Cytotoxic Sesquiterpene Lactones from *Pseudoelephantopus spicatus*

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Five new sesquiterpene lactones, spicatolides D−H (**1**−**5**), along with four known compounds, pitocarphin D (**6**), 8α-acetoxy-10α-hydroxy-13-*O*-methylhirsutinolide (**7**), spicatolide A (**8**), and 13-*O*-methylvernojalcanolide 8-*O*-acetate (**9**), were isolated from an ethyl acetate extract of the aerial parts of *Pseudoelephantopus spicatus*. The structures of the new compounds were elucidated on the basis of spectroscopic data interpretation. All of the compounds isolated were evaluated for their cytotoxic effects against five human cancer cell lines. Compounds **1**, **3**, and **4** showed cytotoxicity (IC_{50} < 5 μ g/mL) against the Hep3B and MCF-7 cancer cell lines.

In the course of our search for anticancer candidate compounds from Taiwanese folk medicines, a crude extract of *Pseudoelephantopus spicatus* (Juss.) C. F. Baker (Asteraceae) was found to show cytotoxicity against the MCF-7 human breast cancer cell line. In Taiwan, *P. spicatus* is used for the treatment of nephritis, edema, dampness, chest pain, fever, and coughing due to pneumonia.[1] A crude extract of *P. spicatus* was reported to exhibit significant hepatoprotective effects.[1,2] In a previous study, fractionation of this plant has resulted in the isolation of several sesquiterpene lactones with weak cytotoxicity.[3]

In order to elucidate the cytotoxic components of *P. spicatus* in more detail, two germacranolide-type sesquiterpenes with a C-1/C-4 hemiketal linkage, spicatolides D and E (**1**, **2**), a germacranolide with a C-6/C-10 ketal linkage, spicatolide F (**3**), a germacranolide with C-1/C-4 hemiketal and C-6/C-10 ketal linkages, spicatolide G (**4**), and a sesquiterpene with two lactone rings, spicatolide H (**5**), along with four known compounds, piptocarphin D (**6**),[4] 8α-acetoxy-10α-hydroxy-13-*O*-methylhirsutinolide (**7**),[5] spicatolide A (**8**),[6] and 13-*O*-methylvernojalcanolide 8-*O*-acetate (**9**),[5] were isolated from *P. spicatus*. The isolation, structure elucidation, and cytotoxicity evaluation of these isolated compounds are described herein.

Results and Discussion

Spicatolide D (**1**) was obtained as a colorless oil. The HRESIMS indicated the molecular formula to be $C_{16}H_{22}O_7$, with eight degrees of unsaturation. The IR spectrum suggested the presence of a hydroxyl group (3445 cm^{-1}) and an α,β-unsaturated γ-lactone ring (1740 cm^{-1}). In the ^{13}C NMR and DEPT spectra (Table 1), resonances for three methyls (including one methoxyl carbon), four methylenes (one of which was oxygenated), and two methines (one oxygenated sp^3 carbon and one olefinic carbon), together with signals for seven quaternary carbons (one hemiketal carbon, one carbonyl carbon, two oxygenated sp^3 carbons, and three olefinic carbons), were observed. These data suggested that compound **1** contains three rings, two sets of double bonds, and one carbonyl group. The carbon skeleton of **1** was suggested by several diagnostic HMBC correlations (H-2/C-1, C-3; H-3/C-4; H-15/C-3, C-4, C-5; H-5/C-6, C-7, C-15; H-13/C-7, C-11, C-12, methoxyl carbon; H-8/C-7; H-14/C-1, C-9, C-10) and $^1H-^1H$ COSY (H-2/H-3; H-8/H-9) (Figure 1). The HMBC correlations suggested that an α,β-unsaturated γ-lactone ring (δ_C 144.1, 154.6, 129.1, 167.6) may be placed at C-6, C-7, C-11, and C-12, with a methoxyl group (δ_H 3.37, δ_C 58.8) attached at C-13, adjacent to the α,β-unsaturated γ-lactone ring. The gross structure of **1** was revealed as a germacranolide-type sesquiterpene, on the basis of the above analysis.[6,7] In the ^1H NMR spectrum (Table 2), H-8 exhibited a strong coupling with OH-8 (J = 12.0 Hz) and H-2 showed long-range coupling with OH-1, which suggested C-8 and C-1 (δ_C 110.1, a hemiketal carbon) to both be bonded to a hydroxyl group. In considering the degrees of unsaturation and molecular formula, an ether linkage was placed between the hemiketal carbon C-1 and C-4, and C-10 (δ_C 77.7) was assigned as a hydroxylated quaternary carbon.

