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## New Mosquito Larvicidal Tetranortriterpenoids from *Turraea wakefieldii* and *Turraea floribunda*

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The crude methanol extracts of the root barks of *Turraea wakefieldii* and *Turraea floribunda* were found to show mosquito larvicidal activity against third-instar larvae of *Anopheles gambiae sensu stricto*. Four new limonoids comprising a vilasininoid **1** and three havanensinoids **2–4** were isolated from the chloroform fractions of the methanol extracts of *T. wakefieldii* and *T. floribunda*, respectively. The structures of the compounds were elucidated by NMR spectroscopy. Compounds **1**, **2**, and **4** had LD<sub>50</sub> values of 7.1, 4.0, and 3.6 ppm, respectively, and were more potent than azadirachtin, which had an LD<sub>50</sub> value of 57.1 ppm when tested against larvae of *A. gambiae*.

**KEYWORDS:** *Turraea wakefieldii*; *T. floribunda*; *Anopheles gambiae*; limonoids; mosquito larvicidal activity

### INTRODUCTION

Plants in the family Meliaceae are characterized by the presence of limonoids (tetranortriterpenoids) that exhibit a wide range of anti-insect effects (1–4). In eastern Africa, a number of Meliaceae plants are used in traditional medicine, including *Turraea floribunda* (Hochstetter), a small tree or shrub, which is used as an emetic and a purgative (5). *Turraea wakefieldii* Oliv., a plant native to Kenya, is not known for any traditional uses (6). The root barks of these plants contain limonoids (7–10), some of which are mosquito larvicides (10). We now report on the structures of four new limonoids that were isolated from the root barks of *T. wakefieldii* and *T. floribunda* and their activities against larvae of *Anopheles gambiae sensu stricto*.

### MATERIALS AND METHODS

**Plant Material.** The root barks of *T. wakefieldii* and *T. floribunda* were collected from Shimba Hills National Park, Kwale, southern coast of Kenya, and were identified by S. G. Mathenge of the Botany Department, University of Nairobi. Voucher specimens WM 3/99 and 89/401 of *T. wakefieldii* and *T. floribunda*, respectively, have been deposited in the herbarium of that department.

**Insects.** Larvae of *A. gambiae* used for bioassays were obtained from a colony maintained at the International Centre of Insect Physiology

and Ecology (ICIPE) Insect Mass Rearing Unit. This strain of *A. gambiae* was originally obtained from Njage village, 70 km from Ifakara, southeastern Tanzania. The colony has been reared under laboratory conditions since April 1996. Larvae were allowed to emerge from eggs in plastic containers filled with distilled water and were transferred to larger pans at densities of 200–300 at second-instar stage. Larvae were fed on Tetramin fish food, and the water temperature was maintained between 28 and 30 °C throughout larval development.

**Mosquito Larvicidal Assay.** The effects of different doses of methanol and chloroform extracts and isolated limonoids on larval mortality after 24 h were determined and LD<sub>50</sub> values computed. Azadirachtin, a potent anti-insect naturally occurring limonoid, was tested as a positive control (1). The standard World Health Organization (WHO) larvicidal assay procedure was used (10, 12). Briefly, 1 mL of standard w/v concentrate of each test material in acetone was made up to 20 mL with distilled water in a 100 mL beaker in three replicates. Control solutions with 1 mL of acetone were similarly prepared. Twenty late third-instar larvae each were transferred into the test and control solutions, and larval mortality was recorded after 24 h. Probit analysis was used to estimate LD<sub>50</sub> values of extracts and limonoids (SAS version 8.0).

**Data Analysis.** A two-way analysis of variance was carried out to evaluate the combined effects of the extracts, limonoids, and dose on larval mortality, respectively. In addition to single factors, that is, extract or limonoid and dose, the effect of interaction between the test material (extract, limonoid) and the doses was evaluated. Mean mortalities induced by each dose for each extract and limonoid were compared by Student–Newman–Keuls (SNK) test ( $\alpha = 0.05$ ).

