New and Bioactive Sesquiterpenes from *Schisandra sphenanthera*

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Three new sesquiterpenes, schisansphenins A (**1**) and B (**2**) and (−)-γ-cuparenol (**3**), were isolated from an acetone extract of the fruits of *Schisandra sphenanthera*. The known compound **4** was isolated for the frist time from a natural source. The structures of the isolated compounds were elucidated through extensive spectroscopic analyses, particularly 2D-NMR experiments (^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY). A plausible biogenetic pathway for schisansphenin B (**2**) is proposed. Compounds **2** and **3** significantly reduced activation of NF-AT and NF-κB in the luciferase-reporter assay.

Introduction. – As interest in traditional Chinese medicine has increased in recent years, the usage of important Chinese medicinal plants, including *Schisandra*, has attracted great attention among the public and herbalists [1]. The genus of *Schisandra* comprising 30 species of scandent and twining woody vines is widely distributed in East Asia, especially in southeastern and southcentral China [2]. The fruits of *S. sphenanthera* REHDER et E. H. WILSON are used as antitussive, tonic, and sedative agents under the name of Nanwuweizi in traditional Chinese medicine, together with the fruits of *S. chinensis* BAILL. (Beiwuweizi) [3]. In the 2005 edition of the Chinese Pharmacopoeia, the fruits of *S. chinensis* and *S. sphenanthera* were listed as Fructus *Schisandrae chinensis* and Fructus *Schisandrae sphenantherae*, respectively [4]. Continued investigation of the constituents of *Schisandra* [5–8] led to the isolation of three new sesquiterpenes, *i.e.*, schisansphenins A (**1**) and B (**2**), and (−)-γ-cuparenol (**3**) (*Fig. 1*)[1]),

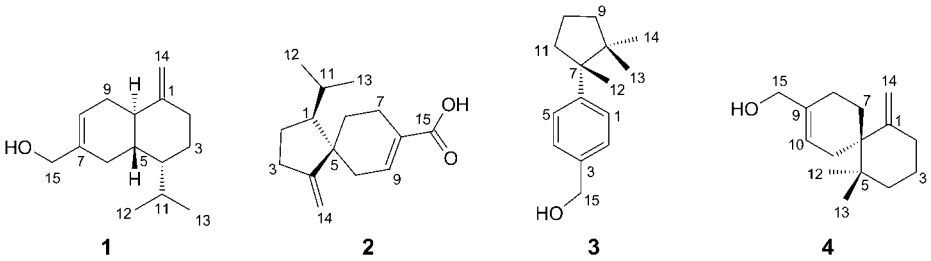


Fig. 1. *Compounds* **1**–**4**[1]) isolated from *S. sphenanthera*

[1]) Trivial atom numbering, for systematic names, see *Exper. Part.*

which were isolated from the fruits of *S. sphenanthera* together with twelve known compounds, among them compound **4** which was isolated for the first time from a natural source. The structures of these compounds were elucidated through extensive spectroscopic analyses, such as 2D-NMR experiments (^{1}H,^{1}H-COSY, HMQC, HMBC, and NOESY), and confirmed by HR-ESI-MS. We report herein the isolation, structural elucidation, and immuno-modulatory activities of compounds **1**–**4**.

Results and Discussion. – *Structure Elucidation*. Compound **1**[1]) was obtained as a colorless oil and possessed the molecular formula $C_{15}H_{24}O$ as revealed by its HR-ESI-MS (*m*/*z* 243.1725 ($[M+Na]^+$)) and NMR data (*Tables 1* and *2*). The IR spectrum showed absorption bands at 3369 (OH), 1647 (C=C), and 885 (=CH) cm^{-1}. In the NMR spectra, the assignments were guided by DEPT, HMQC, ^{1}H,^{1}H-COSY, as well as HMBC experiments. The ^{13}C-NMR data (*Table 2*) showed the presence of 15 C-atoms, which were attributed by DEPT analysis to five CH (δ(C) 26.3, 44.3, 44.8, 46.6, and 123.7), six CH_2 (δ(C) 25.3, 26.0, 26.5, 36.2, 67.5, and 103.4), and two Me groups (δ(C) 15.1 and 21.5), and to two quaternary C-atoms (δ(C) 138.0 and 152.9). The MS and NMR data suggested the presence of a sesquiterpenoid skeleton. The ^{1}H-NMR spectrum (*Table 1*) revealed the presence of one olefinic H-atom at δ(H) 5.81 (br. *s*, H–C(8)) connected to C(8) (δ(C) 123.7), an exocyclic CH_2= group at δ(H) 4.53 and 4.64 (2*s*, CH_2(14)) attached to C(1) (δ(C) 152.9), two isopropyl Me groups at δ(H) 0.71

