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Ring A-seco mosquito larvicidal limonoids from *Turraea wakefieldii*

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Abstract

Five novel limonoids, **1**–**5**, were isolated from the root bark of *Turraea wakefieldii* and were characterized as tecleaninoid derivatives. This is the first report of the natural occurrence of tecleanin-type limonoids with a five-membered-ring A-seco structure for which we propose the name neotecleanins. The relative stereochemical structures of compounds **1**–**5** were established on the basis of NMR spectroscopy. The absolute stereochemical structure of **5** was confirmed by X-ray diffraction methods. In mosquito larvicidal assays, compounds **1**, **2** and **4** showed dose-dependent larvicidal activity against larvae of *Anopheles gambiae s.s.*

Keywords: *Turraea wakefieldii*; Meliaceae; Limonoids; Larvicidal activity; *Anopheles gambiae s.s*; Tecleaninoids; Neotecleanins

1. Introduction

Considerable interest has been shown in the limonoids of the genus *Turraea*, comprising mainly shrubs distributed widely in East Africa. Like many other limonoids isolated from Meliaceae, they exhibit a wide variety of biological properties including insect-antifeedant, insecticidal and antimicrobial activity (Champagne et al., 1992). In previous studies, limonoids from a number of *Turraea* species have been reported (Akinniyi et al., 1986; Adul et al., 1993; Bentley et al., 1995; Fraser et al., 1994; Torto et al., 1995, 1996; Mulholland et al., 1998). *Turraea wakefieldii* Oliv., closely related to *T. floribunda*, is a shrub native to Kenya (Kokwaro, 1976; Styles and White, 1991) and in continuing our studies of limonoids in this genus, we report the isolation of five novel tecleanin-type limonoids from *T. wakefieldii*.

2. Results and discussion

The methanol extract of the air-dried root-bark of *T. wakefieldii* was fractionated between water and chloroform. Chromatography of the chloroform fraction on silica followed by semi-preparative HPLC yielded five new limonoids **1**–**5**. The structure and relative stereochemistry of all the compounds were elucidated using ^{1}H, ^{13}C, gradient COSY, gradient HMQC, gradient HMBC experiments and by selected 1D gradient NOE spectroscopy (GOESY). The structure of compound **5** was completed using X-ray diffraction methods and the absolute stereochemistry of **1**–**4** was assumed to be identical. The structures of compounds **1**–**5** (Fig. 1) were all found to be similar in structure to tecleanin (**6**), isolated previously from *Teclea grandifolia* and *Teclea oubanguiensi* (Ayafor et al., 1981, 1986). The characteristics of rings B–D were all present in the NMR spectra but the A-seco ring part of these compounds showed some unique features. For compounds **1**–**4**, only four methyl singlets at 4α (C-28), 4β (C-29), 8β (C-30) and 13α (C-18) were observed, with the absence of a methyl group at 10 (C-19) and the presence of an extra methylene group directly attached to the C-1 carbonyl.

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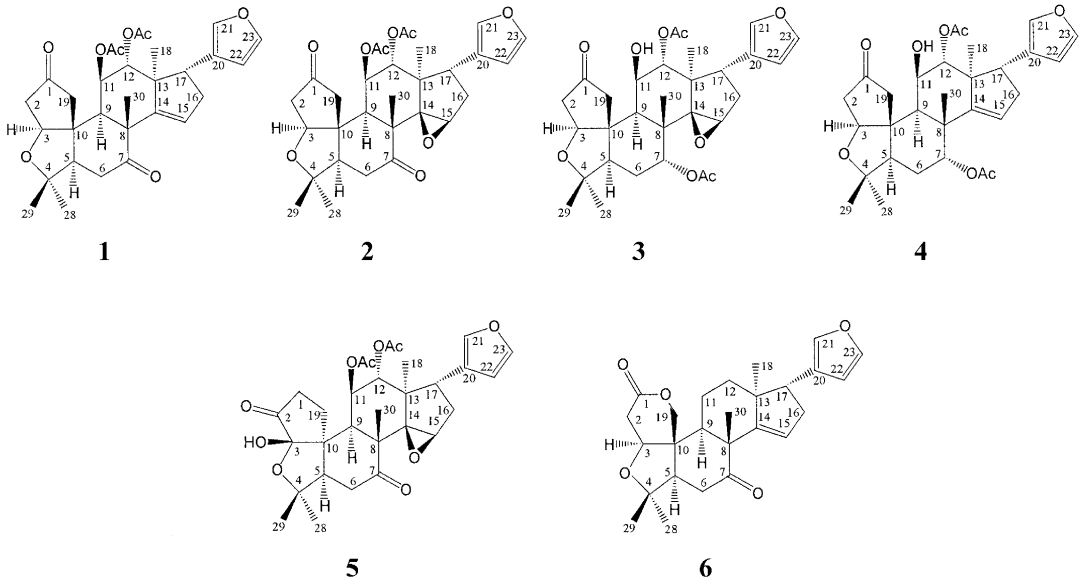