**Extraction and Isolation from *T. wakefieldii*.** A limonoid-bearing fraction (12 mg), obtained from previous work on the plant (10), was analyzed by semipreparative LC on a 250 mm × 10 mm i.d. ultrasphere

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Table 1.  $^1\text{H}$  NMR Data of Limonoids 1–4<sup>a</sup>

proton	1	2	3	4
H-1	4.72, t (2.7)	4.87, t (3.0)	4.87, t (3.0)	5.19, t (3.0)
2 $\alpha$	2.10, dt (16.7, 2.4)	2.10, dt (17.4, 2.2)	2.13, dt (11.4, 2.0)	1.99, m
2 $\beta$	2.24, dt (16.7, 3.3)	2.18, dt (17.4, 2.2)	2.16, dt (11.4, 2.0)	2.22, m
3	4.96, t (2.8)	3.75, dd (10.2, 3.0)	3.75, dd (10.2, 3.0)	3.72, dd (12.0, 3.0)
5	2.72, d (12.5)	2.94, dd (12.0, 2.2)	2.94, dd (12.0, 2.2)	2.90, dd (12.1, 3.0)
6	4.18, dd (2.9, 12.5)	1.72–1.85, m	1.72–1.85, m	1.72–1.96, m
7	4.22, d (2.8)	4.68, m	4.68, m	3.82, m
9	2.58, d (11.4)	3.30, d (3.9)	3.28, d (3.6)	2.63, d (2.4)
11 $\alpha$	1.35, m	5.12, t (3.6)	5.11, t (3.3)	5.66, t (3.0)
11 $\beta$	1.60, m			
12 $\alpha$	1.80, m			
12 $\beta$	1.60, m	4.86, d (3.0)	4.83, d (3.0)	5.10, d (3.6)
14				3.20, s
15	5.64, m (1.9)	3.61, s	3.63, s	
16 $\alpha$	2.61, dd (11.0, 14.9)	2.05, br d (15.3)	1.88, br d (16.3)	2.58, s
16 $\beta$	2.45, ddd (3.4, 7.2, 15.4)	2.32, ddd (3.9, 7.2, 15.1)	2.32, br d (3.4, 7.0, 14.9)	2.49, m
17	2.87, dd (7.4, 10.9)	2.75, br d (9.3)	2.77, br d (9.3)	3.94, br d (10.2)
18	0.87, s	1.06, s	1.04, s	0.83, s
19	1.02, s	1.19, s	1.14, s	1.06, s
21	7.29, br s	7.07, m	7.04, m	7.22, m
22	6.31, br s	6.41, m	6.38, m	6.26, m
23	7.40 t, 1.4	7.28, m	7.24, m	7.40, m
28	3.61, s			
29	1.23, s	1.20, s	1.17, s	1.27, m
30	1.14, s	1.33, s	1.30, s	1.35, s
C1–OCOCH <sub>3</sub>	2.03, s	2.14, s	2.13, s	2.15, s
C7–OCOCH <sub>3</sub>		2.06, s	2.11, s	
C11–OCOCH <sub>3</sub>		2.16, s	2.03, s	2.03, s
C3–OCOCH <sub>2</sub> CH <sub>3</sub>	2.3–2.4, m			
C3–OCOCH <sub>2</sub> CH <sub>3</sub>	1.15, t (7.6)			
C12–OCOCH(CH <sub>3</sub> ) <sub>2</sub>		2.61, m		2.52, t (9.7)
C12–OCOCH(CH <sub>3</sub> ) <sub>2</sub>		1.24, d (7.0)		1.16, d (7.0)
C12–OCOCH(CH <sub>3</sub> ) <sub>2</sub>		1.22, d (7.0)		1.14, d (7.0)
C12–OCOCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>			2.36, m	
C12–OCOCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>			1.24–1.48, m	
C12–OCOCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>			1.60, d (7.0)	
C12–OCOCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>			0.94, t (7.4)	
COOCH <sub>3</sub>		3.65, s	3.63, s	3.63, s
3-OH		2.60, br d (10.2)	2.60, br d (10.2)	2.50, br d (9.9)
7-OH	2.14, br s			2.20, d (3.0)

<sup>a</sup> Spectra were recorded in CDCl<sub>3</sub> at 500 MHz. Multiplicity, *J* (hertz) in parentheses. Assignments confirmed by 2D TCOSY and COSY

ODS column (Beckman) and detected at 215 nm. Elution of the fraction using 50% CH<sub>3</sub>CN–water at 3 mL/min yielded **1** (4 mg), which was tested for mosquito larvicidal activity.

