Phytoquinoids and Secoprezizaane-Type Sesquiterpenes from *Illicium arborescens*

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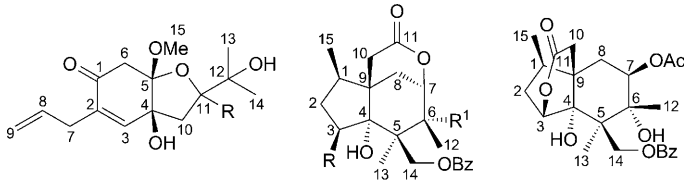
A phytochemical investigation of the MeOH extract of *Illicium arborescens* yielded the two new phytoquinoid epimers, 2,3-didehydro-5-*O*-methyl-11-epiillifunone E (**1**) and 2,3-didehydro-5-*O*-methyl-illifunone E (**2**), as well as five new sesquiterpene lactones (8,9-secoprezizaane-type sesquiterpenes). Two of them, *i.e.*, **3** and **4**, were minwanensin-type sesquiterpenes, the other two, *i.e.*, **5** and **6**, had the anisatin-type (or floridanolide type) skeleton, and the fifth, *i.e.*, **7**, was a dunnianin-type sesquiterpene. Their structures were established by analyses of 1D- and 2D-NMR, HR-MS, and chemical evidence. The *in vitro* cytotoxic activity of compounds **1**–**7** was tested against four human tumor cell lines, including HeLa (cervical epitheloid), WiDr (colon), Daoy (medulloblastoma), and Hep2 (liver carcinoma) human-tumor cells.

Introduction. – The plants of *Illicium* (star-anise), belonging to the sole genus of the family Illiciaceae, are evergreen, toxic shrubs. About 40 species have been found in eastern North America, Mexico, the West Indies, and eastern Asia. The highest concentration of species is in northern Myanmar and southern China, where nearly 35 species have been described [1]. Secoprezizaane-type sesquiterpenes, prenylated phenylpropanes, and sesqui-neolignans are the secondary metabolites of the *Illicium* plants[1]). These compounds belong to unique structural types and occur exclusively in *Illicium* species. They are considered to be characteristic chemical markers of *Illicium* species. From a chemical point of view, the *Illicium* species are interesting sources, rich in the biosynthetically unique sesquiterpenes of the secoprezizaane, anislactone, and *allo*-cedrane type. Secoprezizaane-type sesquiterpenes can be categorized into four subgroups according to their C-skeletons, namely the anisatin [3][4], pseudoanisatin [5], majucin [6], and minwanensin subgroup [7]. A rare cage-like secoprezizaane-type sesquiterpene and cycloparvifloralone [8][9] have been added as new sub-types. It is noteworthy that an anislactone-type compound with two types of γ-lactones have a different C-skeleton from that of secoprezizaane-type sesquiterpenes found in the *Illicium* plants, and have been so far isolated only from *I. anisatum* as minor constituents [10]. Tetracyclic sesquiterpene hemiketals possess a rare *allo*-cedrane C-

[1]) For reviews of chemical constituents of the *Illicum* species, see [2].

skeleton [11]. Merrilactones are di-γ-lactones with an oxetane ring [12][13]. Anisatin is well-known as one of the neurotoxic components in the *Illicium* plants [4], whereas merrilactone A [13], an anislactone-type sesquiterpene isolated from *I. merrillianum*, and jiadifenin [14], a majucin-type sesquiterpene isolated from *I. jiadifengpi*, exhibited neurite outgrowth-promoting activity in the primary cultured rat cortical neurons. Additionally, bicycloillicinone asarone acetal [3] and tricycloillicinone [15], prenylated C_6-C_3 compounds isolated from *I. tashiroi*, are able to enhance choline acetyltransferase (ChAT) activity in the primary cultured P10 rat septal neurons. Sesquiterpenes, anisatin, neoanisatin [5][16], and 2-oxyneoanisatin [17], of the fruits have been shown to be responsible for *Illicium* toxicity. The root bark of *I. anisatum* was also suggested to contain a convulsion-causing component [18]. In addition, a group of prenylated C_6-C_3 compounds have been shown to be representative for the additional chemical constituents [19]. Hence, the diversity of sesquiterpenes and their fascinating biological activities have inspired us to undertake a systematic study of *I. arborescens*, which is an evergreen tree indigenous to Taiwan. We now describe the isolation and structural determination of the two new prenylated C_6-C_3 compounds **1** and **2** and of the five sesquiterpene lactones **3**–**7**.



1 R = β-H
2 R = α-H

3 R = OH, R^1 = H
4 R = AcO, R^1 = H
5 R = R^1 = OH
6 R = AcO, R^1 = OH

7

Results and Discussion. – A combination of silica gel and reversed-phase column chromatography (*RP-18*) and prep. HPLC of the MeOH extract of the aerial parts of *I. arborescens* gave two new phytoquinoid epimers, 2,3-didehydro-5-*O*-methyl-11-epi-illifunone E (**1**), 2,3-didehydro-5-*O*-methyllillifunone E (**2**), and five new sesquiterpene lactones (8,9-secoprezizaane-type sesquiterpenes), 14-*O*-benzoylminwanensin (**3**), 3-*O*-acetyl-14-*O*-benzoylminwanensin (**4**), (3β)-14-*O*-benzoyl-10-deoxy-3-hydroxyfloridanolide (**5**), 3-*O*-acetyl-14-*O*-benzoyl-10-deoxyfloridanolide (**6**), and 7-*O*-acetyldunnianin (**7**).

