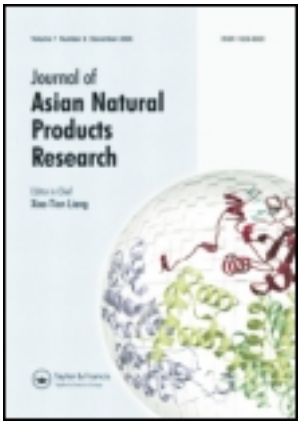

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Two new compounds from *Khaya senegalensis*

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Two new compounds, khayseneganin I (**1**) and 2α,3α,16β-trihydroxy-20-acetoxy-20(*R*)-pregnane (**2**), along with six known compounds, 2α,3α,20-trihydroxy-16β-acetoxy-20(*R*)-pregnane (**3**), 2α,3β-dihydroxypregnan-16-one-2β,19-hemiketal (**4**), (+)-catechin (**5**), ivorenolide A (**6**), luteolin-7-*O*-α-L-rhamnoside (**7**), and (−)-5′-methoxy-isolariciresinol-2a-*O*-β-D-xylopyranoside (**8**), were isolated from the leaves and twigs of *Khaya senegalensis*. The structures of new compounds were elucidated by 2D NMR spectroscopy and MS. Selected compounds (**2**–**8**) were evaluated for their antimicrobial activities and compounds **5** and **7** showed weak antimicrobial activities against MRSA 92# and MRSA 98#.

Keywords: *Khaya senegalensis*; limonoid; steroid; antimicrobial activitites

1. Introduction

The genus *Khaya* (Meliaceae family), comprising eight species, is distributed extensively in tropical Africa and Madagascar [1]. The bark of this genus has been used in traditional medicine for the treatment of fever and malaria in Africa [2]. Modern pharmaceutical studies revealed that crude extracts of the bark of this genus showed cytotoxic, antifungal, anti-inflammatory, and antimalarial activities [3,4]. Previous chemical investigations on this genus have afforded a series of rings B,D-*seco* limonoids [5–7]. Recently, our group has reported an array of secondary metabolites with fascinating structural features and significant biological activities from Meliaceae family [8–14]. In continuation of our studies, we now report the isolation of two new compounds, khayseneganin I (**1**) and 2α,3α,16β-trihydroxy-20-acetoxy-20(*R*)-pregnane (**2**), along with six known compounds, 2α,3α,20-trihydroxy-16β-acetoxy-20(*R*)-pregnane (**3**) [15], 2α,3β-dihydroxypregnan-16-one-2β,19-hemiketal (**4**) [16], (+)-catechin (**5**) [17], ivorenolide A (**6**) [18], luteolin-7-*O*-α-L-rhamnoside (**7**) [19], and (−)-5′-methoxy-isolariciresinol-2a-*O*-β-D-xylopyranoside (**8**) [20], from the leaves and twigs of *Khaya senegalensis* (Desr.) A. Juss [1] (Figure 1). Selected compounds (**2**–**8**) were screened for their antimicrobial activities.

2. Results and discussion

Khayseneganin I (**1**) was obtained as a white powder and its molecular formula was determined as $C_{28}H_{36}O_{11}$ in

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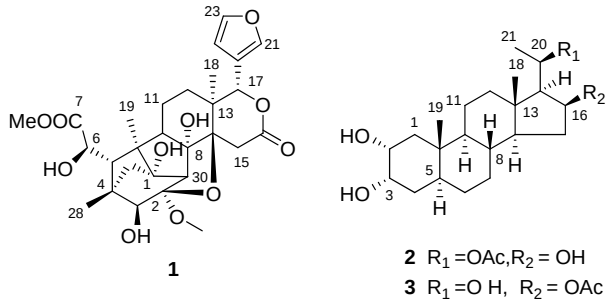


