

## Chromomoric Acid Derivatives from *Tectona philippinensis*

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Received August 15, 2007

The air-dried leaves of *Tectona philippinensis*, an endemic and endangered Philippine medicinal tree, afforded four new chromomoric acid derivatives (**1**, **2**, **3a**, and **3b**). Their structures were elucidated by extensive 1D and 2D NMR spectroscopy. Antimicrobial testing was carried out on **1–3** against a panel of bacteria and fungi.

*Tectona philippinensis* Benth. & Hook. represents one of only three species of the genus *Tectona* (family Verbenaceae). This hardwood tree species, locally known as Philippine teak, is endemic to the Philippines, where it occurs in only three locations, namely, the San Juan and Lobo regions of Batangas, and Ilin Island, Occidental Mindoro. Its wood is valued in the construction industry, and unabated cutting for this purpose has resulted in the Philippine teak being listed as endangered.<sup>1</sup> A conservation program for this tree, however, is now in place for the Batangas population.<sup>2</sup> Similar efforts have also been initiated in Mindoro, where its population is more fragmented and of much lower count.<sup>3</sup>

Wood and leaves of this tree are also of medicinal value. The charred wood, soaked in poppy juice and made into a paste, was used to relieve the swelling of eyelids, while a decoction of the leaves is used to treat blood-related disorders.<sup>4</sup> To date, there is no reported chemical study on the plant.

We report here the isolation and structure elucidation of four new chromomoric acid derivatives (**1**, **2**, **3a**, and **3b**) from the leaves of *T. philippinensis* from Ilin Island, Mindoro. Chromomoric acids are a group of natural products that are metabolites of linolenic acid and structurally resemble prostaglandins. They were previously reported as constituents of *Chromolaena morii*,<sup>5</sup> *C. chasleae*,<sup>6</sup> *Schistostephium*,<sup>7</sup> *Montanoa*,<sup>8</sup> and *Inulanthera calva*.<sup>9</sup> These compounds were also reported to possess antibiotic and antihypertensive activities.<sup>10</sup> They bear structural resemblance to marine prostanoid clavulones, which exhibit antitumor and anti-inflammatory activity.<sup>11,12</sup>

Silica gel chromatography of the dichloromethane extract of *T. philippinensis* afforded four new chromomoric acid derivatives. The <sup>1</sup>H NMR spectrum of **1** (Table 1) indicated resonances for an allylic methyl group at  $\delta$  1.96, olefinic protons at  $\delta$  7.06, 6.23, 6.21, and 7.59, and an oxymethine proton at  $\delta$  4.45. The remaining resonances were attributed to methine and methylene protons in **1**.

The <sup>13</sup>C NMR spectrum of **1** (Table 1) gave resonances for 18 carbons with the following functionalities: conjugated ketone carbonyls at  $\delta$  200.94 and 204.79; a lactone carbonyl at  $\delta$  177.09; vinylic carbons at  $\delta$  145.76, 131.07, 131.60, and 164.80; an oxymethine carbon at  $\delta$  80.05; an oxygenated quaternary carbon at  $\delta$  79.69; a methine carbon at  $\delta$  56.17; and seven methylene carbons.

The HRESIMS of **1** gave a pseudomolecular ion of  $m/z$  343.1521 [M + Na]<sup>+</sup>, which corresponded to a molecular formula of C<sub>18</sub>H<sub>24</sub>O<sub>5</sub>. The molecular formula indicated an index of hydrogen

deficiency of seven. With five double bonds deduced from the three carbonyls and two olefins, the compound is bicyclic.

The COSY spectrum of **1** indicated four isolated spin systems as follows: a methyl group on a disubstituted double bond, H<sub>3</sub>-18/H-17/H-16; a methine adjacent to a methylene, H-13/H<sub>2</sub>-14; an isolated disubstituted double bond, H-10/H-11; and a chain of six methylenes and one oxymethine, H<sub>2</sub>-2/H<sub>2</sub>-3/H-4/H<sub>2</sub>-5/H<sub>2</sub>-6/H<sub>2</sub>-7/H<sub>2</sub>-8 (Figure 1).

