Three New Fukiic Acid Esters, Cimicifugic Acids A, B and C, from *Cimicifuga Simplex* WORMSK.

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Three new fukiic acid esters, cimicifugic acids A (1), B (2), and C (3), were isolated along with fukinolic acid (4), fukiic acid (5), caffeic acid (6), ferulic acid (7), isoferulic acid (8), 3,4-dimethoxycinnamic acid (9) and *p*-coumaric acid (10) from *Cimicifuga simplex*. Their structures were identified as 2-feruloyl, 2-isoferuloyl, and 2-*p*-coumaroyl fukiic acid (1—3) on the basis of spectroscopic and chemical data.

Key words *Cimicifuga simplex*; Ranunculaceae; fukiic acid ester; caffeic acid; *p*-coumaric acid

The rhizomes of some *Cimicifuga* (*C.*) species including *C. simplex* (Ranunculaceae) have been used as anti-inflammatory, antipyretic and analgesic agents in traditional Chinese medicine.[1] The following constituents of *C. simplex* have been reported: highly oxygenated 9,19-cyclolanostane triterpenic glycosides,[2] cimifugin, visnagin, 3,4-dimethoxycinnamic acid,[3] and 2-hydroxy-7-methyl-9*H*-carbazol.[4]

In the couse of our studies on the water-soluble constituents of *C. simplex*, we obtained three new fukiic acid derivatives, cimicifugic acids A (**1**), B (**2**), and C (**3**), along with fukinolic acid (**4**), fukiic acid (**5**), caffeic acid (**6**), ferulic acid (**7**), isoferulic acid (**8**), 3,4-dimethoxycinnamic acid (**9**), and *p*-coumaric acid (**10**). The present report deals with the isolation of these constituents and their structural elucidation.

The crude new compounds were obtained as arginine and histidine salts by repeated chromatography on octadecylsilanized silicic acid (ODS) and silica-gel (SiO_2) columns from the water-soluble portion of the underground parts of *C. simplex*, as described in detail in the experimental section. After treatment of the salts of amino acids and esters with dilute HCl, cimicifugic acids A (**1**), B (**2**), and C (**3**) were obtained by repeated column chromatography and HPLC.

Cimicifugic acid A (**1**), was a pale yellow powder, $[\alpha]_D$ +47.8°, UV λ_{max} 328 nm (log ε 4.28), and its molecular formula was determined as $C_{21}H_{20}O_{11}$ by positive high resolution secondary ion mass spectroscopy (pos. HR-SI-MS) *m/z*: 449.1076 $(M+H)^+$, and ^{13}C-NMR spectral data. The IR spectrum exhibited hydroxyl (3400 cm^{-1}), bicarboxylic, conjugated carboxyl (1714 and 1629 cm^{-1}) and aromatic ring (1600 and 1516 cm^{-1}) bands.

The ^{1}H-NMR spectrum (CD_3OD) showed signals assignable to an isolated methylene [δ 2.93 ppm (J=13.6 Hz) and δ 3.06 (J=13.6 Hz) as AB type quartets, 2H-4], a carbinyl methine [δ 5.67, br s, H-2], a feruloyl moiety [δ 6.51 (d, J=16.0 Hz, H-2″); δ 7.79 (d, J=16.0 Hz, H-3″); δ 3.91 (3H, s, OCH_3), δ 7.24 (d, J=2.4 Hz, H-2‴), δ 7.13 (dd, J=8.5, 2.4 Hz, H-6‴), δ 6.84 (d, J=8.5 Hz, H-5‴)], and a 1,3,4-trisubstituted benzene moiety [δ 6.76 (d, J=2.2 Hz, H-2′), δ 6.61 (dd, J=8.0, 2.2 Hz, H-6′), δ 6.66 (d, J=8.0 Hz, H-5′] as shown in Table 1.

