Monoterpene Lactones from the Seeds of *Nephelium lappaceum*[§]

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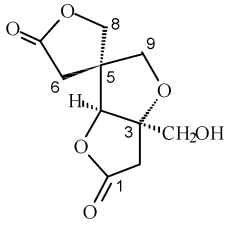
Dichloromethane extracts of the seeds of *Nephelium lappaceum* afforded two new diastereomeric monoterpene lactones, **1** and **2**, and the known butenolide siphonodin (**3**), as well as kaempferol 3-*O*-*β*-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside. Compounds **1** and **2** represent a new monoterpene skeleton, and their structures were elucidated by extensive 1D and 2D NMR spectral data interpretation. Antimicrobial testing was carried out on **1** and **2** against a panel of bacteria and fungi.

Nephelium lappaceum L. (Sapindaceae), commonly known as "rambutan", is cultivated in the Philippines and other tropical regions for its fruit, which is sold commercially. The seeds are bitter and narcotic, with the roots used for treating fevers, the leaves as poultices, and the bark as an astringent. The fruit is recommended for severe dysentery and as an astringent, an antifebrile, and a warm carminative in dyspepsia.[1] Chemical studies on the fruit have led to the identification of volatile constituents.[2,3] The seeds of the plant afforded type II cyanolipids, which were found to have insecticidal activity.[4,5]

We report here the isolation, structure elucidation, and antimicrobial testing of **1** and **2** from the seeds of *N. lappaceum*. These lactones represent a new monoterpene skeleton. Siphonodin (**3**)[6] and kaempferol 3-*O*-*β*-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside[7] were also obtained from the seeds of the plant.



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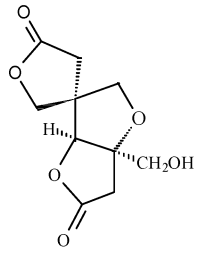

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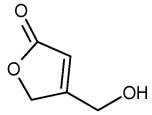

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Seeds from commercial *N. lappaceum* fruits were air-dried, ground, and extracted with dichloromethane. Subsequent silica gel chromatography afforded two new monoterpene lactones (**1** and **2**) in a 10:7 mixture, along with the known butenolide siphonodin (**3**). The mixture of **1** and **2** was separated into the individual diasteroisomers by HPLC on a Chirobiotic T column.

Table 1. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) Spectroscopic Data for **1** and **2** ($CDCl_3$)

	1		**2**	
position	δ_H	δ_C	δ_H	δ_C
1		173.3		173.4
2	2.70 d (19.2), 2.79 d (19.2)	37.0	2.68 d (19.2), 2.82 d (19.2)	36.9
3		89.0		89.2
4	4.78 (br s)	87.7	4.78 (br s)	88.6
5		51.6		50.9
6	2.42 d (18.4), 3.02 d (18.4)	31.7	2.61 d (18.0), 2.75 dd (18.0, 1.2)	38.3
7		174.2		174.4
8	4.26 d (9.6), 4.34 dd (9.6, 1.2)	74.6	4.15 d (10.4), 4.63 d (10.4)	69.0
9	3.80 dd (9.6, 1.2), 4.12 d (9.6)	74.1	3.85 d (9.6, 1.2), 4.05 d (9.6)	74.6
10	3.72 (11.6), 3.86 (11.6)	64.5	3.73 (11.6), 3.83 (11.6)	64.3

The ^{1}H NMR (Table 1) and 2D HSQC spectra of the major lactone isomer **1** indicated resonances for five sets of methylene protons (*δ* 2.42/3.02, 2.70/2.79, 3.72/3.86, 3.80/4.12, and 4.34/4.26) and a methine proton at *δ* 4.78 (br s). Unusually, no vicinal couplings were present, but small *W*-couplings were observed between the protons resonating at *δ* 4.74 and 4.12, and *δ* 4.34 and 3.80. The ^{13}C NMR (Table 1) and DEPT spectra indicated 10 carbons for a possible monoterpene: two carbonyl carbons of esters (*δ* 173.3 and 174.2); two nonoxygenated (*δ* 31.7 and 37.0) and three oxygenated (*δ* 74.6, 74.1, and 64.5) methylene carbons; one oxygenated methine carbon (*δ* 87.7); one nonoxygenated (*δ* 51.6) and one oxygenated (*δ* 89.0) quaternary carbon.

