# New Sesquiterpene Lactones from the Aerial Parts of *Pseudoelephantopus spicatus*

Hamad Hassan Issa,[a,b] Sue-Ming Chang,[a] Yu-Liang Yang,[a] Fang-Rong Chang,*[,a] and Yang-Chang Wu*[,a]

[a] *Graduate Institute of Natural Products, Kaohsiung Medical University; Kaohsiung 807, Taiwan: and* [b] *Department of Sciences, School of Education, Arts and Sciences, The State University of Zanzibar; P.O.Box 146, Zanzibar, Tanzania.*

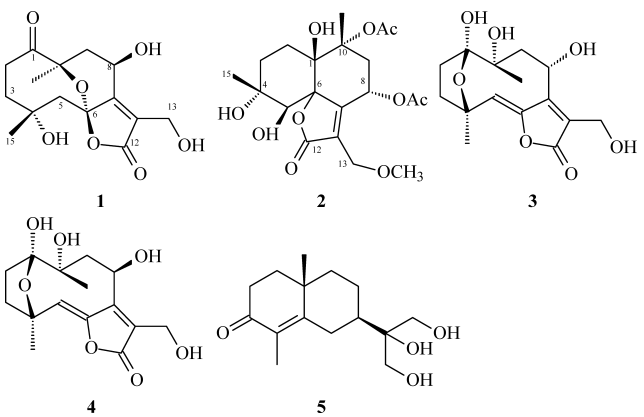
Received May 15, 2006; accepted July 27, 2006

**Two new sesquiterpene lactones, spicatolide C (1) and spicatocadinanolide A (2), have been isolated along with the known piptocarphol isomers (3, 4) and one eudismane type sesquiterpene (5) from the EtOAc extract of the aerial parts of *Pseudoelephantopus spicatus*. The structures and the relative stereochemistries of the new metabolites were determined by spectroscopic methods.**

**Key words** sesquiterpene lactone; *Pseudoelephantopus spicatus*; Taiwanese folk medicine; germacranolide; spicatolide

*Pseudoelephantopus spicatus* (Juss.) C. F. Baker is an essential component of "Teng-Khia-U"; a folk medicine that has been widely used in Taiwan for the treatment of inflammatory conditions such as edema, chest pain, nephritis, fever and coughing associated with pneumonia, scabies and anthralgia due to wounding. The crude extract of this composite of Chinese traditional medicine has been demonstrated to have significant hepatoprotective effects in experimental models.[1,2] The earlier studies of this plant by Ragasa *et al.* had resulted with the isolation of previously unknown types of germacranolides with a unique C-6/C-10 ketal linkage instead of a mostly encountered elephantopus type sesquiterpenes with a C-1/C-4 linkage.[3,4] The structures of these new types of germacranolides were elucidated with the help of X-ray crystallographic studies and named spicatolides A and B.[3]

In the course of our search for anti-inflammatory candidates from Taiwanese folk medicine, we have investigated the extracts of *P. spicatus*, and isolated a new germacranolide, spicatolide C (**1**) and a new cadinanolide, spicatocadinanolide A (**2**), along with three known sesquiterpenoids (**3**—**5**).[5—7] Herein, the isolation and structure elucidation of the two new compounds are described. Structures of new metabolites were determined by spectroscopic techniques and by comparison with the spectroscopic data from literature.



**1** **2** **3** **4** **5**

## Results and Discussion

The aerial parts of *P. spicatus* were extracted with MeOH and partitioned between EtOAc and $H_2O$. The EtOAc extract was further partitioned between *n*-hexane and MeOH/$H_2O$. The resulting MeOH/$H_2O$ extract was found to be moderately cytotoxic against MC-F7 cell lines ($IC_{50}$=16.8 $\mu$g/ml). Fractionation and purification of this extract by combination of chromatographic methods led to the isolation of compounds **1**—**5**. Identification of the known compounds **3**—**5** was accomplished by comparison of their spectral data with those reported in the literature.[5—7]

Compound **1** was isolated as a colorless glass and was assigned molecular formula $C_{15}H_{20}O_7$ with six degrees of unsaturation, as deduced by HR-ESI-MS at *m*/*z* 335.1108 $[M+Na]^+$ (Calcd for $C_{15}H_{20}O_7Na$, 335.1107). The UV spectrum displayed absorption maxima at $\lambda_{max}$ 207 nm. The IR absorption indicated the presence of an $\alpha,\beta$-unsaturated $\gamma$-lactone (1768, 1697 $cm^{-1}$), a carbonyl absorption (1705 $cm^{-1}$), and a hydroxyl group (3388 $cm^{-1}$).