The presence of the feruloyl moiety was confirmed by the rotating-frame Overhauser enhancement (ROE) between the methoxy group and H-2‴. This evidence suggested that **1** is an ester between ferulic and fukiic acids [2,3-dihydroxy-4-(3,4-dihydroxyphenyl)-3-carboxy butyric acid].[5,6] This suggestion was supported by the presence of a base peak at *m/z* 177 (feruloyl) and a peak at *m/z* 123 (3,4-dihydroxybenzyl moiety) in the pos. SI-MS spectrum. The chemical shift (δ 5.67) of H-2 of **1**, a shift (Δ1.17 ppm) from H-2 (δ 4.50) of **5** to that of **1**, and a cross-peak between H-2 and C-1″ in the heteronuclear multiple bond connectivity (HMBC) spectrum of **1** confirmed that the feruloyl group was linked to C-2 of the fukiic acid moiety.

Hydrolysis of **1** with 2 N NaOH gave ferulic acid (**7**) and fukiic acid (**5**), in a molar ratio (1 : 1). The latter **5**, a white powder, $[\alpha]_D$ +29.9°, had a UV λ_{max} 248 nm (log ε 4.02), 291 (4.13), 332 (4.26), and its molecular formula was determined as $C_{11}H_{12}O_8$ by pos. HR-SI-MS *m/z*: 273.0536 $(M+H)^+$. Data on the ^{1}H-NMR, ^{13}C-NMR and IR spectra of **5** were identical with those of fukiic acid, obtained by alkaline hydrolysis of fukinolic acid (**4**).

Fukiic acid (**5**) has been isolated from *Petasites japonicus* (Compositae)[7] and *Piscidia erythrina* (Leguminosae) as the 1-monomethyl ester,[8] the absolute configuration of which has been determined to be 2*S* and 3*R*.[9] Because the reported $[\alpha]_D$ value was +40.5° for the methy ester of **5**, and our value was +29.9° for the free acid (**5**), the same stereochemistry was concluded to be present. Thus, the structure of cimicifugic acid A (**1**) was established as 2-feruloyl fukiic acid, as shown in Figs. 1, 2.

Cimicifugic acid B (**2**), was a pale yellowish powder, $[\alpha]_D$ +42.5°, UV λ_{max} 313 nm (log ε 4.27) and its molecular formula was determined as $C_{21}H_{20}O_{11}$ by pos. HR-SI-MS *m/z*: 449.1078 $(M+H)^+$ and ^{13}C-NMR spectral data. The IR spectrum exhibited hydroxyl (3400 cm^{-1}), bicarboxylic, conjugated carboxyl (1715 and 1609 cm^{-1}), and aromatic ring (1515 cm^{-1}) bands. The ^{1}H-NMR spectrum (CD_3OD) was similar to that of **1**, except for the signal patterns due to H-2‴, H- 5‴, and H-6‴ as shown in Table 1. These data suggested that **2** has isoferulic acid in the place of

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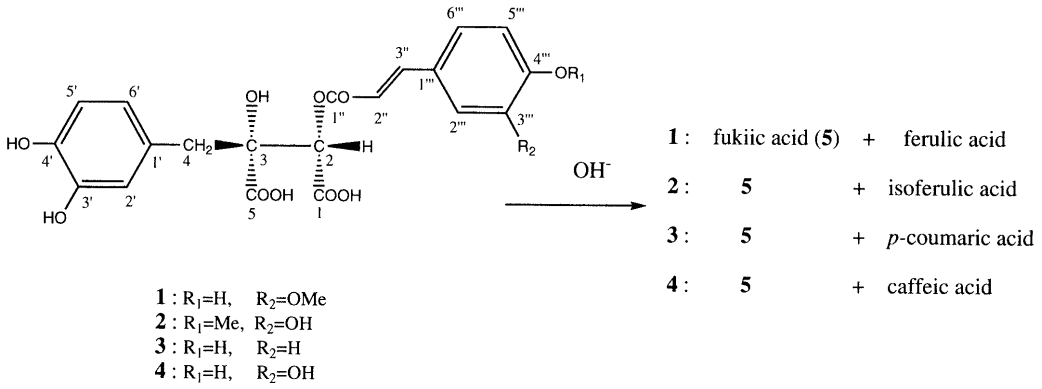

Fig. 1. Structures of Cimicifugic Acid A (**1**), B (**2**), C (**3**) and Fukinolic Acid (**4**)

