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Senegalensions A–C, Three Limonoids from *Khaya senegalensis*

Chun-Mao Yuan,[a, b] Yu Zhang,[a] Gui-Hua Tang,[a] Shun-Lin Li,[a] Ying-Tong Di,[a] Li Hou,[a] Jie-Yun Cai,[a] Hui-Ming Hua,*[b] Hong-Ping He,*[a] and Xiao-Jiang Hao*[a]

Limonoids, possessing highly complicated structures and broad range of bioactivities, have been becoming a hot topic in the field of natural products and synthetic chemistry.[1] In recent years, many new tetranortriterpenoids with unique skeletons and significant biological activities have been obtained.[2] The genus *Khaya* (Meliaceae) is distributed extensively in tropical regions of Africa and Madagascar,[3] and their barks were used as a traditional medicine for the treatment of fever and malaria in Africa.[4] Investigations on the genus have led to the isolation of a variety of rings B,D-*seco* limonoids such as mexicanolides, phragmalins, rearranged phragmalins, and angolensates.[5] Our previous studies on different genera of the Meliaceae family collected from several locations have resulted in a series of new limonoids.[6] In further study, senegalensions A (**1**; Figure 1) and B (**2**) with a unique C2–C4 linkage and senegalension C (**3**), possessing a rare spiro ring system with an unprecedented tricyclo[3.3.1.0^{1,10}]nonane moiety, were isolated from the leaves and twigs of *K. senegalensis*. Their structures were elucidated by extensive spectroscopic analysis and computational methods. A plausible biosynthetic pathway of them was also proposed.

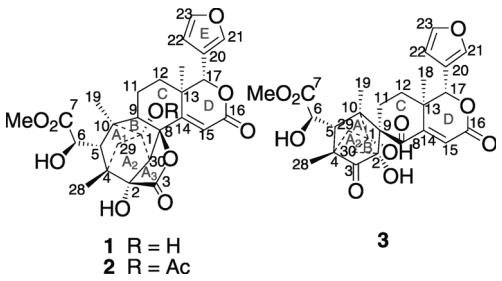

Figure 1. Structures of senegalensions A–C.

Senegalension A (**1**) was shown to have the molecular formula $C_{27}H_{30}O_{10}$ from high-resolution electron-spray ionization mass spectrometry (HR-ESI-MS) [*m*/*z* 537.1725 ([*M*+Na]$^+$)], revealing 13 degrees of unsaturation. The ^{1}H and ^{13}C NMR spectroscopy data (Table 1), along with DEPT experiments, showed the presence of 27 carbon signals, including four methyl carbon atoms (one methoxy group), three methylenes, nine methines (four olefinic ones), and 11 quaternary carbons (two olefinic and three carbonyl ones). Apart from seven degrees of unsaturation consumed by a characteristic β-furan ring, three carbonyl groups, and a double bond, the remaining six degrees of unsaturation suggested compound **1** to be hexacyclic.

Further analysis of the ^{13}C NMR and HMBC spectra (Figure 2a) indicated that **1** was related to khayanolide C,[7] sharing the same A_1, B, C, D, and E rings. The most striking characteristic of **1** was the formation of a five-membered A_2 ring with a "loose end" of C3 through the ring contraction of a six-membered A_2 ring as those in khayanolide C.[7] The HMBC correlations of a proton signal at $\delta = 6.27$ ppm (s, OH) with C2, C3, C4, and C30 indicated a unique linkage of C3, C4, and C30 to C2. Moreover, a five-membered ring (A_2) was assigned by HMBC correlations of a proton resonance at $\delta = 5.42$ ppm (s, OH) with C1, C29, and C30, along with HMBC correlations of H_3C28 with C2, C4, and C29. The "loose end" of ester carbonyl C3 and the severely downfield-shifted signal at $\delta = 88.9$ ppm (C8),[8] coupled with the remaining one degree of unsaturation, revealed that an unusual 3,8-γ-lactone ring (A_3) was formed between C3 and C8 through an ester bond. Thus, the above evidence assigned the planar structure of **1**.

