

Medicinal Foodstuffs. XVIII.¹⁾ Phytoestrogens from the Aerial Part of *Petroselinum crispum* MILL. (PARSLEY) and Structures of 6''-Acetylapiin and a New Monoterpene Glycoside, Petroside

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In the course of our screening for natural estrogenic compounds from Occidental medicinal herbs, the extracts of several herbs were found to show proliferative activity in MCF-7 (an estrogen-sensitive breast cancer cell line). Among these active herbs, the methanolic extract from the aerial parts of *Petroselinum crispum* (parsley) showed potent estrogenic activity, which was equal to that of isoflavone glycosides from soybean. Through bioassay-guided separation, we isolated several flavone glycosides and a new flavone glycoside, 6''-acetylapiin, with estrogenic activity together with a new monoterpene glucoside, petroside. The structures of 6''-acetylapiin and petroside were characterized by the chemical and physicochemical evidence. Estrogenic activities of these flavone glycosides were found to be enhanced by removal of their glycoside moieties. The EC₅₀ values (concentration needed to enhance the MCF-7 proliferation 50% compared to non-estrogen treated cell) of their aglycones are as follows, apigenin (1.0 μM), diosmetin (2.9 μM), and kaempferol (0.56 μM). The estrogenic activities of these flavones are nearly equal to those of the isoflavones, daidzein (0.61 μM) and genistein (0.60 μM). The methanolic extract of parsley, apiin, and apigenin restored the uterus weight in ovariectomized mice when orally administered for consecutive 7 days.

Key words phytoestrogen; *Petroselinum crispum*; 6''-acetylapiin; petroside; flavone glycoside; breast cancer cell line

Insufficiency of internal estrogen (estrone, estradiol) secretion is known to cause several physical disorders shown in postmenopausal women, such as osteoporosis, blood cholesterol elevation, and symptoms of menopause (hot flashes and depression). Synthetic estrogen (ethynyl estradiol, 17 β -estradiol) replacement therapy was reported to have a curative effect on these conditions. Recently, the estrogenic activity of isoflavones, lignans, and coumarins, which are widely distributed in vegetables, fruits, and medicinal plants, has been investigated; these compounds are generically called phytoestrogens.²⁾ Isoflavones, representative phytoestrogens, were shown to prevention of bone loss³⁾ and arteriosclerosis,⁴⁾ and to have cardioprotective⁵⁾ and anticarcinogenic⁶⁾ activities.

In a search for new phytoestrogens effective in preventing postmenopausal diseases, the estrogenic activities of many Occidental medicinal herbs were examined using the proliferation activity of MCF-7 (an estrogen sensitive cell line).⁷⁾ The methanolic extract from several of these herbs was found to possess estrogenic activity in the range of 1 to 10 $\mu\text{g}/\text{ml}$ (Table 1). Particularly, the extracts of the aerial part of *Petroselinum crispum* MILL. (parsley, cultivated in U.S.A. and Japan) showed reproducible estrogenic activity comparable to that of the isoflavone glucoside fraction⁸⁾ from soybean. Through bioassay-guided separation, we isolated two benzoxoles, myristicin (3)⁹⁾ and apiole (4),⁹⁾ two furocoumarins, cnidilin (5)¹⁰⁾ and isoimperatorin (6),¹⁰⁾ and four flavone glycosides, apigetrin (7),¹¹⁾ apiin (8),¹²⁾ diosmetin 7-*O*- β -D-glucopyranoside (9),¹³⁾ and kaempferol 3-*O*- β -D-glucopyranoside (10)¹⁴⁾ together with a new glycoside, 6''-acetylapiin (1), from American dried parsley. A new monoterpene glucoside called petroside (2) was isolated from Japanese fresh parsley together with 8 and icaraside F₂ (11). This paper deals with the isolation of phytoestrogens from American

and Japanese parsley and the structure elucidation of the two new glycosides, 1 and 2.

Estrogenic Activities of Medicinal Herbs We tested the estrogenic activities of various medicinal herbs originating in European and South Asian countries. Table 1 shows the MCF-7 proliferative activities of the methanolic extract (at 1.0 and 10 $\mu\text{g}/\text{ml}$) from twelve Occidental herbs. The extracts of agrimony, raspberry, dill, vervain, and parsley exhibited estrogenic activities at 1.0 $\mu\text{g}/\text{ml}$. Among them, the extract from parsley showed reproducible and potent estrogenic activity equal to that of the isoflavone glycoside fraction of soybean.⁸⁾

Bioassay-Guided Separation of the Constituents from Parsley The methanolic extract from American dried parsley was separated into ethyl acetate- and water-soluble fractions which showed estrogenic activity (Table 2). An ethyl acetate-soluble extract was separated into five fractions by silica gel column chromatography. Fractions 1, 2, 4, and 5 with the activity were further subjected to SiO₂ and reversed phase column chromatography, and finally HPLC. Two benzoxoles, myristicin (3, 0.0051%) and apiole (4, 0.0016%), were isolated from fraction 1, and two furocoumarins, cnidilin (5, 0.0010%) and isoimperatorin (6, 0.0021%), were obtained from fraction 2. Known flavone glycosides, apigetrin (7, 0.0037%), apiin (8, 0.0063%), diosmetin 7-*O*- β -D-glucopyranoside (9, 0.0013%), and kaempferol 3-*O*- β -D-glucopyranoside (10, 0.0024%) and a new flavone glycoside, 6''-acetylapiin (1, 0.0075%) was isolated from fraction 4 and 5. From the water-soluble extract, 8 (1.5%) was also isolated using similar separation procedures. Furthermore, we isolated a new monoterpene glucoside, petroside (2, 0.0004%), from the methanolic extract of Japanese fresh parsley, which also showed potent estrogenic activity, together with 8