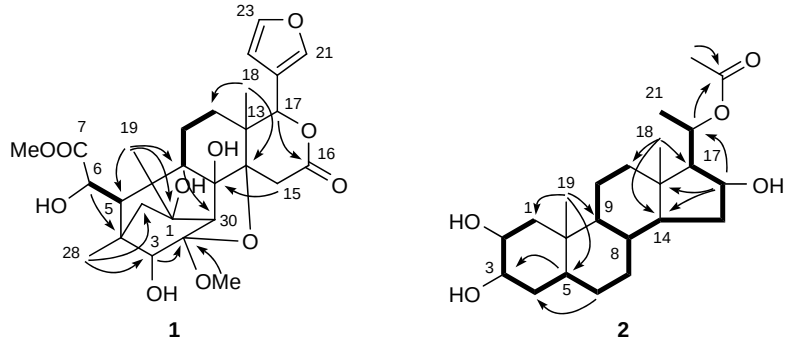
Figure 1. The structures of compounds **1**–**3**.

agreement with molecular ion peak $[M]^+$ at *m/z* 548.2259 in HR-EI-MS. The ^{13}C NMR data along with distortionless enhancement by polarization transfer experiments (Table 1) showed 28 carbon signals, including 5 methyls (two methoxyls), 4 methylenes, 9 methines (three olefilic ones), and 10 quaternary carbons (a olefilic and two carbonyl ones). From the ^1H and ^{13}C NMR data, the presence of a typical β-furan ring, a C-29-methylene (δ_H 1.50, d, $J = 11.6$ Hz and 1.89 d, $J = 11.6$ Hz; δ_C 45.1), and the fragment $MeO_2CCH(OH)$ – implied that **1** is a phragmalin-type limonoid [11]. Further analysis of the ^1H and ^{13}C NMR spectra of **1** indicated that **1** is similar to khayseneganin E [21]. The chemical shift differences resulted from the absence of an oxygenated methine resonance and the presence of an *O*-methyl signal (δ_H 3.31, s; δ_C 50.7) at C-2 and a quaternary carbon signal (δ_C 106.7) in **1**. This assumption was supported by the key HMBC correlations (Figure 2) from an *O*-methyl (δ_H 3.31), H-3 (δ_H 3.37), and H-30 (δ_H 2.68) to C-2 (δ_C 106.7). Therefore, the gross structure of **1** was constructed as depicted.

The relative stereochemistry of **1** was assigned from the ROESY spectrum (Supporting Information). The ROESY correlations of H-5/H-11β and H-12β/H-17 indicated that these groups were cofacial, and were assigned arbitrarily as β-oriented. The ROESY cross-peaks of Me-19/H-9, Me-18/H-9, H-3/H_2-29, and

Table 1. ^1H (400 Hz) and ^{13}C NMR (125 Hz) spectroscopic data for compound **1** in CD_3OD.

Position	δ_H (*J*, Hz)	δ_C	Position	δ_H (*J*, Hz)	δ_C
1		85.4	15a	3.12 (1H, d, 18.8)	37.1
2		106.7	15b	3.45 (1H, d, 18.8)	
3	3.37 (1H, s)	84.6	16		174.9
4		43.5	17	5.87 (1H, s)	82.6
5	2.79 (1H, d, 8.0)	41.5	18	1.07 (3H, s)	16.0
6	4.17 (1H, d, 8.0)	72.4	19	1.23 (3H, s)	18.5
7		177.1	20		122.4
8		87.6	21	7.57 (1H, s)	142.6
9	2.13 (1H, m)	57.1	22	6.50 (1H, s)	111.3
10		61.2	23	7.51 (1H, s)	144.1
11α	1.80 (1H, m)	17.2	28	1.05 (3H, s)	20.0
11β	1.87 (1H, m)		29a	1.50 (1H, d, 11.6)	45.1
12α	0.93 (1H, m)	28.0	29b	1.89 (1H, d, 11.6)	
12β	1.94 (1H, m)		30	2.68 (1H, s)	74.0
13		38.9	2-OCH_3	3.31 (3H, s)	50.7
14		84.6	7-OCH_3	3.74 (3H, s)	52.6



1 **2**

Figure 2. The key HMBC and $^1H-^1H$ COSY correlations of compounds **1** and **2**.

