DOI:10.1021/jf9009894

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY
ARTICLE

Geranylated Flavonoids from the Roots of *Campylotropis hirtella* and Their Immunosuppressive Activities

Qing-Yao Shou,[†] Run-Zhong Fu,[#] Qing Tan,[†] and Zheng-Wu Shen*[,†,‡]

[†]School of Pharmacy, Shanghai University of Traditional Chinese Medicine, Shanghai 201203, People's Republic of China, [#]Lu-Wan Senior High School, Shanghai 200023. People's Republic of China, and [‡]Basilea Pharmaceutical China Ltd., Haimen, Jiangsu 226100, People's Republic of China

In an effort to identify new immunosuppressive agents from natural sources, 12 new geranylated flavonoids, 5,7,4′-trihydroxy-3′-[7-hydroxy-3,7-dimethyl-2(*E*)-octenyl]isoflavone (**1**), a racemate of 5,7,2′,4′-tetrahydroxy-3′-[7-hydroxy-3,7-dimethyl-2(*E*)-octenyl]isoflavanone (**2**), 2″(*S*)-5,7-dihydroxy-[2″-methyl-2″-(4-methyl-3-pentenyl)pyrano]-5″,6″:3′,4′-isoflavone (**3**), (2″*S*,3″*R*,4″*S*)-5,7,3″,4″-tetrahydroxy[2″-methyl-2″-(4-methyl-3-pentenyl)pyrano]-5″,6″:3′,4′-isoflavone (**4**), a racemate of 3′-geranyl-5,7,2′,4′-tetrahydroxyisoflavanone (**5**), a racemate of 3′-geranyl-4′-methoxy-5,7,2′-trihydroxyisoflavanone (**6**), 3′-geranyl-5,7,4′,5′-tetrahydroxyisoflavone (**8**), 3′-geranyl-5,7,2′,5′-tetrahydroxyisoflavone (**9**), 3′-geranyl-4′-methoxy-5,7,2′-trihydroxyisoflavone (**10**), 2(*R*),3(*R*)-3′-geranyl-2,3-*trans*-5,7,4′-trihydroxyflavonol (**12**), (2*R*,3*R*)-6-methyl-3′-geranyl-2,3-*trans*-5,7,4′-trihydroxyflavonol (**13**), and 5,7-dihydroxy-4′-*O*-geranylisoflavone (**14**), were isolated from the roots of *Campylotropis hirtella* (Franch.) Schindl. together with three previously described flavonoids. Their structures were elucidated by spectroscopic measurements, including two-dimensional nuclear magnetic resonance (NMR) techniques. The immunosuppressive effects of these compounds were assessed using mitogen-induced splenocyte proliferation, and the cytotoxicity of the compounds was also examined. The IC_{50} values of the compounds were found to be in the range of 1.49−61.23 μM for T lymphocyte suppression and 1.16−73.07 μM for B lymphocyte suppression. An analysis of their structure−activity relationships revealed that an isoflavonoid carbon skeleton with a C10 substituent at the C3′ position was necessary for the activity. As many of the compounds exhibited good immunosuppressive activities, they may be promising as novel immunosuppressive agents.

KEYWORDS: ***Campylotropis hirtella*; geranylated flavonoids; Leguminosaea; immunosuppressants**

INTRODUCTION

Immunosuppressive agents are used in the treatment of patients who have received organ grafts and for managing autoimmune chronic inflammatory disorders. In the 1970s and 1980s, the discovery of CsA and tacrolimus (FK506) enabled the successful transplantation of major organs in human patients. However, neither drug can be used for managing transplantation tolerance for the long term (*1*, *2*). These drugs cause systemic immunosuppression which greatly increases the risks of tumors and lethal fungal infections. Therefore, new immunosuppressants that possess better therapeutic effects or those which can be combined with currently used drugs for longer time maintenance and reduced side effects are of great interest.

Traditional Chinese medicines (TCM) have been used for centuries in China to treat various immune-mediated disorders (*3*). The search for immunosuppressants from TCM has led to the discovery of triptolide from an antirheumatic Chinese herb *Tripterygium wilfordii* Hook. f. (TWHF), which has shown strong immunosuppressive and anti-inflammatory activities (*4*−*6*). As part of an effort to discover promising immunosuppressants from TCM, *Campylotropis hirtella* (Franch.) Schindl. which belongs to the plant family Leguminosaea was chosen for investigation. The roots of *C. hirtella* are traditionally used in Chinese folklore to treat diseases such as irregular menstruation, dysmenorrhea, metrorrhagia and metrostaxis, and gastric ulcers (*7*). Phytochemical analysis of the plant led to the isolation of a series of structurally related flavonoids. Herein, we report the isolation and structural characterization of 12 novel geranylated flavonoids and three known flavonoids from the roots of *C. hirtella*. All of these compounds were tested to assess their cytotoxicity and inhibition of mitogen-induced splenocyte proliferation.

MATERIALS AND METHODS

General Procedures. Optical rotation was measured with a Perkin-Elmer 341MC polarimeter. UV spectra were recorded with a Shimadzu UV−VIS 2201 spectrometer. CD spectra were measured on a JASCO DIP-360 J-500C polarimeter at room temperature. The IR spectra were acquired using a Shimadzu FTIR-8400S spectrometer. LR-EIMS spectra were obtained with a MAT-95 spectrometer, HR-EIMS spectra with a

*To whom correspondence should be addressed. Telephone: +86-513-82198001. Fax: +86-513-82198003. E-mail: jeff_shen_1999@yahoo.com.

 Published on Web 07/02/2009 © 2009 American Chemical Society

Table 1. ^{1}H NMR Spectroscopic Data of Compounds **1**−**6**[a] (δ values in parts per million and J values in hertz)

	1	2	3	4	5	6
2	8.12 s	a 4.60 (dd, 4.8, 11.2)	8.20 s	8.08 s	a 4.64 (dd, 4.8, 11.6)	a 4.56 (dd, 5.2, 11.2)
		b 4.70 (dd, 7.6,11.2)			b 4.77 (dd, 4.8, 11.6)	b 4.70 (dd, 7.2, 11.2)
3		4.17 (dd, 5.2, 7.6)			4.00 (t, 4.8)	4.14 (dd, 5.2, 7.6)
6	6.27 (d, 2.0)	5.97 (d, 2.0)	6.29 brs	6.22 (d, 2.0)	5.95 brs	5.91 brs
8	6.40 (d, 2.0)	5.97 (d, 2.0)	6.42 brs	6.34 brs	5.95 brs	5.92 brs
2′	7.35 (d, 2.0)		7.29 (d, 2.0)	7.61 brs		
5′	6.89 (d, 8.4)	6.43 (d, 8.0)	6.79 (d, 8.4)	6.81 (d, 8.4)	6.39 (d, 8.8)	6.47 (d, 8.8)
6′	7.27 (dd, 2.4, 8.4)	6.93 (d, 8.0)	7.36 (dd, 1.6, 8.4)	7.34 (brd, 7.6)	7.12 (d, 8.4)	7.12 (d, 8.8)
1″	3.38 (d, 7.2)	3.43 (d,7.2)			3.45 (d, 6.8)	3.43 (d, 7.2)
2″	5.40 (t, 7.2)	5.25 (t, 7.2)			5.24 brs	5.23 brs
3″			5.75 (d, 10.0)	3.65 (d, 8.4)		
4″	2.05 m	1.93 m	6.49 (d, 10.0)	4.58 (d, 8.4)	2.08 m	2.08 m
5″	1.52 m	1.46 m			2.09 m	2.09 m
6″	1.41 m	1.36 m			5.03 brs	5.07 brs
7″			1.71 m	1.85 m		
8″	1.12 s	1.13 s	2.13 m	2.17 m	1.57 s	1.60 s
9″			5.13 (t, 7.2)	5.16 (t, 7.2)		
11″			1.57 s	1.65 s		
3″-Me	1.73 s	1.77 s			1.80 s	1.81 s
6″-Me			1.40 s	1.19 s		
7″-Me	1.12 s	1.13 s			1.65 s	1.66 s
10″-Me			1.64 s	1.69 s		
4′-OMe						3.76 s

[a] **1**−**3** in Me_2CO-d_6, **4** in CD_3OD (400 MHz), and **5** and **6** in $CDCl_3$ (400 MHz).