Structure Elucidation. Compounds **1** and **2** represent phenylpropane derivatives with a prenyl unit cyclized to a tetrahydrofuran ring and have the same molecular formula $C_{15}H_{22}O_5$ judging from the molecular-ion peak at *m*/*z* 305.1366 ($[M+Na]^+$) in the mass spectra and 15 C-atom signals in the ^{13}C-NMR spectra (*Table 1*). The IR spectra of **1** and **2** showed absorption bands at 3470–3475 and 1685–1690 cm^{-1}, indicating the presence of an OH group and an α,β-conjugated CO group. The ^{1}H- and ^{13}C-NMR spectra of both **1** and **2** (*Tables 2* and *1*, resp.) resembled those of 2,3-didehydro-4,5-di-*O*-methylillifunone E [19], except for the appearance of only one MeO signal (δ(H) 3.99; δ(C) 48.8) instead of the two MeO groups in 2,3-didehydro-

Table 1. *^{13}C-NMR Spectral Data of Compounds* **1**–**7** *in $CDCl_3$*[a])

	1	**2**	**3**	**4**	**5**	**6**	**7**
C(1)	195.1 (*s*)	195.0 (*s*)	37.3 (*d*)	39.5 (*d*)	37.2(*d*)	37.7 (*d*)	39.8 (*d*)
C(2)	135.7 (*s*)	132.4 (*s*)	40.3 (*t*)	41.0 (*t*)	40.8 (*t*)	40.1 (*t*)	41.3 (*t*)
C(3)	144.6 (*d*)	141.8 (*d*)	77.8 (*d*)	80.7 (*d*)	77.3 (*d*)	77.4 (*d*)	80.9 (*d*)
C(4)	76.6 (*s*)	77.0 (*s*)	84.2 (*s*)	81.1 (*s*)	86.4 (*s*)	86.5 (*s*)	82.0 (*s*)
C(5)	103.9 (*s*)	102.5 (*s*)	45.0 (*s*)	44.6 (*s*)	48.2 (*s*)	48.3 (*s*)	48.2 (*s*)
C(6)	41.2 (*t*)	40.1 (*t*)	43.2 (*d*)	39.6 (*s*)	76.3 (*s*)	77.4 (*s*)	78.0 (*d*)
C(7)	32.9 (*t*)	32.9 (*t*)	80.4 (*d*)	74.1 (*d*)	82.8 (*d*)	82.9 (*d*)	77.0 (*d*)
C(8)	134.4 (*d*)	134.4 (*d*)	32.0 (*t*)	33.2 (*t*)	27.7 (*t*)	27.7 (*t*)	29.5 (*t*)
C(9)	117.6 (*t*)	117.6 (*t*)	45.5 (*s*)	45.0 (*s*)	45.0 (*s*)	45.8 (*s*)	45.4 (*s*)
C(10)	39.4 (*t*)	38.3 (*t*)	34.2 (*t*)	35.6 (*t*)	34.0 (*t*)	34.1 (*t*)	35.5 (*t*)
C(11)	82.4 (*d*)	83.9 (*d*)	170.0 (s)	170.0 (*s*)	171.1 (*s*)	171.1 (*s*)	170.2 (*s*)
C(12)	71.0 (*s*)	70.8 (*s*)	12.2 (*q*)	12.5 (*q*)	22.4 (*q*)	23.5 (*q*)	23.9 (*q*)
C(13)	24.6 (*q*)	24.5 (*q*)	19.3 (*q*)	20.7 (*q*)	13.3 (*q*)	13.7 (*q*)	15.0 (*q*)
C(14)	26.6 (*q*)	26.8 (*q*)	63.9 (*t*)	65.5 (*t*)	64.3 (*t*)	63.6 (*t*)	64.6 (*t*)
C(15)	48.8 (*q*)	49.1 (*q*)	14.8 (*q*)	14.8 (*q*)	14.6 (*q*)	14.4 (*q*)	14.9 (*q*)
CO			166.4 (*s*)	166.4 (*s*)	166.4 (*s*)	166.1 (*s*)	166.1 (*s*)
C(1′)			130.3 (*s*)	130.3 (*s*)	130.3 (*s*)	130.0 (*s*)	130.3 (*s*)
C(2′,6′)			129.9 (*s*)	129.2 (*s*)	129.9 (*s*)	129.5 (*s*)	129.9 (*s*)
C(3′,5′)			128.6 (*s*)	128.6 (*s*)	128.6 (*s*)	128.6 (*s*)	128.6 (*s*)
C(4′)			133.0 (*s*)	132.9 (*s*)	133.0 (*s*)	133.2 (*s*)	133.0 (*s*)
AcO				170.3 (*s*)		171.2 (*s*)	170.7 (*s*)
				21.4 (*q*)		21.7 (*q*)	21.6 (*q*)

[a]) Assignments on the basis of HMQC and HMBC data. *s*: C_q, *d*: CH, *t*: CH_2; *q*: Me.

4,5-di-*O*-methylillifunone E. The position of the MeO group was determined as follows. The MeO signal (δ(H) 3.99) of **1** was correlated with the C(5) signal (δ(C) 103.9) in the HMBC spectrum. The CH_2 group of **1** (δ(H) 3.04 and 2.73) and the trisubstituted olefinic CH group (δ(H) 6.46) showed an HMBC with the downfield C-atom signal (δ(C) 76.6) supporting the position of the tertiary OH group at C(4). All of the C-atom signals of **1** appeared at similar values to those of 2,3-didehydro-4,5-di-*O*-methylillifunone E with respect to C(4) and C(5), except for the two signals of C(10) and C(11), suggesting a stereoisomer of 2,3-didehydro-4,5-di-*O*-methylillifunone E. The C(11) signal of **1** was shifted upfield by 1.3 ppm and the C(10) signal shifted downfield by 3.2 ppm in the ^{13}C-NMR spectrum, and H–C(11) of **1** was shifted downfield by 0.27 ppm in the ^{1}H-NMR spectrum, compared with 2,3-didedydro-4,5-di-*O*-methylillifunone E. Thus, compound **1** was concluded to be an 11-epimer of 4-*O*-demethoxy-2,3-didehydro-4,5-di-*O*-methylillifunone E. A careful investigation of the NMR data of **2** revealed similarity to those of 2,3-didehydro-4,5-di-*O*-methylillifunone E and differences from those of **1** concerning C(11), suggesting that **2** is a stereoisomer of **1**. Inspection of the NOESY data (*Fig. 1*) established the relative configuration at C(11) of **1** and **2** as follows. Assuming that the MeO group (δ(H) 3.99) of **1** is in β-orientation, cross-peaks H–C(11)/H–C(10) (δ(H) 2.24)/MeO were observed, whereas Me(13) (δ(H) 1.08) of **2** showed a NOESY correlation with the β-MeO (δ(H) 3.43). According to the above NMR data, compound **2** was determined to be the 11-epimer of **1**, *i.e.*, 2,3-didehydro-5-*O*-methylillifunone E.

