Iriformonins E – K, C_{22}-Trinorsesterterpenoids from the Sponge *Ircinia formosana*

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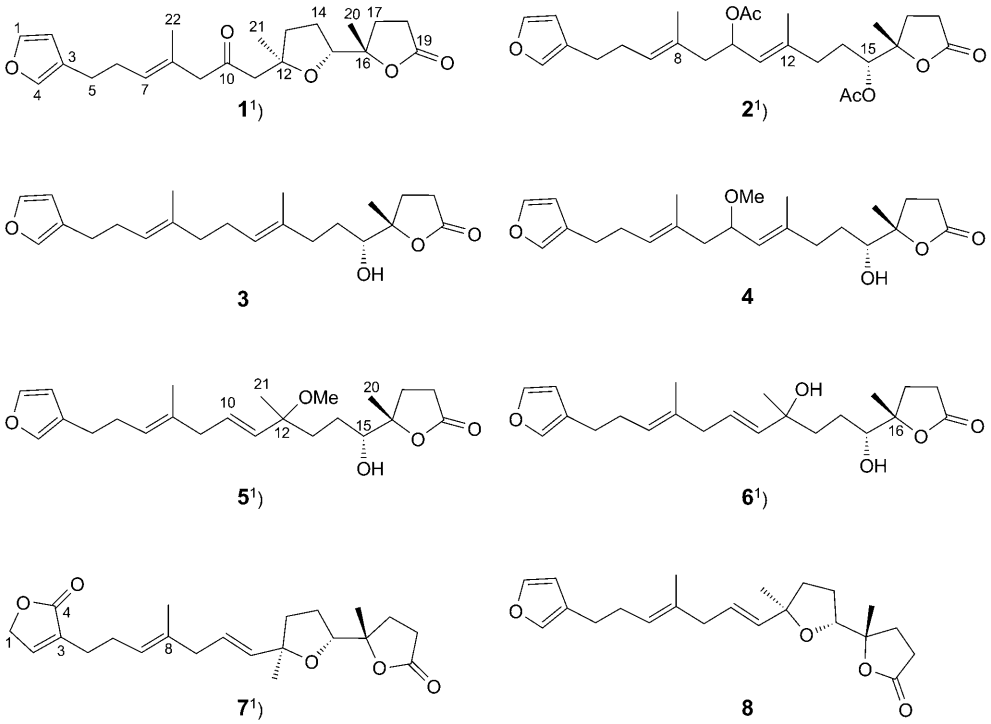
Chemical investigation of the sponge *Ircinia formosana* resulted in the isolation of seven new linear C_{22}-sesterterpenoids, iriformonins E – K (**1** – **7**) in addition to iriformonin A (**8**), a previously isolated furanosesterterpenoid (= a furan-moiety-containing sesterterpenoid) from the same species. The structures were determined by interpretation of HR-ESI-MS and 2D-NMR spectra. The structure of iriformonin A (**8**) was revised. Compound **5** exhibited significant inhibition of peripheral blood mononuclear cell proliferation induced by phytohemaglutinin.

Introduction. – Sponges (poriferans) are simple sedentary marine organisms that produce a wide variety of secondary metabolites that may act as a chemical defense against microorganisms and predators [1][2]. Sponges of the genus *Ircinia* produce and exude low-molecular-mass volatile compounds (*e.g.*, Me_2S and Me–N=C=S) that give them an unpleasant garlic odor [3]. Several steroid [4], sphingolipid [5], alkaloid [6], hydroquinone [7], and cyclic hexapeptide [8] derivatives have been isolated from this genus, in addition to furanosesterterpenes (= a furan-moiety-containing sesterterpenes) which are considered as one of the major constituents [9][10]. Furanosesterterpenes were frequently isolated from other marine sponge genera such as *Spongia*, *Spongionella*, *Cacospongia*, *Dysidea*, *Sarcotragus*, *Amphimedon*, and *Hippospongia* and have chemotaxonomic importance [11][12]. Some furanosesterterpenes were reported to possess antimicrobial [13], cytotoxic [14][15], and inhibition of lymphocytic proliferation activities [14]. In a search for bioactive marine metabolites from the local fauna, a chemical investigation of a new collection of *Ircinia formosana* was carried out, which resulted in the isolation of seven new C_{22}-furanosesterterpenoids, iriformonins E – K[1]) (**1** – **7**), in addition to iriformonin A (**8**), previously isolated from the same species [16]. The biological activities of sesterterpenes **1** – **7** were tested against HSV-1 and evaluated with peripheral blood mononuclear cell proliferation induced by phytohemaglutinin.

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



1[1]) **2**[1]) **3** **4**

5[1]) **6**[1])

7[1]) **8**

Results and Discussion. – Solvent fractionation and multiple chromatographic separations over normal-phase and reversed-phase silica gel of the lipophilic extract of *Ircinia formosana* afforded seven new irciformonins E–K (**1**–**7**). The HR-ESI-MS data of **1** revealed a molecular-ion peak at *m*/*z* 397.1989 ($[M+Na]^+$) suggesting a molecular formula $C_{22}H_{30}O_5$ and eight degrees of unsaturations. The IR spectrum displayed absorption bands diagnostic for a 5-membered lactone (1768 cm^{-1}) and C=O (1710 cm^{-1}) functionalities. The ^{1}H-NMR data (*Table 1*) disclosed signals of an O-bearing CH group at δ(H) 3.99 (H–C(15)), an olefin moiety at δ(H) 5.28 (*t*, J = 6.5 Hz, H–C(7)), and three signals of a monosubstituted furan at δ(H) 7.34, 7.22, and 6.28 (3 br. *s*), consistent with a low-resolution EI-MS fragment ion at *m*/*z* 67 ($C_4H_3O^+$). The ^{13}C-NMR spectrum (*Table 2*) revealed a ketone (δ(C) 208.3), lactone C=O (δ(C) 177.0), tri-substituted olefin (δ(C) 129.2 (CH), 129.6 (C)), as well as a furan moiety (δ(C) 142.7 (CH), δ(C) 111.0 (CH), δ(C) 138.9 (CH), and δ(C) 124.7 (C)) [17]. The CH_2 group at δ(H) 2.50 (*m*, CH_2(5)) exhibited HMBCs to C(2), C(4), and an olefinic CH (C(7)), a NOESY correlation to H–C(4) of the furan ring, as well as a COSY cross-peak to the adjacent CH_2 at δ(H) 2.32 (CH_2(6)) (*Figs. 1* and *2*). The vinylic Me at δ(H) 1.60 correlated with the olefinic CH at δ(C) 129.2 (C(7)) proving the presence of the structural unit furanyl–CH_2CH_2CH=C(Me)– that was further supported by an EI-MS fragment ion at *m*/*z* 135. The tertiary Me at δ(H) 1.35 (Me(20)) correlated to a CH_2 at δ(C) 28.7 (C(17)) whose H-atoms (δ(H) 2.27 and 1.85) ^3J-correlated with a quaternary C–O moiety at δ(C) 87.4 (C(16)) and coupled with CH_2

