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Research Note

Antimicrobial Terpenoids from *Lansium domesticum*

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The air-dried fruit peel of *Lansium domesticum* Correa afforded five onoceroid triterpenes: 3β-hydroxyonocera-8(26),14-dien-21-one (1), α,γ-onoceradienedione (2), lansiolic acid (3), lansionic acid (4) and lansioside C (5), while the air-dried seeds afforded 3 and germacrene D (6). Antimicrobial tests on 1-6 gave the following results. 2 has high activity against *P. aeruginosa*, while 1, 3, 4 and 5 have moderate activities against this microorganism. 5 and 4 have moderate and low activities against *B. subtilis,* respectively, while 3 and 5 have low activities against *S. aureus.* All the compounds tested have moderate activities against *C. albicans* and *A. niger*, and low activities against *T. mentagrophytes*.

Key Words: *Lansium domesticum*, Meliaceae, lansones, antifungal, antibacterial

Abbreviations: HMBC - heteronuclear multiple bond coherence, HSQC - heteronuclear multiple quantum coherence, NMR - nuclear magnetic resonance, NOESY - nuclear Overhauser effect spectrometry

INTRODUCTION

As part of our continuing investigations of medicinal plants currently in use as folk remedies in the Philippines, we investigated *Lansium domesticum,* commonly known as lansones. It is cultivated for its fruit which is sold commercially. The fruit peel is dried, then burned to drive away mosquitoes. The fruit skin is used as an arrow poison. The seeds are used as febrifuge, vermifuge, antipyretic and anthelmintic (Quisumbing 1978).

Earlier studies on *Lansium domesticum* reported the isolation of onoceroid triterpenes (Tanaka et al. 2002, Nishizawa et al. 1984), lansiosides (Nishizawa et al. 1982, 1983) and lansic acid (Nishizawa et al. 1986). Lansionic acid and 3β-hydroxyonocera-8(26),14-dien-21-one exhibited mild toxicity against brine shrimp (*Artemisia salina*) (Tanaka et al. 2002), while lansiolic acid was reported to have insect feeding deterrent activity as well as antimalarial activity (Arnason et al. 2004). Lansioside A effectively inhibited the leukotriene D4 induced contraction of guinea pig ileum in 2.4 ppm concentration (Nishizawa et al. 1983). The α-reductase inhibitors lansic acid and lansiosides A-C are effective in controlling male hormone-type baldness, acne and prostate hypertrophy (Miyamoto and Hamanaka 1985).

We report here the isolation, identification and antimicrobial assay of five onoceroid triterpenes (**1**-**5)** from the fruit peel, and **3** and a sesquiterpene (**6**) from the seeds of *L. domesticum*. To the best of our knowledge, this is the first report on the antimicrobial activities of **1**-**5**.