The $^1$H-NMR spectrum indicated the presence of two singlet methyl groups, one allylic carbinol proton at $\delta$ 5.26 (ovlp), an isolated methylene protons at $\delta$ 2.01 (d, $J$=16.4 Hz) and 1.90 (d, $J$=16.4 Hz), and an allylic methylene protons on an oxygen bearing carbon at $\delta$ 5.02 (d, $J$=13.2 Hz) and 4.98 (d, $J$=13.2 Hz), consistent with the basic skeleton of spicatolide type lactones isolated earlier from *P. spicatus*.[3] The $^{13}$C- and DEPT NMR analyses indicated fifteen carbon resonances attributed to seven quaternary, one methine, five methylene, and two methyl carbons. The presence of $\alpha,\beta$-unsaturated $\gamma$-lactone ring was further supported by low-field carbon signals at $\delta$ 170.7 (s, C-12), 126.1 (s, C-11) and 165.0 (s, C-7). Thus, three double bond equivalents were attributed to two carbonyl groups and a double bond. To account for the remaining degrees of unsaturation, it was suggested that **1** is a tricyclic sesquiterpene lactone related to spicatolide A.[3] The basic spicatolide skeleton was also supported by the COSY (H-2/H-3; H-8/H-9) and HMBC (H-2/C-1, C-3; H-3/C-2, C-4; H-15/C-3, C-4, C-5; H-13/C-11, C-12, C-7; H-8/C-7, C-9; H-14/C-1, C-9, C-10) correlations. The relative stereochemistry of **1** was elucidated by the observed correlations of the NOESY cross peaks between H-8 and H-14. Hence, the structure of compound **1** is elucidated as shown and named spicatolide C.

* To whom correspondence should be addressed. e-mail: yachwu@kmu.edu.tw.

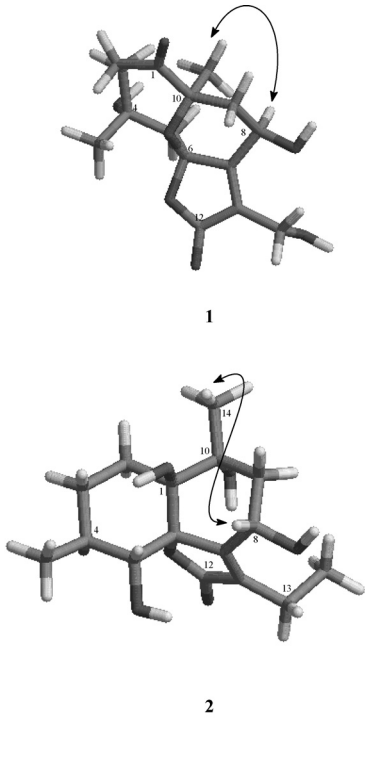
Table 1. NMR Data of Specatolide C (**1**) in $C_5D_5N$ ($J$=Hz)

