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Research Note

A Triterpene from *Musa errans*

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The dichloromethane extract of the corm of *Musa errans*, an endemic form of wild banana afforded 31-norcyclolaudenone (1), squalene, and a mixture of stigmasterol and sitosterol. The structure of 1 was elucidated by extensive 1D and 2D NMR spectroscopy. Antimicrobial tests on 1 indicated that it has low activity against *C. albicans; E. coli, P. aeruginosa,* and *T. mentagrophytes*; and inactive against *B. subtilis, S. aureus,* and *A. niger.*

Key Words: *Musa errans*, Musaceae, 31-norcyclolaudenone

INTRODUCTION

Musa errans, a form of wild banana is endemic to the Philippines. It is also known as *Musa troglodytarium* Blanco. The leaves are used as topicals for chest pains. The juice of the corms is used as antituberculars. The sap is vulnerary and it is used in the treatment of gonorrhoea (Quisumbing 1978). A new subspecies of *Musa acuminata* has been described and named *M. acuminata ssp. Errans* (Valmayor 2001). There are no reported studies on *Musa errans,* but a number of studies were reported on its congener *Musa acuminata* and *Musa sapientum*. *Musa acuminata* afforded oxabenzochrysenones (Opitz et al. 2002), 4'-hydroxy-anigorootin, 4, 4'-dihydroxy-anigorootin, 3,3'-bishydroxy anigorufone (Otalvaro et al. 2002), and (S)-(+)-6-methoxy-α-methyl-2-naphthaleneacetic acid, which is an anti-inflammatory drug (Abad et al. 2000). *Musa sapientum* afforded 6 triterpenes: cyclomusalenol, cyclomusalenone, 24-methylenecycloartanol, stigmast-7-methylenecycloartanol, stigmast-7-en-3-ol, lanosterol, and β-amyrin and eight flavonoids: quercetin and its 3-O-galactoside, 3-O-glucoside, and 3-O-rhamnosyl glucoside (Zeid and Abou 1999), 4-epicycloeucalenone, and 4-epicyclomusalenone (Akihisa et al. 1997), (24S)-14α,24-dimethyl-9β,19-cyclo-5α-cholest-25-en-3β-ol, (24S)-24-methyl-Δ25-sterols and their 24-methylene isomers, 4,4-dimethyl-, 4α-methyl-, and 4-demethyl sterols, 3-oxo-4α-methylsteroids (Akihisa et al. 1986) and 31-norcyclolaudenone (Knapp and Nicholson 1970; Desai et al. 1982). The corm of banana was reported to have the following constituents: α-pinene, β-pinene, β-myrcene, limonene, α-cubebene, α-copaene, α-cedrene, β-caryophyllene, and α-humulene (Ndiege at al. 1991). The dichloromethane extracts of banana "dwarf Cavendish" afforded by GC-MS fatty acids and sterols as major compounds and aromatic compounds, fatty alcohols, and alkanes as minor compounds (Oliveira et al. 2006).

We now report the isolation, structure elucidation, and antimicrobial test results of **1** from the corm of *Musa errans.*

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MATERIALS AND METHODS

General Experimental Procedures

NMR spectra were recorded on a Bruker Avance 400 in $CDCl_3$ at 400 MHz for ^1H and 100 MHz for ^{13}C. Column chromatography was performed with silica gel 60 (70-230 mesh), while the TLC was performed with plastic backed plates coated with silica gel F_{254}. The plates were visualized with vanillin-H_2SO_4 and warming.

Sample Collection

The corm part of *Musa errans* was collected in August 2005 in Negros Occidental. It was identified as *Musa errans* (Blco.) Teod. Var. botoan Teod. with control number 058 by Danilo N. Tandang and noted by Dr. Domingo A. Madulid of the Philippine National Museum.

Isolation

About 1 kg of the corm of *Musa errans* was cut and ground in an osterizer, then air-dried. The air-dried corm (286 g) were ground in an osterizer, soaked in dichloromethane for 3 days, then filtered. The filtrate was concentrated under vacuum to afford a crude extract (2 g), which was chromatographed in increasing proportions of acetone in dichloromethane at 10 % increment. The dichloromethane fraction was rechromatographed in 5 % ethyl acetate in petroleum ether, then petroleum ether to afford squalene (18 mg). The 10 % acetone in dichloromethane fraction was rechromatographed in 10 % ethyl acetate in petroleum ether, then 2.5 % ethyl acetate in petroleum ether to afford a **1** (10 mg). The 30 % acetone in dichloromethane fractions were rechromatographed (2x) in 15 % ethyl acetate in petroleum ether to afford a mixture of stigmasterol and sitosterol (24 mg).