Table 1. *^{1}H-NMR Spectroscopic Data* (300 MHz, $CDCl_3$) *for Irciformonins* **1**–**7**[1]). δ in ppm, J in Hz.

	1	**2**[a])	**3**	**4**[b])	**5**[c])	**6**	**7**
H–C(1)	7.34 (br. *s*)	7.31 (br. *s*)	7.33 (br. *s*)	7.29 (br. *s*)	7.31(br. *s*)	7.34 (br. *s*)	4.77 (br. *s*, 2 H)
H–C(2)	6.28 (br. *s*)	6.25 (br. *s*)	6.28 (br. *s*)	6.24 (br. *s*)	6.25 (br. *s*)	6.27 (br. *s*)	7.12 (br. *s*)
H–C(4)	7.22 (br. *s*)	7.18 (br. *s*)	7.21 (br. *s*)	7.17 (br. *s*)	7.18 (br. *s*)	7.20 (br. *s*)	–
CH_2(5)	2.47–2.53 (*m*)	2.40 (*t*, *J* = 7.8)	2.39–2.43 (*m*)	2.40 (*t*, *J* = 7.4)	2.38–2.44 (*m*)	2.45 (*t*, *J* = 7.7)	2.34 (*d*, *J* = 6.0)
CH_2(6)	2.30–2.35 (*m*)	2.18–2.24 (*m*)	2.21–2.27 (*m*)	2.21–2.26 (*m*)	2.17–2.23 (*m*)	2.23–2.28 (*m*)	2.25–2.30 (*m*)
H–C(7)	5.28 (*t*, *J* = 6.5)	5.18 (*t*, *J* = 6.3)	5.16 (*t*, *J* = 6.6)	5.16 (*t*, *J* = 6.5)	5.15 (*t*, *J* = 6.5)	5.19 (*t*, *J* = 6.7)	5.10 (br. *t*, *J* = 6.8)
CH_2(9)	3.08 (*s*)	2.23–2.28 (*m*), 2.10–2.14 (*m*)	2.00 (*t*, *J* = 7.0)	2.18–2.24 (*m*), 2.02–2.08 (*m*)	2.69 (*d*, *J* = 6.5)	2.68 (*d*, *J* = 5.7)	2.70 (*d*, *J* = 5.3)
H–C(10)	–	5.59 (br. *td*, *J* = 7.0, 7.0)	2.04–2.10 (*m*)	3.95–4.01 (*m*)	5.49 (*dt*, *J* = 15.5, 6.5)	5.55–5.61 (*m*)	5.50–5.56 (*m*)
CH_2(11)	2.64 (*d*, *J* = 13.8), 2.56–2.62 (*m*)	5.08 (*d*, *J* = 7.5)	5.16 (*t*, *J* = 6.6)	5.01 (*d*, *J* = 8.8)	5.34 (*dd*, *J* = 15.5, 1.0)	5.48 (*d*, *J* = 15.6)	5.40 (*d*, *J* = 15.2)
CH_2(13)	1.86–1.91 (*m*)	1.97–2.03 (*m*)	2.21–2.47 (*m*), 2.04–2.10 (*m*)	2.18–2.24 (*m*), 2.02–2.07 (*m*)	1.82–1.87 (*m*), 1.56–1.62 (*m*)	1.78–1.84 (*m*), 1.57–1.63 (*m*)	1.87–1.94 (*m*), 1.65–1.71 (*m*)
CH_2(14)	1.88–1.94 (*m*), 1.67–1.73 (*m*)	1.65–1.71 (*m*), 1.60–1.66 (*m*)	1.52–1.58 (*m*), 1.37–1.43 (*m*)	1.39–1.45 (*m*),	1.59–1.65 (*m*), 1.36–1.42 (*m*)	1.57–1.63 (*m*), 1.37–1.43 (*m*)	1.86–1.92 (*m*), 1.73–1.77 (*m*)
H–C(15)	3.96–4.02 (*m*)	4.99 (*dd*, *J* = 9.9, 2.0)	3.64 (*d*, *J* = 10.1)	3.59 (*d*, *J* = 9.9)	3.58 (*d*, *J* = 9.8)	3.65 (*d*, *J* = 9.4)	3.98 (br. *t*, *J* = 6.5)
CH_2(17)	2.24–2.31 (*m*), 1.82–1.88 (*m*)	2.18–2.24 (*m*), 1.87–1.93 (*m*)	2.38–2.44 (*m*), 1.79–1.85 (*m*)	2.37–2.43 (*m*), 1.78–1.84 (*m*)	2.34–2.40 (*m*), 1.76–1.82 (*m*)	2.42–2.48 (*m*), 1.78–1.83 (*m*)	2.22–2.28 (*m*), 1.24–1.28 (*m*)
CH_2(18)	2.53–2.59 (*m*)	2.58 (*t*, *J* = 8.1)	2.58–2.64 (*m*)	2.55–2.61 (*m*)	2.54–2.61 (*m*)	2.61–2.67 (*m*)	2.66–2.72 (*m*), 2.49–2.55 (*m*)
Me(20)	1.35 (*s*)	1.37 (*s*)	1.35 (*s*)	1.31 (*s*)	1.32 (*s*)	1.35 (*s*)	1.35 (*s*)
Me(21)	1.25 (*s*)	1.60 (*s*)	1.61 (*s*)	1.64 (*s*)	1.22 (*s*)	1.30 (*s*)	1.29 (*s*)
Me(22)	1.60 (*s*)	1.61 (*s*)	1.58 (*s*)	1.57 (*s*)	1.54 (*s*)	1.56 (*s*)	1.55 (*s*)

[a]) Two AcO groups appear at δ(H) 2.06 (*s*) and 1.95 (*s*). [b]) One MeO group appears at δ(H) 3.12 (*s*). [c]) One MeO group appears at δ(H) 3.20 (*s*).