**Extraction and Isolation from *T. floribunda*.** LC-MS analysis of a fraction (40 mg) obtained from previous work on *T. floribunda* (8) revealed the presence of three components, which displayed fragment ions the patterns of which were similar to those of limonoids previously reported from the plant. The three components were isolated by semipreparative LC on a 250 mm × 4.6 mm i.d., 5  $\mu$ m, ODS column (Beckman) using 50% acetonitrile–water at a flow rate of 2 mL/min at 215 nm to yield **2** (4 mg), **3** (1 mg), and **4** (2 mg).

NMR experiments on compound **1** were recorded on a Bruker 500 MHz Avance spectrometer, whereas **2**–**4** were recorded on a Bruker DRX-500 MHz spectrometer.  $^1\text{H}$  and  $^{13}\text{C}$  spectra were measured at 500 and 125 MHz, respectively, in CDCl<sub>3</sub>. Two-dimensional (2D)  $^1\text{H}$ – $^1\text{H}$ -TOCSY and  $^1\text{H}$ – $^{13}\text{C}$ -ROESY spectra were acquired with 80 and 300 ms mixing times, respectively. LC-MS analysis was carried out on a VG Platform II mass spectrometer interfaced with a Beckman System Gold 126 HPLC equipped with a model 168 diode array detector module. HREIMS spectra were recorded on a Finnigan MAT 95Q Hybrid Sector (ThermoFinnigan, San Jose, CA), EI = 70 eV and a mass resolving power of 5000.

Semipreparative HPLC of the fraction from *T. wakefieldii* was carried out on a Varian 5000 LC with a UV detector (215 nm). Semipreparative HPLC of the fraction from *T. floribunda* and analytical LC of all the limonoids were carried out on a Beckman System Gold 126 equipped with a model 168 diode array detector. The IR spectrum of compound **4** was recorded with a Shimadzu FT-IR 8101 spectrometer.

**Compound 1:** colorless prisms, 4 mg, mp 187–188 °C; HREIMS,  $m/z$   $\text{M}^+$  calcd for C<sub>31</sub>H<sub>42</sub>O<sub>7</sub>, 526.2931; found, 526.2922; EIMS (70 eV),  $m/z$  (relative intensity) 526 [ $\text{M}^+$ ] (18), 508 [ $\text{M} - \text{H}_2\text{O}]^+$  (21), 466 [ $\text{M} - \text{AcOH}]^+$  (100), 448 [ $\text{M} - \text{AcOH} - \text{H}_2\text{O}]^+$  (35), 434 [ $\text{M} - \text{H}_2\text{O} - \text{C}_3\text{H}_6\text{O}_2]^+$  (80);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see **Tables 1** and **2**, respectively.

**Compound 2:** white amorphous solid; 4 mg; mp 222–223 °C; HREIMS,  $m/z$   $\text{M}^+$  calcd for C<sub>37</sub>H<sub>50</sub>O<sub>13</sub>, 702.3251; found, 702.3205; MS (EI) (70 eV),  $m/z$  (relative intensity) 702 [ $\text{M}^+$ ] (28), 684 [ $\text{M} - \text{H}_2\text{O}]^+$  (8), 642 [ $\text{M} - \text{AcOH}]^+$  (20), 614 [ $\text{M} - \text{C}_4\text{H}_8\text{O}_2]^+$  (40), 572 [ $\text{M} - \text{C}_4\text{H}_8\text{O}_2 - \text{H}_2\text{O}]^+$  (20), 554 [ $\text{M} - \text{C}_4\text{H}_8\text{O}_2 - \text{AcOH}]^+$  (86), 494 [ $\text{M} - \text{C}_4\text{H}_8\text{O}_2 - 2\text{AcOH}]^+$  (100);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see **Tables 1** and **2**, respectively.