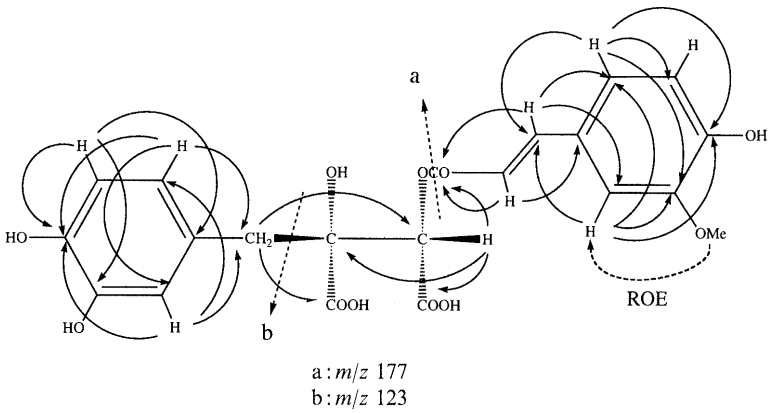

Fig. 2. MS Spectrum Fragment Ions, HMBC Correlations and ROE of Cimicifugic Acid A (**1**)

the ferulic acid of **1**. The isoferulic acid moiety was confirmed by the ROE between the methoxy group (δ 3.90) and H-5‴ (δ 6.97). A base peak at *m/z* 177 and a peak at *m/z* 123 in the pos. SI-MS spectrum of **2** like that of **1** supported the suggestion that **2** possesses isoferuloyl and 3,4-dihydroxy-benzyl moieties. The chemical shift (δ 5.66) of H-2 of **2**, a cross-peak between H-2 and C-1″ in the HMBC spectrum of **2**, and a shift (Δ1.16 ppm) from H-2 of **5** to that of **2** confirmed that the isoferuloyl group was linked to C-2 of the fukiic acid moiety.

Hydrolysis of **2** with 2 N NaOH gave isoferulic acid (**8**), and fukiic acid (**5**) in a molar ratio (1 : 1). Thus, the structure of **2** was established as 2-isoferuloyl fukiic acid, as shown in Fig. 1.

Cimicifugic acid C (**3**), was a pale yellowish powder, $[\alpha]_D$ +38.7°, UV λ_{max} 313 nm (log ε 4.27) and its molecular formula was determined as $C_{20}H_{18}O_{10}$ by pos. HR-SI-MS *m/z*: 419.0978 $(M+H)^+$ and ^{13}C-NMR spectral data. The IR spectrum exhibited hydroxyl (3405 cm^{-1}), bicarboxylic, conjugated carboxyl (1713 cm^{-1}) and aromatic ring (1605, 1516 cm^{-1}) bands. The ^{1}H-NMR spectrum (CD_3OD) of **3** was similar to those of **1** and **2**, except for a pair of characteristic doublets (AA′XX′ type) of δ 6.83 (J=8.4 Hz) and δ 7.52 (J=8.4 Hz) attributed to 4H on a *p*-hydroxyphenyl ring in the place of a methoxy and the 3H aromatic signals of **1** and **2**. A base peak at *m/z* 147 and a peak at *m/z* 123 in the pos. SI-MS spectrum suggested that **3** possesses a *p*-coumaroyl group and a 3, 4-dihydroxybenzyl moiety. The chemical shift (δ 5.65) of H-2 of **3**, a shift (Δ1.15 ppm) between H-2 of **3** and that of **5**, and a cross-peak between H-2 and C-1″ in the HMBC spectrum of **3** like those of **1** and **2**, confirmed that the *p*-coumaroyl group of **3** was linked to C-2 of the fukiic acid moiety.

Hydrolysis of **3** with 2 N NaOH gave *p*-coumaric acid (**10**) and fukiic acid (**5**) in a molar ratio (1 : 1) as in **1** and **2**. Thus, the structure of cimicifugic acid C (**3**) was established as 2-*p*-coumaroyl fukiic acid, as shown in Fig. 1.

