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Three New Modified Limonoids from *Khaya senegalensis*

Munehiro Nakatani,*[,†] Samir A. M. Abdelgaleil,[‡] Shihata M. I. Kassem,[‡] Ken Takezaki,[§] Hiroaki Okamura,[†] Tetsuo Iwagawa,[†] and Matsumi Doe[⊥]

Department of Chemistry and Bioscience, Faculty of Science, Kagoshima University, 1-21-35 Korimoto, Kagoshima 890-0065, Japan, Department of Pesticide Chemistry, Faculty of Agriculture, Alexandria University, Alexandria, Egypt, Kagoshima Prefectural Agricultural Experiment Station, 5500 Kamifukumoto-cho, Kagoshima 890-0116, Japan, and Analytical Division, Graduate School of Science, Osaka City University, 3-3-7 Sugimoto, Sumiyoshi-ku, Osaka 558-8585, Japan

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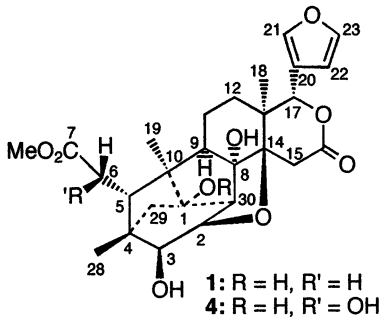
Three new rings B/D opened limonoids, two rearranged phragmalin limonoids named khayanolides D and E (**1** and **2**), and one limonoid glucoside named khayanoside (**3**) were isolated as insect antifeedants from the stem bark of Egyptian *Khaya senegalensis*. The structures of these compounds were established by spectroscopic methods, including 2D NMR and CD.

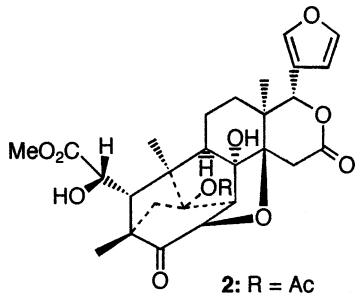
Khaya senegalensis (Desr.) A. Juss. (Meliaceae) is an African mahogany native to the sub-Sahara savannah from Senegal to Uganda and is the source of one of the most popular traditional medicines in Africa.[1] Recently, we reported the isolation of some mexicanolide-type and rearranged phragmalin-type rings B and D opened limonoids from the ether and acetone extracts of the stem bark.[2−5]

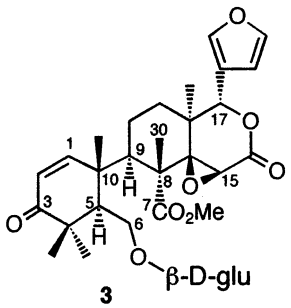
We have now isolated three additional new limonoids (**1**−**3**) from the acetone extract by droplet countercurrent chromatography (DCCC), followed by HPLC separation. In this paper, we report the isolation, structure elucidation, and antifeedant and antiviral activities of compounds **1**−**3**.

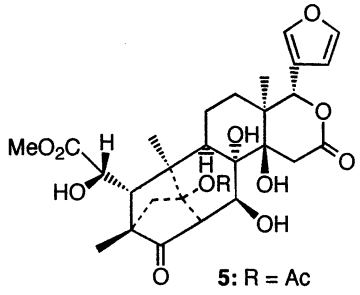
Khayanolide D (**1**) was isolated as an amorphous powder, and its molecular formula was established as $C_{27}H_{34}O_9$ by HRFABMS and NMR data. The UV (211 nm) and the IR (3650−3200, 1741, 1724, 1618, and 875 cm^{-1}) indicated the presence of carbon−carbon double bond, hydroxyl, and ester carbonyl groups. The ^1H NMR spectrum (Table 1) of **1** was very similar to that of a rearranged phragmalin-type limonoid, khayanolide B (**4**),[4] except for lack of the C-6 methine proton signal at δ 4.21 in **4** and the presence of new methylene proton signals in **1** assigned to H_2-6 at δ 2.39 (dd, J = 16.9 and 12.0 Hz) and 2.19 (dd, J = 16.9 and 2.9 Hz). The presence of a similar ether linkage between C-2 and C-14 as in **4** was determined from NOE correlations between the H-2 and H-15β signals and between the H-15α and 13-Me (Me-18) proton signals, from which the configuration of the 8-OH group was determined to be α. Other NOE correlations confirmed that **1** had the same stereochemistry as **4** at positions C-3−C-5, C-9, and C-10 in the tricyclo[4.2.1.1]decane ring system. Thus, khayanolide D was identified as the 6-dehydroxy derivative of khayanolide B.