Q-TOF Micro LC-MS-MS spectrometer, and ESI-MS spectra with an Agilent 1100 1946D LC-MS spectrometer. NMR spectra were acquired on a Varian INOVA 400 MHz spectrometer with TMS as the internal standard. Column chromatography (CC) separations were carried out using silica gel H60 (300−400 mesh, Qingdao Haiyang Chemical Group Corp.).

Plant Material. The roots of *C. hirtella* (Franch.) Schindl. were collected from Chuxiong, Yunnan Province, People's Republic of China, in November 2006 and authenticated by Z. Xiujia of Shanghai University of TCM. A voucher specimen has been deposited in the herbarium of the Shanghai University of TCM.

Extraction and Isolation. The powdered air-dried roots of *C. hirtella* (2 kg) were extracted with ethanol. The ethanol extract was suspended in H_2O and extracted in successive steps using CH_2Cl_2, EtOAc, and *n*-BuOH. The CH_2Cl_2 portion was evaporated under reduced pressure to afford a brownish crude extract (65.3 g). The crude extract was subjected to silica gel CC, eluted with a petroleum ether/EtOAc gradient to give Fr.1 (8.6 g), Fr.2 (14.7 g), and Fr.3 (9.5 g). These fractions were then subjected to repeated silica gel CC using petroleum ether with EtOAc or petroleum ether with acetone as the eluent, which yielded compounds **11** (18 mg), **12** (24 mg), and **13** (12 mg) from Fr.1, compounds **5** (14 mg), **7** (305 mg), **8** (106 mg), **9** (11 mg), **10** (23 mg), **14** (16 mg), and **15** (33 mg) from Fr.2, and compounds **1** (26 mg), **2** (105 mg), **3** (74 mg), **4** (26 mg), and **6** (9 mg) from Fr.3.

Compound 1: white solid; mp 179.3−180.9 °C; UV λ_{max} (MeOH) 260.0 nm (log ε 4.61); IR ν_{max} (KBr) 3362, 2968, 2935, 1653, 1616, 1506, 1446, 1364, 1263, 1198, 1047, 825, 669 cm^{-1}; ^{1}H NMR (**Table** 1); ^{13}C NMR (**Table** 2); HR-EIMS m/z 424.1890 $[M]^+$ (calcd for $C_{25}H_{28}O_6$ 424.1886); EIMS m/z (relative intensity) 424 (3.5), 406 (39), 338 (16), 337 (22), 335 (22), 321 (33), 284 (100), 283 (73), 153 (60); ESI-MS m/z 407.1 $[M - OH]^+$.

Compound 2: pale yellow oil; $[\alpha]_D^{25} = 27.1°$ (c 1.05, MeOH); UV λ_{max} (MeOH) 290.0 nm (log ε 4.58); IR ν_{max} (KBr) 3367, 2966, 2931, 1699, 1684, 1635, 1452, 1379, 1269, 1184, 1465, 835, 800 (cm^{-1}); ^{1}H NMR (**Table** 1); ^{13}C NMR (**Table** 2); HR-EIMS m/z 442.1989 $[M]^+$ (calcd for $C_{25}H_{30}O_7$ 442.1992); EIMS m/z (relative intensity) 442 (5), 424 (49), 300 (42), 299 (45), 153 (100); ESI-MS m/z 425.2 $[M - OH]^+$.

Compound 3: pale yellow oil; $[\alpha]_D^{25} = 4.3°$ (c 1.14, MeOH); CD λ_{max} (MeOH) $\Delta\varepsilon_{262}$ 4.85, $\Delta\varepsilon_{249}$ −4.60; UV λ_{max} (MeOH) 255.0 nm (log ε 4.44); IR ν_{max} (KBr) 3339, 2924, 1652, 1618, 1578, 1491, 1448, 1360, 1306, 1261, 1180, 1045, 831 cm^{-1}; ^{1}H NMR (**Table** 1); ^{13}C NMR(**Table** 2); HR-EIMS m/z 404.1635 $[M]^+$ (calcd for $C_{25}H_{24}O_5$ 404.1624); EIMS m/z (relative intensity) 404 (5), 322 (23), 321 (100); ESI-MS m/z 405.2 $[M+H]^+$.

Compound 4: pale yellow oil; $[\alpha]_D^{25} = 5.3°$ (c 1.07, MeOH); CD λ_{max} (MeOH) $\Delta\varepsilon_{263}$ 0.39, $\Delta\varepsilon_{254}$ −0.43; UV λ_{max} (MeOH) 260.0 nm (log ε 4.56); IR ν_{max} (KBr) 3379, 2972, 2935, 1650, 1616, 1576, 1495, 1448, 1364, 1306, 1286, 1258, 1194, 1076, 829, 679 cm^{-1}; ^{1}H NMR (**Table** 1); ^{13}C NMR (**Table** 2); HR-EIMS m/z 438.1677 $[M]^+$ (calcd for $C_{25}H_{26}O_7$ 438.1679); EIMS m/z (relative intensity) 438 (3), 421 (11), 420 (19), 338 (22), 337 (28), 301 (19), 300 (61), 299 (100), 271 (27), 270 (41), 153 (16); ESI-MS m/z 439.0 $[M+H]^+$.

Compound 5: pale yellow oil; $[\alpha]_D^{25} = 4.2°$ (c 1.21, MeOH); UV λ_{max} (MeOH) 290.0 nm (log ε 4.40); IR ν_{max} (KBr) 3304, 2926, 1639, 1614, 1450, 1379, 1263, 1614, 1450, 1379, 1263, 1182, 1163, 1091, 835 cm^{-1}; ^{1}H NMR (**Table** 1); ^{13}C NMR (**Table** 2); HR-EIMS m/z 424.1882 $[M]^+$ (calcd for $C_{25}H_{28}O_6$ 424.1886); EIMS m/z (relative intensity) 424 (12), 301 (46), 201 (26), 187 (36), 179 (31), 153 (100), 123 (60), 91 (35), 69 (80); ESI-MS m/z 425.4 $[M+H]^+$.

Compound 6: pale yellow oil; $[\alpha]_D^{25} = 5.7°$ (c 0.96, MeOH); UV λ_{max} (MeOH) 290.0 nm (log ε 4.40); IR ν_{max} (KBr) 3342, 2966, 2925, 1643, 1634, 1495, 1454, 1385, 1315, 1275, 1227, 1182, 1163, 1094, 835, 756 cm^{-1}; ^{1}H NMR (**Table** 1); ^{13}C NMR (**Table** 2); HR-EIMS 438.2057 m/z $[M]^+$ (calcd for $C_{26}H_{30}O_6$ 438.2042); EIMS m/z (relative intensity) 438 (28), 353 (25), 327 (15), 316 (29), 315 (100), 286 (14), 215 (22), 204 (11), 203 (31), 201 (26), 189 (30), 153 (48); ESI-MS m/z 439.4 $[M+H]^+$.

