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Novel linear C_{22}-sesterterpenoids from sponge *Ircinia formosana*

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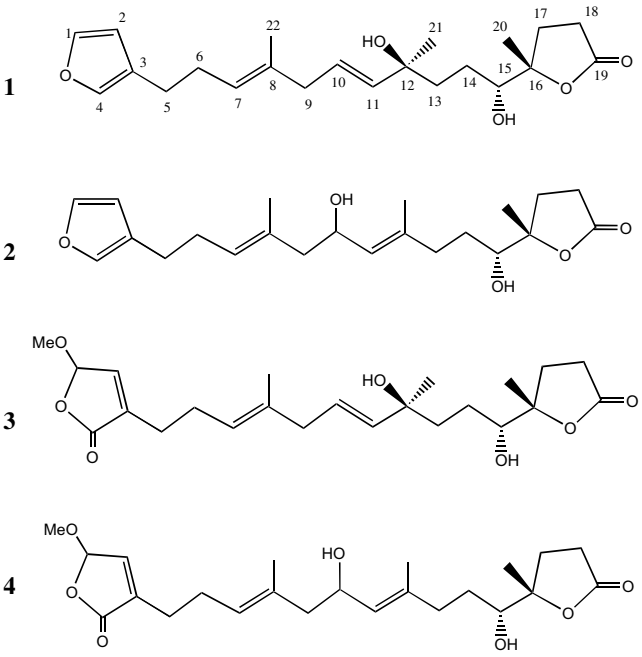
Abstract—Four unprecedented C_{22}-sesterterpenes, irciformonins A–D (**1**–**4**), have been isolated from the marine sponge *Ircinia formosana*, collected off the coast of eastern Taiwan. The structures of the isolated metabolites were established on the basis of extensive spectral analysis, primarily 1- and 2D-NMR. Compounds **3** and **4** exhibited significant cytotoxicity against human colon (WiDr) tumor cells.

Sponges have provided the largest number of marine natural products, many of which have interesting biomedical potential.[1] Furanosesterterpenes were frequently isolated from marine sponges of genera *Ircinia, Spongia, Spongionella, Cacospongia, Dysidea, Sarcotragus, Amphimedon*, and *Hippospongia* and have chemotaxonomic importance.[2–6] Some furanosesterterpenes were reported to exhibit antibacterial, antiviral, cytotoxic, antispasmodic, and anti-inflammatory activities.[7,8] Chemical investigation of *Ircinia formosana*[9] afforded four novel linear C_{22}-sesterterpenes, designated irciformonins A–D (**1**–**4**). Two of the isolated metabolites **1** and **2** have diene linear structures with a furan ring at one end and a five-membered lactone ring at the other, while **3** and **4** are devoid of furan rings, having a lactone at either side of the linear skeleton.

The sponge material (175 g) was exhaustively extracted with acetone, and the resulting residue (2 g) was partitioned between EtOAc–H_2O (1:1) to afford an EtOAc extract (1.8 g). The latter was chromatographed on a silica gel column using a gradient of *n*-hexane/EtOAc to give 16 fractions (F1–F16). Fractions F3, F9, F10, and F16 were separately fractionated on Sephadex LH-20 using CH_2Cl_2/MeOH, followed by purification by NP-HPLC using *n*-hexane/$CHCl_3$/MeOH (17:17:1) as a solvent to yield **1** (21 mg), **4** (18 mg), **3** (7 mg), and **2** (12 mg), respectively.



Irciformonin A (**1**), $[\alpha]_D^{25}$ +1 (acetone), isolated as a yellowish white powder, had the composition of $C_{22}H_{32}O_5$ as deduced from HREIMS (*m*/*z* 399.2149 $[M+Na]^+$).[10] The IR spectrum revealed hydroxyl (3443 cm^{-1}), lactone ring (1767 cm^{-1}) and double bond (1638, 941 cm^{-1}) functionalities. The ^1H NMR spectrum (Table 1) revealed three furan singlets (δ_H 7.34, 7.20, 6.28), a trans-disubstituted olefin (δ_H 5.54 and 5.44, each d,