H_2-29/H-30 implied the α-orientation of those groups. Therefore, the relative stereochemistry of compound **1** was established as shown.

Compound **2** possessed the molecular formula $C_{23}H_{38}O_5$ as evidenced by the positive HR-ESI-MS at *m/z* 417.2617 $[M + Na]^+$ with five degrees of unsaturations. Extensive analysis of the 1D and 2D NMR data showed a high similarity between **2** and 2α,3α,20-trihydroxy-16β-acetoxy-20(*R*)-pregnane (**3**) [15] except for the location of the acetyl. The acetyl was assigned at C-20 and a hydroxyl was located at C-16, which were readily confirmed by the HMBC correlations from H-20 (δ_H 5.18, dq, $J = 11.7$, 5.9 Hz) to CH_3COO-20 (δ_C 170.9) and C-16 (δ_C 71.9) as well as the $^1H-^1H$ COSY cross-peaks of H-17 (δ_H 1.41, dd, $J = 11.7$, 7.8 Hz) with H-16 (δ_H 4.25, td, $J = 7.8$, 4.4 Hz) and H-20, and of Me-21 with H-20 (Figure 2). Comparison of the ^1H NMR spectrum of **2** with azedarcrcbol [22], H-16 (δ_H 5.71, td, $J = 7.8$, 4.4 Hz) was almost the same as that of azedarcrcbol. In addition, the coupling constant ($J = 7.8$) between H-16 with H-17 was in good agreement with Ref. [15,22,23], indicating that the H-16 was assigned as α-orientated. Therefore, the hydroxyl at C-16 was assigned as β-orientated. Accordingly, the compound was established as 2α,3α,16β-trihydroxy-20-acetoxy-20(*R*)-pregnane (Table 2).

Selected compounds (**2**–**8**) were screened for their antimicrobial activities against four microorganisms, *Pseudomonas*

Table 2. ^1H (400 Hz) and ^{13}C NMR (125 Hz) spectroscopic data for compound **2** in $CDCl_3$.

Position	δ_H (*J*, Hz)	δ_C	Position	δ_H (*J*, Hz)	δ_C
1	1.18 (1H, m) 1.64 (1H, m)	40.4	13		42.2
2	3.67 (1H, m)	68.8	14	0.86 (1H, m)	53.6
3	3.88 (1H, s)	69.0	15	1.14 (1H, m) 2.22 (1H, dt, 13.3, 7.8)	37.3
4	1.45 (1H, m) 1.51 (1H, m)	34.1	16	4.25 (1H, td, 7.8, 4.4)	71.9
5	1.44 (1H, m)	38.1	17	1.41 (1H, dd, 11.7, 7.8)	60.2
6	1.11(1H, m) 1.24 (1H, m)	27.5	18	0.79 (3H, s)	13.7
7	0.86 (1H, m) 1.61(1H, m)	31.7	19	0.75 (3H, s)	12.3
8	1.38 (1H, m)	34.2	20	5.18 (1H, dq, 11.7, 5.9)	70.4
9	0.73 (1H, m)	54.2	21	1.28 (1H, d, 5.9)	19.9
10		36.8	20-OAc		170.9
11	1.47 (1H, m) 1.22 (1H, m)	20.5		1.99 (3H, s)	21.5
12	1.08 (1H, m) 1.76 (1H, d, 12.7)	39.5			

aeruginosa, *Staphylococcus aureus*, MRSA (methicillin-resistant *Staphylococcus aureus*) 92#, and MRSA 98#. The minimum inhibitory concentrations (MICs) of these compounds were determined by the 2-fold dilution method [24,25]. The results revealed that compounds **5** and **7** showed weak antimicrobial activities against MRSA 92# and MRSA 98# with an MIC value of 25 μg/ml (Table 3).