Compound 8: pale yellow oil; UV λ_{max} (MeOH) 260.0 nm (log ε 4.54); IR ν_{max} (KBr) 3371, 2964, 2922, 2854, 1650, 1614, 1574, 1514, 1441, 1367, 1306, 1283, 1177, 1051, 839, 822, 793 cm^{-1}; ^{1}H NMR (**Table** 3); ^{13}C NMR (**Table** 2); HR-EIMS m/z 422.1719 $[M]^+$ (calcd for $C_{25}H_{26}O_6$ 422.1729); EIMS m/z (relative intensity) 422 (16), 353 (27), 337 (14), 300 (67), 153 (93), 123 (49), 69 (100); ESI-MS m/z 423.3 $[M+H]^+$.

Compound 9: pale yellow oil; UV λ_{max} (MeOH) 265.1 nm (log ε 4.75); IR ν_{max} (KBr) 3352, 2924, 2854, 1649, 1610, 1500, 1448, 1364, 1306, 1259, 1200, 1167, 1150, 1094, 1051, 1038, 820 cm^{-1}; ^{1}H NMR (**Table** 3); ^{13}C NMR (**Table** 2); HR-EIMS m/z 422.1743 $[M]^+$ (calcd for $C_{25}H_{26}O_6$ 422.1729); EIMS m/z (relative intensity) 353 (10), 299 (38), 128 (38), 86 (100), 69 (79), 59 (61), 55 (85); ESI-MS m/z 423.0 $[M+H]^+$.

Compound 10: pale yellow oil; UV λ_{max} (MeOH) 260.0 nm (log ε 4.76); IR ν_{max} (KBr) 3361, 2923, 2852, 1651, 1614, 1549, 1445, 1361, 1307, 1261, 1103, 1051, 818, 789 cm^{-1}; ^{1}H NMR (**Table** 3); ^{13}C NMR (**Table** 2); HR-ESIMS m/z 459.1762 $[M+Na]^+$ (calcd for $C_{26}H_{28}O_6Na$ 459.1784); ESI-MS m/z 437.4 $[M+H]^+$.

Compound 12: yellow oil; $[\alpha]_D^{25} = 4.5°$ (c 1.27, MeOH); CD λ_{max} (MeOH) $\Delta\varepsilon_{328}$ 0.92, $\Delta\varepsilon_{293}$ −3.30; UV λ_{max} (MeOH) 280.0 nm (log ε 4.49);

Table 2. ^{13}C NMR Spectroscopic Data of Compounds **1**−**6**, **8**−**10**, and **12**−**14**[a] (δ values in parts per million)

	1	2	3	4	5	6	8	9	10	12	13	14
2	153.5	70.4	153.9	153.8	70.0	70.2	153.5	155.5	154.6	83.5	83.6	152.9
3	123.7	46.1	123.1	123.2	45.6	46.1	124.0	123.9	123.6	72.5	72.6	124.0
4	181.0	197.9	181.0	181.0	197.5	197.8	181.1	182.3	182.1	196.1	196.2	181.1
5	163.3	164.9	163.2	162.7	165.1	164.9	162.7	162.7	162.7	164.0	161.3	162.6
6	99.2	96.2	99.2	99.0	97.2	97.1	98.9	100.5	100.7	97.3	104.9	99.7
7	164.4	167.0	164.4	164.9	166.6	166.5	165.0	163.5	164.7	166.0	163.8	163.2
8	93.8	95.1	93.9	93.7	95.8	95.8	93.6	94.5	94.6	96.2	95.5	94.3
9	158.4	163.8	158.9	158.5	163.4	163.6	158.5	158.2	158.3	163.5	160.9	159.4
10	105.5	102.2	105.4	105.1	102.0	102.5	105.2	105.7	105.3	100.4	100.7	106.5
1′	122.5	114.6	123.6	123.5	115.1	115.3	121.7	112.7	113.3	128.1	128.3	122.9
2′	130.5	154.1	127.4	128.7	154.2	154.2	121.0	154.5	155.2	129.7	129.8	130.3
3′	128.1	116.3	121.1	124.6	115.8	117.2	128.5	117.4	120.1	127.5	127.5	115.1
4′	155.3	155.8	153.6	152.9	155.5	158.1	143.5	157.5	159.7	155.9	155.8	158.3
5′	114.9	107.7	115.9	116.5	108.9	103.9	144.6	109.5	104.0	116.5	116.5	115.1
6′	127.9	126.5	130.3	129.7	126.1	126.8	113.6	128.2	127.7	127.3	127.3	130.3
1″	28.3	22.7			23.0	22.8	28.3	23.1	27.4	29.9	30.3	65.2
2″	122.7	122.8	99.0	80.3	121.7	122.0	122.8	121.9	122.7	121.4	121.4	119.5
3″	136.1	135.3	130.0	73.2	139.4	138.3	135.6	139.1	135.6	139.4	143.6	141.8
4″	40.3	40.4	122.7	69.1	39.9	40.0	39.7	39.6	40.1	39.9	39.9	39.8
5″	22.7	22.5			26.6	26.7	27.9	26.0	27.0	26.6	26.6	26.5
6″	43.7	43.7			124.0	124.2	124.2	124.1	124.7	123.9	124.0	124.0
7″	69.5	69.4	41.4	38.7	132.3	132.0	131.0	132.3	131.5	132.4	132.4	132.1
8″	29.0	29.0	22.8	21.1	18.0	17.9	16.6	18.0	17.9	18.0	18.0	18.0
9″			124.3	124.3								
10″			131.4	131.2								
11″			17.0	16.5								
6-Me											7.7	
3″-Me	15.5	15.6			16.5	16.4	15.0	16.6	16.4	16.5	16.5	17.0
6″-Me			26.3	17.1								
7″-Me	29.0	29.0			26.0	25.9	24.7	26.6	23.0	25.9	25.9	26.0
10″-Me			25.2	24.7								
4′-OMe						56.0			56.0			

[a] **1**−**3** in Me_2CO-d_6, **4** and **8** in CD_3OD, and others in $CDCl_3$ (100 MHz).

Table 3. ^{1}H NMR Spectroscopic Data of Compounds **8**−**10** and **12**−**14**[a] (δ values in parts per million and *J* values in hertz)

	8	9	10	12	13	14
2	7.96 s	7.94 s	7.90 s	5.01 (d, 11.6)	5.02 (d, 10.8)	8.21 s
3				4.59 (d, 11.6)	4.63 (d, 9.6)	
6	6.20 (d, 2.0)	6.33 brs	6.32 brs	5.90 brs		6.29 brs
8	6.32 (d, 2.0)	6.41 brs	6.39 brs	5.96 brs	6.02 s	6.43 brs
2′	6.72 (d, 1.6)			7.27 brs	7.31 brs	7.54 (d, 8.4)
3′						7.00 (d, 8.4)
5′		6.52 (d, 8.0)	6.54 (d, 8.8)	6.89 (d, 8.0)	6.89 (d, 8.0)	7.00 (d, 8.4)
6′	6.86 (d, 1.6)	6.90 (d, 8.0)	6.97 (d, 8.4)	7.19 (brd, 8.4)	7.23 (brd, 8.0)	7.54 (d, 8.4)
1″	3.34 (d, 7.2)	3.56 (d, 6.8)	3.48 (d, 6.8)	3.33 (d, 7.2)	3.38 (d, 7.6)	4.63 (d, 6.4)
2″	5.32 brs	5.30 brs	5.25 brs	5.36 brs	5.40 brs	5.50 brs
4″	2.08 m	2.08 m	2.03 m	2.01 m	2.02 m	2.08 m
5″	2.10 m	2.10 m	2.06 m	2.09 m	2.09 m	2.10 m
6″	5.06 brs	5.06 brs	5.07 brs	5.03 brs	5.12 brs	5.12, brs
8″	1.55 s	1.59 s	1.57 s	1.54 s	1.49 s	1.60 s
6-Me					1.99 s	
3″-Me	1.71 s	1.84 s	1.80 s	1.69 s	1.73 s	1.77 s
7″-Me	1.58 s	1.67 s	1.63 s	1.59 s	1.57 s	1.65 s
4′-OMe			3.84 s			

[a] **8** in CD_3OD (400 MHz), **9** and **10** in $CDCl_3$ (400 MHz), and others in Me_2CO-d_6.