Fig. 1. Chemical structures of limonoids **1–5** isolated from *Turraea wakefieldii*.

Compound **5**, on the other hand, was hydroxylated at C-3, and contained adjacent methylenes in this region.

The HREIMS of compound **1** gave a molecular ion at *m/z* 524.2405 (calc. 524.2410), corresponding to $C_{30}H_{36}O_8$. The mass spectrum displayed fragment peaks at *m/z* 464 and 404, both corresponding to the sequential loss of two acetic acid moieties. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) showed the presence of a β-substituted furan, two carbonyl groups, a double bond, an ether function, two acetyl and four quaternary methyl groups. Comparison of the NMR spectra of **1** to that of tecleanin **6** suggested that the rearranged A-ring containing a lactone in tecleanin has been replaced by a five-membered ring ketone. In the HMBC spectrum (Fig. 2), the methine proton H-3 (δ 4.35) and methylene protons H_2-19 and H_2-2 all coupled to C-1 at δ 216.6. Also, the coupling of H-5 (δ 2.16) and H-9 (δ 2.77) with C-3, clearly established the A-ring structure. The ketone at C-7 was deduced from HMBC correlations of H-5, H_2-6, H-9 and H_3-30 to δ 206.9. The two acetate groups were placed at C-11 and C-12 based on the COSY correlation of H-11 with H-9 and H-11 with H-12 which were confirmed by HMBC correlations of H-11 and H-12 to δ 171.1 and 171.4 respectively.

The GOESY experiments on **1** involving the top face atoms revealed a twist in the rearranged A-ring structure (Fig. 3). H_3-30 could relax H-12 (which relaxed H-17) and H-6β but is proximal to the back-face proton H-19α, so determining the C-10 relative stereochemistry. This twist is clear in a 3-D model and results in H-19β being situated proximal to the 4β methyl group and H-6β. NOEs were observed between H-3 and H-5, H-9, and the 4α methyl group, which were assigned to be on the back face. As one of the enantiotopic H-2 resonances was relaxed by H-11, this too was determined to be on the back face. H-9 relaxed H_3-18 and because the furanyl protons H-21 and H-23 were relaxed by both H_3-18 and the C-12 acetoxy methyl, these were also verified to be on the back face. This describes a previously undiscovered carbon skeleton for limonoids for which we propose the name neotecleanin. Thus compound **1** was characterized as 11β,12α-diacetoxyneotecleanin.

Compound **2**, $C_{30}H_{36}O_9$ (HREIMS *m/z* 540.2358, calc. 540.2359), was 16 amu greater than that of **1**, and was identified as the epoxide derivative of **1**. The H-15 resonance occurred in compounds **1** and **2** as a singlet at δ 6.32 and 3.81 respectively. Based on similar GOESY results, the stereochemistry of **2** was shown to be the same as **1**. The stereochemistry of the epoxide was deduced, from X-ray crystallographic analysis of **5** (Fig. 4), to be β, in accordance with the orientation described previously for limonoids with a 14, 15 epoxide group (Torto et al., 1996; Tada et al., 1999; Garcez et al., 2000, Simmonds et al., 2001).

Compound **3** was isolated as a white powder. HREIMS of **3** gave *m/z* 542.2524 (calc. 542.2516), corresponding to $C_{30}H_{38}O_9$. The ^1H and ^{13}C NMR spectra of **3** were quite similar to those of **2** except for minor differences. For compound **3** there was only one ketone which was explained by a reduced C-7 ketone (Table 2). This was determined from HMBC correlations of the H_2-2 and H_2-19 methylenes to the C-1 ketone and COSY correlations of H-7 (δ 4.75) to H-6α (δ 1.78) and H-6β (δ 1.89). HMBC correlation of H-12 and H-7 to the acetate carbonyl carbons respectively confirmed the structure and characterized compound **3** as 7α, 12α-diacetoxy-14β, 15β-epoxy-11β-hydroxyneotecleanin.

The ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **4** were similar to those of **3**, with the major differences being the absence of H-15 and C-14 and C-15 resonances

Table 1
^{1}H NMR assignments for limonoids **1–5**

^{1}H	1	2	3	4	5
H-1α					2.71 *ddd*, 3.6 11.7 20.4
H-1β					2.42 *ddd*, 8.2 9.7 19.9
H-2α	2.32[a] *dd*, 3.2 18.8	2.56 *dd*, 3.2 18.2	2.57 *dd*, 3.0 17.1	2.67 *dd*, 3.3 17.3	
H-2β	2.40[a] *d*, 19.0	2.47 *d*, 17.8	2.43 *d*, 16.9	2.40 *d*, 17.3	
3	4.35 *d*, 3.2	4.37 *d*, 3.0	4.32 *d*, 2.9	4.35 *d*, 3.2	
3-OH					3.85 *s*
5	2.16 *dd*, 2.7 13.4	2.25 *dd*, 3.5 15.8	2.25 *dd*, 2.8 13.8	2.29 *dd*, 2.6 13.6	2.80 *t*, 9.4
H-6α	2.44 *dd*, 14.6 2.4	2.44 *dd*, 11.4 3.5	1.78 *dt*, 14.6 2.8	1.80 *dt*, 14.0 2.4	2.46 *dd*, 9.5 14.9
H-6β	2.76 *dd*, ~15	2.92 *dd*, 13.9 15.5	1.89 *dt*, 2.3 14.2	1.92 *dt*, 2.4 14.0	3.13 *dd*, 10.2 15.0
7			4.75 *s*	5.25 *t*, 1.6	
9	2.77 *d*, 4.9	2.97 *d*, 2.0	2.86 *d*, 4.8	2.50 *m*	3.08 *d*, 2.0
11	5.21 *s*	5.02 *d*, 1.4	3.93 *t*, 3.5	3.93 *t*, 4.5	4.97 *s*
11-OH			3.97 br *s*	4.03 *s*	
12	5.21 *s*	5.18 *s*	4.83 *d*, 2.8	4.71 *d*, 3.9	5.24 *s*
15	6.32 *s*	3.81 *s*	3.51 *s*	5.65 *dd*, 1.3 1.7	3.74 *s*
H-16α	2.5 *m*	2.05 *dd*, 11.4 13.6	1.73 *dd*, 11.4 13.4	2.45[a] *m*	2.0 *m*
H-16β	2.5 *m*	2.29 *dd*, 6.7 13.5	2.24 *dd*, 6.3 13.4	2.48[a] *m*	2.26 *dd*, 6.9 13.8
17	3.04 *dd*, 8.8 11.6	2.89 *dd*, 6.6 10.8	2.89 *m* 6.7	3.03 *dd*, 7.8 11.0	2.90 *dd*, 6.5 11.1
18	1.06 3H, *s*	0.98 3H, *s*	1.17 3H, *s*	1.05 3H, *s*	0.94 3H, *s*
H-19α	3.09 *d*, 19.0	2.97 *d*, 19.3	3.43 *d*, 19.0	3.31 *d*, 19.0	2.32 *ddd*, 8.1 10.6 13.8
H-19β	2.61 *d*, 19.2	2.73 *d*, 19.4	2.36 *d*, 18.9	2.33 *d*, 19.6	2.02 *m*
21	7.14 *s*	7.14 *s*	7.16 *s*	7.26 *s*	7.13 *s*
22	6.21 *s*	6.13 *s*	6.15 *s*	6.26 *d*, 0.9	6.14 *s*,
23	7.31 *s*	7.34 *s*	7.38 *t*, 1.4	7.39 *t*, 1.6	7.33 *t*, 1.5
28	1.25 3H, *s*	1.32 3H, *s*	1.26 3H, *s*	1.25 3H, *s*	1.45 3H, *s*
29	1.16 3H, *s*	1.17 3H, *s*	1.11 3H, *s*	1.14 3H, *s*	1.45 3H, *s*
30	1.55 3H, *s*	1.35 3H, *s*	1.24 3H, *s*	1.41 3H, *s*	1.56 3H, *s*
C7-OCOCH_3			2.16 3H, *s*	2.05 3H, *s*	
C11-OCOCH_3	1.94 3H, *s*	2.13 3H, *s*			2.05 3H, *s*
C12-OCOCH_3	1.80 3H, *s*	1.94 3H, *s*	2.09 3H, *s*	2.06 3H, *s*	1.93 3H, *s*