The relative configuration of **1** was established from the NOESY spectrum (Figure 2). The *E* form of $\Delta_{5,6}$ could be inferred by NOE cross-peaks between H-5/H-3 and H-5/H-15. The correlations of H-14/H-2 and H-14/H-9b suggested that the methyl group (H-14) is in the opposite orientation relative to the C-1/C-4 ether linkage. The small coupling (J = 2.0 Hz) of H-8/H-9a indicated the dihedral

Table 1. ^{13}C NMR Spectroscopic Data (100 MHz) of **1**–**4** in $CDCl_3$

carbon	**1**	**2**	**3**	**4**
1	110.1, C	108.6, C	213.5, C	108.6, C
2	32.3, CH_2	31.7, CH_2	40.7, CH_2	32.1, CH_2
3	37.5, CH_2	37.7, CH_2	35.2, CH_2	34.6, CH_2
4	83.1, C	81.9, C	72.0, C	78.0, C
5	123.6, CH	125.6, CH	43.7, CH_2	69.8, CH
6	144.1, C	146.8, C	105.7, C	90.2, C
7	154.6, C	154.2, C	165.7, C	157.5, C
8	64.0, CH	65.8, CH	64.4, CH	69.1, CH
9	38.2, CH_2	38.0, CH_2	42.5, CH_2	40.9, CH_2
10	77.7, C	78.0, C	82.8, C	84.2, C
11	129.1, C	128.4, C	121.9, C	134.2, C
12	167.6, C	169.2, C	168.0, C	166.7, C
13	63.1, CH_2	63.5, CH_2	64.6, CH_2	64.2, CH_2
14	26.1, CH_3	25.6, CH_3	25.7, CH_3	25.6, CH_3
15	29.3, CH_3	28.9, CH_3	31.1, CH_3	26.7, CH_3
OCH_3	58.8, CH_3	58.9, CH_3	59.1, CH_3	59.0, CH_3
$OCOCH_3$				169.7, C
$OCOCH_3$				21.6, CH_3
1′		166.4, C		
2′		139.2, C		
3′		139.4, CH		
4′		11.9, CH_3		
5′		14.6, CH_3		

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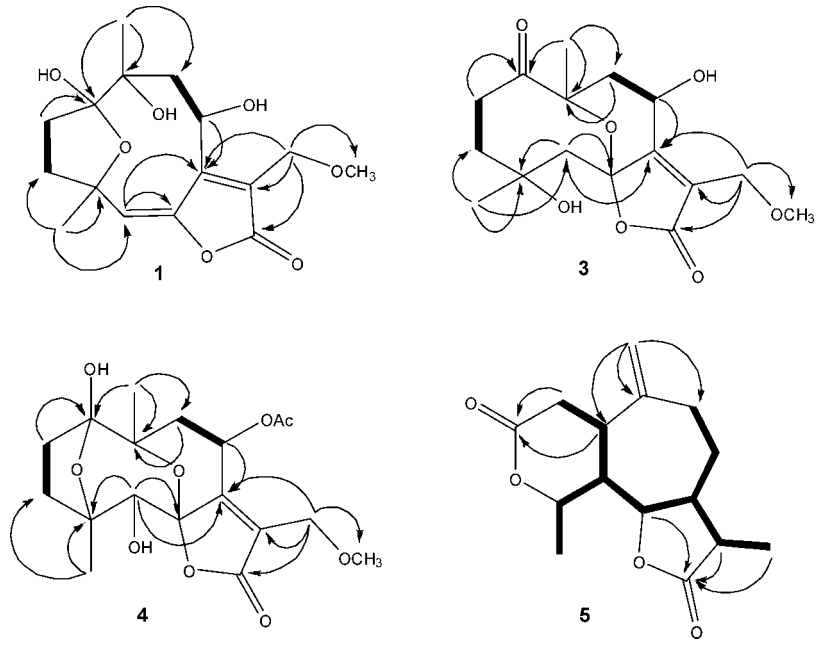


Figure 1. HMBC (arrow) and $^1H-^1H$ COSY (—) correlations of **1**, **3**, **4**, and **5**.