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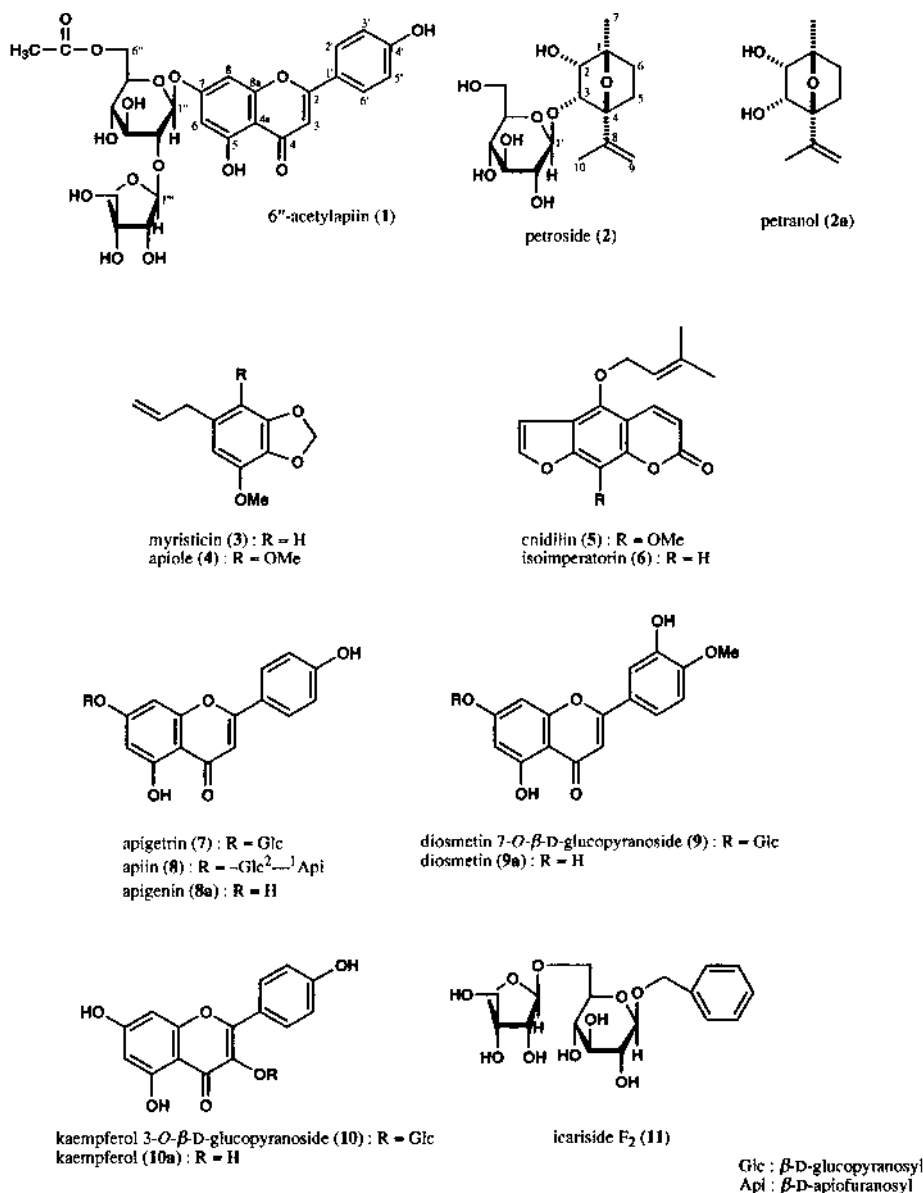


Chart 1. Chemical Constituents Isolated from Parsley

(0.0011%) and icariside F₂ (11, 0.0019%).

Estrogenic Activities of the Constituents from Parsley
Table 3 shows estrogenic activities of constituents from parsley and their derivatives. Benzooxoles (3, 4) and kaempferol 3-O-β-D-glucopyranoside (10) had no activity, and flavone glycosides such as 6''-acetylapiin (1), apiin (8), and diosmetin 7-O-β-D-glucoside (9) showed weak activity at 10 μM but it was not significant. Only apigetrin (7) revealed a potent activity (EC₅₀: 2.2 μM). Apigenin (8a), diosmetin (9a), and kaempferol (10a), the aglycones of 7, 8, 9, and 10, showed significant activities at 1.0 and 10 μM. The ED₅₀ of 8a and 10a were 1.0 and 0.56 μM, respectively, which were nearly equal to those of the isoflavones, daidzein⁸⁾ (0.61 μM) and genistein⁸⁾ (0.69 μM).

Increase by the Methanolic Extracts, Apiin (8), and Apigenin (8a) on Uterus Weight in Ovariectomized Mice
Fig. 1 shows the restoration effects of the methanolic extracts, apiin (8), and apigenin (8a) on uterus weight gain in ovariectomized mice. The mean uterus weight of ovariectomized mice was decreased to 18.6 and 24.2% of sham operated

groups. Consecutive treatment (7 days) of the methanolic extract tended to increase the uterus weight up to 17.5 and 27.0% at a dose of 1000 and 2000 mg/kg, respectively. Apiin (8), a principal flavone glycoside, and apigenin (8a), an aglycone of 8, also restored uterus weight up to 17.4 to 22.4%, and 15.0 to 17.5%, respectively, at a dose of 250 and 500 mg/kg. Although those effects were not significant but weak, all samples showed the activity was dose-dependent.

