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IONONE DERIVATIVES FROM *ALTERNANTHERA SESSILIS*

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The chloroform extract of the air-dried leaves of *Alternanthera sessilis* afforded a mixture of diastereomers of a new ionone derivative **1** whose structures were elucidated by extensive 1D and 2D NMR spectroscopy and mass spectrometry. Oxidative cleavage of **1** to aldehyde **3** with manganese dioxide confirmed diastereoisomerism arose from a racemic side chain chiral centre. Antimicrobial tests on the mixture of diastereomers and the derivative indicated that they have low activities against *Pseudomonas aeruginosa* and *Trichophyton mentagrophytes.*

Keywords: *Alternanthera sessilis*; Amaranthaceae; Ionone derivatives; Antimicrobial

INTRODUCTION

Alternanthera sessilis (Linn.) R. Br. is a very common weed found throughout the Philippines. It is used as a medicinal plant for snake bites and as febrifuge, cholagogue, poultice for boils and a wash for the eyes. It is also eaten as a vegetable by mothers to increase the flow of milk [1]. Previous studies on the plant reported the isolation of 3β-O-β-D-glucopyranosyluronic acid and 28-O-β-D-glucopyranosyloleanolic acid [2], a saponin having oleanolic acid as aglycone and glucose and rhamnose as sugar moieties [3], 24-methylene cycloartanol, cycloeucalenol, sterols and their palmitates [4], lupeol [5], α and β spinasterol [6], sterols, hydrocarbon and esters [7] and lipids, fatty acids and tocopherols [8]. The petroleum ether and benzene extracts of *A. sessilis* were found to inhibit the growth of some human and plant pathogenic bacteria [9].

We now report the isolation and structural elucidation of a mixture of diastereomers of a new ionone derivative (**1a** and **1b**) from the leaves of *A. sessilis.*

RESULTS AND DISCUSSION

Analysis of *A. sessilis* chloroform extracts yielded **1.** The ^{1}H and ^{13}C NMR spectra of **1** (Table I) indicated a relatively low molecular weight compound with ketone, alcohol ($\times$ 3;

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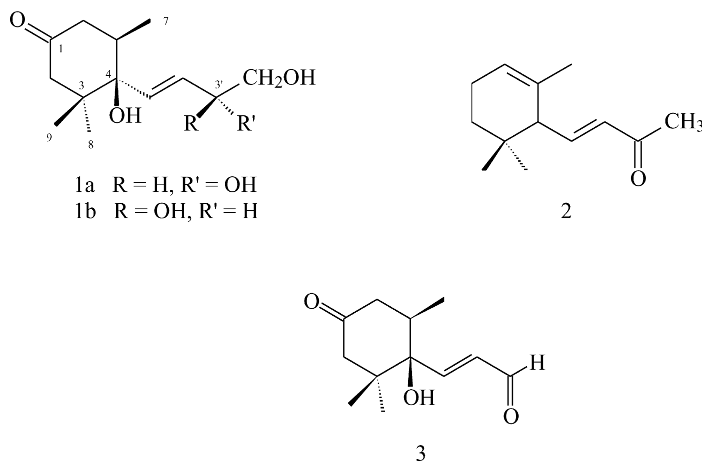

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primary, secondary and tertiary) and disubstituted alkene functionalities. As **1** also showed signals for three methyl groups, terpenoid biosynthesis seemed likely. Careful examination of the ^{1}H and ^{13}C spectra, however, showed that a number of signals appeared to be split into "doublets", though the difference between the "doublets" did not exceed 0.03 ppm in the proton and 0.1 ppm in the carbon spectra. This suggested that **1**, as obtained from *A. sessilis,* is actually a mixture of almost identical diastereoisomers (**1a** and **1b**). We have also recently observed diastereomers in a secoabitane diterpene isolated from *Coleus blumei* [10].