[a] Resonances cannot be assigned unambiguously and those highlighted with the same letter are interchangeable.

corresponding to the epoxy group in compound **3**. Compound **4** had a molecular formula of $C_{30}H_{38}O_8$ (HREIMS *m*/*z* 526.2562, calc. 526.2567), which was 16 amu less than that of **3**, confirming the absence of a 14, 15 epoxy group in **4** and a double bond in its place. Based on similar GOESY results, the stereochemistry of **4** was shown to be the same as **3**.

HRMS of compound **5** showed a peak at *m*/*z* 556.2480 of low intensity (ca. 3% relative abundance). The calculated mass for $C_{30}H_{36}O_{10}$ is 556.2308, which suggested that compound **5** might be unstable. Its ^{1}H and ^{13}C NMR spectra, however, were similar to those of **1**, with major differences being the absence of an H-3 resonance in **5**, a shift of the C-3 resonance from δ 84.4 to 104.2, and a shift of the H-15 resonance from δ 6.32 in **1** to 3.74 in **5**. Since compound **5** was isolated in a very small quantity (<0.5 mg), carbon data could only be detected indirectly and so its NMR spectral data were insufficient to allow for full unambiguous characterization of its structure. Structural characterization was therefore completed by single crystal X-ray diffraction, leading to the structure presented in Fig. 4.

Several A-seco limonoids have been isolated from a number of Meliaceae plants (Ayafor et al., 1981, 1986; Taylor, 1984; Champagne et al., 1992). To the best of our knowledge, the neotecleanins **1–5** reported in the present study represent the first such occurrence of this limonoid skeleton, differing from related limonoids in the five-membered fused A-ring. These compounds may serve as an intermediate in the pathway to the formation of tecleanin and related compounds.

Mosquito larvicidal activity of the three most abundant compounds **1**, **2** and **4** were tested against late third or early fourth-instar larvae of the mosquito *Anopheles gambiae s.s.* in standard bioassays (WHO, 1996). The three compounds showed strong mosquito larvicidal activity: **1** (LD_{50} = 7.83 ppm), **2** (LD_{50} = 7.07 ppm) and **4** (LD_{50} = 7.05 ppm), which shows that epoxidation of the C-14, C-15 double bond or de-acetylation of the 11-acetate group does not alter mosquito larvicidal activity.

3. Experimental

3.1. General experimental procedure

Melting points were determined in capillary tubes with a Gallenkamp melting point apparatus and are

Table 2
^{13}C NMR spectral data for compounds **1–5**

^{13}C	**1**	**2**	**3**	**4**	**5**
1	216.6	215.3	217.1	218.1	31.9
2	45.2	45.3	46.0	46.0	NS
3	84.4	84.3	85.2	85.0	104.2
4	80.9	80.7	81.1	81.1	85.8
5	59.9	62.2	56.4	54.6	58.1
6	38.2	38.2	26.7	26.3	38.9
7	206.9	207.5	75.1	76.0	211.0
8	53.6	51.5	42.7	43.2	49.8
9	47.7	47.1	46.1	45.3	48.1
10	54.2	52.8	53.5	53.9	40.6
11	76.9	77.3	77.4	75.7	75.2
12	83.1	80.6	88.7	91.4	80.2
13	51.3	45.7	45.3	50.6	45.2
14	147.9	67.8	71.3	154.7	68.6
15	131.5	57.0	56.9	124.4	57.4
16	37.9	33.2	33.6	36.9	33.4
17	50.8	41.8	40.5	51.7	41.1
18	17.3	15.7	15.7	16.5	15.7
19	41.6	41.9	43.2	42.9	37.1
20	124.5	122.4	123.3	124.7	122.6
21	140.7	141.0	141.0	140.7	141.1
22	111.8	111.8	112.7	112.2	112.0
23	142.7	143.0	142.7	142.6	142.9
28	31.6	31.2	31.5	31.7	32.6[a]
29	24.2	23.5	23.9	24.2	28.1[a]
30	30.6	22.3	22.3	29.4	21.9
C7-OCO			169.9	170.1	
C7-OCH_3			21.9	21.6[a]	
C11-OCO	171.2	169.9			170.2
C11-OCH_3	21.7	21.4			21.4
C12-OCO	171.4	169.7	173.9	174.2	169.7
C12-OCH_3	21.5	21.4	21.5	21.5[a]	21.5

