

# Khayanolides from African mahogany *Khaya senegalensis* (Meliaceae): A revision

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Methyl 6-hydroxyangolensate

## ABSTRACT

Five khayanolides (1-O-acetylkhayanolide B **1**, khayanolide B **2**, khayanolide E **3**, 1-O-deacetylkhayanolide E **4**, 6-dehydroxylkhayanolide E **5**) were isolated from the stem bark of African mahogany *Khaya senegalensis* (Meliaceae). Their structures and absolute configurations were determined through extensive spectroscopic analyses including MS, NMR, and single-crystal X-ray diffraction experiments. The results established that two previously reported khayanolides, 1 $\alpha$ -acetoxy-2 $\beta$ ,3 $\alpha$ ,6,8 $\alpha$ ,14 $\beta$ -pentahydroxy-[4.2.1<sup>10,30</sup>.1<sup>1,4</sup>]-tricyclomeliac-7-oate **6** and 1 $\alpha$ ,2 $\beta$ ,3 $\alpha$ ,6,8 $\alpha$ ,14 $\beta$ -hexahydroxy-[4.2.1<sup>10,30</sup>.1<sup>1,4</sup>]-tricyclomeliac-7-oate **7**, were, in fact, 1-O-acetylkhayanolide B **1** and khayanolide B **2**, and that the two reported phragmalin derivatives, methyl 1 $\alpha$ -acetoxy-6,8 $\alpha$ ,14 $\beta$ ,30 $\beta$ -tetrahydroxy-3-oxo-[3.3.1<sup>10,2</sup>.1<sup>1,4</sup>]-tricyclomeliac-7-oate **8** and methyl 1 $\alpha$ ,6,8 $\alpha$ ,14 $\beta$ ,30 $\beta$ -pentahydroxy-3-oxo-[3.3.1<sup>10,2</sup>.1<sup>1,4</sup>]-tricyclomeliac-7-oate **9**, were, in fact, khayanolide E **3** and 1-O-deacetylkhayanolide E **4**, respectively. Based on the results from this study and consideration of the biogenetic pathway, the methyl 6-hydroxyangolensate in African mahogany *K. senegalensis* should have a C-6 S configuration while methyl 6-hydroxyangolensate in genuine mahogany *Swietenia* species should have a C-6 R configuration.

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## 1. Introduction

The nomenclature limonoid is derived from the compound limonin, one of the primary bitter components in citrus fruits. The structural determination of limonin in 1960 marked the beginning of limonoid (tetranortriterpenoid) chemistry (Arigoni et al., 1960). These highly oxygenated, modified tetranortriterpenoids are commonly found in plants of the families Meliaceae, Rutaceae, Cneoraceae and Simaroubaceae of the order Ratales. Approximately 1300 limonoids, exhibiting more than 35 different carbon frameworks created through ring fission, re-cyclization, reopening, re-closure, and skeletal rearrangements, have been observed over the past five decades, with new structural types continuing to appear (Rajab et al., 1997; Rogers et al., 1998; Tchuendem et al., 1998; Zhang et al., 2003; Yuan et al., 2005; Fang et al., 2008). Of these four families, Meliaceae is of particular interest because of the abundance and structural diversity of the limonoids present

in its plant members (Connolly, 1983; Taylor, 1984; Champagne et al., 1992; Mulholland et al., 2000).

Specifically, African mahogany *Khaya senegalensis* (Meliaceae) is a rich source of limonoids, exemplifying a wide variety of structural types (Adesida et al., 1971; Mulholland et al., 2000; Abdelgaleil and Nakatani, 2003). Khayanolide limonoid, first isolated from *Khaya senegalensis* in 1996, results from a cleavage between C-1, C-2 and a linkage between C-1, C-30 of phragmalin (Olmo et al., 1996). Past research has characterized the following 11 khayanolide limonoids in two plants of the *Khaya* genus: 1-O-deacetylkhayanolide E from *K. grandifoliola* (Zhang et al., 2008) and khayanolide A, B, C (Abdelgaleil et al., 2001), khayanolide D, E (Nakatani et al., 2002), 1-O-acetylkhayanolide A (Nakatani et al., 2001), 1-O-acetylkhayanolide B (Abdelgaleil et al., 2000), 1 $\alpha$ -acetoxy-2 $\beta$ ,3 $\alpha$ ,6,8 $\alpha$ ,14 $\beta$ -pentahydroxy-[4.2.1<sup>10,30</sup>.1<sup>1,4</sup>]-tricyclomeliac-7-oate **6** (Olmo et al., 1996), 1 $\alpha$ ,2 $\beta$ ,3 $\alpha$ ,6,8 $\alpha$ ,14 $\beta$ -hexahydroxy-[4.2.1<sup>10,30</sup>.1<sup>1,4</sup>]-tricyclomeliac-7-oate **7** and 1 $\alpha$ -acetoxy-3 $\beta$ ,6,8 $\alpha$ -trihydroxy-2 $\alpha$ -methoxy-2 $\beta$ ,14 $\beta$ -epoxy-[4.2.1<sup>10,30</sup>.1<sup>1,4</sup>]-tricyclomeliac-7-oate (Olmo et al., 1997) from *K. senegalensis*. However, the configurations of oxygenated C-6 in these khayanolides were not confirmed, except for that in khayanolide A, which was determined through

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X-ray crystallography and CD study (Abdelgaleil et al., 2001). To investigate the structures of these remaining khayanolides fully, the research reported here studied the stem bark of *K. senegalensis* from the Republic of Guinea, isolating five khayanolides: 1-O-acetylkhayanolide B **1**, khayanolide B **2**, khayanolide E **3**, 1-O-deacetylkhayanolide E **4**, and 6-dehydroxykhayanolide E **5**. The first four compounds were already known but their structures not previously confirmed, while compound **5** was a new discovery. The structures and stereochemistry of **1–5** were subsequently determined through spectroscopic methods, including MS (ESI-MS and HREI-MS), 1D and 2D NMR, and most importantly, X-ray diffraction experiments.