The high resolution MS of **1** gave a molecular ion of *m/z* 242.1518, which corresponded to a molecular formula of $C_{13}H_{22}O_4$, and indicated a possible monocyclic bis-norsesquiterpene. The structure of **1** was determined by 2D NMR analysis as follows. The COSY spectrum indicated three isolated spin systems; an isolated methylene, a methine adjacent to methylene and methyl groups, and a secondary alcohol methine adjacent to the disubstituted double bond and the primary alcohol methylene. The connectivity of these groups was determined by gHMQC and gHMBC spectra (see Table I and Fig. 1), and is as shown in **1**. In particular, the HMBC correlations of the quaternary oxygenated carbon (C-4) to the vinyl protons (H1′/2′) confirmed the attachment of the side chain at C-4. All NMR data is consistent with this structure, which is closely related to the perfume α-ionone (**2**).

The relative stereochemistry of **1** was determined by a combination of coupling constant analyses and NOESY (Fig. 2). The ^{1}H triplet at δ 2.38 (H-6b) is coupled to the geminal proton at δ 2.20 (H-6a) and the methine proton at δ 2.30 (H-5) by 12.1 Hz. The large vicinal coupling constant indicates that H-6b and H-5 are both in axial positions. On the other hand, H-6a is equatorial since the broad doublet indicates very small coupling with H-5. The methyl doublet at δ 0.88 attached to C-5 is therefore in the equatorial position. The NOESY spectrum supported this conclusion as strong correlations are observed between the olefinic proton at δ 5.80 (H-2′) and H-5 and between the methyl singlet attached to C-3 and the olefinic proton at δ 5.82 (H-1′). This indicated that the olefin moiety attached to C-4 is in the

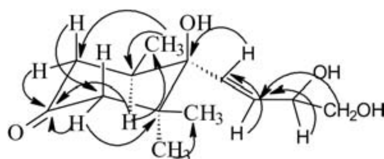

FIGURE 1 HMBC correlations of **1**.

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TABLE I 300 MHz ^{1}H and 75 MHz ^{13}C NMR spectral data of 1 in $CDCl_3$

Position	^{13}C, δ	^{1}H, δ	HMBC correlations
1	211.2	–	H-2a, H-2b, H-5, H-6a, H-6b
2	51.4	2.83 (1H, d, *J*=13.6 Hz), 1.90 (1H, d, *J*=13.6 Hz)	H-6a, H-6b, H-8/9
3	42.5	–	H-2a, H-2b, H-8/9
4	77.3	–	H-2a, H-6a, H-7, H-8/9, H-1’, H-2’
5	36.3*, 36.4*	2.27 (1H, m)	H-6a, H-6b, H-7
6	45.1	2.20 (1H, br, d, *J*=12.1 Hz), 2.38 (1H, t, 12.1 Hz)	H-2a, H-5, H-7
7	15.9	0.87† (3H, d, *J*=6.0 Hz), 0.89† (3H, d, *J*=6.0 Hz)	H-5, H-6a
8	24.4‡	0.94¶ (3H, s)	H-2a, H-2b, H-9
9	24.5‡	0.95¶ (3H, s)	H-2a, H-2b, H-8
1′	134.8*, 134.9*	5.82 (1H, d, *J*=15.8 Hz)	H-2′, H-3′
2′	129.7	5.80 (1H, dd, *J*=4.4, 15.8 Hz)	H-1′, H-4a′, H-4b′
3′	72.6	4.37 (1H, m)	
4′	66.3	3.52 (1H, dd, *J*=7.3, 11 Hz), 3.72 (1H, dd, *J*=3.2, 11 Hz)	

* Carbon doublets due to diastereomerism.
† Proton doublets due to diastereomerism.
‡,¶ May be interchanged.

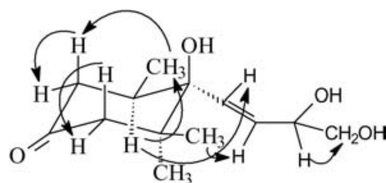

FIGURE 2 NOESY correlations of **1**.

equatorial position and the hydroxyl in the axial position. This is the more stable conformation of **1** since the bulky groups are in the equatorial positions.