Table 1. *^{1}H-NMR Data* (400 MHz, $CDCl_3$) *of* **1**–**4**[1]). δ in ppm, *J* in Hz.

	1	**2**	**3**	**4**
H–C(1)		1.48–1.54 (*m*)	7.36 (*d*, *J* = 8.0)	
CH_2(2) or H–C(2)	2.06 (*d*, *J* = 16.1), 2.36 (*d*, *J* = 13.1)	1.81–1.84 (*m*, 2 H)	7.28 (*d*, *J* = 8.0)	2.08–2.15, 2.20–2.30 (2*m*)
CH_2(3)	2.10–2.18 (*m*)	2.41–2.45, 2.31–2.33 (2*m*)		1.71–1.76, 1.95–2.00 (2*m*)
H–C(4) or CH_2(4)	1.21–1.28 (*m*)		7.28 (*d*, *J* = 8.0)	1.16–1.24, 1.75–1.82 (2*m*)
H–C(5)	1.70–1.76 (*m*)		7.36 (*d*, *J* = 8.0)	
CH_2(6)	1.16–1.18, 1.72–1.82 (2*m*)	1.46–1.54 (*m*)		
CH_2(7)		2.41–2.45 (*m*)		1.50–1.65 (*m*, 2 H)
H–C(8) or CH_2(8)	5.81 (br. *s*)			1.46–1.53, 2.03–2.07 (2*m*)
CH_2(9) or H–C(9)	2.00–2.08, 1.44–1.48 (2*m*)	7.16 (*s*)	1.66–1.68, 1.52–1.58 (2*m*)	
H–C(10) or CH_2(10)	1.80–1.82 (*m*)	2.62, 2.03 (2*d*, each *J* = 10.3)	1.76–1.82 (*m*)	5.62 (*s*)
H–C(11) or CH_2(11)	2.18–2.27 (*m*)	1.71–1.76 (*m*)	2.47–2.54, 1.66–1.73 (2*m*)	2.05–2.10, 2.15–2.20 (2*m*)
Me(12)	0.89 (*d*, *J* = 6.6)	0.88 (*d*, *J* = 6.6)	1.27 (*s*)	0.88 (*s*)
Me(13)	0.71 (*d*, *J* = 6.6)	0.96 (*d*, *J* = 6.6)	1.07 (*s*)	0.83 (*s*)
CH_2(14) or Me(14)	4.64, 4.53 (2*s*)	4.76, 4.61 (2*s*)	0.56 (*s*)	4.48, 4.87 (2*s*)
CH_2(15)	4.00 (br. *s*)		4.67 (*s*)	3.90 (br. *s*)
OH–C(15)			3.59 (*s*)	

Table 2. *^{13}C-NMR Data* (100 MHz. $CDCl_3$) *of* **1**–**4**[a]). δ in ppm.