NS = not seen.
[a] Resonances cannot be assigned unambiguously and those highlighted with the same letter are interchangeable.

uncorrected. IR spectra were recorded with a Shimadzu FT-IR 8101 spectrometer; UV spectra were determined using a Cecil CE 3041 spectrophotometer. Semi-preparative HPLC was carried out on Varian 5000 LC with a UV detector (215 nm). Analytical HPLC was carried out on a Beckman System Gold 126 equipped with a 168 diode array detector. All NMR experiments were recorded using a Bruker 500 MHz Avance NMR Spectrometer. ^{1}H and ^{13}C spectra were measured at 500 MHz and 125 MHz respectively in $CDCl_3$. HREIMS were recorded on a Finnigan MAT 95Q Hybrid Sector (ThermoFinnigan, San Jose, CA), EI = 70 eV and a mass resolving power of 5000.

3.2. Plant material

The root bark of *T. wakefieldii* was collected from Shimba Hills National Park, Kwale, South Coast of Kenya, and was identified by Mr. S.G. Mathenge of the Botany Department, University of Nairobi. A voucher specimen No. WM 3/99 has been deposited in the herbarium of that department.

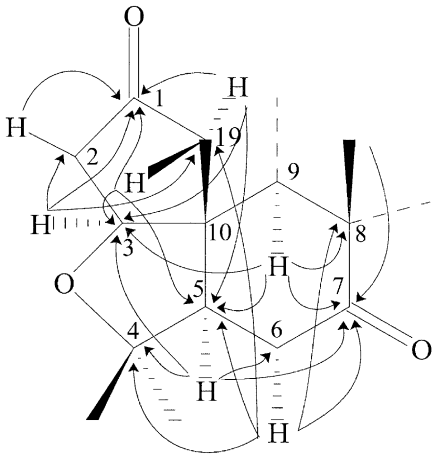

Fig. 2. Long-range HMBC correlations elucidating the neotecleanin ring structure of **1**.

3.3. Extraction and isolation

The air-dried root bark of *T. wakefieldii* (1 kg) was powdered and extracted in the dark by soaking for three weeks with methanol. The methanol extract was concentrated in vacuo to yield a viscous red oil (105 g). Four portions of the oil (22 g each) were partitioned, separately, between water 250 ml and chloroform (200 ml×3). The combined organic layer was concentrated to dryness. The chloroform extract (30 g) was chromatographed on silica gel (81×4.5 cm; 230–400 mesh) using hexane/ethyl acetate gradient. Separation was monitored by thin layer chromatography (TLC). The TLC plates were developed with hexane–ethyl acetate (2:1). The plates were sprayed with Ehrlich's reagent (2% 4-dimethylaminobenzaldehyde in ethanol) and developed in a hydrogen chloride gas chamber (Maier and Grant, 1970), and the stains were compared with those of limonoids previously characterized from *T. floribunda* (Torto et al., 1995, 1996). Fractions that stained for the presence of limonoids similar to those isolated from *T. floribunda* were found predominantly in the 60–75% ethyl acetate–hexane gradient, which consisted of eight (A–H) main fractions. Fraction A, which was eluted with 60% ethyl acetate–hexane, yielded compound **1** (9 mg) after HPLC separation using a Beckman, ultrasphere, ODS (25 cm×10 mm) with 60% CH_3CN-water at a flow rate of 3 ml min^{-1} at 215 nm. Fractions B–E and G contained mixtures of limonoids, which were difficult to separate by further chromatography. Fractions F and H, which were both eluted with 75% ethyl acetate–hexane, were rechromatographed on silica gel using 10% acetone–toluene to give 6 and 12 fractions each, respectively. HPLC separation of fraction six from