Of the three chiral centers in **1**, it did not appear reasonable that inversion of stereochemistry at C-4 or C-5 would produce diastereomeric compounds which differed in ^{13}C chemical shift by less than 0.09 ppm. We therefore reasoned that **1** is an approximately 1:1 mixture of the two diastereomers (**1a** and **1b**) formed by the inversion of the stereochemistry at C-3′. This was confirmed by oxidation at C-3′ of **1** with activated MnO_2 [11]. The product obtained was the aldehyde **3** from oxidative cleavage [12] of the vicinal diol. All NMR data for **3** were consistent with it being a single compound rather than a diastereomeric mixture, thus confirming that isomerism in **1** was due to the essentially racemic C-3′ chiral centre.

Since a previous study reported the antibacterial property of the petrroleum ether and benzene extracts of *A. sessilis* [9], compounds **1** and **3** were tested for possible antimicrobial activity by the agar cup method [13]. Results of the study (Table II) indicated that **1** and **3** were active against the gram-negative bacterium, *P. aeruginosa* and the fungus, *T. mentagrophytes.* Compound **1** showed low activity against *C. albicans* and *A. niger*, while **3** was inactive against these fungi. Both compounds were found inactive against the bacteria, *B. subtilis*, *S. aureus* and *E. coli*.

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EXPERIMENTAL SECTION

General Experimental Procedures

NMR spectra were recorded in $CDCl_3$ on a Bruker AMX at 300 MHz for ^{1}H NMR and 75 MHz for ^{13}C NMR of **1** and Bruker Avance 400 at 400 MHz for ^{1}H NMR and 100 MHz for ^{13}C NMR of **3**. The high resolution EIMS were recorded on a Micromass Autospec mass spectrometer. Column chromatography was performed with silica gel 60 (70–230 mesh); TLC was performed with plastic-backed plates coated with silica gel F_{254}; plates were visualized by spraying with vanillin-H_2SO_4 and warming.

Plant Material

The sample was collected from Baguio City in September 1998. It was identified at the Philippine National Museum as *A. sessilis* (Linn.) R. Br. and a voucher specimen # 037 is kept at the Chemistry Department of De La Salle University.

Extraction and Isolation

The air-dried leaves of *A. sessilis* (300 g) were ground in a blender, then extracted with $CHCl_3$ at room temperature for 2 days. The crude extract (5 g) was dissolved in EtOH, then placed on an ice bath. To the solution was added 4% aqueous $Pb(OAc)_2$ to precipitate the

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TABLE II Antimicrobial test results on **1** and **3**

Sample	Concn. (μg)	*Staphylococcus aureus*		*Escherichia coli*		*Pseudomonas aeruginosa*		*Bacillus subtilis*		*Candida albicans*		*Aspergillus niger*		*Trichophyton mentagrophytes*	
		C.Z.* (mm)	A.I.	C. Z.* (mm)	A.I.	C. Z.* (mm) ± σ	A.I.	C.Z.* (mm)	A.I.	C.Z.* (mm) ± σ	A.I.	C.Z.* (mm) ± σ	A.I.	C.Z.* (mm) ± σ	A.I.
1	70	–	0	–	0	14 ± 0.0	0.4	–	0	12 ± 0.0	0.2	12 ± 0.0	0.2	15 ± 0.9	0.5
3	70	–	0	–	0	13	0.3	–	0	–	0	–	0	14	0.4
Standard Antibiotic	30	30	2.0	30	2.0	20	1.0	40	3.0	25	1.5	16	0.6	38	2.8
		Chloramphenicol		Tetracycline		Tetracycline		Chloramphenicol		Chlotrimazole		Chlotrimazole		Chlotrimazole	

CZ, clear zone; AI, activity index.
* Average of three trials.

more polar components [14]. The mixture was then filtered and the filtrate was concentrated *in vacuo*. The concentrate was extracted with $CHCl_3$, and the extract was dried with anhydrous Na_2SO_4, then filtered. The filtrate was concentrated *in vacuo* to afford the treated extract (1 g) which was subjected to gravity column chromatography (silica gel 60, 70–230 mesh, 40 g) using increasing proportions (10% increment) of Me_2CO in $CHCl_3$ (50 mL) as eluents. The 30% Me_2CO in $CHCl_3$ fraction was again chromatographed (3 ×) in 10% Me_2CO in $CHCl_3$ to afford a mixture of **1a** and **1b** (3.5 mg) after washing with Et_2O. IR ν_{max} (film) cm^{-1}: 3389 (OH), 2961, 2922, 1697 (C=O), 1650 (C=C), 1281, 1078, 1033, 981, 911, 736; ^1H and ^{13}C NMR data are listed in Table I; EIMS *m/z*202.4 (18), 193.1 (14), 157.1 (13), 144.1 (42), 140.1 (34), 126.1 (55), 127.1 (38), 113.1 (100), 109.1 (46), 97.1 (50), 95.0 (46); HREIMS *m/z* 242.151777 (calcd for $C_{13}H_{22}O_4$, 242.151809).