IR ν_{max} (KBr) 3360, 3198, 2952, 2950, 2851, 1641, 1612, 1443, 1358, 1161, 1128, 1110, 1089, 804, 680 cm^{-1}; ^{1}H NMR (**Table** 3); ^{13}C NMR (**Table** 2); HR-EIMS *m*/*z* 424.1875 $[M]^+$ (calcd for $C_{25}H_{28}O_6$ 424.1886); EIMS *m*/*z* (relative intensity) 424 (6), 271 (26), 153 (100), 123 (72), 69 (68); ESI-MS *m*/*z* 425.1 $[M+H]^+$.

Compound 13: yellow oil; $[\alpha]_D^{25}$ = 4.1° (*c* 1.17, MeOH); UV λ_{max} (MeOH) 295.1 nm (log ε 4.89); CD λ_{max} (MeOH) $\Delta\varepsilon_{334}$ 2.44, $\Delta\varepsilon_{294}$ −8.23; IR ν_{max} (KBr) 3363, 2924, 2854, 1636, 1497, 1447, 1352, 1292, 1273, 1182, 1161, 1122, 1097, 824 cm^{-1}; ^{1}H NMR (**Table** 3); ^{13}C NMR (**Table** 2); HR-EIMS *m*/*z* 438.1664 $[M]^+$ (calcd for $C_{26}H_{30}O_6$ 438.1679); EIMS *m*/*z* (relative intensity) 438 (5), 270 (14), 243 (3), 167 (26), 149 (12), 123 (14), 109(11), 72 (55), 59 (100); ESI-MS *m*/*z* 439.0 $[M+H]^+$.

Compound 14: pale yellow oil; UV λ_{max} (MeOH) 260.0 nm (log ε 4.95); IR ν_{max} (KBr) 3362, 2958, 2922, 2851, 1645, 1634, 1614, 1574, 1514, 1470, 1281, 1246, 1173, 1155, 1119, 1045, 1003, 991, 837, 798 cm^{-1}; ^{1}H NMR (**Table** 3); ^{13}C NMR (**Table** 2); HR-TOFMS *m*/*z* $[M+Na]^+$ 429.2855 (calcd for $C_{25}H_{26}O_5Na$ 429.1678); ESI-MS *m*/*z* 407.0 $[M+H]^+$.

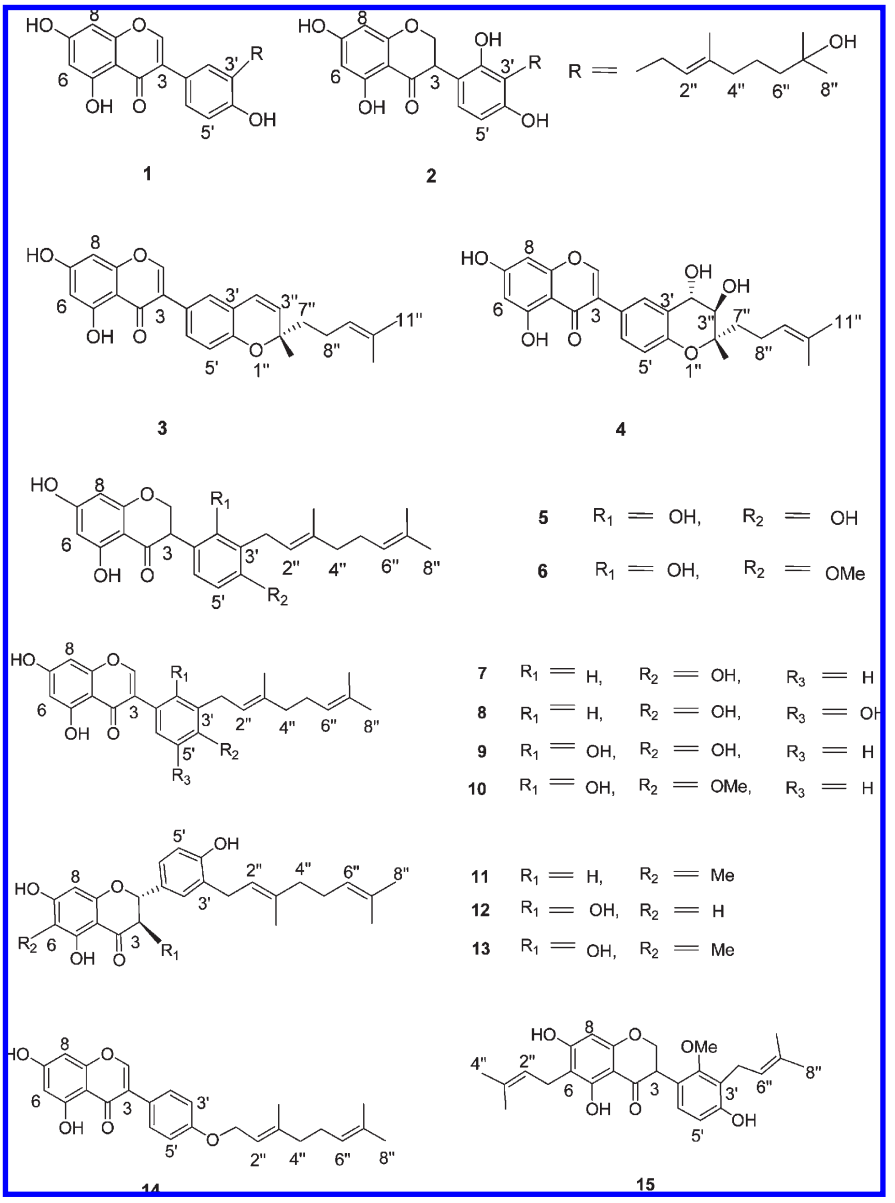

Figure 1. Chemical structures of compounds **1**−**15**.

Bioassays. *Cell Culture and Test Compound Preparation.* Inbred 7−9-week-old BALB/c mice were obtained from the Shanghai Experimental Animal Center of the Chinese Academy of Science. Mice were sacrificed, and their spleens were removed aseptically. A single spleen cell suspension was prepared, and cell debris and clumps were removed. Erythrocytes were lysed with Tris-buffered ammonium chloride [0.155 M NH_4Cl and 16.5 mM Tris (pH 7.2)]. Mononuclear cells were washed and resuspended in RPMI 1640 medium (containing 10% FBS) supplemented with penicillin (100 units/mL) and streptomycin (100 μg/mL). For in vitro assays, the spleens of five mice were pooled for cell separation. All of the compounds were dissolved in pure dimethyl sulfoxide (DMSO) as a stock solution and stored at 4 °C. The stock solution was diluted to the required concentrations with RPMI 1640 medium supplemented with 10% FBS. The final concentration of DMSO in the culture medium was less than 0.01%, which had no influence on the assays.