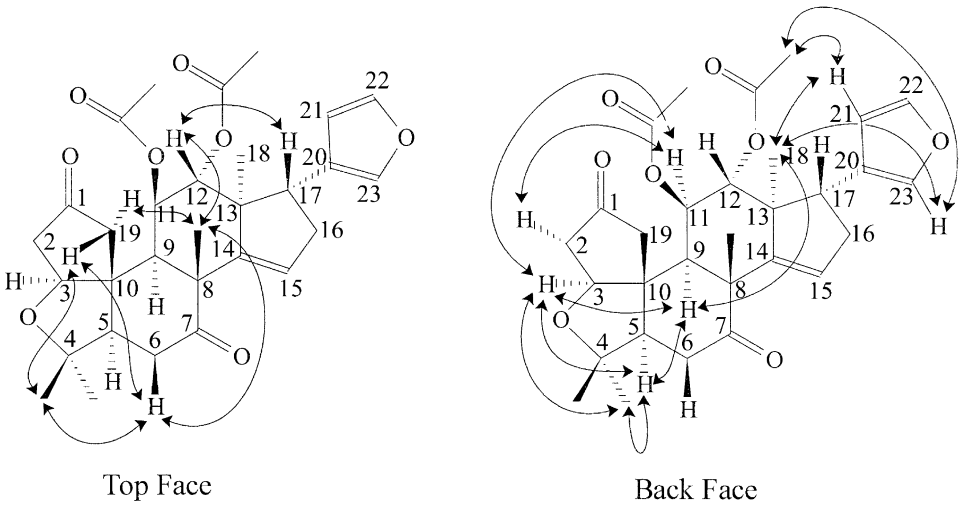

Fig. 3. GOESY correlations on the top face and back face of **1**.

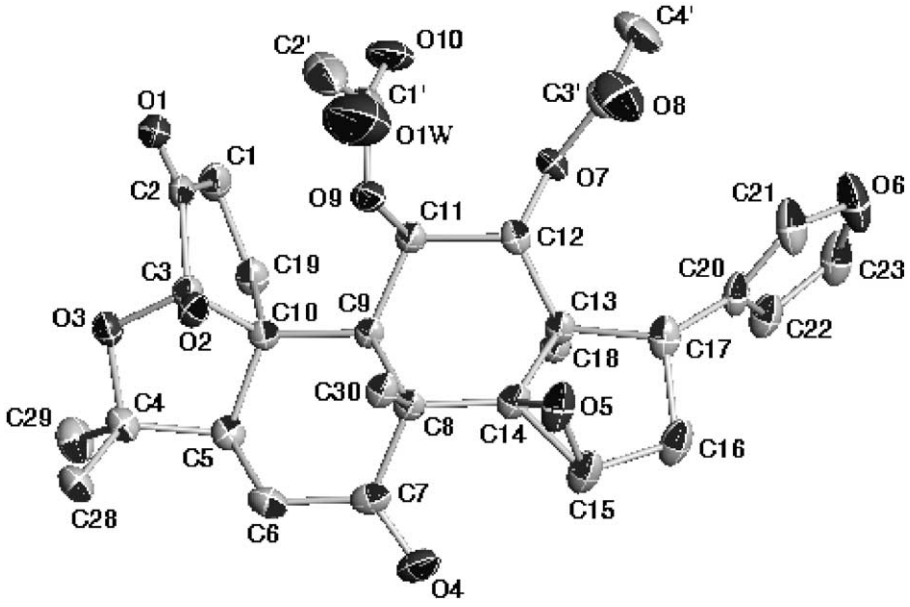

Fig. 4. X-ray diffraction structure of **5**.

F using the same ODS column and conditions as above with 50% CH_3CN–water as the solvent gave **2** (5 mg), while HPLC separation of fraction eight from H, also using the same solvent system at 3 ml min^{-1} gave **3** (0.5 mg), **4** (5 mg) and **5** (0.3 mg).

3.4. 11β, 12α-Diacetoxyneotecleanin (*1*)

White amorphous solid, 9 mg, mp 154–156 °C; $[\alpha]_D^{20}$ ($c = 1.59$, $CHCl_3$) −34.0°; λ_{max}^{MeCN} no absorption above 210 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740, 1715, 1250; ^1H and ^{13}C NMR (Tables 1 and 2). HREIMS m/z 524.2405 (calc. for $C_{30}H_{36}O_8$, 524.2410), EIMS m/z 524 $[M]^+$ (58), 464 (12), 404 (70), 389 (18), 376 (10), 210 (15), 188 (25), 43 (100).