Table 2. *^{1}H-NMR Data of Compounds* **1**–**7** *in $CDCl_3$.* δ in ppm, J in Hz[a]).

	1	**2**	**3**	**4**	**5**	**6**	**7**[b])
H–C(1)			2.24–2.29 (*m*)	2.37–2.44 (*m*)	2.30–2.35 (*m*)	2.24–2.30 (*m*)	2.37–2.43 (*m*)
CH_2(2)			2.50–2.56 (*m*), 1.26 (*t*, *J* = 10.0)	2.60 (*ddd*, *J* = 14.7, 9.9, 5.4)[b]), 1.47 (*dd*, *J* = 14.7, 6.0)	2.56–2.63 (*m*), 1.22 (*m*)	2.72 (*t*, *J* = 9.0), 1.09 (*t*, *J* = 9.0)	2.63 (*t*, *J* = 6.0), 1.40 (*t*, *J* = 6.0)
H–C(3)	6.46 (br. *s*)	6.44 (br. *s*)	4.11 (*d*, *J* = 4.0)	4.59 (*d*, *J* = 5.4)	4.21 (*d*, *J* = 6.0)	5.17 (*d*, *J* = 5.7)	4.54 (*d*, *J* = 8.0)
CH_2(6) or H–C(6)	2.73 (*d*, *J* = 16.5), 3.04 (*d*, *J* = 16.5)	2.64 (*d*, *J* = 15.0), 3.04 (*d*, *J* = 15.0)	2.18 (*d*, *J* = 8.0)	2.40 (*m*)			
CH_2(7) or H–C(7)	2.95 (*dd*, *J* = 12.0, 6.6)	2.98 (*t*, *J* = 5.4)	4.43 (*d*, *J* = 1.6)	5.17 (br. *d*, *J* = 3.9)	4.39 (br. *s*)	4.41 (br. *s*)	5.08 (br. *s*)
H–C(8) or CH_2(8)	5.80–5.84 (*m*)	5.79–2.83 (*m*)	2.03 (*dt*, *J* = 12.0, 3.0)	1.99 (*dd*, *J* = 15.0, 3.9)	2.36 (*d*, *J* = 7.2)	2.26 (*d*, *J* = 8.4)	2.14 (*d*, *J* = 16.0), 1.68 (*dd*, *J* = 16.0, 3.6)
CH_2(9)	5.08 (*d*, *J* = 17.0)	5.08 (*d*, *J* = 17.0)					
CH_2(10)	2.24 (*d*, *J* = 7.5)	2.05 (*dd*, *J* = 12.6, 6.0), 2.37 (*t*, *J* = 12.6)	2.53 (*d*, *J* = 19.6), 3.38 (*dd*, *J* = 2.4, 19.6)	2.68 (*d*, *J* = 20.4), 3.44 (*dd*, *J* = 1.8, 20.4)	2.65 (*d*, *J* = 20.1), 3.40 (*dd*, *J* = 2.4, 20.1)	2.72 (*d*, *J* = 20.1), 2.97 (*d*, *J* = 20.1)	2.68 (*d*, *J* = 20.0), 3.39 (*dd*, *J* = 2.0, 20.0)
H–C(11)	3.99 (*t*, *J* = 7.5)	3.59 (*dd*, *J* = 12.6, 6.0)					
Me(12)			1.22 (*d*, *J* = 8.0)	1.10 (*d*, *J* = 7.5)	1.47 (*s*)	1.63 (*s*)	1.39 (*s*)
Me(13)	1.04 (*s*)	1.08 (*s*)	1.10 (*s*)	1.35 (*s*)	1.29 (*s*)	1.30 (*s*)	1.52 (*s*)
Me(14) or CH_2(14)	1.17 (*s*)	1.20 (*s*)	4.89 (*d*, *J* = 12.0), 4.52 (*d*, *J* = 12.0)	4.53 (*d*, *J* = 12.3), 4.92 (*d*, *J* = 12.3)	4.60 (*d*, *J* = 12.0), 5.16 (*d*, *J* = 12.0)	5.25 (*d*, *J* = 12.0), 4.28 (*d*, *J* = 12.0)	5.01 (*d*, *J* = 12.0), 4.65 (*d*, *J* = 12.0)
Me(15)	3.39 (*s*)	3.43 (*s*)	0.90 (*d*, *J* = 7.8)	0.99 (*d*, *J* = 7.5)	0.86 (*d*, *J* = 8.1)	0.96 (*d*, *J* = 7.2)	0.98 (*d*, *J* = 7.2)
H–C(2′,6′)			7.87 (*d*, *J* = 7.5)	8.06 (*d*, *J* = 7.5)	7.97 (*d*, *J* = 7.5)	7.96 (*d*, *J* = 7.5)	8.02 (*d*, *J* = 7.5)
H–C(3′,5′)			7.52 (*t*, *J* = 7.5)	7.55 (*t*, *J* = 7.5)	7.60 (*t*, *J* = 7.5)	7.57 (*t*, *J* = 7.5)	7.56 (*t*, *J* = 7.5)
H–C(4′)			7.39 (*t*, *J* = 7.5)	7.44 (*t*, *J* = 7.5)	7.47 (*t*, *J* = 7.5)	7.44 (*t*, *J* = 7.5)	7.44 (*t*, *J* = 7.5)
AcO				2.12 (*s*)		2.07 (*s*)	2.15 (*s*)

[a]) Assignments on the basis of COSY and HMBC data. [b]) Measured at 400 MHz.

