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Antifungal limonoids from the fruits of *Khaya senegalensis*

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Abstract

Investigation of the fruits of *Khaya senegalensis* resulted in the isolation of three new mexicanolide limonoids containing a rare conjugated diene lactone system named seneganolide A (**1**), 2-hydroxyseneganolide A (**2**) and 2-acetoxyseneganolide A (**3**). Two known limonoids, 3-deacetyl-7-deacetoxy-7-oxokhivorin (**4**) and methyl 6-hydroxyangolensate (**5**), were also found. The structures of the new compounds were elucidated on the basis of spectral methods. The antifungal activity of compounds **1**, **3** and **5** was tested against the fungus *Botrytis cinerea*.
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1. Introduction

Khaya senegalensis (Desr.) (Meliaceae) is a large tree native to the sub-Sahara savannah from Senegal to Uganda and is used in traditional medicines in Africa [1].

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The decoction of the bark is extensively used as a febrifuge, which could be associated with its use as an antimalarial drug [2]. We have reported the isolation of several types of rings B,D-opened limonoids including methyl angolensates [3], mexicanolides [4,5], and rearranged phragmalins [6,7] from the stem bark ether and acetone extracts of *K. senegalensis*.

In our continuing search for new biologically active limonoids, we have investigated the chemical constituents of the ether extract of fruits of *K. senegalensis* collected from Mbour, Senegal. Three new mexicanolide limonoids named senegano-lide A (**1**), 2-hydroxyseneganolide A (**2**) and 2-acetoxyseneganolide A (**3**) together with two known limonoids, 3-deacetyl-7-deacetoxy-7-oxokhivorin (**4**) and methyl 6-hydroxyangolensate (**5**) have been isolated. The structure of the new compounds were elucidated on the basis of spectral data interpretation. Three of the isolated compounds (**1**, **2** and **5**) were examined for their antifungal activity on the *Botrytis cinerea* (Pers. Fr.).

2. Experimental

2.1. General

^{1}H and ^{13}C-NMR spectra were measured at 600 and 125 MHz at 27 °C in CDCl$_3$ on a JEOL FX-600 spectrometer. Optical rotation was measured at 22 °C using JASCO J-720 spectropolarimeter. IR (KBr) and UV (MeOH) were recorded on JASCO FT/IR 5300 and Shimadzu UV-210A spectrophotometers. HPLC was performed on a Waters µBondapak C$_{18}$ column.

2.2. Plant material

The fruits of *K. senegalensis,* collected in September 2000 from Mbour City, Senegal, were identified by Mr. Ahmed Moharib of Alexandria University. A voucher specimen (KSF-1) is deposited in the Faculty of Science, Kagoshima University.

2.3. Extraction and isolation

The air-dried fruits of *K. senegalensis* (450 g) were extracted with Et$_2$O (2.5 l) at room temperature to yield 5.6 g of extract. The extract was fractionated by droplet countercurrent chromatography (DCCC) using CH$_2$Cl$_2$–MeOH–H$_2$O (5:5:3 v/v) in an ascending mode to give 300 fractions. These fractions were pooled into three fractions, Fr 1 (216–229: 77 mg), Fr 2 (282–293: 1.4 g) and Fr 3 (294–296: 600 mg), on the basis of similar TLC profiles. Fr 1 was purified repeatedly through HPLC with 40–50% H$_2$O/MeOH as solvent to give **2** (1.8 mg). Fr 2 was subjected to HPLC with 30–50% H$_2$O/MeOH to give **1** (24 mg) and **3** (40 mg). Similar purification of Fr 3 with 40–50% H$_2$O/MeOH gave **4** (6.9 mg) and **5** (45 mg).
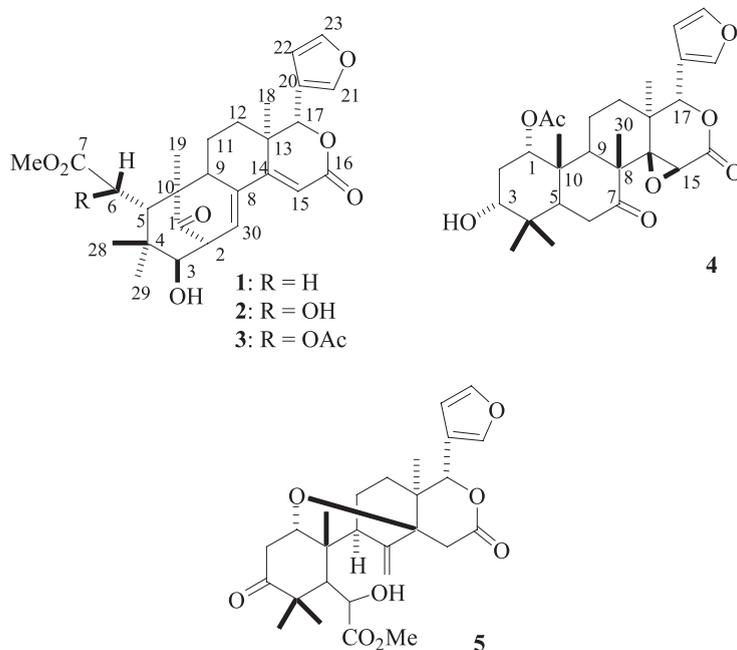
Seneganolide A (**1**). White amorphous powder; $[\alpha]_D$+245° (c 0.30, MeOH); UV max (MeOH): 203 (log ε 4.11), 286 (4.04) nm; IR bands (KBr): 3600–3400, 2949, 1740–1700, 1593, 1261, 1026, 873 cm^{-1}; $C_{27}H_{32}O_7$; HRFABMS m/z: 469.2222 [M+H]$^+$, Δ+0.2 mmu. (calculated for $C_{27}H_{32}O_7$ 468.2148). ^{1}H and ^{13}C-NMR data: see Tables 1 and 2.

