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Antiestrogenic Constituents of the Thai Medicinal Plants *Capparis flavicans* and *Vitex glabrata*

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Antiestrogenic compounds were investigated from Thai indigenous plants for galactogogues since estrogen is reported to suppress lactation in breastfeeding women. The aerial parts of the Thai medicinal plant *Capparis flavicans*, which has traditionally been used to promote lactation, gave the new compound capparoside A (**1**), along with 28 known compounds. The leaves of *Vitex glabrata* belong to the same genus as the chaste tree (*Vitex agnus-castus*), which is used traditionally to support lactation, and afforded the new compounds khainaoside A (**14**), khainaoside B (**15**), and khainaoside C (**16**), together with six known compounds. The isolates were tested for their biological activity using the estrogen-responsive human breast cancer cell lines MCF-7 and T47D. Syringaresinol (**3**) and princepin (**6**), from *C. flavicans*, and khainaoside A (**14**) showed the most potent inhibitory effects on estrogen-enhanced cell proliferation among all compounds isolated. These results suggest that the lactation-promoting properties of *C. flavicans* might be related to the inhibitory effect on excess estrogen of women who experience insufficient breastfeeding and highlight the possibility of using *V. glabrata* leaves for their antiestrogenic properties.

Estrogen acts as a regulator in the milk production process during pregnancy.[1,2] While breastfeeding, women have high levels of prolactin and relatively low levels of estradiol.[3] High levels of estradiol can inhibit lactation due to reduced milk secretion and changes in mammary secretory epithelial morphology.[4,5]

An inadequate milk production has often been observed in mothers who deliver preterm infants.[6,7] Other factors such as diabetes, hypothyroidism, obesity, thecalutein cysts, and polycystic ovarian syndrome may also lead to insufficient milk production.[8] Herbal galactogogues, such as fenugreek (*Trigonella foenum-graecum*) seed, goat's rue (*Galega officinalis*), fennel (*Foeniculum vulgare*) seed, and chaste tree (*Vitex agnus-castus*) seed are used widely today in an attempt to overcome this problem.[9]

While there are indigenous herbal galactogogues available in Thailand, there is limited scientific evidence supporting their use. In a continuation of work on the bioactive constituents of Thai medicinal plants, *Capparis flavicans* and *Vitex glabrata* were investigated as potential herbal galactogogues.

Capparis flavicans Kurz. (Capparidaceae), which is known as "kra-chick" or "ngua-lea" in Thailand,[10] is used as a folk medicine in northeast Thailand for promoting lactation in breastfeeding women.[11] The plant grows in mixed deciduous forests at 40−350 m above sea level in Thailand and Myanmar. As no research on the chemical constituents of this plant has been carried out previously, we have investigated an extract from the aerial parts of *C. flavicans*.

Vitex glabrata R.Br. (Verbenaceae), which is called "khai-nao" in the Thai language,[10] is used for its bark and roots as a folk medicine in Thailand as a tonic, an antidiarrheal agent, and an antipyretic.[12] In previous studies, three ecdysteroids were isolated from the stem bark and root bark of this species.[13,14]

This paper deals with the isolation of constituents from these plants, their structure characterization by spectroscopic methods, and the assessment of antiestrogenic activity of the isolates as an index for their lactation-promoting activity using estrogen-responsive MCF-7 and T47D cell lines.

Results and Discussion

A methanolic extract of the aerial parts of *C. flavicans* was partitioned with ethyl acetate and H_2O to yield ethyl acetate- (22 g) and H_2O-soluble fractions (90 g). Then, the H_2O-soluble components were subjected to HP20 resin column chromatography by elution with H_2O, 50% MeOH, and MeOH to give H_2O (60 g), 50% MeOH (18 g), and MeOH eluates (3 g), respectively. The ethyl acetate-soluble fraction and 50% MeOH eluate suppressed the estrogen-enhanced proliferation in MCF-7 and T47D cells at a concentration of 0.2 μg/mL. Thus, these two fractions were subjected to silica gel column chromatography and HPLC to give 29 pure compounds, including a new compound (**1**). Twenty-eight known compounds were identified as (7*R*,8*R*)-*threo*-guaiacylglycerol 8-*O*-4′-sinapyl ether 7-*O*-β-D-glucopyranoside (**2**),[15] (±)-syringaresinol (**3**),[16] (±)-3′,3″-bisdemethylpinoresinol (**4**),[17] isoamericanol A (**5**),[18] princepin (**6**),[19] (−)-dehydrodiconiferyl alcohol 4-*O*-β-D-glucopyranoside (**7**),[20] (±)-8,8′-dimethoxy-1-*O*-(β-D-glucopyranosyl) secoisolariciresinol (**8**),[21] spionoside B (**9**),[22] tachioside (**10**),[23] koaburaside (**11**),[24] 4-hydroxy-2,6-dimethoxyphenol 1-*O*-β-D-glucopyranoside, leonuriside (**12**),[25] adenosine (**13**),[26] tamarixetin 3-*O*-β-D-galactoside,[27] americanol A,[18] isoprincepin,[19] (−)-5,5′-dimethoxy-4-*O*-(β-D-glucopyranosyl) lariciresinol,[28] alangilignoside B,[29] alangilignoside C,[29] alangilignoside D,[29] (−)-lariciresinol 4-*O*-β-D-glucopyranoside,[30] 3α-*O*-β-D-glucopyranosyl lyoniresinol,[31] icariside B_1,[32] roseoside,[33] isotachioside,[23] vanilloloside,[28] 4-hydroxy-3-methoxyphenyl 1-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside,[34] alangionoside C,[35] and 4-(hydroxymethyl)-2,6-dimethoxyphenyl 1-*O*-β-D-glucopyranoside,[36] respectively, by comparison of their spectroscopic data (especially NMR) and optical rotation with the reported values in the literature.