3. Experimental

3.1 General experimental procedures

Optical rotations were determined on a JASCO P-1020 digital polarimeter (Jasco, Tokyo, Japan). UV spectra were detected on a Shimadzu UV-2401 PC spectrophotometer (Shimadzu, Tokyo, Japan). IR spectra were scanning with Bruker Tensor-27 infrared spectrometer with a KBr disk (Bruker, Karlsruhe, Germany). Brucker HCT/E squire (Bruker, Karlsruhe, Germany) and Waters Autospec Premier P776 spectrum (Waters, Millford, MA, USA) were used to measure ESI-MS, HR-ESI-MS, and HR-EI-MS spectra, respectively. 1D and 2D NMR spectra were recorded on a Bruker AM-400 and DRX-500 spectrometers (Bruker, Karlsruhe, Germany) with trimethylsilyl as internal standard. Column chromatography was performed on silica gel (200–300 and 300–400 mesh; Qingdao Marine Chemical Inc., Qingdao, China), MCI gel CHP 20P (75–150 μm; Mitsubishi Chemical Corporation, Tokyo, Janpan), Sephadex LH-20 (40–70 μm; Amersham Pharmacia Biotech AB, Uppsala, Sweden), and Chromatorex Rp-C_{18} gel (20–45 μm; Merck, Darmstadt, Germany). Thin layer chromatography (TLC plates; Qingdao Marine Chemical Inc., Qingdao, China) spots were visualized under UV light and by dipping into 5% H_2SO_4 in EtOH followed by heating.

3.2 Plant material

The dried leaves and twigs of *K. senegalensis* were collected from Xishuangbanna, Yunnan Province of China in August 2010, and were identified by Mr Yu Chen (Kunming Institute of Botany, Chinese Academy of Sciences). A voucher specimen (H20100808) was deposited at the Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences.

3.3 Extraction and isolation

The air-dried powdered plant material (8.0 kg) was extracted with MeOH (3 × 20 liters) under reflux for three times (4, 3, and 3 h), respectively. The combined MeOH extracts were concentrated under vacuum to give a crude residue (630 g),

Table 3. Antimicrobial activities of **2**–**8**.

Compound	Antimicrobial activities (MICs in μg/ml)			
	S. aureus[a]	*P. aeruginosa*[a]	MRSA 92#[a]	MRSA 98#[a]
2	50	>50	50	50
3	50	>50	>50	>50
4	50	>50	50	50
5	50	>50	50	25
6	>50	>50	>50	50
7	50	>50	25	25
8	50	>50	50	50
Vancomycin hydrochloride[a]	0.78	25	0.78	0.78

Note: *S. aureus*, *Staphylococcus aureus*; *P. aeruginosa*, *Pseudomonas aeruginosa*; MRSA, methicillin-resistant *Staphylococcus aureus.*

[a] Positive control.

which was suspended in water and then partitioned successively with petroleum ether, EtOAc, and *n*-BuOH. The EtOAc portion (200.6 g) was chromatographed on a silica gel column, eluted with petroleum ether–acetone (from 1:0 to 0:1) to yield six fractions (1–6). Fr. 3 (8.1 g) was then separated over an MCI-gel column (MeOH/H_2O from 2:8 to 10:0) to obtain four fractions (Fr. 3A–3D). Fr. 3B (500 mg) was chromatographed on Sephadex LH-20 (MeOH) to obtain Fr. 3B1 (400 mg), which was further purified by a silica gel column ($CHCl_3$:Me_2CO, 100:2) to obtain **4** (10 mg) and **6** (5.2 mg). Fr. 3F (4.0 g) was chromatographed over a silica gel column to yield four fractions (Fr. 3F1–3F4). Fr. 3F1 (400 mg) was purified by Sephadex LH-20 (MeOH) and then chromatographed on a silica gel column ($CHCl_3$:Me_2CO, 9:2) to obtain **2** (10 mg) and **3** (10 mg). Compounds **1** (9 mg) and **5** (60 mg) were isolated from Fr. 3F3 (400.0 mg) by repeated silica gel column chromatography, eluted with $CHCl_3$–Me_2CO (100:20). The *n*-BuOH part (320 g) was chromatographed on a silica gel column, eluted with $CHCl_3$–MeOH (from 9:1 to 0:1) to yield four fractions (M–P). Fraction N (80 g) was chromatographed on a silica gel column, eluted with $CHCl_3$–MeOH (from 7:3 to 6:4) to obtain **7** (58 mg) and **8** (80 mg).