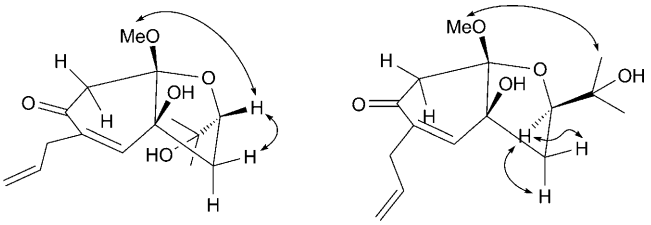


Fig. 1. *Key NOESY correlations and relative configuration of* **1** *and* **2**

Compound **3** was assigned the molecular formula $C_{22}H_{28}O_6$ as determined by HR-ESI-MS (*m/z* 411.1783 ($[M+Na]^+$)), corresponding to nine degrees of unsaturation. Its IR spectrum revealed the presence of OH (3480 cm^{-1}) and ester groups (1740 cm^{-1}). The ^{13}C-NMR spectrum (*Table 1*) displayed 22 C-atom signals, which by applying DEPT were assigned to the resonances of nine CH, four CH_2, and three Me groups. Thus the comparison between the ^{13}C-NMR and DEPT spectra allowed to establish the presence of six quaternary C-atoms. The ^1H-NMR spectrum (*Table 2*) showed a total of 26 H-atoms attached to C-atoms, indicating the presence of two OH groups. Since the benzoyloxy moiety and a CO group account for six degrees of unsaturation for the δ-lactone, compound **3** should be tricyclic. The ^1H-NMR spectrum contained signals due to one tertiary Me group (Me(13) at δ(H) 1.10), two secondary Me groups (Me(12) at δ(H) 1.22 (*d*, $J = 8.0$ Hz) and Me(15) at δ(H) 0.90 (*d*, $J = 7.8$)), two isolated sets of CH_2 groups (CH_2(10) at δ(H) 2.53 and 3.38, and CH_2(14) at δ(H) 4.89 and 4.52), and a secondary alcoholic CH group (H–C(3) at δ(H) 4.11). The large geminal coupling constant between the two H-atoms at C(10) (δ(H) 2.53 and 3.38, $J =$ 19.6 Hz) suggested that compound **3** belongs to the minwanensin-type sesquiterpenes [20] Comparison of the NMR spectra with those of minwanensin isolated previously from *I. minwanense* [21] and its absolute configuration [20] indicated that **3** is 14-*O*-benzoylminwanensin. The signals of the *O*-benzoyl group were present in the ^1H- and ^{13}C-NMR spectra (*Tables 2* and *1*, resp.). In the HMBC spectrum of **3**, correlations were observed between H–C(7) (δ(H) 4.43) and the lactone C(11)=O (δ(C) 170.0), and between CH_2(14) (δ(H) 4.89 and 4.52) and the benzoyloxy CO (δ(C) 166.4), indicating that the δ-lactone ring in the structure bridges C(7) and C(9) and that the benzoyloxyl group is attached to C(14). In addition, the relative configuration of **3** was deduced from a NOESY experiment: H–C(3) was assigned to be α-oriented because of the correlations H–C(3)/H_α–C(2), Me(13), and OH–C(4) (*Fig. 2*). The Me(12) and Me(15) groups were both assigned β-orientation as indicated by the cross-peaks Me(12)/H_β–C(14), and Me(15)/H_α–C(10). The relative α-configuration of Me(13) was indicated by the correlation Me(13)/H–C(3), which was confirmed by the correlation H_β–C(10)/H_α–C(14). A computer-generated perspective model of **3**, obtained by a MM2 force-field calculation, and its key NOESY correlations are illustrated in *Fig. 2*. According to the above analyses, compound **3** was elucidated to be 14-*O*-benzoylminwanensin.

The spectral data of **4** ($C_{24}H_{30}O_7$, two more C-atoms than **3**) resembled those of **3**. Analyses of the 1D- and 2D-NMR data of **4** and comparison of the ^1H-NMR data of **3**

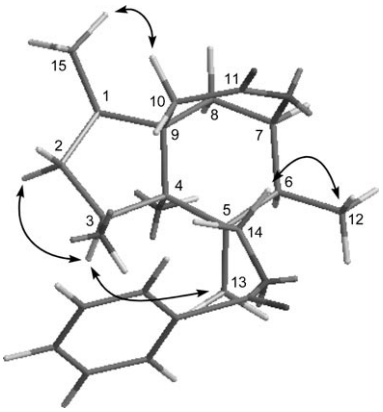


Fig. 2. *Key NOESY correlations of* **3** *and computer-generated perspective model for* **3** (MM2 force-field calculation)

and **4** revealed the following differences. The signal of H–C(3) of **3** at δ(H) 4.11 was shifted downfield to δ(H) 4.59 in **4**. Similarly, in the ^{13}C-NMR spectrum, the signal of C(3) (δ(C) 77.8) was shifted downfield to δ(C) 80.7, indicating that OH–C(3) of **3** is acetylated in **4** (AcO at δ(H) 2.12 and δ(C) 21.4 and 170.3). The relative configuration of **4** was the same as that of **3** according to its NOESY correlations. Thus, compound **4** was deduced to be 3-*O*-acetyl-14-*O*-benzoylminwanensin.