Capparoside A (**1**), $[\alpha]^{25}_D$ −62.8, was assigned the molecular formula $C_{18}H_{26}O_{12}$, from its molecular ion $[M + Na]^+$ peak at *m/z* 457.1331 in the HRFABMS. Its ^{1}H NMR spectrum indicated the presence of a trisubstituted aromatic ring (ABX system) from signals at δ 7.02 (d, J = 9 Hz), 6.96 (d, J = 3 Hz), and 6.31 (dd, J = 9, 3 Hz). Two anomeric proton signals at δ 4.68 (d, J = 8.5 Hz) and 4.30 (d, J = 7.5 Hz) and a methoxy group signal at δ 3.80 were also observed. On acid hydrolysis, **1** gave 1,4-dihydroxy-2-methoxybenzene as its aglycon and two sugar units. The sugar components were identified by GC analysis as D-glucose and D-xylose. The HMBC spectrum revealed connectivities between (i) δ 4.68 (H-1′) and 141.0 (C-1), (ii) δ 4.30 (H-1″) and 69.7 (C-6′), and (iii) δ 3.80 (MeO-2) and 152.1 (C-2). Consequently, the structure of capparoside A (**1**) was established as 2-methoxy-4-hydroxyphenol 1-*O*-β-D-xylopyranosyl-(1→6)-β-D-glucopyranoside.

The methanolic extract of the leaves of *V. glabrata* was partitioned with ethyl acetate and H_2O to yield ethyl acetate- (76 g) and H_2O-soluble fractions (124 g). Next, the H_2O-soluble fraction

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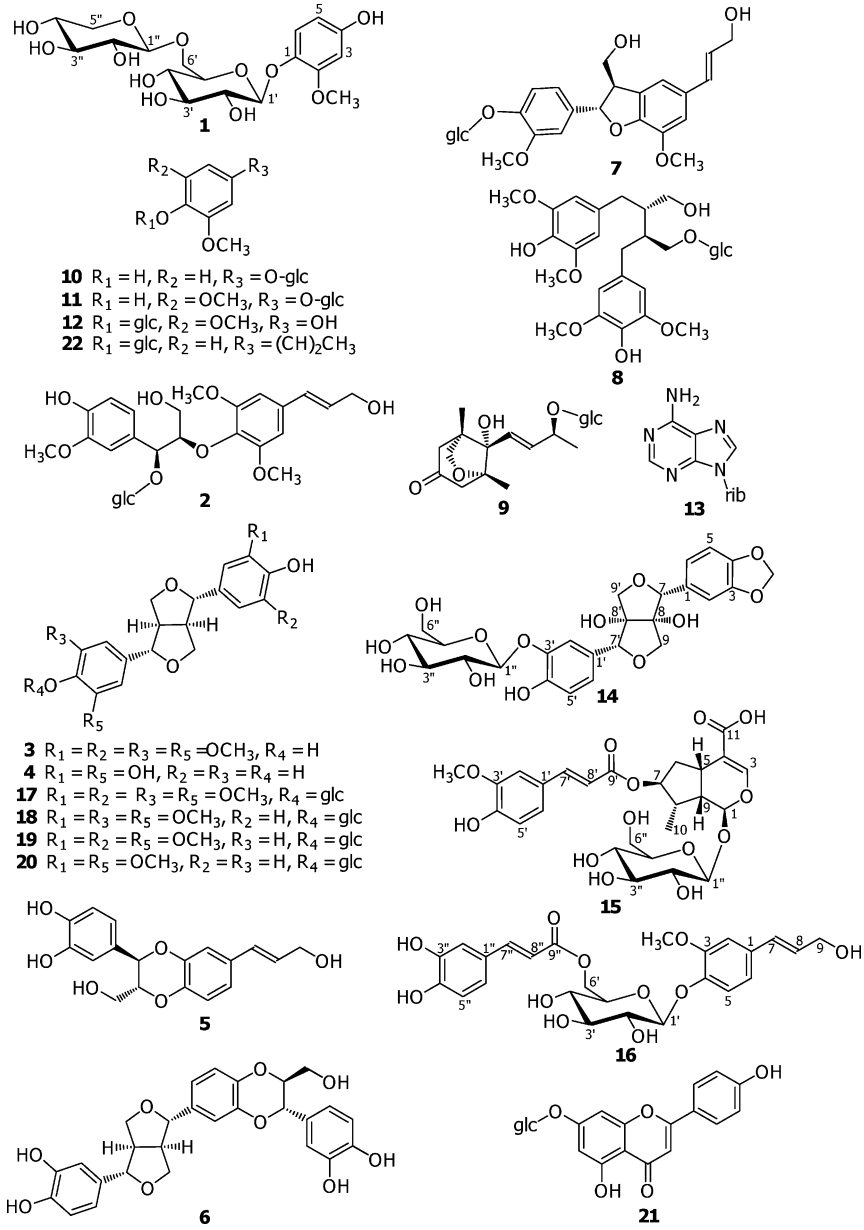
Chart 1. Compounds from *C. flavicans* and *V. glabrata*



was subjected to HP20 resin column chromatography by elution with H_2O, 50% MeOH, and MeOH to give H_2O- (57 g), 50% MeOH- (49 g), and MeOH-soluble fractions (19 g), respectively. The MeOH eluate was subjected to silica gel column chromatography and HPLC to give nine pure compounds, including three new compounds (**14**−**16**). Six known compounds were identified as (−)-syringaresinol 4-*O*-*β*-D-glucopyranoside (**17**),[37] (+) eucommin A (**18**),[38] (±)-isoeucommin A (**19**),[37] (+) pinoresinol 4-*O*-*β*-D-glucopyranoside (**20**),[38] (−)-apigenin 7-*O*-*β*-D-glucopyranoside (**21**),[39] and (−)-isocitruscin C (**22**),[40] respectively, by comparison of their spectroscopic data with literature values.