The <sup>1</sup>H and <sup>13</sup>C NMR connectivities in **1** were verified by HSQC. The structure of **1** was elucidated by analysis of the HMBC 2D NMR data with key HMBC correlations shown in Figure 1. The allylic methyl group (H<sub>3</sub>-18) was long-range correlated to C-17 and C-16, while the vinylic protons (H-17 and H-16) were correlated to the conjugated carbonyl (C-15). Long-range correlations were also observed between H-13 and the conjugated carbonyl (C-12) and the vinylic carbon (C-11). The methylene protons (H<sub>2</sub>-14 and H<sub>2</sub>-8) and the vinylic proton (H-11) were correlated to the methine carbon (C-13). The vinylic protons (H-11 and H-10) were long-range correlated to the quaternary oxygenated carbon (C-9). Correlations were also obtained between the oxymethine proton (H-4) and the lactonic carbonyl (C-1). All long-range correlations were consistent with the structure of **1**.

The relative configuration (Figure 2) of **1** was deduced from NOESY. The methyl protons (H<sub>3</sub>-18) were close in space to the vinylic proton (H-16); thus the double bond was *E*-configured. This was supported by the large coupling constant ( $J = 16$  Hz), which is typical for *trans* coupling. The hydroxy proton (9-OH) was close to the methine proton (H-13); thus these were *cis*-cofacially oriented.

The configuration at C-4, which is remote from the cyclopentenone ring, cannot be determined with confidence. However, there are a number of weaker though clearly observable NOE correlations in the spectrum between the oxymethine proton (H-4) and the methine proton (H-13), hydroxy proton (9-OH), and the  $\delta$  3.50 methylene proton (H-14). Correlations are also observed from a C-2 hydrogen to H-13 and H-14. Assuming these correlations arise from a single conformation, molecular models indicate that only the *R* relative configuration at C-4 allows simultaneous NOE correlations from both H-2 and H-4 to H-13 and H-14 observed in the NOESY spectrum, as depicted in Figure 2, bottom. Possibly, weak hydrogen bonding helps to keep the lactone ring close to the C-9, C13, and C-14 centers long enough for the NOE correlations to be observed.

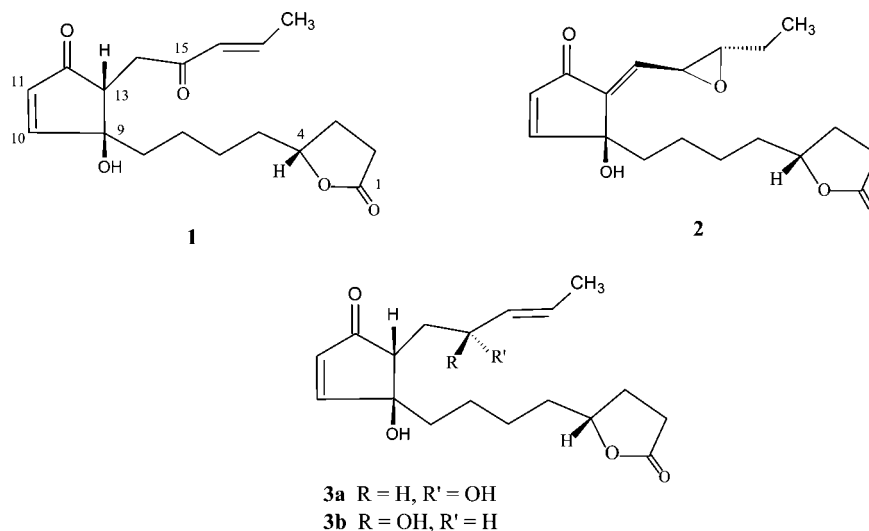
HRESIMS of **2** gave a pseudomolecular ion at  $m/z$  343.1530 [M + Na]<sup>+</sup>, corresponding to a molecular formula of C<sub>18</sub>H<sub>24</sub>O<sub>5</sub>, isomeric with **1**. Comparison of the <sup>1</sup>H NMR spectrum of **2** (Table 1) with that of **1** indicated that the only differences between these compounds lie in the five-carbon side chain. The allylic methyl at  $\delta$  1.96 in **1** was shielded to  $\delta$  1.05 (t) in **2**, suggesting that the methyl group is next to a methylene, and whereas there were two vinylic protons in the side chain of **1**, there was only one vinylic