Antimicrobial Tests

The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *Aspergillus niger* UPCC 4219, *Candida albicans* UPCC 2168, *Bacillus subtilis* UPCC 1295, *Pseudomonas aeruginosa* UPCC 1244, *Escherichia coli* UPCC 1195, *Staphylococcus aureus* UPCC 1143, and *Trichophyton mentagrophyte* UPCC 4193. The test compound was dissolved in 95% ethanol. The antimicrobial assay procedure reported in the literature (Guevara and Recio 1985) was employed. The activity index was computed by subtracting the diameter of the well from the diameter of the clearing zone divided by the diameter of the well.

RESULTS AND DISCUSSION

The ^1H NMR spectrum of **1** (Figure 1) indicated resonances for geminal olefinic protons at δ 4.67 (d, J = 1.6 Hz) and 4.68 (d, J = 1.6 Hz), a cyclopropyl at δ 0.39 (d, J = 4.0 Hz) and 0.61 (d, J = 4.0 Hz), an allylic methyl at δ 1.65 (s), and 5 other methyl groups at δ 0.87 (d, J = 6.8 Hz), 0.89 (s), 0.99 (d, J = 6.4 Hz), 1.00 (s), and 1.01 (d, J = 7.6 Hz). The ^{13}C NMR spectrum of **1** showed resonances for 30 carbons (Table 1) with the following functionalities: a ketone carbonyl at δ 213.33 and olefinic carbons at δ 109.36 and 150.18. The other resonances were attributed to methyl, methylene, and methine carbons. These resonances indicated a triterpene with a cyclopropyl, a ketone carbonyl, and an olefin functionalities.

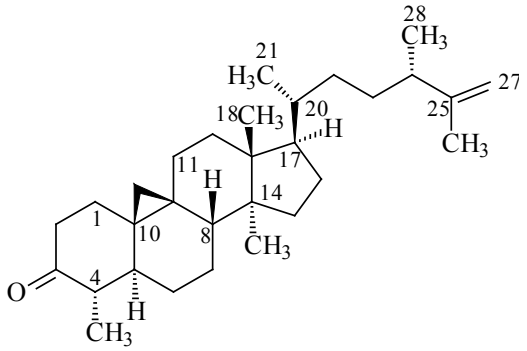

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Figure 1. Structure of 31-norcyclolaudenone (**1**) from *Musa errans*

The COSY spectrum indicated 6 isolated spin systems as follows. H_2-1/H_2-2; H_3-30/H-4/H-5/H_2-6/H_2-7/H-8; H_2-11/H_2-12; H_2-15/H_2-16/H-17/H-20/H_3-21,H_2-22/H_2-23/H-24/H_3-28; H_2-19; H_3-26/H_2-27 (Fig. 2).

The ^1H and ^{13}C connectivities in **1** were verified by HMQC. The structure of **1** was elucidated by analysis of the HMBC 2D NMR data with key HMBC correlations shown in Figure 3. The carbonyl was placed at C-3 due to long-range correlation between the carbonyl and the α-methylene protons at C-2, α-methine protons at C-4, and methyl protons at C-30. The cyclopropylene protons (H_2-19) were attached to C-9 and C-10 since long-range correlations were observed between these protons and C-9 and C-10. The double bond was assigned to C-25 and C-27 due to long-range correlation between these carbons and the allylic methyl C-26. All long-range correlations observed were consistent with the structure of **1**.

The relative stereochemistry of **1** was deduced from NOESY and is as shown in Fig. 3. The C-30 methyl was close in space to H-5, which was in turn close to C-29

Table 1. 400 MHz ^{1}H NMR and 100MHz ^{13}C NMR of 1 in $CDCl_3$

Position	δC 1	δH mult. (J Hz) 1
1	32.77	1.60, 1.89
2	40.97	2.41 (2H)
3	213.33	---
4	50.00	2.24
5	46.06	1.57
6	25.86	0.73 (2H)
7	25.18	1.32, 1.70
8	47.07	1.63
9	24.96	---
10	29.69	---
11	27.20	1.25, 2.05
12	32.85	1.65 (2H)
13	45.33	---
14	48.78	---
15	35.58	1.29 (2H)
16	28.01	1.32, 1.90
17	52.21	1.60
18	17.88	1.00 (s, Me)
19	26.94	0.39 d (4.0) 0.61 d (4.0)
20	36.00	1.36
21	18.33	0.87 d (6.8, Me)
22	33.88	1.35 (2H)
23	31.46	1.45 (2H)
24	41.60	2.10
25	150.18	---
26	18.64	1.65 (s, Me)
27	109.36	4.67 d (1.6) 4.68 d (1.6)
28	20.16	1.01 d (7.6, Me)
29	19.15	0.89 (s, Me)
30	10.73	0.99 d (6.4, Me)