Khainaoside A (**14**), $[\alpha]^{25}_D$ −40.0, was assigned the molecular formula $C_{25}H_{28}O_{13}$, as a result of its molecular ion $[M + Na]^+$ peak at *m*/*z* 559.1447 in the HRFABMS. The ^{1}H NMR spectrum showed two sets of trisubstituted aromatic rings from the signals at *δ* 7.28 (d, *J* = 2 Hz), 6.96 (dd, *J* = 8.5, 2 Hz), and 6.82 (d, *J* = 8.5 Hz) and *δ* 6.93 (d, *J* = 2 Hz), 6.85 (dd, *J* = 8, 2 Hz), and 6.77 (d, *J* = 8 Hz), and two pairs of methylene protons at *δ* 3.96 (d, *J* = 10 Hz) and 4.07 (d, *J* = 10 Hz) and *δ* 3.98 (d, *J* = 10 Hz) and 4.07 (d, *J* = 10 Hz). Three singlet signals at *δ* 4.93, 4.95, and 5.91 (2H) were also observed together with an anomeric proton at *δ* 4.78 (d, *J* = 7.5 Hz). The NMR spectra of **14** suggested it to be a lignan glycoside with a dioxabicyclooctyl unit, and its structure was similar to that of prinsepiol, which was first isolated from *Prinsepia utilus*.[41] Acid hydrolysis of **14** gave 3,4-methylenedioxyprinsepiol as the aglycon and a sugar moiety, which was identified by GC analysis as D-glucose. The HMBC spectrum showed connectivities of five partial structures, namely, (i) *δ* 5.91 (O-CH_2-O) to *δ* 148.7, 148.8 (C-3, C-4); (ii) *δ* 6.93 (H-2) to *δ* 91.0 (C-7); (iii) *δ* 7.28 (H-2′) to *δ* 88.6 (C-7′); and (iv) *δ* 4.78 (H-1″) to *δ* 146.3 (C-3′). The attachment position of the sugar was also confirmed by the NOE enhancement of H-2′ (*δ* 7.28), when the anomeric proton (*δ* 4.78) was irradiated. The absolute configuration of the dioxabicyclooctyl ring was determined to be 7*R*, 8*S*, 7′*R*, and 8′*S* from the negative Cotton effect at 227 nm in the CD spectrum, with reference to the CD data for princepiol and its glucoside.[42] Consequently, the structure of khainaoside A (**14**) was assigned as 3,4-methylenedioxyprinsepiol 3′-*O*-*β*-D-glucopyranoside.

Khainaoside B (**15**), $[\alpha]^{25}_D$ −63.8, was confirmed to have the molecular formula $C_{26}H_{32}O_{13}$ from its molecular ion $[M + Na]^+$ peak at *m*/*z* 575.1768 in HRFABMS. The ^{1}H NMR spectrum indicated *trans* olefinic protons at *δ* 7.58 (d, *J* = 16 Hz) and 6.34 (d, *J* = 16 Hz), ABX-type coupled aromatic protons at *δ* 7.17 (d,

$J = 2$ Hz), 7.06 (dd, $J = 8.5$, 2 Hz), and 6.80 (d, $J = 8.5$ Hz), and two hemiacetal protons at δ 4.69 (d, $J = 8$ Hz) and 5.54 (d, $J = 5$ Hz). The ^{1}H NMR spectrum also suggested the presence of a feruloyl moiety and a sugar in the structure of this compound. The aglycon of **15** was suspected to be a monoterpene from its molecular formula, and the NMR data indicated it to be an iridoid. The NMR data of **15** were similar to those of 7-*O*-*E*-feruloyllloganic acid except for downfield shifts for H-9 (+0.5 ppm) and C-7 (+4.2 ppm) and upfield shifts for H-7 (−0.35 ppm) and C-9 (−3.9 ppm).[43] These effects were due to the configuration of the methyl group at C-8 as found in 8-epiloganin.[44] The HMBC spectrum gave information of connectivities of three partial structures as (i) δ 5.54 (H-1) to δ 99.8, 152.6 (C-1′, C-3); (ii) δ 7.17 (H-2′) to δ 146.7 (C-7′); (iii) δ 7.58 (H-7′) to δ 169.0 (C-9′); and (iv) δ 4.69 (H-1″) to δ 96.0 (C-1). A sugar moiety was identified by GC analysis as D-glucose after an acid hydrolysis of **15**. From this evidence, the structure of khainaoside B (**15**) was assigned as 7-*O*-*E*-feruloyl-8-epiloganic acid.

