An Antifungal Cadinanolide from *Pseudoelephantopus spicatus*

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Received June 4, 2001; accepted July 19, 2001

A new sesquiterpene lactone was obtained from the chloroform extract of *Pseudoelephantopus spicatus*. Its structure was elucidated by extensive one dimentional (1D) and 2D NMR spectroscopy and mass spectrometry. It was found to exhibit moderate antifungal activity against *C. albicans* and *A. niger*, and low activity against *T. mentagrophytes*, *S. aureus*, *E. coli* and *P. aeruginosa*. It was inactive against *B. subtilis*.

Key words *Pseudoelephantopus spicatus*; Compositae; cadinanolide; antifungal

Pseudoelephantopus spicatus ROHR. is an introduced weed of American origin now found throughout the Philippines. It is commonly used as a topical agent and is considered specific for eczema. The leaves are used to treat wounds, reduce inflammations, and are also applied to snake bites.[1] Earlier studies on *P. spicatus* reported the isolation of five germacranolides[2,3] and a cadinanolide.[4] We now report the isolation, structural elucidation and antimicrobial test results of another cadinanolide (**1**) from the same species.

Results and Discussion

The chloroform extract of the air-dried leaves of *P. spicatus* afforded **1**, after chromatography on Si gel. Its structure was elucidated by extensive one dimensional (1D) and 2D NMR spectroscopy and mass spectrometry as follows.

High-resolution electron-impact (HR-EI-MS) of **1** gave an $[M+H]^+$ at *m/z* 485.2033, with the calculated value for $C_{23}H_{33}O_{11}$ being 485.2023. Thus, **1** has an index of hydrogen deficiency of 8. The ^{1}H-NMR spectrum of **1** in C_6D_6 (Table 1) indicated resonances for five methyl singlets, three of which belong to acetates (δ 2.09, 1.95, 1.72); an ethoxy group at δ 1.15 (3H, t, J=7.0 Hz) and δ 3.44 (2H, q, J=7.0 Hz); an allylic carbinyl methylene group at δ 4.62 (1H, J=12.4 Hz) and 4.38 (1H, J=12.4 Hz); two dishielded carbinyl protons at δ 6.15 (1H, s) and 6.01 (1H, dd, J=2.4, 4.0 Hz), and two hydroxyl protons at δ 0.85 and 2.40. The presence of hydroxyl, ether and ester functionalities in **1** are supported by the IR ν_{max} (cm^{-1}) 3471 (br, OH), 1744 and 1733 (C=O), 1255, 1242, 1120, 1090, 1036 and 1025 (C–O). From the correlation spectroscopy (COSY) spectrum, two isolated spin systems were deduced as follows. The carbinyl proton at δ 6.01 is coupled to the methylene protons at δ 3.38 and 2.05 by 2.4 and 4.0 Hz, respectively; and the methylene protons at δ 1.45 and 2.05 are coupled to the protons at δ 1.77 and 2.38. The J mod ^{13}C-NMR spectrum of **1** (Table 1) indicated six methyl groups, five methylene groups (two of which are oxygenated); two oxygenated methine carbons; four oxygenated quaternary carbons; two olefinic carbons; and four carbonyl carbons. ^{13}C and ^{1}H assignments for **1** were verified for ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) NMR spectroscopy. The NMR data for **1** are similar to those reported for cadinanolide,[4,5] except for the replacement of the acetate attached to C-13 by an ethoxy group.

Connectivity for **1** was established by an inverse long-range heteronuclear experiment (HMBC) optimized for J=10 Hz (Table 1). The presence of a third carbocyclic ring in **1** was indicated by the correlation of the hydrogen at δ 2.05 (H-2′) to the carbon at δ 91.5, which was assigned to the oxygenated carbon of the γ-lactone (C-6). Acetate groups were located at C-5 and C-8 due to long range correlations from the two methine hydrogens at δ 6.15, which are connected to the carbon at δ 23.7 (C-15), 157.1 (C-7) and 73.2 (C-4) and 6.01 which are connected to carbons at δ 157.1 (C-7), 91.5 (C-6), 34.1 (C-9) and 84.3 (C-10), respectively. The remaining acetate group was located at C-10 on the basis of the C-10 chemical shift (δ 84.3), the long range correlation from this carbon to H-8. All other long-range correlations were consistent with the structure of **1**.

