d	6.76 (1H, d, 1.8)	4″, 6″, 7″	
3″	144.8 s			114.3 s			
4″	149.0 s			143.9 s			
5″	114.7 d	6.67 (1H, d, 8.4)	1″, 3″	114.5 d	6.66 (1H, d, 8.1)	1″, 3″	
6″	125.3 d	7.02 (1H, dd, 8.4, 2.4)	2″, 4″	121.6 d	6.61 (1H, dd, 8.1, 1.8)	4″	
7″				40.8 t	2.94 (1H, d, 13.8) 3.06 (1H, d, 13.8)	2″, 6″, 3	2
3′ -OMe				55.1 q	3.91 (3H, s)	3′	2′
4′ -OMe				55.0 q	3.89 (3H, s)	4′	5′

A black cohosh powdered extract was redissolved in 80% MeOH/water overnight. After the methanol was removed in vacuo, the resulting aqueous fraction was sequentially partitioned with hexane and *n*-butanol. The *n*-butanol-soluble fraction was separated by a combination of chromatographic procedures to obtain the new compound actaealactone (**1**), along with seven known compounds, protocatechuic acid,[25] protocatechualdehyde,[26] *p*-coumaric acid,[27] caffeic acid,[28] ferulic acid,[28,29] isoferulic acid,[30] and 1-isoferuloyl-β-D-glucopyranoside.[31] Chromatographic purification of the water-soluble fraction yielded a second new compound, cimicifugic acid G (**2**), and eight known compounds, methyl caffeate,[32] ferulate-1-methyl ester,[32] fukinolic acid,[33] cimicifugic acid A,[33] cimicifugic acid B,[33] cimicifugic acid D,[34] cimicifugic acid E,[34] and cimicifugic acid F.[34] The known compounds were identified by comparison of their spectroscopic data (UV, MS, and NMR) with published reports.

Actaealactone (**1**) was isolated as a yellow amorphous powder and gave a molecular ion peak at *m*/*z* 359.0749, corresponding to $[M + 1]^+$ in the positive HRESIMS, establishing the molecular formula $C_{18}H_{14}O_8$. The negative ESIMS of **1** exhibited significant fragment peaks at *m*/*z* 357 $[M - 1]^-$ and 339 $[M - H_2O]^-$. Compound **1** exhibited UV (MeOH) [λ_{max} (log ϵ) 339 (3.34), 295 (3.28), 237 (3.31) nm] absorptions characteristic of a lignan containing a dibenzylbutyrolactone skeleton with a double bond at the 2,6-position of the γ-butyrolactone ring.[35] The ^{1}H NMR spectrum of **1** (Table 1) displayed two sets of typical AMX spin system signals for 1,2,4-trisubstituted phenyl ring protons at δ_H 7.30 (1H, d, J = 2.1 Hz, H-2′), 7.24 (1H, dd, J = 2.1, 8.4 Hz, H-6′), and 6.71 (1H, d, J = 8.4 Hz, H-5′) and at δ_H 7.10 (1H, d, J = 2.4 Hz, H-2″), 7.02 (1H, dd, J = 2.4, 8.4 Hz, H-6″), and 6.67 (1H, d, J = 8.4 Hz, H-5″). The ^{1}H NMR spectrum also showed one olefinic proton signal at δ_H 7.60 (1H, s, H-6) and methylene signals at δ_H 4.69 and 4.43 (each 1H, d, J = 10.5 Hz, H-4). The ^{13}C NMR and DEPT spectrum of **1** showed a total of 18 signals, comprising two carbonyl carbons (δ_C 172.2 and 195.8), two quaternary carbons (δ_C 80.3 and 123.8), one methylene carbon (δ_C 77.7), one methine carbon (δ_C 144.4), and 12 aromatic signal carbons, corresponding to two groups for 1,2,4-trisubstituted phenyl ring carbons at δ_C 124.9 (C-1″), 118.1 (C-2″), 144.8 (C-3″), 149.0 (C-4″), 114.7 (C-5″), 125.3 (C-6″), 124.9 (C-1′), 115.9 (C-2′), 144.5 (C-3′), 151.5 (C-4′), 114.2 (C-5′), and 122.7 (C-6′), respectively. All one-bond H−C correlations were confirmed by the HSQC experiment. The HMBC spectrum (Figure 1) showed the correlations from the olefinic proton (δ_H 7.60) to C-2″, C-6″, C-1, and C-3 and a correlation from the methylene protons (δ_H 4.69 and 4.43) to C-1, C-5, and C-3. In addition, the cross-peaks from H-2′ to C-4′, C-6′, and C-5 and from H-6′ to C-2′, C-4′, and C-5 were also observed in the HMBC spectrum. The olefinic proton signal appeared at a low field (δ_H 7.60) due to the deshielding effect of the β-carbonyl group (C-1),[36] indicating that compound **1** is in the *E*-configuration. The CD spectrum of **1** gave only a weak signal. Although actaealactone has a negative optical rotation, it is not possible to conclude the absolute stereochemistry for C-3 by comparing the optical rotation of **1** with similar compounds; there is not a clear correlation between the optical rotation of related lignans and the absolute stereochemistry of C-3.[35−37] Due to the small amount of **1**, chemical reactions could not be performed on the hindered hydroxyl group. Therefore, we were unable to determine the absolute stereochemistry for C-3 in this study. On the basis of these data, the structure of actaealactone (**1**) was determined to be 2-(3″,4″-dihydroxyphenylmethylene)-3-hydroxy-3-(3′,4′-dihydroxybenzoyl)-γ-butyrolactone.