**Compound 3:** white solid (1 mg), mp 182 °C dec; HREIMS,  $m/z$   $\text{M}^+$  calcd for C<sub>38</sub>H<sub>52</sub>O<sub>13</sub>, 716.3408; found, 716.3414; EIMS,  $m/z$  (relative intensity) 716 [ $\text{M}^+$ ] (24), 698 [ $\text{M} - \text{H}_2\text{O}]^+$  (8), 656 [ $\text{M} - \text{AcOH}]^+$  (18), 614 [ $\text{M} - \text{C}_5\text{H}_{10}\text{O}_2]^+$  (36), 554 [ $\text{M} - \text{C}_5\text{H}_{10}\text{O}_2 - \text{AcOH}]^+$  (84), 494 [ $\text{M} - \text{C}_5\text{H}_{10}\text{O}_2 - 2\text{AcOH}]^+$  (80);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see **Tables 1** and **2**, respectively.

**Compound 4:** white solid (2 mg), mp 180 °C dec; IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  cm<sup>-1</sup>, 1749, 1715; HREIMS,  $m/z$   $\text{M}^+$  calcd for C<sub>35</sub>H<sub>48</sub>O<sub>12</sub>, 660.3146; found, 660.3109; EIMS (70 eV),  $m/z$  (relative intensity) 660 [ $\text{M}^+$ ] (7), 642 [ $\text{M} - \text{H}_2\text{O}]^+$  (12), 600 [ $\text{M} - \text{AcOH}]^+$  (24), 582 [ $\text{M} - \text{H}_2\text{O} - \text{AcOH}]^+$  (12), 572 [ $\text{M} - \text{C}_4\text{H}_8\text{O}_2]^+$  (20), 564 [ $\text{M} - 2\text{H}_2\text{O} - \text{AcOH}]^+$  (22), 512 [ $\text{M} - \text{C}_4\text{H}_8\text{O}_2 - \text{AcOH}]^+$  (10), 494 [ $\text{M} - \text{C}_4\text{H}_8\text{O}_2 - \text{H}_2\text{O}]^+$  (18), 452 [ $\text{M} - \text{C}_4\text{H}_8\text{O}_2 - 2\text{AcOH}]^+$  (10);  $^1\text{H}$  and  $^{13}\text{C}$  NMR data, see **Tables 1** and **2**, respectively.

Table 2.  $^{13}\text{C}$  NMR Data of Limonoids 1–4<sup>a</sup>

carbon	1	2	3	4
1	72.7	74.1 <sup>c</sup>	74.2 <sup>d</sup>	75.5 <sup>e</sup>
2	28.2	27.2	27.2	27.5
3	71.9	73.3	73.2	70.1
4	42.8	40.6	40.2	49.5
5	39.6	32.6	32.5	30.7
6	74.4	25.6	26.7	27.5
7	73.2	74.2	74.1	71.0
8	46.2	40.4	40.8	41.2
9	34.0	40.8	40.8	40.0
10	40.6	40.0	40.7	42.3
11	15.6	74.3 <sup>c</sup>	74.3 <sup>d</sup>	70.8 <sup>e</sup>
12	33.3	79.2	79.3	71.9
13	47.8	48.9	49.0	44.5
14	160.3	73.9	73.9	58.9
15	121.1	63.3	63.3	
16	34.0	32.6	32.6	43.2
17	51.9	40.3	40.0	37.1
18	21.7 <sup>b</sup>	16.9	16.6	21.6
19	15.8	16.9	17.0	17.7
20	124.9	128.0	127.9	123.0
21	140.1	140.5	140.3	140.0
22	111.5	112.3	112.3	111.0
23	143.0	142.4	142.3	143.0
28	78.3	174.9	176.0	174.5
29	19.9	17.9	16.9	16.9
30	26.7	23.9	23.8	20.0
C1-OCO	170.5			
C1-OCH <sub>3</sub>	21.6 <sup>b</sup>			
C3-OCO	174.1			
C3-OCH <sub>2</sub>	28.2			
C3-CH <sub>3</sub>	9.5			
C12-OCO		176.2	176.4	175.9
C12-OCOCH(CH <sub>3</sub> ) <sub>2</sub>		34.4		43.5
C12-OCOCH(CH <sub>3</sub> ) <sub>2</sub>		19.1		18.9
C12-OCOCH(CH <sub>3</sub> ) <sub>2</sub>		18.9		18.9
C12-OCOCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>			41.6	
C12-OCOCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>			25.6	
C12-OCOCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>			17.7	
C12-OCOCH(CH <sub>3</sub> )CH <sub>2</sub> CH <sub>3</sub>			12.0	
C1-OCH <sub>3</sub>		21.6	21.6	21.9
C7-OCH <sub>3</sub>		21.6	21.6	
C11-OCH <sub>3</sub>		21.5	21.5	21.1
C1-OCO		168.6	169.0	168.4
C7-OCO		169.1	169.4	
C11-OCO		169.9	169.9	169.0
OCH <sub>3</sub>		52.1	52.0	52.0