The relative stereochemistry of **1** was determined by nuclear Overhauser effect spectroscopy (NOESY) which indicated correlation through space of the ^{1}H nuclei in the molecule (Table 2). Significant correlations were observed from the hydrogens of the C-10 acetate group to H-3′ (δ 2.38) and H-5, which required the C-1, C-5, C-6, and C-10 and stereochemistries shown in **1**. These NOE correlations were also observed in the rotating frame Overhauser enhancement spectroscopy (ROESY) spectrum of **1** in $CDCl_3$ solvent. The C-4 stereochemistry was assigned on the basis of an NOE from H-3 (δ 1.77) to H-15, and from 4-OH to H-3′ and H-5. The C-8 stereochemistry was determined from the coupling constants from H-8 to the C-9 hydrogens (J=2.4, 4.0 Hz, equatorial coupling), and an NOE from H-8 to H-9, H-9′, H-13 and H-13′.

Pseudoelephantopus spicatus is used to treat wounds and reduce inflammation, hence **1** has antimicrobial activity. Results of the study are presented in Table 3. Among the seven microorganisms tested, **1** was found active against the fungi *C. albicans* and *A. niger* with average antimicrobial indices of 0.55 and 0.7, respectively, at a concentration of 40 μg. It showed low activity against *T. mentagrophytes*, *S. aureus*, *E.*

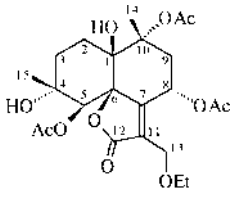

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Table 1. 300 MHz ^{1}H-NMR and 75 MHz ^{13}C-NMR Spectral Data of **1** in C_6D_6

Carbon No.	^{13}C, δ	^{1}H, δ	HMBC correlations
C-1	76.9	—	H-2, H-3, H-9, H-14
C-2	30.5	1.45 (1H, m H-2), 2.05 (1H, m H-2′)	H-3, H-3′
C-3	36.3	1.77 (1H, m H-3), 2.38 (1H, dt, J=4.5, 14 Hz, H-3′)	H-2, H-15
C-4	73.2	—	H-3′, H-5, H-15
C-5	76.6	6.15 (1H, s)	H-3, H-3′, H-15
C-6	91.5	—	H-2′, H-8
C-7	157.1	—	H-5, H-8, H-9′, H-13, H-13′
C-8	66.5	6.01 (1H, dd, J=2.4, 4.0 Hz)	H-9′
C-9	34.1	3.38 (1H, dd, J=2.4, 16 Hz, H-9), 2.05 (1H, dd, J=4.0, 16 Hz, H-9′)	H-8, H-14
C-10	84.3	—	H-2, H-8, H-9′, H-14
C-11	130.6	—	H-13, H-13′
C-12	171.3	—	H-13, H-13′
C-13	61.6	4.39 (1H, J=12.4 Hz, H-13), 4.62 (1H, J=12.4 Hz, H-13′)	CH_2 of OEt, H-13, H-13′, CH_2 of OEt, H-13
C-14	19.8	1.66 (3H, s)	—
C-15	23.7	1.62 (3H, s)	H-3, H-3′, H-5
OEt			
OCH_2	66.5	3.44 (2H, q, J=7.0 Hz)	CH_3 of OEt, H-13, H-13′
CH_3	15.2	1.15 (3H, t, J=7.0 Hz)	
OAc			
C=O, CH_3	168.5, 22.7	2.09 (3H, s)	2.09 CH_3
	171.3, 20.0	1.72 (3H)	1.72 CH_3, H-5
	170.1, 20.9	1.95 (3H, s)	1.95 CH_3, H-8

coli and *P. aeruginosa*. It was inactive against *B. subtilis*.

Experimental

Instrumentation and General Procedure The NMR spectra were recorded in $CDCl_3$ or C_6D_6 with the use of Bruker AMX Fourier Transform 300 MHz and Bruker Avance 400 MHz NMR spectrometers. Mass spectra were recorded on a Micromass AutoSpec spectrometer. Fractions were monitored by TLC and spots were visualized by spraying with vanillin–sulfuric acid and then warming.

Sample Collection The sample was collected from the UP-Diliman campus in August 1997. It was identified as *Pseudoelephantopus spicatus* at the Philippine National Museum and a voucher specimen is kept at the Chemistry Department at De La Salle University.