## 2. Results and discussion

Compound **1** (Fig. 1) was found to have the molecular formula of  $C_{29}H_{36}O_{11}$  as determined through ESI-MS and NMR experiments. Its 1D and 2D NMR spectroscopic data (Tables 1 and 2) (measured in  $CDCl_3$  and trace  $CD_3OD$ ) were in good agreement with those of 1-O-acetylkhayanolide B (obtained in  $CDCl_3$  and trace  $CD_3OD$ ) (Abdelgaleil et al., 2000) and **6** (tested in  $CDCl_3$  and trace  $DMSO-d_6$ ) (Olmo et al., 1996), both isolated from the stem bark of the same species, *K. senegalensis*. All of them (Tables 1 and 2) indicated similar chemical shift values, coupling constants, H–C long-range correlations and coupling patterns. However, the structures of 1-O-acetylkhayanolide B and **6** (Fig. 1) in previous literature were found to differ in their structures (i.e., an ether linkage between C-2 and C-14 in 1-O-acetylkhayanolide B vs. two independent hydroxyl groups at C-2 and C-14 in **6**) and in their stereochemistry (i.e.,  $\beta$ -orientation of the C-3 hydroxyl group and an assumed S configuration of C-6 in 1-O-acetylkhayanolide B vs. an  $\alpha$ -orientation of C-3 hydroxyl and an unresolved configuration of C-6 in **6**). As a result, this research conducted an in-depth 3D structural study of **1** using single-crystal X-ray diffraction analysis to determine its structure and stereochemistry.

The crystal structure of **1** with its atomic labeling shown in Fig. 2 confirmed the structure of 1-O-acetylkhayanolide B as reported by the Nakatani group (Abdelgaleil et al., 2000). This X-ray crystallography, however, did not support 2,14-dihydroxyl groups in **6** as described by the Silva group, perhaps they did not conduct a mass spectroscopic analysis (Olmo et al., 1996). In addition, the stereochemical analysis obtained through X-ray crystallography established that the five-membered-rings **A**<sub>1</sub> (C-1, 29, 4, 5, 10), **B**<sub>1</sub> (C-1, 30, 8, 9, 10) and **B**<sub>2</sub> (C-2, 30, 8, 14 and O) had half-chair conformations and the furan ring E was planar while the six-membered ring **A**<sub>2</sub> had a distorted chair conformation and rings C, D exhibited a chair conformation. The spatial proximity 3.37 Å between H-3 ( $\alpha$ -orientation) and H-5 ( $\beta$ -orientation) results in the observed correlation between H-3 and H-5 in NOESY although they are on different sides of the plane. The 33.2° dihedral angle of H (2)–C (2)–C (3)–H (3) accounted for the coupling constant value  $J_{H-2,H-3} = 6.5$  Hz. Although the  $J_{H-2,H-3}$  value and NOE correlation between H-3 and H-5 were the same, the conclusions from these results reported here differ from those of the Silva group (Olmo et al., 1996), i.e. concerning a boat conformation of the six-membered ring **A**<sub>2</sub> (C-1, 2, 3, 4, 29, 30) and an H-3  $\beta$ -orientation in **6**.

Furthermore, the absolute configuration of C-6 in **1** was confirmed as S on the basis of anomalous scattering of the chlorines of the  $CHCl_3$  molecules in the crystal. The Flack parameter (Flack, 1983) was used to confirm that the correct absolute configuration is the one presented. Based on these results, it was concluded that khayanolide **6** is, in fact, 1-O-acetylkhayanolide B **1**.

Compound **2** (Fig. 1) was found to have the molecular formula of  $C_{27}H_{34}O_{10}$  as determined through ESI-MS and NMR experiments. Its NMR spectroscopic data (Tables 1 and 2) were similar to those of 1-O-khayanolide B **1** except for the absence of the acetyl group at C-1. The low-frequency shift of  $\delta$  84.0 (C-1) in **2** and  $\delta$  91.2 (C-1) in **1** suggests **2** was khayanolide B, a conclusion supported through 2D NMR experiments. In fact, the NMR spectroscopic data of **2** was

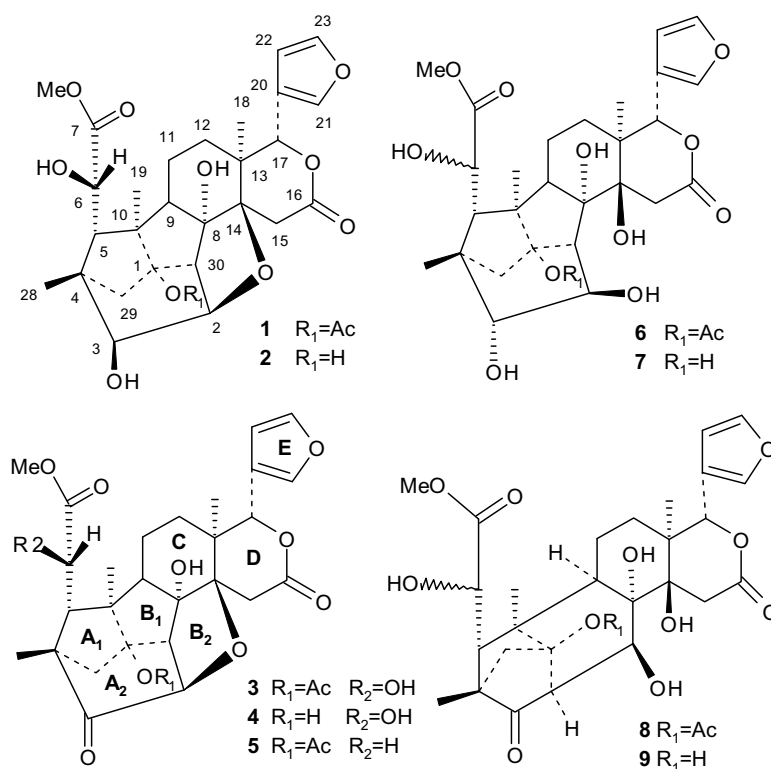


Fig. 1. Structures of isolated khayanolides **1–5** and reported limonoids **6–9** in literature.