Table 2. *^{13}C-NMR Spectroscopic Data* (75 MHz, $CDCl_3$) *for Irciformonins* **1**–**7**[1])[a])

	1	**2**	**3**	**4**	**5**	**6**	**7**
C(1)	142.7 (*d*)	142.6 (*d*)	142.6 (*d*)	142.6 (*d*)	142.6 (*d*)	142.6 (*d*)	70.2 (*t*)
C(2)	111.0 (*d*)	111.1 (*d*)	111.1 (*d*)	111.1 (*d*)	111.1 (*d*)	111.1 (*d*)	144.6 (*d*)
C(3)	124.7 (*s*)	124.8 (*s*)	125.0 (*s*)	124.9 (*s*)	124.8 (*s*)	124.9 (*s*)	133.7 (*s*)
C(4)	138.9 (*d*)	138.9 (*d*)	138.8 (*d*)	138.8 (*d*)	138.8 (*d*)	138.9 (*d*)	174.0 (*s*)
C(5)	24.7 (*t*)	24.9 (*t*)	25.0 (*t*)	24.9 (*t*)	24.9 (*t*)	25.0 (*t*)	25.4 (*t*)
C(6)	28.6 (*t*)	28.4 (*t*)	28.4 (*t*)	28.5 (*t*)	28.6 (*t*)	28.5 (*t*)	25.7 (*t*)
C(7)	129.2 (*d*)	127.4 (*d*)	123.9 (*d*)	126.5 (*d*)	124.9 (*d*)	124.9 (*d*)	123.6 (*d*)
C(8)	129.6 (*s*)	131.3 (*s*)	135.6 (*s*)	132.3 (*s*)	134.3 (*s*)	134.1 (*s*)	135.4 (*s*)
C(9)	55.5 (*t*)	45.2 (*t*)	39.6 (*t*)	45.7 (*t*)	42.7 (*t*)	42.5 (*t*)	42.2 (*t*)
C(10)	208.3 (*s*)	69.6 (*d*)	26.5 (*t*)	76.1 (*d*)	129.6 (*d*)	126.3 (*d*)	125.7 (*d*)
C(11)	51.7 (*t*)	124.5 (*d*)	125.4 (*d*)	126.4 (*d*)	135.1 (*d*)	138.1 (*d*)	136.5 (*d*)
C(12)	82.2 (*s*)	138.6 (*s*)	134.4 (*s*)	138.8 (*s*)	77.1 (*s*)	72.8 (*s*)	83.2 (*s*)
C(13)	37.0 (*t*)	35.6 (*t*)	36.2 (*t*)	36.1 (*t*)	39.8 (*t*)	38.9 (*t*)	37.4 (*t*)
C(14)	27.2 (*t*)	27.7 (*t*)	29.0 (*t*)	29.1 (*t*)	25.4 (*t*)	29.6 (*t*)	27.1 (*t*)
C(15)	83.6 (*d*)	76.0 (*d*)	75.5 (*d*)	75.2 (*d*)	76.3 (*d*)	76.0 (*d*)	82.7 (*d*)
C(16)	87.4 (*s*)	86.2 (*s*)	88.9 (*s*)	89.1 (*s*)	88.9 (*s*)	89.0 (*s*)	87.5 (*s*)
C(17)	28.7 (*t*)	29.9 (*t*)	27.8 (*t*)	27.8 (*t*)	28.5 (*t*)	28.1 (*t*)	29.3 (*t*)
C(18)	29.8 (*t*)	28.7 (*t*)	29.5 (*t*)	29.5 (*t*)	29.4 (*t*)	29.4 (*t*)	29.6 (*t*)
C(19)	177.0 (*s*)	176.1 (*s*)	177.3 (*s*)	177.5 (*s*)	177.4 (*s*)	177.3 (*s*)	177.1 (*s*)
C(20)	23.5 (*q*)	24.8 (*q*)	23.0 (*q*)	23.1 (*q*)	22.6 (*q*)	22.8 (*q*)	23.0 (*q*)
C(21)	27.3 (*q*)	16.7 (*q*)	16.0 (*q*)	16.7 (*q*)	21.5 (*q*)	28.5 (*q*)	27.3 (*q*)
C(22)	16.5 (*q*)	16.4 (*q*)	15.9 (*q*)	16.6 (*q*)	16.2 (*q*)	16.2 (*q*)	16.1 (*q*)
MeO				55.7 (*q*)	50.1 (*q*)		
AcO		20.9 (*q*), 170.3 (*s*)					
AcO		21.2 (*q*), 172.2 (*s*)					

[a]) Assignments were aided by HMQC and DEPT techniques.

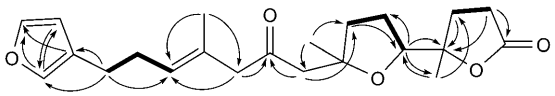


Fig. 1. *Selected HMBC* (H → C) *and COSY* (—) *of* **1**

H-atoms at δ(H) 2.56 (CH_2(18)). The latter H-atoms correlated with a lactone C=O (δ(C) 177.0) indicating a γ-methyl-γ-lactone ring (*m*/*z* 99). The tertiary Me at δ(H) 1.25 (Me(21)) correlated with the CH_2 at δ(C) 37.0 (C(13)). The correlations of CH_2(13) (δ(H) 1.89) with a CH–O at δ(C) 83.6 (C(15)) and of H–C(15) to C(14), a quaternary C–O (C(16)), and Me(20), as well as the COSY cross-peaks of CH_2(13)/CH_2(14)/H–C(15) confirmed the attachment of the tetrahydrofuran unit to the lactone ring through a C(15)–C(16) link. Both the CH_2 at δ(H) 3.08 (CH_2(9)) and 2.64 and 2.59 (CH_2(11)) ^2J-correlated to the ketonic C-atom at δ(C) 208.3 (C(10)), and the correlations Me(22)/C(9), CH_2(9)/C(7), CH_2(11)/C(13), and Me(21)/C(11) and C(13) established the C-atom sequence C(8) to C(12). The geometry of the trisubstituted C=C bond (C(7)) was assigned as (*E*) on the basis of an NOE H–C(7)/CH_2(9) and the high-field resonance of the vinylic Me group (δ(C) 16.5 (C(22))) [9][18]. The NOESY

data confirmed the structure of **1** (*Fig. 2*), and the relationship of the lactone and tetrahydrofuran rings was established by the correlations $CH_2(14)/CH_2(17)$. The NOESY correlations $H-C(15)/H_\beta-C(14)$, Me(21)/ $H_\alpha-C(11)$ suggested an α-configuration for Me(21) and β-configuration for H–C(15). The presence of NOEs between H–C(15) and Me(20) favored β-orientation of the latter. Based on these findings, irciformin E was assigned structure **1**.