Structures of 6''-Acetylapiin (1) and Petroside (2)
6''-Acetylapiin (1) was obtained as a white powder and its IR spectrum showed absorption bands at 1735, 1661, and 1607 cm⁻¹ ascribable to carbonyl, chelated carbonyl, and aromatic ring functions, and strong absorption bands at 3415 and 1078 cm⁻¹ suggestive of its oligoglycosidic structure. The UV spectrum of 1 showed absorption maxima (log ε) at 334 (4.1) and 269 (4.0) nm, which suggested the flavone structure. The negative- and positive-ion FAB-MS of 1 showed quasimolecular ions at *m/z* 605 (M-H)⁻ and *m/z* 607 (M+H)⁺, respectively, and high-resolution MS analysis revealed the molecular formula of 1 to be C₃₄H₃₀O₁₅. Fur-

Table 1. Proliferative Activities of Methanolic Extracts from Occidental Herbs on MCF-7

Source	Conc. ($\mu\text{g/ml}$)	Proliferation (% of control)
Rosemary, leaf (<i>Rosmarinus officinalis</i>)	1 10	103.0 $\pm$ 3.2 122.5 $\pm$ 5.3*
Agrimony, aerial part (<i>Agrimonia eupatoria</i>)	1 10	117.0 $\pm$ 2.5** 158.3 $\pm$ 2.8**
Raspberry, leaf (<i>Rubus idaeus</i>)	1 10	163.3 $\pm$ 3.2** 177.7 $\pm$ 4.2**
Lemon verbena, leaf (<i>Aloysia triphylla</i>)	1 10	102.1 $\pm$ 3.5 125.5 $\pm$ 3.3**
Pennyroyal, leaf (<i>Mentha pulegium</i>)	1 10	99.1 $\pm$ 2.9 130.4 $\pm$ 6.0**
Dill, seed (<i>Anethum graveolens</i>)	1 10	128.4 $\pm$ 4.1** 146.8 $\pm$ 2.3**
Fennel, fruit (<i>Foeniculum vulgare</i>)	1 10	108.3 $\pm$ 3.6 126.5 $\pm$ 2.5**
Alfalfa, aerial part (<i>Medicago sativa</i>)	1 10	110.7 $\pm$ 5.0 139.4 $\pm$ 3.0**
Vervain, leaf (<i>Verbena officinalis</i>)	1 10	147.1 $\pm$ 5.0** 152.1 $\pm$ 5.0**
Borage, aerial part (<i>Borago officinalis</i>)	1 10	109.0 $\pm$ 4.6 141.9 $\pm$ 1.5**
Mugwort, aerial part (<i>Artemisa vulgaris</i>)	1 10	113.0 $\pm$ 4.3 133.0 $\pm$ 7.3**
American Parsley, aerial part (<i>Petroselinum crispum</i>)	1 10	134.7 $\pm$ 5.1** 156.0 $\pm$ 5.4**
Soybean isoflavone glycoside fraction	1 10	130.1 $\pm$ 4.9** 157.1 $\pm$ 14.8**

Each value represents mean with the S.E. of 6 experiments. Asterisks denote significant differences from the control *: $p < 0.05$, **: $p < 0.01$, respectively.

Table 2. Proliferative Activities of Extracts and Fractions of Parsley

Sample	Conc. ($\mu\text{g/ml}$)	Proliferation (% of control)
American Parsley		
MeOH extract	1 10	134.7 $\pm$ 5.1** 156.0 $\pm$ 5.4**
AcOEt-soluble extract	2.5 5 10	114.8 $\pm$ 4.5 118.3 $\pm$ 7.3 146.2 $\pm$ 10.2**
H ₂ O-soluble extract	2.5 5 10	116.5 $\pm$ 10.2 119.2 $\pm$ 6.4 133.5 $\pm$ 6.6**
Fr. 1	2.5 5 10	119.2 $\pm$ 2.8** 125.6 $\pm$ 4.3** 146.1 $\pm$ 4.1**
Fr. 2	2.5 5 10	117.8 $\pm$ 3.7* 124.3 $\pm$ 5.4** 145.3 $\pm$ 5.8**
Fr. 3	2.5 5 10	115.1 $\pm$ 3.2 121.9 $\pm$ 2.1* 117.0 $\pm$ 8.5
Fr. 4	2.5 5 10	120.7 $\pm$ 2.5** 120.8 $\pm$ 3.2** 129.0 $\pm$ 2.6**
Fr. 5	2.5 5 10	159.8 $\pm$ 4.9** 159.4 $\pm$ 3.1** 176.0 $\pm$ 4.0**
Japanese Parsley		
MeOH extract	1 10	145.5 $\pm$ 4.5** 158.7 $\pm$ 2.4**

Each value represents mean with the S.E. of 6 experiments. Asterisks denote significant differences from the control *: $p < 0.05$, **: $p < 0.01$, respectively.