Fukinolic acid (**4**), a pale yellowish powder, $[\alpha]_D$ +51.2°, UV λ_{max} 332 nm (log ε 4.26), was obtained as a major component together with **1**, **2**, and **3**. Its molecular formula was determined as $C_{20}H_{18}O_{11}$ by pos. HR-SI-MS *m/z*: 435.0934 $(M+H)^+$ and ^{13}C-NMR spectral data. A base peak at *m/z* 163 and a peak at *m/z* 123 showed that **4** possesses caffeic acid and 3,4-dihydroxybenzyl moieties. Hydrolysis of **4** with 2 N NaOH gave caffeic acid (**6**), and fukiic acid (**5**) in a molar ratio (1 : 1). The identification of **4** as fukinolic acid was made by direct comparison of HPLC and spectral data with those of an authentic sample obtained from *Petasites japonicus*.

Fukiic acid (**5**), caffeic acid (**6**), ferulic acid (**7**), isoferulic acid (**8**) and 3,4-dimethoxy cinnamic acid (**9**), and *p*-coumaric acid (**10**) were isolated from the water-soluble fraction independently from their esters (**1**—**4**), and identified by direct comparison with authentic specimens.

Fukiic acid (**5**) has been reported as a phosphorus (P) uptake component in Pigeon peas grown in soils with a low P content.[10] Ferulic acid (**7**) and isoferulic acid (**8**) have been reported to exhibit hypothermic, antipyretic,

Table 1. $^{1}H^{a)}$ and $^{13}C^{b)}$-NMR Spectral Data of **1**, **2**, **3** and **4**

Proton No.	1	2	3	4	Carbon No.	1	2	3	4
H-1	—	—	—	—	C-1	170.65	170.09	170.54	170.90
H-2	5.67 br s	5.66 br s	5.65 br s	5.65 br s	C-2	77.52	77.75	77.60	78.28
H-3	—	—	—	—	C-3	80.10	79.43	80.13	80.15
H-4	2.93 d (13.6)	2.92 d (13.7)	2.92 d (13.8)	2.92 d (13.5)	C-4	42.20	42.21	42.22	42.22
	3.06 d (13.6)	3.04 d (13.7)	3.04 d (13.8)	3.03 d (13.5)					
H-5	—	—	—	—	C-5	174.99	175.21	174.98	174.78
H-1′	—	—	—	—	C-1′	127.95	128.04	127.99	128.04
H-2′	6.76 d (2.2)	6.75 d (2.3)	6.75 d (2.3)	6.74 d (2.2)	C-2′	118.82	118.85	118.85	118.83
H-3′	—	—	—	—	C-3′	145.67	145.72	145.72	145.71
H-4′	—	—	—	—	C-4′	145.27	145.30	145.30	145.30
H-5′	6.66 d (8.0)	6.64 d (7.9)	6.65 d (8.2)	6.64 d (8.2)	C-5′	115.96	115.94	115.96	115.94
H-6′	6.61 dd (8.0, 2.2)	6.60 dd (7.9, 2.3)	6.60 dd (8.2, 2.3)	6.59 dd (8.2, 2.2)	C-6′	123.06	123.02	123.06	123.05
H-1″	—	—	—	—	C-1″	168.21	168.07	168.26	168.29
H-2″	6.51 d (16.0)	6.47 d (15.9)	6.48 d (16.0)	6.42 d (16.0)	C-2″	114.53	114.96	114.27	114.26
H-3″	7.79 d (16.0)	7.75 d (15.9)	7.79 d (16.0)	7.72 d (16.0)	C-3″	148.16	147.82	147.90	148.22
H-1‴	—	—	—	—	C-1‴	127.69	128.91	127.19	127.79
H-2‴	7.24 d (2.4)	7.14 d (2.5)	7.52 d (8.4)	7.10 d (2.3)	C-2‴	111.94	115.32	131.38	115.32
H-3‴	—	—	6.83 d (8.4)	—	C-3‴	149.38	148.08	116.90	146.85
H-4‴	—	—	—	—	C-4‴	150.81	151.74	161.46	149.83
H-5‴	6.84 d (8.5)	6.97 d (8.2)	6.83 d (8.4)	6.80 d (8.3)	C-5‴	116.53	112.64	116.90	116.55
H-6‴	7.13 dd (8.5, 2.4)	7.11 dd (8.2, 2.5)	7.52 d (8.4)	7.01 dd (8.3, 2.3)	C-6‴	124.36	123.06	131.38	123.25
OCH_3	3.91 s	3.90 s	—	—	OCH_3	56.52	56.43	—	—