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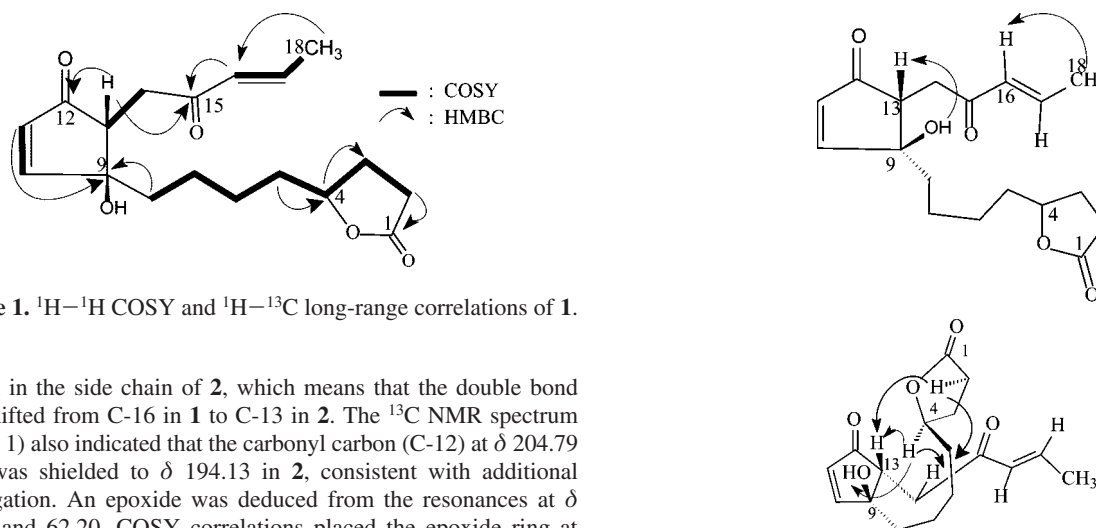
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**Table 1.** 400 MHz  $^1\text{H}$  and 100 MHz  $^{13}\text{C}$  NMR Data of **1** and **2** in  $\text{CDCl}_3$ 

position	$\delta_{\text{C}}$ , <b>1</b>	$\delta_{\text{H}}$ mult. <sup>a</sup> (J Hz), <b>1</b>	$\delta_{\text{C}}$ , <b>2</b>	$\delta_{\text{H}}$ mult. <sup>a</sup> (J Hz), <b>2</b>
1	177.09		177.2	
2	28.77	2.50 (2H), dd (6.8, 9.6)	28.77	2.50 (2H), dd (6.8, 9.6)
3	27.90	1.80, 2.30	27.99	1.80, 2.30
4	80.05	4.45	80.54	4.45
5	35.35	1.55, 1.65	35.42	1.55, 1.70
6	25.84	1.30, 1.30	25.60	1.40, 1.40
7	23.84	1.20, 1.20	24.25	1.20, 1.20
8	39.08	1.18, 1.50	40.08	1.92, 1.95
9	79.69		78.59	
10	164.8	7.59 d (6.0)	161.64	7.37 d (6.4)
11	131.6	6.21 d (6.0)	134.45	6.36 d (6.4)
12	204.79		194.13	
13	56.17	2.90 dd (2.4, 11.6)	143.13	
14	36.06	2.69 dd (11.6, 19.2), 3.50 dd (2.4, 19.2)	129.95	6.40 d (5.6)
15	200.95		55.01	3.73 dd (2.4, 5.2)
16	131.07	6.23 dq (1.6, 16)	62.20	3.00 dt (2.4, 5.4)
17	145.76	7.06 dq (6.8, 16)	24.96	1.70
18	18.48	1.96 Me, dd (1.6, 6.8)	9.64	1.05 Me, t (7.0)
OH		4.58 s		3.53 s

<sup>a</sup> Multiplet unless otherwise indicated.**Figure 1.**  $^1\text{H}$ – $^1\text{H}$  COSY and  $^1\text{H}$ – $^{13}\text{C}$  long-range correlations of **1**.

proton in the side chain of **2**, which means that the double bond had shifted from C-16 in **1** to C-13 in **2**. The  $^{13}\text{C}$  NMR spectrum (Table 1) also indicated that the carbonyl carbon (C-12) at  $\delta$  204.79 in **1** was shielded to  $\delta$  194.13 in **2**, consistent with additional conjugation. An epoxide was deduced from the resonances at  $\delta$  55.01 and 62.20. COSY correlations placed the epoxide ring at C-15/C-16 (Figure 3).