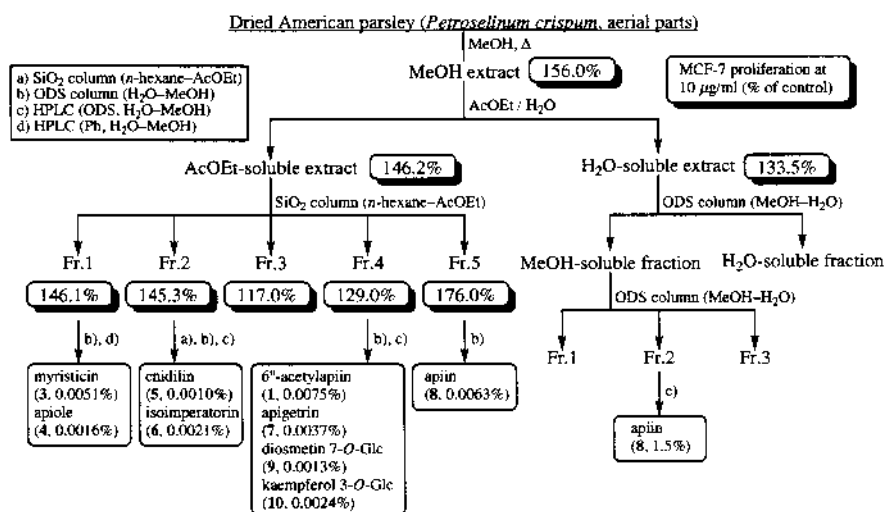


Chart 2

thermore, fragment ion peaks at m/z 473 ($\text{M}-\text{C}_5\text{H}_9\text{O}_4$)⁻ and 269 ($\text{M}-\text{C}_{13}\text{H}_{21}\text{O}_{10}$)⁻, which were derived by cleavage of the glycoside linkage at the 2''- and 7-positions, were observed in the negative-ion FAB-MS of **1**. The proton and carbon signals in the ¹H-NMR (DMSO-*d*₆) and ¹³C-NMR (Table 4) spectra¹⁶⁾ of **1**, were similar to those of apiin (**8**), except for the signals due to an acetyl moiety [δ 2.01 (s)] in **1**. Comparison of the ¹H- and ¹³C-NMR data for **1** with those for **8** revealed an acylation shift around the 6''-position of **1**. In the HMBC experiment on **1**, HMBC correlations were observed between the 1''-proton and the 7-carbon, between the 1'''-pro-

ton and the 2''-carbon, and between the 6''-protons and the acetyl carbonyl carbon. Finally, deacetylation of **1** with sodium methoxide furnished **8** and consequently, the structure of 6''-acetylapiin was determined to be apigenin 7-*O*- β -D-apiofuranosyl(1 $\rightarrow$ 2)-6''-*O*-acetyl- β -D-glucopyranoside (**9**).

Petroside (**2**) was isolated as a white powder. The IR spectrum of **2** showed absorption bands assignable to hydroxyl and *exo*-methylene functions at 3410, 1649, 1452, and 1078 cm⁻¹. The electron impact (EI) MS of **2** showed a molecular ion peak at m/z 346 (M⁺) in addition to a fragment ion peak at m/z 183 (100). The molecular formula C₁₆H₂₆O₈ of **2**

was confirmed from the molecular ion peak and by high-resolution MS measurement. Enzymatic hydrolysis of **2** with β -glucosidase liberated a new monoterpene aglycone petranol (**2a**), while acid hydrolysis of **2** with 5% aqueous sulfuric

Table 3. Estrogenic Activities of Constituents Isolated from Parsley

Source	Conc. (μ M)	Proliferation (% of control)	EC ₅₀ (μ M)
6''-Acetylapiin (1)	0.1	103.3 $\pm$ 6.0	—
	1	116.7 $\pm$ 6.5	
	10	119.2 $\pm$ 3.0	
Myristicin (3)	0.1	94.4 $\pm$ 5.8	—
	1	102.4 $\pm$ 5.4	
	10	92.7 $\pm$ 5.4	
Apiole (4)	0.1	89.9 $\pm$ 3.6	—
	1	83.3 $\pm$ 5.0	
	10	92.9 $\pm$ 4.5	
Apigetrin (7)	0.1	102.6 $\pm$ 5.5	2.2
	1	98.7 $\pm$ 11.5	
	10	206.4 $\pm$ 7.0**	
Apiin (8)	0.1	96.8 $\pm$ 8.8	—
	1	106.5 $\pm$ 6.4	
	10	115.1 $\pm$ 17.7	
Apigenin (8a)	0.1	109.3 $\pm$ 3.5	1.0
	1	148.0 $\pm$ 3.6**	
	10	200.1 $\pm$ 6.2**	
Diosmethin 7- <i>O</i> - β -D-glucopyranoside (9)	0.1	100.8 $\pm$ 10.3	—
	1	102.3 $\pm$ 12.4	
	10	112.7 $\pm$ 12.8	
Diosmethin (9a)	0.1	91.4 $\pm$ 5.8	—
	1	127.9 $\pm$ 5.4**	
	10	133.9 $\pm$ 2.3**	
Kaempferol 3- <i>O</i> - β -D-glucopyranoside (10)	0.1	95.9 $\pm$ 3.8	—
	1	89.1 $\pm$ 6.4	
	10	100.7 $\pm$ 3.3	
Kaempferol (10a)	0.1	130.2 $\pm$ 1.8**	0.56
	1	182.3 $\pm$ 4.0**	
	10	199.6 $\pm$ 4.9**	
Daidzein	0.1	135.0 $\pm$ 4.3**	0.61
	1	170.9 $\pm$ 3.7**	
	10	170.1 $\pm$ 7.4**	
Genistein	0.1	141.7 $\pm$ 9.4**	0.60
	1	171.5 $\pm$ 5.8**	
	10	197.2 $\pm$ 12.4**	

Each value represents mean with the S.E. of 6 experiments. Asterisks denote significant differences from the control **: $p < 0.01$.

acid-1,4-dioxane furnished D-glucose, which was identified by GLC analysis of the thiazolidine derivative.¹⁵⁾ The ¹H-NMR (CD₃OD) and ¹³C-NMR (Table 4) spectra of **2a**, which were assigned on the basis of various NMR experiments,¹⁶⁾ indicated the presence of two tertiary methyls [δ 1.36, 1.79 (both s, 7, 10-H₃)], two methylenes [δ 1.28 (m), 2.12 (ddd-like) (5-H₂), 1.45 (m), 2.26 (ddd-like) (6-H₂)], two methines bearing hydroxyl group [δ 3.52 (d-like, 3-H), 3.72 (d, J =10.6 Hz, 2-H)], and *exo*-methylene [δ 4.82, 4.86 (both brs, 9-H₂)] together with three quaternary carbons (1, 4, 8-C). The plane structure of **2a** was constructed on the basis of ¹H-¹H correlation spectroscopy (H-H COSY) and the heteronuclear multiple bond correlation (HMBC) experiment on **2a** shown in Fig. 2. Thus, the H-H COSY experiment on **2a** indicated the presence of two partial structures shown by thick lines (C-2—C-3 and C-5—C-6). In the HMBC experiment, long-range correlations were observed between the following protons and carbons of **2a** (7-H₃ and 1, 2, 6-C; 10-H₃ and 4, 8, 9-C), so that the connectivities of the quaternary carbons in **2a** were identified. The above-mentioned evidence led us to confirm that the plane structure of **2a** was 1,4-epoxy-*p*-menth-8-en-2,3-diol.