<sup>a</sup> Spectra were recorded in  $\text{CDCl}_3$  at 125.67 MHz. <sup>b–e</sup> Resonances cannot be assigned unambiguously, and those denoted highlighted with the same letter are interchangeable.

## RESULTS AND DISCUSSION

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra (Tables 1 and 2) of the four compounds (Figure 1) contained resonances typical of limonoids including a  $\beta$ -substituted furan and ester functions (7–11).

The HREIMS of compound 1 indicated a molecular formula of  $\text{C}_{31}\text{H}_{42}\text{O}_7$ . The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of 1 were similar to that of 1,3-diacetylvilasinin (5) (Figure 1), a known compound isolated previously from the stem bark of *Turraea holstii* (11). The major difference was the replacement of one of the acetate signals in the  $^1\text{H}$  NMR spectrum of 5 with propanoyl signals ( $\delta_{\text{H}}$  2.3–2.4 and 1.15 and  $\delta_{\text{C}}$  28.2, 9.5, and 174.1) ascribed to C-3 in 1. The 3 $\alpha$ -propanoyl ester group in 1 was deduced from the HMBC spectrum correlation of H-3 $\beta$  and the protons of the propanoyl group with the carbon resonance at  $\delta$  174.1, clearly establishing the attachment of the propanoyl ester at C-3. The relative stereochemical assignment of 1 was achieved using one-dimensional (1D) gradient NOE spectroscopy (GOESY) NMR experiments (13) and is shown in Figure 2. By connecting

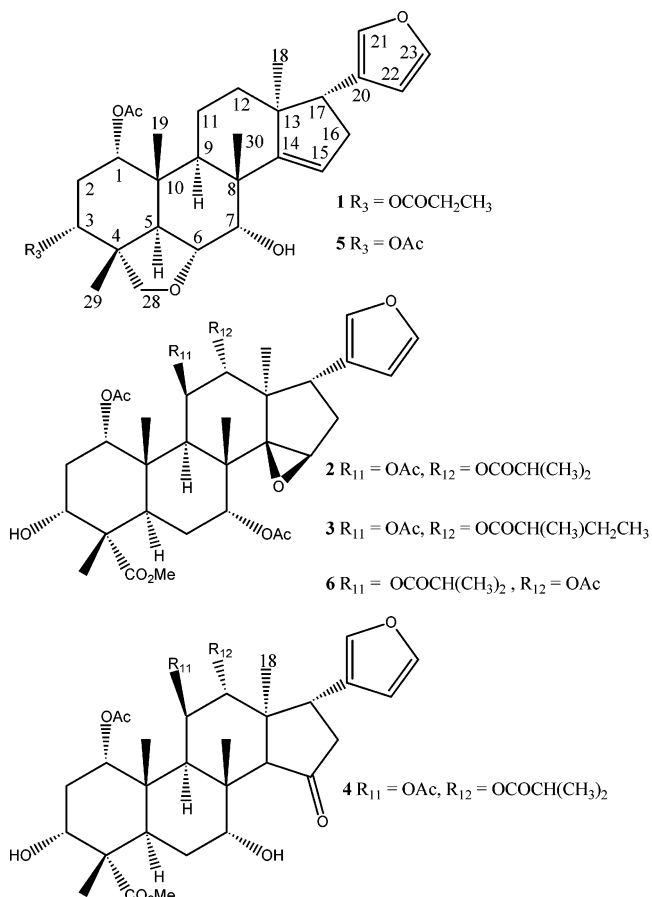


Figure 1. Chemical structures of limonoids 1, isolated from *T. wakefieldii*, and 2–4, isolated from *T. floribunda*.