**Table 1**<sup>1</sup>H chemical shifts for isolated **1–5** and reported **6–9** in literatures.

Number	<b>1</b> <sup>a</sup>	<b>6</b> <sup>b,e</sup>	<b>2</b> <sup>a</sup>	<b>7</b> <sup>c,e</sup>	<b>3</b> <sup>a</sup>	<b>8</b> <sup>b,e</sup>	<b>4</b> <sup>a</sup>	<b>9</b> <sup>c,e</sup>	<b>5</b> <sup>d</sup>
2	4.43 (dd, 6.5,9.5)	4.45 (dd, 6.7, 9.5)	4.26 (dd, 6.5,9.5)	4.28 (dd, 7.2,9.2)	4.34 (d, 10.5)	4.40 (d, 10.4)	4.24 (d, 10.4)	4.37 (d, 10.3)	4.42 (d, 10.5)
3	3.39 (d, 6.5)	3.39 (brd, 6.7)	3.20 (d, 6.5)	3.21 (dd, 7.2,9.0)					
5	2.99 (d, 8.0)	2.93 (d, 7.0)	3.00 (d, 7.8)	2.88 (d, 6.8)	2.87 (brd, 4.8)	3.07 (d, 8.4)	3.08 (d, 8.7)	3.25 (d, 8.0)	3.47 (dd, 11.0, 4.0)
6	4.10 (d, 8.0)	4.15 (d, 7.0)	4.12 (d, 7.8)	4.03 (dd, 6.8,3.6)	4.32 (d, 4.8)	4.13 (8.4,5.0)	4.25 (d, 8.7)	4.22 (dd, 8.0, 5.0)	2.46 (dd, 11.0, 17.0) 2.31 (dd, 4.0, 11.0)
9	2.17 (d, 7.0)	2.16 (brd, 7.0)	2.20 (d, 7.6)	2.03 (brd, 9.0)	2.20 (d, 9.3)	2.30 (brd 8.4)	2.28 (d, 9.3)	2.28 (brd, 10.0)	2.27 (brd, 9.5)
11 $\alpha$	1.78 (m)	–	1.85 (m)	1.74 (m)	1.86 (m)	1.64–1.72 (m)	2.04 (m)	1.77–1.83 (m)	1.98 (m)
$\beta$	1.56 (m)	–	1.75 (m)	1.60 (m)	1.53 (m)	1.90 (m)	1.81 (m)	1.90 (m)	1.52 (m)
12 $\alpha$	0.90 (m)	0.81 (m)	0.91 (m)	0.62 (brd, 12.0)	0.83 (m)	0.74 (brd, 10.8)	0.91 (m)	0.85 (brd, 9.0)	1.04 (m)
$\beta$	1.78 (m)	–	1.81 (m)	1.78 (m)	1.63 (m)	1.64–1.72 (m)	1.81 (m)	1.77–1.83 (m)	1.85 (m)
15 $\alpha$	2.97 (d, 17.5)	3.04 (d, 18.7)	3.04 (d, 17.5)	2.86 (d, 18.5)	3.06 (d, 18.6)	2.96 (d, 18.8)	3.06 (d, 18.8)	3.10 (d, 18.6)	3.14 (d, 18.5)
$\beta$	2.68 (d, 17.5)	2.69 (d, 18.7)	2.67 (d, 17.5)	2.63 (d, 18.5)	2.58 (d, 18.6)	2.72 (d, 18.8)	2.69 (d, 18.8)	2.69 (d, 18.6)	2.76 (d, 18.5)
17	5.58 (s)	5.50 (s)	5.55 (s)	5.61 (s)	5.44 (s)	5.26 (s)	5.43 (s)	5.40 (s)	5.55 (s)
18	1.02 (s)	1.05 (s)	1.02 (s)	0.96 (s)	1.03 (s)	1.03 (s)	1.03 (s)	1.11 (s)	1.10 (s)
19	1.20 (s)	1.21 (s)	1.24 (s)	1.07 (s)	1.34 (s)	1.33 (s)	1.24 (s)	1.33 (s)	1.26 (s)
21	7.39 (s)	7.33 (m)	7.41 (s)	7.58 (m)	7.34 (s)	7.68 (m)	7.41 (d, 1.7)	7.59 (m)	7.39 (s)
22	6.34 (s)	6.33 (m)	6.34 (s)	6.45 (m)	6.30 (s)	6.47 (m)	6.35 (d, 1.7)	6.47 (m)	6.35 (s)
23	7.33 (s)	7.37 (m)	7.35 (s)	7.62 (m)	7.33 (s)	7.67 (m)	7.39 (d, 1.7)	7.56 (m)	7.37 (s)
28	0.99 (s)	1.03 (s)	0.95 (s)	0.95 (s)	1.27 (s)	0.92 (s)	0.91 (s)	0.99 (s)	0.99 (s)
29	1.75 (d, 12.0)	2.23 (d, 12.2)	1.70 (d, 12.0)	1.16 (d, 12.0)	2.08 (d, 12.6)	2.04 (d, 10.0)	1.72 (d, 12.0)	1.73 (d, 12.6)	1.93 (d, 13.5)
	2.21 (d, 12.0)	1.74 (d, 12.2)	2.13 (d, 12.0)	1.68 (d, 12.0)	2.75 (d, 12.6)	2.55 (d, 10.0)	2.03 (d, 12.0)	2.11 (d, 12.6)	3.14 (d, 13.5)
30	3.10 (d, 9.5)	3.15 (d, 9.5)	2.88 (d, 9.5)	2.48 (d, 9.2)	3.28 (d, 10.5)	3.27 (d, 10.5)	2.73 (d, 10.4)	2.88 (d, 10.3)	3.36 (d, 10.5)
OMe	3.70 (s)	3.69 (s)	3.62 (s)	–	3.69 (s)	3.61 (s)	3.62 (s)	3.66 (s)	3.67 (s)
OAc	1.97 (s)	1.97 (s)			2.01 (s)	2.02 (s)			2.02 (s)