The ¹H-NMR (CD₃OD) and ¹³C-NMR (Table 4) spectra¹⁶⁾ of **2** showed signals assignable to a petrol moiety [δ 1.35, 1.82 (both s, 7, 10-H₃), 1.35, 2.13 (both m, 6-H₂), 1.51 (dt-like), 2.32 (m) (5-H₂), 3.62 (m, 2-H), 4.04 (d, J =8.6 Hz, 3-H), 4.83, 5.14 (both brs, 9-H₂)] and a β -D-glucopyranosyl moiety [δ 4.42 (d, J =7.6 Hz, 1'-H)]. In the HMBC experiment on **2**, long-range correlations were observed between 1'-H and 3-C. The stereostructure of **2** was characterized by a phase-sensitive NOESY experiment on **2**, which showed NOE correlations between the 2-proton and the 3-proton, between the 2-proton and the 7-protons, between the 2-proton and the 6 β -proton, between the 3-proton and the 1'-proton, between the 6 β -proton and the 7-protons, and between the 7-protons and the 10-protons. Finally, the absolute stereostructure of **2** was identified using the glycosylation shift rule,¹⁷⁾ which is achieved by comparison of the 3-carbon signal in the ¹³C-NMR spectrum for **2a** with that for **2**. Thus, the shift value [$\Delta\delta = \delta$ glucoside (**2**) - δ alcohol (**2a**)] is 6.5 ppm, which indicates the absolute configuration of the 3-position to be *R*. On the basis of the above evidence, the structure of

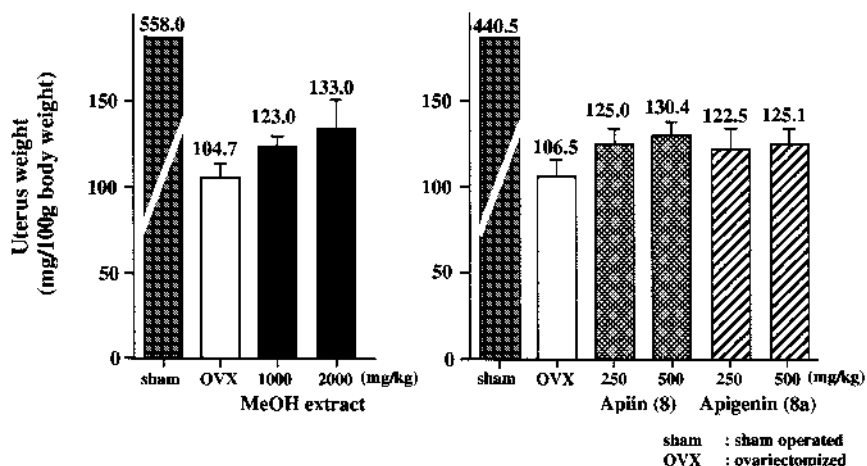


Fig. 1. Effects of Methanolic Extract of Parsley, Apiin (**8**), and Apigenin (**8a**) on Uterus Weight Decrease in Ovariectomized Mice

Mice aged 5 weeks were ovariectomized and kept for 7 d. Samples suspended in 5% Acaica solution were given to mice for the next 7 d. On the last day, mice were sacrificed and the uterus was removed. Each column represents mean with the S.E. of 5 to 7 mice.

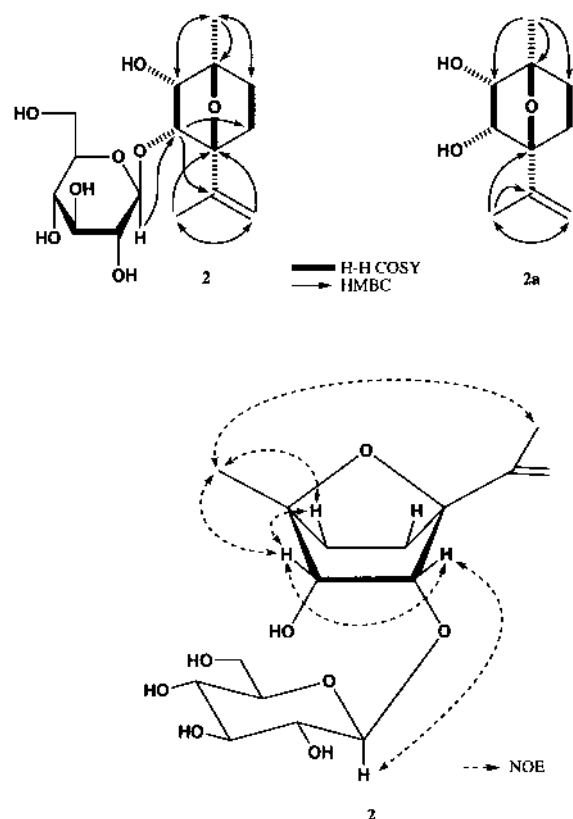


Fig. 2

Table 4. ^{13}C -NMR Data for 6''-Acetylapiin (1), Apiin (8), Petroside (2), Petranol (2a) (68 MHz, CD_3OD , $\text{DMSO}-d_6$)