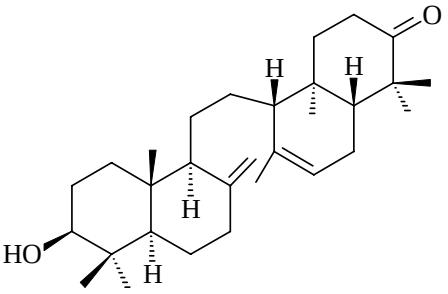
MATERIALS AND METHODS

General Experimental Procedures

Nuclear magnetic resonance (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 fruits of *Lansium domesticum* were obtained from the province of Laguna in August. They were identified as *Lansium domesticum* Correa at the Philippine National Museum and a voucher specimen # 80 is kept at the Chemistry Department, De La Salle University.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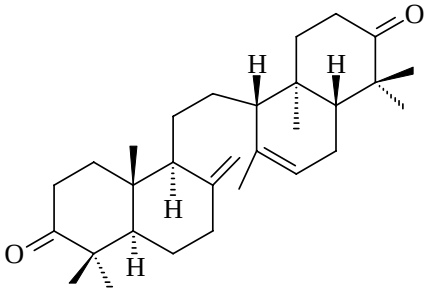
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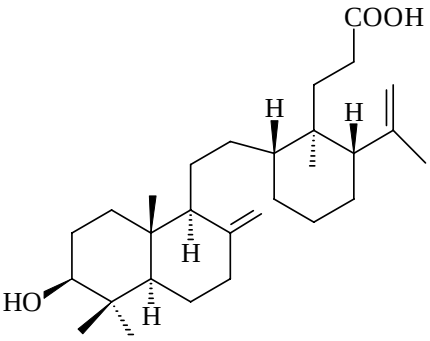
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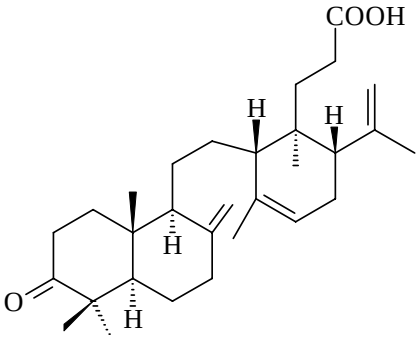
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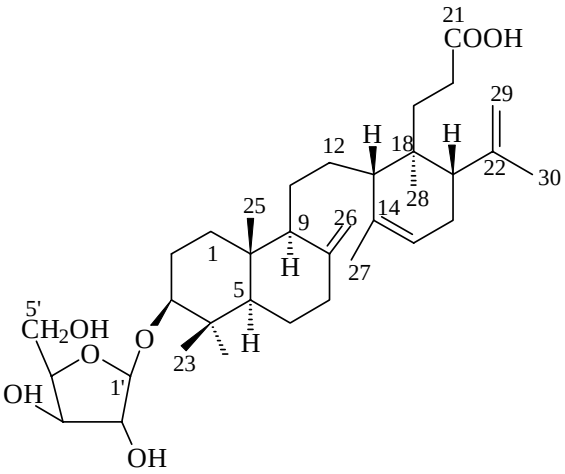
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12
H
18
H
22
30
28
25
9
26
14
1
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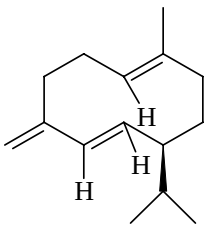
27
5
5'
CH2OH
O
O
23
H
OH
1'
OH

5


H
H
H

6

Isolation

The fruit peel of *L. domesticum* were air-dried (480 g) and ground in an osterizer, soaked in dichloromethane for 3 d and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (84 g) which was chromatographed on silica gel with increasing proportions of acetone in dichloromethane (10% increments) as eluents. The dichloromethane and 10% acetone in dichloromethane fractions were combined, then rechromatographed in 10% ethyl acetate in petroleum ether. Fractions 1-5 were rechromatographed (2x) in 10% ethyl acetate in petroleum ether to afford **2** (25.6 mg). Fractions 10-14 were rechromatographed (3x) in 15% ethyl acetate in petroleum ether to afford **1** (7.6 mg). Fractions 20-25 were rechromatographed (3x) in 15% ethyl acetate in petroleum ether (2x) to afford **4** (10 mg). The 40 % acetone in dichloromethane fractions were rechromatographed (4x) in diethyl ether: acetonitrile: dichloromethane (1.5:1.5:7) to afford **3** (5.2 mg). The 90 % acetone in dichloromethane and acetone fractions were rechromatographed (5x) in 45% acetone in dichloromethane to afford **5** (14.1 mg) after repeated trituration with petroleum ether.

The seeds of *L. domesticum* were air-dried (130 g) and ground in an osterizer, soaked in dichloromethane for 3 d and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (4.5 g) which was chromatographed on silica gel with increasing proportions of acetone in dichloromethane (10% increments) as eluents. The dichloromethane fraction was rechromatographed (3x) in petroleum ether to afford **6** (11.4 mg). The 40 % acetone in dichloromethane fractions were rechromatographed (5x) in diethyl ether: acetonitrile: dichloromethane (1.5:1.5:7) to afford **3** (2.0 mg).

Antimicrobial Tests

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 5 d-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, DIFCO Laboratories, Detroit, Michigan), glucose yeast peptone agar (GYP) (Kreger-van Rij 1984) and potato dextrose agar (PDA, DIFCO Laboratories, Detroit, Michigan), respectively. About 5 mL of corresponding medium, autoclaved and cooled to about 45 ^{0}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. Thirty (30) 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 ^{0}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.