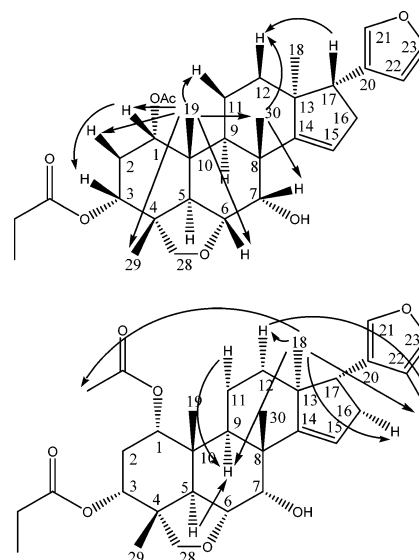


Figure 2. GOESY correlations on the top and back faces of 1.

the resonances on the top face, the relative stereochemistry of C-1, -3, -4, -6, -7, -8, -10, and -17 was possible. In addition, the assignment of diastereotopic protons at C-2, -11, and -12 was deduced. The results on the back face were complementary and also allowed assignment of the relative stereochemistry at C-5, -9, and -13 as well as assignment of the H-16 protons, confirming the structure 1 as 1 $\alpha$ -acetoxy-3 $\alpha$ -propanoyloxyvilasinin.

Compounds 2–4 were all isolated from the root bark of *T. floribunda*. The HREIMS of compound 2 indicated a molecular

**Table 3.** Mosquito Larvicidal Activity (Percent Mortality  $\pm$  SE) of Extracts from the Root Barks of *T. wakefieldii* (Tw) and *T. floribunda* (Tf) against Third–Fourth-Instar *A. gambiae* s.s.<sup>a</sup>

extract	dose								LD <sub>50</sub>
	10 ppm	30 ppm	50 ppm	100 ppm	125 ppm	250 ppm	500 ppm	1000 ppm	
Tf-MeOH	2 $\pm$ 1aC	2 $\pm$ 1abC	9 $\pm$ 2abBC	20 $\pm$ 6aB	95 $\pm$ 3aA	100 $\pm$ 0aA	100 $\pm$ 0aA		107.8
Tf-CHCl <sub>3</sub>	2 $\pm$ 1aF	6 $\pm$ 2 aEF	12 $\pm$ 2aE	22 $\pm$ 2aD	46 $\pm$ 2aC	80 $\pm$ 2bB	93 $\pm$ 2bA	100 $\pm$ 0aA	48.0
Tw-MeOH	2 $\pm$ 1aC	2 $\pm$ 1abC	2 $\pm$ 1bcC	2 $\pm$ 1abC	11 $\pm$ 1dB	15 $\pm$ 2dB	15 $\pm$ 2dB	93 $\pm$ 3aA	579.8
Tw-CHCl <sub>3</sub>			3 $\pm$ 1bc E	5 $\pm$ 2bE	23 $\pm$ 3cD	44 $\pm$ 3cC	75 $\pm$ 0cB	93 $\pm$ 3aA	192.0

<sup>a</sup> Means followed by the same lower case letter within the same column and by the same upper case letter within the same row are not significantly different ( $\alpha = 0.05$ ) (SNK).

**Table 4.** Mosquito Larvicidal Activity (Percent Mortality  $\pm$  SE) of Limonoids Isolated from the Root Barks of *T. wakefieldii* and *T. floribunda* against Third–Fourth-Instar *A. gambiae* s.s.<sup>a</sup>

limonoid	dose							LD <sub>50</sub>
	2.5 ppm	3.5 ppm	5 ppm	10 ppm	50 ppm	70 ppm	100 ppm	
<b>1</b>	4 $\pm$ 1Ad	7 $\pm$ 1Bc	16 $\pm$ 2Cb	85 $\pm$ 4Ba				7.1
<b>2</b>	10 $\pm$ 2Ac	50 $\pm$ 4Ab	58 $\pm$ 4Bb	100 $\pm$ 0Aa				4.0
<b>4</b>	7 $\pm$ 1Ad	50 $\pm$ 2Ac	85 $\pm$ 4Ab	97 $\pm$ 2Aa				3.6
azadirachtin	5 $\pm$ 5Aa	5 $\pm$ 1Ba	8 $\pm$ 1CDa	10 $\pm$ 1Ca	50 $\pm$ 2 b	63 $\pm$ 1 c	84 $\pm$ 4 d	57.1

