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Limonoids from the stem bark of *Khaya grandifoliola*

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Abstract

Three limonoids, deacetylkhayanolide E (**1**), 6*S*-hydroxykhayalactone (**2**), and grandifolide A (**3**), along with three known ones, were isolated from stem bark of the Nigerian medicinal plant *Khaya grandifoliola*. Their structures were characterized on the basis of the application of spectroscopic methods. *In vitro* antimicrobial and cytotoxic activities of the isolates were also tested, but were all found to be inactive.

Keywords: Meliaceae; Limonoids; Deacetylkhayanolide E; 6S-Hydroxykhayalactone; Grandifolide A; *Khaya grandifoliola*

1. Introduction

Some plant species in the Meliaceae and Rubiaceae families, e.g. *Entandrophragma utile*, *Azadirachta indica*, *Morinda lucuda*, and *Khaya* species, produce structurally diverse limonoids with important antiplasmodial and cytotoxic activities (Obih et al., 1985; Bray et al., 1990; Weenen et al., 1990). There are eight species of *Khaya* growing in tropical regions (Chen et al., 1997), and *K. grandifoliola* has been used for treatment of fever and malaria in Nigeria. In particular, a crude extract of stem bark showed *in vitro* mollusc-killing properties (Makanga and Odyek, 1989), trypanocidal activity against *Trypanosoma brucei brucei* (Owolabi et al., 1990), and antimalarial activity against *Plasmodium bergei bergei* (Makinde et al., 1988; Awe and Makinde, 1991; Awe et al., 1997). Recent investigations of both the stem bark and the seeds of this plant have led to isolation of eight limonoids and one flavanol (Agbedahunsi and Elujoba, 1998; Tchuendem et al., 1998; Bickii et al., 2000). In the present paper, we report the isolation and structural elucidation of six additional limonoids, deacetylkhayanolide E (**1**), 6*S*-hydroxykhayalactone (**2**), grandifolide A (**3**), khayanolide A (**4**, Abdelgaleil et al., 2001), anthothecanolide (**5**, Tchimene et al., 2005), and 3-*O*-acetylanthothecanolide (**6**, Tchimene et al., 2005), of which compounds **1**–**3** are new. The *in vitro* antimicrobial and cytotoxic activities of compounds **1**–**6** were also tested, but all tested compounds were inactive.

2. Results and discussion

Deacetylkhayanolide E (**1**) had a molecular formula of $C_{27}H_{32}O_{10}$ as deduced from HREIMS at m/z 516.1979 $[M]^+$ (calcd. 516.1995). Its IR spectrum showed the presence of hydroxyl (3498 cm^{-1}) and carbonyl (1732 and 1713 cm^{-1}) groups. The spectroscopic data of compound **1** (EIMS, IR, and NMR) were very similar to those of khayanolide E (Nakatani et al., 2002). Comparison of the ^1H and ^{13}C NMR spectroscopic data (Tables 1 and 2) of **1** with those of khayanolide E showed that the only structural difference was the absence of the 1-*O*-acetyl group in **1**, and this was supported by its molecular composition.

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Table 1
^{1}H NMR spectroscopic data for compounds **1**–**6** (400 MHz)