| Position | $^{13}$C-NMR | $^{1}$H-NMR | HMBC ($^{1}$H→$^{13}$C) |
|---|---|---|---|
| 1 | 214.9 (s) | | |
| 2 | 40.6 (t) | 1.89 m, 2.90 m | C-1, C-3, C-10 |
| 3 | 35.8 (t) | 2.33 m, 3.47 m | C-2, C-4, C-15 |
| 4 | 71.4 (s) | | |
| 5 | 43.7 (t) | 3.29 (dd, 6.0, 12.4)<br>1.76 (d, 12.4) | C-4, C-6, C-15 |
| 6 | 106.3 (s) | | |
| 7 | 165.0 (s) | | |
| 8 | 64.8 (d) | 5.26 ovlp | C-7, C-9, C-11 |
| 9 | 44.2 (t) | 2.01 (d, 16.4)<br>1.90 (d, 16.4) | C-1, C-8, C-10, C-14 |
| 10 | 82.8 (s) | | |
| 11 | 126.1 (s) | | |
| 12 | 170.7 (s) | | |
| 13 | 54.2 (t) | 5.02 (d, 13.2)<br>4.98 (d, 13.2) | C-7, C-11, C-12 |
| 14 | 25.6 (q) | 1.35 s | C-1, C-9, C-10 |
| 15 | 31.8 (q) | 1.28 s | C-3, C-4, C-5 |

Table 2. NMR Data of Cadinanolide (**2**) in $CD_3OD$ ($J$=Hz)

| Position | $^{13}$C-NMR | $^{1}$H-NMR | HMBC ($^{1}$H→$^{13}$C) |
|---|---|---|---|
| 1 | 78.2 (s) | | |
| 2 | 30.7 (t) | 2.20—2.23 (dd, 2.4, 4.4)<br>1.79 (dq, 4.8, 14.8, 10) | C-1, C-3 |
| 3 | 36.5 (t) | 1.67—1.70 (dq, 2.4, 5.2)<br>2.33 (ddd, 4.8, 14, 9.6) | C-2, C-4, C-15 |
| 4 | 74.3 (s) | | |
| 5 | 75.9 (d) | 4.64 (s) | C-4, C-6, C-15 |
| 6 | 93.3 (s) | | |
| 7 | 160.3 (s) | | |
| 8 | 66.5 (d) | 6.04 (dd, 2.4, 4.0) | C-7, C -11 |
| 9 | 35.2 (t) | 1.95 (d, 3.6)<br>2.00 (d, 4.0) | C-1, C-7, C-8, C-10, C-14 |
| 10 | 86.3 (s) | | |
| 11 | 129.0 (s) | | |
| 12 | 174.2 (s) | | |
| 13 | 64.0 (t) | 4.36 (d, 12.4)<br>4.18 (d, 12.4) | C-7, C-11, C-12, O<u>C</u>H$_3$ |
| 14 | 20.2 (q) | 1.63 (s) | |
| 15 | 21.1 (q) | 1.40 (s) | |
| OCH$_3$ | 58.4 (q) | 3.31 (s) | C-13 |
| 1-O<u>C</u>OCH$_3$ | 171.1 (s) | | |
| | 23.2 (q) | 2.18 (s) | 1-OC<u>O</u>CH$_3$ |
| 2-O<u>C</u>OCH$_3$ | 171.5 (s) | | |
| | 21.1 (q) | 2.05 (s) | 2-OC<u>O</u>CH$_3$ |

The molecular formula of compound **2** was shown to be $C_{19}H_{26}O_{10}$ on the basis of HR-ESI-MS, IR and NMR data. The IR data displayed absorption bands attributable to $\gamma$-lactone (1743 cm$^{-1}$), carbonyl (1705 cm$^{-1}$), and hydroxyl group (3412 cm$^{-1}$). The UV absorption displayed maxima at 215 nm suggesting an $\alpha,\beta$-unsaturated $\gamma$-lactone unit. The $^{13}$C- and DEPT spectra showed 20 carbons assignable to three ester carbonyls, two olefinic carbons, four oxygenated quaternary carbons, two oxygenated methine carbons, four methylenes, one methoxyl, and four methyl singlets (two of them acetyl methyl groups). These spectroscopic features were consistent with cadinanolide type sesquiterpene lactones.[8,9] The $^{1}$H–$^{1}$H COSY data showed proton connectivities for H-2/H-3 (vicinal methylene units) and H-8/H-9. Analysis of HMBC established connection of an isolated carbinol proton signal at $\delta$ 4.64 (H-5) to three oxygenated