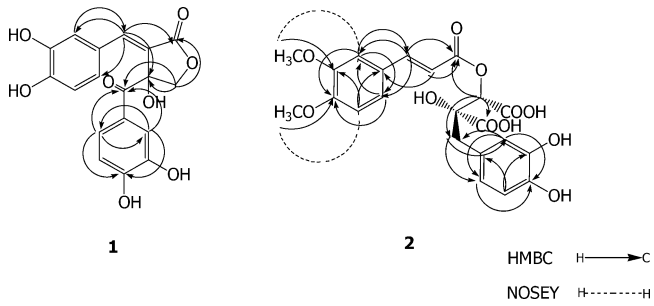

Figure 1. Key HMBC and NOESY correlations of compounds **1** and **2**.

Cimicifugic acid G (**2**) was isolated as a yellow amorphous powder and gave a molecular peak at *m*/*z* 463.1252 corresponding to $[M + 1]^+$ in the positive HRESIMS and establishing the molecular formula $C_{22}H_{22}O_{11}$. The negative ESIMS of **2** exhibited

significant fragment peaks at *m/z* 461 $[M - 1]^-$ and 253 $[M - 208]^-$. The ^1H and ^{13}C NMR data (Table 1) of **2**, assigned by 1D and 2D NMR techniques including HSQC, HMBC, and NOESY, were similar to those of fukinolic acid[33] except for two additional signals at δ_H 3.91 and 3.89 for methoxy groups, indicating that **2** is a fukiic acid ester derivative. The ^1H and ^{13}C NMR data of compound **2** showed signals indicating that the compound is comprised of fukiic acid and 3,4-dimethoxycinnamoyl moieties. The cross-peaks from H-2′ (δ_H 7.30) to C-4′, C-6′, and C-7′; H-5′ (δ_H 7.02) to C-1′ and C-3′; H-6′ (δ_H 7.25) to C-2′ and C-4′; H-7′ (δ_H 7.82) to C-2′, C-6′, and C-9′; and H-8′ (δ_H 6.58) to C-1′ and C-9′ for the 3,4-dimethoxycinnamoyl moiety were observed in the HMBC spectrum (Figure 1). In addition, the fukiic acid moiety was confirmed by the correlations from H-2″ (δ_H 6.76) to C-4″, C-6″, and C-7″; H-5″ (δ_H 6.66) to C-1″ and C-3″; H-6″ (δ_H 6.61) to C-4″; H-7″ (δ_H 2.98 and 3.06) to C-2″, C-6″, and C-3; and H-2 (δ_H 5.66) to C-9′, C-7″, C-1, C-3, and C-4 in the HMBC experiment. Two methoxy groups were located by the analysis of the HMBC and NOESY spectra (Figure 1). In the HMBC spectrum, a methoxy proton (δ_H 3.91) showed a correlation with C-3′ (δ_C 149.5), while another methoxy proton (δ_H 3.89) showed a correlation with C-4′ (δ_C 151.7). In the NOESY experiment, the correlations between the methoxy protons (δ_H 3.91) and the proton signal at δ_H 7.30 (H-2′) and between the methoxy protons (δ_H 3.89) and the proton signal at δ_H 7.02 (H-5′) were observed. This suggested that two methoxy groups are from the 3,4-dimethoxycinnamoyl moiety (Figure 1). The site of esterification of fukiic acid by the (*E*)-3,4-dimethoxycinnamoyl moiety was confirmed by the correlation between the proton at δ_H 5.66 (H-2) and the carbon δ_C 166.6 (C-9′) in the HMBC spectrum. This correlation is commonly found in fukiic acid or piscidic acid ester derivatives from this species.[33,34] Because **2** has a positive optical rotation that is similar to fukinolic acid and cimicifugic acids A and B,[33] the absolute configuration of **2** was deduced to be 2*R*, 3*S*.[33,34] Thus, compound **2** was determined to be (2*R*,3*S*)-2-*O*-(3′,4′-dimethoxy-*E*-cinnamoyl)-3-hydroxy-3-[(3″,4″-dihydroxyphenyl)methyl]butanedioic acid.