Khainaoside C (**16**), $[\alpha]^{25}_D$ −20.8, was assigned the molecular formula $C_{25}H_{28}O_{11}$, due to its molecular ion $[M + Na]^+$ peak at *m*/*z* 527.1514 in the HRFABMS. The ^{1}H NMR spectrum showed two sets of trisubstituted aromatic rings from the signals at δ 7.02 (d, $J = 8.5$ Hz), 6.99 (d, $J = 2$ Hz), and 6.78 (dd, $J = 8.5$, 2 Hz) and δ 7.04 (d, $J = 2$ Hz), 6.93 (dd, $J = 8.5$, 2 Hz), and 6.79 (d, $J = 8.5$ Hz). Two pairs of *trans*-olefinic protons at δ 6.40 (brd, $J = 16$ Hz) and 6.07 (dt, $J = 16$, 6 Hz) and δ 7.53 (d, $J = 16$ Hz) and 6.24 (d, $J = 16$ Hz) were observed, together with an anomeric proton at δ 4.85 (d, $J = 7$ Hz). A singlet methoxy signal was also found at δ 3.85. The NMR spectra of **16** were similar to those of dracunculifoside D isolated from *Baccharis dracunculifolia* except for the loss of one methoxy signal.[45] The HMBC spectrum showed connectivities of four partial structures, namely, (i) δ 4.85 (H-1′) to δ 147.3 (C-4); (ii) δ 3.85 (OMe-3) to δ 151.0 (C-3); (iii) δ 6.99 (H-2) to δ 131.0 (C-7); (iv) δ 7.04 (H-2″) to δ 147.1 (C-7″); (v) δ 7.53 (H-7″) to δ 168.9 (C-9″); and (vi) δ 4.36, 4.50 (H-6′) to δ 168.9 (C-9″). On acid hydrolysis, **16** gave caffeic acid and coniferyl alcohol as aglycons and a sugar unit, which was identified by GC analysis as D-glucose. Consequently, the structure of khainaoside C (**16**) was assigned as {3-methoxy-4-[(6-*O*-(*E*)-caffeoyl)-β-D-glucopyranosyl]}phenyl-1-propen-3-ol.

Estrogenic activity based on stimulatory effects on MCF-7 and T47D cell proliferation was tested with increasing concentrations of all 38 compounds obtained in this study at 0.1, 1, 10, and 100 μM, and their stimulatory activity on cell proliferation was determined by comparison with a positive control, estradiol (E_2), at concentrations ranging from 1 to 100 pM. The bioassay results indicated that no compound exhibited significant stimulatory effects on MCF-7 and T47D cell proliferation (data not shown).

Antiestrogenic activities based on MCF-7 and T47D cell proliferation were also investigated for all 38 compounds. Estradiol (E_2) at 100 pM was used initially to enhance cell proliferation, and each compound was tested at 0.1, 1, 10, and 100 μM. Twenty-two compounds showed significant antiestrogenic effects in this assay (Table 3). Lignans **3**, **6**, and **14** exhibited strong inhibitory activity on E_2-enhanced cell proliferation for both cell lines, and low concentrations were found for their iEqE$_1$ values (**3**, 5 and 7 μM; **6**, 7 and 6 μM; **14**, <0.1 and 3 μM for MCF-7 and T47D, respectively). Those compounds showing antiestrogenic activity were observed to commonly bear a dioxabicyclooctyl ring in their structure. Although **4** has an analogous structure to **3**, the two catechol groups in the structure led to a lower activity when compared to two 3,5-dimethoxypyrogalloyl groups. The analogous lignan glycosides, **17**−**20**, showed high activity particularly against MCF-7 cells, with low iEqE$_1$ values. The phenylpropanoid glucosides (**16**, **22**) also displayed high activity against MCF-7 cells, whereas the phenol glucosides (**1**, **10**−**12**) showed lower activity for the cells. Some samples constantly suppressed E_2 activity to a level less than 10 or 50 pM at the concentrations tested and were expressed as strong (S) or mild (M), respectively.

Table 1. NMR Data of Compound **1** in MeOH-d_4 (^{1}H and ^{13}C at 400 and 100.4 MHz, respectively)

position	δ_H (J in Hz)	δ_C
1		141.0
2		152.1
3	6.96, d (3)	102.1
4		154.9
5	6.31, dd (9,3)	107.8
6	7.02, d (9)	120.7
1′	4.68, d (8.5)	104.2
2′	3.41, t (8.5)	74.9
3′	3.42, t (8.5)	77.8
4′	3.39, t (8.5)	71.5
5′	3.48, m	77.3
6′	3.75, dd (11.5,6)	69.7
	4.06, dd (11.5,2)	
1″	4.30, d (7.5)	105.3
2″	3.19, dd (9,7.5)	75.1
3″	3.30, t (9)	77.6
4″	3.48, m	71.2
5″	3.15, dd (11.5,10)	66.8
	3.84, dd (11.5,5.5)	
MeO	3.80, s	56.6

Lignans having a dioxabicyclooctyl ring, such as syringaresinol (**3**) and pinoresinol, have been reported to be transformed to the mammalian lignans enterodiol and enterolactone by human gut microflora.[46] These metabolites were recognized by their suppressing effect on E_2-enhanced MCF-7 proliferation in nude mice.[47] Recently, a clear positive association between lignans and plasma sex hormone-binding globulin (SHBG) levels was found by using urinary enterodiol and enterolactone. The urinary lignans were also negatively associated with plasma testosterone levels. These findings suggested that lignans promote greater binding of E_2 to SHBG and reduce the amount of free E_2.[48]

This is the first phytochemical study of any plant part of *C. flavicans* and of the leaves of *V. glabrata* and also the first investigation of the estrogenic/antiestrogenic activities of isolates from these species. These results highlight potential new uses for these taxa. On the basis of the effects of the compounds found in these assays, it is possible that *C. flavicans* and *V. glabrata* promote lactation by inhibiting the effect of estradiol in breastfeeding women. However, a number of hormones and mechanisms are also involved in lactation such as the dopaminergic system, thyroid hormones, and prolactin.[49] Other mechanisms might promote such activity, and detailed pharmacological and toxicological experiments would be needed to confirm this hypothesis.

Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-360 digital polarimeter. UV spectra were obtained using a Hitachi U2010 spectrophotometer, and CD spectra were recorded on a JASCO J-20A spectropolarimeter. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL JNM-α 400 instrument with chemical shifts in δ (ppm) using tetramethylsilane (TMS) as an internal standard, and coupling constants, *J*, are in hertz. Mass spectra were recorded using JEOL JMS 700 mass spectrometers. HPLC was carried out with a JASCO model 887-PU pump and an 875-UV variable-wavelength detector with reversed-phase columns (TSK-GEL ODS, 5 μm, 2 × 25 cm, Tosoh Chemicals Co., Ltd., at 9 mL/min with detection at 205 nm; Develosil-Lop-ODS, 12−20 μm, 5 × 50 cm × 2, Nomura Chemical Co., Ltd., at 45 mL/min with detection at 205 nm; and Ascentis Phenyl, 5 μm, 2.2 × 25 cm, Supelco at 9 mL/min with detection at 205 nm). Gas chromatography (GC) was performed on a Hitachi G-3000 gas chromatograph.

Chemicals. Fetal bovine serum (FBS) was purchased from Gibco (Grand Island, NY). Eagle's MEM and RPMI media were purchased from Nissui Pharmaceutical Co., Ltd. (Tokyo, Japan). Antibiotics were purchased from Meiji Seika Kaisha Ltd. (Tokyo, Japan). L-Glutamine

Table 2. NMR Data for Compounds **14**−**16** in MeOH-d_4 (^{1}H and ^{13}C at 400 and 100.4 MHz, respectively)

	14		**15**		**16**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		132.3	5.54, d (5)	96.0		133.9
2	6.93, d (2)	109.4			6.99, d (2)	111.8
3		148.7[b]	7.43, s	152.6		151.0
4		148.8[b]		113.5		147.3
5	6.77, d (8)	108.5	3.10, ddd (8.5,8,7.5)	31.8	7.02, d (8.5)	118.4
6	6.85, dd (8,2)	122.0	1.99, dt (15,7.5)	39.1	6.78, dd (8.5,2)	120.5
			2.25, ddd (15,8.5,3,5)			
7	4.93, s	91.0	4.93, ddd (7.5,5,3.5)	82.7	6.40, br, d (16)	131.0
8		89.0	2.45, quin,d (8,5)	43.1	6.07, dt (16,6)	129.0
9	3.98, d (10)	76.6	2.60, ddd (8,8,5)	43.3	4.14, dd (6,1.5)	63.7
	4.07, d (10)[a]					
10			1.12, d (8)	14.3		
11				170.6		
1′		130.0		127.8	4.85, d (7)	102.7
2′	7.28, d (2)	118.6	7.17, d (2)	111.9	3.56, dd (9,7)	74.9
3′		146.3		149.4	3.48, t (9)	77.9
4′		148.2		150.6	3.38, t (9)	72.1
5′	6.82, d (8.5)	116.5	6.80, d (8.5)	116.5	3.67, m	75.6
6′	6.96, dd (8.5,2)	124.3	7.06, dd (8.5,2)	124.1	4.36, dd (12,7.5)	64.6
					4.50, dd (12,2.5)	
7′	4.95, s	88.6	7.58, d (16)	146.7		
8′		89.0	6.34, d (16)	115.8		
9′	3.96, d (10)	77.0		169.0		
	4.07, d (10)[a]					
1″	4.78, d (7.5)	104.2	4.69, d (8)	99.8		127.8
2″	3.47, dd (9,7.5)	74.9	3.21, dd (9,8)	74.8	7.04, d (2)	115.2
3″	3.38, t (9)	77.7	3.38, t (9)	78.0		146.9
4″	3.45, t (9)	71.5	3.25, t (9)	71.8		149.7
5″		78.4	3.28, m	78.4	6.79, d (8.5)	116.6
6″	3.68, dd (12,5.5)	62.7	3.65, dd (12,6.5)	62.9	6.93, dd (8.5,2)	123.1
	3.89, dd (12,2)		3.90, dd (12,2)			
7″					7.53, d (16)	147.1
8″					6.24, d (16)	115.1
9″						168.9
OCH_2O	5.91, s	102.5				
MeO			3.88, s	56.5	3.85, s	56.8

[a] Signals can be interchanged in the same column. [b] Signals can be interchanged in the same column.