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR connectivities in **2** were verified by HSQC. The structure of **2** was elucidated by analysis of the HMBC 2D NMR data with key HMBC correlations shown in Figure 3. Thus, the epoxide was placed at C-15/C-16 due to long-range correlations between H<sub>3</sub>-18 and C-17 and C-16. Long-range correlations were also observed between the epoxide proton (H-16) and the epoxide carbon (C-15) and vinylic carbon (C-14). H-14 was also correlated

**Figure 2.** (Top) NOESY correlations of **1**. (Bottom) Key NOESY correlations from the lactone ring protons in **1**.

to the vinylic carbon (C-13), carbonyl carbon (C-12), and quaternary oxygenated carbon (C-9). Another vinylic proton (H-11) was long-range correlated to C-13, C-12, C-9, and C-10. All long-range correlations were consistent with the structure of **2**.

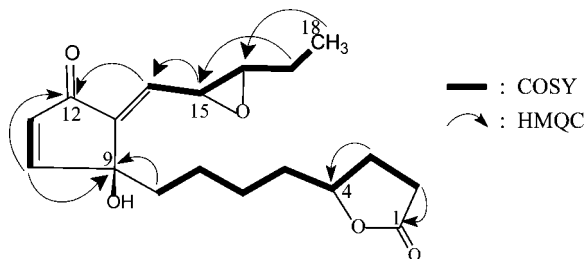


Figure 3.  $^1\text{H}$ – $^1\text{H}$  COSY and  $^1\text{H}$ – $^{13}\text{C}$  long-range correlations of **2**.

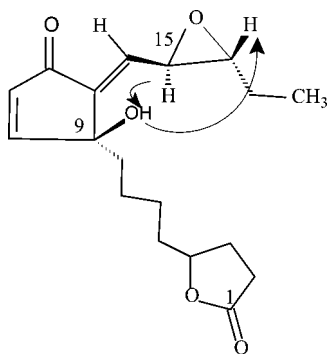


Figure 4. NOESY correlations of **2**.

The relative configuration (Figure 4) of **2** was deduced as follows. The coupling constant ( $J = 2.4$  Hz) between the two epoxide protons (H-15 and H-16) indicated a *trans* configuration for the epoxide ring.<sup>13</sup> In the NOESY spectrum, weak correlations were observed between the C-9 hydroxy proton and both epoxide protons, but not H-14. Thus, the double bond has an *E* configuration.

Moreover, these two NOE correlations to the C-9 OH suggest the epoxide ring has the relative configurations shown in Figure 4, as models indicated greater steric interactions and hydrogen–hydrogen separations in the isomer with inverted C-15/C-16 configurations. The alternative epoxide configuration cannot be unequivocally rejected, however. No correlations were observed between the oxymethine H-4 and the cyclopentenone ring and nearby protons, probably due to differing steric effects, but we assume the same configuration at C-4 in **2** as in **1**.

The HRESIMS of **3** gave a pseudomolecular ion at  $m/z$  345.1684  $[\text{M} + \text{Na}]^+$ , which corresponded to a molecular formula of  $\text{C}_{18}\text{H}_{26}\text{O}_5$ , two hydrogens more than **1** and **2**. The  $^1\text{H}$  NMR spectrum of **3** (Table 2) indicated resonances for a mixture of two compounds in a 2:1 ratio as deduced from the integrals and disparity in single hydrogen peaks. The spectrum showed resonances for two closely spaced doublets of vinylic protons at  $\delta$  7.54 ( $J = 6.4$  Hz) and 7.53 ( $J = 6.4$  Hz), 6.19 (d,  $J = 6.4$  Hz), and 6.20 ( $J = 6.4$  Hz); two closely spaced doublet of doublets of vinylic protons at 5.59 ( $J = 6.4, 15.6$  Hz) and 5.52 ( $J = 6.4, 15.6$  Hz); and allylic methyl groups at  $\delta$  1.71 Me, dd ( $J = 1.2, 7.6$  Hz) and 1.70 Me, dd ( $J = 1.2, 7.6$  Hz). Two closely spaced doublets of doublets  $\alpha$  to the carbonyl group were found at  $\delta$  2.57 ( $J = 2.4, 9.6$  Hz) and 2.67 ( $J = 3.6, 8.8$  Hz), and two oxymethine multiplets were detected at  $\delta$  4.17 and 4.50. These duplicated proton signals suggested a mixture of diastereomers. The  $^1\text{H}$  NMR spectrum also indicated the following overlapping proton resonances:  $\delta$  4.45 (oxymethine), 5.73 (vinylic), 2.51 ( $\alpha$ -methylene) and 2.31, 1.83 ( $\beta$ -methylene). The rest of the resonances were attributed to methylene protons.