The antioxidant activities of **1** and **2** were measured in the DPPH free-radical assay. Both compounds showed antioxidant activity with IC_{50} values of 26 and 37 μM, respectively. The known compounds were also screened for their antioxidant activities in the DPPH assay, and cimicifugic acid A (**3**), cimicifugic acid B (**4**), and fukinolic acid (**5**) were active, with IC_{50} values of 12, 21, and 23 μM, respectively. The IC_{50} value of gallic acid, a positive control, was determined to be 29.9 μM.

According to the chemical structure, actaealactone (**1**) is identified as a member of dibenzyl-γ-butyrolactones type of lignans.[38] Considering that black cohosh is rich in phenylpropanoids such as caffeic acid and isoferulic acid, actaealactone may be formed by two phenylpropanoid units via phenol oxidation coupling, as in the case of lignans. This could be further verified by the isolation of several phenylpropanoid esters from black cohosh.[18] Cimicifugic acid G (**2**), a phenylpropanoid ester dimer, probably forms between fukiic acid and (*E*)-3,4-dimethoxycinnamic acid via esterification. Fukiic acid and (*E*)-3,4-dimethoxycinnamic acid have been previously isolated from the Asian *Actaea* species, but no ester-linked-dimer derivative of these two compounds has been reported.[33]

Compounds **1** and **2** were evaluated for their capacity to stimulate MCF-7 cell proliferation due to structural similarity to enterolactone and fukinolic acid, respectively. Enterolactone and fukinolic acid have been reported to have a stimulating effect on MCF-7 cell proliferation.[17,39] The growth effects on MCF-7 cells of actaealactone and cimicifugic acid G were examined as previously described.[17,39] Estradiol and enterolactone were also tested as positive controls in this assay. It was found that the maximum cell proliferation of MCF-7 cells was obtained with 3.7 nM estradiol (3.6-fold). Both compounds **1** and **2** induced only a slight increase in cell proliferation of 1.24-fold (5 μg/mL, 14 μM) ($p = 0.003$) and 1.14-fold (10 μM) ($p = 0.82$), respectively, when compared with untreated cells. At higher concentration, compound **2** was cytotoxic to MCF-7 cells; 30 μM induced only 32% growth inhibition. Enterolactone stimulated cell proliferation 1.84-fold (5 μg/mL, 16.8 μM), when compared with untreated cells.

Experimental Section

General Experimental Procedures. Melting points were determined on a Mel-Temp II melting point apparatus (Laboratory Devices Inc., Holliston, MA) and are uncorrected. Optical rotations were measured on a JASCO DIP-140 digital polarimeter (Rudolph Research Analytical, Flanders, NJ). UV spectra were measured on a Lambda 2 UV/vis spectrophotometer (Perkin-Elmer, Boston, MA). ^1H NMR and ^{13}C NMR spectra were recorded using a Bruker AVANCE 300 MHz NMR spectrometer, operating at 300 and 75 MHz, respectively. All NMR spectra were obtained in CD_3OD, with chemical shifts expressed in δ and coupling constant (*J*) in hertz. ESIMS was performed with a ThermoQuest Finnigan LCQ instrument (San Jose, CA) equipped with Xcalibur software. Samples were dissolved in MeOH and introduced by direct injection. The capillary voltage was 10 V, the spray voltage was 4.5 kV, and the tube lens offset was 0 V. The capillary temperature was 230 °C. HRESIMS was performed on a 70-SE-4F mass spectrometer (Micromass). Samples were dissolved in MeOH. HPLC analyses were carried out on a Waters 2695 separations module equipped with a Waters 996 photodiode array detector and Waters Empower[32] software using a Phenomenex Aqua C_{18} column (4.6 × 250 mm, 5 μm) and a solvent system of 5:95 to 50:50 MeCN/aqueous acetic acid (1% v/v) in a linear gradient. The flow rate was 1 mL/min, and the column was used at room temperature and 30 min run time for analysis of subfractions. Preparative HPLC was carried out using a Waters 600 controller with a Waters 486 tunable absorbance detector and a Phenomenex Nucleosil C_{18} column (21.1 × 250 mm, 10 μm), eluting with solvent system I [MeCN/0.1% aqueous acetic acid (9:1)], solvent system II [MeOH/0.1% aqueous acetic acid (1:3)], solvent system III [gradients of MeOH in 0.1% aqueous acetic acid from 33% to 50% MeOH in linear gradient], and solvent system IV [MeCN/0.1% aqueous acetic acid (2:8)]. The flow rate was 10 mL/min. The column was conducted at room temperature and 60 min run time. TLC analyses were performed on silica gel 60 F_{254} plates (Merck, Darmstadt, Germany), with compounds visualized by spraying with a vanillin solution (1 g of vanillin in 10 mL of concentrated H_2SO_4 and 90 mL of EtOH). Sephadex LH-20 (25−100 μm; Pharmacia Fine Chemicals, Piscataway, NJ), C_{18} reversed-phase silica gel (40 μm; J. T. Baker, Phillipsburg, NJ), and Diaion HP-20 (Supelco, Bellefonte, PA) were used for column chromatography.