Cytotoxic Assay. Cytotoxicity was assessed by the MTT assay. Briefly, splenic lymphocytes were cultured in triplicate for 48 h with compounds **1**−**15** or cyclosporin A. The cells cultured with media alone were used as controls. MTT (5 mg/mL) reagent was added 4 h before the end of culture, and the supernatants were discarded; then the cells were lysed with DMSO. OD values were read at 570 nm, and the percentage of cell death was calculated.

Proliferation Assay. Splenic lymphocytes were cultured in triplicate for 48 h with 5 μg/mL ConA or 10 μg/mL LPS with compounds **1**−**15** or cyclosporin A. Cells were pulsed with 0.5 μCi of [^{3}H]thymidine per well for 8 h and harvested onto glass filters. The incorporated radioactivity was then counted using a Beta Scintillation Counter (MicroBeta Trilux, PerkinElmer Life Sciences, Boston, MA).

Statistical Methods. The 50% cytotoxic concentration (CC_{50}) and the 50% inhibitory concentration (IC_{50}) were determined by using Origin (Microcal Software).

RESULTS AND DISCUSSION

A total of six new isoflavones (**1**, **3**, **4**, and **8**−**10**), four isoflavanones (**2**, **5**, **6**, and **14**), two flavonols (**12** and **13**), and three known compounds (**7**, **11**, and **15**) were isolated from the roots of *C. hirtella* (**Figure** 1).

Compound **1** was a white solid. Its HR-EIMS spectrum exhibited a molecular ion peak at m/z 424.1890 $[M]^+$, which suggested a molecular formula of $C_{25}H_{28}O_6$. The analysis of its ^{1}H NMR and ^{13}C NMR spectral data (**Tables** 1 and 2) indicated the presence of an isoflavone skeleton (δ_C 181.0, δ_C 153.5, δ_C 123.7, δ_H 8.12, s) and a 5,7-dihydroxy substitution (δ_C 99.2,

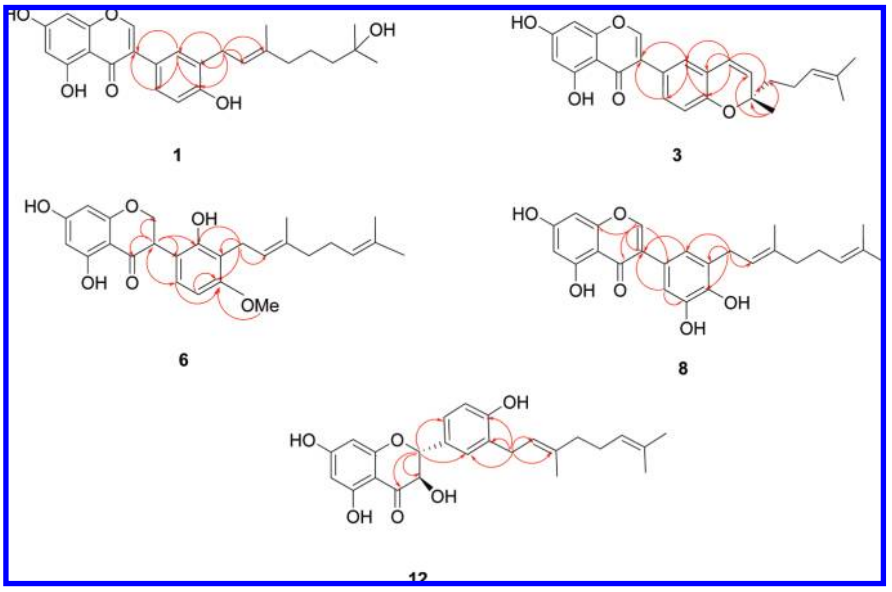

Figure 2. Key HMBC correlations of compounds **1**, **3**, **6**, **8**, and **12** (H−C).

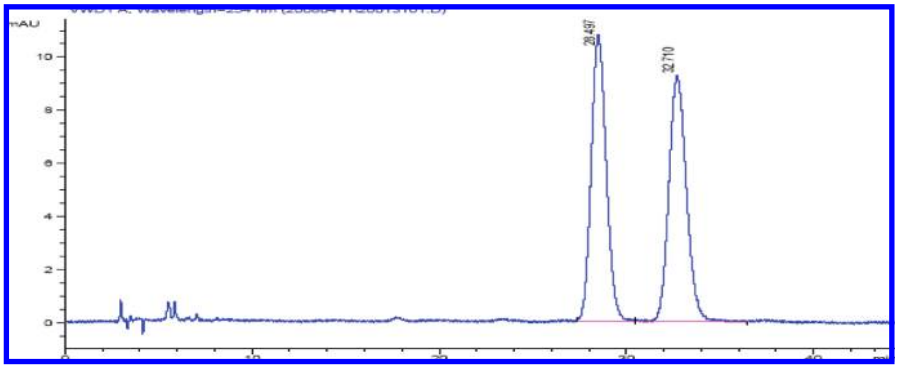

Figure 3. HPLC analysis of compound **2** (chiral column, AD-H; flow, 1.0 mL/min; eluent, 7:3 *n*-hexane/2-propanol mixture).

δ_C 93.8, δ_H 6.27, δ_H 6.40, d, J = 2.0 Hz). The ^{1}H NMR signals of **1** at δ_H 1.12 (two tertiary methyls), δ_H 5.40 (t, J = 7.2 Hz), δ_H 2.05 (2H, m), δ_H 1.52 (2H, m), and δ_H 1.41 (2H, m) as well as the ^{13}C signal of a quaternary carbon at δ_C 69.5 (C-7″) demonstrated the presence of a 7-hydroxy-3,7-dimethyl-2(*E*)-octenyl group (*8*). The *E* configuration of the double bond at position 2″ was confirmed by the upfield signals of the carbon 3″-Me at δ_C 15.5 due to γ-gauche effects. In addition, three protons appearing as an ABX spin system [δ_H 6.89 (d, J = 8.4 Hz), δ_H 7.27 (dd, J = 8.4, 2.4 Hz), and δ_H 7.35 (d, J = 2.0 Hz)] on the B ring and a downfield proton at δ_H 13.05 that can be assigned to the C-5−OH group chelated to C-4 were observed.

In the HMBC spectrum (**Figure** 2), the doublet at δ_H 7.35 which correlated with δ_C 130.5 in the HSQC spectrum was correlated with δ_C 123.7 (C-3), allowing the assignment of the δ_H 7.35 to H-2′. The HMBC spectrum also identified correlations between the doublet at δ_H 3.38 (H-1″) and the carbon resonance at δ_C 130.5 (C-2′), δ_C 128.1 (C-3′), and δ_C 155.3 (C-4′), which allowed us to unequivocally assign the 7-hydroxy-3,7-dimethyl-2(*E*)-octenyl group at the C-3′ position. Consequently, the remaining hydroxyl substituent could be placed at only C-4′. The EIMS data that exhibited ions at *m*/*z* 284 and 153 supported the substitution of the 5,7-dihydroxy A ring and the 3′-geranyl-4′-hydroxy B ring. Hence, the structure of compound **1** was concluded to be 5,7,4′-trihydroxy-3′-[7-hydroxy-3,7-dimethyl-2(*E*)-octenyl]-isoflavone. All the ^{1}H and ^{13}C NMR spectroscopic signals of **1** were assigned on the basis of DEPT, ^{1}H−^{1}H COSY, HSQC, and HMBC spectra.