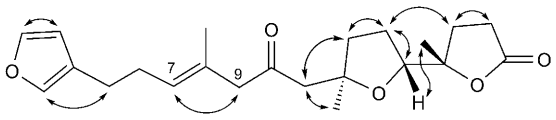


Fig. 2. *Key NOESY correlations of* **1**[1])

The molecular formula of **2** was determined as $C_{26}H_{36}O_7$ from HR-ESI-MS at m/z 483.2362 ($[M+Na]^+$) and ^{13}C-NMR data (*Table 2*). The IR spectrum showed absorption bands characteristic of a γ-lactone (1768 cm^{-1}) and ester (1738 cm^{-1}) groups. The ^{13}C-NMR data indicated the presence of a monosubstituted furan ring (δ(C) 142.6, 111.1, 124.8 and 138.9; EI-MS fragment ion at m/z 67) and a lactone ring (δ(C) 176.1), two tri-substituted olefin moieties (δ(C) 127.4 (CH) and 131.3 (C), and 124.5 (CH) and 138.6 (C)), in addition to two Ac groups (δ(C) 170.3 and 20.9, and 172.2 and 21.2) and two CH–O groups (δ(C) 69.6 (C(10)) and 76.0 (C(15))). The ^{1}H-NMR (*Table 1*) revealed two AcO (δ(H) 2.06 and 1.95), along with three quaternary Me groups (δ(H) 1.37, 1.60, and 1.67), and two CH–O groups (δ(H) 5.59 and 4.99). The HMBC and COSY data (*Fig. 3*) established the presence of the structural unit furanyl–CH_2CH_2CH=C(Me)–, identical with that of **1**. The CH–O group at δ(H) 5.59 (br. *td*, $J=7.0$, 7.0 Hz, H–C(10)) correlated to C(8) (δ(C) 131.3), the quaternary olefinic C(12) (δ(C) 138.6), and the Ac C=O (δ(C) 170.3), while the Me at δ(H) 1.60 (Me(21)) correlated to the olefinic C(11) and to C(13). On the other hand, the CH–O at δ(H) 4.99 (*dd*, $J=9.9$, 2.0 Hz) was assigned to H–C(15) on the basis of its correlation to C(16) as well as its ^3J-correlation to $CH_2(17)$ (δ(C) 29.9) and Me(20) (δ(C) 24.8). Furthermore, the COSY plot revealed the cross-peaks $CH_2(5)/CH_2(6)/H-C(7)$, $CH_2(9)/H-C(10)/H-C(11)$, and $CH_2(13)/CH_2(14)/H-C(15)$ confirming the sequence of the linear C-chain C(12) to C(15), that was also suggested by the upfield resonance of C(15) (δ(C) 76.0) relative to that of **1** (δ(C) 83.6).

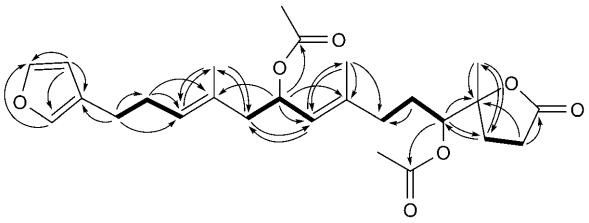


Fig. 3. *Key HMBC* (H → C) *and COSY* (—) *of* **2**

The molecular formula $C_{22}H_{32}O_4$ was assigned to **3** as indicated by the HR-ESI-MS and ^{13}C-NMR data (unsaturation degree 7). The IR spectrum revealed the presence of OH (3448 cm^{-1}) and lactone (1764 cm^{-1}) groups. The NMR data (*Tables 1* and *2*) suggested a closely similar structure to that of **2**, except for the presence of one OH group in **3** instead of two AcO groups in **2**. The CH−O at δ(H) 3.64 (*d*, $J = 10.1$ Hz, H−C(15)) correlated to the quaternary C−O at δ(C) 88.9 (C(16)), while the Me(20) at δ(H) 1.35 correlated with the CH−O at δ(C) 75.5 (C(15)) and 88.9 (C(16)) and CH_2(17) at δ(C) 27.8, thereby locating an OH group at C(15) (EI-MS fragment at *m*/*z* 243 ($[M - OH]^+$)). The signal at δ(H) 5.16 (*t*, $J = 6.6$ Hz, 2 H) was assigned to two olefinic H-atoms of two tri-substituted C=C bonds, both flanked by two CH_2 groups. Apparently, one of the olefinic H-atoms belonged to H−C(7) due to its COSY cross-peak with H−C(6) as well as the HMBCs CH_2(5) (δ(H) 2.41)/C(7) (δ(C) 123.9), H−C(7)/C(22) (δ(C) 15.9), and Me(22) (δ(H) 1.58)/C(8) (δ(C) 135.6) and C(9) (δ(C) 39.6). The same olefinic signal (δ(H) 5.16) correlated with Me(21) (δ(C) 16.0) and C(9), while Me(21) (δ(H) 1.61) correlated with C(11) (δ(C) 125.4), C(12) (δ(C) 134.4) and C(13) (δ(C) 36.2) suggesting a C(11)=C(12) bond. This was confirmed by COSY cross-peaks between CH_2(9), CH_2(10), and CH_2(11) and EI-MS fragment ions at *m*/*z* 135 and 157 resulting from fission between C(8) and C(9) and between C(12) and C(13), respectively. The geometry of both C=C bonds were (*E*) as deduced from the chemical shifts of C(22) and C(21). NOESY Correlations H−C(15)/Me(20) suggested their β-orientation and hence, an α-orientation of OH−C(15).

