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Khayanolides, rearranged phragmalin limonoid antifeedants from *Khaya senegalensis*

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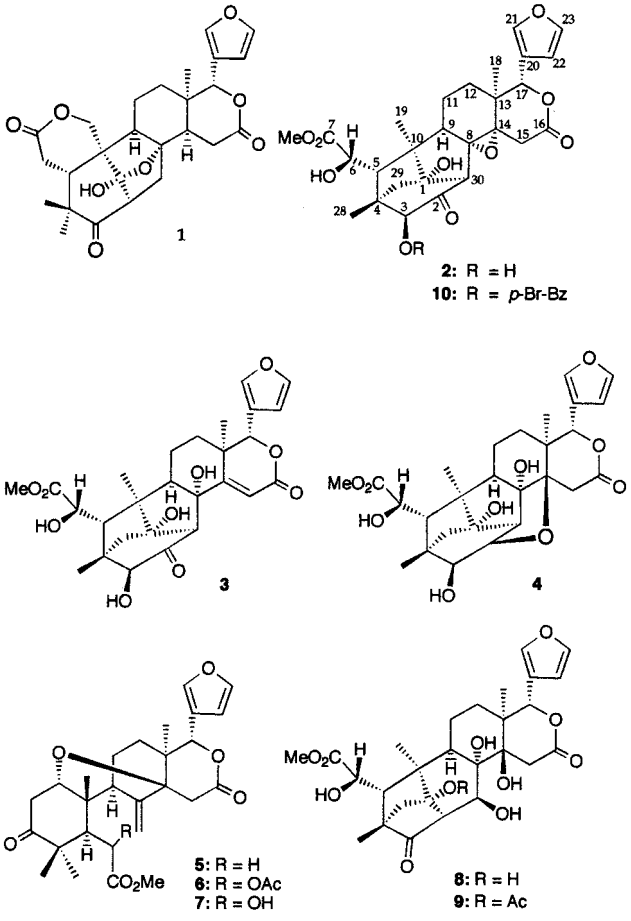
Abstract—Three new rearranged phragmalin-type limonoids, named khayanolides A, B and C, were isolated as insect antifeedant together with four known rings B,D-*seco* compounds, seneganolide, methyl angolensate and its 6-hydroxy and 6-acetoxy derivatives from the ether extract of the stem bark of *Khaya senegalensis*. The structure of new compounds was elucidated by spectroscopic means and the absolute structure of khayanolide A was established by X-ray analysis and CD study. The antifeedant activity of the isolated compounds was also reported.

1. Introduction

Meliaceae plants are attracting considerable interest, because of their significant biological activities. We have reported many limonoid antifeedants from Meliaceae plants of *Trichilia roka*,[1] *Melia azedarach*[2] and *M. toosendan*.[3] *Khaya senegalensis* (Desr.) A. Juss. is a large meliaceous tree native to the sub-Sahara savannah from Senegal to Uganda[4] and one of the most popular traditional medicines in Africa. The decoction of the bark is extensively used as febrifuge which could be associated with its use as an anti-malarial drug.[5] This genus is a main African mahogany closely related to the South American genus *Swietenia*, which is one of the main source of rings B,D-*seco* limonoids such as mexicanolides[6] having a bicyclo[3.3.1]-ring system. Several types of rings B,D-*seco* limonoids containing mexicanolides and their ring A bridged phragmalin limonoids have been also reported from *K. senegalensis*.[7–9]

During our study on limonoid antifeedants from Meliaceae plants, we have found out the ether extract of the stem bark of *K. senegalensis* collected at Alexandria, Egypt, to have potent activity against the Japanese insect pest *Spodoptera littoralis* (Boisduval). Recently, we have reported the isolation and structure of a novel mexicanolide-type limonoid, seneganolide (**1**),[10] from the ether extract. The continuous study of the complex mixture by droplet countercurrent



Keywords: khayanolides A, B and C; rearranged phrogmalin limonoids; seneganolide; methyl angolensate; methyl 6-hydroxy and 6-acetoxyangolensates; X-ray analysis; CD study; *Khaya senegalensis*; Meliaceae.