<sup>a</sup> Means followed by the same upper case letter within the same column and by the same lower case letter within the same row are not significantly different ( $\alpha = 0.05$ ) (SNK) (DF = 9 for limonoid and dose interaction [see Discussion, mosquito larvicidal activity]; doses 50, 70, and 100 ppm were for azadirachtin only and so were excluded in the analysis).

formula of C<sub>37</sub>H<sub>50</sub>O<sub>13</sub>. <sup>1</sup>H and <sup>13</sup>C NMR spectra (**Tables 1** and **2**) of the compound were almost identical to those of the limonoid 1 $\alpha$ ,7 $\alpha$ -12 $\alpha$ -triacetoxo-4 $\alpha$ -carbomethoxy-11 $\beta$ -(2-methylpropanoyloxy)-14 $\beta$ ,15 $\beta$ -epoxyhavanensin **6**, previously isolated from the bark of the plant (7). The major difference was location of the isopropyl ester group, which was ascribed to C-11 in **6**, with a  $\beta$  stereochemistry, but attached to C-12 in **2**. In the HMBC spectrum of **2**, the H-12 at  $\delta_H$  4.86, the methyl signals from the isopropyl group ( $\delta_H$  1.22 and 1.24), and H-2' ( $\delta_H$  2.61) all coupled with the carbonyl ( $\delta_C$  169.99). ROESY correlation of H-17 (2.75 ppm) and H-12 indicated the relative stereochemistry of the 12-ester to be  $\alpha$ . DQF-COSY and HSQC experiments showed the following connectivities: C-3–C-2–C-1 for ring A, C-5–C-6–C-7 for ring B, and C-9–C-11–C-12 for ring C. The feature of an epoxide attached at C-14 and C-15 (H-15,  $\delta_H$  3.61; C-14 and C-15,  $\delta_C$  74.13 and 65.23, respectively) (4, 9, 10) was confirmed by HMBC correlations between H-17 ( $\delta_H$  2.85) and C-15 ( $\delta_C$  65.23). Accordingly, compound **2** was assigned as 1 $\alpha$ ,7 $\alpha$ ,11 $\beta$ -triacetoxo-4 $\alpha$ -carbomethoxy-12 $\alpha$ -(2-methylpropanoyloxy)-14 $\beta$ ,15 $\beta$ -epoxyhavanensin.

Compound **3** had spectroscopic properties very similar to those of **2**. The HREIMS was 14 amu higher than that of **2** (M<sup>+</sup>, 702). A prominent ion peak at  $m/z$  614 [M – 102] was consistent with a loss of C<sub>5</sub>H<sub>10</sub>O<sub>2</sub>, compatible with 2-methylbutanoic acid, a side chain at the C-12 position previously reported for related limonoids (8). The <sup>1</sup>H NMR spectrum of **3** contained signals at  $\delta$  1.60 (3H, d), 0.94 (3H, t), and 1.24–1.48 (2H) compared to signals at  $\delta$  1.22 (3H, d) and 1.24 (3H, d) in **2**. Analysis of the DEPT spectra of **3** showed an additional methylene carbon at  $\delta$  25.56. These observations and correlations from the DQ-COSY confirmed that the isobutyl group in **2** has been replaced with a 2-methylbutyl moiety in **3**. Comparison of the NMR spectroscopic data of **3** with those of **2**, and to those of previously reported limonoids (8, 9), suggested the location of the 2-methylbutanoyloxy group at C-12. Within compound **3**, HMBC cross-peaks were observed between H-12 (4.83 ppm), H-2' (2.36 ppm), and H-3' (1.24–1.48 ppm) and the ester carbonyl at  $\delta_C$  176.4. ROESY correlations were similar

to those of **2** and supported the characterization of compound **3** as 1 $\alpha$ ,7 $\alpha$ ,11 $\beta$ -triacetoxo-4 $\alpha$ -carbomethoxy-12 $\alpha$ -(2-methylbutanoyloxy)-14 $\beta$ ,15 $\beta$ -epoxyhavanensin.