The molecular formula of **4** was determined as $C_{23}H_{34}O_5$ on the basis of HR-ESI-MS (unsaturation degree 7). The ^{13}C-NMR data (*Table 2*) revealed the presence of furan and 5-membered lactone rings, of two tri-substituted C=C bonds similar to those of **2** and **3**, of two CH−O groups (δ(C) 75.2 and 76.1), and of one MeO group (δ(C) 55.7). The CH−O signal at δ(H) 3.59 (*d*, $J = 9.9$ Hz) was assigned to H−C(15) as indicated by its large coupling constant (*Table 1*) as well as the HMBCs CH_2(17) and Me(20)/C(15), and Me(20)/C(16) (δ(C) 89.1 (C)) and C(17) (δ(C) 27.8). The CH−O at δ(H) 3.98 (*m*) correlated with MeO, the quaternary C(8) (δ(C) 132.3), and C(12) (δ(C) 138.8) and had a COSY cross peak with H−C(11) (δ(H) 5.01 (*d*, $J = 8.8$ Hz)), implying attachment of the MeO group at C(10). Additionally, the HMBC spectrum showed the correlations MeO (δ(H) 3.12 (*s*))/C(10) and Me(21)/C(11) and C(13). To determine the absolute configuration at C(15), *Mosher*'s method was attempted but was unsuccessful, presumably because **4** is unstable. The proposed structure of **4** is to be considered as tentative.

The molecular formula $C_{23}H_{34}O_5$ was assigned to **5** from its HR-ESI-MS, implying that it is isomeric with **4**. The HMBCs Me(22) (δ(H) 1.54 (*s*))/C(7) (δ(C) 124.9 (CH)) and C(9) (δ(C) 42.7), and H−C(7) (δ(H) 5.15 (*t*))/C(22) (δ(C) 16.2) (data in *Tables 1* and *2*), along with an EI-MS fragment ion at *m*/*z* 135 (C(8)−C(9) fission), confirmed the C(7)=C(8) bond. HMBC Data confirmed the OH substitution at C(15) as in **3** and **4** (MS; *m*/*z* 373 ($[M - OH]^+$)). The quaternary C−O at δ(C) 77.1 (C(12)) correlated to H-atoms at δ(H) 1.85 and 1.59 (CH_2(13)), 1.22 (Me(21)), 3.20 (MeO), as well as two *trans*-olefinic H-atoms at δ(H) 5.49 (*dt*, $J = 15.5$, 6.5 Hz, H−C(10)) and 5.34 (*dd*, $J =$ 15.5, 1.0 Hz, H−C(11)). Additionally, H−C(10) correlated to the quaternary C(8) (δ(C) 134.3) and C(12), while H−C(11) correlated to the allylic positions C(9) and C(21) (δ(C) 21.5) suggesting a C(8), C(12) connection *via* an (*E*)-allyl moiety

C(9)–C(10)=C(11). The structure was further verified by the HMBCs Me(22) (δ(H) 1.54)/C(7) and C(9), and Me(21)/C(11) and C(13) (δ(C) 39.8), by the COSY cross-peaks CH_2(9)/H–C(10)/H–C(11) and CH_2(13)/CH_2(14)/H–C(15), and by EI-MS fragment ions at *m/z* 175 and 215 generated by C(11)–C(12) fission. The coupling constant of J(10,11) = 15.5 Hz points to an (*E*) geometry of this C=C-bond.

The spectroscopic data (*Tables 1* and *2*) of irciformonin J (**6**), molecular formula $C_{22}H_{32}O_5$, revealed a structure similar to that of **5**, except for the absence of the MeO group. The ^{1}H-NMR spectrum showed one CH–O group at δ(H) 3.65 (*d*, J = 9.4 Hz, H–C(15)) and an olefinic H-atom at δ(H) 5.19 (*t*, J = 6.7 Hz, H–C(7)), as well as *trans*-olefinic H-atoms at δ(H) 5.58 (*m*, H–C(10)) and 5.48 (br. *d*, J = 15.6 Hz, H–C(11)). The COSY and HMBC data verified a structure similar to **5** but with an OH group instead of the MeO group at C(12). The J value of H–C(15) and the chemical shifts of C(15), C(16), and C(20) resembled those of compounds **3**–**5**, suggesting the same configuration at C(15) and C(16). This led to structure proposal **6** for irciformonin J. Although structure **6** seemed identical to that reported earlier from our group for irciformonin A [16], the two compounds differed in their NMR data of C(12), C(15), and H–C(15) that resonated at lower field in the case of irciformonin A (δ(C) 83.3 and 82.6, and δ(H) 4.00, resp.) relative to those of **6** (δ(C) 72.8, 76.0, and δ(H) 3.65). We believe that irciformonin A (**8**) possessing a tetrahydrofuran ring underwent spontaneous hydration after running the NMR spectra and before the determination of its HR-ESI-MS. This caused the previously incorrect identification of **8** to be **6**. The authors regret that the NMR and MS data of **8** were not concomitant in its first isolation [16]. Irciformonin A was also isolated during the present study and identified by comparison with published data [16]; subsequent hydration was induced by traces of dilute HCl which produced **6** by opening of the tetrahydrofuran ring of **8**.