The HREIMS of **1** gave a molecular ion of *m/z* 228.0634, which corresponded to a molecular formula of $C_{10}H_{12}O_6$. The molecular formula indicated an index of hydrogen deficiency of five. With two double bonds deduced from the ester carbonyls, the compound is tricyclic. The structure of **1** was elucidated by analysis of the HMBC 2D NMR data with key HMBC correlations shown in Figure 1. The quaternary carbon signal at *δ* 51.6 was assigned to C-5 by long-range correlations to the three methylene protons (H_2-6, H_2-8, H_2-9). The second quaternary carbon at *δ* 89.0 was assigned to C-3 by long-range correlations to the methine protons (H-4, H-9′) and methylene protons (H_2-10). All other long-range correlations observed were consistent with the structure of **1**.

[§] This paper is dedicated to Bro. Andrew Gonzalez, FSC, president emeritus of De La Salle University, on the occasion of his retirement, for his vision in bringing DLSU to research university status.

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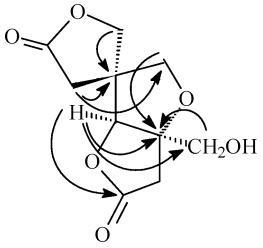

Figure 1. Key $^{1}H-^{13}C$ HMBC long-range correlations for **1**.

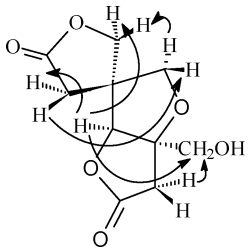

Figure 2. Key NOESY NMR correlations for **1**.

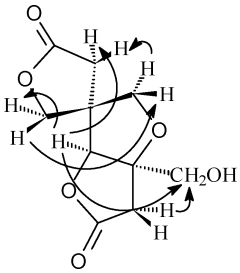

Figure 3. Key NOESY NMR correlations for **2**.

The stereochemistry of **1** was determined by an analysis of its NOESY 2D NMR data. Proton H-2′ is close to H_2-10, which is in turn close to H-4, which is also near H-8′, indicating that these protons are on the same face of **1**. It was found that H-8 is on the same face as H-9′, while H-9 is on the same face as H-6. Thus, the relative stereochemistry of **1** is as drawn in Figure 2. The trivial name lappaceolide A is suggested for the compound.

Compound **2** was obtained as a diastereomer of **1**. Table 1 presents the ^{1}H NMR and ^{13}C NMR spectroscopic data of **2**. The principal difference between these two compounds is that C-6 (δ 31.7) of **1** is shielded, whereas C-8 (δ 69.0) of **2** is shielded, owing to the proximity to the lower lactone oxygen. The remaining ^{13}C NMR resonances in both compounds were quite similar. The stereochemistry of **2** was confirmed by an analysis of NOESY 2D NMR data. Proton H-2′ is close to H-10, which is in turn close to H-4, which also correlated with H-8′ and H-6′, indicating that these protons are on the same face of **2**. H-8 correlated with H-9, and H-9′ with H-6. Thus, the relative stereochemistry of **2** (lappaceolide B) is as drawn in Figure 3.

It is proposed that the diastereomers **1** and **2** may result from dimerization of **3**, which was also present in the seed extract of *N. lappaceum*. Attack from C-4 of **3** onto the α,β-unconjugated system of a second butenolide molecule, followed by conjugate addition of the alcohol functional group from the second butenolide back onto the first, would result in either **1** or **2**. This raises the possibility that **1** and **2** are artifacts of the isolation, but this appears unlikely, since the diastereomeric mixture of **1** and **2** and the individual isomers are all optically active.

The antimicrobial potential of **1** and **2** was tested using an agar well method. Results of the study (Table 2) indicated that they were active against *C. albicans* with activity index (AI) of 0.3 at 30 μg, while the standard antibiotic Canesten at 0.2 g (1% chlotrimazole) gave an AI of 0.8. Compared to the standard antibiotic, **1** and **2** were relatively inactive against the bacteria (*E. coli, P. aeruginosa, S. aureus, B. subtilis*) and fungi (*C albicans*, *T. mentagrophytes, A. niger*) investigated.

Table 2. Antimicrobial Test Results of **1** and **2**

organism	sample	quantity (μg)	zone of inhibition[b] (mm)	AI
E. coli	**1**	30	11	0.1
	2	30	11	0.1
	chloramphenicol	30	23	2.8
P. aeruginosa	**1**	30	12	0.2
	2	30	12	0.2
	chloramphenicol	30	14	1.3
S. aureus	**1**	30	11	0.1
	2	30	11	0.1
	chloramphenicol	30	25	3.2
B. subtilis	**1**	30		0
	2	30		0
	chloramphenicol	30	20	2.3
C. albicans	**1**	30	13	0.3
	2	30	13	0.3
	Canesten	0.2 g[a]	18	0.8
T. mentagrophytes	**1**	30	14	0.4
	2	30	13	0.3
	Canesten	0.2 g[a]	55	4.5
A. niger	**1**	30	12	0.2
	2	30	11	0.1
	Canesten	0.2 g[a]	23	1.3

[a] Contains 1% chlotrimazole. [b] Average of three trials.