	1 ^{b)}	8 ^{b)}		2 ^{a)}	2a ^{a)}
C-1			C-1	86.7	86.9
C-2	164.3	164.3	C-2	74.8	74.6
C-3	103.1	103.1	C-3	79.7	73.2
C-4	181.9	181.9	C-4	90.5	91.8
C-4a	161.3	161.4	C-5	30.3	30.3
C-5	99.3	99.4	C-6	30.0	29.3
C-6	162.4	162.7	C-7	20.3	20.4
C-7	94.8	94.8	C-8	145.9	146.7
C-8	156.8	156.9	C-9	111.5	110.6
C-8a	105.4	105.4	C-10	19.4	19.2
C-1'	121.0	121.0	Glc-1'	104.2	
C-2'	128.5	128.5	-2'	75.4	
C-3'	115.9	115.9	-3'	78.3	
C-4'	161.1	161.1	-4'	71.5	
C-5'	115.9	116.0	-5'	78.2	
C-6'	128.5	128.5	-6'	62.8	
Glc-1''	98.0	98.2			
-2''	75.7	75.9			
-3''	76.1	76.1			
-4''	70.0	69.8			
-5''	76.1	77.0			
-6''	63.3	60.6			
Api-1'''	108.7	108.7			
-2'''	76.4	76.7			
-3'''	79.2	79.1			
-4'''	73.9	73.9			
-5'''	64.1	64.2			
Ac-1	170.6				
-2	20.4				

a) In CD_3OD , b) in $\text{DMSO}-d_6$.

petroside (2) was determined as shown.

Experimental

The following instruments were used to obtain physical data: specific rotations, Horiba SEPA-300 digital polarimeter ($l=5\text{ cm}$); UV spectra, Shimadzu UV-1200 spectrometer; IR spectra, Shimadzu FTIR-8100 spectrometer; ^1H -NMR spectra, JEOL EX-270 (270 MHz) and JNM-LA500 (500 MHz) spectrometer; ^{13}C -NMR spectra, JEOL EX-270 (68 MHz) and JNM-LA500 (125 MHz) spectrometers with tetramethylsilane as an internal standard; MS and high-resolution MS, JEOL JMS-SX 102A mass spectrometer and JMS-GCMATE; HPLC, Shimadzu LC-10AS chromatograph.

The following experimental conditions were used for chromatography: normal-phase column chromatography; silica gel BW-200 (Fuji Silysia Chemical, Ltd., 150–350 mesh), reversed-phase column chromatography; Chromatorex ODS DM1020T (Fuji Silysia Chemical, Ltd., 100–200 mesh); TLC, pre-coated TLC plates with silica gel 60F₂₅₄ (Merck, 0.25 mm) (normal-phase) and silica gel RP-18 60F₂₅₄ (Merck, 0.25 mm) (reversed-phase); HPTLC, pre-coated TLC plates with silica gel RP-18 60WF_{254S} (Merck, 0.25 mm) (reversed-phase). Detection was done by spraying with 1% $\text{Ce}(\text{SO}_4)_2$ –10% aqueous H_2SO_4 , followed by heating.

Preparation of Herbal Extract Dried herbs were purchased from Tochimoto Tenkaido Co., Ltd. (Osaka, Japan). Each herb was extracted with 5 volumes of MeOH 3 times at 80 °C for 3 h, and the extract was evaporated under reduced pressure at under 40 °C to give the MeOH extract.

Cell Culture MCF-7 cells¹⁸⁾ were kindly provided by the Institute of Development, Aging and Cancer, Tohoku University. Cells were maintained in Dulbecco's modified Eagle medium (D-MEM, Gibco BRL, Life Technologies) supplemented with 10% fetal calf serum (FCS, Biowhittaker) and an antibiotic [penicillin (100 unit/ml) and streptomycin (100 $\mu\text{g}/\text{ml}$) mixture (Gibco BRL, Life Technologies)], and subcultured every 3 or 4 d.

Estrogenic Activity of Herb Estrogenic activity was investigated with MCF-7 according to the previous report.¹⁹⁾ MCF-7 collected by trypsinization was washed (80 $\times$ g, 3 min, 4 °C) 3 times with phenol red-free D-MEM. Cells (2000 cells/100 μl) were suspended in phenol red-free D-MEM containing 5% estrogen free FCS (charcoal and dextran treated FCS),²⁰⁾ and were seeded in a 96-well culture plate. After 24 h culture, the medium was changed with 90 μl fresh medium and 10 μl sample solution, and the cells were continuously cultured for 4 days. Proliferation of cells was assessed by MTT assay.²¹⁾ Each samples were dissolved in dimethyl sulfoxide (DMSO) and diluted with medium. The final concentration of DMSO was adjusted to 0.1 or 0.01% in each experiment.

Measurement of Uterus Weight Increase in Ovariectomized Mice Mice aged 5 weeks (Kiwa Laboratory Animals, Wakayama, Japan) were ovariectomized and were kept for 7 d. The methanolic extracts, apigenin, and apiin were suspended with 5% Acacia in water, and given to ovariectomized mice for 7 consecutive days. On the last day, mice were sacrificed by cervical vertebra dislocation and the uterus was removed. The uterus was weighed immediately and its ratio to total body weight was calculated.

Statistical Analysis Dunnett's multiple range test²²⁾ was used to evaluate any significant difference from the control group.