The relative configuration of **1** was deduced by the ROESY experiment (Figure 2b). As shown in ROESY spectrum, correlations of Me28/H6, H6/H5, H5/H11β, and H12β/H17 indicated that these groups were cofacial, and they were arbitrarily assigned as β-oriented. The ROESY cross-peaks observed from H9 to Me18, Me19, OH1, and H30, and from OH2 to H30 indicated α orientation of those groups. Therefore, the relative stereochemistry of compound **1** was established as shown. However, the observed ROESY

[a] Dr. C.-M. Yuan,[+] Dr. Y. Zhang,[+] Dr. G.-H. Tang, Dr. S.-L. Li, Dr. Y.-T. Di, L. Hou, J.-Y. Cai, Prof. H.-P. He, Prof. X.-J. Hao
State Key Laboratory of Phytochemistry and Plant Resources in West China
Kunming Institute of Botany, Chinese Academy of Sciences
Lanhei road 132, Heilongtan Kunming Yunnan 650201 (China)
Fax: (+86) 871-5223070
E-mail: hehongping@mail.kib.ac.cn
haoxj@mail.kib.ac.cn

[b] Dr. C.-M. Yuan,[+] Prof. H.-M. Hua
Key Laboratory of Structure-Based Drug Design & Discovery
Ministry of Education
Shenyang Pharmaceutical University
Wenhua road 103, Shenhe district
Shenyang Liaoning 110016 (China)
Fax: (+86) 2423986465
E-mail: huimhua@163.com

[+] These authors have contributed equally to this work.

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Table 1. ^{1}H and ^{13}C NMR spectroscopy assignments of **1**–**3**.[a]

Position	**1**		**3**	
	δ_H	δ_C	δ_H	δ_C
1		90.1		80.1
2		85.2		83.4
3		175.2		210.3
4		48.8		52.7
5	1.95 (br s)	44.6	2.24 (br s)	49.9
6	4.26 (t, 4.4)	70.0	4.23 (dd, 4.5, 2.1)	69.4
7		175.1		175.1
8		88.9		203.8
9	2.13 (m)	46.7		67.9
10		51.7		57.4
11α	1.61 (m)	15.0	2.29 (m)	21.4
11β	1.72 (m)		2.10 (m)	
12α	1.20 (m)	26.0	1.45 (m)	29.6
12β	1.77 (m)		1.93 (m)	
13		38.6		38.9
14		164.5		158.6
15	6.24 (s)	114.7	6.06 (s)	118.3
16		164.3		164.1
17	5.21 (s)	81.9	5.54 (s)	80.2
18	1.16 (s, 3H)	20.1	0.89 (s, 3H)	17.4
19	0.88 (s, 3H)	14.5	1.19 (s, 3H)	14.9
20		119.9		119.6
21	7.80 (br s)	141.9	7.81 (br s)	141.8
22	6.58 (br s)	110.3	6.58 (d, 1.5)	110.1
23	7.69 (br s)	143.4	7.71 (t-like, 1.5)	143.7
28	1.08 (s, 3H)	11.0	1.06 (s, 3H)	14.2
29 a	1.91 (d, 9.4)	43.5	1.43 (d, 10.8)	52.9
29 b	2.32 (d, 9.4)		3.07 (d, 10.8)	
30 a	2.80 (s)	60.4	1.54 (m)	50.9
30 b			2.34 (m)	
7OMe	3.63 (s, 3H)	51.9	3.53 (s, 3H)	52.0
1OH	5.42 (s)		5.15 (s)	
2OH	6.27 (s)		5.09 (s)	
6OH	5.60 (d, 4.4)		5.68 (d, 4.5)	

[a] Recorded in DMSO at 500 MHz (^{1}H NMR) or 150 MHz (^{13}C NMR spectra). Chemical shifts and coupling constants (in parentheses) are given in ppm and Hz, respectively.