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Table 1. ^{1}H NMR data (500 MHz, $CDCl_3$)[a] of **1**–**4**[b]

	1	2	3	4
1	7.34 s	7.35 s	5.75 d (1.0)	5.73 s
2	6.28 s	6.28 s	6.77 d (1.0)	6.80 s
4	7.20 s	7.22 s		
5	2.45 m	2.47 t	2.35 m	2.36 m
6	2.26 m	1.30 m	2.28 m	2.32 m
7	5.17 t (7.0)	5.27 t (6.5)	5.10 t (7.0)	5.21 br d (7.5), 2H
9	2.62 d (6.0)	2.13 d (6.5)	2.65 d (6.5)	2.16 d (8.1)
10	5.54 d (15.5)	4.42 q (6.9)	5.52 m	4.47 m
11	5.44 d (15.5)	5.19 d (7.8)	5.45 d (15.5)	5.21 br d (7.5)
13	1.93 m 1.72 m	2.28 m 1.45 m	1.92 m 1.70 m	2.28 m 1.40 m
14	1.94 m 1.73 m	2.27 m 2.18 m	1.95 m 1.72 m	2.30 m 2.16 m
15	4.00 m	3.64 dd (8.7, 0.9)	4.00 t (7.0)	3.60 dd (8.7, 0.9)
17	2.65 m 2.58 m	2.64 m 1.79 m	2.61 m 2.57 m	2.63 m 1.81 m
18	2.30 m 1.89 m	2.35 m 1.57 m	2.30 m 1.88 m	2.37 m 1.60 m
20	1.36 s	1.33 s	1.36 s	1.34 s
21	1.30 s	1.62 s	1.30 s	1.63 s
22	1.55 s	1.67 s	1.65 s	1.65 s
–OMe			3.55 s	3.55 s

[a] Chemical shifts are in ppm and coupling constants in Hz are in parentheses.
[b] Assignments made using COSY and HMQC.

$J = 15.5$ Hz), a trisubstituted olefin (δ_H 5.17, t, $J = 7.0$ Hz), an oxymethine (δ_H 4.00, m), and three methyls (δ_H 1.55, 1.36, 1.30). The ^{13}C NMR spectrum (Table 2) displayed signals for monosubstituted furan (δ_C 142.5, 138.8, 124.8, 111.1), three olefinic CH (δ_C 124.6, 125.9, 136.3), an oxymethine (δ_C 82.6, C-15), two oxy-quaternary carbons (δ_C 83.3, 87.5, C-12, C-16), a carbonyl (δ_C 177.1, C-19), and six aliphatic methylenes. The EIMS confirmed the presence of a furan ring [m/z 67 (C_4H_3O) and m/z 81 (C_5H_5O)] and a methylated five-membered lactone ring (m/z 99, $C_5H_7O_2$, base peak). The furan carbon signal at δ_C 138.8 (C-4) showed HMBC correlations with furan proton at δ_H 6.28 (H-2) and with the methylene signal at δ_H 2.45 (H-5) (Fig. 1). The methylene (H-5) had COSY correlation with another methylene at δ_H 2.26 (H-6), which in turn showed COSY correlation to an olefinic triplet at δ_H 5.17 (H-7) as well as HMBC correlation with the quaternary olefinic at δ_C 134.5 (C-8). The HMBC correlations between the methyl singlet H-22/C-8 and C-7 together with the fragment ion at m/z 135 confirmed the presence of the partial structure **A**. The methyl signal at δ_H 1.36 (H-20) had ^2J-correlation to the quaternary oxygenated carbon at δ_C 87.5 (C-16) and ^3J-correlation to the oxymethine at δ_C 82.6 (C-15). The oxymethine at δ_H 4.00 showed ^2J-correlation with C-16 and ^3J-correlation with C-17. The COSY correlations between H-15/H-14/H-13 and HMBC correlations between H-13/C-15; H-14/C-12; H-21/C-12, C-13, together with the mass fragment ion at m/z 201, suggested the occurrence of the partial structure **B** or **C**. However, the IR adsorption band at 1767 cm^{-1} excluded the structure C from consideration. It was assumed that the two partial structures **A** and **B** were connected through an *E*-allylic group (C-9 to C-11). This was proved by HMBC correlations between

