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Structure Determination of Grandifotane A from *Khaya grandifoliola* by NMR, X-ray Diffraction, and ECD Calculation

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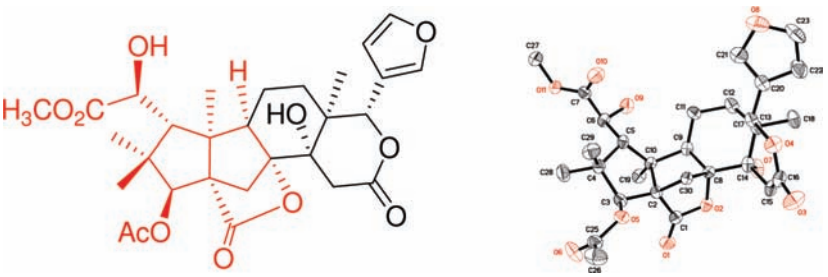
ABSTRACT



Grandifotane A (1), a limonoid with an unprecedented carbon skeleton, was isolated from the stem bark of *Khaya grandifoliola*. The structure of 1 with the absolute configuration was determined by spectroscopic data, X-ray crystallography, and ECD calculation. A biogenetic route for grandifotane A (1) involving an enzymatic Baeyer−Villiger oxidation as the key step is proposed.

Limonoids, a series of highly oxygenated and structurally modified tetranortriterpenoids exhibiting a variety of biological significance, have been attracting great interest in natural products and synthetic chemistry.[1] Recently, the endeavors made by Corey,[2] Ley,[3] and their co-workers have led to the successful syntheses of several complex limonoids. Phytochemical studies on the plants of the Meliaceae family conducted by our research group have afforded an array of structurally fascinating limonoids, some of which showed remarkable bioactivities.[4] The genus *Khaya* is the main source of African mahogany,[5] and there are eight species in this genus growing over the tropical zone.[6] The plant of *Khaya grandifoliola* (Meliaceae) has been traditionally

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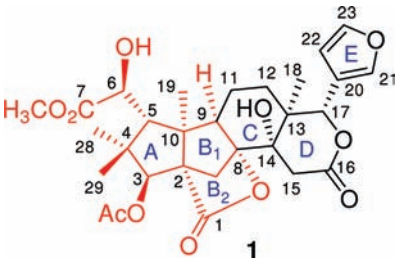
applied in Africa for the treatment of malaria.[7] Investigations on the stem bark and seeds of this plant have led to the isolation of eight limonoids and one flavanol.[8] Recently, three new limonoids have been isolated by us from the stem bark of *K. grandifoliola*.[9] In continuation, grandifotane A (**1**), a limonoid with an unprecedented carbon skeleton, was isolated from the same sample. We present herein the details of isolation and structural elucidation of grandifotane A (**1**).



The air-dried powder of the stem bark of *K. grandifoliola* (2.5 kg) was percolated with 85% EtOH at room temperature (3 × 3 L), and the crude extract (156 g) was partitioned between H_2O and EtOAc. The EtOAc soluble fraction (58 g) was subjected to silica gel chromatography (petroleum ether/acetone, from 50/1 to pure acetone, v/v) to give five fractions (1−5). Fraction 3 was extensively separated over columns of silica gel and Sephadex LH-20 to obtain **1** (13 mg).

Grandifotane A (**1**)[10] was obtained as colorless crystals (in MeOH) that gave a $[M]^+$ at *m/z* 560.2265 in the HREIMS consistent with an elemental composition of $C_{29}H_{36}O_{11}$ (calcd 560.2258) requiring 12 degrees of unsaturation. The positive mode of ESIMS at *m/z* 583.2 $[M + Na]^+$ and the negative mode of ESIMS at *m/z* 605.3 $[M + HCOO]^-$ further secured this assignment. The IR spectrum of **1** showed absorptions at 3480 (hydroxy), 1780 (five-membered lactone), and 1735 cm^{-1} (esters and/or six-membered lactone). The ^1H NMR spectrum (Table 1) of **1** revealed the presence of four tertiary methyls (at δ 0.89, 1.01, 1.28, 1.30, each 3H, s), an acetyl (at δ 2.08, 3H, s), and a methoxyl (at δ 3.82, 3H, s). The ^1H NMR also displayed the feature of a β-substituted furan ring (Table 1). The ^{13}C NMR spectrum (Table 1) showed 29 well-resolved resonances (two overlapped with the solvent signals of $CDCl_3$ were assigned to C-3 at δ 77.3 and C-17 at δ 77.7 by HSQC spectrum) in agreement with HREIMS analysis, and the DEPT experiments/HSQC indicated that 34 of the 36 hydrogen atoms were directly attached to carbons (CH_3 × 6, CH_2 × 4, CH × 8, C × 11). The aforementioned analyses and biogenetic reasoning suggested that **1** bears the nature of a limonoid.