Note:  $\delta$  in ppm relative to the internal TMS, multiplicities (s, single; d, double; t, triplet; m, multiplet; br, broad; and coupling constants ( $J$  in Hz) are given in brackets. Assignments are based on extensive 2D NMR data including COSY, HMQC and HMBC and NOESY. “–” data are absent. The assignments of H-2, 30 of **8**, **9** from previous references were exchanged for comparison with a underline marker.

<sup>a</sup> In CDCl<sub>3</sub> and trace CD<sub>3</sub>OD.

<sup>b</sup> In CDCl<sub>3</sub> and trace DMSO–D<sub>6</sub>.

<sup>c</sup> In DMSO–D<sub>6</sub>.

<sup>d</sup> In CDCl<sub>3</sub>.

<sup>e</sup> Data are from references.

in good agreement with those reported for khayanolide B (Abdelgaleil et al., 2001) and khayanolide **7** (Olmo et al., 1996), both isolated from the stem bark of the same species, *K. senegalensis*. Since the only difference between **7** and **6** was a substituent group change at C-1 and khayanolide **6** was found to be the same as **1** through X-ray crystallography analysis, it was concluded based on spectroscopic correlations that khayanolide **7** was, in fact, khayanolide B **2**. In addition, the biogenetic pathway indicated the configuration of oxygenated C-6 of **2** is *S*, the same as for **1**.

Compound **3** (Fig. 1) was found to have the molecular formula of C<sub>29</sub>H<sub>34</sub>O<sub>11</sub> as determined through ESI-MS and NMR experiments (Tables 1 and 2). Its NMR spectroscopic data were in good agreement with those of khayanolide E (Nakatani et al., 2002) and the phragmalin derivative methyl 1 $\alpha$ -acetoxy-6,8 $\alpha$ ,14 $\beta$ ,30 $\beta$ -tetrahydroxy-3-oxo-[3.3.1<sup>10,2,1,4</sup>]-tricyclomeliac-7-oate **8** (Fig. 1) (Olmo et al., 1997), both being isolated from the stem bark of the same plant species, *K. senegalensis*. However, the following differences between khayanolide E and **8** were reported in past studies: Khayanolide E has an ether linkage between C-2 and C-14 and a proposed 6 *S* configuration, while **8** has two independent hydroxyl groups at C-14 and C-30 and an unresolved C-6 configuration; furthermore, khayanolide E was a khayanolide limonoid, while **8** was

a phragmalin limonoid because of the locations of C-2 and C-30. In addition, khayanolide E and **8** had essentially identical spectroscopic properties. These structural differences resulted in an in-depth 3D structure study of **3** using single-crystal X-ray diffraction analysis.

The crystal structure of **3** with its atomic labeling shown in Fig. 3 confirmed the structure of khayanolide E as reported by the Nakatani group (Nakatani et al., 2002), indicating that khayanolide **8** was the same as khayanolide E **3**. The reasons that the Silvo group (Olmo et al., 1997) concluded that this was phragmalin **8**, and not khayanolide **3**, were perhaps due to the following three factors: (1) they did not observe a molecular ion peak in the EI-MS spectrum (Olmo et al., 1997); (2) they did not consider other possibilities besides phragmalin as being compatible with the 2D NMR spectroscopic data and (3) they did not take into account the important HMBC correlations between H-30 (1H,  $\delta$  3.28, d,  $J$  = 10.5) and C-9 ( $\delta$  55.1, CH), and between H-2 (1H,  $\delta$  4.34, d,  $J$  = 10.5) and C-4 ( $\delta$  51.4, C), observed in both the experiments reported here and those of the Nakatani group. These three factors were critical in determining the structural similarities and differences between khayanolides and phragmalins.

**Table 2**<sup>13</sup>C NMR chemical shifts for isolated **1–5** and reported **1–4** as well as **6–9** in literatures.