Table 3. Inhibitory Activities of Isolates against E_2-Enhanced MCF-7 and T47D Cell Proliferation[a]

	MCF-7				T47D			
compound	$iEqE_{50}$ (μM)	$iEqE_{10}$ (μM)	$iEqE_1$ (μM)	IL[b]	$iEqE_{50}$ (μM)	$iEqE_{10}$ (μM)	$iEqE_1$ (μM)	IL[b]
1	<0.1	0.3	>100		<0.1	>100	>100	M[c]
2	<0.1	6	>100		<0.1	>100	>100	M[c]
3	2	4	5		<0.1	1	7	
4	0.2	31	94		0.2	26	73	
5	<0.1	6	>100		<0.1	9	>100	
6	0.3	4	7		0.4	1	6	
7	0.3	>100	>100		<0.1	63	>100	
8	<0.1	0.8	93		0.6	>100	>100	
9	<0.1	<0.1	15		0.2	61	>100	
10	<0.1	0.2	>100		<0.1	10	>100	
11	<0.1	<0.1	>100	S[d]	2	37	70	
12	<0.1	<0.1	>100		0.4	39	>100	
13	<0.1	<0.1	>100	S[d]	2	69	>100	
14	<0.1	<0.1	<0.1		<0.1	<0.1	3	
15	<0.1	<0.1	>100	S[d]	<0.1	<0.1	>100	S[d]
16	4	4	5		<0.1	<0.1	>100	S[d]
17	<0.1	<0.1	<0.1		<0.1	19	>100	
18	<0.1	<0.1	>100	S[d]	<0.1	2	>100	
19	<0.1	<0.1	<0.1		<0.1	<0.1	>100	S[d]
20	<0.1	<0.1	<0.1		<0.1	<0.1	>100	S[d]
21	<0.1	<0.1	>100	S[d]	<0.1	<0.1	2	
22	<0.1	<0.1	0.4		<0.1	>100	>100	M[c]
Tam[e]	0.1	0.2	0.4		0.1	0.7	9	

[a] $iEqE_{50}$, $iEqE_{10}$, and $iEqE_1$ represent the concentrations of the compound that inhibited the cell proliferation enhanced by 100 pM of E_2 to levels equivalent to those induced by 50, 10, and 1 pM of E_2 treatment, respectively. These values were determined by linear regression analysis using four different concentrations. [b] IL Inhibitory level of the compound. [c] Mild inhibition (M), more than 50% inhibition through the concentrations tested. [d] Strong inhibition (S), more than 90% inhibition through the concentrations tested. [e] Tamoxifen.

was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). 17β-Estradiol and dextran-coated charcoal (DCC) were purchased from Sigma Chemicals (St. Louis, MO).

Plant Materials. The aerial parts of *Capparis flavicans* Kurz. were collected at Khon Kaen Province, Thailand, in August 2006, and the leaves of *Vitex glabrata* R.Br. were collected at Chantaburi Province,

Thailand, in November 2007. The plants were identified by Dr. T. Thitimetharoj, a botanist from the Faculty of Pharmaceutical Sciences, Khon Kaen University, Thailand, and voucher specimens numbered PL-001 and PL-003, respectively, were deposited at the herbarium of the Faculty of Pharmaceutical Sciences, Khon Kaen University.

Extraction and Isolation. The dried aerial parts of *C. flavicans* (2.6 kg) were extracted three times with hot MeOH (3 × 20 L) by refluxing for 6 h. The extracts were combined and concentrated under reduced pressure at 60 °C to yield 130 g of dried extract. This concentrated extract was suspended in water (1.5 L) and partitioned with EtOAc (6 × 1.5 L) to yield dried EtOAc- (22 g) and H_2O-soluble (90 g) fractions. The H_2O-soluble fraction was subjected to HP20 column chromatography using H_2O, 50% MeOH, and MeOH as eluents (5 L each) to yield H_2O (60 g), 50% MeOH (18 g), and MeOH eluates (3 g). The EtOAc-soluble fraction (22 g) was chromatographed on a silica gel column (6 × 50 cm) and fractionated (300 mL for each fraction) using a chloroform−MeOH gradient solvent system (99:1, 98:2, 95:5, 90:10, 5 L each). Fractions were collected and pooled by TLC analysis to afford 15 combined fractions. From these combined fractions, fraction 5 [0.6 g: eluted with chloroform−MeOH (99:1)] was subjected to semipreparative HPLC [column: Tosoh, TSKgel ODS-100 V, 2 × 25 cm; solvent: MeOH−water (40:60); detector: 205 nm] to give **3** (2 mg; t_R 28 min). Fraction 15 [4.4 g: eluted with chloroform−MeOH (9:1)] was subjected to preparative HPLC [column: Nomura Chemical, Develosil-Lop-ODS, 5 × 50 cm × 2; solvent: acetonitrile−water (18:82 → 38:62); detector: UV 205 nm] to afford 20 fractions. From these fractions, fractions 9 (52 mg; t_R 190 min), 11 (46 mg; t_R 230 min), 13 (45 mg; t_R 270 min), 14 (36 mg; t_R 300 min), 16 (50 mg; t_R 390 min), and 17 (53 mg; t_R 420 min) were subjected to semipreparative HPLC [column: Supelco, Ascentis Phenyl, 2.2 × 25 cm; detector: UV 205 nm] to yield **4** [50 mg; t_R 32 min with acetonitrile−water (17:83)], tamarixetin 3-*O*-*β*-D-galactoside [2 mg; t_R 110 min with acetonitrile−water (15:85)], **5** [2 mg; t_R 145 min with acetonitrile−water (15:85)], americanol A [7 mg; t_R 72 min with acetonitrile−water (20:80)], isoprincepin [5 mg; t_R 100 min with acetonitrile−water (22.5:77.5)], and **6** [7 mg; t_R 56 min with MeOH−water (50:50)], respectively.