**1**

**2**

⟷ NOESY

Fig. 1. Key NOESY Correlations of **1** and **2**

quaternary carbons (C-4/C-6/C-15) and the methylene carbon (C-3). The low-field methine signal at $\delta$ 6.04 (H-8) was found to correlate to the other two oxygenated quaternary carbons (C-6/C-10) and two olefinic carbons (C-7/C-11). Thus, the cadinanolide skeleton for structure **2** was confirmed. The free hydroxyl at C-1 and two acetoxy functions were positioned from the chemical shifts of C-1, C-4, C-5, C-8, and C-10 and biogenetic cosiderations.[9] NOESY experiments consolidated stereochemical determination of compound **2**. The existence of NOESY correlations (Fig. 1) of H-14 to H-8 and the absence of correlation to C-15, verified the $\beta$-configurations of C-14 and C-15 methyl groups and the stereochemistry of the C-8 hydroxyl group. The configuration for C-8 acetoxyl was suggested by the equatorial coupling of H-8 proton to C-9 hydrogens ($J$=2.2, 4.0 Hz). Thus, the structure of compound **2** is elucidated as shown and named spicatocadinanolide A.

Compounds **1**—**4** were evaluated for their cytotoxic activity against A549, MCF7, HepG2 and MDA-MB-231 cancer cell lines. The isomeric mixture of piptocarphol isomers (**3**, **4**) displayed the strongest activity against MCF7, HepG2 and MDA-MB-231 cell lines with an $IC_{50}$ 11.20, 16.48 and 14.71 $\mu$g/ml, respectively. The rest of the compounds were not active at 20 $\mu$g/ml. These compounds were also screened for the inhibitory effects on superoxide anion generation and elastase release by human neutrophils in response to fMLP/CB but no activity could be observed.

## Experimental

**General Experimental Procedures** The optical rotation values were recorded with a Jasco-P-1020 polarimeter. UV spectra were taken using a Jasco V-530 UV/VIS spectrometer. IR spectra were measured on a Mattson Genesis II$^{TM}$ FT-IR spectrometer. NMR spectra were taken on Varian Unity-Plus 400 MHz FT-NMR spectrometers. Mass spectral data were recorded on

a VG Biotech Quattro 5022 Mass spectrometer. Silica gel 60 (Mark, 70—230, 230—400 mesh) and Versa flash RP-18 column were used for column chromatography. TLC were carried out on precoated silica Kieselgel 60, F254 plates, and TLC plates were visualized by spraying with Dragendroff's reagent or 50% $H_2SO_4$ aqueous solution followed by heating.

**Plant Material** The aerial parts of *Pseudoelephantopus spicatus* (JUSS.) C. F. BAKER were collected in Feb. 2004. A voucher specimen is deposited in Graduate Institute of Natural Products (No. Pseudoelephantopus 1), Kaohsiung Medical University, Taiwan.

**Extraction and Isolation** The aerial parts of *Pseudoelephantopus spicatus* (JUSS.) C. F. BAKER (10.0 kg) was 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) layer (70 g, wet weight). The later was subjected to a silica gel column using step gradient elution with *n*-hexane–$CHCl_3$ and $CHCl_3$–MeOH to afford 14 fractions (Fr. 1—15). Based on TLC examination and cytotoxicity results, Fr. 8 and Fr. 9 were selected and combined (1.57 g) for further purification. Fractionation on a silica gel column using step gradient elution with $CHCl_3$/MeOH with increasing polarity afforded six fractions (IH-1—IH-6). Fraction IH-3 was subjected to HPLC separation (Hypersil ODS, 250×20 mm, ACN–$H_2O$ (1.5 : 10), flow rate=2 ml/min) to afford spicatolide C (**1**) (7 mg, $t_R$=7.2 min). Fractions 1—2 were combined (C7, 900 mg) and subjected to a Versa Flash column, RP-18, $H_2O$/MeOH (1 : 2/1 : 1/0 : 100) to afford 14 fractions (IH-9-1—IH-9-14). Fraction IH-9-1 was combined with IH-9-2 and subjected to HPLC separation (Develosil C30-UG-5, 250×20 mm, ACN–$H_2O$ (1.5 : 10), flow rate=2 ml/min) to yield isomeric mixture of piptocarphol **3** and **4** (80 mg, $t_R$=23.4 min), a new cadinanolide **2** (4.0 mg, $t_R$=51.7 min) and tri hydroxylated eudismane **5** (4 mg, $t_R$=57.7 min).