Chemical shifts are in δ values and are followed by multiplicities and J values (in Hz). *a*) Obtained on a JEOL ALPHA-400 in CD_3OD solution. *b*) Measured at 100.4 MHz in CD_3OD solution.

analgesic, and antiedematous effects,[11] and potent inhibition of murine interleukin- 8 to act as the main components of the anti-inflammatory rhizoma of *Cimicifuga* species,[12] and sodium isoferulate has been reported to be an anti-inflammatory agent in a *C. heracleifolia* decoction.[13] Caffeic acid derivatives are ubiquitous plant constituents with potent antioxidant properties. Many crude drugs, which contain high concentrations of caffeic acid derivatives, can be applied to the skin for protection as cosmetic lotions, act as inhibitors of lipid peroxidation in foods, or protect against various inflammatory diseases.[14] It is notable that the rhizomes of *C. simplex* contain fukiic acid esters (**1**—**4**) along with caffeic acid, ferulic acid, isoferulic acid, dimethyl caffeic acid and other acids.

Experimental

The instruments used in this investigation were as follows : a Yanagimoto micromelting apparatus (for melting points, uncorrected); a Shimadzu UV-2100 (for UV spectra, measured at 25 °C); a JASCO digital polarimeter (for specific rotation, measured at 25 °C); a Perkin-Elmer 1720X-FT IR spectrometer (for IR spectra); a Hitachi M-80 spectrometer (for MS spectra); and a Varian Gemini-200, Varian Gemini-300BB and a JEOL α-400 instrument (for NMR spectra, measured in CD_3OD using tetramethylsilane as an internal standard and D_2O solution, on the δ scale). Chromatography was carried out on Diaion HP-20 (Nippon Rensui Co.), silica-gel (Wakogel C-200) and ODS-A YMC columns. HPLC was carried out using a JASCO PU-980 pump equipped with a JASCO 875-UV detector, operated at 254 and 280 nm, and a JASCO Model 807-IT integrator. TLC was carried out on precoated Kieselgel 60F_{254} (Merck) or RP-8F_{254} (Merck) reversed-phase plates with $CHCl_3$–MeOH (1 : 99) and MeOH–H_2O (1 : 1) as developing solvents, and spots were detected by 40% H_2SO_4 followed by heating, or under a "National" UV (6 W) lamp.

Isolation of 1—5 and Five Cinnamic Acid Derivatives (6—10) The dried rhizomes (1.1 kg) of *C. simplex*, which was transplanted from Shiga Prefecture in Japan, and cultivated in the research station of Osaka University of Pharmaceutical Sciences for several years, were extracted with MeOH (5 l × 3) at room temperature overnight. The MeOH solution was concentrated *in vacuo* to yield a viscous extract (*ca.* 300 ml). The concentrated extract was suspended in water (100 ml) and the mixture was shaken with *n*-BuOH–EtOAc (1 : 1) (200 ml) three times.

The water-soluble layer was chromatographed on a Diaion HP-20 column (500 ml, i.d. 5.5 × 70 cm). After elution with H_2O (3 l), the adsorbed fraction was eluted with MeOH (500 ml). The MeOH eluate was concentrated *in vacuo* to give a gummy extract (3.5 g) which was rechromatographed on an ODS column (80 g, i.d. 3.0 × 20 cm) and eluted with MeOH–H_2O (2 : 1 to 1 : 0). The fraction eluted with MeOH–H_2O (2 : 1) (2.5 g) was chromatographed on a SiO_2 column (90 g, i.d. 4.0 × 20 cm) and eluted with $CHCl_3$–MeOH (10 : 1 to 1 : 5).