3.5. 11β, 12α-Diacetoxy-14β, 15β-epoxyneotecleanin (*2*)

White amorphous solid, 5 mg, mp 161–162 °C; λ_{max}^{MeCN} 213 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1749, 1734, 1716, 1236; ^1H and ^{13}C NMR (Tables 1 and 2). HREIMS *m/z* 540.2358 (calc. for $C_{30}H_{36}O_9$, 540.2359), EIMS *m/z* 540 $[M]^+$ (16), 480 (3), 465 (5), 421 (29), 374 (14), 332 (8), 43 (100).

3.6. 7α, 12α-Diacetoxy-14β, 15β-epoxy-11β-hydroxy-neotecleanin (*3*)

White amorphous solid, 0.5 mg, mp 180–182 °C; λ_{max}^{MeCN} no absorption above 210 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}:

3570, 1740, 1715, 1250; ^{1}H and ^{13}C NMR (Tables 1 and 2). HREIMS *m/z* 542.2524 (calc. for $C_{30}H_{38}O_9$, 542.2516), EIMS *m/z* 542 $[M]^+$ (38), 524 (6), 482 (36), 422 (52), 376 (28), 256 (30), 175 (50), 43 (100).

3.7. 7α, 12α-Diacetoxy-11β-hydroxyneotecleanin (*4*)

White amorphous solid, 5 mg, mp 186–187 °C; λ_{max}^{MeCN} no absorption above 210 nm; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3570, 1740, 1715, 1250; ^{1}H and ^{13}C NMR (Tables 1 and 2). HREIMS *m/z* 526.2562 (calc. for $C_{30}H_{38}O_8$, 526.2567), EIMS *m/z* 526 $[M]^+$ (16), 466 (100), 448 (9), 446 (14), 406 (14), 43 (27).

3.8. 11β, 12α-Diacetoxy-1-deoxo-14β, 15β-epoxy-3β-hydroxy-2-oxo-neotecleanin (*5*)

Colorless needle-like crystals from MeCN–H_2O, 0.3 mg, mp 179–180 °C; ^{1}H and ^{13}C NMR (Tables 1 and 2). HREIMS *m/z* 556.2480 (calc. for $C_{30}H_{36}O_{10}$, 556.2308). X-ray diffraction: compound **5** crystallized by slow evaporation from 50% acetonitrile–water in the orthorhombic crystal system. X-ray measurements were made at 100 K using a Bruker Proteum diffractometer with a 6 kW Cu rotating anode and Osmic beam focus, and using graphite monochromatised Cu-K_α radiation ($\lambda = 1.54184$ Å). The crystal data are: Space group $P2_12_12_1$, $a = 7.040$ (2), $b = 14.822$ (5), $c = 26.721$ (8) Å, $V = 2788.24$ (15) Å^3, $Z = 4$, $d_x = 1.364$ g cm^{-3}, [μ(Cu-$K\alpha$) = 0.870 mm^{-1}]. Crystal dimensions: 0.4×0.4×0.01 mm. A total of 5222 independent reflections were measured, $R_1 = 0.0421$. A weighting scheme of the form $w = 1/[\sigma^2(F_o^2) + (0.0723P)^2 + 0.5586P]$ where $P = (F_o^2 + 2F_c^2)/3$ was applied. Structure solution and refinement used the SHELX package (version 5.03) comprising SHELXS-97 (Sheldrick, 1997a) and SHELXL-97 (Sheldrick, 1997b); absorption corrections were applied to the data using SADABS (Sheldrick, 1997c). Hydrogen atoms were included in calculated positions with isotropic thermal parameters and refined as riding atoms. A molecule of water was also found in the asymmetric unit bonded to the carbonyls of the C-11 and C-12 acetoxy substituents: O10...O1W = 2.81 (5) Å, O8...O1W = 2.88 (4) Å. Hydrogen bonding was also observed between the C-3 tertiary alcohol and the C-7 keto-group. O2...O4 = 2.72 (4) Å. Crystallographic data for the structural analysis has been deposited with the Cambridge Crystallographic Data Centre, UK, (CCDC #200999). Use the full literature citation when ordering material.

3.9. Mosquito larvicidal assays

Mosquito larvicidal assays followed standard procedures (WHO, 1996). One millilitre of a standard w/v concentrate of each compound in acetone was made to 100 ml with distilled water. Twenty late third or early fourth-instar larvae of *Anopheles gambiae s.s.* were exposed to varying doses of the compounds. Experiments were performed at 25 °C and replicated five times. Mortality was observed after 24 h.