Table 2. ^{13}C NMR data (125 MHz, $CDCl_3$)[a] of **1**–**4**[b]

C atom	1	2	3	4
1	142.5 d	142.9 d	102.4 d	102.8 d
2	111.1 d	111.1 d	142.2 d	142.8 d
3	124.8 s	124.7 s	138.0 s	138.2 s
4	138.8 d	139.0 d	171.4 s	171.6 s
5	24.9 t	24.9 t	25.3 t	25.2 t
6	28.4 t	29.4 t	27.0 t	26.0 t
7	124.6 d	128.2 d	123.2 d	128.0 d
8	134.5 s	132.3 s	135.7 s	133.7 s
9	42.3 t	48.2 t	42.2 t	48.2 t
10	125.9 d	65.6 d	125.6 d	66.1 d
11	136.3 d	128.0 d	136.6 d	126.5 d
12	83.3 s	137.7 s	83.4 s	138.0 s
13	37.3 t	28.5 t	37.3 t	28.0 t
14	27.0 t	36.1 t	27.0 t	36.3 t
15	82.6 d	75.5 d	82.7 d	75.5 d
16	87.5 s	88.7 s	87.5 s	89.0 s
17	29.5 t	27.8 t	29.3 t	29.0 t
18	29.6 t	28.8 t	29.4 t	29.5 t
19	177.1 s	177.4 s	177.0 s	177.2 s
20	22.8 q	22.9 q	22.9 q	22.9 q
21	27.2 q	16.2 q	27.2 q	16.5 q
22	16.1 q	16.7 q	16.5 q	16.8 q
OMe			57.0 q	57.2 q

[a] Multiplicity determined by DEPT.
[b] Assignments made using HMQC and HMBC.

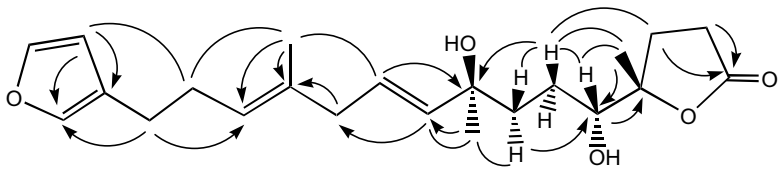
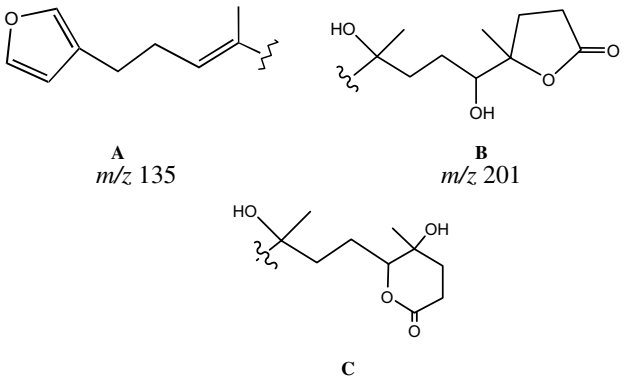


Figure 1. Selected HMBC (arrows) and NOESY (curved lines) correlations of **1**.

C-9/H-22, H-11; C-11/H-21; C-12/H-10, as well as a NOESY correlation between H-10/H-22 and H-11/21. Compound **1** could not afford its acetate upon acetylation, nor did benzoylation succeed because of a bulky tertiary group near the secondary hydroxyl at C-15. Likewise, that compound **1** did not undergo mosher reaction hampered the determination of the chirality of C-15. The *E*-geometry of the trisubstituted double bond C-7/C-8 was assigned on the basis of the upfield resonance of the vinylic methyl carbon (δ_C 16.1, C-22).[11] The relative configuration at C-12, C-15, and C-16 was tentatively determined through a molecular model as well as the NOESY correlations between H_β-14/H-15, H-20; H-15/H-20; H_α-13/H-21, favoring the β-orientation of the OH at C-12, H-15, and H-20, as well as the α-orientation of H-21 and the OH at C-15. A computer-modeled structure of **1**, generated by CS Chem 3D version 9.0 using MM2 force field calculation for energy minimization, is shown in Figure 2.