Table 1. ^1H and ^{13}C NMR Spectroscopic Data of **1**[a]

position	δ_H (mult; *J*, Hz)	δ_C
1		172.5
2		66.4
3	5.40 (s)	77.3
4		43.8
5	2.48 (d, 10.6)	52.9
6	4.23 (dd, 10.6, 5.8)	69.7
7		174.5
8		94.7
9	2.16 (ddd, 12.4, 5.9, 2.4)	52.1
10		51.2
11α	2.23 (m)	24.1
11β	1.51 (dd, 14.2, 2.4)	
12α	1.51 (dd, 14.2, 2.4)	29.5
12β	1.98 (ddd, 14.2, 14.2, 4.3)	
13		40.4
14		72.4
15α	2.80 (d, 18.6)	37.6
15β	3.10 (d, 18.6)	
16		169.0
17	5.60 (s)	77.7
18	1.01 (3H, s)	16.2
19	1.28 (3H, s)	22.1
20		120.8
21	7.43 (s)	140.9
22	6.42 (d, 0.9)	110.0
23	7.41 (t, 1.5)	143.5
28	0.89 (3H, s)	26.9
29	1.30 (3H, s)	26.4
30α	2.38 (d, 10.8)	40.8
30β	2.22 (dd, 10.8, 2.4)	
3-OAc		170.3
	2.08 (3H, s)	21.0
6-OH	2.44 (d, 5.8)	
7-OMe	3.82 (3H, s)	53.0
14-OH	2.79 (s)	

[a] Data were recorded in $CDCl_3$ at 400 (^1H) and 100 MHz (^{13}C).

Comparison of the ^{13}C NMR data of **1** with that of a known limonoid, anthothecanolide, indicated that they shared the same rings C, D, and E, which were confirmed by 1D and 2D NMR spectra (see Figure 1). Accordingly, the key point for the structure determination was restricted to the construction of the western hemisphere of **1**, a tricyclic system (A, B_1, and B_2 rings). In the HMBC spectrum (Figure 1A), the correlations from H_3-19 to C-2 (δ 66.4), C-9 (δ 52.1), and C-10 (δ 51.2), and from H_2-30 to C-2, C-8 (δ 94.7), and C-9, allowed the construction of the five-member

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(10) **Grandifotane A (1):** colorless crystal (MeOH); mp 172−174°C; $[\alpha]^{20}_D$ −3 (*c* 0.050, $CHCl_3$); IR (KBr) ν_{max} 3480, 2954, 2923, 2850, 1780, 1735, 1463, 1378, 1236, 1033, 759 cm^{-1}; for ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS *m/z* 583.2 $[M + Na]^+$, 1143.3 $[2M + Na]^+$, 605.3 $[M + HCOO]^-$; EIMS *m/z* 560 $[M]^+$ (4), 446 (20), 422 (100), 404 (17), 360 (21), 345 (21), 188 (51), 157 (24), 95 (22); HREIMS *m/z* 560.2265 $[M]^+$ (calcd for $C_{29}H_{36}O_{11}$ 560.2258) .

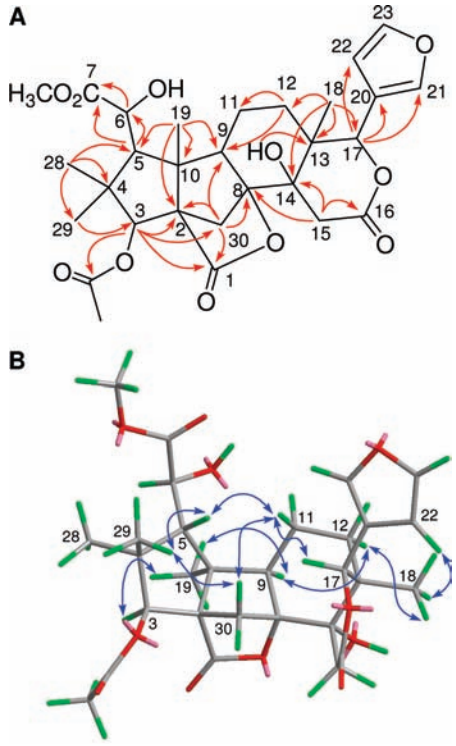


Figure 1. (A) Selected HMBC correlations (H→C) of **1**; (B) key ROESY correlations (H↔H) of **1**.