Table 2. ^1H NMR Spectroscopic Data (400 MHz, *J* in Hz) of **1**−**4** in $CDCl_3$

position	1	2	3	4
2	1.89 (1H, dddd, *J* = 12.8, 12.0, 6.8, 2.4)	1.90 (1H, m)	1.84 (1H, m)	1.50 (1H, dd, *J* = 12.4, 4.4)
	1.98 (1H, dd, *J* = 12.8, 6.8)	1.97 (1H, m)	2.59 (1H, m)	1.95 (1H, dd, *J* = 12.4, 7.6)
3a	2.15 (1H, dd, *J* = 12.8, 6.8)	2.15 (1H, m)	2.33 (1H, ddd, *J* = 14.0, 10.4, 4.0)	2.15 (1H, dd, *J* = 13.0, 4.4)
3b	2.42 (1H, ddd, *J* = 12.8, 12.0, 6.8)	2.42 (1H, ddd, *J* = 14.0, 12.4, 7.2)	3.26 (1H, dd, *J* = 10.4, 7.6)	2.35 (1H, dd, *J* = 13.0, 7.6)
5	5.82 (1H, s)	5.83 (1H, s)	1.81 (2H, dd, *J* = 16.4)	3.53 (1H, s)
8	5.45 (1H, ddd, *J* = 12.0, 11.0, 2.0)	6.57 (1H, d, *J* = 8.8)	4.66 (1H, ddd, *J* = 12.0, 4.4, 2.0)	6.12 (1H, d, *J* = 10.0)
9a	1.94 (1H, dd, *J* = 16.0, 2.0)	1.93 (2H, m)	1.38 (1H, dd, *J* = 14.0, 4.4)	2.22 (1H, d, *J* = 16.6)
9b	2.52 (1H, dd, *J* = 16.0,11.0)		3.06 (1H, dd, *J* = 14.0, 12.0)	2.55 (1H, dd, *J* = 16.6, 10.0)
13a	4.21 (1H, d, *J* = 12.4)	4.25 (1H, d, *J* = 10.8)	4.31 (1H, d, *J* = 12.8)	4.27 (1H, d, *J* = 13.2)
13b	4.25 (1H, d, *J* = 12.4)	4.28 (1H, d, *J* = 10.8)	4.32 (1H, d, *J* = 12.8)	4.34 (1H, d, *J* = 13.2)
14	1.21 (3H, s)	1.22 (3H, s)	1.48 (3H, s)	1.19 (3H, s)
15	1.64 (3H, s)	1.56 (3H, s)	1.25 (3H, s)	1.57 (3H, s)
OCH_3	3.37 (3H, s)	3.41 (3H, s)	3.47 (3H, s)	3.40 (3H, s)
$OCOCH_3$				2.15 (3H, s)
3′		7.03 (1H, q, *J* = 7.2)		
4′		1.82 (3H, d, *J* = 7.2)		
5′		1.83 (3H, s)		
OH/C-1	4.51 (1H, d, *J* = 2.4)			
OH/C-8	6.09 (1H, d, *J* = 12.0)			
OH/C-10	4.81 (1H, s)			

angle to be close to 90°. The large coupling ($J_{H\text{-}8/H\text{-}9b}$ = 11.0 Hz) and NOE between H-8/H-9b and H-8/H-13 were used to deduce the β-equatorial orientation of H-8. The formation of a hydrogen bond between the oxygen of the C-1/C-4 ether and OH-8 (ca. 1.6 Å) could be supported by a strong *anti*-vicinal coupling ($J_{H\text{-}8/OH\text{-}8}$ = 12.0 Hz), which also confirmed the β-equatorial orientation of H-8. Thus, the structure of **1** was determined as shown, and this compound has been named spicatolide D.

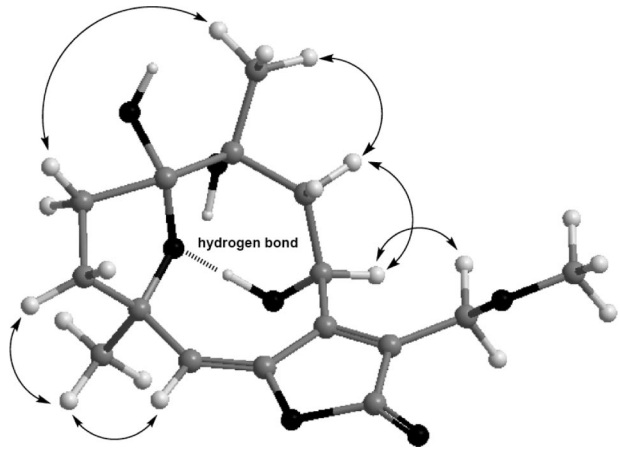
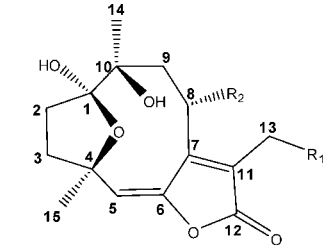
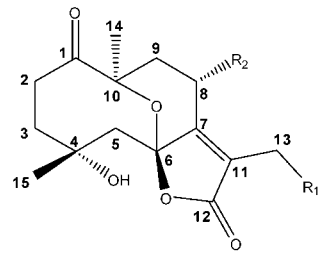


Figure 2. Key NOE correlations of **1**.