The IR and UV spectra of **2** ($C_{29}H_{34}O_{11}$) were similar to those of **1**, but the CD spectrum showed the presence of a keto group at 308 nm. The ^1H and ^{13}C NMR spectra (Table 1) showed the presence of additional acetyl and keto carbonyl groups at δ 2.07 and 169.3 and δ 204.8, respectively. The spectra resembled very closely those of the 1-acetate of a phragmalin limonoid, methyl 1α,6,8α,14β,30β-









* Corresponding author. Tel: +81-99-285-8114. Fax: +81-99-285-8117. E-mail: nakat@mail.sci.kagoshima-u.ac.jp.
† Kagoshima University.
‡ Alexandria University.
§ Kagoshima Prefectural Agricultural Experiment Station.
⊥ Osaka City University.

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Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR Spectral Data of Compounds **1**, **2**, and **3**[a]

	1		**2**		**3**	
C no.	δ_H (mult.)	δ_C (mult.)	δ_H (mult.)	δ_C (mult.)	δ_H (mult.)	δ_C (mult.)
1		84.7 (s)		91.4 (s)	6.68 (d, 10.5)	152.3 (d)
2	4.52 (dd, 9.5, 6.6)	72.5 (d)	4.38 (d, 10.5)	74.0 (d)	5.93 (d, 10.5)	126.5 (d)
3	3.43 (d, 6.6)	78.4 (d)		204.8 (s)		203.1 (s)
4		43.8 (s)		52.3 (s)		45.4 (s)
5	3.30 (dd, 12.0, 2.8)	34.7 (d)	3.02 (d, 4.9)	42.1 (d)	1.98 (br d. 4.5)	50.2 (d)
6α	2.39 (dd, 16.9, 12.0)	34.0 (d)	4.34 (d, 4.9)	71.7 (d)	4.53 (dd, 10.1, 1.9)	67.2 (d)
6β	2.19 (dd, 16.9, 2.9)				3.59 (dd, 10.1, 4.5)	
7		174.7 (s)		174.9 (s)		176.6 (s)
8		87.5 (s)		87.7 (s)		50.9 (s)
9	2.11 (br d, 8.8)	55.6 (d)	2.36 (br d, 9.5)	57.6 (d)	3.03 (br d, 11.0)	43.1 (d)
10		57.1 (s)		62.1 (s)		43.6 (s)
11α	1.91 (m)	16.4 (t)	1.92 (ddt, 9.3, 5.5, 13.5)	16.7 (t)	1.55 (dd, 13.5, 7.0)	21.8 (t)
11β	1.52 (br d, 14.5)		1.54 (ddd, 13.5, 4.4, 2.0)		1.46 (m)	
12α	1.02 (br d, 14.7)	26.0 (t)	0.94 (ddd, 14.4, 5.5, 2.0)	32.9 (t)	1.38 (m)	32.7 (t)
12β	1.86 (dt, 3.9, 14.7)		1.71 (ddd, 14.4, 13.5, 4.4)		1.78 (br dd, 13.2, 5.2)	
13		38.0 (s)		37.5 (s)		38.1 (s)
14		81.6 (s)		83.3 (s)		68.1 (s)
15α	3.14 (d, 18.8)	32.3 (t)	3.08 (d, 18.8)	32.5 (t)	3.66 (s)	51.5 (d)
15β	2.76 (br d, 18.8)		2.64 (br d, 18.8)			
16		169.8 (s)		170.0 (s)		166.4 (s)
17	5.65 (s)	80.6 (d)	5.52 (s)	79.9 (d)	5.43 (s)	78.4 (d)
18	1.10 (s)	14.4 (q)	1.10 (s)	14.2 (q)	1.22 (s)	19.5 (q)
19	0.94 (s)	17.3 (q)	1.41 (s)	20.1 (q)	1.12 (s)	17.9 (q)
20		120.8 (s)		120.6 (s)		120.0 (s)
21	7.45 (m)	141.1 (d)	7.40 (br s)	141.1 (d)	7.38 (br s)	141.0 (d)
22	6.40 (d, 2.0)	110.2 (d)	6.36 (br d, 1.5)	110.1 (d)	6.33 (br d, 1.5)	109.9 (d)
23	7.39 (t, 1.7)	142.7 (d)	7.39 (t, 1.7)	142.9 (d)	7.40 (t, 1.7)	143.2 (d)
28	0.92 (s)	18.7 (q)	1.26 (s)	15.9 (q)	1.20 (s)	24.2 (q)
29_{Pro-R}	1.39 (d, 12.0)	44.9 (t)	2.12 (d, 12.7)	40.7 (t)	1.12 (s)	23.5 (q)
29_{Pro-S}	1.74 (d, 12.0)		2.78 (d, 12.7)			
30	2.63 (d, 9.5)	64.4 (d)	3.37 (d, 10.5)	59.7 (d)	1.25 (s)	14.9 (q)
OMe	3.72 (s)	52.0 (q)	3.75 (s)	52.5 (q)	3.79 (s)	53.6 (q)
OAc			2.07 (s)	21.8 (q)		
Glu 1′					4.34 (d, 7.8)	101.7 (d)
2′					3.24 (dd, 9.0, 7.8)	73.9 (d)
3′					3.55 (t, 9.1)	76.5 (d)
4′					3.32 (t, 9.1)	71.0 (d)
5′					3.48 (ddd, 9.4, 7.6, 2.7)	76.3 (d)
6′					3.93 (m,), 3.57 (m)	63.0 (t)