Num.	<b>1</b> <sup>a</sup>	<b>1</b> <sup>a,f</sup>	<b>6</b> <sup>b,f</sup>	<b>2</b> <sup>a</sup>	<b>2</b> <sup>a,f</sup>	<b>7</b> <sup>c,f</sup>	<b>3</b> <sup>a</sup>	<b>3</b> <sup>a,f</sup>	<b>8</b> <sup>c,f</sup>	<b>4</b> <sup>a</sup>	<b>4</b> <sup>a,f</sup>	<b>9</b> <sup>c,f</sup>	<b>5</b> <sup>d</sup>
1	91.2	91.4	91.1	84.0	84.3	83.5	90.8	91.4	90.1	84.0	84.8	83.6	90.9
2	72.1	72.0	72.0	72.3	72.2	72.3	74.3	74.0	74.1	74.8	75.8	74.5	74.5
3	78.2	78.1	78.1	78.6	78.5	78.2	205.8	204.8	205.4	206.7	207.3	206.5	204.0
4	43.7	44.1	44.1	42.7	42.7	42.7	51.4	52.3	50.5	49.4	51.8	49.3	51.8
5	39.1	39.4	39.1	40.9	40.9	40.8	40.9	42.1	40.0	42.2	43.1	41.9	35.3
6	71.1	71.6	71.5	71.3	71.6	71.4	70.7	71.7	69.6	70.2	72.7	70.0	34.0
7	175.3	175.2	175.1	175.3	175.4	175.1	174.4	174.9	173.4	174.2	175.9	173.6	172.5
8	86.6	86.3	86.8	86.9	87.0	86.6	86.8	87.7	86.6	87.1	88.5	86.8	87.8
9	55.2	55.8	55.9	55.5	56.1	55.3	55.1	57.6	54.5	55.3	56.8	54.6	55.8
10	61.0	61.2	60.9	59.3	59.4	59.2	61.6	62.1	60.9	59.5	59.4	59.3	59.4
11	16.3	16.4	16.4	16.3	16.5	16.4	16.4	16.7	16.4	16.1	17.1	16.2	16.3
12	26.1	26.1	26.0	26.1	26.0	25.9	26.0	26.9	26.4	26.4	26.6	26.3	25.9
13	37.6	37.7	37.5	37.6	37.7	37.1	37.5	37.5	37.0	37.4	38.4	36.9	37.7
14	81.5	81.6	81.5	81.5	81.5	80.7	83.5	83.3	83.4	83.4	84.3	83.0	83.5
15	31.9	31.9	32.0	32.0	32.0	32.3	32.7	32.5	33.0	33.7	34.2	33.1	32.7
16	171.6	170.9	170.3	171.5	171.4	170.5	171.6	170.0	169.7	172.0	170.4	169.9	169.6
17	81.1	80.9	80.5	81.1	81.0	81.0	80.9	79.9	80.0	81.2	80.7	80.2	80.1
18	14.4	14.4	14.4	14.4	14.4	14.5	14.2	14.2	14.7	13.9	14.6	14.6	14.3
19	18.0	18.0	18.1	17.8	17.7	18.6	18.6	20.1	18.0	17.2	21.0	18.3	18.5
20	120.5	120.6	120.6	120.5	120.7	121.3	120.4	120.6	120.7	120.8	121.9	120.7	120.5
21	141.0	141.0	140.9	141.0	140.9	141.2	141.1	141.1	141.7	141.2	141.7	141.5	141.2
22	110.0	110.0	110.1	110.0	110.0	110.5	110.0	110.1	110.5	110.0	110.9	110.4	110.1
23	142.7	142.7	142.6	142.7	142.6	143.2	142.9	142.9	143.5	142.8	143.4	143.3	142.8
28	19.0	19.2	19.3	19.1	19.2	19.7	15.3	15.9	15.1	14.4	16.2	15.3	15.0
29	41.3	41.2	41.1	44.5	44.6	45.3	40.8	40.7	40.7	44.0	46.5	44.3	40.6
30	58.5	58.8	58.7	63.2	63.3	63.1	58.5	59.7	58.1	63.2	65.1	62.7	60.0
OCH <sub>3</sub>	52.2	52.5	52.2	52.2	52.1	51.4	52.2	52.5	51.8	51.3	52.2	51.6	52.0
OCOCH <sub>3</sub>	171.0	170.7	170.4				171.2	–	170.2				170.1
OCOCH <sub>3</sub>	21.7	21.7	22.0				21.6	–	22.0				21.9

Note:  $\delta$  in ppm relative to the internal TMS. “–” data are absent. Assignments are based on DEPT, COSY, HMQC and HMBC. The assignments of C-2, 30 of **8** and **9** from references were exchanged with a underline marker for comparison.

<sup>a</sup> In CDCl<sub>3</sub> and trace CD<sub>3</sub>OD.

<sup>b</sup> In CDCl<sub>3</sub> and trace DMSO-*d*<sub>6</sub>.

<sup>c</sup> In DMSO-*d*<sub>6</sub>.

<sup>d</sup> In CDCl<sub>3</sub>.

<sup>e</sup> In C<sub>5</sub>D<sub>5</sub>N.

<sup>f</sup> Data are from references.

Compound **4** (Fig. 1) was found to have the molecular formula of C<sub>27</sub>H<sub>32</sub>O<sub>10</sub> as determined through ESI-MS and NMR experiments. Its NMR spectroscopic data (Tables 1 and 2) were similar to those of khayanolide **E** **3** except for the absence of an acetyl group at C-1. The low-frequency shift of  $\delta$  84.0 (C-1) in **4** and  $\delta$  90.8 (C-1) in **3** suggested **4** was 1-O-deacetylkhayanolide **E**, a conclusion supported through 2D NMR experiments. In fact, the NMR spectroscopic data of **4** were in good agreement with those reported for 1-O-deacetylkhayanolide **E** (Zhang et al., 2008) and the phragmalin derivative 1 $\alpha$ ,6,8 $\alpha$ ,14 $\beta$ ,30 $\beta$ -pentahydroxy-3-oxo-[3.3.1<sup>10,2.1</sup><sup>1,4</sup>]-tricyclicmeliac-7-oate **9** (Olmo et al., 1997). Since the only difference between **9** and **8** was a substituent group change at C-1 and phragmalin **8** was found to be the same as **3** through X-ray crystallography analysis, it was concluded based on spectroscopic correlations that phragmalin **9** was, in fact, 1-O-deacetylkhayanolide **E** **4**. In addition, the biogenetic pathway indicates the configuration of oxygenated C-6 of **4** was *S*, the same as for **3**. Since different solvents were used in the research on these compounds, the NMR spectroscopic data of **4** assigned through 2D NMR experiments are listed for comparison in Tables 1 and 2.