The  $^{13}\text{C}$  NMR spectrum of **3** (Table 2) indicated 28 carbon resonances with the following functionalities (chemical shift of minor isomer in brackets): a lactone carbonyl at  $\delta$  177.2; an oxymethine at  $\delta$  80.72; a conjugated ketone carbonyl at  $\delta$  204.9 (206.0); four vinylic carbons, one at 164.3 and three at  $\delta$  127.90 (126.96), 133.27 (132.62), and 131.60 (131.88); a quaternary

Table 2. 400 MHz  $^1\text{H}$  and 100 MHz  $^{13}\text{C}$  NMR Data of **3a** and **3b** in  $\text{CDCl}_3$

position	$\delta_{\text{C}}$ , <b>3a</b>	$\delta_{\text{H}}$ mult. <sup>a</sup> ( $J$ Hz), <b>3a</b>	$\delta_{\text{C}}$ , <b>3b</b>	$\delta_{\text{H}}$ mult. <sup>a</sup> ( $J$ Hz), <b>3b</b>
1	177.20		177.20	
2	28.75	2.51 (2H), dd (6.8, 9.6)	28.75	2.51 (2H), dd (6.8, 9.6)
3	27.93	1.83, 2.30	27.93	1.83, 2.30
4	80.72	4.45	80.72	4.45
5	35.40	1.60, 1.70	35.40	1.60, 1.70
6	25.72	1.40, 1.40	25.72	1.40, 1.40
7	24.02	1.13, 1.50	24.02	1.13, 1.50
8	39.65	1.40, 1.87	39.08	1.43, 1.85
9	80.23		80.44	
10	131.60	6.19 d (6.4)	131.88	6.20 d (6.4)
11	164.30	7.54 d (6.4)	164.30	7.53 d (6.4)
12	204.90		206.00	
13	60.46	2.57 dd (2.4, 9.6)	57.00	2.67 dd (3.6, 8.8)
14	30.10	1.55, 2.16	29.43	1.85, 2.04
15	74.23	4.17	71.03	4.50
16	133.27	5.59 dd (6.4, 15.6)	132.62	5.52 dd (6.4, 15.6)
17	127.90	5.73 dq (7.6, 15.6)	126.96	5.73 dq (7.6, 15.6)
18	17.57	1.71 Me, dd (1.2, 7.6)	17.60	1.70 Me, dd (1.2, 7.6)
15-OH		3.65 br s		2.78 br s
9-OH		4.10 br s		4.10 br s

<sup>a</sup> Multiplet unless otherwise indicated.

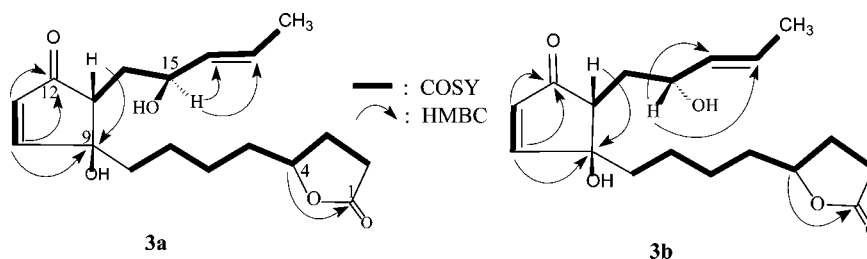
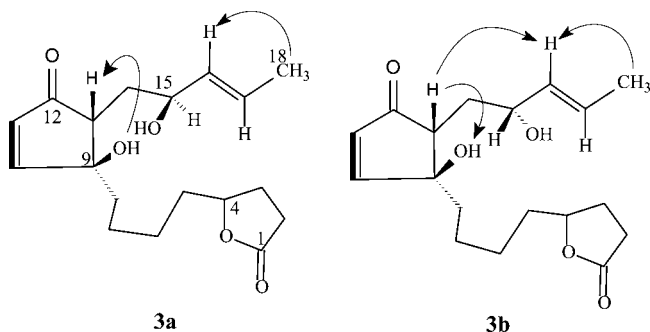


Figure 5.  $^1\text{H}$ – $^1\text{H}$  COSY and  $^1\text{H}$ – $^{13}\text{C}$  long-range correlations of **3a** and **3b**.