Extraction and Isolation. i) **American Parsley** The dried aerial parts of *Petroselinum crispum* MILL. cultivated in U.S.A. (900 g, purchased from Tochimoto Tenkaido Co., Ltd., Osaka) were powdered and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (320.9 g, 36%), and the MeOH extract (300 g) was partitioned in an AcOEt– H_2O (1:1, v/v) mixture. Removal of the solvent from the AcOEt-soluble and H_2O -soluble fractions under reduced pressure yielded the AcOEt-soluble extract (77.8 g, 8.6%) and the H_2O -soluble extract (202.2 g, 24%). The AcOEt-soluble extract (72.8 g) was separated by normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Ltd., 810 g), n -hexane–AcOEt (4:1 $\rightarrow$ 2:1, v/v) $\rightarrow$ CHCl_3 –MeOH (30:1 $\rightarrow$ 15:1 $\rightarrow$ 5:1, v/v) $\rightarrow$ MeOH] to afford seven fractions [fr. 1 (8.1 g), fr. 2 (6.9 g), fr. 3 (25.7 g), fr. 4 (7.7 g), fr. 5 (6.8 g)]. Fraction 1 (6.8 g) was separated by reversed-phase silica gel column chromatography [Chromatorex DM1020T (Fuji Silysia Ltd., 120 g), MeOH– H_2O (70:30 $\rightarrow$ 95:5, v/v) $\rightarrow$ CHCl_3 –MeOH– H_2O (6:4:1, v/v) $\rightarrow$ MeOH] and HPLC [YMC-Pack Ph (250 $\times$ 20 mm i.d., YMC Co., Ltd.), MeOH– H_2O (60:40, v/v)] to give myristicin (3, 46 mg, 0.0051%) and apiol (4, 15 mg, 0.0016%). Fraction 2 (6.0 g) was separated by normal-phase [200 g, n -hexane–AcOEt (9:1 $\rightarrow$ 5:1 $\rightarrow$ 3:1 $\rightarrow$ 2:1, v/v) $\rightarrow$ MeOH], reversed-phase silica gel column chromatography [50 g, MeOH– H_2O (70:30 $\rightarrow$ 80:20 $\rightarrow$ 95:5, v/v) $\rightarrow$ CHCl_3 –MeOH– H_2O (6:4:1, v/v) $\rightarrow$ MeOH], and HPLC [YMC-Pack ODS-A (250 $\times$ 20 mm i.d., YMC Co., Ltd.), MeOH– H_2O (75:25, v/v)] to give cnicidin (5, 8.9 mg, 0.0010%) and isoimperatorin (6, 19 mg, 0.0021%). Fraction

4 (7.0 g) was separated by reversed-phase silica gel column chromatography [200 g, MeOH-H₂O (50:50→70:30→90:10→95:5, v/v)→CHCl₃-MeOH-H₂O (6:4:1, v/v)→MeOH] to give fr. 4-1 (1.3 g), 4-2 (783 mg), 4-3 (158 mg), 4-4 (535 mg, fatty acid mixture), 4-5 (592 mg), 4-6 (2.9 g, daucosterin), 4-7 (569 mg). Fraction 4-1 (450 mg) was purified by HPLC [MeOH-H₂O (45:55, v/v)] to give apigetrin (**7**, 33 mg, 0.0037%), diosmetin 7-*O*-β-D-glucopyranoside (**9**, 12 mg, 0.0013%), and kaempferol 3-*O*-β-D-glucopyranoside (**10**, 21 mg, 0.0024%). Fraction 4-2 (175 mg) was purified by HPLC [MeOH-H₂O (60:40, v/v)] to give 6"-acetylapiin (**1**, 68 mg, 0.0075%). Fraction 5 (5.7 g) was separated by reversed-phase silica gel column chromatography [180 g, MeOH-H₂O (50:50→60:40→70:30→90:10, v/v)→CHCl₃-MeOH-H₂O (6:4:1, v/v)→MeOH] to give apiin (**8**, 56 mg, 0.0063%). The H₂O-soluble fraction (23.3 g) was separated by reversed-phase silica gel column chromatography [700 g, MeOH-H₂O (30:70→50:50→70:30)→MeOH→CHCl₃-MeOH-H₂O (6:4:1, v/v)→MeOH] to give **8** (1.3 g, 1.5%). The known compounds (**3**–**10**) were identified by comparison of their physical data ([α]_D, ¹H-NMR, ¹³C-NMR) with reported values.^{9–14)}

6"-Acetylapiin (**1**): A white powder, [α]_D²⁵ -151.6° (*c*=0.4, MeOH). High-resolution positive-ion FAB-MS: Calcd for C₃₄H₃₁O₁₅ (M+H)⁺: 607.1658. Found: 607.1671. UV [λ_{max}^{MeOH} nm (log ε)]: 334 (4.1), 269 (4.0). IR (KBr): 3415, 2926, 1735, 1661, 1607, 1078 cm⁻¹. ¹H-NMR (270 MHz, DMSO-*d*₆) δ: 2.01 (3H, s, Ac-2), 5.19 (1H, d, *J*=7.6 Hz, 1"-H), 5.36 (1H, br s, 1"-H), 6.43 (1H, d-like, 6-H), 6.77 (1H, d-like, 8-H), 6.84 (1H, s, 3-H), 6.94 (2H, d, *J*=8.9 Hz, 3', 5'-H), 7.94 (2H, d, *J*=8.9 Hz, 2', 6'-H). ¹³C-NMR (DMSO-*d*₆, 68 MHz) δ_c: given in Table 4. Negative-ion FAB-MS: *m/z* 605 (M-H)⁻, 473 (M-C₃H₅O₄)⁻, 269 (M-C₁₃H₂₁O₁₀)⁻. Positive-ion FAB-MS: *m/z* 607 (M+H)⁺.