The molecular formula of **5** was determined to be C<sub>29</sub>H<sub>34</sub>O<sub>10</sub> through ESI-MS, HR-EI-MS and NMR data. The strong IR absorption peaks (3430, 1736, 1703 cm<sup>−1</sup>) indicated the presence of hydroxyl, ketone, and ester carbonyl groups. The NMR spectroscopic data were similar to those of **3**, except for the presence of the C-6 methine (<sup>1</sup>H NMR: 1H,  $\delta$  4.32, d, *J* = 4.8 Hz; <sup>13</sup>C NMR:  $\delta$  70.7 CH) in **3** and the methylene assigned to C-6 (<sup>1</sup>H NMR 1H,  $\delta$  2.46, dd, *J* = 11.0, 17.0 Hz and 1H,  $\delta$  2.31, dd, *J* = 4.0, 17.0 Hz; <sup>13</sup>C NMR:  $\delta$  34.0, CH<sub>2</sub>) in **5**, suggesting **5** is a 6-dehydroxy derivative of **3**. This conclusion was supported through COSY, HMQC, HMBC and NOESY

experiments and finally confirmed through single-crystal X-ray diffraction (Fig. 4). The full assignments of the NMR data of 6-dehydroxykhayanolide **E** **5** are listed in Tables 1 and 2.

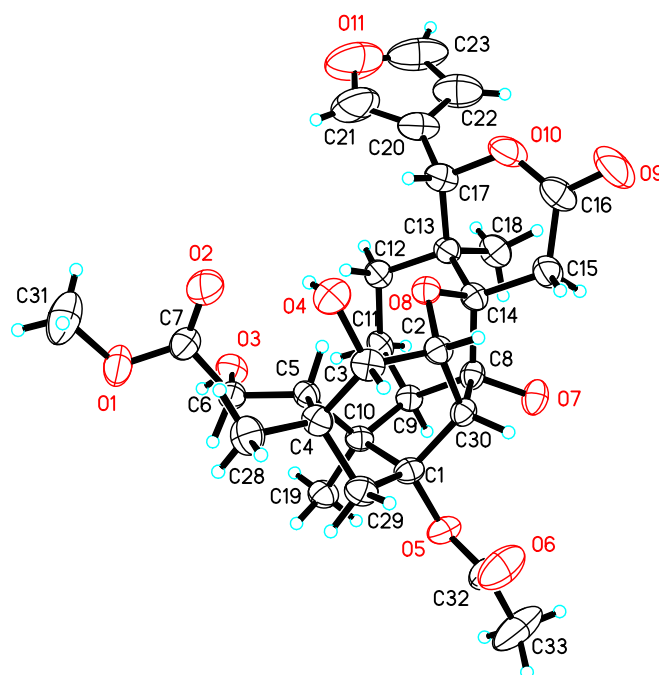


Fig. 2. The crystal structure of **1** with the atom-numbering scheme.

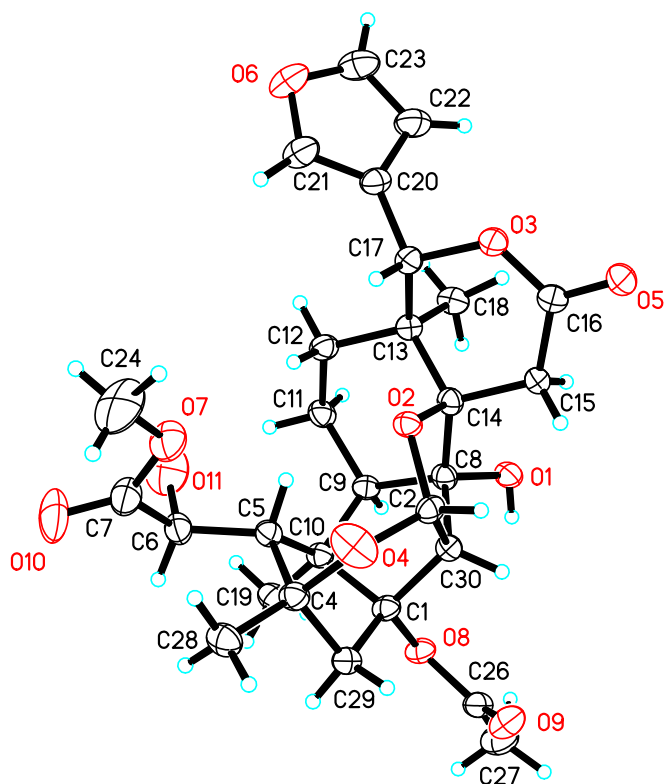


Fig. 3. The crystal structure of **3** with the atom-numbering scheme.