**Figure 6.** NOESY correlations of **3**.

oxygenated carbon at  $\delta$  80.23 (80.44); an oxymethine carbon at  $\delta$  74.23 (71.03); a methyl carbon at  $\delta$  17.57 (17.60); a methine carbon at  $\delta$  60.46 (57.00); and five methylene carbons at  $\delta$  35.4, 25.72, 24.02, 39.65 (39.08), and 30.10 (29.43). As with the  $^1\text{H}$  NMR, the  $^{13}\text{C}$  NMR spectrum suggested a diastereomeric mixture.

The COSY spectrum indicated that **3** again differed from **1** only in the five-carbon side chain, with the two isolated spin systems of **1** (H-13/H<sub>2</sub>-14 and H-16/H-17/H<sub>3</sub>-18) now linked in **3** through an oxymethine proton at C-15 (Figure 5).

The structure of **3** was elucidated by analysis of the HMBC 2D NMR data with key HMBC correlations shown in Figure 5. The cyclopentenone was deduced from the long-range correlations between the vinylic protons (H-10 and H-11) and the carbonyl carbon (C-12). Long-range correlations were also observed between these protons and the quaternary oxygenated carbon (C-9); thus this carbon has to be part of the cyclopentenone ring. The oxymethine proton (H-15) showed long-range correlations to the vinylic carbons (C-16 and C-17); thus this proton has to be allylic. The oxymethine proton (H-4) was long-range correlated to the lactonic carbonyl (C-1).

The large difference in the proton shift from  $\delta$  4.17 (H-15) in **3a** to  $\delta$  4.50 in **3b** and the change in chemical shifts of nearby protons (H-16, H-14, and H-13) suggested that the diastereomerism

may have resulted from the inversion of configuration at C-15. This assumption was supported by the shielding of the carbon resonances from  $\delta$  74.23 in **3a** to  $\delta$  71.03 in **3b** and the change in chemical shifts of neighboring carbons (C-17, C-16, C-14, and C-13). This was confirmed by oxidation of **3** with activated  $\text{MnO}_2$ ,<sup>14</sup> yielding compound **1**.

The relative configuration of **3a** (Figure 6) was deduced from the NOESY spectrum as follows. The geometry of the double bond was assigned *E* since NOESY correlations were observed between the allylic methyl (H<sub>3</sub>-18) and the upfield vinylic proton (H-16). This was further supported by a 16 Hz coupling constant between the two vinylic protons. H-13 and the 9-OH were again *cis*-oriented since H-13 showed a NOESY correlation with 9-OH. For **3b**, the vinyl proton (H-16) correlated with H-13, but this correlation was not observed for the major isomer. This correlation favors the relative configurations of **3a** and **3b** as shown in Figure 6, if the C-15 hydroxy group at least weakly hydrogen bonds to the C-12 carbonyl in both isomers. Again, no correlations were observed between H-4 and the cyclopentenone ring and nearby protons, but we assume the same configuration at C-4 in **3a** and **3b** as in **1**.

As part of our continuing search for antimicrobial compounds from Philippine medicinal plants, **1**, **2**, and the mixture of **3a** and **3b** were tested for their antimicrobial potentials against seven microorganisms. Results of the study (Table 3) indicated that all the compounds tested were slightly active against the bacteria *E. coli* and *P. aeruginosa* with an activity index (AI) of 0.2 at a concentration of 30  $\mu\text{g}$ , while the standard antibiotic chloramphenicol gave an AI of 2.8 and 1.3, respectively. Compounds **1**, **2**, and the mixture of **3a** and **3b** were also active against the fungus *T. mentagrophytes* with AI of 0.3, 0.2, and 0.1, respectively at a concentration of 30  $\mu\text{g}$ , while the standard antibiotic Canesten indicated an AI of 4.5. Compounds **1** and **2** were moderately active against the fungus *C. albicans* with an AI of 0.3, while the mixture of **3a** and **3b** gave an AI of 0.2. Canesten, the standard antibiotic against *C. albicans*, gave an AI of 0.8.