	1	**2**	**3**	**4**
C(1)	152.9 (*s*)	54.0 (*d*)	127.3 (*d*)	148.8 (*s*)
C(2)	36.2 (*t*)	24.4 (*t*)	126.3 (*d*)	32.1 (*t*)
C(3)	26.0 (*t*)	29.8 (*t*)	137.8 (*s*)	23.4 (*t*)
C(4)	46.6 (*d*)	159.3 (*s*)	126.3 (*d*)	36.9 (*t*)
C(5)	44.8 (*d*)	46.2 (*s*)	127.3 (*d*)	37.3 (*s*)
C(6)	26.5 (*t*)	24.0 (*t*)	147.3 (*s*)	45.1 (*s*)
C(7)	138.0 (*s*)	21.0 (*t*)	50.4 (*s*)	23.7 (*t*)
C(8)	123.7 (*d*)	129.5 (*s*)	44.3 (*s*)	25.6 (*t*)
C(9)	25.3 (*t*)	142.2 (*d*)	39.7 (*t*)	136.3 (*s*)
C(10)	44.3 (*d*)	36.9 (*t*)	19.7 (*t*)	122.3 (*d*)
C(11)	26.3 (*d*)	28.0 (*d*)	36.8 (*t*)	28.7 (*t*)
C(12)	21.5 (*q*)	21.2 (*q*)	24.3 (*q*)	24.9 (*q*)
C(13)	15.1 (*q*)	23.6 (*q*)	23.5 (*q*)	23.0 (*q*)
C(14)	103.4 (*t*)	104.4 (*t*)	26.4 (*q*)	110.5 (*t*)
C(15)	67.5 (*t*)	171.9 (*s*)	65.2 (*t*)	67.3 (*t*)

[a]) Assignments were supported by HMBC and DEPT techniques.

(*d*, $J = 6.6$ Hz, Me(13)) and 0.89 (*d*, $J = 6.6$ Hz, Me(12)), attached to CH(11) (δ(C) 26.3), besides a OH–CH_2 group at δ(H) 4.00 (br. *s*, CH_2(15)) attached to a C=C bond C(7) at δ(C) 138.0), as confirmed by the HMBC (*Fig. 2*) and HMQC data. The connectivities CH_2(2)/CH_2(3)/H–C(4)/H–C(11)/Me(12)/Me(13), CH_2(6)/H–C(5)/H–C(10)/CH_2(9)/H–C(8), and H–C(5)/H–C(4) were deduced by COSY cross-peaks (*Fig. 2*). The ^3J(C,H) and ^2J(C,H) correlations CH_2(2)/C(1) and C(10), CH_2(14)/C(1), C(2) and C(10), H–C(15)/C(6), C(7), and C(8), CH_2(6)/C(7) and C(8) in the HMBC experiments secured the assignment of all quaternary C-atoms (*Fig. 2*). The relative configuration of **1** was determined by interpretation of its NOESY data (*Fig. 3*) which revealed the correlations H–C(2)/H–C(14), H–C(5)/H–C(6), Me(12)/Me(13), H–C(8)/CH_2(15), H–C(11)/CH_2(15), and H–C(10)/Me(12) and Me(13). However, no correlation between H–C(5) and H–C(10) was observed. This finding established the *trans* ring junction of the cadinene skeleton. Accordingly, compound **1** was identified as schisansphenin A with a yet unknown absolute configuration.

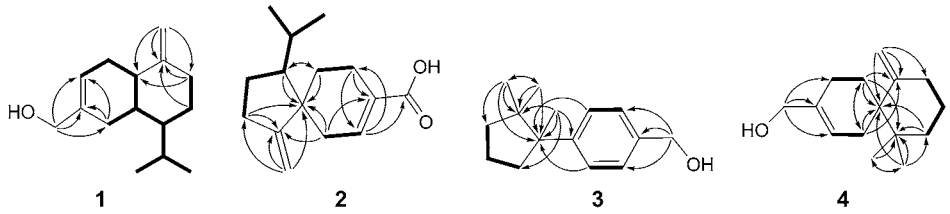
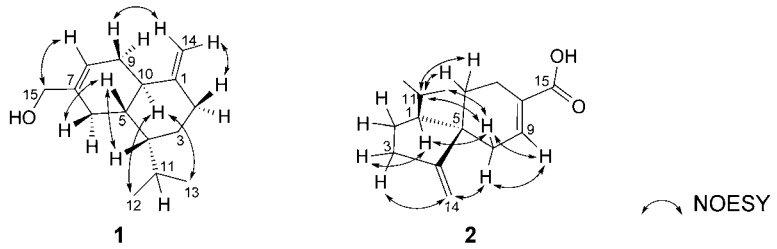


Fig. 2. *Selected HMBC* (arrows) *and COSY* (bold line) *features of* **1**–**4**

The molecular formula of **2**[1]) was determined as $C_{15}H_{22}O_2$ from its HR-ESI-MS (*m*/*z* 257.1517 ($[M + Na]^+$)). The IR spectrum showed absorption bands at 3427 (OH),