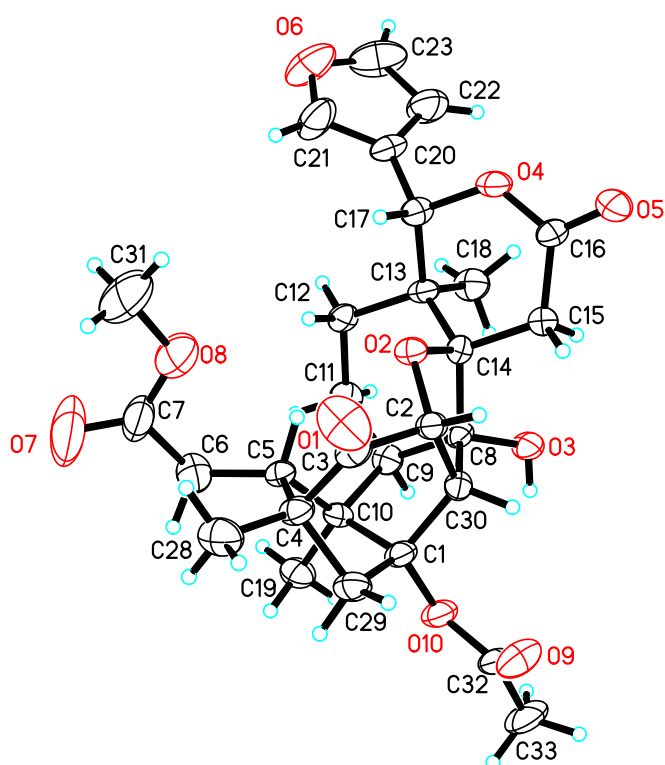


Fig. 4. The crystal structure of **5** with the atom-numbering scheme.

### 3. Concluding remarks

Overall, the X-ray crystallography results reported here confirm the structure and the stereochemistry of 1-*O*-deacetylkhayanolide **B** **1** and khayanolide **E** **3** was reported by the Nakatani group, estab-

lishing that the two reported khayanolides, 1 $\alpha$ -acetoxy-2 $\beta$ ,3 $\alpha$ ,6,8 $\alpha$ ,14 $\beta$ -pentahydroxy-[4.2.1<sup>10,30,1<sup>4</sup></sup>]-tricyclomeliac-7-oate **6** and 1 $\alpha$ ,2 $\beta$ ,3 $\alpha$ ,6,8 $\alpha$ ,14 $\beta$ -hexahydroxy-[4.2.1<sup>10,30,1<sup>4</sup></sup>]-tricyclomeliac-7-oate **7** were, in fact, 1-*O*-acetylkhayanolide **B** **1** and khayanolide **B** **2**, respectively, and that the two reported phragmalin derivatives, methyl 1 $\alpha$ -acetoxy-6,8 $\alpha$ ,14 $\beta$ ,30 $\beta$ -tetrahydroxy-3-oxo-[3.3.1<sup>10,2,1<sup>4</sup></sup>]-tricyclomeliac-7-oate **8** and methyl 1 $\alpha$ ,6,8 $\alpha$ ,14 $\beta$ ,30 $\beta$ -pentahydroxy-3-oxo-[3.3.1<sup>10,2,1<sup>4</sup></sup>]-tricyclomeliac-7-oate **9** were, in fact, khayanolide **E** **3** and 1-*O*-deacetylkhayanolide **E** **4**, respectively. All the C-6 configurations in these khayanolides were determined to be *S*, the same as that of khayanolide **A**.

On the contrary, all the oxygenated C-6 configurations in the mexicanolide limonoids from the original mahogany *Swietenia* genus were reported to be *R*, the same as that of swietenine isolated from *Swietenia macrophylla* and confirmed to have a 6 *R* configuration through X-ray crystallography (McPhail and Sim, 1964). Past research has shown that methyl 6-hydroxyangolensate is a common precursor (Connolly et al., 1964; Taylor, 1984) of further derived C-6 oxygenated limonoids such as mexicanolides, phragmalins, and khayanolides. However, the C-6 configuration of methyl 6-hydroxyangolensate was not fully analyzed in the past because its stereochemistry was unable to be determined through routine spectroscopic analysis. Considering that the configuration of the oxygenated C-6 does not change during its transformation from methyl 6-hydroxyangolensate to mexicanolides, phragmalins, and khayanolides, it is reasonable to assume the methyl 6-hydroxyangolensate from the *K. senegalensis* (Adesida et al., 1967) should have a 6 *S* configuration while methyl 6-hydroxyangolensate (Saad et al., 2003) from the *Swietenia* species should have a 6 *R* configuration. The methyl 6*S*-hydroxyangolensate from *K. senegalensis* was proposed by Taylor 40 years ago, though it was not verified at that time (Adesida et al., 1967; Adesogan and Taylor, 1968). The results reported here not only confirm the C-6 *S* configuration assignment of the khayanolides but also indirectly support the structure of methyl 6 *S*-hydroxyangolensate deduced by Taylor group. The configuration difference of oxygenated C-6 in B,*D*-seco limonoids imply a significant chemotaxonomy between the African mahogany *Khaya* genus and the genuine mahogany *Swietenia* genus.

### 4. Experimental

#### 4.1. General

Optical rotation: Perkin–Elmer-341 polarimeter. IR spectra: Nicolet-Magna-750-FTIR spectrometer, KBr pellets; NMR spectra: Varian Mercury plus 300, Bruker-DRX-400 or Bruker-AV-500 or AV-300 instruments, in CDCl<sub>3</sub> or CDCl<sub>3</sub>+CD<sub>3</sub>OD with TMS as internal reference; Mass spectral: EI-MS (HREI-MS); Finnigan MAT-95 mass spectrometer; ESI-MS; LCQ-Deca or Waters Q-TOF micro™ mass spectrometer. X-ray diffraction: Rigaku AFC8S diffractometer with Mercury CCD detector or Bruker Smart Apex CCD.

#### 4.2. Plant material

The stem bark of *K. senegalensis* (495 g) was collected from the maritime plains near Conakry, Republic of Guinea in November 2005, and was identified by Dr. Youssef Koita, a botanist in the department Sante Communautaire, Ministere de Sante Publique, Republic of Guinea. A voucher specimen 018/INSP/2005 has been deposited in the Ministry of Public Health, Republic of Guinea.