Extraction and Isolation The air-dried leaves (500 g) of *P. spicatus* were extracted with $CHCl_3$ to afford a crude extract (20 g), which was treated with aqueous $Pb(OAc)_2$ to precipitate the pigments.[6] The treated extract (5 g) was chromatographed in silica gel (70—230 mesh) using increasing proportions of EtOAc in petroleum ether (10% increments). The EtOAc fractions were rechromatographed (2 times) in CH_2Cl_2: Et_2O: CH_3CN (8 : 1 : 1) to afford **1**.

Compound **1**: Colorless oil; $[\alpha]_D$=−32° ($CHCl_3$, c=0.7); IR (KBr) ν_{max} 3471 (br, OH), 1744, 1733 (C=O), 1255, 1242, 1120, 1090, 1036, 1025 (C-O); ^{1}H- and ^{13}C-NMR data are listed in Table 1. EI-MS m/z 485.2 $[M+H]^+$ (7), 364.2 (30), 318.1 (32), 276.2 (100), 258.2 (47), 233.1 (30), 216.1 (22), 177.1 (19), 99.0 (51); HR-EI-MS m/z 485.2033 $[M+H]^+$ ($C_{23}H_{33}O_{11}$ requires 485.2023).

Antimicrobial Test The microorganisms used in these tests are *Staphylococcus aureus* UPCC 143, *Bacillus subtilis* UPCC 1, *Escherichia coli* UPCC 195, *Pseudomonas aeruginosa* UPCC 244, *Candida albicans* UPCC 2168, *Aspergillus niger* UPCC 4063 and *Trichophyton mentagrophytes* UPCC 4193.

A microbial suspension containing approximately 10^7 cells/ml was prepared for each test organism for 24-h agar culture using 0.1% peptone water. One-tenth (0.1 ml) of the bacterial suspension was transferred into prepoured 30 ml deep nutrient agar plate, the yeast suspension into glucose yeast peptone agar plate and the fungal suspension on potato dextrose agar plate. About 5 ml of the corresponding medium melted and cooled to about 45 °C was immediately poured into the plate. The plate was swirled to distribute the microbial cells evenly on the plate. After the overlay agar has solidified, three 1-cm diameter holes were bored from equidistant points using a sterile cork borer.

Two-tenths (0.2 ml) portions of **1** were placed in duplicate holes per organism. A similar volume of the solvent ethanol and of the corresponding antibiotic for each test organism was placed in the remaining two wells on the plate. Plates were incubated at room temperature to prevent evaporation of liquid on the petri dishes that may cause interference in distribution of organisms on the surface. Bacterial and yeast plates were checked after 24 h, while the mold plate was checked after 3 d. Clearing zones were measured in millimeter (mm). The average for the compounds was taken and the antimicrobial activity index (AI) was computed as the millimeter clearing zone minus millimeter hole divided by millimeter hole.

Table 2. Selected NOESY Correlation Data of **1** in C_6D_6

Hydrogen No.	NOESY correlation
4-OH	H-3′, H-5
H-3′	H-2, 10 OAc
H-5	1-OH, H-3′, 10-OAc
H-8	H-9, H-9′, H-13, H-13′
H-14	H-9, H-9′
H-15	H-2, H-3

Table 3. Antimicrobial Test Results of **1**

Test organism	Sample	Amount of sample, μg	Clearing zone, mm	AI[a]
P. aeruginosa	**1**	40	12, 12	0.2
	Tetracycline	30	20	1.0
B. subtilis	**1**	40	—	0
	Chloramphenicol	30	23	1.3
E. coli	**1**	40	11, 11	0.1
	Tetracycline	30	18	0.8
S. aureus	**1**	40	12, 12	0.2
	Chloramphenicol	30	24	1.4
C. albicans	**1**	40	16, 15	0.55
	Chlotrimazole	30	25	1.5
T. mentagrophytes	**1**	40	12, 12	0.2
	Chlotrimazole	30	35	2.5
A. niger	**1**	40	16, 18	0.7
	Cycloheximide	30	30	2.0

a) Antimicrobial Index.

Acknowledgements The authors would like to acknowledge the University of the Philippines-Natural Sciences Research Institute (UP-NSRI) for conducting the antimicrobial tests. A research grant from the College Re-

search Office and University Research Coordination Office of De La Salle University is gratefully acknowledged.

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