Elution with $CHCl_3$–MeOH (1 : 2 to 1 : 5) provided a gummy extract (1.8 g). This was diluted with 1 N HCl 15 ml and H_2O 50 ml, and the diluted solution was rechromatographed on a Diaion HP-20 column (i.d. 3 × 20 cm). The fraction eluted with H_2O gave arginine (main component) and histidine (minor component), which were identified by TLC, ^{1}H-NMR spectra, and amino acid analysis. The fraction eluted with MeOH–H_2O (5 : 1 to 1 : 0) (450 mg) was subjected to preparative HPLC [column, Cosmosil 5C18-AR (5 μm, i.d. 10.0 × 250 mm) and Cosmosil 10Ph (10 μm, i.d. 8.5 × 250 mm); solvent, MeCN–1% AcOH (5 : 95 to 15 : 85); column temperature, 40 °C; effluent rate, 2 ml/min]. The components eluted with MeCN–1% AcOH (5 : 95) afforded, in order of their t_R values, fukiic acid (**5**, 20 mg), caffeic acid (**6**, 46 mg), *p*-coumaric acid (**10**, 7 mg), ferulic acid (**7**, 38 mg), fukinolic acid (**4**, 70 mg) and isoferulic acid (**8**, 20 mg). The components eluted with MeCN–1% AcOH (15 : 85) afforded, in order of their t_R values, cimicifugic acid C (**3**, 18 mg), cimicifugic acid A (**1**, 50 mg), cimicifugic acid B (**2**, 18 mg), and 3,4-dimethoxycinnamic acid (**9**, 5 mg).

Caffeic acid (**6**), ferulic acid (**7**), isoferulic acid (**8**), 3,4-dimethoxycinnamic acid (**9**), and *p*-coumaric acid (**10**), were identified by comparison with authentic samples (^{1}H- and ^{13}C-NMR spectra, HPLC t_R values, mp, and IR).

Fukiic Acid (**5**): Oily syrup, $[\alpha]_D$ +29.9° (c = 0.59, MeOH at 25 °C), UV λ_{max}^{MeOH} nm (log ε): 332 (4.26), 291 (4.13), 248 sh (4.02). $C_{11}H_{12}O_8$ pos. HR-SI-MS m/z: 273.0536 [(M+H)$^+$, error: 0.5 m.m.u.], pos. SI-MS m/z: 273 (M+H)$^+$ (23.9), 166 (100), 147 (11.9), 123 (27.5), 86 (18.9). IR (KBr) cm^{-1}: 3450 (OH), 3400—2450 (OH, COOH), 1732 (C=O), 1615, 1520 (aromatic ring), 1471. ^{1}H-NMR (CD_3OD) δ: 2.95 (1H, d, J = 13.8 Hz, H-4), 3.09 (1H, d, J = 13.8 Hz, H-4), 4.50 (1H, s, H-2), 6.59 (1H, dd, J = 7.8, 2.4 Hz, H-6′), 6.65 (1H, d, J = 7.8 Hz, H-5′), 6.74 (1H, d, J = 2.4 Hz, H-2′). ^{13}C-NMR (CD_3OD) δ: 174.76 (C-1), 76.48 (C-2), 81.34 (C-3), 42.10 (C-4), 176.05 (C-5), 128.70 (C-1′), 118.79 (C-2′), 145.58 (C-3′), 145.03 (C-4′), 115.92 (C-5′), 123.00 (C-6′). The physical, ^{1}H- and ^{13}C-NMR spectral data of **5** in the free acid form were recorded for the first time.

Cimicifugic Acid A (**1**): A pale powder obtained by recrystallization from $CHCl_3$–MeOH, $[\alpha]_D$ +47.8° (c=0.90, MeOH at 25 °C). UV λ_{max}^{MeOH} nm (log ε): 328 (4.28), 287 sh (4.08), 234 (4.16). $C_{21}H_{20}O_{11}$ pos. HR-SI-MS m/z: 449.1076 $[(M+H)^+$, error: −0.6 m.m.u.], pos. SI-MS m/z: 449 $(M+1)^+$ (8.2), 194 (12.0), 177 (100), 123 (15.6), 86 (18.3), 70 (20.9). IR (KBr) cm^{-1}: 3400 (OH), 1714, 1629, (conjugated C=O), 1600, 1516 (aromatic ring), 1449. ^{1}H-NMR and ^{13}C-NMR (CD_3OD) δ: Table 1.