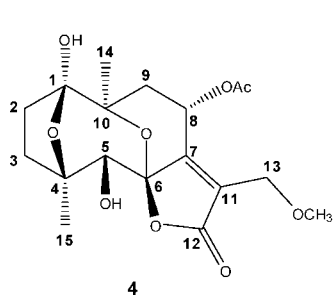
Compound **2** was obtained as a colorless oil. The HRESIMS exhibited a $[M + Na]^+$ peak at *m/z* 431.1675, consistent with the molecular formula, $C_{21}H_{28}O_8$. The IR spectrum showed absorptions at 3540, 1770, 1750, 1724, and 1679 cm^{-1}, and the UV spectrum revealed an absorption maximum at 280 nm. The NMR data of **2** (Tables 1 and 2), which were similar to those of **1**, were again consistent with a germacranolide-type sesquiterpene.[6,7] The ^1H NMR spectrum of **2** (Table 2) revealed a relatively low-field methine signal at δ 6.57 (1H, d, *J* = 8.8 Hz, H-8), in comparison with that of **1**. From an analysis of the ^1H NMR, ^{13}C NMR, and HMBC spectra (Tables 1 and 2, Figure 1), a tiglate ester group (δ_H 1.82, 1.83, 7.03; δ_C 11.9, 14.6, 139.4, 139.2, 166.4)[5] was assigned as being linked to C-8. The relative configuration of **2** was proposed as the same as **1** on the basis of NOESY correlations.





1 $R_1 = OCH_3$ $R_2 = OH$
2 $R_1 = OCH_3$ $R_2 = OCOC(CH_3)CHCH_3$ (*E* form)
6 $R_1 = OAc$ $R_2 = OH$
7 $R_1 = OCH_3$ $R_2 = OAc$

3 $R_1 = OCH_3$ $R_2 = OH$
8 $R_1 = OAc$ $R_2 = OAc$



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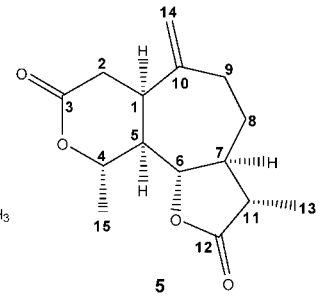

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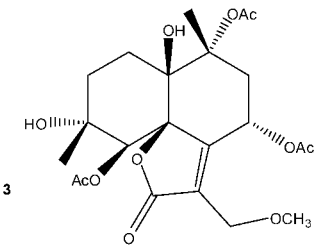

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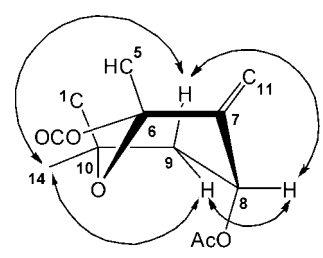
Thus, the structure of **2** was established as shown and has been named spicatolide E.