Compound **2**, obtained as pale yellow oil, had a $[M]^+$ at 442.1989 corresponding to a molecular formula of $C_{25}H_{30}O_7$. The ^{1}H NMR spectrum of compound **2** (**Table** 1) showed three protons at δ_H 4.17 (1H, dd, J = 5.2, 7.6 Hz), δ_H 4.60 (1H, dd, J = 4.8, 11.2 Hz), and δ_H 4.70 (1H, dd, J = 7.6, 11.2 Hz), which typically can be assigned to H-3 and H_2-2 of an isoflavanone skeleton (*9*). The ^{13}C NMR spectrum exhibited signals at δ_C 70.4 (C-2), δ_C 46.1 (C-3), and δ_C 197.9 (C-4) in good agreement with the structure of the skeleton. Compared with **1**, the same substitution with the 5,7-dihydroxy groups and a 7-hydroxy-3,7-dimethyl-2(*E*)-octenyl group were also observed. Two ortho-coupled doublets at δ_H 6.93 (d, J = 8.0 Hz) and δ_H 6.43 (d, J = 8.0 Hz) and the HMBC correlations (**Figure** 2) of δ_H 6.93 with δ_C 46.2 (C-3) indicated the B ring of compound **2** was substituted at the −2′, −3′, and −4′ positions. The 7-hydroxy-3,7-dimethyl-2(*E*)-octenyl group was determined to be at position C-3′ by the correlations between δ_H 3.43 (H-1″) and δ_C 154.1 (C-2′), δ_C 116.3 (C-3′), and δ_C 155.8 (C-4′) in the HMBC spectrum. Consequently, the two remaining hydroxyl groups were assigned to C-2′ and C-4′. The CD spectrum showed no Cotton effect at 320 nm, suggesting that **2** might be a racemate, which was confirmed by the separation of enantiomers of compound **2** with a chiral column (**Figure** 3). Thus, compound **2** was established as a racemate of 5,7,2′,4′-tetrahydroxy-3′-[7-hydroxy-3,7-dimethyl-2(*E*)-octenyl]isoflavanone.

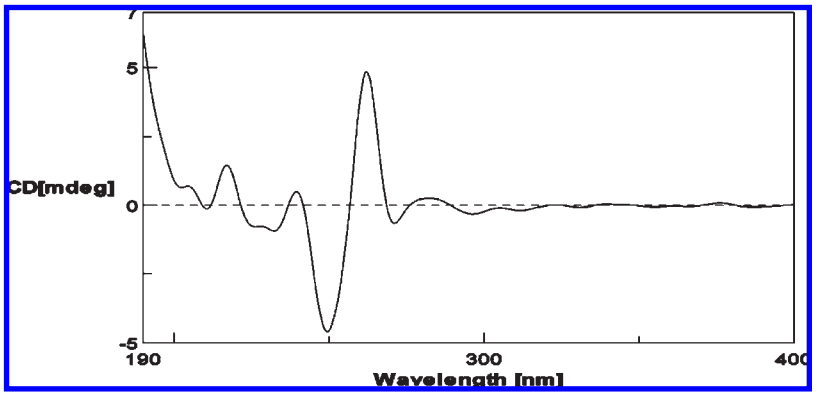

Figure 4. CD spectrum of compound **3**.

Compound **3**, isolated as pale yellow oil, showed a molecular ion peak at m/z 404.1635, indicating a molecular formula of $C_{25}H_{24}O_5$. Analysis of the NMR spectroscopic data (**Tables** 1 and 2) established the skeleton of the isoflavone with a 5,7-dihydroxy substitution that was the same as that for compound **1**. The B ring of compound **3** appeared as an ABX system at δ_H 7.29 (d, J = 2.0 Hz), δ_H 6.79 (d, J = 8.4 Hz), and δ_H 7.36 (dd, J = 1.6, 8.4 Hz) in the ^{1}H NMR. A pyran substituent present on the B ring accounted for δ_C 153.6, δ_C 99.0 as well as δ_H 6.49 (d, J = 10.0 Hz), and δ_H 5.75 (d, J = 10.0 Hz). From the HMBC spectrum (**Figure** 2), correlations were observed between δ_H 6.49 (H-4″) and δ_C 127.4 (C-2′), δ_C 121.1 (C-3′), and δ_C 153.6 (C-4′); thus, the alkenyl group of the pyran substituent was attached to C-3′. Additional signals included an olefinic proton at δ_H 5.13 (1H, brs), four coupled aliphatic protons at δ_H 2.13 (2H, m, H-8″) and δ_H 1.71 (2H, m, H-7″), and three singlets corresponding to methyl groups at δ_H 1.40, δ_H 1.57, and δ_H 1.64. One of the methyl groups was confirmed to be positioned on the oxygen-bearing carbon according to the correlations with δ_C 99.0 (C-2″), δ_C 130.0 (C-3″), and δ_C 41.1 (C-7″) in the HMBC spectrum. The remaining signals suggested a 4-methyl-3-pentenyl group as the second substituent of the oxygen-bearing quaternary carbon. The CD spectrum (**Figure** 4) showed a positive Cotton effect at 262 nm and a negative Cotton effect at 249 nm, suggesting a *S* configuration at C-2″ (*10*). In conclusion, the structure of compound **3** was elucidated as 2″(*S*)-5,7-dihydroxy-[2″-methyl-2″-(4-methyl-3-pentenyl)pyrano]-5″,6″:3′,4′-isoflavone.

Compound **4**, isolated as pale yellow oil, had a molecular weight of m/z 438.1677, indicating a molecular formula of $C_{25}H_{26}O_7$. The ^{13}C NMR spectra (**Table** 2) of **4** and **3** were very similar except that signals for C-3″ and C-4″ shifted from δ_C 130.0 and 122.7 to δ_C 73.2 and 69.1 respectively, which indicated that the double bond between C-3″ and C-4″ in compound **3** was replaced by a glycol unit in **4**. In the ^{1}H NMR spectrum, two ortho-coupled doublets at δ_H 4.58 (d, J = 8.4 Hz) and δ_H 3.65 (d, J = 8.4 Hz) supported the presence of a glycol unit. The NOESY spectrum (**Figure** 5) exhibited a correlation between H-4″ and H-(2″-Me), indicating that these protons were on the same side of the molecule; further NOESY correlation between H-3″ and H-7″/H-8″ placed them on the opposite face of the molecule. It is likely that compound **3** is a biosynthetic precursor of compound **4**, so the *S* configuration at C-2″ was suggested, which was also confirmed by the CD spectrum showing a positive Cotton effect at 263 nm and a negative Cotton effect at 254 nm. According to the analysis described above, we concluded that compound **4** had the 2″-*S*, 3″-*R*, 4″-*S* configuration. Thus, compound **4** was deduced to be (2″*S*,3″*R*,4″*S*)-5,7,3″,4″-tetrahydroxy[2″-methyl-2″-(4-methyl-3-pentenyl)pyrano]-5″,6″:3′,4′-isoflavone.

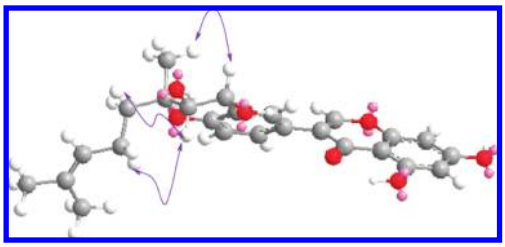

Figure 5. Key NOESY correlations of compound **4** (H—H).