Oxidation

The mixture of diastereoisomers of **1** (1.5 mg) in chloroform (0.5 mL) was stirred overnight with activated MnO_2 (20 mg) prepared [11] from $KMnO_4$ and $MnCl_2$. The product aldehyde **3** (1 mg) was purified by passing the reaction product through a small silica gel column eluted with $Me_2CO/CHCl_3$ (1:1). IR ν_{max} (film) cm^{-1}: 3417 (OH), 2972, 2923, 2851, 1698 (C=O), 1668 (C=C), 1455, 1114, 1024, 991, 824, 732; ^1H NMR ($CDCl_3$) δ 0.89 (3H, d, $J = 7.6$ Hz, H-7), 0.98 (3H, s, H-8), 1.04 (3H, s, H-9), 1.86 (1H, s, OH), 1.99 (1H, dd, $J = 2$, 13.8 Hz, H-2a), 2.28 (1H, m H-6a), 2.42 (2H, m, H-5, -6b), 2.85 (1H, d, $J = 13.8$ Hz, H-2b), 6.45 (1H, dd, $J = 7.6$, 15.8 Hz, H2′), 6.87 (1H, d, $J = 15.8$ Hz), 9.66 (1H, d, $J = 7.6$ Hz); ^{13}C NMR ($CDCl_3$) δ 15.9 (C-7), 24.4 (C-8), 24.5 (C-9), 36.1 (C-5), 42.8 (C-3), 44.7 (C-6), 51.1 (C-2), 78.4 (C-4), 133.3 (C-2′), 159.1 (C-1′), 192.2 (C-3′), 213.2 (C-1); EIMS *m/z* 210 (4), 154 (100), 126 (23), 112 (84), 111 (38), 97 (66), 95 (21); HREIMS *m/z* 210.1250 (calcd for $C_{12}H_{18}O_3$, 210.1256).

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Antimicrobial Test

The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These are *Pseudomonas aeruginosa* UPCC 1244, *Bacillus subtilis* UPCC 1149, *Escherichia coli* UPCC 1195, *Staphylococcus aureus* UPCC 1143, *Candida albicans* UPCC 2168, *Trichophyton mentagrophytes* UPCC 4193 and *Aspergillus niger* UPCC 3701.

Microbial suspension containing approximately 6×10^8 cells/mL was prepared from each test organism for 24-h culture of *S. aureus*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *C. albicans* and from a 5-day-old *A. niger* and *T. mentagrophytes*. The suspending medium used for each microbial suspension was 0.1% peptone water. One-tenth (0.1) mL of the bacteria, yeast and molds were transferred into pre-poured nutrient agar (NA, DISCO Laboratories, Detroit, Michigan), glucose yeast peptone agar (GYP) [15] and potato dextrose agar (PDA, DISCO Laboratories, Detroit, Michigan), respectively. About 5 mL of corresponding medium, autoclaved and cooled to about 45°C was poured into the 90 mm petri dish. The plate was swirled to distribute the microbial cells evenly on the plate and the agar overlay was allowed to solidify. Three 10 mm wells were cut from equidistant points of the seeded agar plates using sterile cork borer. Seventy (70) μg of samples dissolved in 95% EtOH were transferred in each well. For the standard agent, 30 μg were used.

The NA, GYP and PDA-based cultures were incubated at 30 ± 1°C for 24, 48 and 72 h, respectively. Antimicrobial effects were determined by measuring the zone of the growth inhibition represented by a clear zone in mm. The average diameter of the clear zones was used to calculate an antimicrobial index [13].

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