RESULTS AND DISCUSSION

Five onoceroid triterpenes: 3β-hydroxyonocera-8(26),14-dien-21-one (**1**), α,γ-onoceradienedione (**2**), lansiolic acid (**3**), lansionic acid (**4**), and lansioside C (**5**) were isolated from the air-dried peel of *L. domesticum*, while the air-dried seeds afforded germacrene D (**6**) and **3**. The structures of **1-4** and **6** were deduced by comparison of their ^{1}H and ^{13}C NMR spectral data with those reported in the literature for 3β-hydroxyonocera-8(26),14-dien-21-one (Tanaka et al. 2002), α,γ-onoceradienedione (Nishizawa et al. 1984), lansiolic acid, lansionic acid (Tanaka et al. 2002) and germacrene D (Nishizawa et al. 1986), respectively. Only the structure of **5** in $CDCl_3$ was elucidated by 1D and 2D NMR spectroscopy as follows.

The ^{1}H NMR spectrum of **5** gave resonances for carbinyl protons at δ 3.21 (H-3), 4.50 (H-1'), 3.50 (H-2'), 3.61 (H-3'), 3.72 (H-4'), 3.37 (H-5'a) and 4.06 (H-5'b); olefinic protons at δ 4.57 (H-26a) and 4.79 (H-26b), 5.36 (H-15), 4.83 (H_2-29); methyl singlets at δ 0.80 (H-23), 1.00 (H-24), 0.67 (H-25), and allylic methyl singlets at δ 1.72 (H-27) and 1.74 (H-30).

The ^{13}C NMR spectrum gave resonances for the carbinyl carbons at δ 89.7 (C-3), 72.7 (C-2'), 74.6 (C-3'), 69.7 (C-4'), 64.3 (C-5') and an anomeric carbon at δ 104.6 (C-1'); olefinic carbons at δ 148.0 (C-8), 106.8 (C-26), 136.0 (C-14), 121.6 (C-15), 147.7 (C-22) and 113.9 (C-29). The rest of the carbon resonances accounted for the remaining methyl, methylene, and methine carbons in the molecule.

The ^{1}H and ^{13}C assignments of **5** (Table 1) were verified by HSQC and their connectivities were verified by HMBC (Table 1). The glycoside was attached to C-3 due to long-range correlations between the carbinyl carbon at δ 89.7 (C-3) and the anomeric proton at δ 4.50 (H-1'), and the anomeric carbon at δ 104.6 (C-1') and the carbinyl proton at δ 3.21 (H-3). An allylic methyl was located at C-30 since long-range correlations were observed between the carbon at δ 22.8 (C-30) and the methylene olefinic protons at δ 4.83 (H_2-29). The other allylic methyl was placed at C-27

Table 1. 400 MHz ^{1}H NMR and 100MHz ^{13}C NMR, HMBC and NOESY correlations of 5 in $CDCl_3$

Position	δ_C	δ_H mult. (J Hz)	HMBC Correlations	NOESY Correlations
1	37.1	0.65		
2	26.5	1.70, 1.90		
3	89.7	3.21 (dd, 3.3, 11.7)	H-23, H-24, H-1'	H-5, H-23, H-24
4	39.1		H-23, H-24	
5	54.8	1.20	H-23, H-24, H-25	
6	23.8	1.90		
7	38.0	2.30	H-26a, H-26b	
8	148.0		H-9	
9	58.1	1.50	H-25, H-26a, H-26b	
10	39.3		H-23, H-24, H-25	
11	26.3	1.70		
12	27.4	1.21		
13	48.4	1.80	H-28	
14	136.0		H-27	
15	121.6	5.36 (s, br)	H-27	H-27, H-16
16	29.6	1.68, 2.19		
17	49.2	2.20	H-30	
18	38.7			
19	32.5	1.70		
20	28.3	1.68		
21	178.1			
22	147.7		H-30	
23	16.1	0.80 (s, Me)		
24	28.3	1.00 (s, Me)		
25	14.6	0.67 (s, Me)		
26	106.8	4.57 (s, br),	H-26b	
		4.79 (s, br)	H-26a	
27	22.85	1.72 (s, Me)		
28	16.4	0.80 (s, Me)		
29	113.9	4.83 (2H, s, br)	H-30	
30	22.8	1.74 (s, Me)		
1'	104.6	4.50 (d, 5.7)	H-2', H-3	H-2'
2'	72.7	3.50 (t, 6.3)		H-1', H-3'
3'	74.6	3.61 (t, 7.2)		H-2', H-4'
4'	69.7	3.72 (m)		H-3', H-5a', H-5b'
5'	64.3	3.37 (dd, 8.4, 11.4)		H-4', H-5b' H-4', H-5a'
		4.06 (dd, 3.3, 11.1)		