1 **2**

Fig. 3. *Key NOESY correlations of* **1** *and* **2**

2924 (CH), 1642 (C=CCOOH), and 675 (=CH) cm^{-1}. The ^{1}H-NMR data of **2** (*Table 1*) revealed the obvious signals of an isopropyl group (δ(H) 0.88 and 0.96 (each *d*, J = 6.6 Hz, Me(12), Me(13)) and 1.71–1.76 (*m*, H–C(11)), an olefinic H-atom at a trisubstituted C=C bond (δ(H) 7.16 (*s*, H–C(9))), and an exocyclic CH_2= group (δ(H) 4.61 and 4.76 (2*s*, CH_2(14)). The ^{13}C-NMR data of **2** (*Table 2*) showed signals of two Me groups (δ(C) 21.2 (C(12)) and 23.6 (C(13))), of a trisubstituted olefin moiety (δ(C) 129.5 (C(8)) and 142.2 (C(9))), of a methylidenecyclopentane moiety (δ(C) 159.3 (*s*, C(4)) and 104.4 (*t*, C(14))), and of a carboxylic acid group (δ(C) 171.9 (C(15))). Further were identified by DEPT analysis two CH (δ(C) 28.0 and 54.0) and five CH_2 groups (δ(C) 21.0, 24.0, 24.4, 29.8, and 36.9) and one quaternary C-atom (δ(C) 46.2). The spiro nature of the bicyclic sesquiterpene core of **2** was deduced from the presence of δ(C) 46.2 (C(5)) [9]. The connectivities CH_2(3)/CH_2(2)/H–C(1)/H–C(11)/Me(12)/Me(13), CH_2(6)/CH_2(7), and H–C(9)/CH_2(10) were easily deduced from the ^{1}H,^{1}H-COSY cross-peaks (*Fig. 2*). The location of a carboxylic acid group at C(8) was deduced from the HMBC cross-peak of H–C(9) (δ(H) 7.16, (*s*)) with C(15) (δ(C) 171.9). The structure of **2** was confirmed by further HMBC data as shown in *Fig. 2*. NOESY Correlations of **2** also suggested that the C=C bonds were located at C(4)=C(14) and C(8)=C(9). Additionally, the NOESY correlations CH_2(14)/H–C(3) and H_β–C(10), H_α–C(10)/H–C(11), H–C(9), Me(12) and Me(13), and H–C(11)/CH_2(6) clearly established the relative configuration at C(1) and C(5) (*Fig. 3*). Thus, on the basis of above evidence, the structure of **2** was elucidated, apart from its absolute configuration, and **2** was named schisansphenin B.

Compound **3**[1]) was obtained as colorless oil exhibiting $[\alpha]_D^{25} = -89$ (c = 0.13, CH_2Cl_2). The IR spectrum showed absorption bands at 3414 (OH), 2949 (CH), 1457 (C=C), and 1105 (C–O) cm^{-1}. The ^{1}H- and ^{13}C-NMR and DEPT spectra of **3** (*Tables 1* and *2*) revealed the conspicuous signals of three Me groups (δ(H) 0.56 (*s*, Me(14)), 1.07 (*s*, Me(13)), and 1.27 (*s*, Me(12)); δ(C) 23.5 (C(13)), 24.3 (C(12)), and 26.4 (C(14))) and of a 1,4-disubstituted benzene ring (δ(H) 7.28 (*d*, J = 8.0 Hz, H–C(2), H–C(4)) and 7.36 (*d*, J = 8.0 Hz, H–C(1), H–C(5)); δ(C) 126.3 (C(2), C(4)) and 127.3 (C(1), C(5))). In addition, the ^{13}C-NMR and DEPT spectra (*Table 2*) showed the presence of four CH_2 groups (δ(C) 19.7, 36.8, 39.7, and 65.2) and two quaternary C-atoms (δ(C) 44.3 and 50.4). The ^{1}H,^{1}H-COSY cross-peaks H–C(1)/H–C(2), H–C(4)/H–C(5), CH_2(9)/CH_2(10)/CH_2(11) and the ^3J(C,H) and ^2J(C,H) correlations H–C(1) and H–C(5)/C(7), Me(12)/C(6), C(7), C(8), and C(11), Me(13)/C(7), C(8), and C(14), Me(14)/C(7), C(8), C(9), and C(13), and CH_2(15)/C(2), C(3), and C(4) in the HMBC