Compound **5** has a molecular formula $C_{22}H_{28}O_7$, as determined by HR-ESI-MS (*m*/*z* 427.1730 ($[M+Na]^+$)). The spectral data of **5** indicated the presence of a benzoyloxy group (1740 cm^{-1}; δ(H) 7.60 (*t*, *J* = 7.5 Hz, 2 H), 7.47 (*t*, *J* = 7.5 Hz, 1 H), and 7.97 (*d*, *J* = 7.5 Hz, 2 H) at a CH_2 group (δ(H) 4.60 and 5.16 (each *d*, *J* = 12.0, 2 H); δ(C) 166.4, 130.3, 129.9, 128.6, 133.0, and 64.3) which was supported by the observation of a prominent peak at *m*/*z* 122 ($PhCOOH^+$) in the EI-MS. Moreover, the presence of two tertiary Me groups at δ(H) 1.47 and 1.29, a secondary Me group at δ(H) 0.86, and two sets of CH_2 groups suggested that **5** was either a floridanolide-type [21][22] or a dunnianin-type sesquiterpene [23][24], which differ in the lactone-ring type (at the C(11), C(7) or the C(11), C(3) position). The NMR data of compound **5** indicated that it is an analog of **3**, *i.e.*, the signals of the cyclopentane ring and benzoyloxy moiety, as well as the connection of the δ-lactone ring between C(7) and C(11), have similarities to each other as shown in *Tables 1* and *2*. Comparison of NMR data of **5** with those of **3** revealed a different signal for the Me(12) group at the six-membered ring: The Me(12) group at C(6) of **5** was observed at δ(H) 1.47 (*s*) and at δ(C) 22.4, the shifts resulting from the presence of an additional OH group at C(6) (δ(C) 76.3) instead of the secondary CH(6) of **3** (δ(H) 2.18; δ(C) 43.2). An HMBC cross-peak supported the tertiary alcoholic Me group at C(6) by showing a ^2J correlation with C(6) (δ(C) 76.3) and a ^3J correlation with C(7) (δ(C) 82.8) and C(5) (δ(C) 48.2). The relative configuration of Me(12) at C(6) was assigned to be β as in **3** according to the NOESY spectrum of **5**. Thus, **5** is a 6-hydroxy derivative of **3**, *i.e.*, 14-*O*-benzoyl-6-hydroxyminwanensin or (3β)-14-*O*-benzoyl-10-deoxy-3-hydroxyfloridanolide.

Compound **6** was assigned the molecular formula $C_{24}H_{30}O_8$ on the basis of the HR-ESI-MS (*m*/*z* 469.1840 ($[M+Na]^+$)). Compound **6** is most likely a monoacetate of **5**. The ^{1}H-NMR spectrum showed a downfield-shifted resonance at δ(H) 5.17 for

H–C(3) suggesting that O–C(3) is acetylated in **6**. This structure was confirmed by analyses of the 2D-NMR data. The relative configuration of **6** and **5** was the same according to the NOESY data. Thus, the structure of **6** was elucidated to be (3β)-3-(acetyloxy)-14-*O*-benzoyl-10-deoxyfloridanolide.

The molecular formula of compound **7** was established as $C_{24}H_{30}O_8$, based on the analysis of the EI-MS and ^{13}C-NMR data, and confirmed by HR-ESI-MS (*m*/*z* 469.1837). The IR spectrum of **7** showed absorption bands for OH groups (3480 cm^{-1}), a γ-lactone moiety (1783 cm^{-1}), and an AcO group (1740 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra of **7** (*Tables 2* and *1*, resp.) indicated a secoprezizaane sesquiterpene [25]. A characteristic lactone signal at δ(C) 170.2 was observed in the ^{13}C-NMR spectrum. In addition, the ^{1}H- and ^{13}C-NMR data of **7** revealed similarities with those of 3-*O*-acetyl-14-*O*-benzoylminwanensin (**4**), except for the connection of a δ-lactone ring between C(11) and C(3) and an AcO–C(7) moiety in **7**. The *s* for H–C(10) in the anisatin series is missing, and, as found in the dunnianins, two signals with a large geminal coupling constant (^2J(10a,10b)) were observed at δ(H) 3.39 (*dd*, ^2J = 20.0 Hz, ^4J = 2.0 Hz (*W*-coupling with H$_\alpha$–C(8)), H$_\beta$–C(10)) and 2.68 (*d*, ^2J = 20 Hz, H$_\alpha$–C(10)). According to the coupling constants of H–C(3), the pseudoanisatin- and dunnianin-type compounds differ significantly due to the different geometry of the cyclopentane ring: ^3J(3,2α) and ^3J(3,2β) amount to 7.7 and 2.8 Hz in pseudoanisatin, but to 5–7 and $<$1 Hz in the dunnianin series [21][24]. The ^{1}H-NMR of **7** revealed that H–C(3) appeared as *d* (J = 7.0 Hz) resonating at δ(H) 4.54. The H–C(3) signal showed ^2J correlations in the HMBC plot with δ(C) 82.0 (C(4)) and 41.3 (C(2)) and ^3J-correlations with δ(C) 39.8 (C(1)), 45.4 (C(9)), 48.2 (C(5)), and 170.2 (C(11)=O). The position of the AcO group at C(7) was confirmed by 1D- and 2D-NMR spectra. The coupling constants of CH$_2$(10) and H–C(3) and HMBC correlations also supported the structure of **7**. The relative configuration of **7** and dunnianin, which was previously reported from *I. floridanum* [24], has to be the same according to its NOESY data and combined with a computer-generated perspective model of **7** (*Fig. 3*). Therefore, compound **7** was elucidated as the 3,11-lactone 7-*O*-acetyldunnianin.