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References

Adul, G.O., Bentley, M.D., Benson, B.W., Huang, F-Y., Gelbaum, L., Hassanali, A., 1993. Two new prieurianin class limonoids from *Turraea mombassana*. Journal of Natural Products 56, 1414–1417.

Akinniyi, J.A., Connolly, J.D., Mulholland, D.A., Rycroft, D.S., Taylor, D.A.H., 1986. Limonoid extractives from *Turraea floribunda* and *Turraea obtusifolia*. Phytochemistry 25, 2187–2189.

Ayafor, J.F., Sondengam, B.L., Connolly, J.D., Rycroft, D.S., Okogun, J.I., 1981. Tetranortriterpenoids and related compounds. Part 26. Tecleanin, a possible precursor of limonin, and other new tetranortriterpenoids from *Teclea grandifolia* Engl. (Rutaceae). Journal of Chemical Society Perkin I 1750–1753.

Ayafor, J.F., Sondengam, B.L., Bilon, A.N., Connolly, J.D., 1986. Limonoids of *Teclea oubanguiensis*. Journal of Natural Products 49, 583–587.

Bentley, M.D., Adul, G.O., Alford, A.R., Huang, F-Y., Gelbaum, L., Hassanali, A., 1995. An insect antifeedant limonoid from *Turraea nilotica*. Journal of Natural Products 58, 748–750.

Champagne, D.E., Koul, O., Isman, M.B., Scudder, G.G.E., Towers, G.H.N., 1992. Biological activity of the Rutales. Phytochemistry 31, 377–394.

Fraser, L.A., Mulholland, D.A., Nair, J.J., 1994. Limonoids from the seed of *Turraea floribunda*. Phytochemistry 35, 455–458.

Garcez, F.R., Garcez, W.S., Roque, N.F., Castellano, E.E., Zukerman-Schpector, J., 2000. 7β-Oxygenated limonoids from *Trichilia elegans* ssp. *elegans*. Phytochemistry 55, 733–740.

Kokwaro, J.O., 1976. Medicinal Plants of East Africa. East African Literature Bureau, Nairobi, Kenya.

Maier, V.P., Grant, E.R., 1970. Specific thin-layer chromatography of limonin, a citrus bitter principle. J. Agr. Food Chem. 18 (2), 250–252.

Mulholland, D.A., Monkhe, T.V., Coombes, P.H., Rajab, M.S., 1998. Limonoids from *Turraea holstii* and *Turraea floribunda*. Phytochemistry 49, 2585–2590.

Sheldrick, G.M., 1997a. SHELXS-97, Program for Automatic Solution of Crystal Structures. University of Göttingham, Göttingham, Germany.

Sheldrick, G.M., 1997b. SHELXL-97, A Program for Crystal Structure Refinement. University of Göttingham, Göttingham, Germany.

Sheldrick, G.M., 1997c. SADABS: A Program for Absorption Correction of Crystallographic Data. University of Göttingham, Göttingham, Germany.

Simmonds, M.S.J., Stevenson, P.C., Porter, E.A., Veitch, N.C., 2001. Insect antifeedant activity of three new tetranortriterpenoids from *Trichilia pallida*. Journal of Natural Products 64, 1117–1120.

Styles, B.T., White, F., 1991. Flora of Tropical East Africa. A A Balkema/Rotterdam/Bookfield.

Tada, K., Takido, M., Kitanaka, S., 1999. Limonoids from fruit of *Melia toosendan* and their cytotoxic activity. Phytochemistry 51, 787–791.

Taylor, D.A.H., 1984. The chemistry of the limonoids from Meliaceae. In: Hertz, W., Grisebach, H., Kirby, G. (Eds.), Progress in the Chemistry of Organic Natural Products, Vol. 45. Srpinger, New York, pp. 1–102.

Torto, B., Bentley, M.D., Cole, J.W., Hassanali, A., Huang, F-Y., Gelbaum, L., Vanderveer, D.G., 1995. Limonoids from *Turraea floribunda*. Phytochemistry 40, 239–243.

Torto, B., Nyandat, E., Hassanali, A., Bentley, M.D., 1996. A limonoid from *Turraea floribunda*. Phytochemistry 42, 1235–1237.

WHO, 1996. Report of the WHO Informal Consultation on the evaluation and testing of insecticides. Geneva.