Compound **5**, obtained as pale yellow oil, had a molecular formula of $C_{25}H_{28}O_6$, determined by HR-EIMS (m/z 424.1886 $[M]^+$). ^{1}H NMR and ^{13}C NMR spectroscopic data of **5** (**Tables** 1 and 2) resembled those of **2** except for the terminal portion of the C_{10} side chain at C-3′. The ^{1}H NMR signals of **5** resonating at δ_H 1.57, δ_H 1.65, δ_H 1.80 (three tertiary methyls), δ_H 5.03 (1H, brs), δ_H 5.24 (1H, brs), δ_H 2.08 (2H, m), δ_H 2.09 (2H, m), and δ_H 3.45 (2H, d, J = 7.2 Hz) established the C_{10} side chain to be a geranyl unit. Thus, compound **5** was identified as 3′-geranyl-5,7,2′,4′-tetrahydroxyisoflavanone. The separation of enantiomers of compound **5** with a chiral column indicated the presence of a racemate.

Compound **6**, pale yellow oil, had a molecular ion $[M]^+$ at m/z 438.2057, in agreement with the formula $C_{26}H_{30}O_6$. The ^{1}H NMR spectrum (**Table** 1) of compound **6** was very similar to that of compound **5**, except for the presence of the singlet at δ_H 3.76 (3H, OMe), which was supported by the ^{13}C NMR spectrum with a resonance at δ_C 56.0. In the HMBC spectrum of **6**, the correlation of methoxyl protons (δ_H 3.76, 3H, s), H-5′ (δ_H 6.47, d, J = 8.8 Hz), and H-6′ (δ_H 7.12, d, J = 8.8 Hz) with C-4′ (δ_C 158.1) was observed, suggesting that the methoxyl group was located at C-4′. This was supported by the NOESY correlation of the methoxyl protons with H-5′. Compound **6** could also be separated with a chiral column in a fashion similar to that used for compound **5**, which indicated that **6** was a racemate. Thus, compound **6** was identified as a racemate of 3′-geranyl-4′-methoxy-5,7,2′-trihydroxyisoflavanone.

Compound **8** was isolated as yellowish oil, with the molecular formula $C_{25}H_{26}O_6$, (HR-EIMS m/z 422.1719 $[M]^+$). The 5,7-dihydroxy A ring structure for an isoflavone was established according to the analysis of the ^{1}H and ^{13}C NMR spectroscopic data (**Tables** 2 and 3). Signals that can be assigned to a geranyl side chain and two meta-coupled doublets (J = 1.6 Hz) at δ_H 6.72 and δ_H 6.86 on the B ring were also observed in the ^{1}H NMR spectrum. The HMBC correlations among δ_H 6.72 (H-2′), δ_H 6.86 (H-6′), and δ_C 124.0 (C-3) indicated the B ring of compound **8** was substituted at positions 3′, 4′, and 5′. The HMBC spectrum placed the geranyl group at C-3′, as H-1″ (δ_H 3.34, d, J = 7.2 Hz)

correlated with three nearby quaternary carbons (δ_C 121.0, C-2′; δ_C 128.5, C-3′; δ_C 143.5, C-4′). The two remaining hydroxyl groups were unequivocally placed at C-4′ and C-5′. Thus, the structure of compound **8** was determined to be 3′-geranyl-5,7,4′,5′-tetrahydroxyisoflavone.

Compound **9** had a molecular formula, $C_{25}H_{26}O_6$, identical to that of compound **8** (HR-EIMS m/z 422.1743 $[M]^+$). The ^1H NMR and ^{13}C NMR spectral data (**Tables** 2 and 3) of **9** were closely related to those of **8**. The major difference in the ^1H NMR signals was the replacement of the two meta-coupled doublets of **8** on the B ring with two ortho-coupled doublets at δ_H 6.52 (1H, d, J = 8.0 Hz) and δ_H 6.90 (1H, d, J = 8.0 Hz) for **9**. The HMBC correlations between the doublet at δ_H 6.90 (H-6′) and the carbon resonances δ_C 123.9 (C-3) implied a 2′, 3′, and 4′ substitution on the B ring. A 3′-geranyl substituent was confirmed by HMBC correlations. Thus, the identity of compound **9** was 3′-geranyl-5,7,2′,5′-tetrahydroxyisoflavone.

Compound **10** was obtained as pale yellow oil. Its HR-ESIMS spectrum displayed a molecular ion $[M+Na]^+$ at m/z 459.1762, in agreement with the formula $C_{26}H_{28}O_6$. The ^1H NMR spectrum (**Table** 3) of compound **10** differed from that of compound **9** only in the presence of a singlet at δ_H 3.84 (3H, OMe), which was supported by the ^{13}C NMR spectrum with a resonance at δ_C 56.0. The location of the methoxyl group was assigned to C-4′ on the basis of the HMBC correlations between the methoxyl protons at δ_H 3.84, H-5′ at δ_H 6.54, and H-6′ at δ_H 6.97 and C-4′ (δ_C 155.9). Thus, the structure of compound **10** was 3′-geranyl-4′-methoxy-5,7,2′-trihydroxyisoflavone.

Compound **12**, obtained as a yellow oil, had the molecular formula $C_{25}H_{28}O_6$ (HR-EIMS m/z 424.1875 $[M]^+$). In the ^1H NMR spectrum (**Table** 3), signals of an AB system at δ_H 5.01 (1H, d, J = 11.6 Hz) and δ_H 4.59 (1H, d, J = 11.6 Hz) were assigned to the trans-diaxial H-2 and H-3 atoms of the C ring of a flavonol (*11*). This was supported by the ^{13}C NMR spectrum showing the C-2 and C-3 signals at δ_C 83.5 and δ_C 72.4, respectively. The ^1H NMR spectrum also showed the presence of a 5,7-dihydroxy A ring and a geranyl group which was assigned to C-3′ by HMBC correlations. Signals at δ_H 7.27 (brs), δ_H 6.89 (d, J = 8.0 Hz), and δ_H 7.19 (brd, J = 8.4 Hz) were attributed to aromatic protons H-2′, H-5′, and H-6′ on the B ring. The presence of a positive Cotton effect at 328 nm and a negative Cotton effect at 293 nm in the CD spectrum suggested a 2*R*, 3*R* absolute configuration for compound **12** (*12*). Thus, the structure of compound **12** was determined to be (2*R*,3*R*)-3′-geranyl-2,3-*trans*-5,7,4′-trihydroxyflavonol.

Compound **13** was obtained as a yellow oil. Its HR-EIMS spectrum displayed a molecular ion $[M]^+$ at m/z 438.1664, in agreement with the formula $C_{26}H_{30}O_6$. The ^1H NMR spectrum (**Table** 3) of compound **13** differed from that of compound **12** only by the presence of the singlet at δ_H 1.99 (3H, Me), and the absence of a signal at δ_H 5.90 (H-6), which was supported by the ^{13}C NMR spectrum. The CD curve of this compound showed a positive Cotton effect at 334 nm and a negative Cotton effect at 294 nm, similar to those of compound **12**. Thus, the structure of compound **13** was determined to be (2*R*,3*R*)-6-methyl-3′-geranyl-2,3-*trans*-5,7,4′-trihydroxyflavonol.

Compound **14**, yellow oil, had an ion peak at m/z 429.2855 $[M + Na]^+$ (calcd 429.1678), corresponding to the molecular formula $C_{25}H_{26}O_5$. The analysis of the NMR spectrum (**Tables** 2 and 3) showed that compound **14** was also an isoflavone with a 5,7-dihydroxy substitution. Doublets (2H, each, J = 8.4 Hz) of an AA′BB′ spin system at δ_H 7.00 and δ_H 7.54 suggested a 4′-monosubstitution on the B ring. The presence of a geranyl unit was evident from the ^1H NMR and ^{13}C NMR spectrum, and its attachment to the oxygen at C-4′ was derived from NOESY experiments, which revealed connections between the oxymethylene protons at C-1″ and the aromatic proton at C-3′ and C-5′. Thus, compound **14** was identified as 5,7-dihydroxy-4′-*O*-geranylisoflavone.