experiments secured the assignment of all quaternary C-atoms of **3** (*Fig. 2*). The structure and spectroscopic data were found to be identical with those of γ-cuparenol [10]. However, γ-cuparenol showed a positive optical rotation ($[\alpha]_D^{27} = +92$) and had a (7*S*) configuration. Thus, the negative optical rotation of **3** ($[\alpha]_D^{25} = -89$) established its (7*R*) configuration [11].

The HR-ESI-MS analysis of **4**[1]) revealed a molecular-ion peak at *m*/*z* 243.1725 corresponding to the molecular formula $C_{15}H_{24}O$, in agreement with the NMR data. The colorless oil showed a negative optical rotation. The IR spectrum exhibited bands at 3442 (OH), 2930 (CH), 2056, 1634 (C=C), and 1385 (=CH) cm^{-1}. The ^{13}C-NMR spectrum of **4** (*Table 2*) displayed the signals of one CH, eight CH_2, and two Me groups and of four quaternary C-atoms indicating a sequiterpene skeleton with 15 C-atoms. From HMBC data, the ^{1}H,^{13}C-NMR long-range correlations Me(13) (δ(H) 0.83)/C(4), C(5), and C(6), and Me(12) (δ(H) 0.88)/C(4), C(5), and C(6), CH_2(3)/C(1), and CH_2(8)/C(9) were deduced (*Fig. 2*). The presence of the OH–CH_2(15) group (δ(H) 3.90 (br. *s*)) was confirmed by DEPT and HMQC analyses. The ^{1}H,^{1}H-cosy cross-peaks CH_2(2)/CH_2(3)/CH_2(4), CH_2(7)/CH_2(8), and H–C(10)/CH_2(11) were observed. The relative configuration of **4** was deduced from a NOESY experiment, establishing the correlations H–C(10)/CH_2(15), Me(12)/Me(13), CH_2(4), and CH_2(11), and Me(13)/H_β–C(7). The above spectroscopic data established the structure of **4** to be 7,7-dimethyl-11-methylenespiro[5.5]undec-2-ene-3-methanol, identical with chamigrenol whose absolute configuration was not elucidated, however [12]. Compound **4** was isolated for the first time from a natural source.

The eleven known compounds were determined to be macelignan [13], 1-(3,4-dimethoxyphenyl)-4-(3,4-methylenedioxyphenyl)-2,3-dimethylbutane [14], chicanine [15], *meso*-dimethyl dihydroguaiaretic acid [16], schisantherin D [17], 6-*O*-benzoylgomisin O [18], *erythro*-austrobailignan-6 [19], gomisin B [20], schisantherin A [21][22], gomisin G [23], and gomisin C [20] by comparison of their spectral data with literature values. Mostly, sesquiterpenes are derived from the single acyclic precursor farnesyl diphosphate (FPP). The cyclizations of FPP may occur at the distal C=C bond to generate either the ten-membered ((*E*,*E*)-germacradienyl cation) or eleven-membered (*trans*-humulyl cation) ring, but topological constraints prevent cyclization at the central C=C bond without prior isomerization of FPP to the *cisoid* nerolidyl diphosphate (NPP), thereby permitting the formation of the six-membered ring (bisabolyl cation) [5]. We propose a possible biogenetic pathway for the formation of schisansphenin B as shown in the *Scheme* [20][24][25].

To evaluate a potential immune-modulatory activity, compounds **1**–**4** were tested on NF-AT and NF-κB activation by using the luciferase-reporter assay [26][27]. As shown in *Table 3*, the results indicated that PHA significantly induced NF-AT and NF-κB activation in Jurkat cells ($p < 0.0001$). Cyclosporin A (0.25 μM) and pyrrolidine dithiocarbamate PDTC (50 μM) significantly interrupted the luciferase activities ($p < 0.001$). PHA-Induced luciferase activity was significantly decreased by compounds **2** and **3** ($p < 0.001$), while NF-AT and NF-κB activation were not affected by compounds **1** and **4**. These results suggested that compounds **2** and **3** may be potential immune-modulatory agents.