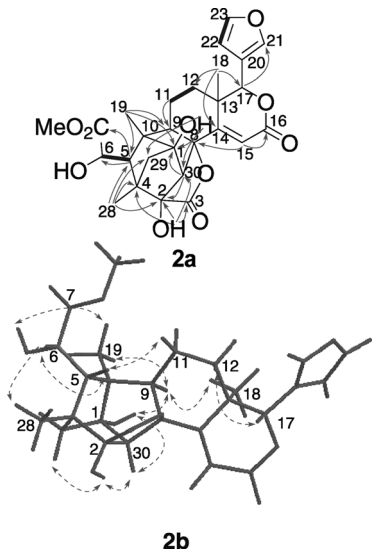


Figure 2. a) ^{1}H–^{1}H COSY (bold bonds) and selected HMBC (H→C); b) ROESY (H←-→H) of compound **1**

correlations of H17/H21 and H22 indicated that several interconversional conformations coexist in **1** due to a variation of the dihedral angle C13-C17-C20-C22.[9]

To allocate the absolute configuration and to model its conformational constitution, we obtained two stable conformations (I and II) of **1** through calculations with the Gaussian 03 package at the B3LYP/6-31G* level.[10] Moreover, the potential energy surface (PES) was scanned, which started from the optimized geometry of conformation I and varied the dihedral angle C13-C17-C20-C22 (Figure 3). The results exhibited only two minima and therefore suggested that the occurrence of the comparatively rapid interconversion of I

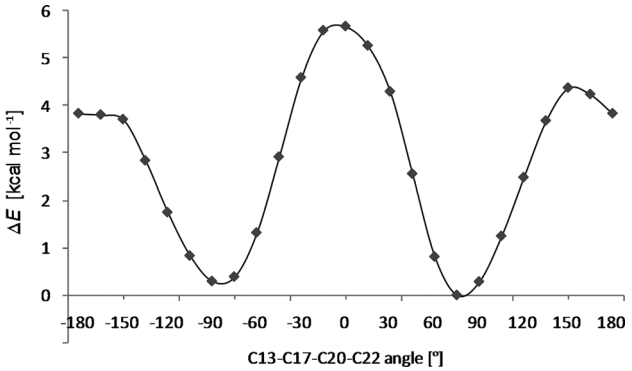


Figure 3. HF/6-31G* PES scan with respect to the dihedral angle C12-C17-C20-C22 of senegalonoid A (**1**).

and II in solution at room temperature was a logical process. Subsequently, the "self-consistent reaction field" method (SCRF) was employed to perform the optical rotation calculation of the two major conformers of compound **1** in methanol at the B3LYP/6-31G** level. As shown in Table 2, the computed optical rotation values for conformers I and II were +44.0° and +19.5°, respectively. The summed [α] value of I and II according to the Boltzmann formula was +31.3°, which was matched up to the experimental value (+46.7), and thus the absolute configuration of **1** was assigned as 1*S*,2*S*,4*R*,5*S*,6*S*,8*R*,9*R*,10*S*,13*R*,17*R*,30*S*, which was also in agreement with the proposed biosynthetic pathway.

Senegalension B (**2**) had a molecular formula of $C_{29}H_{32}O_{11}$ from HR-ESI-MS, with 42 mass units more than **1**. The 1D NMR spectroscopy data (see the Supporting In-

Table 2. Computed energy, populations, and optical rotation for compound **1**.