Compound **3** was formulated as $C_{16}H_{22}O_7$ with six degrees of unsaturation by HRESIMS. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) indicated resonances for a methoxy group (δ_H 3.47, δ_C 59.1), a ketone carbonyl (δ_C 213.5), an oxygenated methine carbon (δ_C 64.4), five methylene carbons (δ_C 35.2, 40.7, 42.5, 43.7, 64.6), two quaternary olefinic carbons (δ_C 121.9, 165.7), three quaternary oxygenated carbons (δ_C 72.0, 82.8, 105.7), an ester carbonyl carbon (δ_C 168.0), and two methyl carbons (δ_C 25.7, 31.1). These observations, along with the UV absorbance (218 nm) and IR spectroscopic (3474, 1780, 1748, 1724, 1679 cm^{-1}) parameters, revealed that **3** is similar in structure to spicatolide A (**8**), a germacranolide-type sesquiterpene with an α,β-unsaturated γ-lactone ring.[6] The relatively high-field methine signal at δ 4.66 (ddd, J = 12.0, 4.4, 2.0 Hz, H-8) was consistent with a hydroxy group at C-8 in **3**. The HMBC correlations (Figure 1) between the methoxy group and the C-13 oxygenated methylene indicated C-13 to be substituted by a methoxy group, compared with the *O*-acetyl group in **8**. The relative configuration in **3** was determined by NOE correlations and $^1H-^1H$ coupling constants. The values of $J_{H\text{-}8/H\text{-}9}$ (12.0, 4.4 Hz) and the NOE correlation of H-8/H-5 supported a β-axial configuration of H-8. The orientation of the methyl group (H-15) could be assigned as β due to the NOE interaction between H-15/H-2. By comparing the NMR data of **3** with those of **8**, the molecular structure of **3** was regarded as being consistent with that of **8**, which was deduced by X-ray crystallography.[6] The absolute configuration in **3** was revealed by circular dichroism. The CD curve of **3** (MeOH, c 3.0 × 10^{-3}) showed an n → π* Cotton effect at Δ_{245} +1.5 and a π → π* Cotton effect at Δ_{215} –3.4. According to an empirical observation for the absolute configuration of α,β-unsaturated γ-lactones,[8] the negative π → π* Cotton effect was consistent with the absolute configuration of C-6 as *S*. This result was in agreement with experimental and literature CD data of compound **8** (MeOH, c 2.5 × 10^{-3}, Δ_{250} +7.6, Δ_{212} –9.1, experimental data; MeOH, c 2.1 × 10^{-3}, Δ_{247} +3.7, Δ_{212} –9.8, literature data).[6] Accordingly, the structure of **3** was elucidated as shown, and this compound has been named spicatolide F.

Spicatolide G (**4**) was assigned the molecular formula $C_{18}H_{24}O_9$ on the basis of HRESIMS. The IR spectrum displayed absorption bands attributable to hydroxyl group (3430 cm^{-1}), γ-lactone (1748 cm^{-1}), and ester (1724 cm^{-1}) functionalities. The UV spectrum showed a maximum at 235 nm, consistent with the presence of an α,β-unsaturated γ-lactone moiety. The ^{13}C NMR and DEPT spectra (Table 1) exhibited 18 carbon signals corresponding to four methyls (δ_C 21.6, 25.6, 26.7, 59.0), four methylenes (δ_C 32.1, 34.6, 40.9, 64.2), two oxygenated methines (δ_C 69.1, 69.8), and eight quaternary carbons (δ_C 78.0, 84.2, 90.2, 108.6, 134.2, 157.5, 166.7, 169.7). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **4** were close to those of **7**[5] except for the signals at positions C-5 and C-6. The chemical shifts of C-5 (δ_H 3.53, δ_C 69.8) and C-6 (δ_C 90.2) indicated that they are an oxygenated methine and a ketal quaternary carbon, respectively. Moreover, compound **4** was attributed with one more degree of unsaturation than compound **7**. This revealed that there should be another ether linkage in **4** in addition to the C-1/C-4 ether linkage, occurring at either C-5/C-10 or C-6/C-10. In the NOESY spectrum (Figure 3), the key correlation of H-2/H-14 proved that the orientations of the C-1/C-4 ether linkage and H-14 are the same as those in **7**. Should compound **4** possess a C-5/C-10 ether linkage, H-5 would be in the α-equatorial orientation. However, the important NOE correlation between H-3 and H-5 indicated that H-5 is in an α-axial orientation, from which information on the second ether linkage was deduced to be between C-6 and C-10.

The doublet coupling of H-8 (J = 10.0 Hz) with H-9b indicated the dihedral angle of H-8/H-9a to be close to 90°. Hence, the relative configuration of H-8 in **4** could be proposed to be the same as in **1**, and this was confirmed by the NOE correlation of H-8/H-9. Furthermore, when considering the biogenetic pathway of **4**, the 10β-hydroxy group of **1** may undergo a nucleophilic attack on C-6 to form the C-10/C-6 ether linkage, and this lends support for the conformational elucidation of **4**. The absolute configuration in **4** was suggested by circular dichroism. An n → π* Cotton effect at



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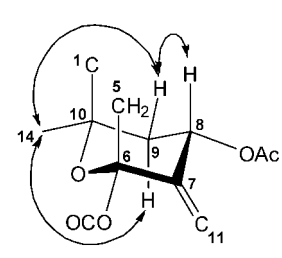

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Figure 3. Key NOE correlations of partial structures of **4** and **8**.