ring B_1 with Me-19 attached to C-10. The HMBC correlations of H_3-28 (or H_3-29) with C-3 (δ 77.3), C-4 (δ 43.8), and C-5 (δ 52.9) indicated an in-order linkage of C-3 to C-5, and also attached Me-28 and Me-29 to C-4. The linkage of C-5 and C-10 was established by the HMBC correlations from H-5 to C-10, and from H_3-19 to C-5 and C-10. The HMBC correlations from H-3 to C-2 and C-30 (δ 40.8) enabled the linkage of C-2 and C-3 to form the five-membered ring A. A $MeO_2CCH(OH)-$ moiety was appended to ring A at C-5 by the HMBC correlations from H-5 to C-6 (δ 69.7) and C-7 (δ 174.5), from H-6 to C-5 and C-7, and from CH_3O to C-7. The acetoxy was located at C-3 by the HMBC correlation between H-3 and its ester carbonyl at δ 170.3. The HMBC correlations from H-3 and H_2-30 to C-1 at δ 172.5 revealed the linkage of C-1 and C-2. The terminus of C-1 ester carbonyl was most likely to form the ring B_2 of a five-membered lactone with the oxygenated C-8, which was severely downfield shifted at δ 94.7, to consume the remaining one degree of unsaturation. The planar structure of **1** was therefore assigned.

The relative configuration of **1** was partially assigned by the ROESY spectrum (Figure 1B). The ROESY correlations of H-3/H_3-19, H_3-19/H-9, H-9/H-12α, and H-12α/H_3-18 revealed that H-3, CH_3-19, H-9, CH_3-18, and furan ring were cofacial, and were arbitrarily assigned in an α-orientation. In consequence, the ROESY cross-peaks of H-5/H-11β, H-5/H_3-29, H_3-29/H-30β, H-30β/H-11β, and H-11β/H-17 indicated that H-5, H-17, and CH_3-29 were β-oriented. The relative configurations of 6-OH and 14-OH, and the fused-manner of rings B_1 and B_2 were left unassigned by the available ROESY spectroscopic data. Fortunately, the successful performance of a single crystal X-ray diffraction (Figure 2) allowed the definite assignment of the relative configuration of **1**.

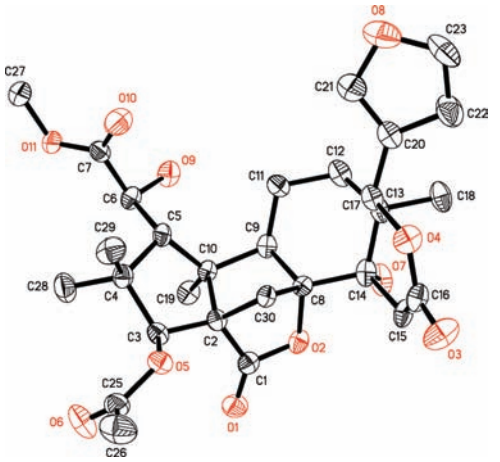


Figure 2. Single-crystal X-ray structure of **1**.

To determine its absolute configuration, the CD spectrum of **1** was measured (in CH_3OH), which was dominated by a strong negative Cotton effect at $\lambda_{max} = 217$ nm ($\Delta\varepsilon = -24.4$) (Figure 3). However, due to the facts that no proper model compounds were found for reference,