#### 4.3. Extraction and isolation

To air dried and powdered stem bark of *K. senegalensis* (495 g) was added MeOH (3 l) in three batches with the whole heated until reflux began thus using a Soxhlet extractor being maintained for



24 h (each). The methanol-soluble portions of the combined extracts was dried on a rotary evaporator under reduced pressure to give a brown residue (60 g), which was suspended in H<sub>2</sub>O (300 ml) and partitioned between H<sub>2</sub>O and EtOAc (300 × 3 ml). The concentrated EtOAc solubles (15 g) were separated on a silica gel (32–63 μm) column eluted with CHCl<sub>3</sub>/acetone with gradient increasing of acetone (30:1 CHCl<sub>3</sub>:acetone to acetone) to obtain six fractions (F1–F6). F5 (3.5 g) was further separated into five fractions using a silica gel column (CHCl<sub>3</sub>/MeOH 50:1–4:1). Repeated chromatography over reversed phase C-18 silica gel (40–63 μm) and Sephadex LH-20 afforded compound **1** (35 mg) eluted by MeOH:H<sub>2</sub>O (2:1) from sub-fraction F5-3, **4** (7 mg) eluted by MeOH:H<sub>2</sub>O (1:1) from sub-fraction F5-4, **2** (12 mg) eluted by MeOH:H<sub>2</sub>O (1:2) from sub-fraction F5-5. F3 (2.7 g) was further purified by a silica gel column (CHCl<sub>3</sub>/EtOAc 20:1–2:1) to give five fractions. A crystal mixture of **3** and **5** (35 mg) obtained from sub-fraction F3-3 was subjected to reversed phase C-18 column eluted by gradient CH<sub>3</sub>CN:MeOH:H<sub>2</sub>O (15:15:70, 18:18:64, 20:20:60, 25:25:50, 30:30:40 and pure MeOH). Compound **3** (11 mg) was obtained from CH<sub>3</sub>CN:MeOH:H<sub>2</sub>O 20:20:60 fractions while **5** (12 mg) was obtained from the MeOH fractions. Compounds **1**–**5** were re-crystallized in the solvents CHCl<sub>3</sub>, or CHCl<sub>3</sub>/MeOH, or MeOH/H<sub>2</sub>O, or CHCl<sub>3</sub>/CH<sub>3</sub>COCH<sub>3</sub>.

#### 4.4. 1-O-acetylkhyanolide B (**1**)

Colorless block-shaped crystals (CHCl<sub>3</sub>); ESI-MS *m/z*: 561.22 [M+H]<sup>+</sup>, 1121.42 [2M+H]<sup>+</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Tables 1 and 2.

#### 4.5. Khyanolide B (**2**)

Colorless needles from MeOH/H<sub>2</sub>O; ESI-MS *m/z*: 519.22 [M+H]<sup>+</sup>, 541.20 [M+Na]<sup>+</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Tables 1 and 2.

#### 4.6. Khyanolide E (**3**)

Colorless block-shaped crystals from CHCl<sub>3</sub>/MeOH; ESI-MS *m/z*: 559.3 [M+H]<sup>+</sup>, 1117.5 [2M+H]<sup>+</sup>, 1139.4 [2M+Na]<sup>+</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Tables 1 and 2.

#### 4.7. 1-O-deacetylkhyanolide E (**4**)

Colorless needles from CHCl<sub>3</sub>/CH<sub>3</sub>COCH<sub>3</sub>; ESI-MS *m/z*: 517.14 [M+H]<sup>+</sup>, 539.11 [M+Na]<sup>+</sup>, 1033.27 [2M+H]<sup>+</sup>, 1055.23 [2M+Na]<sup>+</sup>; for <sup>1</sup>H and <sup>13</sup>C NMR data, see Tables 1 and 2.

#### 4.8. 6-Dehydroxylkhyanolide E (**5**)

Colorless block-shaped crystals from chloroform and methanol; [α]<sub>D</sub><sup>20</sup> 26 (0.14, CHCl<sub>3</sub>); IR ν<sub>max</sub> (KBr) cm<sup>−1</sup>: 3430 (br), 2923, 1736 (s), 1703 (s), 1465, 1383, 1242 (s), 1167, 1014, 875; ESI-MS (positive ion model) *m/z*: 543.2 [M+H]<sup>+</sup>, 1085.5 [2M+H]<sup>+</sup>, 1107.5 [2M+Na]<sup>+</sup>; ESI-MS (negative ion model) *m/z*: 587.4 [M+HCOOH−H]<sup>−</sup>, 1083.4 [2M−H]<sup>−</sup>; EI-MS *m/z* (rel. int.): 542 [M]<sup>+</sup> (65), 446 (48), 404 (100), 386 (19), 358 (37), 344 (49), 312 (13), 238 (53), 215 (37), 182 (18), 125 (21); HREI-MS *m/z* 542.2151 [M]<sup>+</sup> (calcd. for C<sub>29</sub>H<sub>34</sub>O<sub>10</sub>, 542.2152). For <sup>1</sup>H and <sup>13</sup>C NMR spectroscopic data, see Tables 1 and 2.

### Supplementary material

CCDC contain the supplementary crystallographic data for this paper. These data can be obtained free of charge at via [www.ccdc.ca-](http://www.ccdc.ca-)

[m.ac.uk/conts/retrieving.html](http://m.ac.uk/conts/retrieving.html) (or from the CCDC, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; e-mail: [desposit@ccdc.cam.ac.uk](mailto:desposit@ccdc.cam.ac.uk)).

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