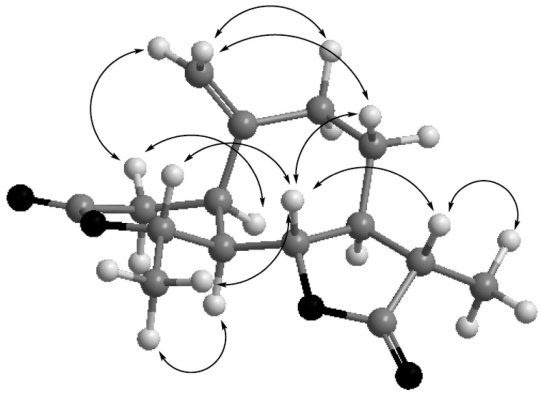

Figure 4. Key NOE correlations of **5**.

Δ_{245} +2.6 and a $\pi \rightarrow \pi^*$ Cotton effect at Δ_{210} –6.0 of **4** indicated the absolute configuration of C-6 to be *S*.[6,8] In comparison with the conformation of **8** deduced by X-ray crystallography,[6] the coupling constants ($J_{H\text{-}8/H\text{-}9}$ = 12.0, 6.0 Hz in **8**; $J_{H\text{-}8/H\text{-}9}$ = 10.0 Hz in **4**) and NOE correlations between H-8/H-9 (Figure 3) indicated that the chair form (C-6/C-7/C-8/C-9/C-10/O) in **8** is changed to the boat form in **4**. Thus, the structure of **4** was established as shown, and this substance was named spicatolide G.

Spicatolide H (**5**) was obtained as colorless oil and formulated as $C_{15}H_{20}O_4$ with six degrees of unsaturation by HRESIMS. The IR data displayed absorption bands attributable to a δ-lactone and a γ-lactone at 1776 and 1739 cm^{-1}, respectively. Fifteen carbon signals, including two methyls, four methylenes (including one terminal olefinic carbon), six methines (including two oxygenated methines), and three quaternary carbons (including two ester carbonyl carbons and one olefinic carbon) were observed in the ^{13}C NMR and DEPT spectra. The gross structure of **5** was suggested by several ^{1}H−^{1}H COSY (H-15/H-4/H-5/H-1/H-2; H-5/H-6/H-7/H-8/H-9; H-13/H-11/H-7) and HMBC (H-2/C-3; H-1/C-3; H-6/C-12; H-13/C-12; H-14/C-1, C-9, C-10) correlations (Figure 1). This suggested that **5** incorporates two lactone rings, one seven-membered ring, and one terminal double bond.

The relative configuration of **5** was revealed by NMR ^{1}H−^{1}H coupling constants and NOE correlations. The allylic methine (H-1) showed a quartet pattern with coupling constants of 6.4 Hz ($J_{H\text{-}1/H\text{-}2a} = J_{1\text{-}H/H\text{-}2b} = J_{H\text{-}1/H\text{-}5}$), which suggested that H-1 is in the equatorial orientation. The axial−axial couplings, $J_{H\text{-}4/H\text{-}5}$ = 8.8 Hz, $J_{H\text{-}6/H\text{-}5,\ H\text{-}7}$ = 10.0, 8.4 Hz, and $J_{H\text{-}7/H\text{-}11}$ = 11.6 Hz, proved that five methines (H-4, H-5, H-6, H-7, H-11) are all in axial configurations. The NOE correlations (Figure 4) revealed H-4, H-6, and H-11 to be in β-axial orientations. Two methyl groups, H-13 and H-15, were assigned in an α-equatorial orientation. The ring junctions were deduced to be *cis* (H-1/H-5) and *trans* (H-6/H-7), respectively. Thus, the structure of **5** was determined as shown and has been named spicatolide H.

Compounds **1**–**9** were evaluated against the HepG2 and Hep3B human hepatocellular carcinoma, MCF-7 and MDA-MB-231 human breast carcinoma, and A-549 human lung carcinoma cell lines. Compounds **1**, **3**, and **4** showed inhibition against Hep3B cells, with IC_{50} values of 4.8 ± 0.2, 3.2 ± 0.2, and 6.1 ± 0.2 μ g/mL, and against MCF-7 cells, with IC_{50} values of 5.1 ± 0.2, 2.4 ± 0.1, and 4.8 ± 0.1 μ g/mL, respectively. None of the compounds showed activity toward the HepG2, MDA-MB-231, and A-549 cell lines (IC_{50} > 5 μg/mL).