3.3.1 *Khayseneganin I (**1**)*

Khayseneganin I (**1**) is a white amorphous power; $[\alpha]_D^{16}$ + 47.7 (*c* 0.16, CH_3OH); UV (MeOH) λ_{max} nm 209, IR (KBr) ν_{max} 3433, 1727, 1634, 1246, 1167, 1036 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 1; positive ESI-MS: *m/z* 571 $[M + Na]^+$; HR-EI-MS: *m/z* 548.2259 $[M]^+$ (calculated for $C_{28}H_{36}O_{11}$, 548.2258).

3.3.2 *2α,3α,16β-trihydroxy-20-acetoxy-20(R)-pregnane (**2**)*

2α,3α,16β-trihydroxy-20-acetoxy-20(*R*)-pregnane (**2**) is a white amorphous power; $[\alpha]_D^{19}$ + 56.9 (*c* 0.2, CH_3OH); IR (KBr) ν_{max} 3435, 2927, 1715, 1381, 1246, 1035, 615 cm^{-1}; ^1H and ^{13}C NMR spectral data, see Table 2; positive ESI-MS: *m/z* 417 $[M + Na]^+$; HR-ESI-MS: *m/z* 417.2617 $[M + Na]^+$ (calculated for $C_{23}H_{38}O_5Na$, 417.2616).

3.4 *Antimicrobial assays*

The MICs of selected compounds against *S. aureus* (ATCC25923), *P. aeruginosa* (ATCC27853) (National Institutes for Food and Drug Control (NIFDC), China), MRSA 92#, and MRSA 98# (Clinically isolated strains, from Kunming General Hospital of Chengdu Military Command) were determined by the twofold dilution method [22]. The strains used in antimicrobial tests were obtained from the Research Center of Natural Medicine, Clinical School of Kunming General Hospital of Chengdu Military Command. Antimicrobial tests were performed according to the previously described method [25].

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References

[1] H. Peng and J.M. David, *Flora of China (Zhongguo Zhiwu Zhi)* (Science Press, Beijing, 2008), Vol. 11, p. 116.
[2] J.M. Dalziel, *The Useful Plants of West Tropical Africa*, (The Crown Agents for the Colonies, London, 1937), p. 325.
[3] J.M. Agbedahunsi, F.A. Fakoya, and S.A. Adesanya, *Phytomedicine* **11**, 504 (2004).
[4] S.A.M. Abdelgaleil, F. Hashinaga, and M. Nakatani, *Pest Manage. Sci.* **61**, 186 (2005).
[5] M. Nakatani, S.A.M. Abdelgaleil, H. Okamura, T. Iwagawa, A. Sato, and M. Doe, *Tetrahedron Lett.* **41**, 6473 (2000).