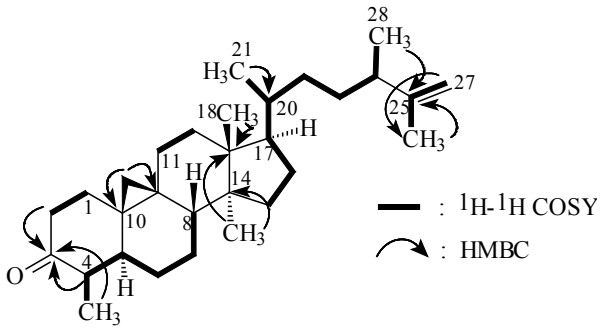

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Figure 2. ^{1}H-^{1}H COSY and Key ^{1}H-^{13}C long-rang range correlations for **1**

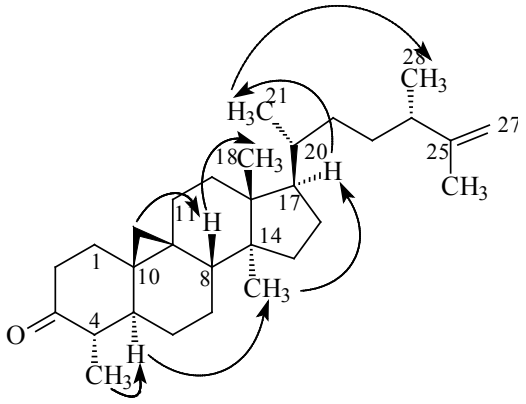

Figure 3. Key NOESY correlations for **1**

methyl, which was close to H-17, which was in turn close to C-21 methyl, which was finally close to C-28 methyl. Thus, they are on the same face of the molecule. On the opposite face of the molecule are the cyclopropylene protons that were close to H-8, which was in turn close to C-18 methyl. Literature search revealed that **1** is 31-norcyclolaudenone, which was reported to have been previously isolated from *Musa sapientum* (Knapp et al. 1970, Desai et al. 1982).

As part of our continuing search for possible antimicrobial compounds, **1** was tested for its antimicrobial potential against the following bacteria: *Bacillus subtilis* and *Staphylococcus aureus* (gram-positive), *Pseudomonas aeruginosa,* and *Escherichia coli* (gram-negative) and fungi: *Candida albicans* (yeast), *Trichophyton mentagrophytes* (parasitic), and *Aspergillus niger* (mold). The agar well method was employed. The microorganisms tested were chosen based on their availability in the culture collection of the University of the Philippines-Natural Sciences Research Institute (UP-NSRI).

Results of the antimicrobial tests (Table 2) indicated that **1** at a concentration of 30 µg was slightly active against the following bacteria: *E. coli* with an activity index (AI) of 0.1, while the standard antibiotic chloramphenicol indicated an AI of 2.8; *P. aeruginosa* with an AI of 0.1, while the standard antibiotic showed an AI of 1.3 and fungi: *C. albicans* with an AI of 0.2, while the standard antibiotic Canesten indicated an AI of 0.8; and *T. mentagrophytes* with an AI of 0.1, while the standard antibiotic chlotrimazole gave an AI of 4.5. Compound **1** was inactive against *B. subtilis, S. aureus,* and *A. niger.*

Table 2. Antimicrobial Test Results on 1

Organism	Sample (30 µg)	Clearing Zone (mm)			Antimicrobial Index (AI)
		Replicate 1	Replicate 2	Replicate 3	
E. coli	**1**	12	11	11	0.1
	Chloramphenicol	23			2.8
P. aeruginosa	**1**	11	11	11	0.1
	Chloramphenicol	14			1.3
S. aureus	**1**	-	-	-	0
	Chloramphenicol	25			3.2
B. subtilis	**1**	-	-	-	0
	Chloramphenicol	20			2.3
C. albicans	**1**	12	12	12	0.2
	Canesten, 0.2 ga	18			0.8
T. mentagrophytes	**1**	12	11	11	0.1
	Canesten, 0.2 ga	55			4.5
A. niger	**1**	-	-	-	0
	Canesten, 0.2 ga	23			1.3

[a] Contains 1% chlotrimazole

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