Plant Material. A standardized black cohosh (*A. racemosa*) extract (lot number: 9-2044) was supplied by PureWorld Botanicals Inc., South Hackensack, NJ.

Extraction and Isolation. The standardized black cohosh powdered extract (0.5 kg) was re-extracted with 80% MeOH/water at room temperature overnight (12 h). After the MeOH was removed in vacuo, the resulting aqueous solution was sequentially partitioned with hexane and *n*-BuOH. The hexane, *n*-BuOH, and aqueous extracts were concentrated in vacuo at 40 °C. A part (30 g) of the residue from the *n*-BuOH fraction was fractionated over Diaion HP-20 (600 g), eluting with water/MeOH (1:1), MeOH, and acetone to give four fractions (B_{1-4}). Fraction B_2 (3.2 g) was chromatographed over C_{18} silica gel (120 g) by elution with gradients of MeCN in 0.1% aqueous acetic acid (5% to 50% MeCN) to obtain 10 subfractions (B_{2a-j}). Fraction B_{2b} (270 mg) was further separated by C_{18} silica gel (12 g) column chromatography with gradients of MeCN in 0.1% aqueous acetic acid (5% to 15% MeCN) to obtain three fractions (B_{2b1-3}). Fraction B_{2b2} (20.3 mg) was further purified by preparative HPLC eluting with solvent system I to yield protocatechuic acid (4.4 mg) and protocatechualdehyde (2.3 mg). Fraction B_{2d} (390 mg) was chromatographed over Sephadex LH-20 (80 g) by elution with MeOH/H_2O (9:1) to yield caffeic acid (290 mg). Fraction B_{2e} (50 mg) was chromatographed over Sephadex LH-20 (10 g) by elution with MeOH/H_2O (9:1) to obtain six subfractions (B_{2e1-6}). Fraction B_{2e4} (9.3 mg) was rechromatographed over Sephadex LH-20 (20 g) eluted with MeOH/H_2O (9:1) to yield 1-isoferuloyl-β-D-glucopyranoside (1.6 mg). Fraction B_{2f} (150 mg) was chromatographed over Sephadex LH-20 (30 g) using MeOH/H_2O (9:

1) to obtain eight subfractions (B_{2f1-8}). Fractions B_{2f3} (4 mg) and B_{2f8} (2 mg) were rechromatographed over Sephadex LH-20 (2 g) with MeOH/H_2O (9:1) to yield *p*-coumaric acid (0.8 mg) and **1** (1.1 mg), respectively. Fraction B_{2h} (82 mg) was purified by preparative HPLC eluting with solvent system II to give ferulic acid (32.2 mg) and isoferulic acid (12 mg). Fraction B_{2i} (100 mg) was chromatographed over Sephadex LH-20 (20 g) eluted with MeOH/H_2O (9:1) to yield isoferulic acid (84.2 mg).