Conformation	Senegalonoid A (**1**)		
	I	II	Exptl.
Relative energy [kcal mol^{-1}][a]	0.00	0.20	
Equilibrium populations (%)	52.0	48.0	
$[\alpha]_D$	+44.0	+19.5	
Sum of $[\alpha]_D$[b]	+31.3		+46.7

[a] Lowest energy conformation was used as the reference zero point; the geometries were obtained in the gas phase at the B3LYP/6-31G* level. [b] The Boltzmann formula was used to produce the sum of two different conformational optical rotations.

formation) of **2** were almost the same as those of **1**, except for an additional acetyl located at C1, which was supported by the HMBC correlations observed from $H_3$19 and H30 to C1, along with the severely downfield ^{13}C signal at $\delta=$ 94.4 ppm (C1). The relative configuration of **2** was identical to that of **1** by analysis of the ROESY spectrum.

Senegalension C (**3**) was found to have the molecular formula $C_{27}H_{30}O_{10}$ from the HR-ESI-MS [*m*/*z* 537.1725 ([*M*+Na]$^+$)] with 13 degrees of unsaturation. The IR spectrum revealed the existence of hydroxy (3439 cm^{-1}) and carbonyl (1724 cm^{-1}) functionalities. From the ^1H and ^{13}C NMR spectroscopy data (Table 1), the existence of a typical β-furan ring, a 29-methylene [$\delta=$1.43 (d, H29), 3.07 ppm (d, H29); 52.9 ppm (C29)], and the fragment Me-CO_2CH(OH) implied that **3** should be a phragmalin-type limonoid.[11]

Detailed analysis of 2D NMR spectra (Figure 4a) allowed the assignments of an A_1 ring with the moiety Me-CO_2CH(OH) at C5 and an α,β-unsaturated δ-lactone D ring with a β-furan ring at C17, which were the same as those of khayanolide C.[7] The most significant features were the replacement of two typical sp^3 methines of C9 and C30 in khayanolide C with two signals of an sp^3 quaternary carbon C9 ($\delta=$67.9 ppm) and an sp^3 methylene C30 [$\delta=$1.54 (m, H30), 2.34 ppm (m, H30); 50.9 ppm] in **3**. The HMBC cross-peaks from $H_2$11 to C2, C8, C9, and C10, and from $H_3$19 and OH2 to C9 revealed a connection of C2, C10, C8, and C11 to C9. In the HMBC spectrum, two hydroxy correlations from the proton at $\delta=$5.15 ppm (s, OH) to C1, C10, C29, and C30, and from the proton at $\delta=$5.09 ppm (s, OH) to C2, C3, C9, and C30 strongly supported the key connectivities of C29 to C10 and C30 through C1, and C30 to C9 and C3 through C2. Furthermore, the construction of the tricyclo[3.3.1.0^{1,10}]nonane moiety (A_1, A_2, and B rings) was revealed based on the HMBC correlations of $H_3$19 with C1, C5, C9, and C10, and $H_3$28 with C3, C4, C5, and C29. The HMBC correlations of H11/C9 and C8, H15/C8, and $H_3$18/C12, C13, and C14, as well as the correlations of ^1H-^1H COSY (H11/H12) allowed the establishment of ring C with a carbonyl at C8. Taken together, the tricyclic moiety and ring C joined at C9 to form the unusual spiro ring system. Thus the gross structure of **3** was elucidated as depicted, which represented the first phragmalin-type limonoid with an unprecedented spiro ring system.