Spicatolide C (**1**): Colorless glass (7 mg); mp 169—170 °C; $[\alpha]_D^{26}$ −103.6° (*c*=0.7, MeOH); IR $\nu_{max}$ (neat) $cm^{-1}$: 3388, 1768, 1705, 1697; UV (MeOH) $\lambda_{max}$ nm: 207 (log $\varepsilon$ 4.35); $^1$H- and $^{13}$C-NMR: see Table 1; ESI-MS *m/z* (rel int): 336 ($[M+Na+H]^+$, 20), 335 ($[M+Na]^+$, 90), 313 ($[M+H]^+$, 18), 207 (8), 168 (10); HR-ESI-MS *m/z*: 335.1108 $[M+Na]^+$ (Calcd for $C_{15}H_{20}O_7Na$, 335.1107).

Spicatocadinanolide A (**2**): Pale yellow oil (4 mg); $[\alpha]_D^{26}$ −25° (*c*=0.2, MeOH); IR $\nu_{max}$ (neat) $cm^{-1}$: 3412, 1743, 1705; UV $\lambda_{max}$ (MeOH) nm: 215 (log $\varepsilon$ 4.52); $^1$H- and $^{13}$C-NMR : see Table 1. ESI-MS *m/z* (rel int): 452 ($[M+Na+H]^+$, 20), 451 ($[M+Na]^+$, 85), 437 ($[M+H]^+$, 20), 413 (25), 409 (30), 381 (80), 367 (15), 353 (28); HR-ESI-MS *m/z*: 551.1582 $[M+Na]^+$ (Calcd for $C_{20}H_{28}O_{10}Na$, 451.1580).

**Antiinflammatory and Cytotoxicity Assays** The assays were performed using methods described previously.[10,11]

**Acknowledgments** We gratefully acknowledge the financial support for this project from the National Science Council and National Science Technology Program/Biotechnology and Pharmaceuticals, Taiwan, ROC.

**References**

1) Tsai C. C., Lin C. C., *J. Ethnopharmacol.*, **64**, 85—89 (1999).
2) Lin C. C., Tsai C. C., Yen M. H., *J. Ethnopharmacol.*, **45**, 113—123 (1995).
3) Ragasa C. Y., Padolina W. G., Yamauchi T., Otsuka H., Yamasaki K., Satoshi T., *Phytochemistry*, **33**, 627—629 (1993).
4) Pollora G. C., Bardón A., Catálan C. A. N., Gedris T. E., Herz W., *Biochem. Systematics Ecol.*, **31**, 397—405 (2003).
5) Bardon A., Montanaro S., Catalan C. A. N., Daiz J. G., Herz W., *Phytochemistry*, **34**, 253—259 (1993).
6) Herz W., Kulanthaivel P., *Phytochemistry*, **22**, 1286—1287 (1983).
7) Ahmed A. A., Mahmoud A. A., Ali E. T., Tzakou O., Couladis M., Mabry T. J., *Phytochemistry*, **59**, 851—856 (2002).
8) Ragasa C. Y., Rideout J. A., *Chem. Pharm. Bull.*, **49**, 1359—1361 (2001).
9) Jakupovic J., Schmeda-Hirschmann G., Schuster A., Zdero C., Bohlmann F., King R. M., Robinson H., Pickardt J., *Phytochemistry*, **25**, 145—158 (1986).
10) Lan Y. H., Chia Y. C., Chang F. R., Hwang T. L., Liaw C. C., Wu Y. C., *Helv. Chim. Acta*, **88**, 905—909 (2005).
11) Hsu H. F., Chang C. L., Wu C. C., Chang F. R., Wu Y. C., *J. Agric. Food Chem.*, **53**, 6117—6125 (2005).