2-Hydroxyseneganolide A (**2**). White amorphous powder; $[\alpha]_D$ +264° (c 0.09, MeOH); IR bands (KBr): 3600–3400, 1716, 1263, 1026, 873 cm^{-1}; $C_{27}H_{32}O_8$; HRFABMS m/z: 485.2170 [M+H]$^+$, Δ−0.5 mmu. (calculated for $C_{27}H_{32}O_8$ 484.2097). ^{1}H and ^{13}C-NMR data: see Tables 1 and 2.

2-Acetoxyseneganolide A (**3**). White amorphous powder; $[\alpha]_D$+269° (c 0.31, MeOH); UV max (MeOH): 203 (log ε 4.08), 284 (3.95) nm; IR bands (KBr): 3600–3400, 2953, 1740–1700, 1595, 1269, 1030, 873 cm^{-1}; $C_{29}H_{34}O_9$; HRFABMS m/z: 527.2281[M+H]$^+$, Δ−0.4 mmu. (calculated for $C_{29}H_{34}O_9$ 526.2203). ^{1}H and ^{13}C-NMR data: see Tables 1 and 2.

3. Results and discussion

Seneganolide A (**1**) was obtained as an amorphous powder. Its molecular formula $C_{27}H_{32}O_7$ was shown by HRFABMS. The IR spectrum exhibited characteristic absorption bands for hydroxyl (3600–3400 cm^{-1}), carbonyl groups (1740–1700 cm^{-1}) and furan ring (873 cm^{-1}). Strong absorption bands at 203 nm (log ε 4.11) and 286 (4.04) in the UV spectrum suggested the presence of conjugated double bond and enone groups. ^{1}H and

Table 1
¹H-NMR data of compounds **1**–**3**

Proton	1	2	3
2	3.07 *d* (5.9)	3.01 *d* (6.1)	3.08 *d* (5.8)
3	3.77 *d* (5.2)	3.52 *s*	3.68 *s*
5	2.85 *dd* (9.7, 1.5)	2.76 *s*	3.04 *s*
6	2.46 *dd* (16.8, 9.7)	4.35 *s*	5.44 *s*
	2.35 *dd* (17.2, 1.6)		
9	2.27 *br dt* (12.2, 2.8)	2.25 *dd* (13.0, 2.9)	2.28 *br dd* (14.7, 2.9)
11 α	1.73 *ddd* (13.2, 9.1, 4.3)	1.78 *m*	1.86 *m*
11β	1.47 *ddd* (14.5, 12.1, 5.2)	1.30 *ddd* (25.7, 14.0, 4.4)	1.49 *ddd* (21.2, 13.0, 4.7)
12α	1.26 *ddd* (14.1, 11.0, 5.3)	1.21 *dd* (14.2, 4.1)	1.25 *dd* (13.7, 8.9)
12β	1.90 *dt* (14.1, 4.5)	1.93 *dt* (14.4, 4.1)	2.01 *dt* (13.7, 4.2)
15	6.31 *s*	6.26 *s*	6.31 *s*
17	5.14 *s*	5.06 *s*	5.12 *s*
18	1.05 *s*	1.01 *s*	1.05 *s*
19	1.20 *s*	1.42 *s*	1.22 *s*
21	7.50 *d* (1.5)	7.48 *dd* (1.6, 0.7)	7.52 *br d* (1.0)
22	6.48 *br d* (1.0)	6.45 *br d* (1.0)	6.49 *br d* (1.0)
23	7.42 *t* (1.6)	7.40 *t* (1.6)	7.44 *t* (1.6)
28	0.92 *s*	0.95 *s*	1.06 *s*
29	0.72 *s*	0.97 *s*	1.00 *s*
30	6.67 *dd* (6.0, 2.7)	6.64 *dd* (6.0, 2.9)	6.67 *dd* (6.0, 2.9)
OMe	3.69 *s*	3.77 *s*	3.74 *s*
OAc			2.18 *s*

Measured in $CDCl_3$ at 600 MHz; *J* values (in Hz) are presented in parentheses.