A *m/z* 135 **B** *m/z* 201 **C**

Irciformonin B (**2**), $[\alpha]_D^{25}$ +3.1 (acetone), possessed a molecular formula $C_{22}H_{32}O_5$ (HREIMS), the same as that of **1**.[12] Detailed inspection of the NMR spectral data and the EIMS fragmentation pattern revealed the same C_{22}-sesterterpene skeleton with the partial structure **A** as well as the five-membered lactone ring. The ^{1}H NMR spectrum revealed only two distant olefinic protons [δ_H 5.27 (t, $J = 6.5$ Hz, H-7); δ_H 5.19 (d, $J = 7.8$ Hz, H-11)] belonging to two trisubstituted double bonds, besides three methyl singlets (δ_H 1.33, 1.62, 1.67) and two oxymethines [δ_H 4.42 (q, $J = 6.9$ Hz, H-10); δ_H 3.64 (dd, $J = 8.7$, 0.9 Hz, H-15)]. The HMBC correlations of H-10 with two quaternary olefinic carbons at δ_C 132.3 (C-8) and δ_C 137.7 (C-12) along with COSY correlations of H-10/H-9 and H-10/H-11 located a hydroxyl group between the two double bonds. This was confirmed through HMBC correlations between the methyl at δ_H 1.67 (H-22) and C-7, C-9 and between a methyl at δ_H 1.62 (H-21) and C-11, C-13. Thus the two double bonds were positioned at C-7/C-8 and C-11/C-12 and the two hydroxyls at C-10 and C-15. The NOESY correlations between H-15/H-20, H_β-14 were in agreement with the β-orientation of H-15 and H-20. A computer generated 3D chemical model for **2** is shown in Figure 2 by using MM2 force field calculation in agreement with the β-hydroxyl at C-10.

The molecular formula of irciformonin C (**3**) was deduced as $C_{23}H_{34}O_7$ (HREIMS, *m/z* 445.2200).[13] Comparison of the spectral data of **3** with those of **1** suggested that it had the partial structure **B** present in **1**. Moreover, the 1- and 2-D NMR data proved that the composition of the straight chain part is similar in both compounds despite the absence of the furan ring in **3**. In addition to three olefinic signals (H-7, H-10, H-11) of the straight chain, the ^{1}H NMR spectrum revealed an olefinic signal at δ_H 6.77 (d, $J = 1.0$ Hz, H-2) that was spin-coupled with an oxymethine at δ_H 5.75 (d, $J = 1.0$ Hz, H-1). The latter oxymethine proton was ^3J-correlated with a methoxyl carbon at δ_C 57.0 (δ_H 3.55), a carbonyl at δ_C 171.4 (C-4) and with a quaternary olefinic carbon at δ_C 138.0 (C-3). In the HMQC spectrum, the proton at δ_H 5.57 was directly attached to a downfield-shifted carbon at δ_C 102.4 (C-1) indicating a hemiacetal moiety. The ^2J-correlation δ_H 6.77 (H-2) with the signal at δ_C 102.4 (C-1) and δ_C 138.0 (C-3) suggested the presence of a methoxylated five-membered unsaturated lactone ring (furanone ring). This was confirmed by EIMS fragment at *m/z* 113 ($C_5H_5O_3$) and in accordance with the presence of seven degree of unsaturation. The furanone ring was attached to the straight carbon chain through C-3, as verified by ^3J-correlations between H-5 (δ_H 2.35) with each of C-2 and C-4. The NOESY spectrum exhibited correlations between OCH_3/H-2; H-6/H-2, H-22; H-7/H-5, H-9; H-15/H_β-14, H-20; H_α-13/H-21 and H-14/H-17. A molecular model