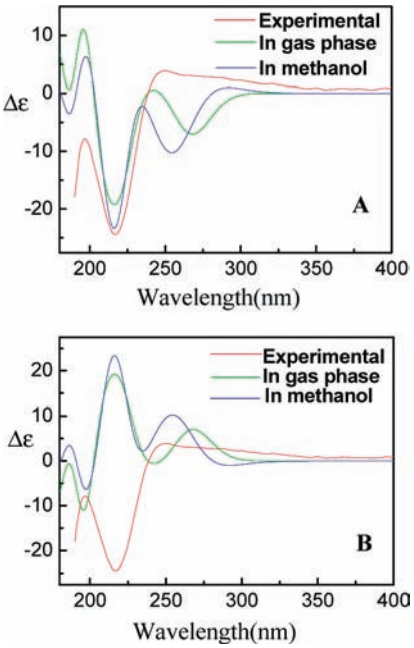


Figure 3. (A) ECD spectra of **1**: experimental ECD (red); calculated ECD in gas phase (green) and in MeOH (blue). (B) ECD spectra of the enantiomer of **1**: experimental ECD (red); calculated ECD in gas phase (green) and in MeOH (blue).

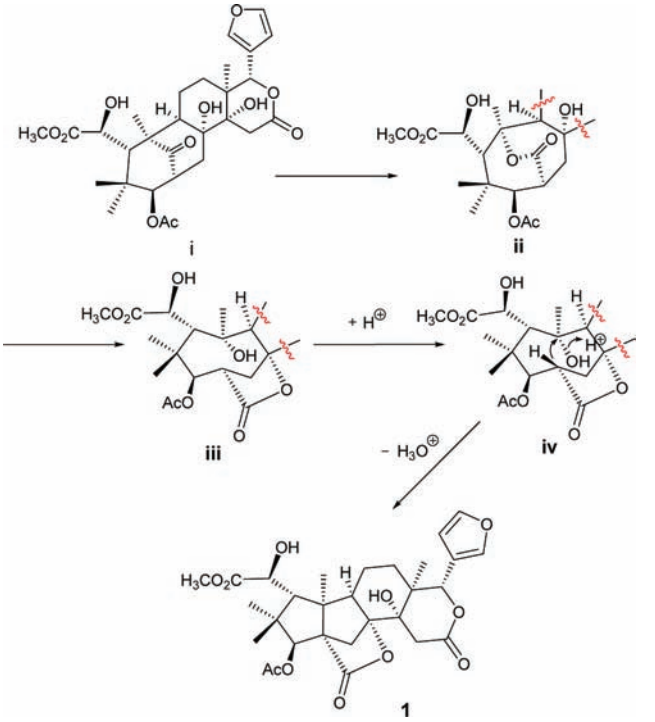
and the absence of applicable exciton coupling in the CD spectrum of **1**, the absolute configuration of **1** cannot be resolved directly by the analysis of its CD curves. To solve this matter, the calculation of electronic circular dichroism (ECD) by using time-dependent density functional theory (TDDFT), which has greatly enhanced the value of ECD in determining absolute configuration in recent years,[11] was applied in combination with the experimental CD data. The calculated ECD data of **1** and its enantiomer in both the gas phase and CH_3OH were shown in Figure 3. The calculated ECD of **1** matchs very well with the experimental ECD, while the calculated ECD of its enantiomer is opposite to the experimental one, allowing the assignment of the absolute configuration of **1** as depicted.

The possible biosynthetic pathway of **1** is postulated in Scheme 1. The biosynthetic precursor of **1** is proposed to be a mexicanolide-type limonoid **i**, which was transformed into a key intermediate **ii** by an enzymatic Baeyer−Villiger oxidation.[12] The **ii** would then undergo an intramolecular ester exchange reaction to yield **iii**, which then protonated to give intermediate **iv**. After a long shift of Wagner−Meerwein rearrangement,[13] the intermediate **iv** was transformed into **1** by the loss of one molecule of water and formation of a C2−C10 bond in a simultaneous manner to keep the required stereochemistry.

On biogenetic reasoning, the absolute configuration of **1** assigned by CD analysis is consistent with those of mexicanolide-type limonoids, whose absolute configuration has been unambiguously determined,[14] further supporting the absolute configuration of **1**.

Grandifotane A (**1**) has a complex hexacyclic carbon skeleton without precedent among the known limonoid families. We propose to name this limonoid scaffold grandifotane.

Scheme 1. The Plausible Biosynthetic Pathway of **1**



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Supporting Information Available: Experimental procedures; copies of 1D and 2D NMR spectra, IR and MS data, and X-ray crystallographic data of **1**. This material is available free of charge via the Internet at http://pubs.acs.org.

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