Scheme. *Plausible Biogenetic Pathway for Schisansphenin B* (**2**)

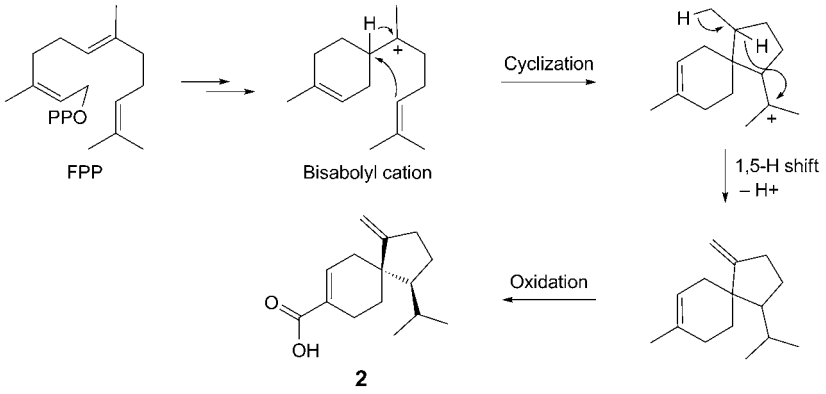


Table 3. *Effects of Compounds* **1**–**4** *on NF-AT and NF-κB Activation Determined by the Luciferase-Reporter Assay*[a])

	Luciferase activity (relative light units)	
	resting cells	PHA-activated cells
DMSO (0.1%)	73.0 ± 6.6	4916.3 ± 117.7[b])
Cyclosporin A (0.25 μM)	65.0 ± 10.1	737.4 ± 101.5
1	73.7 ± 8.5	4712.3 ± 234.4
2	52.3 ± 3.0	2698.3 ± 129.8[c])
3	64.3 ± 7.8	3765.3 ± 177.9[c])
4	59.3 ± 3.1	4631.3 ± 365.9
NF-κB		
DMSO (0.1%)	108.3 ± 7.0	3648.0 ± 250.2[b])
PDTC[d]) (50 μM)	121.5 ± 11.5	280.5 ± 75.8
1	108.0 ± 1.4	4089.5 ± 191.6
2	114.0 ± 2.8	2227.0 ± 21.2[c])
3	113.5 ± 12	2511.7 ± 206.4[c])
4	111.5 ± 6.4	3694.5 ± 729

[a]) Amount of compound tested, 10 μg/ml; the data is the mean ± s.d. of three independent determinations. [b]) $p < 0.0001$: *vs.* cells treated with DMSO. [c]) $p < 0.001$: *vs.* cells treated with DMSO and PHA. [d]) PDTC = pyrrolidine dithiocarbamate

Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; *Merck*); FC = flash chromatography. HPLC: *Hitachi-L-6250* system; flow rate 2 ml/min; UV detection at 254 and 220 nm); *LiChrospher® Si 60* (5 μm, *250-10*; *Merck*) and *LiChrospher® 100 RP-18e* (5 μm, *250-10*; *Merck*). Optical rotation: *Jasco-DIP-1000* polarimeter. UV Spectra: *Hitachi-U-3210* spectrophotometer; λ_{max} (log ε) in nm. CD Spectra: *Jasco-J-720* spectrophotometer. IR Spectra: *Hitachi-T-2001* spectrophotometer; $\tilde{\nu}$ in cm^{-1}. ^1H- and ^{13}C-NMR Spectra: *Bruker-FT-400* spectrometer; at 400 and 100 MHz, resp.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS: *VG-Quattro-5022* spectrometer; in *m*/*z*. ESI-MS: *Bruker-Daltonics-ApexII* spectrometers; in *m*/*z*.