[a] Measured in $CDCl_3$. Chemical shift valuues are in ppm (δ) from TMS, and J values (in Hz) are presented in parentheses.

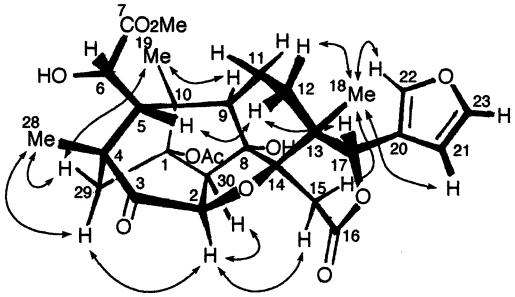

Figure 1. Significant NOE correlations in **2**.

pentahydroxy-3-oxo[3.3.1,^{10,2}1^{1,4}]tricyclomeliac-7-oate (**5**),[6] isolated from *K. senegalensis* collected in Brazil. However, the HMBC correlations of a proton signal at δ 3.37 (d, J = 10.5 Hz, H-30) with the C-9, C-10, and C-29 signals at δ 57.6, 62.1, and 40.7 and a *W*-type long-range coupling of the 30-H signal with the 9-H signal at δ 2.36 (br d, J = 9.5 Hz) indicated that **2** was also a rearranged phragmalin. An ether linkage between C-2 at δ 74.0 and C-14 at δ 83.3 was indicated by the significant downfield shift of the H-5 signal at δ 3.02 (δ 2.43 in a 2-keto-3-ol compound, khayanolide C[4]). The NOE correlations (Figure 1), particularly correlations of the H-2 signal at δ 4.38 (d, J = 10.5 Hz) with the H-15β at δ 2.64 (br d, J = 18.8 Hz) and H_{Pro-S} -29 signals at 2.78 (d, J = 12.7 Hz), provided conclusive proof for the structure. The stereochemistry of **2** was similar to that of **5**, and the configuration at C-6 was assumed to be *S*, the same as that of known limonoids[3,4] isolated from the same specimen.