The HR-ESI-MS data of **7** demonstrated a molecular-ion peak at *m/z* 397.1991 ($[M+Na]^+$) suggesting a molecular formula $C_{22}H_{30}O_5$. The ^{13}C-NMR data (*Table 2*) displayed a C=O at δ(C) 177.1, diagnostic of a 5-membered lactone ring as in **1**–**6**, along with an additional C=O at δ(C) 174.0, and three C=C bonds at δ(C) 144.6 (CH), 133.7 (C), 123.6 (CH), 135.4 (C), 125.7 (CH), and 136.5 (CH). The HMBC data of **7** (*Fig. 4*) verified the presence of a γ-lactone ring linked with the tetrahydrofuran ring as in compound **1**. The olefinic H-atoms at δ(H) 5.10 (br. *t*, J = 6.8 Hz, H–C(7)), 5.53 (*m*, H–C(10)), and 5.40 (*t*, J = 15.2 Hz, H–C(11)) (*Table 1*) were assigned with the aid of HMBC and COSY data. The CH_2O signal at δ(H) 4.77 (br. *s*, CH_2(1)) showed a COSY cross-peak with the olefinic signal at δ(H) 7.12 (br. *s*, H–C(2)) as well as HMBCs with the olefinic quaternary C-atom at δ(C) 133.7 (C(3)), and the C=O at δ(C) 174.0 (C(4)). We suggest that C(3) of the α,β-unsaturated γ-lactone ring of **7** is connected with C(5). This finding was supported by the HMBCs CH_2(5)/C(2) and C(4), and

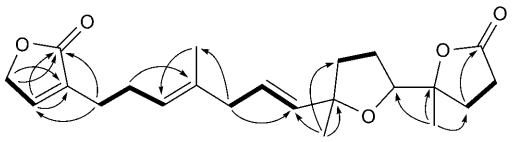


Fig. 4. *Selected HMBC* (H → C) *and COSY* (—) *of* **7**

H–C(2)/C(3) and C(4). The configuration of **7** was tentatively assigned by comparing the ^{1}H- and ^{13}C-NMR data with those of **1**.

The isolated sesterterpenes **1**–**7** were tested against HSV-1 *in vitro*. They exhibited very weak activity as compared with acyclovir. A preliminary study on resting cells and cells activated with PHA (phytohemagglutinin) were tested with compounds **1**–**7** at a concentration of 100 μg/ml (*Table 3*). The inhibition of cell proliferation was determined by the uptake of tritiated thymidine. Among them, compound **5** exhibited significant inhibition on peripheral blood mononuclear cell (PBMC) proliferation induced by PHA.

Table 3. *Effects of Compounds* **1**–**7** *on PBMC* (peripheral blood mononuclear cell) *Proliferation Induced by PHA* (phytohemagglutinin)

Compound [100 μg/ml]	Activity [%]		
	Resting[a])	PHA [0.5 μg/ml]	PHA [5 μg/ml]
1	−73.0 ± 6.7	13.7 ± 4.4	−48.2 ± 4.1
2	−16.3 ± 1.5	−12.9 ± 1.4	−12.5 ± 1.0
3	84.4 ± 21.6	17.5 ± 7.4	−23.2 ± 1.6
4	−22.0 ± 2.6	−39.6 ± 3.1	−47.8 ± 6.7
5	69.1 ± 6.8	−22.8 ± 5.6	−72.3 ± 0.9
6	6.5 ± 7.7	−15.7 ± 4.5	−1.4 ± 3.4
7	74.6 ± 2.7	−6.6 ± 1.5	−35.7 ± 2.4
IL-2 [10 U/ml]	95.3 ± 10.1	208 ± 25.7	208 ± 25.7
Cyclosporine A [2.5 μg/ml]	−15.9 ± 4.4	−92.2 ± 6.8	−92.2 ± 6.8

[a]) Negative values represent inhibitory activity; positive values represent enhancement of proliferation.

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Experimental Part

General. Prep. TLC: pre-coated silica gel plates (SiO_2; silica gel *60 F-254*, 1 mm; *Merck*). Column chromatography (CC): SiO_2 *60* (*Merck*) and *Sephadex LH-20* (*Amersham Pharmacia Biotech AB*, Uppsala, Sweden). HPLC: *Hitachi* system; *LiChrospher® Si 60* (5 μm, 250–10; *Merck*) and *LiChrospher® 100 RP-18e* (5 μm, 250–10; *Merck*) for normal and reversed-phase, resp. Optical rotations: *Jasco-DIP-1000* polarimeter. UV Spectra: *Hitachi-U-3210* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra: *Hitachi-T-2001* spectrophotometer; $\tilde{\nu}_{max}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR, COSY, HMQC, HMBC, and NOESY Experiments: *Bruker-FT-300* spectrometer; at 300 (^{1}H) and 75 MHz (^{13}C); $CDCl_3$ solns.; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. EI-MS: *VG-Quattro-5022* mass spectrometer; in *m*/*z* (rel. %). HR-ESI-MS: *Shimadzu-LCMS-2010A* spectrometer; in *m*/*z* (rel. %).

Animal Material. The sponge *Ircinia formosana* was collected by scuba diving off the coast of eastern Taiwan, at a depth of 20 m, in June 2005, and frozen shortly after collection. A specimen (GSPN-11) and a photo are deposited with the School of Pharmacy, College of Medicine, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation. The sponge material (wet weight 500 g) was chopped and exhaustively extracted with CH_2Cl_2/acetone/MeOH 1:1:1 at r.t. The resulting extract was filtered and concentrated under vacuum then partitioned between AcOEt/H_2O to provide the AcOEt extract. The latter (25 g) was separated by flash CC (SiO_2, hexane/CH_2Cl_2 20:1 → 0:1, then CH_2Cl_2/MeOH 100:1 → 5:1): *Fractions 1–20*. *Fr. 3* (eluted with CH_2Cl_2/MeOH 90:1; 3.04 g) was subjected to CC (SiO_2, hexane/AcOEt

25:1 → 0:1, then AcOEt/MeOH 100:1 → 1:1): irciformonin A (= rel-*(2*R*,2′*S*,5′*S*)-5′-[(1*E*,4*E*)-7-(furan-3-yl)-4-methylhepta-1,4-dien-1-yl]hexahydro-2,5′-dimethyl[2,2′-bifuran]-5(2* H*)-one*; **8**; 453 mg) and a mixture *Fr. 3.M*. *Fr. 3.M* (938 mg) was subjected to CC (SiO_2, hexane/AcOEt 25:1 → 0:1, then AcOEt/MeOH 100:1 → 40:1). Fraction *Fr. 3.M.12* (eluted with AcOEt/MeOH 80:1; 520 mg) was repeatedly subjected to reversed-phase HPLC (MeOH/H_2O/MeCN 3:1:1): **1** (10 mg), **2** (50 mg), **3** (22 mg), **4** (89 mg), and **5** (84 mg). *Fr. 5* (eluted with CH_2Cl_2/MeOH 70:1; 4.8 g) subjected to CC (SiO_2, hexane/AcOEt 50:1 → 0:1, then AcOEt/MeOH 100:1 → 10:1). The fraction eluted with AcOEt/MeOH 40:1 was purified by CC (*Sephadex LH-20*, CH_2Cl_2/MeOH 1:1): *Fractions 5.L$_1$* and *5.L$_2$*. *Fr. 5.L$_2$* (3.9 g) was subjected to CC (SiO_2, hexane/AcOEt/MeOH gradient), and the fraction eluted with AcOEt/MeOH 60:1 (1.53 g) was subjected to reversed-phase HPLC (MeOH/H_2O/MeCN 65:30:5) followed by normal-phase HPLC (hexane/CH_2Cl_2/MeOH 15:5:1) and purification by prep. TLC (SiO_2, hexane/BuOH 4:1): **6** (17 mg). *Fr. 9* (eluted with CH_2Cl_2/MeOH 30:1; 1.4 g) was subjected to CC (SiO_2, hexane/AcOEt 20:1 → 0:1), and the fraction eluted with hexane/AcOEt 3:1 was purified by reversed-phase HPLC (MeOH/H_2O/MeCN 55:40:5): **7** (4 mg).