Plant Material. The fruits of *Schisandra sphenanthera* were purchased from Zu-Nan. A voucher specimen (code CP99-1) was deposited with the School of Pharmacy, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The fruits (2.2 kg) were extracted three times at r.t. with acetone (10 l and 3 d each time), and the combined extract was concentrated. The residue was partitioned between hexane/MeOH/H_2O 4:3:1. The resulting MeOH/H_2O extract (69.6 g) was partitioned between CH_2Cl_2/H_2O 1:1 and the org. phase concentrated to give the CH_2Cl_2 extract (28.9 g). The CH_2Cl_2 extract was subjected to FC (SiO_2, hexane/AcOEt 30:1 → 1:3 and AcOEt/MeOH 30:1 → 3:1): *Fractions 1–10*. *Fr. 2* was subjected to HPLC (*Si 60*, hexane/AcOEt 6:1): **1** (4.1 mg) and **4** (10.3 mg). *Fr. 4* was purified by CC (*Sephadex LH-20*, MeOH (2 l): *Fr. 4.1–4.5*. *Fr. 4.5* (84.7 mg) was subjected to HPLC (*Si 60*, hexane/AcOEt 3:1): **2** (4.3 mg) and **3** (13.8 mg).

Schisansphenin A (= rel-*(4a*R,*8*R,*8a*S*)-1,4,4a,5,6,7,8,8a-Octahydro-5-methylene-8-(1-methylethyl)-naphthalene-2-methanol*; **1**): Colorless oil. $[\alpha]_D^{23} = -46$ ($c = 0.05$, CH_2Cl_2). IR (CH_2Cl_2): 3369 (OH), 2928 (CH), 1647 (C=C), 885 (=CH). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 243.1725 ($[M+Na]^+$, $C_{15}H_{24}NaO^+$; calc. 243.1728).

Schisansphenin B (= rel-*(1*R,*5*R*)-1-Methylene-4-(1-methylethyl)spiro[4.5]dec-7-ene-8-carboxylic Acid*; **2**): Colorless oil. $[\alpha]_D^{25} = +48$ ($c = 0.01$, CH_2Cl_2). IR (CH_2Cl_2): 3427 (OH), 2924 (CH), 1642 (C=C), 675. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 257.1517 ($[M+Na]^+$, $C_{15}H_{22}NaO_2^+$; calc. 257.1519).

(−)-γ-Cuparenol (= *4-[(1*R*)-1,2,2-Trimethylcyclopentyl]benzenemethanol*; **3**): Colorless oil. $[\alpha]_D^{25} = -89$ ($c = 0.13$, CH_2Cl_2). IR (CH_2Cl_2): 3414 (OH), 2949 (CH), 1457 (C=C), 1105 (C–O). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. HR-ESI-MS: 219.1740 ($[M+H]^+$, $C_{15}H_{23}O^+$; calc. 219.1750).

*(6*R *or 6*S*)-7,7-Dimethyl-11-methylenespiro[5.5]undec-2-ene-3-methanol* (**4**): Colorless oil. $[\alpha]_D^{25} = -21.3$ ($c = 0.03$, CH_2Cl_2). IR (CH_2Cl_2): 3442 (OH), 2930 (CH), 1634 (C=C), 1385 (C=CH). ^{1}H- and ^{13}C-NMR: *Table 1* and *2*. HR-ESI-MS: 243.1725 ($[M+Na]^+$, $C_{15}H_{24}NaO^+$; calc. 243.1728).

Luciferase-Reporter Assay [26][27]. Jurkat cells ($5 \cdot 10^4$), a T lymphocytes cell line, were stably transfected with pGL4.30 (luc2P/NF-AT-RE/Hygro) or pGL4.30 (luc2P/NF-κB-RE/Hygro), seeded into 96-well plates and cultured with PHA (phytohemaglutin; 5 μg/ml) in the presence or absence of compounds **1**, **2**, **3**, or **4** (10 μg/ml) for 4 h. NF-AT Inhibitor cyclosporin A (0.25 μM) and NF-κB inhibitor pyrrolidine dithiocarbamate (PDTC; 50 μM) were utilized as positive controls [26]. Total cell lysates were extracted with 1X reporter lysis buffer (*Promega*, USA), then 10 μg of total cell lysates were used to determine the luciferase activity by the luciferase assay system (*Promega*, USA). Data are presented as mean ± s.d., and the differences between groups were assessed with *Student*'s *t*-test at a significant level of $p < 0.05$.

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Received May 18, 2011