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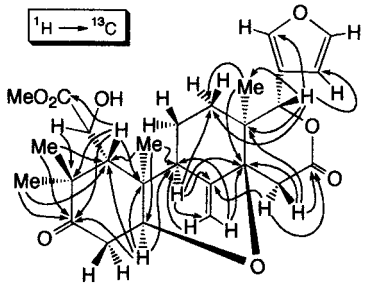


Figure 1. Selected HMBC correlations in **7**.

chromatography (DCCC) followed by HPLC purification, resulted in the isolation of three new antifeeding limonoids having rings B,D-seco structure together with three known rings B,D-seco compounds, methyl angolensate (**5**)[11] and its 6-hydroxy (**7**) and 6-acetoxy (**6**) derivatives.[11,12] In this paper, we report the isolation and structure elucidation of three novel rearranged phragmalin-type limonoids, named khayanolides A (**2**), B (**4**) and C (**3**), and the antifeedant activity of the isolated compounds against the third instar larvae of *S. littoralis* by a conventional leaf disk method.[13]

2. Results and discussion

The ether extract (3.9 g) of the stem bark was divided into eight fractions by droplet countercurrent chromatography (DCCC) with descending mode and each of the fractions was purified by reversed-phase HPLC to give one mexicanolide oxygenated at C-19 (Me), seneganolide (**1**; 5 mg),[10] and three novel rearranged phragmalin-type limonoids, khayanolides A (**2**; 17 mg), C (**3**; 3 mg) and B (**4**; 4.5 mg), together with three known limonoids of methyl angolensate (**5**; 2.5 mg),[11] methyl 6-acetoxyangolensate (**6**; 33 mg) and methyl 6-hydroxyangolensate (**7**; 6 mg).[11,12] In preference to elucidating the structures of the new compounds (**2**–**4**), the ^1H and ^{13}C NMR spectra of the known compounds (**5**–**7**) were re-examined by 2D NMR studies such as ^1H–^1H COSY, ^1H–^{13}C COSY (HMQC) and ^1H–^{13}C long-range COSY (HMBC), and their relative stereochemistry were also elucidated with NOESY spectra. The ^1H–^{13}C HMBC and ^1H–^1H NOE correlations in methyl 6-hydroxyangolensate (**7**) were illustrated in Figs. 1 and 2.

Khayanolide A (**2**) was isolated as colorless prisms from ethyl acetate, mp 237–239°C; $[\alpha]_D$=+62° (MeOH), and it was shown to have the molecular formula $C_{27}H_{32}O_{10}$ (12 unsaturations) by accurate mass measurement (HRFAB-MS: *m/z* 517.2092 $[M+1]^+$; Δ +1.8 mmu). The UV maximum at 211 nm and the IR absorption at 3550–3350, 1735, 1720 (sh), 1618, 1028 and 875 cm^{-1} showed the presence of carbon–carbon double bond and hydroxyl, carbonyl and/or ester groups. The CD spectrum showed the presence of carbonyl group ($\Delta\epsilon$ +0.1, 294 nm; n–π^* of C=O). From the ^1H and ^{13}C NMR data, it was evident that five of the elements of unsaturation were present as double bonds: two carbon–carbon double bonds (as a furan ring) and three CO (as one ketone and two esters). Thus, the molecule is heptacyclic. It was also clear from the NMR data (Table 1) that **2** contained 4×CH_3 (three tertiary, and one methoxy), 4×CH_2, 9×CH (three olefinic) and 10 carbons (one olefinic and one keto and two ester carbonyls) not bonded to hydrogen. The presence of a β-furyl moiety and one methoxycarbonyl group was also apparent from the spectra.

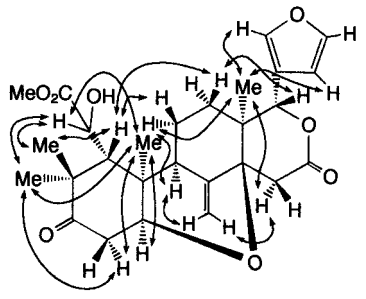


Figure 2. Significant NOE correlations in **7**.