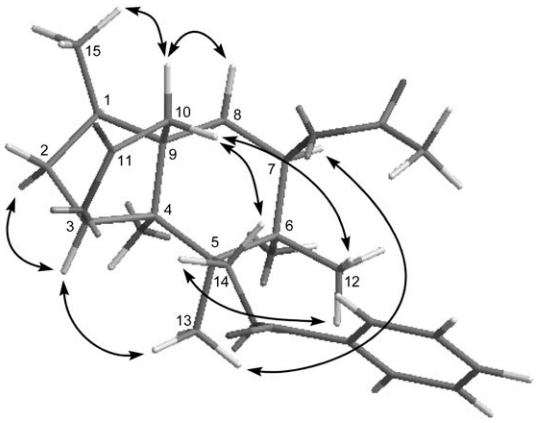


Fig. 3. *Key NOESY correlations of* **7** *and computer-generated perspective model for* **7** (MM2 force-field calculation)

In conclusion, two phytoquinoid epimers (see **1** and **2**), two minwanensin-type sesquiterpenes (see **3** and **4**), two anisatin-type sesquiterpenes (see **5** and **6**), and one dunnianin-type sesquiterpene (see **7**) were isolated from *I. arborescens*. This plant is rich in anisatin-type sesquiterpenes and pseudoanisatin-type sesquiterpenes. This is the first report of minwanensin-type sesquiterpenes in this plant. Most of the minwanensin-type sesquiterpenes have been isolated from *I. minwanense* [20]. According to the co-occurrence of these sesquiterpenes, *I. arborescens* is likely to be taxonomically closely related to the *I. minwanense*.

Cytotoxicity Assays. The *in vitro* cytotoxic activities of **1**–**7** were investigated against human Hela (cervical-epitheloid carcinoma), WiDr (colon adenocarcinoma), Daoy (medulloblastoma), and Hep2 (liver carcinoma) tumor cells, by using the MTT assay. The results are shown in *Table 3*. Among the compounds tested, **3** and **6** exhibited weak cytotoxic activity against Hela, WiDr, and Hep2 tumor cell lines.

Table 3. *Cytotoxicity Data* (IC_{50} [μg/ml]) *of* **1**–**7** *against Human Tumor Cells*

	HeLa[b])	WiDr[b])	Daoy[b])	Hep2[b])
1	(–)[a])	(–)	(–)	(–)
2	(–)	(–)	(–)	(–)
3	9.0	7.1	11.2	10.9
4	17.7	15.2	16.2	11.5
5	17.2	(–)	(–)	(–)
6	5.1	6.3	10.9	6.24
7	(–)	(–)	(–)	(–)
Mitomycin C	0.16	0.10	0.12	0.14

[a]) (–) = Inactive, *i.e.*, $IC_{50} > 20$ μg/ml. [b]) HeLa = human-cervical-epitheloid carcinoma, WiDr = human-colon carcinoma, Daoy = human medulloblastoma, Hep2 = human-liver carcinoma.

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Experimental Part

General. Column chromatography (CC): silica gel *60* (SiO_2; *Merck*) and *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*, Uppsala, Sweden). HPLC: *Hitachi-L-6250* intelligent pump, *Hitachi-L-4000 H* UV detector, *Lichrosorb Si-60* (7 μm, 250 mm × 10 mm) column; *Lichrosorb RP-18* (7 μm, 250 mm × 10 mm) column. Optical rotations: *Jasco-DIP-1000* polarimeter. UV Spectra: *Hitachi-U-3210* spectrometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi-T-2001* spectrometer; in cm^{-1}. ^1H- and ^{13}C-NMR, COSY, HMQC, HMBC, and NOESY experiments: *Bruker-FT-300* spectrometer or *Varian-Unity-Inova-400* FT-NMR spectrometers at 300 or 400 (^1H) and 75 or 100 MHz (^{13}C); $SiMe_4$ as internal standard; δ in ppm, coupling constants J in Hz. HR-ESI-MS: *Finingan-Mat-95S* mass spectrometer; in m/z.

Plant Material. Illicium arborescens was collected from Ping-tong County on July 9, 2005, and was identified by one of the authors (*S.-Y. C.*). A voucher specimen was deposited with the School of Pharmacy, College of Medicine, National Taiwan University, Taiwan.

Extraction and Isolation. The dried aerial parts of *Illicium arborescens* (4.2 kg) were powdered and extracted with acetone/MeOH 1:1 at r.t. After evaporation of the solvent, the crude extract (300 g) was

partitioned between hexane and MeOH/H_2O (4 : 3 : 1). The MeOH-soluble portion (200 g) was subjected to CC (*Sephadex LH-20*, MeOH): *Fractions I-1 – I-5*. *Fr. I-2* was subjected to CC (SiO_2, step gradient hexane/AcOEt/MeOH 70 : 1 : 0, 0 : 0 : 1, and 0 : 3 : 1): *Frs. I-2-1 – I-2-4*. *Fr. I-2-3* (2.5 g) was applied to CC (SiO_2, hexane/AcOEt/MeOH 50 : 1 : 0, 0 : 1 : 0, and 0 : 3 : 1): *Frs. I-2-3-1 – I-2-3-5*. *Fr. I-2-3-2* (181.0 mg) was further separated by HPLC (*RP-18*, MeOH/H_2O/MeCN 3 : 6 : 1) and further purified by HPLC (*RP-18*, MeOH/H_2O/MeCN 2 : 7 : 1): **1** (3 mg). *Fr. I-2-3-3* (291.0 mg) was separated by HPLC (*RP-18*, MeOH/H_2O/MeCN 3 : 6 : 1) and further purified by HPLC (*RP-18*, MeOH/H_2O/MeCN 4 : 3 : 3): **2** (5 mg). *Fr. I-2-3-4* (570 mg) was further separated by CC (*Sephadex LH-20*, MeOH/CH_2Cl_2 1 : 1) followed by HPLC (*Si-60*, hexane/AcOEt 2 : 1): **4** (5 mg) and **6** (3 mg). *Fr. I-2-3-5* (90 mg) was separated by HPLC (*RP-18*, MeOH/H_2O/MeCN 4 : 3 : 3): **5** (4 mg). *Fr. I-2-4* (2.2 g) was applied to CC (*Sephadex LH-20*, MeOH/CH_2Cl_2 1 : 1): *Frs. I-2-4-1 – I-2-4-4*. *Fr. I-2-4-4* (80 mg) was subjected to CC (SiO_2, hexane/AcOEt/MeOH 50 : 1 : 0, 0 : 1 : 0, 0 : 3 : 1): *Frs. I-2-4-4-1 – I-2-4-4-3*. Further purification of *Fr. I-2-4-4-3* (43.0 mg) by HPLC (*RP-18*, MeOH/H_2O/MeCN 50 : 40 : 5) yielded **7** (2 mg) and **3** (1.5 mg).