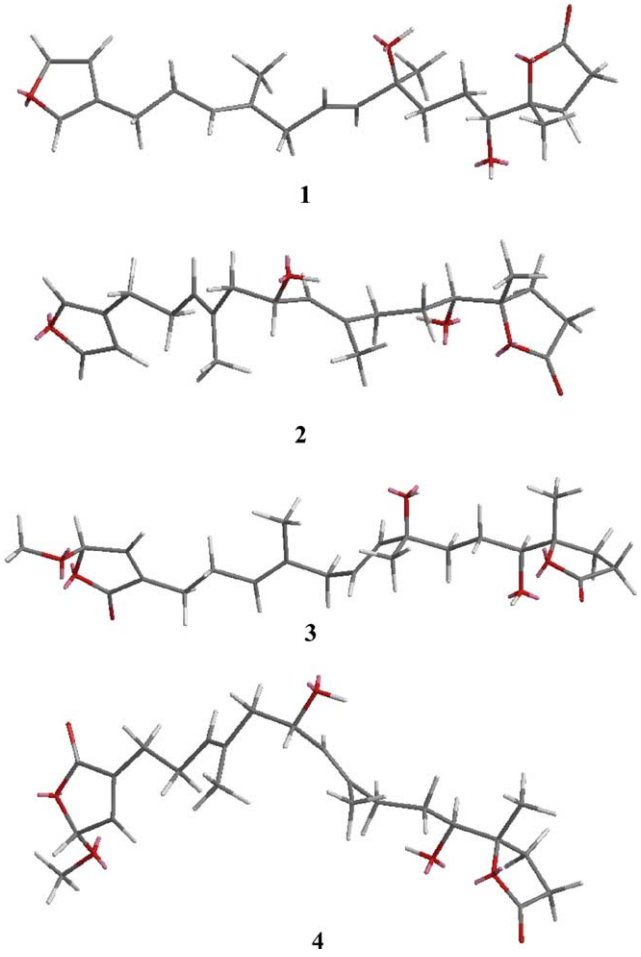


Figure 2. Computer-generated perspective models for **1**–**4** using MM2 force field calculation.

Table 3. Cytotoxicity of compounds **1**–**4** against human tumor cells (ED_{50}, μg/mL)[a]

Compound	hepa59T/VGH[b]	WiDr[c]	A-549[d]	MCF-7[e]
1	(—)[f]	9.46	9.58	(—)
2	(—)	(—)	(—)	(—)
3	18.1	6.7	11.8	(—)
4	(—)	4.9	13.3	(—)
Doxorubicin	0.11	0.12	0.21	0.12

[a] The concentration that inhibits 50% of the growth of human tumor cell lines after 72 h exposure according to the mentioned reference.
[b] Human liver carcinoma.
[c] Human colon adenocarcinoma.
[d] Human lung carcinoma.
[e] Human breast adenocarcinoma.
[f] ED_{50} >20 μg/mL.

of **3** indicates its lowest energy after MM2 force field calculation, as shown in Figure 2. A final proof of the structure was obtained by the EIMS fragment ion at *m*/*z* 181 ($C_{10}H_{13}O_3$) containing the furanone ring, which was produced by fission between C-8/C-9. It is worthy to note that some sesterterpenes with a similar hydroxy furanone ring have been isolated from sponges of the genus *Sarcotragus*, but with C-2 attachment to the linear chain.[8]

The elemental composition of irciformonin D (**4**),[14] $C_{23}H_{34}O_7$, was established by HREIMS. Detailed study of the NMR data (Tables 1 and 2) as well as 2D-NMR spectra (HMQC, HMBC, COSY, and NOESY) suggested the presence of two lactone rings identical to those in **3**. However, the straight chain carbon skeleton was proved to be identical to that of **2**. The proposed structure was confirmed by the EIMS fragment ions at *m*/*z* 113 (unsaturated lactone ring), *m*/*z* 99 (lactone ring, base peak), in addition to two fragment ions at *m*/*z* 181 and 241 resulting from fission between C-8/C-9. Molecular modeling by MM2 force field calculation also agreed with the structure of **4** (Fig. 2).