All protons directly bonded to carbon atoms were first assigned by the HMQC spectrum and then the subsequent 2D NMR studies elucidated that **2** was a phragmalin-type compound derived via a mexicanolide. Thus, a triplet proton (*J*=4.5 Hz, H-6) at δ 4.35, attached to a carbon adjacent to an ester carbonyl, was changed to doublet by the addition of D_2O and further coupled with a doublet proton (*J*=4.2 Hz, H-5) at δ 1.77. The presence of this moiety and a characteristic H-17 singlet at δ 5.50 strongly suggested that **2** was a rings B,D-seco limonoid, and the absence of signals due to two tertiary methyls to be at 4β (C-29) and 8β (C-30) in the basic limonoid skeleton and the presence of the proton signals to be assigned to 29-methylene at δ 2.11 and 2.33 (each d, *J*=12.7 Hz), supported that **2** was a phragmalin-type compound such as **8**, isolated from *K. senegalensis* collected in Brazil,[8] rather than being any mexicanolide. Except for **8** and its 1-*O*-acetate (**9**),[9] all of the phragmalins so far isolated have been, however, reported to be present as the 1,8,9- or 8,9,14-orthoacetates.[7] Compound **2** exhibited similar NMR spectra to **8** except for the presence of an epoxide group at δ_C 75.7 s (C-8) and 63.8 s (C-14), but the long range ^1H–^{13}C correlations (Fig. 3), particularly the correlations of the H-5, H-9, H-17 and H-30 with many carbons, provided conclusive proof that **2** was a rearranged phragmalin limonoid.

The observed long-range correlations of the H-5 signal at δ 1.77 with the ^{13}C signals at δ 19.0 q, 57.3 s, 85.2 d and 86.3 s led to their assignments as C-28, C-10, C-3 and C-1, respectively. A methine proton at δ 2.83 (br s, H-30) attached to a carbon at δ 61.6 adjacent to a carbonyl at δ 210.4 (C-2) showed HMBC correlations with the C-1 and C-10 quaternary carbons and another methine carbons at δ 54.5 d and 85.2 d to be assigned to C-9 and C-3, respectively. Further, the 29-methylene protons showed correlations with C-3, C-4, C-30 and 4-Me (C-28). These findings clearly characterized the first molecular fragment, the left-hand tricyclo decane ring system including Me-28 in the molecule, in which the $C_1 \rightarrow C_2$ bond in phragmalin was shifted to C-30, as shown in **2**. The methine proton at δ 1.94 (ddd, *J*=13.4, 5.6 and 1.0 Hz, H-9) showed correlations with the ^{13}C signals at δ 18.1 q (C-19), 31.4 t (C-12), 45.2 d (C-5) and the epoxide carbons at δ 75.7 s (C-8) and 63.8 s (C-14), the latter signal of which was correlated to methylene

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectral data of khayanolides A (**2**), C (**3**) and B (**4**) (measured in $CDCl_3$ containing a small amount of CD_3OD. Chemical shift valuues are in ppm from TMS, and *J* values (in Hz) are presented in parentheses)

No C	**2**		**3**		**4**	
	δ_H, mult.	δ_C, mult.	δ_H, mult.	δ_C, mult	δ_H, mult.	δ_C, mult.
1		86.3 s		87.4 s		84.3 s
2		210.4 s		210.0 s	4.50 dd (9.5, 6.8)	72.2 d
3	3.83 br s	85.2 d	3.56 br s	84.2 d	3.41 d (6.8)	78.5 d
4		43.3 s		42.6 s		42.7 s
5	1.77 d (4.2)	45.2 d	2.43 d (3.4)	44.8 d	3.05 d (7.3)	40.9 d
6	4.35 t (4.5)	72.4 d	4.36 br d (3.4)	72.4 d	4.21 d (7.3)	71.6 d
7		174.8 s		175.3 s		175.4 s
8		75.7 s		87.1 s		87.0 s
9	1.94 ddd (13.4, 5.6, 1.0)	54.5 d	1.94 m	58.9 d	2.09 d (8.1)	56.1 d
10		57.3 s		59.4 s		59.4 s
11α	1.17 m	19.0 t	0.80 ddt (12.2, 5.2, 13.7)	23.7 t	1.86 m	16.5 t
11β	1.04 dq (2.4, 13.4)		1.65 ddt (13.7, 4.9, 5.2)		1.74 m	
12α	1.20 m	31.4 t	1.20 ddd (14.4, 12.2, 5.2)	32.5 t	0.97 m	26.0 t
12β	1.47 ddd (14.6, 3.4, 2.4)		1.76 ddd (14.4, 5.4, 5.2)		1.85 m	
13		37.1 s		37.8 s		37.7 s
14		63.8 s		165.4 s		81.5 s
15α	3.13 d (18.7)	36.4 t	6.14 s	119.8 d	3.14 d (18.8)	32.0 t
15β	2.53 d (18.7)				2.78 d (18.8)	
16		169.8 s		165.8 s		171.4 s
17	5.50 s	76.7 d	5.28 s	80.1 d	5.61 s	81.0 d
18	1.11 s	16.1 q	1.27 s	23.9 q	1.10 s	14.4 q
19	1.33 s	18.1 q	1.32 s	22.2 q	1.21 s	17.7 q
20		120.3 s		120.2 s		120.7 s
21	7.45 br s	141.2 d	7.53 t (0.7)	141.7 d	7.45 br t (0.7)	140.9 d
22	6.42 br dd (1.8, 0.9)	109.9 d	6.50 dd (1.5, 0.7)	110.4 d	6.41 br dd (1.7, 0.7)	110.0 d
23	7.45t (1.8)	143.3 d	7.44 t (1.5)	143.0 d	7.40 t (1.7)	142.6 d
28	1.40 s	19.0 q	1.39 s	20.0 q	1.09 q	19.2 q
$29_{pro\text{-}R}$	2.11 d (12.7)	44.3 t	1.92 d (12.6)	43.9 t	1.88 d (12.2)	44.6 t
$29_{pro\text{-}S}$	2.33 d (12.7)		2.43 d (12.6)		1.37 d (12.2)	
30	2.83 br s	61.6 d	3.24 t (2.1)	66.1 d	2.60 d (9.5)	63.3 d
OMe	3.77 s	52.6 q	3.78 s	52.5 q	3.77 s	52.1 q
6-OH	2.58 br d (5.4)		1.78			