2,3-Didehydro-5-O-*methyl-11-epiillifunone E* (= rel-*(2*R,*3a*R,*7a*R*)-3,3a,7,7a-Tetrahydro-3a-hydroxy-2-(1-hydroxy-1-methylethyl)-7a-methoxy-5-(prop-2-en-1-yl)benzofuran-6(2*H*)-one*; **1**): Colorless amorphous solid. $[\alpha]_D^{25} = -102$ ($c = 0.1$, CH_2Cl_2). UV (MeOH): 208 (4.10), 240 (3.28). IR (neat): 3475, 1690, 1649. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 2* and *1*, resp. HR-ESI-MS: 305.1366 ($[M+Na]^+$, $C_{15}H_{22}NaO_5^+$; calc. 305.1365).

2,3-Didehydro-5-O-*methylillifunone E* (= rel-*(2*R,*3a*S,*7a*S*)-3,3a,7,7a-Tetrahydro-3a-hydroxy-2-(1-hydroxy-1-methylethyl)-7a-methoxy-5-(prop-2-en-1-yl)benzofuran-6(2*H*)-one*; **2**): Colorless amorphous solid. $[\alpha]_D^{25} = -68$ ($c = 0.1$, CH_2Cl_2). UV (MeOH): 206 (4.12), 245 (3.28). IR (neat): 3472, 1685, 1648. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 2* and *1*, resp. HR-ESI-MS: 305.1362 ($[M+Na]^+$, $C_{15}H_{22}NaO_5^+$; calc. 305.1365).

14-O-*Benzoylminwanensin* (= rel-*(4*R,*5*S,*6*S,*6a*R,*7*S,*9*R,*9a*R*)-6-[(Benzoyloxy)methyl]hexahydro-6a,7-dihydroxy-5,6,9-trimethyl-4*H*-4,9a-methanocyclopent[*d*]oxocin-2(1*H*)-one*; **3**): Colorless amorphous solid. $[\alpha]_D^{25} = -112.2$ ($c = 0.1$, CH_2Cl_2). UV (MeOH): 206 (4.1), 235 (3.28), 275 (3.3). IR (neat): 3480, 2920, 1740, 1720. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 2* and *1*, resp. HR-ESI-MS: 411.1783 ($[M+Na]^+$, $C_{22}H_{28}NaO_6^+$; calc. 411.1782).

3-O-*Acetyl-14*-O-*benzoylminwanensin* (= rel-*(4*R,*5*S,*6*S,*6a*R,*7*S,*9*R,*9a*R*)-7-(Acetyloxy)-6-[(benzoyloxy)methyl]hexahydro-6a-hydroxy-5,6,9-trimethyl-4*H*-4,9a-methanocyclopent[*d*]oxocin-2(1*H*)-one*; **4**): Colorless amorphous solid. $[\alpha]_D^{25} = -70$ ($c = 0.1$, CH_2Cl_2). UV (MeOH): 207 (4.23), 235 (3.28). IR (neat): 3500, 2930, 1730, 1720, 1649. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 2* and *1*, resp. HR-ESI-MS: 453.1887 ($[M+Na]^+$, $C_{24}H_{30}NaO_7^+$; calc. 453.1889).

(3β)-14-O-*Benzoyl-10-deoxy-3-hydroxyfloridanolide* (= rel-*(4*R,*5*R,*6*R,*6a*R,*7*S,*9*R,*9a*R*)-6-[(Benzoyloxy)methyl]hexahydro-5,6a,7-trihydroxy-5,6,9-trimethyl-4*H*-4,9a-methanocyclopent[*d*]oxocin-2(1*H*)-one*; **5**): Colorless amorphous solid. $[\alpha]_D^{25} = -52$ ($c = 0.6$, CH_2Cl_2). UV (MeOH): 230 (3.28). IR (neat): 3480, 1740, 1649. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 2* and *1*, resp. HR-ESI-MS: 427.1730 ($[M+Na]^+$, $C_{22}H_{28}NaO_7^+$; calc. 427.1733).

3-O-*Acetyl-14*-O-*benzoyl-10-deoxyfloridanolide* (= rel-*(4*R,*5*R,*6*R,*6a*R,*7*S,*9*R,*9a*R*)-7-(Acetyloxy)-6-[(benzoyloxy)methyl]hexahydro-5,6a-dihydroxy-5,6,9-trimethyl-4*H*-4,9a-methanocyclopent[*d*]oxocin-2(1*H*)-one*; **6**): Colorless amorphous solid. $[\alpha]_D^{25} = -72$ ($c = 0.1$, CH_2Cl_2). UV (MeOH): 235 (3.28), 275 (3.20). IR (neat): 3477, 1716, 1649. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 2* and *1*, resp. HR-ESI-MS: 469.1840 ($[M+Na]^+$, $C_{24}H_{30}NaO_8^+$; calc. 469.1838).