[6] B. Zhang, S.P. Yang, S. Yin, C.R. Zhang, Y. Wu, and J.M. Yue, *Phytochemistry* **70**, 1305 (2009).
[7] T. Yuan, R.X. Zhu, H. Zhang, A. Odeku Oluwatoyin, S.P. Yang, S.G. Liao, and J.M. Yue, *Org. Lett.* **12**, 252 (2010).
[8] Y.T. Di, H.P. He, H.Y. Liu, P. Yi, Z. Zhang, Y.L. Ren, J.S. Wang, Q.Y. Sun, F.M. Yang, X. Fang, S.L. Li, H.J. Zhu, and X.J. Hao, *J. Nat. Prod.* **70**, 1352 (2007).
[9] X. Fang, Y.T. Di, H.P. He, H.Y. Liu, Z. Zhang, Y.L. Ren, Z.L. Gao, S. Gao, and X.J. Hao, *Org. Lett.* **10**, 1905 (2008).
[10] J.L. Yin, Y.T. Di, X. Fang, E.D. Liu, H.Y. Liu, H.P. He, S.L. Li, S.F. Li, and X.J. Hao, *Tetrahedron Lett.* **52**, 3083 (2011).
[11] C.M. Yuan, Y. Zhang, G.H. Tang, S.L. Li, Y.T. Di, L. Hou, J.Y. Cai, H.M. Hua, H.P. He, and X.J. Hao, *Chem. Asian J.* **7**, 2024 (2012).
[12] J.Y. Cai, Y. Zhang, S.H. Luo, D.Z. Chen, G.H. Tang, C.M. Yuan, Y.T. Di, S.H. Li, X.J. Hao, and H.P. He, *Org. Lett.* **14**, 1523 (2012).
[13] X.Y. Wang, G.H. Tang, C.M. Yuan, Y. Zhang, T. Zou, C. Yu, Q. Zhao, X.J. Hao, and H.P. He, *Fitoterapia* **85**, 64 (2013).
[14] C.M. Yuan, Y. Zhang, G.H. Tang, Y. Li, H.P. He, S.F. Li, L. Hou, X.Y. Li, Y.T. Di, S.L. Li, H.M. Hua, and X.J. Hao, *Planta Med.* **79**, 163 (2013).
[15] S.M. Yang, W.W. Fu, D.X. Wang, C.H. Tan, and D.Y. Zhu, *J. Asian Nat. Pro. Res.* **10**, 459 (2008).
[16] M.T. Pupo, P.C. Vieira, J.B. Fernandes, M.F. Das, G.F. da Silva, and E.R. Fo, *Phytochemistry* **45**, 1495 (1997).
[17] H.N.R. Seto, F. Nanjo, and Y. Hara, *Biosci. Biotechnol. Biochem.* **61**, 1434 (1997).
[18] B. Zhang, Y. Wang, S.P. Yang, Y. Zhou, W.B. Wu, W. Tang, J.P. Zuo, Y. Li, and J.M. Yue, *J. Am. Chem. Soc.* **134**, 20605 (2012).
[19] K.R. Markham, C. Vilain, and B.P.J. Molloy, *Phytochemistry* **24**, 2607 (1985).
[20] V. Vecchietti, G. Ferrari, F. Orsini, and F. Pelizzoni, *Phytochemistry* **18**, 1847 (1979).
[21] C.M. Yuan, Y. Zhang, G.H. Tang, Y.T. Di, M.M. Cao, X.Y. Wang, G.Y. Zuo, S.L. Li, H.M. Hua, H.P. He, and X.J. Hao, *J. Nat. Prod.* **76**, 327 (2013).
[22] M. Nakatani, H. Takao, I. Miura, and T. Hase, *Phytochemistry* **24**, 1945 (1985).
[23] T. Nakanishi, M. Kobayashi, H. Murata, and A. Inada, *Chem. Pharm. Bull.* **36**, 4148 (1988).
[24] S.Y. Xu, R.L. Bai, and X. Chen, *Pharmacological Experiment Methodology*, 3rd ed. (People's Medical Publishing House, Beijing, 2002), p. 1647.
[25] G.H. Tang, Y. Zhang, Y.C. Gu, S.F. Li, Y.T. Di, Y.H. Wang, C.X. Yang, G.Y. Zuo, S.L. Li, H.P. He, and X.J. Hao, *J. Nat. Prod.* **75**, 996 (2012).