**Table 3.** Antimicrobial Test Results on **1**, **2**, and a Mixture of **3a** and **3b**

organism	sample (30 $\mu\text{g}$ )	clearing zone (mm)			antimicrobial index (AI)
		replicate 1	replicate 2	replicate 3	
<i>E. coli</i>	<b>1</b>	12	12	12	0.2
	<b>2</b>	12	12	12	0.2
	<b>3a</b> and <b>3b</b>	12	12	12	0.2
	chloramphenicol	23			2.8
<i>P. aeruginosa</i>	<b>1</b>	12	12	12	0.2
	<b>2</b>	12	12	12	0.2
	<b>3a</b> and <b>3b</b>	12	12	12	0.2
	chloramphenicol	14			1.3
<i>S. aureus</i>	<b>1</b>				0
	<b>2</b>				0
	<b>3a</b> and <b>3b</b>				0
	chloramphenicol	25			3.2
<i>B. subtilis</i>	<b>1</b>				0
	<b>2</b>				0
	<b>3a</b> and <b>3b</b>				0
	chloramphenicol	20			2.3
<i>C. albicans</i>	<b>1</b>	13	13	13	0.3
	<b>2</b>	13	13	13	0.3
	<b>3a</b> and <b>3b</b>	12	12	12	0.2
	Canesten, 0.2 g <sup>a</sup>	18			0.8
	<b>1</b>	13	13	13	0.3
<i>T. mentagrophytes</i>	<b>2</b>	12	12	12	0.2
	<b>3a</b> and <b>3b</b>	11	11	11	0.1
	Canesten, 0.2 g <sup>a</sup>	55			4.5
	<b>1</b>				0
	<b>2</b>				0
<i>A. niger</i>	<b>3a</b> and <b>3b</b>				0
	Canesten, 0.2 g <sup>a</sup>	23			1.3

<sup>a</sup> Contains 1% clotrimazole.

## Experimental Section

**General Experimental Procedures.** Optical rotations were measured on a Perkin-Elmer 341 polarimeter. IR spectra were recorded on a Perkin-Elmer 1600 Fourier transform IR spectrometer. UV spectra were measured on a U-2000 Hitachi UV-vis spectrometer. NMR spectra were recorded on a Bruker Avance 400 in CDCl<sub>3</sub> at 400 MHz for <sup>1</sup>H NMR and 100 MHz for <sup>13</sup>C NMR spectra. The high-resolution ESIMS were recorded on a Bruker BioApex 47e FTMS. Column chromatography was performed on Si gel 60 (70–230 mesh); TLC was performed on plastic-backed plates coated with Si gel F<sub>254</sub>; plates were visualized by spraying with vanillin sulfuric acid and warming.

**Plant Material.** The plant material was collected from Ilin, Mindoro, in June 2006. It was identified as *Tectona philippinensis* by one of the authors (E.M.), and a voucher specimen (#102) is kept at the Chemistry Department, De La Salle University—Manila.

**Extraction and Isolation.** The air-dried leaves (3.5 kg) of *T. philippinensis* were ground in an Osterizer, soaked in CH<sub>2</sub>Cl<sub>2</sub> for three days, and then filtered. The filtrate was concentrated under vacuum to afford a crude extract (350 g), which was chromatographed in increasing proportions of acetone in CH<sub>2</sub>Cl<sub>2</sub> at 10% increments. The 50% acetone in CH<sub>2</sub>Cl<sub>2</sub> fraction was rechromatographed in 30% EtOAc in petroleum ether (3×), followed by rechromatography in MeCN–Et<sub>2</sub>O–CH<sub>2</sub>Cl<sub>2</sub> (0.5:0.5:9) (8×) to afford **1** (20 mg, colorless oil). The 60–70% acetone in CH<sub>2</sub>Cl<sub>2</sub> fraction was rechromatographed (10×) in MeCN–Et<sub>2</sub>O–CH<sub>2</sub>Cl<sub>2</sub> (0.5:0.5:9). The less polar fractions afforded **2** (15 mg, colorless oil), while the more polar fractions afforded **3a** and **3b** (25 mg, colorless oil).

**Oxidation.** The mixture of diastereomers of **3** in CHCl<sub>3</sub> was stirred with activated MnO<sub>2</sub> prepared<sup>5</sup> from KMnO<sub>4</sub> and MnCl<sub>2</sub>. The product **1** was purified by passing the reaction mixture through a small Si gel column eluted with Me<sub>2</sub>CO–CHCl<sub>3</sub> (1:1).