The third compound, named khayanoside (**3**), was isolated as an amorphous powder, and the molecular formula $C_{33}H_{44}O_{13}$ was determined from a pseudomolecular ion $[M + Na]^+$ in the HRFABMS and NMR data. The UV and IR spectra supported the presence of lactone, ester, and α,β-unsaturated ketone functions. ^{1}H and ^{13}C NMR spectra (Table 1) of **3** showed the presence of one ether-linked terminal β-glucopyranose moiety, which was elucidated from the characteristic chemical shifts of the anomeric proton (δ 4.34) and carbon (δ 101.7) signals and the observed vicinal coupling constants of $J \approx 9$ Hz between the trans diaxial oxymethine protons of H-2′ and H-3′, H-3′ and H-4′, and H-4′ and H-5′ together with J = 7.8 Hz between H-1′ and H-2′.

The NMR data of the aglycone part showed the presence of six methyls (five tertiary and one methoxy), three methylenes, 10 methines (five olefinic), and nine quaternary carbons (one olefinic and one keto and two ester carbonyls). From the spectra, the aglycone part is pentacyclic. The ^{1}H NMR spectrum showed a characteristic H-15 epoxide proton as singlet at δ 3.66 and five methyls due to the basic limonoid skeleton at δ 1.12, 1.12, 1.20, 1.22, and 1.25. The characteristic H-17 singlet at δ 5.43 confirmed the lactonic ring D. A conjugated enone system confirmed by the NMR spectra [δ_H 6.68 and 5.93 (each d, J = 10.5 Hz); δ_C 152.3 d, 126.5 d, and 203.1 s] was assigned to the

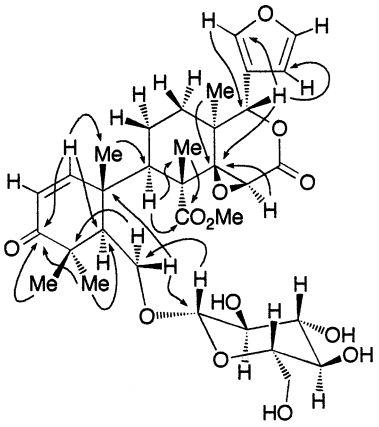

Figure 2. Selected HMBC correlations in **3**.

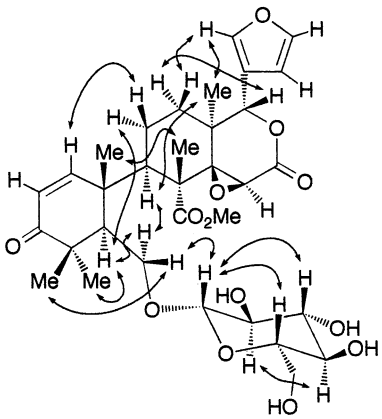

Figure 3. Significant NOE correlations in **3**.

ring A by the HMBC correlations (Figure 2). The HMBC spectrum also clarified the nature of the opened ring B at C_6-C_7. These NMR data and NOE correlations (Figure 3) demonstrated that the aglycone part of **3** was the same of secomahoganin, isolated from Japanese *Swietenia mahogani*.[7]

The location of the sugar moiety on the aglycone was subsequently deduced from the 2D NMR data. Thus, in the HMBC spectrum of **3**, correlation signals were observed between H-1′ (δ 4.34) of glucose and C-6 (δ 67.2) of the aglycon. Thus, **3** was determined to be 7-*O*-β-D-glucopyranosylsecomahoganin. Khayanoside (**3**) is the first example of a glycoside of the ring-B seco limonoid.

Insect antifeedant and antiviral activities of the new compounds were studied. Antifeedant activity was tested against the third-instar larvae of *Spodoptera littoralis* (Boisduval) by the leaf disk method.[8] Khayanolide E (**2**) was active at 100 ppm, with 50 ppm corresponding to a concentration of ca. 1 μg/leaf cm^2, but khayanolide D (**1**) and khayanoside (**3**) showed weak activities at 1000 ppm. Antiviral activity against HIV-1 replication was tested on the inhibition of virus-induced cytopathicity in MT-4 cells,[9] but the tested compounds showed no anti-HIV activity at a concentration of 100 μg/mL.