The 50% MeOH-soluble (18 g) fraction was chromatographed by silica gel column chromatography (6 × 50 cm) and fractionated (300 mL for each fraction) using a chloroform−MeOH−H_2O gradient solvent system (80:20:0, 80:20:0.2, 7:13:8, 65:35:10, 5 L each). Fractions were collected and pooled by TLC analysis to afford 13 combined fractions. From these combined fractions, fraction 3 [0.4 g: eluted with chloroform−MeOH (8:2)] was subjected to semipreparative HPLC [column: Supelco, Ascentis Phenyl, 2.2 × 25 cm; solvent: acetonitrile−water (15:85); detector: UV 205 nm] to give 5,5′-dimethoxy-4-*O*-(*β*-D-glucopyranosyl)lariciresinol (2 mg; t_R 58 min), alangilignoside C (20 mg; t_R 81 min), and alangilignoside D (8 mg; t_R 89 min). Fraction 4 [1.5 g: eluted with chloroform−MeOH−water (8:2:0.2)] was subjected to semipreparative HPLC (column: Supelco, Ascentis Phenyl, 2.2 × 25 cm; detector: UV 205 nm) with various solvent systems to afford pure compounds as follows: acetonitrile−water (12.5:87.5) solvent system gave 3α-*O*-(*β*-D-glucopyranosyl) lyoniresinol (88 mg; t_R 64 min), alangionoside C (6 mg; t_R 54 min), **8** (10 mg; t_R 74 min), **2** (2 mg; t_R 94 min), lariciresinol 4-*O*-*β*-D-glucopyranoside (6 mg; t_R 96 min), **7** (8 mg; t_R 104 min), and alangilignoside B (4 mg; t_R 130 min); acetonitrile−water (10:90) yielded icariside B_1 (9 mg; t_R 36 min) and roseoside (24 mg; t_R 38 min); acetonitrile−water (5:95) gave **10** (18 mg; t_R 26 min), isotachioside (12 mg; t_R 35 min), vanilloloside (6 mg; t_R 48 min), **11** (11 mg; t_R 35 min), **12** (25 mg; t_R 58 min), **13** (14 mg; t_R 56 min), and 4-(hydroxymethyl)-2,6-dimethoxyphenyl 1-*O*-*β*-D-glucopyranoside (20 mg; t_R 80 min). In addition, on using an ODS column [Tosoh, TSKgel ODS-100 V, 2 × 25 cm; solvent, MeOH−water (10:90); detector 205 nm], fraction 4 also gave **9** (26 mg; t_R 20 min). Fraction 7 [1.4 g: eluted with chloroform−MeOH−water (7:13:8)] was subjected to semipreparative HPLC [column: Tosoh, TSKgel ODS-100 V, 2 × 25 cm; solvent: acetonitrile−water (5:95); detector: 205 nm] to give **1** (2 mg; t_R 34 min) and 4-hydroxy-3-methoxyphenyl 1-*O*-*β*-D-xylopyranosyl-(1→6)-*β*-D-glucopyranoside (2 mg; t_R 32 min). All known compounds were identified by comparison with published data.[15–36]

The dried leaves of *V. glabrata* (3.0 kg) were extracted three times with hot MeOH using the same method as described above for *C. flavicans*. Extracts were combined and concentrated to give 566 g of a dried extract. A part of this concentrated extract (200 g) was suspended in water (1.5 L) and partitioned with EtOAc (6 × 1.5 L) to yield dried EtOAc- (76 g) and H_2O-soluble (124 g) fractions. The H_2O-soluble fraction was subjected to HP20 column chromatography using H_2O, 50% MeOH, and MeOH as eluents (5 L each) to yield H_2O (57 g), 50% MeOH (49 g), and MeOH extracts (19 g). The MeOH eluate (19 g) was separated by silica gel column chromatography (6 × 50 cm) and fractionated (300 mL for each fraction) using a chloroform−MeOH−H_2O gradient solvent system (9:1:0, 8:2:0, 8:2:0.2, 8:3:0.3, 65:35:10, 5 L each). Fractions were collected and pooled by TLC analysis to afford 14 combined fractions. From these combined fractions, fraction 5 [0.3 g: eluted with chloroform−MeOH−H_2O (8:2:0)] was subjected to semipreparative HPLC [column: Tosoh, TSKgel ODS-100 V, 2 × 25 cm; solvent: acetonitrile−water (25:75); detector: 205 nm] to give **17** (69 mg; t_R 15 min), **18** (24 mg; t_R 17 min), and **22** (20 mg; t_R 29 min). Fraction 6 [0.5 g: eluted with chloroform−MeOH−H_2O (8:2:0)] was subjected to semipreparative HPLC [column: Tosoh, TSKgel ODS-100 V, 2 × 25 cm; solvent: MeCN−water (20:80); detector: 205 nm] to give **19** (6 mg; t_R 32 min) and **20** (16 mg; t_R 34 min). Fraction 9 [1.9 g: eluted with chloroform−MeOH−H_2O (8:2:0)] was subjected to semipreparative HPLC [column: GL Sciences, Inertsil ODS-EP, 2 × 25 cm; solvent: acetonitrile−water (20:80); detector: 205 nm] to give **21** (12 mg; t_R 44 min). Fraction 8 [1.2 g: eluted with chloroform−MeOH−H_2O (8:2:0)] was subjected to preparative HPLC [column: Tosoh, TSKgel ODS-80-TS, 6 × 60 cm × 2; solvent: acetonitrile−water (20:80 → 30:70); detector: UV 205 nm] to afford **15** (38 mg; t_R 246 min) and 12 fractions. From these fractions, fractions 6 (26 mg; t_R 146 min) and 7 (33 mg; t_R 160 min) were subjected to semipreparative HPLC [column: Tosoh, TSKgel ODS-100 V, 2 × 25 cm; detector: 205 nm] to yield **14** [8 mg; t_R 22 min with acetonitrile−water (22.5:77.5)] and **16** [3 mg; t_R 22 min with acetonitrile−water (20:80)]. All known compounds were identified by comparison with published data.[37–40]