The cytotoxic activity of the isolated sesterterpenes were tested in vitro against human liver carcinoma (hepa59T/VGH), human colon adenocarcinoma (WiDr), human lung carcinoma (A-549), and human breast adenocarcinoma (MCF-7). Table 3 revealed that compounds **3** and **4** had a mild activity against human colon adenocarcinoma (WiDr) tumor cells. None of the compounds showed growth inhibition toward other cell lines.

Cytotoxicity assay: The bioassay used against all tumor cell lines was based on the MTT assay method.[15] Doxorubicin was used as a standard compound.

Acknowledgments

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References and notes

1. Faulkner, D. J. *Nat. Prod. Rep.* **2001**, *18*, 1–49.
2. Höller, U.; König, G. M.; Wright, A. D. *J. Nat. Prod.* **1997**, *60*, 832–835.
3. Carotenuto, A.; Conte, M. R.; Fattorusso, E.; Lanzotti, V.; Magno, S. *Tetrahedron* **1995**, *51*, 10751–10758.
4. Carotenuto, A.; Fattorusso, E.; Lanzotti, V.; Magno, S.; Carnuccio, R.; D'Acquisto, F. *Tetrahedron* **1997**, *53*, 7305–7310.
5. El Sayed, K. A.; Mayer, A. M. S.; Kelly, M.; Hamann, M. T. *J. Org. Chem.* **1999**, *64*, 9258–9260.
6. Tsoukatou, M.; Siapi, H.; Vagias, C.; Roussis, V. *J. Nat. Prod.* **2003**, *66*, 444–446.
7. Issa, H. H.; Tanaka, J.; Higa, T. *J. Nat. Prod.* **2003**, *66*, 251–254.
8. Liu, Y.; Mansoor, T. A.; Hong, J.; Lee, C.-O.; Sim, C. J.; Im, K. S.; Kim, N. D.; Jung, J. H. *J. Nat. Prod.* **2003**, *66*, 1451–1456.
9. The sponge *Ircinia formosana* was collected by Scuba diving off the coast of eastern Taiwan, at a depth of 20 m, in June 2003 and frozen shortly after collection. A reference sample and a photograph are deposited at the Institute of Marine Resources, National Sun Yat-sen University (GSPN-11).
10. Amorphous powder, $[\alpha]_D^{25}$ +1 (*c* 1.5, acetone); IR (neat) ν_{max} 3443, 1767, 1638, 1372, 1208, 1084, 1026, 941, 662 cm^{-1}; ESIMS *m*/*z* 399 $[M+Na]^+$; HRESIMS *m*/*z* 399.2149 ($[M+Na]^+$, calcd for $C_{22}H_{32}O_5Na$, 399.2147).
11. Liu, Y.; Bae, B. H.; Alam, N.; Hong, J.; Sim, C. J.; Lee, C.-O.; Im, K. S.; Jung, J. H. *J. Nat. Prod.* **2001**, *64*, 1301–1304.
12. Amorphous powder, $[\alpha]_D^{25}$ +3.1 (*c* 3, acetone); IR (neat) ν_{max} 3422, 2928, 1761, 1651, 1076, 1026, 943, 774 cm^{-1}; ESIMS *m*/*z* 399 $[M+Na]^+$; HRESIMS *m*/*z* 399.2150 ($[M+Na]^+$, calcd for $C_{22}H_{32}O_5Na$, 399.2147).
13. Amorphous powder, $[\alpha]_D^{25}$ +0.5 (*c* 2.67, acetone); ESIMS *m*/*z* 445 $[M+Na]^+$; HRESIMS *m*/*z* 445.2200 ($[M+Na]^+$, calcd for $C_{23}H_{34}O_7Na$, 445.2202).
14. Amorphous powder, $[\alpha]_D^{25}$ +2.3 (*c* 1.67, acetone); ESIMS *m*/*z* 445 $[M+Na]^+$; HRESIMS *m*/*z* 445.2201 ($[M+Na]^+$, calcd for $C_{23}H_{34}O_7Na$, 445.2202).
15. Shen, Y. C.; Li-Tung Wang, L. T.; Khalil, A. T.; Kuo, Y. H. *Chem. Pharm. Bull.* **2004**, *52*, 402–405.