Table 4. Cytotoxicity and Immunosuppressive Activities of Compounds **1**−**15**[a]

		proliferative responses of lymphocytes			
		ConA		LPS	
compound	CC_{50} (μM)	IC_{50} (μM)	SI[b]	IC_{50} (μM)	SI[b]
1	34.20	1.65	20.73	1.42	24.08
2	91.11	8.03	11.35	3.69	24.69
3	121.41	3.02	40.20	3.02	40.20
4	44.34	4.95	8.96	2.74	16.18
5	16.06	1.49	10.78	2.62	6.13
6	113.86	8.74	13.03	34.36	3.31
7	38.94	5.42	7.18	1.53	25.45
8	5.76	22.27	0.26	1.16	4.97
9	85.17	8.15	10.45	3.82	22.30
10	212.25	16.76	12.66	9.83	21.59
11	506.73	10.39	48.77	22.68	22.34
12	124.22	28.16	4.41	73.07	1.70
13	121.92	35.94	3.39	62.79	1.94
14	78.13	61.23	1.28	16.60	4.70
15	78.49	6.96	11.28	23.40	3.35
CsA	0.88	0.01	88.00	0.07	12.57

[a] The data shown here were from a representative experiment repeated three times with similar results. [b] The selectivity index (SI) was determined as the CC_{50}/IC_{50} value.

The known compounds (**7**, **11**, and **15**) were identified by comparison of their physical, MS, and NMR data with literature values of myrsininone A (**7**) (*13*), myrsininone B (**11**) (*13*), and 2′-methoxy-6,3′-diprenyl-6,8,4′-trihydroxyisoflanone (**15**) (*14*).

All of the flavonoids (**1**−**15**) elucidated above were investigated for their cytotoxicity and immunosuppressive activities toward mitogen-induced splenocyte proliferation in vitro. The results were summarized in **Table** 4. According to the results, most of the compounds exhibited notable immunosuppressive activity on ConA as well as LPS-induced splenocyte proliferation in vitro. Compounds **1**−**10**, **14**, and **15**, all isoflavonoids, had average IC_{50} values of 12.39 μM (T lymphocyte suppression) and 8.68 μM (B lymphocyte suppression). However, compounds **11**−**13** were flavonoids which were less active with average IC_{50} values of 24.83 μM (T lymphocyte suppression) and 52.85 μM (B lymphocyte suppression). Although compound **7** has an identical LHS with **14**, compound **7** was more potent than **14**, with IC_{50} values of 5.42 μM (T lymphocyte suppression) and 1.53 μM (B lymphocyte suppression) as compared with IC_{50} values of 61.23 μM (T lymphocyte suppression) and 16.60 μM (B lymphocyte suppression) for **14**. This indicated that an isoflavonoid with a C_{10} substituent at the C-3′ position is crucial for activity.

As many of the tested isoflavonoids had a C_{10} substituent at the C-3′ position, variations around the carbon skeleton allowed an analysis of their structure−activity relationships. The replacement of a methoxy group at position 4′ with a hydroxyl group increased both activity and toxicity (**6** vs **5** and **10** vs **9**). The presence of a hydroxyl group at position 5′ results in a marked increase in toxicity but was deleterious for activity (**7** vs **8**). A hydroxyl group at position 2′ increased both activity and toxicity (**7** vs **9**). The existence of a $\Delta^{2,3}$ double bond decreased toxicity. The hydration of the $\Delta^{6'',7''}$ double bond does not affect the activity (**1** vs **7**). The establishment of a chromene ring between C-4′ and C-3″ decreased the toxicity but did not affect the activity (**3** vs **7**).

This investigation allowed the identification of a series of new geranylated flavonoids from *C. hirtella* (Franch.) Schindl. Many

of the compounds exhibited good immunosuppressive activity. Although the activities are lower than that of CsA, the toxicity is even lower, which indicated good safety indexes. According to an analysis of structure−activity relationships, an isoflavonoid carbon skeleton with a C_{10} substituent at the C-3′ position was necessary for the activity. Further chemical modifications of this type of molecule could pave the way for the development of more potent immunosuppressants. The lower cytotoxicity of these compounds makes them good candidates for in vivo tests for immunosuppressants.

ACKNOWLEDGMENT

This work was supported by a grant from the Ministry of Personnel of the People's Republic of China ("2006 returnee's research funding").

LITERATURE CITED

(1) Toungouz, M.; Donckier, V.; Goldman, M. Tolerance induction in clinical transplantation: The pending questions. *Transplantation* **2003**, *75* (Suppl. 9), 58S–60S.

(2) Offermann, G. Immunosuppression for long-term maintenance of renal allograft function. *Drugs* **2004**, *64*, 1325–1338.

(3) Ramgolam, V.; Ang, S. G.; Lan, Y. H.; Loh, C. S.; Yah, H. K. Traditional Chinese medicines as immunosuppressive agents. *Ann. Acad. Med. Singapore* **2000**, *29*, 11–16.

(4) Chen, B. J. Triptolide, a novel immunosuppressive and anti-inflammatory agent purified from a Chinese herb *Tripterygium Wilfordii* Hook. f. Leuk. *Lymphoma* **2001**, *42*, 253–265.

(5) Qiu, D. M. Immunosuppressive and anti-inflammatory mechanisms of triptolide, the principal active diterpenoid from the Chinese medicinal herb *Tripterygium Wilfordii* Hook. f. *Drugs R&D* **2003**, *4*, 1–18.

(6) Lu, H.; Hanchida, M.; Enosawa, S.; Li, X. K.; Suzuki, S.; Koyangagi, H. Immunosuppressive effect of triptolide. *Transplant. Proc.* **1999**, *31*, 2056–2057.

(7) Pharmacopoeia Committee of the People's Republic of China Edita Chinese material medica. In *Pharmacopoeia of the People's Republic of China*; People′s Hygiene Press: Beijing, 1977; pp 30−31.

(8) Asai, T.; Hara, N.; Kobayashi, S.; Kohshima, S.; Fujimoto, Y. Geranylated flavanones from the secretion on the surface of the immature fruits of *Paulownia tomentosa*. *Phytochemistry* **2008**, *69*, 1234–1241.

(9) Miyase, T.; Sano, M.; Yoshino, K.; Nonaka, K. Antioxidants from *Lespedeza homoloba*. *Phytochemistry* **1999**, *52*, 311–319.

(10) Iwata, N.; Wang, N. L.; Yao, X. S.; Kitanaka, S. Structures and histamine release inhibitory effects of prenylated orcinol derivatives from *Rhododendron dauricum*. *J. Nat. Prod.* **2004**, *67*, 1106–1109.

(11) Benavides, A.; Bassarello, C.; Montoro, P.; Vilegas, W.; Piacente, S.; Pizza, C. Flavonoids and isoflavonoids from *Gynerium sagittatum*. *Phytochemistry* **2007**, *68*, 1277–1284.

(12) Islam, M. T.; Tahara, S. Dihydroflavonols from *Lannea coromandelica*. *Phytochemistry* **2000**, *54*, 901–907.

(13) Kang, L.; Zhou, J. X.; Shen, Z. W. Two novel antibacterial flavonoids from *Myrsine africana* L. *Chin. J. Chem.* **2007**, *25*, 1323–1325.

(14) Komatsu, M.; Yokoe, I.; Shirataki, Y. Studies on the constituents of Sophora species. Constituents of the parts of *Sophora tomentosa* L. (2). *Chem. Pharm. Bull.* **1978**, *26*, 3863–3870.

Received March 25, 2009. Revised manuscript received May 12, 2009. Accepted June 18, 2009.