¹³C-NMR data (Tables 1 and 2) indicated the existence of five methyls (four tertiary and one methoxy), three methylenes, 10 methines (five olefinic) and nine non-protonated carbon atoms (three olefinic, one keto and two ester carbonyls). NMR data also revealed that seven elements of unsaturation were present as double bonds: four carbon–carbon double bonds and three CO, which suggests a pentacyclic structure for the molecule.

All of the proton-bearing carbons were assigned by the HMQC experiment. Extensive studies using spin-decoupling, ¹H–¹H COSY and HMBC spectra revealed **1** to be a mexicanolide-type limonoid. A singlet at δ 5.14 and a signal at δ 2.85 coupled with signals at δ 2.46 and 2.35 were assigned to H-17 and H-5, respectively. A methine proton at δ 2.27 (H-9) was coupled to a methylene proton at δ 1.47 (H-11β) which in turn coupled with two protons of the adjacent methylene at 1.26 (12α) and 1.90 (12β) according to the structure of the C-9–C-12 fragment. A methine proton at δ 3.07 (H-2) attached to a carbon at δ 56.5 adjacent to a carbonyl at δ 213.9 (C-1) showed HMBC correlations with the ¹³C-NMR signals at δ 213.9, 80.2, 39.5, 51.2, 131.2 and 134.9 led to their assignments as C-1, C-3, C-4, C-10, C-30 and C-8, respectively. A methine proton at δ 2.85 (H-5) exhibited HMBC correlations with ¹³C-signals at δ 15.9 (Me-19), 51.2 (C-10), 39.5 (C-4), 80.2 (C-3), 32.7 (C-6), 173.9 (C-7), 53.6 (C-9), 26.1 (Me-28) and 14.2 (Me-29). These findings clearly characterized the second fragment, the dicyclo[3.3.1]decane ring system [2,8], including Me-28 and Me-29, and the side chain at C-5 of the molecule. Further, an olefinic proton at δ 6.31 (H-15) coupled to the ¹³C signals at δ 164.8 (C-16), 79.8 (C-17), 37.5 (C-13) and 134.9 (C-8). These correlations characterized the third fragment, C-8 to C-17 of C and D

Table 2
^{13}C-NMR data of compounds **1**–**3**

Carbon	1	2	3
1	213.9 s	215.0 s	212.8 s
2	56.5 d	56.6 d	56.5 d
3	80.2 d	80.3 d	80.5 d
4	39.5 s	39.4 s	39.6 s
5	40.9 d	45.2 d	44.3 d
6	32.7 t	72.1 d	72.2 d
7	173.9 s	176.0 s	170.8 s
8	134.9 s	134.6 s	135.2 s
9	53.6 d	55.0 d	54.9 d
10	51.2 s	51.6 s	51.6 s
11	21.3 t	22.1 t	22.0 t
12	32.7 t	33.3 t	33.4 t
13	37.5 s	37.5 s	37.6 s
14	160.7 s	161.7 s	160.8 s
15	112.3 d	111.8 d	112.7 d
16	164.9 s	165.5 s	164.8 s
17	79.8 d	79.7 d	79.9 d
18	21.9 q	22.3 q	22.4 q
19	15.9 q	16.2 q	15.4 q
20	120.3 s	119.6 s	120.2 s
21	141.5 d	141.4 d	141.3 d
22	110.2 d	110.1 d	110.2 d
23	143.2 d	143.2 d	143.2 d
28	26.1 q	25.6 q	25.7 q
29	14.2 q	16.0 q	16.0 q
30	131.2 d	132.5 d	131.5 d
OMe	52.0 q	53.1 q	52.9 q
OCOCH$_3$			169.9 s
OCOCH$_3$			20.9 q

Measured in CDCl$_3$ at 150 MHz.

rings of the molecule. The most interesting finding in this compound is the presence of a rare conjugated diene lactone, which indicated from the down-field proton signals at δ 6.31 (H-15) and 6.67 (H-30) and the ^{13}C signals at δ 112.3 (C-15), 160.7 (C-14), 134.9 (C-8) and 131.2 (C-30).