Experimental Section

General Experimental Procedures. ^{1}H and ^{13}C NMR spectra were measured at 500 and 125 MHz at 45 °C on a JEOL FX-500 spectrometer. IR and UV spectra were recorded on JASCO FT/IR 5300 and Shimazu UV-210A spectrophotometers. Specific rotations and CD spectra were measured using JASCO DIP-370S and JASCO J-720 spectropolarimeters. DCCC was used in ascending mode and HPLC was performed on Waters μBondapak C_{18} column.

Plant Material. The stem bark of *Khaya senegalensis* was collected in January 1999 at Alexandria, Egypt, and identified by Mr. Ahmed Moharib of Alexandria University. A voucher specimen (AAU-A1) is deposited in the Faculty of Agriculture, Alexandria University.

Extraction and Isolation. After extraction with hexane, followed by ether, the dried stem bark (910 g) was extracted with acetone (3 L) to yield 19 g of material. The acetone extract was fractionated by DCCC using CH_2Cl_2−MeOH−H_2O (5:5:3 v/v) in ascending mode to give five limonoid fractions of 108, 149, 321, 117, and 319 mg. The third fraction was purified through HPLC with 40−50% H_2O−MeOH as the solvent to give compounds **1** (1.5 mg) and **2** (2 mg) together with khayalactol (34 mg),[3] seneganolide (27 mg),[2] and 2-hydroxyseneganolide (5 mg).[5] Compound **3** (5 mg) was obtained together with 1-*O*-acetylkhayanolides A (7 mg)[5] and B (24 mg)[3] from the first fraction through HPLC with 35−50% H_2O−MeOH.

Khayanolide D (1): amorphous powder; $[\alpha]_D$ +4° (MeOH; *c* 0.09); UV (MeOH) λ_{max} 211 (ϵ 3500) nm; IR (KBr) ν_{max} 3600−3200, 1742, 1725, 1638, and 1618 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m/z* 503.2276 [M + H]$^+$ (calcd for $C_{27}H_{35}O_9$, 503.2281).

Khayanolide E (2): amorphous powder; $[\alpha]_D$ +21° (MeOH; *c* 0.12); UV (MeOH) λ_{max} 211 (ϵ 4000) nm; IR (KBr) ν_{max} 3600−3200, 1732, 1638, and 1618 cm^{-1}; CD (MeOH) $\Delta\epsilon_{215}$ +0.07 ($\pi-\pi^*$ of furan) and $\Delta\epsilon_{308}$ 0.08 (n−π^* of C=O); ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m/z* 559.2158 [M + H]$^+$ (calcd for $C_{29}H_{35}O_{11}$, 559.2180).

Khayanoside (3): amorphous powder; $[\alpha]_D$ −23° (MeOH; *c* 0.26); UV (MeOH) λ_{max} 211 (ϵ 4000) nm; IR (KBr) ν_{max} 3600−3200, 1736, 1726, 1674, 1638, and 1618 cm^{-1}; ^{1}H NMR and ^{13}C NMR, see Table 1; HRFABMS *m/z* 671.2681 [M + Na]$^+$ (calcd for $C_{33}H_{44}O_{13}Na$, 671.2679).

Antifeedant Test. The antifeeding potential of the isolated compounds was assessed by presenting them, on leaf disks of a Chinese cabbage, to the third-instar larvae of *Spodoptera littoralis* (Boisduval) and visually comparing the treated and untreated disks eaten by the larvae. The feeding assay terminated after the larvae had eaten approximately 50% of one of the disks.

Antiviral Assay. The inhibitory activity on HIV-1-induced cytopathic effects in MT-4 cells was measured by the method reported previously.[5] Cytotoxicity of the compounds was evaluated in parallel with their anti-HIV-1 activity. Compounds **1**−**3** showed no activity at 100 μg/mL.

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