Acid Hydrolysis of Glycosides (1 and 14−16). Identification of sugars was conducted according to a previously reported procedure.[50] Compound **1** (1 mg) was heated to 100 °C with dioxane (0.05 mL) and 5% H_2SO_4 (0.05 mL) for 1 h. After dilution with H_2O, the reaction mixture was extracted twice with EtOAc, and the H_2O layer was passed through an Amberlite IRA-60E column. The H_2O eluate was concentrated, and the residue was treated with D-cysteine (0.05 mg) in H_2O (0.03 mL) and pyridine (0.015 mL) to 60 °C for 1 h with stirring. After the solution had been concentrated and the reaction mixture dried, pyridine (0.015 mL), hexamethyldisilazane (0.015 mL), and trimethylsilyl chloride (0.015 mL) were added to the residue. The reaction mixture was then heated at 60 °C for 30 min, and the supernatant was analyzed by GC. GC conditions: column Supelco SPB-l, 0.25 mm × 27 m; column temp: 230 °C; carrier gas: N_2; t_R (min): D-glucose (18.8), L-glucose (17.7), D-xylose (10.4), and L-xylose (9.7). The t_R of D-glucose and D-xylose were detected from **1**. Acid hydrolysis of **14−16** was performed by the same manner used for **1**, and the t_R of D-glucose was detected in these compounds.

Capparoside A (1): colorless, amorphous powder; $[\alpha]^{25}_D$ −62.8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 222 (sh) (2.76), 285 (2.49), 308 (sh) (2.13) nm; ^{1}H and ^{13}C NMR, Table 1; HRFABMS *m/z* 457.1331 $[M + Na]^+$ (calcd for $C_{18}H_{26}O_{12}$ Na, 457.1322).

Khainaoside A (14): brown, amorphous powder; $[\alpha]^{25}_D$ −40.0 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 230 (4.26), 282 (3.96), 317 (sh) (3.18) nm; CD (*c* 0.2 mM, MeOH) $[\theta]_{227}$ −5798; ^{1}H and ^{13}C NMR, Table 2; HRFABMS *m/z* 559.1447 $[M + Na]^+$ (calcd for $C_{25}H_{28}O_{13}$ Na, 559.1428).

Khainaoside B (15): brown, amorphous powder; $[\alpha]^{25}_D$ −63.8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 234 (4.39), 299 (sh) (4.15), 356 (4.29) nm; CD (*c* 0.4 mM, MeOH) $[\theta]_{235}$ −18450; ^{1}H and ^{13}C NMR, Table 2; HRFABMS *m/z* 575.1768 $[M + Na]^+$ (calcd for $C_{26}H_{32}O_{13}$ Na, 575.1741).

Khainaoside C (16): brown, amorphous powder; $[\alpha]^{25}_D$ −20.8 (*c* 0.2, MeOH); UV (MeOH) λ_{max} (log ε) 250 (4.08), 264 (4.03), 298 (sh) (4.01), 329 (4.03) nm; CD (*c* 0.02, MeOH); ^{1}H and ^{13}C NMR, Table 2; HRFABMS *m/z* 527.1514 $[M + Na]^+$ (calcd for $C_{25}H_{28}O_{11}$ Na, 527.1529).

Cell Culture. MCF-7 and T47D human breast cancer cells were purchased and cultured as described in a previous report.[51]

Cell Proliferation Assay. The cell proliferation assay was performed as described in a previous report.[51] Fluorescence was measured at 590 nm with excitation at 550 nm using a Wallac 1420 ARVOsx multilabel counter (Perkin-Elmer Inc., Wellesley, MA) to evaluate cell concentrations. Estrogenic activity of isolates was determined by this bioassay.

Antiestrogenic Assay. Antiestrogenic assay was conducted according to the procedure for the cell proliferation assay with minor modifications. MCF-7 or T47D cells were seeded at a density of 1×10^4 cells/well in 96-well plates in 90 μL of 5% DCC-treated, FBS-supplemented RPMI phenol red-free medium. Then, 5 μL of estradiol (E_2) at a concentration of 20 nM was added into each well, and the plates were incubated at 37 °C with 5% CO_2 for 1 h. A 5 μL portion of each test compound was added to each well with concentrations ranging from 2 to 2000 μM and incubated in a CO_2 incubator for 96 h. In all experiments, 5 μL of serially diluted tamoxifen at concentrations from 2 to 200 μM were used as positive controls. Antagonistic effects of samples were evaluated from the cell populations, and iEqE values of each sample ($iEqE_{50}$, $iEqE_{10}$, and $iEqE_1$) were determined for the required concentrations to inhibit the E_2 effect ($iEqE_{50}$; $iEqE_{10}$; and $iEqE_1$, the concentration suppressing the E_2 effect to the equivalent level of 50; 10; and 1 pM, respectively). When samples constantly suppressed E_2 activity to the level less than 10 or 50 pM through the concentrations tested, they were categorized as strong (S) or mild (M), respectively.

Data and Statistical Analysis. Statistical differences were determined by analysis of variance followed by Dunnett's multiple comparison test. Statistical significance was established at the $p < 0.05$ level.

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Supporting Information Available: ^{1}H, ^{13}C, and HMBC spectra of **1** and **14**−**16**. This material is available free of charge via the Internet at http://pubs.acs.org.

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