Relative stereochemistry of the dicyclo[3.3.1]decane ring in seneganolide A (**1**) was elucidated by NOE studies [2,8]. The W-type long-range coupling between H-9 and H-30, and NOE of H-9 with Me-19 and H-5 with H-11 indicating that ring C is present in a skew boat form. NOE correlations (Fig. 1) of H-17 with H-12β and H-11β, and H-5 with H-11β suggested a β orientation of these protons. On the other hand, the NOE correlations of H-9 with Me-18, H-12α, H-11α and Me-19 indicated α orientation of these protons (Fig. 1).

The second compound, 6-hydroxyseneganolide A (**2**), C$_{27}$H$_{32}$O$_8$, was isolated as an amorphous powder. Compound **2** showed IR and NMR spectra similar to those of **1** except for the presence of an additional hydroxyl group at C-6. The presence of only two methylene carbons in DEPT spectrum, a down-field ^{13}C signal δ 72.1 and a proton signal at δ 4.35 indicated a hydroxyl group. The location of this hydroxyl group at C-6 was
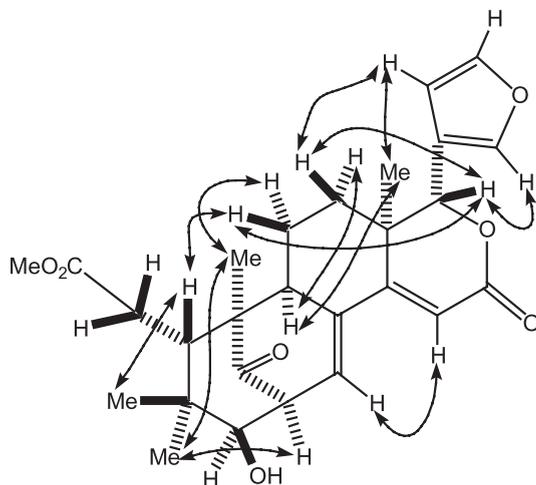
Fig. 1. Significant NOE correlations in **1**.

confirmed from the lack of multiplicity of H-5, which appeared as a singlet in **2** instead of a doublet of doublets as in **1** and from HMBC correlations of H-5 with ^{13}C signals at δ 72.1 (C-6) and H-6 with ^{13}C signals at δ 45.2 (C-5) and 39.4 (C-4). Therefore, compound **2** is the 6-hydroxy derivative of **1**.

Compound **3** ($C_{29}H_{34}O_9$) named 6-acetoxyseneganolide A, showed IR and UV spectra closely related to those of **1**. ^{1}H and ^{13}C-NMR data of **3** were similar to those of **2** except for the presence of acetoxy group instead of hydroxyl group at C-6 of side chain. This was indicated from the appearance of two additional ^{13}C signals at δ 169.7 *s* and 20.9 *q* and proton signal at δ 2.18 of acetoxy group and HMBC correlations (Fig. 2) of H-6 at δ 5.44
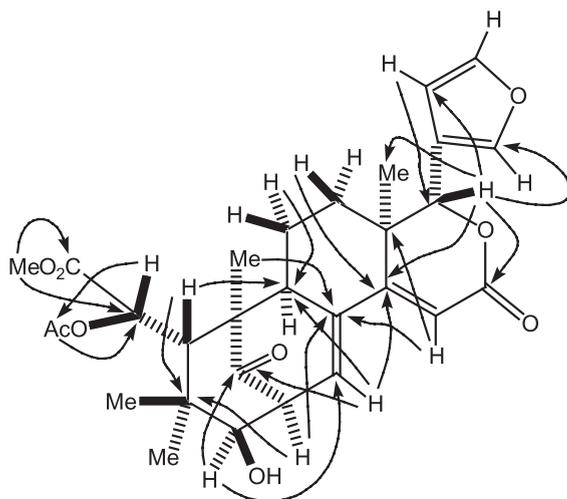
Fig. 2. Selected HMBC correlations in **3**.

with carbon signal at δ 169.7 of acetoxy group and proton signal at δ 2.18 of acetoxy group with carbon signals at δ 72.2 (C-6). In addition to some changes of proton chemical shifts of H-6 and H-5 to be at δ 5.44 and 3.04 in **3** instead of 4.35 and 2.76, respectively.

The structure of known limonoids (**4** and **5**) was established by ^{1}H and ^{13}C-NMR analysis and HRFABMS analysis, as well as by comparison of NMR data with those reported in the literature [8,9].

Radial growth technique [10] was used to evaluate the antifungal activity of three of isolated compounds seneganolide A (**1**), 2-acetoxyseneganolide A (**3**) and methyl 6-hydroxyangolensate (**5**) on the fungus *B. cinerea*. Compound **3** at concentrations of 1000 and 1500 ppm showed an inhibition of mycelial growth of 61.50% and 68.33%, respectively, without significant differences from **1** at 1000 ppm (60.83%) and **5** at 1500 ppm (65.33%).

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