7-O-*Acetyldunnianin* (= rel-*(1*R,*4a*S,*6*S,*7*S,*8*S,*8a*S,*9*S*)-6-(Acetyloxy)-8-[(benzoyloxy)methyl]hexahydro-7,8a-dihydroxy-7,8,9-trimethyl-1,4a-ethano-4a*H*-2-benzopyran-3(4*H*)-one*; **7**): Colorless amorphous solid. $[\alpha]_D^{25} = -58$ ($c = 0.1$, CH_2Cl_2). UV (MeOH): 245 (3.28). IR (neat): 3480, 2920, 1783, 1740. ^{1}H- and ^{13}C-NMR ($CDCl_3$): *Tables 2* and *1*, resp. HR-ESI-MS: 469.1837 ($[M+Na]^+$, $C_{24}H_{30}NaO_8^+$; calc. 469.1838).

Cytotoxicity Assay. Cytotoxicity was tested against HeLa (human-cervical-epitheloid carcinoma), WiDr (human-colon adenocarcinoma), Daoy (medulloblastoma), and Hep2 (liver carcinoma) tumor cells. The assay procedure with MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide) was carried out as previously described [26]. The cells were cultured in *RPMI-1640* medium. After seeding of cells in a 96-well microplate for 4 h, 20 µl of sample were placed in each well and incubated at

37° for 3 d, and then 20 µl of MTT were added, and the mixtures were left for 5 h. After removing the medium and putting DMSO (200 µl/well) into the microplate with shaking for 10 min, the formazan crystals were redissolved, and their absorbance was measured on a microtiter plate reader (*Dynatech*, *MR 7000*) at a wavelength of 550 nm.

REFERENCES

[1] R. M. K. Saunders, *Bot. J. Linn. Soc.* **1995**, *117*, 333.
[2] Y. Fukuyama, J.-M. Huang, 'Illicium, Pimpinella and Foeniculum', Ed. M. M. Jodral, CRC, Florida FL, 2004, p. 31; Y. Fukuyama, J.-M. Huang, 'Studies in Natural Products Chemistry', Ed. Atta-ur-Rahman, Elsevier, Amsterdam, 2005, Vol. 32, p. 395.
[3] Y. Fukuyama, Y. Hata, M. Kodama, *Planta Med.* **1997**, *63*, 275.
[4] J. F. Lane, W. T. Koch, N. S. Leeds, G. Gorin, *J. Am. Chem. Soc.* **1952**, *74*, 3211.
[5] K. Yamada, S. Takada, S. Nakamura, Y. Hirata, *Tetrahedron* **1968**, *24*, 199.
[6] I. Kouno, H. Irie, N. Kawano, Y. Katsube, *Tetrahedron Lett.* **1983**, *24*, 771.
[7] C.-S. Yang, I. Kouno, N. Kawano, S. Sato, *Tetrahedron Lett.* **1988**, *29*, 1165.
[8] J.-L. Wang, C.-S. Yang, R.-N. Yan, B. Yao, X.-B. Yang, *Zhongguo Yaoxue Zazhi* **1994**, *29*, 693.
[9] T. J. Schmidt, *J. Nat. Prod.* **1999**, *62*, 684.
[10] I. Kouno, K. Mori, S. Okamoto, S. Sato, *Chem. Pharm. Bull.* **1990**, *38*, 3060.
[11] T. J. Schmidt, E. Müller, F. R. Froncezk, *J. Nat. Prod.* **2001**, *64*, 411.
[12] J.-M. Huang, C.-S. Yang, H. Wang, Q.-M. Wu, J.-L. Wang, Y. Fukuyama, *Chem. Pharm. Bull.* **1999**, *47*, 1749.
[13] J.-M. Huang, R. Yokoyama, C.-S. Yang, Y. Fukuyama, *Tetrahedron Lett.* **2000**, *41*, 6111.
[14] R. Yokoyama, J.-M. Huang, C.-S. Yang, Y. Fukuyama, *J. Nat. Prod.* **2002**, *65*, 527.
[15] Y. Fukuyama, N. Shida, M. Kodama, H. Chaki, T. Yugami, *Chem. Pharm. Bull.* **1995**, *43*, 2270.
[16] K. Yamada, S. Takada, S. Nakamura, Y. Hirata, *Tetrahedron* **1968**, *24*, 1267.
[17] I. Kouno, K. Mori, T. Akiyama, M. Hashimoto, *Phytochemistry* **1991**, *30*, 351.
[18] N. Kawano, A. Matsuo, *Yakugaku Zasshi* **1958**, *78*, 1220.
[19] I. Kouno, S. Shimamoto, Z.-H. Jiang, T. Tanaka, *Phytochemistry* **1997**, *46*, 1389.
[20] R. Yokoyama, J.-M. Huang, A. Hosoda, K. Kino, C.-S. Yang, Y. Fukuyama, *J. Nat. Prod.* **2003**, *66*, 799.
[21] T. J. Schmidt, H. M. Schmidt, E. Müller, W. Peters, F. R. Fronczek, A. Truesdale, N. H. Fischer, *J. Nat. Prod.* **1998**, *61*, 230.
[22] J.-L. Wang, C.-S. Yang, R.-N. Yan, B. Yao, X.-B. Yang, *Acta Pharm. Sin.* **1994**, *29*, 693.
[23] I. Kouno, N. Kawano, C.-S. Yang, *J. Chem Soc., Perkin Trans. 1* **1988**, 1537.
[24] T. J. Schmidt, W. Peters, F. R. Fronczek, N. H. Fischer, *J. Nat. Prod.* **1997**, *60*, 783.
[25] I. Kouno, N. Baba, M. Hashimoto, N. Kawano, M. Takahashi, H. Kaneto, C.-S. Yang, *Chem. Pharm. Bull.* **1990**, *38*, 422.
[26] Y.-C. Shen, Y.-S. Lin, Y.-B. Cheng, K.-C. Cheng, A. T. Khalil, Y.-H. Kuo, C.-T. Chien, Y.-C. Lin, *Tetrahedron* **2005**, *61*, 1345.

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