A portion (30 g) of the residue from the water extract was fractionated over Diaion HP-20 (600 g), eluting in turn with water, water/MeOH (1:1), MeOH, and acetone to give seven combined fractions (W_{1-7}). Fraction W_3 (2.12 g) was chromatographed over C_{18} silica gel (100 g), eluted with gradients of MeCN in 0.1% aqueous acetic acid (5% to 35% MeCN), to afford eight combined fractions (W_{3a-h}). Fractions W_{3c} (170 mg), W_{3d} (70.9 mg), W_{3e} (82.0 mg), and W_{3g} (94.9 mg) were subjected to preparative HPLC eluting with solvent system III to yield crude fukinolic acid (102.2 mg), crude cimicifugic acid D (6.4 mg), crude cimicifugic acid A (39.1 mg), and crude cimicifugic acid B (46.6 mg), respectively. The crude fukinolic acid, cimicifugic acid D, cimicifugic acid A, and cimicifugic acid B were further purified by preparative HPLC eluting with solvent system IV to yield fukinolic acid (54.4 mg), cimicifugic acid D (2.2 mg), cimicifugic acid A (19.4 mg), and cimicifugic acid B (30.1 mg), respectively.

Fraction W_4 (120.4 mg) was chromatographed over C_{18} silica gel (8 g), eluted with gradients of MeCN in 0.1% aqueous acetic acid (10% to 40% MeCN), to yield four combined fractions (W_{4a-d}). Fraction W_{4d} (20.2 mg) was subjected to preparative HPLC eluting with the solvent system III to obtain crude cimicifugic acid E (6.1 mg) and crude cimicifugic acid F (11.4 mg). The crude cimicifugic acids E and F were further purified by preparative HPLC using solvent system IV, to afford cimicifugic acid E (3.8 mg) and cimicifugic acid F (8.7 mg). Fraction W_5 (84.4 mg) was chromatographed over C_{18} silica gel (40 g), eluted with gradients of MeCN in 0.1% aqueous acetic acid (15% to 50% MeCN), to obtain eight combined fractions (W_{5a-g}). Fraction W_{5d} (32.8 mg) was chromatographed over Sephadex LH-20 (5 g), eluted with MeOH−H_2O (9:1), to obtain 10 fractions (W_{5d1-10}). Fraction W_{5d6} (6 mg) was further purified by preparative HPLC eluting with solvent system IV to yield **2** (2.1 mg). Fraction W_6 (355 mg) was passed over Sephadex LH-20 (80 g), eluting with MeOH−H_2O (9:1), to obtain 10 combined fractions (W_{6a-j}). Fraction W_{6b} (10.4 mg) and fraction W_{6f} (15.1 mg) were further purified by preparative HPLC, eluting with solvent system II, to afford methyl caffeate (4.3 mg) and ferulate-1-methyl ester (1.1 mg), respectively.

Actaealactone (1): yellow, amorphous powder; mp 149−150 °C; $[\alpha]^{25}_D$ −23.6 (*c* 0.0005, MeOH); UV (MeOH) λ_{max} (log ϵ) 339 (3.34), 295 (3.28), 237 (3.31) nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; positive HRESMS *m*/*z* 359.0774 $[M + H]^+$ (calcd for $C_{18}H_{15}O_8$, 359.0767).

Cimicifugic Acid G (2): yellow, amorphous powder; mp 112−113 °C; $[\alpha]^{25}_D$ +22.4 (*c* 0.001, MeOH); UV (MeOH) λ_{max} (log ϵ) 328 (3.17), 290 sh (3.06), 225 (3.10) nm; ^{1}H NMR and ^{13}C NMR data, see Table 1; positive HRESMS *m*/*z* 463.1252 $[M + H]^+$ (calcd for $C_{22}H_{23}O_{11}$, 463.1240).

DPPH Free-Radical Scavenging Assay. The DPPH assay was performed on fractions and purified isolates as previously described, with 400 μM DPPH.[40]

Cell Culture Assay. MCF-7 cells (human breast cancer, estrogen-sensitive cells) were obtained from the ATCC (Manassas, VA). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) fetal bovine serum (FBS) (Gibco BRL) in a humidified incubator at 37 °C, with a 5% CO_2 atmosphere.

The MCF-7 cells were transferred to phenol red-free DMEM containing 2 mM L-glutamine and 5% charcoal-stripped serum and passaged at least two times and washed two times with phosphate-buffered saline prior to being seeded. Cells were seeded into a 24-well plate at a density of 2×10^4 cells and allowed to attach for 24 h in phenol red-free DMEM containing 2 mM l-glutamine and 5% charcoal-striped serum. The medium was then replaced with fresh medium with or without the indicated test compounds at a range of concentrations. After 4 days, the numbers of attached viable cells were counted using a Coulter counter, model Z_F (Coulter Electronics Inc., Hialeah, FL). Cell viability was calculated by comparing cell counts in treated samples relative to cell counts in the untreated group.

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Supporting Information Available: An HPLC chromatogram of the black cohosh extract used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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