**Antimicrobial Test.** The microorganisms used 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 suspensions were prepared from 24-h-old cultures of the bacteria and yeast and from 5-day-old culture of the molds. The suspending medium used was 0.1% peptone water. One tenth (0.1) mL aliquots of the bacterial, yeast, and mold suspensions were transferred into pre-poured nutrient agar (NA), glucose yeast peptone (GYP) agar, and potato dextrose agar (PDA) plates, respectively. Five of the corresponding media, melted and cooled to 45 °C, were poured into the agar plate and swirled to distribute the inoculum evenly on the agar surface. Three equidistant wells were made on the agar plate using a cork borer (10 mm). Two hundred (200) μL of the sample was placed in each hole. The solvent used for the samples was 95% EtOH. The plates were incubated at room temperature. NA and GYP plates were observed after 24–48 h, and PDA plates were observed after 3–5 days. The clearing zone was measured in millimeters, and the average diameter of the clearing zones was calculated. The antimicrobial index was computed by subtracting the diameter of the well from the diameter of the clearing zones divided by the diameter of the well.

**(5R)-5-(4-((1S,5R)-1-hydroxy-4-oxo-5-[(3E)-2-oxopent-3-en-1-yl]cyclopent-2-en-1-yl)butyl)dihydrofuran-2(3H)-one (1):** colorless oil; [α]<sub>D</sub><sup>20</sup> +60 (c 0.019, CHCl<sub>3</sub>); IR (neat) ν<sub>max</sub> (cm<sup>-1</sup>) 3435 (OH), 1769,

1710 (C=O), 1184, 1119 (C–O), 2936, 2864, 1458, 1357; UV (EtOH) λ<sub>max</sub> 245 (ε 2395) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; HRESIMS *m/z* 343.1521 [M + Na]<sup>+</sup> (C<sub>18</sub>H<sub>24</sub>O<sub>5</sub>Na requires 343.1516).

**(5R)-5-(4-((1S,5E)-5-((2S,3S)-3-ethoxyiran-2-yl)methylidene)-1-hydroxy-4-oxocyclopent-2-en-1-yl)butyl)dihydrofuran-2(3H)-one (2):** colorless oil; [α]<sub>D</sub><sup>20</sup> +20 (c 0.0084, CHCl<sub>3</sub>); IR (neat) ν<sub>max</sub> (cm<sup>-1</sup>) 3428 (OH), 1770, 1706 (C=O), 1219, 1184, 1020 (C–O); 2938, 2865, 1481, 1335; UV (EtOH) λ<sub>max</sub> 250 (ε 2156) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 1; HRESIMS *m/z* 343.1530 [M + Na]<sup>+</sup> (C<sub>18</sub>H<sub>24</sub>O<sub>5</sub>Na requires 343.1516).

**(5R)-5-(4-((1S,5R)-1-hydroxy-5-[(2R,3E)-2-hydroxypent-3-en-1-yl]-4-oxocyclopent-2-en-1-yl)butyl)dihydrofuran-2(3H)-one (3a) and (5R)-5-(4-((1S,5R)-1-hydroxy-5-[(2S,3E)-2-hydroxypent-3-en-1-yl]-4-oxocyclopent-2-en-1-yl)butyl)dihydrofuran-2(3H)-one (3b):** colorless oil; IR (CCl<sub>4</sub>) ν<sub>max</sub> (cm<sup>-1</sup>) 3042 (OH), 1768, 1706 (C=O), 1184, 1017 (C–O), 2938, 2863, 1455, 1347; UV (EtOH) λ<sub>max</sub> 245 (ε 2450) nm; <sup>1</sup>H and <sup>13</sup>C NMR data, see Table 2; HRESIMS *m/z* 345.1684 [M + Na]<sup>+</sup> (C<sub>18</sub>H<sub>26</sub>O<sub>5</sub>Na requires 345.1673).

**Acknowledgment.** A research grant from the University Research Coordination Office of De La Salle University is gratefully acknowledged. The antimicrobial tests were conducted at the University of the Philippines—Natural Sciences Research Institute. The plant materials were provided by the Mindoro Biodiversity Conservation Foundation, Inc. (MBCFI) through its Conservation of the Philippine Teak Project in Ilin Island, Mindoro.

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NP0704304