Irciformonin E (= rel-*(2*R*,2′*S*,5′*S*)-5′-[(4*E*)-7-(Furan-3-yl)-4-methyl-2-oxohept-4-en-1-yl]hexahydro-2,5′-dimethyl[2,2′-bifuran]-5(2* H*)-one*; **1**): Colorless powder. $[\alpha]_D^{25} = -3.5$ ($c = 1.0$, CH_2Cl_2). IR (CH_2Cl_2): 1768 (lactone), 1710 (C=O), 1063 (C−O), 943 (furan). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 397 (M^+), 191, 183, 135, 99 (100), 67. HR-ESI-MS: 397.1989 ($[M+Na]^+$, $C_{22}H_{30}NaO_5^+$; calc. 397.1991).

Irciformonin F (= rel-*(5*R*)-5-[(1*S*,4*E*,8*E*)-1,6-Bis(acetyloxy)-11-furan-3-yl)-4,8-dimethylundeca-4,8-dien-1-yl]dihydro-5-methylfuran-2(3* H*)-one*; **2**): Colorless powder. $[\alpha]_D^{25} = -4.2$ ($c = 4.6$, CH_2Cl_2). IR (CH_2Cl_2): 1768 (lactone), 1738 (C=O, ester), 1240, 1022, 943, 735. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 460 (M^+), 400 ($[M-AcOH]^+$), 342 ($[M-2\ AcOH]^+$), 209, 135, 99 (100), 67. HR-ESI-MS: 483.2362 ($[M+Na]^+$, $C_{26}H_{36}NaO_7^+$; calc. 483.2359).

Irciformonin G (= rel-*(5*R*)-5-[(1*S*,4*E*,8*E*)-11-(Furan-3-yl)-1-hydroxy-4,8-dimethylundeca-4,8-dien-1-yl]dihydro-5-methylfuran-2(3* H*)-one*; **3**): Colorless powder. $[\alpha]_D^{25} = +5.4$ ($c = 2.4$, CH_2Cl_2). IR (CH_2Cl_2): 3448 (OH), 1764 (lactone), 1071, 941. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 360 (M^+), 343 ($[M-OH]^+$), 157, 135, 99 (100), 67. HR-ESI-MS: 361.2380 ($[M+H]^+$, $C_{22}H_{33}O_4^+$; calc. 361.2379).

Irciformonin H (= rel-*(5*R*)-5-[(1*S*,4*E*,8*E*)-11-(Furan-3-yl)-1-hydroxy-6-methoxy-4,8-dimethylundeca-4,8-dien-1-yl]dihydro-5-methylfuran-2(3* H*)-one*; **4**): Colorless powder. $[\alpha]_D^{25} = +5.7$ ($c = 7.0$, CH_2Cl_2). IR (CH_2Cl_2): 3424 (OH), 1765 (lactone), 1076, 942. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 390 (M^+), 359 ($[M-MeO]^+$), 343 ($[M-MeO-OH]^+$), 241, 149, 135, 99 (100), 67. HR-ESI-MS: 413.2301 ($[M+Na]^+$, $C_{23}H_{34}NaO_5^+$; calc. 413.2304).

Irciformonin I (= rel-*(5*R*)-5-[(1*S*,5*E*,8*E*)-11-(Furan-3-yl)-1-hydroxy-4-methoxy-4,8-dimethylundeca-5,8-dien-1-yl]dihydro-5-methylfuran-2(3* H*)-one*; **5**): Colorless powder. $[\alpha]_D^{25} = +2.3$ ($c = 7.8$, CH_2Cl_2). IR (CH_2Cl_2): 3426 (OH), 1764 (lactone), 1072, 943. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 390 (M^+), 359 ($[M-MeO]^+$), 343 ($[M-MeO-OH]^+$), 233, 209, 203, 135, 99 (100), 67. HR-ESI-MS: 413.2301 ($[M+Na]^+$, $C_{23}H_{34}NaO_5^+$; calc. 413.2304).

Irciformonin J (= rel-*(5*R*)-5-[(1*S*,5*E*,8*E*)-11-(Furan-3-yl)-1,4-dihydroxy-4,8-dimethylundeca-5,8-dien-1-yl]dihydro-5-methylfuran-2(3* H*)-one*; **6**): Colorless powder. $[\alpha]_D^{25} = +2.2$ ($c = 0.9$, CH_2Cl_2). IR (CH_2Cl_2): 3422 (OH), 1760 (lactone), 1072, 942, 736. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 376 (M^+), 343 ($[M-2\ OH]^+$), 195, 135, 99 (100), 67. HR-ESI-MS: 399.2142 ($[M+Na]^+$, $C_{22}H_{32}NaO_5^+$; calc. 399.2147).

Irciformonin K (= rel-*(2*R*,2′*S*,5*S*)-5-[(1*E*,4*E*)-7-(2,5-Dihydro-2-oxofuran-3-yl)-4-methylhepta-1,4-dien-1-yl]hexahydro-2,5′-dimethyl[2,2′-bifuran]-5(2* H*)-one*; **7**): Colorless powder. $[\alpha]_D^{25} = +19.6$ ($c = 0.1$, CH_2Cl_2). UV (EtOH): 241 (3.20). IR (CH_2Cl_2): 1763 (lactone), 1075. ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*. EI-MS: 397 (M^+), 191, 183, 135, 99 (100), 67. HR-ESI-MS: 397.1991 ($[M+Na]^+$, $C_{22}H_{30}NaO_5^+$; calc. 397.1984).