Cimicifugic Acid B (**2**): A pale yellow powder obtained from $CHCl_3$–MeOH, $[\alpha]_D$ +42.5° (c=0.51, MeOH at 25 °C), UV λ_{max}^{MeOH} nm (log ε): 313 (4.27), 286 sh (4.16), 224 (4.23). $C_{21}H_{20}O_{11}$ pos. HR-SI-MS m/z: 449.1078 $[(M+H)^+$, error: −0.4 m.m.u.], pos. SI-MS m/z: 449 $(M+1)^+$ (7.6), 225 (12.1), 177 (25.9), 133 (100), 123 (5.4), 86 (3.0). IR (KBr) cm^{-1}: 3400 (OH), 1715, 1609 (conjugated C=O), 1515 (aromatic ring), 1443. ^{1}H-NMR and ^{13}C-NMR (CD_3OD) δ: Table 1.

Cimicifugic Acid C (**3**): A pale yellow powder obtained from $CHCl_3$–MeOH, $[\alpha]_D$ +38.7° (c=0.34, MeOH at 25 °C). UV λ_{max}^{MeOH} nm (log ε): 313 (4.27), 286 sh (4.16), 224 (4.23). $C_{20}H_{18}O_{10}$ pos. HR-SI-MS m/z: 419.0978 $[(M+H)^+$, error: 0.1 m.m.u.], pos. SI-MS m/z: 419 $(M+1)^+$ (15.0), 163 (9.1), 147 (100), 123 (22.0), 86 (35.8), 70 (35.5). IR (KBr) cm^{-1}: 3405 (OH), 1713, 1630, 1605 (conjugated C=O), 1516 (aromatic ring), 1447. ^{1}H-NMR and ^{13}C-NMR (CD_3OD) δ: Table 1.

Fukinolic Acid (**4**): A pale yellow powder obtained from $CHCl_3$–MeOH, $[\alpha]_D$ +51.2° (c=0.59, MeOH at 25 °C). $[\alpha]_D$ +71.2° (c=1.0, H_2O at 20 °C). UV λ_{max}^{MeOH} nm (log ε): 332 (4.26) , 291 (4.13), 248 sh (4.02). $C_{20}H_{18}O_{11}$ pos. HR-SI-MS m/z: 435.0934 $[(M+H)^+$, error: 0.8 m.m.u], pos. SI-MS m/z: 435 $(M+H)^+$ (22.4), 163 (100), 123 (11.3), 86 (17.4). IR (KBr) cm^{-1}: 3438 (OH), 1713, 1605 (conjugated C=O), 1523 (aromatic ring), 1447. **4** was identified as fukinolic acid by direct comparison with an authentic sample obtained from *Petasites japonicus*. ^{1}H-NMR and ^{13}C-NMR (CD_3OD) δ: Table 1.

Hydrolysis of 1, 2, 3, and 4 **1** (25 mg) was dissolved in 2 N NaOH (2 ml) under a nitrogen atmosphere, and stirred for 5 h at 25 °C. After acidifying the solution to pH 2.5 with 2 N HCl, it was shaken with EtOAc (30 ml × 3). After washing the combined EtOAc layers, followed by drying over Na_2SO_4, the organic layer was evaporated *in vacuo*. The residue was recrystallized from MeOH–H_2O to give yellowish crystals of ferulic acid (**7**, 7 mg), mp 172—174 °C, which was identified by comparison of ^{1}H- and ^{13}C-NMR and IR spectra, and the HPLC t_R with those of an authentic sample.

The above aqueous layer was subjected to Diaion HP-20 column chromatography (i.d. 1 × 10 cm). After elution with H_2O (300 ml), the absorbed fraction was eluted with H_2O–MeOH (1:4 to 0:1). The H_2O–MeOH (1:4) eluate was subjected to preparative HPLC [column, Cosmosil 5C_{18}-AR (5 μm, i.d. 10.0 × 250 mm); solvent, MeCN–1% AcOH (10:90); column temperature, 40 °C; effluent rate, 2 ml/min] to give fukiic acid (**5**, 10 mg), which was identified by direct comparison with an authentic specimen obtained by alkaline hydrolysis of fukinolic acid (**4**).

Under the same treatment with 2 N NaOH as for **1**, **2** (15 mg) provided **8** (4 mg) and **5** (9 mg), **3** (13 mg) provided **10** (3 mg) and **5** (5 mg), and **4** (25 mg) provided **6** (6 mg) and **5** (9 mg).

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References and Notes

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