Experimental Section

General Experimental Procedures. Melting points were measured on a Fisher-Johns melting point apparatus. Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 Fourier transform IR spectrometer. NMR spectra were recorded on a Bruker Avance 400 in $CDCl_3$ at 400 MHz for ^{1}H and 100 MHz for ^{13}C. The high- and low-resolution EIMS were recorded on a Micromass Autospec mass spectrometer. Column chromatography was performed with silica gel 60 (70−230 mesh), while TLC was performed with plastic-backed plates coated with silica gel F_{254}. The plates were visualized with vanillin-H_2SO_4 and warming. HPLC was performed on a Waters 501 pump with a Gilson 132 RI detector and an Astec Chirobiotic T column (250 × 4.6 mm) in ethyl acetate solvent at 0.5 mL/min flow rate.

Plant Material. Air-dried fruit peel and seeds of *Nephelium lappaceum* were obtained at Laguna, Philippines, in June 2004. The plant was identified at the Philippine National Museum by Noe B. Gapas, and a voucher specimen (#85) is kept at the Chemistry Department, De La Salle University.

Extraction and Isolation. The air-dried seeds (980 g) of *N. lappaceum* were ground in an osterizer, soaked in dichloromethane for 3 days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (300 g), which was chromatographed on silica gel using increasing proportions of acetone in dichloromethane (10% increments) as eluents. The 70% to 90% acetone in dichloromethane fractions were combined and rechromatographed (3×) in diethyl ether−acetonitrile−dichloromethane (2:2:6) to afford **1**−**3**. The mixture was triturated several times with petroleum ether, then rechromatographed in diethyl ether−acetonitrile−dichloromethane (2:2:6). This procedure was repeated (7×) to afford **3** (colorless oil, 1 mg) and a mixture of **1** and **2** (50 mg). The mixture of **1** and **2** was separated into the individual diastereomers by HPLC on a Chirobiotic T column to afford **1** (2.5 mg) and **2** (3.5 mg).

In a separate extraction procedure, seeds (850 g) of *N. lappaceum* were ground, soaked in acetone for 3 days, and then

filtered. After solvent removal, the crude acetone extract was partitioned between hexane and MeOH. The residue from the MeOH fraction was recrystallized from aqueous MeOH to yield kaempferol 3-*O*-β-D-glucopyranoside-7-*O*-α-L-rhamnopyranoside (yellow powder, mp 265−266 °C, 4.1 g).

Lappaceolide A (1): colorless needles ($CHCl_3$); mp 123−124 °C; $[\alpha]_D$ −10° (*c* 0.001, acetone); IR (neat) ν_{max} 3440 (br, OH), 1775 (ester), 1183, 1020 (C−O) cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 228 [M^+] (2), 197 (100), 170 (12), 153 (9), 140 (7), 123 (10), 111 (8); HREIMS *m/z* 228.0636 [M^+] ($C_{10}H_{12}O_6$ requires 228.0634).

Lappaceolide B (2): colorless needles ($CHCl_3$); mp 124−125 °C; $[\alpha]_D$ +16° (*c* 0.003, acetone); ^1H NMR and ^{13}C NMR data, see Table 1; EIMS *m/z* 228 [M^+] (75), 210 (27), 197 (100), 170 (5), 153 (9), 140 (7), 123 (10), 111 (10); HREIMS *m/z* 228.0629 [M^+] ($C_{10}H_{12}O_6$ requires 228.0634).

Antimicrobial Tests. The microorganisms used in these tests were obtained from the University of the Philippines Culture Collection (UPCC). These were *Escherichia coli* UPCC 1195, *Pseudomonas* aeruginosa UPCC 1244, *Staphylococcus aureus* UPCC 1143, *Bacillus subtilis* UPCC 1295, *Candida albicans* UPCC 2168, *Trichophyton mentagrophyte* UPCC 4193, and *Aspergillus niger* UPCC 4219. The test compounds were dissolved in 95% ethanol. An antimicrobial assay procedure reported in the literature[8] was employed.

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Supporting Information Available: NMR spectra for compounds **1** and **2**. This information is available free of charge via the Internet at http://pubs.acs.org.

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