Experimental Section

General Experimental Procedures. Optical rotation values were recorded on a JASCO P-1020 polarimeter. UV spectra were taken using a JASCO V-530 UV/vis spectrometer. CD spectra were obtained on a JASCO-715 spectropolarimeter. IR spectra were measured on a Mattson Genesis II FT-IR spectrometer. NMR spectra were taken on a Varian Unity-Plus 400 MHz FT-NMR spectrometer. Mass spectrometric data were recorded on a VG Biotech Quattro 5022 EI-mass spectrometer. Silica gel 60 (Merck, 70–230 and 230–400 mesh) and a Versa flash RP-18 system were used for column chromatography. TLC was carried out on precoated silica Kieselgel 60 F254 plates, and TLC plates were visualized by spraying with Dragendorff's reagent or 50% H_2SO_4 aqueous solution followed by heating.

Plant Material. The aerial parts of *Pseudoelephantopu spicatus* (Juss.) C. F. Baker were collected in Tainan County, Taiwan, in February 2004. The plant material was identified by Dr. Ming-Ho Yen. A voucher specimen is deposited at the Graduate Institute of Natural Products (No. P1), Kaohsiung Medical University, Kaohsiung, Taiwan.

Extraction and Isolation. The aerial parts of *P. spicatus* (10.0 kg) were extracted with MeOH (10 L × 5) and concentrated under reduced pressure. The MeOH extract (ca. 250 g, wet weight) was partitioned between EtOAc and H_2O (1:1) to yield two extracts. The EtOAc extract (ca. 150 g) was then partitioned into *n*-hexane (45 g) and MeOH−H_2O (8:2) (100 g). The latter was subjected to passage over a silica gel column using step gradient elution, with mixtures of *n*-hexane−$CHCl_3$ and $CHCl_3$−MeOH, to afford 15 fractions (A−O). Fraction C (3.5 g) was separated on a Sephadex LH-20 column (5 × 23 cm), with $CHCl_3$−EtOAc (1:1), to give 11 major subfractions. Fraction C-4 was separated on a Sephadex LH-20 column (5 × 23 cm, eluting with EtOAc−MeOH, 1:1), then a silica gel column (eluting with EtOAc−acetone, 6:1) to afford seven major subfractions (C-4-5-1 through C-4-5-7). Fractions C-4-5-1 and C-4-5-2 were purified by HPLC (MeCN−H_2O, 2:3) to yield spicatolide D (**1**) (11.7 mg) and pitocarphin D (**6**) (5.5 mg), respectively. Fraction C-4-5-4 was separated by HPLC (MeCN−H_2O, 1:4) to obtain spicatolide A (**8**) (7.4 mg). Fraction C-4-5-6 was further separated by HPLC (MeCN−H_2O, 1:1) to give spicatolide F (**3**) (3.7 mg) and 13-*O*-methylvernojalcanolide 8-*O*-acetate (**9**) (5.2 mg). Fraction C-4-4 was chromatographed over a silica gel column to afford six major subfractions (C-4-4-1 through C-4-4-6). Fraction C-4-4-6 was purified by HPLC (MeCN−H_2O, 1:4) to provide spicatolide E (**2**) (3.5 mg). Fraction D (3.7 g) was purified on a Sephadex LH-20 column (5 × 23 cm), which was eluted with $CHCl_3$−EtOAc in the ratio of 1:1 to give seven major subfractions (D1–7). Fraction D-3 was chromatographed on a Sephadex LH-20 column (2 × 35 cm), by elution with MeOH, to give six major subfractions, D-3-1 to D-3-6. Fraction D-3-2 was chromatographed over a silica gel column to give five major subfractions (D-3-2-1 to D-3-2-5). Fraction D-3-2-3 was subjected to HPLC (MeCN−H_2O, 1:9) to give spicatolide G (**4**) (6.8 mg) and 8α-acetoxy-10α-hydroxy-13-*O*-methylhirsutinolide (**7**) (20.5 mg). Fraction D-2 was separated on a Sephadex LH-20 column (2 × 35 cm), using MeOH−$CHCl_3$ (1:1) for elution, to give three major subfractions (D-2-1 to D-2-3). Fraction D-2-2 was chromatographed over a silica gel column to give 10 major subfractions (D-2-2-1 to D-2-2-10). Fraction D-2-2-5 was subjected to HPLC (MeCN−H_2O, 3:97) to give spicatolide H (**5**) (10.8 mg).