due to long-range correlations between the methyl protons at δ 1.72 (H-27) and the olefinic carbons at δ 136.0 (C-14) and 121.6 (C-15). All long-range correlations observed are consistent with the structure of **5**.

The relative stereochemistry of **5** was deduced from NOESY (Table 1) as follows. The resonance at δ 4.06 (H-5'b) is close to δ 3.37 (H-5'a) and 3.72 (H-4') which, in turn, is close to δ 3.61 (H-3'), in turn close to δ 3.50 (H-2'), which is finally close to the anomeric proton at δ 4.50 (H-1') . This anomeric proton is close to the carbinyl proton at δ 3.21 (H-3) of the triterpene which, in turn, is close to the methine proton at δ 1.20 (H-5). Thus, **5** was identical to the structure of lansioside C (Nishizawa et al. 1983).

As part of our continuing studies on potential antimicrobial compounds from Philippine medicinal plants, the antimicrobial potential of **1-6** was tested by the use of the agar well method. Results of the tests are presented in Table 2. Compound **2** indicated high activity against *P. aeruginosa*. The rest of the compounds tested, except **6**, have moderate activities against this microorganism. All the compounds tested are inactive against *E. coli*. Compounds **1**, **2** and **6** are inactive against *S. aureus* and *B. subtilis*, **3** and **5** have low activities against *S. aureus*, while **5** has moderate activity against *B. subtilis*. Compound **4** has low activity against *B. subtilis* and is inactive against *S. aureus.* All the compounds tested have moderate activities against the fungi (*C. albicans* and *A. niger*) and low activities against the fungus (*T. mentagrophytes*). Although the compounds tested have antimicrobial activities, they have lower activities than the standard antibiotics against the microorganisms tested.

CONCLUSION

The fruit peel of *L. domesticum* afforded five onoceroid triterpenes (**1-5**), while the air-dried seeds afforded **3** and a

Table 2. Antimicrobial test results on 1-6.

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)	A.I.	C.Z.* (mm)	A.I.	C.Z.* (mm)	A.I.	C.Z.* (mm)	A.I.	C.Z.* (mm)	A.I.
1	60	-	0	11	0.1	12	0.2	-	0	14	0.4	15	0.5	13	0.3
2	60	-	0	-	0	13	0.3	-	0	13	0.3	12	0.2	13	0.3
3	60	12	0.2	11	0.1	12	0.2	13	0.3	14	0.4	14	0.4	14	0.4
4	60	-	0	12	0.2	12	0.2	13	0.3	12	0.2	14	0.4	15	0.5
5	60	19	0.9	12	0.2	12	0.2	26	1.6	13	0.3	14	0.4	20	1.0
6	60	-	0		0.2	11	0.1	-	0	14	0.4	13	0.3	13	0.3
Standard Antibiotic		25 Chloramphenicol[a]	3.2	23 Chloramphenicol[a]	2.8	8 Chloramphenicol[a]	0.3	20 Chloramphenicol[a]	2.3	10 Chlotrimazole[b]	0.7	10 Chlortrimazole[b]	0.7	50 Chlortrimazole[b]	7.3

C.Z. - clear zone, *Average of three trials, A.I. - activity index, [a]chloramphenicol at 30 µg, [b]chlotrimazole at 5

sesquiterpene (**6**) which were tested for antimicrobial properties. Results of the study indicated that **2** has the same activity as the standard antibiotic, chloramphenicol against *P. aeruginosa*. The rest of the triterpenes have moderate activities against *P. aeruginosa*, while **5** has moderate activity against *B. subtilis*. All the compounds tested have moderate antifungal activities against *C. albicans* and *A. niger*. Thus, these triterpenes can prevent infections caused by these microorganisms.

ACKNOWLEDGMENT

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