Compound **4** HREIMS indicated a molecular formula of C<sub>35</sub>H<sub>48</sub>O<sub>12</sub> and had spectroscopic characteristics similar to those of **2**. The major differences were found in the attachments at C-7 and C-15. The H-7 resonance was shifted upfield from  $\delta$  4.68 to 3.82, supporting replacement of the acetate group at C-7 $\alpha$  with a hydroxy group (**Tables 1** and **2**), which was confirmed by COSY, HSQC, and HMBC correlations. The NMR spectrum of **4** also lacked the H-15 resonance, corresponding to the 14 $\beta$ ,15 $\beta$ -epoxy group in **3** (**Tables 1** and **2**). Because compound **4** was isolated in a very small quantity, carbon data could be detected only indirectly from its HSQC spectrum. The HSQC spectrum showed that the C-14 and C-16 resonances in **4** occurred at 58.9 and 43.5 ppm, respectively, whereas they were at 74.1 and 33.3 ppm in **2**, indicating that a keto group was attached at C-15 (11). The H-17 resonance was shifted downfield from  $\delta$  2.77 in **3** to 3.94 in **4**, with the C-17 resonance at 40.0 ppm occurring at 37.1 ppm in **4**, further supporting the presence of a keto group attached at C-15 (11). Because it was not possible to detect strong correlations above  $\delta$  200 in the HSQC spectrum, confirmation of the keto group at C-15 was deduced from the IR spectrum of **4**, which showed an absorption at 1749 cm<sup>–1</sup>, consistent with the presence of a five-ring ketone. These data identified **4** as 1 $\alpha$ ,11 $\beta$ -diacetoxo-4 $\alpha$ -carbomethoxy-7 $\alpha$ -hydroxy-12 $\alpha$ -(2-methylpropanoyloxy)-15-oxohavanensin.

Mosquito larvicidal activity was carried out on the crude methanol and chloroform fractions and limonoids isolated from *T. wakefieldii* and *T. floribunda*. Analysis of variance of single factors, that is, extract ( $F = 509.5$ ; DF = 3,  $p < 0.0001$ ) and dose ( $F = 1321.1$ , DF = 7;  $p < 0.0001$ ) and limonoid ( $F = 297.9$ ; DF = 3;  $p < 0.0001$ ) and (dose,  $F = 670.7$ ; DF = 6;  $p < 0.0001$ ), was highly significant. In addition, the effect of the interaction between the extracts or limonoids and doses ( $F = 94.9$ ; DF = 21;  $p < 0.0001$ ) ( $F = 99.3$ ; DF = 9;  $p < 0.0001$ ), respectively, was highly significant. For both plants, fractionation of the extracts increased mosquito larvicidal activity, as



shown by the LD<sub>50</sub> values (Table 3). Further fractionation of the chloroform fractions led to the isolation of limonoids **1**, **2**, and **4**, which showed more potent mosquito larvicidal activity than the crude extracts (Tables 3 and 4). No bioassays were carried out on **3** due to paucity of material. The vilasinin derivative **1** had an LD<sub>50</sub> value of 7.1 ppm against larvae of *A. gambiae* (Table 4), similar to the activity shown by the ring A-seco limonoids also isolated from the same plant (10). These limonoids are less potent as mosquito larvicides than the haveninsin-type limonoids **2** and **4**, which had LD<sub>50</sub> values of 4.0 and 3.6 ppm, respectively. Azadirachtin, widely used for insect control (1), used as a positive control in this study, had an LD<sub>50</sub> value of 57.1 ppm and was relatively less potent than these limonoids isolated from the two *Turraea* species. These results provide further evidence of previously reported mosquito larvicidal activity of limonoids from *Turraea* species.

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