Proton	**1**[a]	**2**[a]	**3**[a]	**3**[b]	**4**[a]	**5**[c]	**6**[a]
2	4.83 *d* (10.4)						
3		4.01 *s*	5.74 *s*	4.74 *s*	4.25 *s*	3.60 *s*	5.73 *s*
5	4.09 br *s*	4.22 *d* (1.6)	3.32 *d* (7.9)	2.38 *d* (4.0)	2.61 *d* (6.5)	2.26 *dd* (9.6, 7.7)	2.26 *dd* (9.0, 7.2)
6	4.90 br *s*	4.92 br *s*	4.93 *d* (7.9)	4.38 br *s*	4.86 *d* (6.5)	2.70 *dd* (15.6, 9.6)	2.92 *dd* (14.2, 9.0)
						2.41 *dd*(15.6, 7.7)	2.74 *dd* (14.2, 7.2)
9	3.07 *d* (8.7)	2.44[h]	2.78 *m*	1.61[l]	2.49 *dd* (13.2, 5.6)	2.48 br *d* (12.0)	3.22 br *d* (11.4)
11	2.16[d]	2.15 *m*	2.32 *m*	1.88 *m*	1.52[n]	1.65 *m*	2.32 *m*
	1.92[e]	2.04 *m*	2.02 *m*	1.59[l]	1.33 *m*	1.39[o]	2.00[p]
12	2.22[d]	1.35 *m*	1.87[j]	1.82 br *d* (14.9)	1.60 *m*	1.69 *m*	1.97[p]
	1.08 br *d* (11.2)	1.10 *dd* (14.3, 8.7)	1.37 *m*	1.33 *m*	1.47[n]	1.39[o]	1.54 br *d* (14.7)
14		1.89[i]	2.42[k]				
15	3.83 *d* (18.6)	3.60 *dd* (15.0, 13.0)	3.48 *d* (19.1)	2.86[m] (2H)	3.52 *d* (18.2)	3.35 *d* (19.2)	3.80 *d* (18.7)
	3.36 *d* (18.6)	3.00 *dd* (15.0, 6.0)	3.02 *dd* (19.1, 8.0)		3.15 *d* (18.2)	2.73 *d* (19.2)	3.15 *d* (18.7)
17	5.85 *s*	5.37 *s*	6.10 *s*	5.32 *s*	5.87 *s*	5.56 *s*	6.11 *s*
18	1.39 *s*	0.87 *s*	1.02 *s*	0.98 *s*	1.18 *s*	0.98 *s*	0.75 *s*
19	1.94 *s*	1.92 *s*	2.11 *s*	1.39 *s*	1.94 *s*	4.42 *d* (11.6)	4.83 *d* (11.2)
						4.15 *d* (11.6)	4.59 *d* (11.2)
21	7.65[f]	7.65 br *s*	7.53 *br s*	7.49 *br s*	7.35 br *s*	7.62 br *s*	7.66 br *s*
22	6.55 br *s*	6.51 *d* (1.2)	6.49 *d* (1.0)	6.40 *d* (1.2)	6.45 *d* (1.2)	6.48 *d* (1.2)	6.54 *dd* (1.7, 0.6)
23	7.64[f]	7.63 *t* (1.6)	7.61 *t* (1.5)	7.43 *t* (1.7)	7.61 *t* (1.7)	7.52 *t* (1.8)	7.60 *t* (1.8)
28	1.72 *s*	1.89 *s*	1.23 *s*	0.95 *s*	1.66 *s*	0.94 *s*	1.18 *s*
29	2.67 *d* (12.2)	2.60 *d* (13.7)	1.83 *s*	1.30 *s*	2.79 *d* (12.1)	1.29 *s*	1.64 *s*
	2.17 *d* (12.2)	2.46 *d* (13.7)			2.40 *d* (12.1)		
30	3.49 *d* (10.4)	4.03 *d* (12.6)	3.60 *d* (14.1)	2.53 *d* (14.3)	3.41 *s*	2.93 *d* (13.9)	3.59 *d* (13.8)
		2.78 *d* (12.6)	2.41[k]	1.57[l]		1.50 *dd* (13.9, 1.5)	2.40 *dd* (13.8, 1.4)
–COO*Me*	3.73 *s*	3.92 *s*	3.83 *s*	3.81 *s*	3.67 *s*		
CH_3COO–			1.74 *s*	2.15 s			1.74 *s*
1-OH	6.99 br *s*	8.05[A]		3.90 br *s*			7.23 br *s*
OH	7.57[g] (6-OH)	8.04[A] (3-OH)		4.76 br *s* (2-OH)			10.08 br *s* (2-OH)
	7.36 br *s* (8-OH)	7.23 br *s* (6-OH)		2.88[m] (6-OH)			7.08 *s* (14-OH)

[A] Interchangeable signals.
[a] Recorded in C_5D_5N.
[b] Recorded in $CDCl_3$.
[c] Recorded in CD_3OD.
[d-p] Overlapping signals.

The structure of compound **1** was further confirmed by 2D NMR spectra, including HSQC, HMBC, and NOESY spectra (Supplementary data). Compound **1** was therefore determined to be deacetylkhayanolide E.

6*S*-Hydroxykhayalactone (**2**) showed a molecular formula of $C_{27}H_{34}O_{10}$ as determined by HREIMS at *m*/*z* 518.2146 $[M]^+$ (calcd. 518.2152), which is 16 mass units more than that of khayalactone (Tchuendem et al., 1998). The spectroscopic data (EIMS, IR, and NMR) of **2** were very similar to those of khayalactone, indicating that the structures of both compounds were related, and the only difference is likely the presence of one more hydroxyl group in **1**. The NMR spectroscopic data further indicated that compound **2** possessed one more oxygenated methine (δ_H 4.92, br *s*; δ_C 73.4) and one fewer methylene group than khayalactone, supporting the above deduction. In the HMBC, the correlations of proton signal (δ_H 4.92, br *s*) to C-4 (δ_C 47.3), C-5 (δ_C 44.1), C-7 (δ_C 176.7), and C-10 (δ_C 56.6) established that the additional hydroxyl group in **2** was located at C-6. Comparison of the chemical shifts of both H-6 and C-6 of **2** with those of compound **1** suggested that the 6-OH had the *S*-configuration. The structure of **2** was thus elucidated, and confirmed by HMQC, HMBC, and NOESY spectra.

Grandifolide A (**3**) was determined to have a molecular formula $C_{29}H_{38}O_{11}$ by HREIMS at *m*/*z* 562.2392 $[M]^+$ (calcd. 562.2414). The NMR spectroscopic data (Tables 1 and 2, in C_5D_5N) of compound **3** were very similar to those of 3-*O*-acetylanthothecanolide (**6**, Tchimene et al., 2005), indicating that they were structurally related analogs. In the HMBC spectrum, the correlations of OMe (δ_H 3.83, *s*)/C-7 (δ_C 175.8) and H_3-19 (δ_H 2.11, *s*)/C-10 (δ_C 46.2) showed that the partial structure of **3** (C-5 to C-7, C-5 to C-10, and C-10 to C-19) is the same as that of compound **2**. The proton signal at δ_H 2.42 was assignable to H-14 by its HMBC correlations with C-8 (δ_C 79.2), C-13 (δ_C 35.7), and C-15 (δ_C 28.5), indicating that the C-14 was a methine. The relative stereochemistry of compound **3** was confirmed by NOESY spectroscopic analysis (Supplementary data). The complete assignments of the ^{1}H and ^{13}C NMR spectroscopic data of **3** were therefore fully achieved by HMQC spectroscopy.