ii) **Japanese Parsley** The fresh aerial parts of Japanese parsley (2.2 kg) cultivated in Chiba Prefecture, Japan were cut and extracted three times with MeOH under reflux. Evaporation of the solvent under reduced pressure provided the MeOH extract (120 g, 5.5%), and 100 g of this extract was partitioned in an AcOEt-H₂O (1:1, v/v) mixture. Removal of the solvent from the AcOEt-soluble and H₂O-soluble fractions under reduced pressure yielded the AcOEt-soluble extract (14.5 g, 0.8%) and H₂O-soluble extract (85.5 g, 4.7%). The H₂O-soluble extract (85.5 g) was separated by reversed-phase silica gel column chromatography [Chromatorex DM1020T (Fuji Silysia Ltd., 120 g), H₂O→MeOH→CHCl₃-MeOH-H₂O (5:5:1, v/v)→MeOH→acetone→MeOH] to afford seven fractions [fr. 1 (11.1 g), fr. 2 (184 mg), fr. 3 (99 mg), fr. 4 (70 mg), fr. 5 (67 mg), fr. 6 (63 mg), fr. 7 (49 mg)]. Fraction 1 (10 g) was separated by normal-phase silica gel column chromatography [BW-200 (Fuji Silysia Ltd., 500 g), CHCl₃-MeOH-H₂O (15:3:1, lower layer→6:4:1, v/v) to give fr. 1-1 (790 mg), 1-2 (406 mg), 1-3 (496 mg), 1-4 (269 mg), 1-5 (3.2 g), 1-6 (1.8 g), and 1-7 (1.2 g). Fraction 1-2 (400 mg) was separated by reversed-phase silica gel column chromatography [10 g, H₂O→MeOH] and purified by HPLC [YMC-Pack ODS-AL (250×20 mm i.d.), MeOH-H₂O (50:50, v/v)] to give petroside (**2**, 10 mg, 0.0006%). Fraction 1-4 (260 mg) was separated by reversed-phase silica gel column chromatography [10 g, H₂O→MeOH] and purified by HPLC [MeOH-H₂O (35:65, v/v)] to give icarisside F₂ (**11**, 24 mg, 0.0015%). Fraction 1-6 (1.8 g) was separated by reversed-phase silica gel column chromatography [90 g, H₂O→MeOH] and purified by HPLC [MeOH-H₂O (45:55, v/v)] to give apiin (**8**, 318 mg, 0.0193%). The known compounds (**8**, **11**) were identified by comparison of their physical data ([α]_D, ¹H-NMR, ¹³C-NMR) with reported values.^{12,23)}

Petroside (**2**): A white powder, [α]_D²⁵ -13.8° (*c*=0.3, MeOH). High-resolution EI-MS: Calcd for C₁₆H₂₆O₈ (M⁺): 346.1627. Found: 346.1631. IR (KBr): 3410, 2928, 1649, 1452, 1078 cm⁻¹. ¹H-NMR (270 MHz, CD₃OD) δ: 1.35, 1.82 (3H each, both s, 7, 10-H₃), 1.35, 2.13 (1H each, both m, 6-H₂), 1.51 (1H, dt-like), 2.32 (1H, m) (5-H₂), 3.62 (1H, m, 2-H), 4.04 (1H, d, *J*=8.6 Hz, 3-H), 4.42 (1H, d, *J*=7.6 Hz, 1'-H), 4.83, 5.14 (1H each, both brs, 9-H₂). ¹³C-NMR (68 MHz, CD₃OD) δ_c: given in Table 4. EI-MS: *m/z* 346 (M⁺, 3.8), 183 (100.0).

Deacetylation of 6"-Acetylapiin (1**)** A solution of **1** (1 mg) in 0.1% NaOMe-MeOH (1.0 ml) was stirred at room temperature for 15 min. The reaction solution was neutralized with Dowex HCR W2 (H⁺ form) and the insoluble portion was removed by filtration. After removal of the solvent *in vacuo* from the filtrate, the crude product was purified by normal-phase silica gel column chromatography [1 g, *n*-hexane-AcOEt (1:1)] to give apiin (**8**, 1 mg), which was identified by comparison of physical data ([α]_D, ¹H-NMR) and TLC with an authentic sample.

Enzymatic Hydrolysis of Petroside (2**) Given Petranol (**2a**)** A solution of **2** (6 mg) in 0.2 M acetate buffer (pH 4.4, 1.0 ml) was treated with β-glucosidase (Oriental Yeast Co., Japan, 6.0 mg) and the reaction mixture was

stirred at 38 °C for 12 h. The reaction mixture was then poured into H₂O and the whole was extracted with AcOEt. The AcOEt extract was washed with sat. aq. NaHCO₃ and brine, then dried over MgSO₄ and filtered. After removal of the solvent under reduced pressure, the residue (3 mg) was purified by silica gel column chromatography [5.0 g, *n*-hexane-AcOEt (1:1)] to give **2a** (2 mg).

Petranol (**2a**): A white powder, [α]_D²⁷ +18.7° (*c*=0.1, MeOH). High-resolution EI-MS: Calcd for C₁₀H₁₆O₃ (M⁺): 184.1099. Found: 184.1101. IR (KBr) 3436, 2361, 1385, 1117 cm⁻¹. ¹H-NMR (270 MHz, CD₃OD) δ: 1.28 (1H, m), 2.12 (1H, ddd-like) (5-H₂), 1.36, 1.79 (3H each, both s, 7, 10-H₃), 1.45 (1H, m), 2.26 (1H, ddd-like) (6-H₂), 3.52 (1H, d-like, 3-H), 3.72 (1H, d, *J*=10.6 Hz, 2-H), 4.82, 4.86 (1H each, both brs, 9-H₂). ¹³C-NMR (CD₃OD, 68 MHz) δ_c: given in Table 4.

Acid Hydrolysis of Petroside (2**)** A solution of petroside (**2**, 2 mg) in 5% aqueous H₂SO₄-1,4-dioxane (1:1, v/v, 2 ml) was heated under reflux for 1 h. After cooling, the reaction mixture was neutralized with Amberlite IRA-400 (OH⁻ form) and the residue was removed by filtration. After removal of the solvent from the filtrate *in vacuo*, the residue was passed through a Sep-Pak C₁₈ cartridge with H₂O and MeOH. The H₂O eluate was concentrated and the residue was treated with L-cysteine methyl ester hydrochloride (4 mg) in pyridine (0.5 ml) at 60 °C for 1 h. After reaction, the solution was treated with *N,O*-bis(trimethylsilyl)trifluoroacetamide (0.2 ml) at 60 °C for 1 h. The supernatant was then subjected to GLC analysis to identify the derivatives of D-glucose from **2**; GLC conditions: Supelco STBTM-1, 30 m×0.25 mm (i.d.) capillary column, column temperature 230 °C, He flow rate 15 ml/min, *t*_R: 24.2 min.

References and Notes

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