Cell Culture and Viruses. Vero cells were cultured in minimal essential medium (MEM; *Gibco*, Grand Island, NY) supplemented with 10% fetal calf serum (FCS; *Hyclone*, Logan, UT), 100 U/ml of penicillin, and 100 μg/ml of streptomycin and incubated at 37° in a 5% CO_2 incubator. To prepare HSV-1 (KOS strain, VR-1493, ATCC) stocks, Vero cells were infected by HSV-1 at a multiplicity of infection of

3 plaque forming units (PFU)/cell and harvested at 24 h post-infection and centrifuged at 1500 *g* at 4° for 20 min. The supernatant was collected and stored at −70° for use.

Plaque Reduction Assay. The assay followed procedures described previously [19]. Vero cells ($3.5 \cdot 10^5$/dish) were incubated with 100 PFU of HSV-1, and various compounds (100 μM) or acyclovir (2.5 μM) were added to the cells. The viruses were absorbed for 1 h at 37°, and 1% methylcellulose was added to each well. After 5 d, the virus plaques formed in HeLa cells were counted by crystal-violet staining. The activities of various compounds and acyclovir for inhibition of plaque formation were calculated. Acyclovir was used as a positive control.

Lymphoproliferation Test. The lymphoproliferation test was modified from that previously described [20][21]. The density of PBMC was adjusted to $2 \cdot 10^6$ cells/ml before use. Cell suspension (100 μl) was applied into each well of a 96-well flat-bottomed plate (*Nunc 167008*, *Nunclon*; Roskilde, Denmark) with or without phytohemaglutinin (*Sigma*). Various compounds were added to the cells at 100 μM. The plates were incubated in a 5% CO_2 air humidified atmosphere at 37° for 3 d. Subsequently, tritiated thymidine (1 μCi/well, *NEN*) was added into each well. After 16 h incubation, the cells were harvested on glass-fiber filters by an automatic harvester (*Multimash 2000*; *Dynatech*, Billingshurst, U.K.). Radioactivity on the filters was measured by scintillation counting. Interleukin 2 (IL-2) and cyclosporine A were used as positive and negative standard compounds, resp.

REFERENCES

[1] J. W. Blunt, B. R. Copp, W. P. Hu, M. H. G. Munro, P. T. Northcote, M. R. Prinsep, *Nat. Prod. Rep.* **2007**, *24*, 31.
[2] R. W. Newbold, P. R. Jensen, W. Fenical, J. R. Pawlik, *Aquat. Microb. Ecol.* **1999**, *19*, 279.
[3] J. Pawlik, G. McFall, S. Zea, *J. Chem. Ecol.* **2002**, *28*, 1103.
[4] Y. Venkateswarlu, M. V. R. Reddy, M. R. Rao, *J. Nat. Prod.* **1996**, *59*, 876.
[5] G. W. Zhang, X. O. Ma, C. X. Zhang, J. Y. Su, W. C. Ye, X. Q Zhang, X. S. Yao, L. M. Zeng, *Helv. Chim. Acta.* **2005**, *88*, 885.
[6] K. Kondo, H. Shigemori, Y. Kikuchi, M. Ishibashi, T. Sasaki, J. Kobayashi, *J. Org. Chem.* **1992**, *57*, 2480.
[7] Y. Venkateswarlu, M. V. R. Reddy, *J. Nat. Prod.* **1994**, *57*, 1286.
[8] C. M. S. Mau, Y. Nakao, W. Y. Yoshida, P. J. Scheuer, *J. Org. Chem.* **1996**, *61*, 6302.
[9] R. J. Capone, J. K. MacLeod, *Aust. J. Chem.* **1987**, *40*, 1327.
[10] Y. Liu, S. Zhang, P. J. M. Abreu, *Nat. Prod. Rep.* **2006**, *23*, 630.
[11] K. A. El Sayed, A. M. S. Mayer, M. Kelly, M. T. Hamann, *J. Org. Chem.* **1999**, *64*, 9258.
[12] M. Tsoukatou, H. Siapi, C. Vagias, V. Roussis, *J. Nat. Prod.* **2003**, *66*, 444.
[13] K. K. H. Ang, M. J. Holmes, T. Higa, M. T. Hamann, U. A. K. Kara, *Antimicrob. Agents Chemother.* **2000**, *44*, 1645.
[14] S. Rifai, A. Fassouane, P. Pinho, A. Kijjoa, N. Nazareth, M. S. J. Nascimento, W. Herz, *Mar. Drugs* **2005**, *3*, 15.
[15] K. Choi, J. Hong, C. O. Lee, D. Kim, C. J. Sim, K. S. Im, J. H. Jung, *J. Nat. Prod.* **2004**, *67*, 1186.
[16] Y. C. Shen, K. L. Lo, Y. C. Lin, A. T. Khalil, Y. H. Kuo, P. S. Shih, *Tetrahedron Lett.* **2006**, *47*, 4007.
[17] Y. Liu, B. H. Bae, N. Alam, J. Hong, C. J. Sim, C.-O. Lee, K. S. Im, J. H. Jung, *J. Nat. Prod.* **2001**, *64*, 1301.
[18] Y. Liu, T. A. Mansoor, J. Hong, C.-O. Lee, C. J. Sim, K. S. Im, N. D. Kim, J. H. Jung, *J. Nat. Prod.* **2003**, *66*, 1451.
[19] Y. C. Kuo, L. C. Lin, W. J. Tsai, C. J. Chou, S. H. Kung, Y. H. Ho, *Antimicrob. Agents* **2002**, *46*, 2854.
[20] Y. C. Kuo, N. S. Yang, C. J. Chou, L. C. Lin, W. J. Tsai, *Mol. Pharmacol.* **2000**, *58*, 1057.
[21] Y. C. Kuo, C. K. Lu, L. W. Huang, Y. H. Kuo, C. Chang, F. L. Hsu, T. H. Lee, *Planta Med.* **2005**, *71*, 412.

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