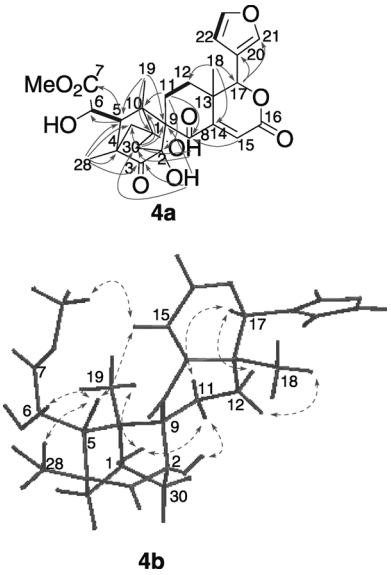
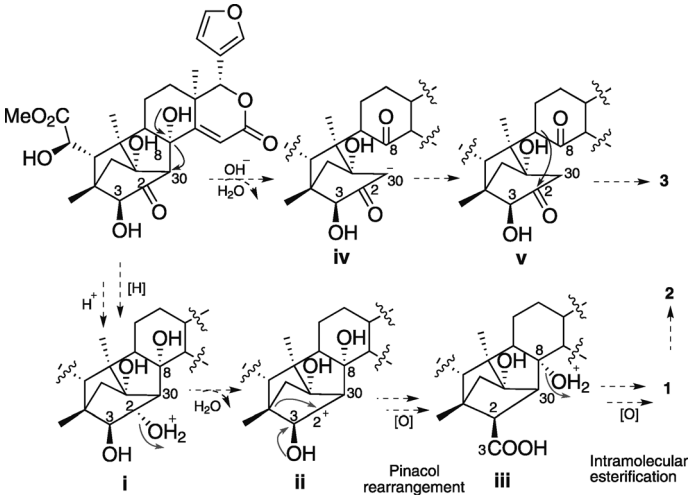


Figure 4. a) ^1H–^1H COSY (bold bonds) and selected HMBC (H→C); b) ROESY (H←-→H) of compound **3**.

The relative stereochemistry of **3** was assigned by the ROESY spectrum (Figure 4b). Strong ROESY cross-peaks observed between H5 with Me28 and H6 indicated that those three groups were on the same side of the molecule and arbitrarily assigned in a β-orientation, whereas Me19 and OH1 were α-oriented, as deduced from the ROESY correlations of Me19 with OH1. Furthermore, the key ROESY cross-peaks of both OH1 and OH2 with H11α, and H15 with H5 and OMe7, fixed the spiro ring system as shown in Figure 4b. Meanwhile, the C ring adopted a boat conformation by the ROESY correlations of H17/H12β and H11β. Accordingly, the relative configuration of **3** was established as depicted.

As shown in Scheme 1, khayanolide C might be the biosynthetic precursor of **1**, **2**, and **3**. As for **1** and **2**, the crucial step was pinacol rearrangement and oxidation from **ii** to **iii**. Compound **1** was produced after intramolecular esterification and oxidation, which then converted to **2** after acetylation. As for **3**, the key intermediate **v** could undergo aldol condensation to yield **3**.



Scheme 1. Plausible biosynthetic pathway of **1**–**3** from khayanolide C.

Compounds **1**–**3** were evaluated for their cytotoxicities against five cell lines (HL-60, SMMC-7721, A-549, MCF-7, and SW480) by using the MTT method.[12] Compound **1** showed moderate cytotoxicities against MCF-7 and SW480 with IC_{50} values of 16.1 and 19.0 μM, and weak cytotoxicities against HL-60 and A-549 with IC_{50} values of 40.0 and 39.7 μM, respectively. Compounds **2** and **3** were inactive against the above cancer cells ($IC_{50}>40$ μM).

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Experimental Section

The air-dried powder of the plant material (8.0 kg) was extracted with MeOH three times, followed by combination, concentration, and suspension in water. It was subsequently partitioned successively with PE (petroleum ether), EtOAc, and *n*BuOH. The EtOAc part (200.6 g) was chromatographed on a silica gel column eluted with petroleum ether/acetone (from 1:0 to 1:1) and then $CHCl_3/CH_3OH$ (from 1:1 to 0:1), to yield six fractions (A1–A6). Fraction A4 (15.0 g) was chromatographed over a RP-18 column ($MeOH/H_2O$ from 2:8 to 10:0) and further purified on Sephadex LH-20 (MeOH) to yield **1** (10.8 mg) and **2** (30.5 mg). Fraction A5 (16.2 g) was subjected to a RP-18 column ($MeOH/H_2O$ from 3:7 to 1:1) and then separated on repeated silica gel columns to obtain **3** (6.2 mg).

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Keywords: biosynthesis · computational chemistry · limonoids · natural products · structure elucidation

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