Spicatolide D (1): colorless oil; $[\alpha]^{25}_D$ −5.83 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 280 (4.26) nm; IR (neat) ν_{max} 3455, 1765, 1740, 1640, 1225 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 326 [M]$^+$ (25), 309 (30), 218 (65), 163 (100); HRESIMS *m/z* 349.1239 [M + Na]$^+$ (calcd for $C_{16}H_{22}O_7Na$, 349.1263).

Spicatolide E (2): colorless oil; $[\alpha]^{25}_D$ −17.2 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 280 (4.34) nm; IR (neat) ν_{max} 3540, 1770, 1750, 1724, 1679, 1250, 1237, 1197, 1037 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 408 [M]$^+$ (15), 391 (30), 272 (40), 229 (80), 188 (100); HRESIMS *m/z* 431.1675 [M + Na]$^+$ (calcd for $C_{21}H_{28}O_8Na$, 431.1688).

Spicatolide F (3): colorless oil; $[\alpha]^{25}_D$ −21.2 (*c* 0.31, MeOH); UV (MeOH) λ_{max} (log ε) 218 (4.16) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 245 (+1.5), 215 (–3.4) nm; IR (neat) ν_{max} 3474, 1780, 1748, 1724, 1679, 1250, 1237, 1197, 1037 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 326 [M]$^+$ (50), 257 (38), 135.9 (70), 99 (100); HRESIMS *m/z* 349.1243 [M + Na]$^+$ (calcd for $C_{16}H_{22}O_7Na$, 349.1263).

Spicatolide G (4): colorless oil; $[\alpha]^{25}_D$ +34.8 (*c* 0.08, MeOH); UV (MeOH) λ_{max} (log ε) 235 (4.33) nm; CD (MeOH) λ_{max} ($\Delta\varepsilon$) 245 (+2.6), 210 (–6.0) nm; IR (neat) ν_{max} 3430, 1780, 1748, 1724, 1250, 1237, 1197, 1037 cm^{-1}; ^{13}C and ^{1}H NMR data, see Tables 1 and 2; EIMS (70 eV) *m/z* 384 [M]$^+$ (45), 305 (60), 153 (90); HRESIMS *m/z* 407.1239 [M + Na]$^+$ (calcd for $C_{18}H_{24}O_9Na$, 407.1318).

Spicatolide H (5): colorless oil; $[\alpha]^{25}_D$ +23.6 (*c* 0.13, MeOH); UV (MeOH) λ_{max} (log ε) 213 (4.29) nm; IR (neat) ν_{max} 2954, 1776, 1739, 1456, 1180 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.25 (3H, d, *J*

= 6.8 Hz, H-13), 1.31 (1H, m, H-8a), 1.50 (1H, d, J = 6.4 Hz, H-15), 2.07 (1H, m, H-7), 2.09 (1H, m, H-5), 2.11 (1H, m, H-8b), 2.12 (1H, m, H-9a), 2.22 (1H, dq, J = 11.6, 6.8 Hz, H-11), 2.55 (1H, m, H-9b), 2.62 (1H, dd, J = 17.2, 6.4 Hz, H-2a), 2.71 (1H, dd, J = 17.2, 6.4 Hz, H-2b), 2.96 (1H, q, J = 6.4 Hz, H-1), 3.74 (1H, dd, J = 10.0, 8.4 Hz, H-6), 4.52 (1H, qd, J = 8.8, 6.4 Hz, H-4), 4.87 (1H, s, H-14a), 5.12 (1H, s, H-14b); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 13.0 (q, C-13), 20.6 (q, C-15), 31.2 (t, C-8), 33.8 (t, C-2), 37.1 (t, C-9), 37.5 (d, C-1), 41.9 (d, C-11), 45.6 (d, C-5), 48.3 (d, C-7), 76.3 (d, C-4), 83.1 (d, C-6), 114.7 (t, C-14), 147.6 (s, C-10), 170.7 (s, C-3), 177.5 (s, C-12); EIMS (70 eV) m/z 264 $[M]^+$ (40), 236 (100); HRESIMS m/z 287.1263 $[M + Na]^+$ (calcd for $C_{15}H_{20}O_4Na$, 287.1259).

Cytotoxicity Assay. The assays were performed using a previously described method.[9] Doxorubicin was used as a positive control with IC_{50} values of 0.06 ± 0.01 and 0.38 ± 0.00 μg/mL against the Hep3B and MCF-7 cell lines, respectively.

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