protons at δ 3.13 and 2.53 (each d, *J*=18.7 Hz, H_2-15) attached to a carbon (δ 36.4) adjacent to a lactone carbonyl at δ 169.8 s (C-16). The H-17 signal at δ 5.50 coupling with furan carbons at δ 141.2 d (C-21) and 109.9 d (C-22) was also coupled to the ^{13}C signals at δ 16.1 q (C-18), 31.4 t (C-12) and 63.8 s (C-14). These correlations characterized the second fragment of the molecule, C-8–C-17 of the C and D rings in the limonoid skeleton.

Relative stereochemistry of the tricyclo[4.2.1^{10,30}.1^{1,4}]-decane ring was elucidated by NOE and decoupling studies. The $H_{pro\text{-}R}$ signal of the 29-methylene at δ 2.11 showed NOE correlations (Fig. 4) with signals at δ 3.83 (br s, H-3) and δ 2.83 (br s, H-30) and the other $H_{pro\text{-}S}$ signal at δ 2.33, with the 4-Me (C-28) and 10-Me (C-19) signals, respectively. Irradiation of the H-30 signal coalesced the H-9 signal to double doublet. This W-type long-range coupling between the H-9 and H-30 signals and an NOE correlation observed between H-30 and H-15β (δ 2.53, d, *J*=18.7 Hz) signals clarified the stereochemistry of the tricyclodecane system. A model for the molecule of **2** also accounted for well these observations. In conclusion, we proposed structure **2** for khayanoside A except for the stereochemistry at C-6. This novel rearranged carbon skeleton is the third example in nature[8,9] but their stereochemistry at C-6 had not been established.

Finally, the absolute stereochemistry of **2** including C-6 was determined by X-ray structure determination and CD study. The relative stereochemistry of **2** was first determined by a crystal X-ray analysis (Fig. 5) and then the absolute structure was confirmed by exciton chilarity method,[14] because benzoylation of **2** with *p*-Br-benzoyl chloride gave the 3-*O*-benzoate (**10**) only in a low yield. In the CD spectrum (Fig.

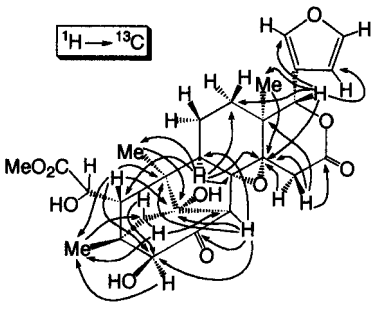


Figure 3. Selected HMBC correlations in **2**.

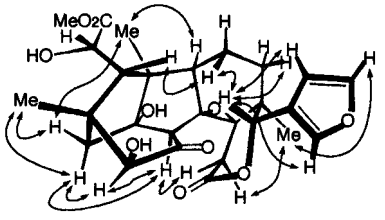


Figure 4. Significant NOE correlations in **2**.

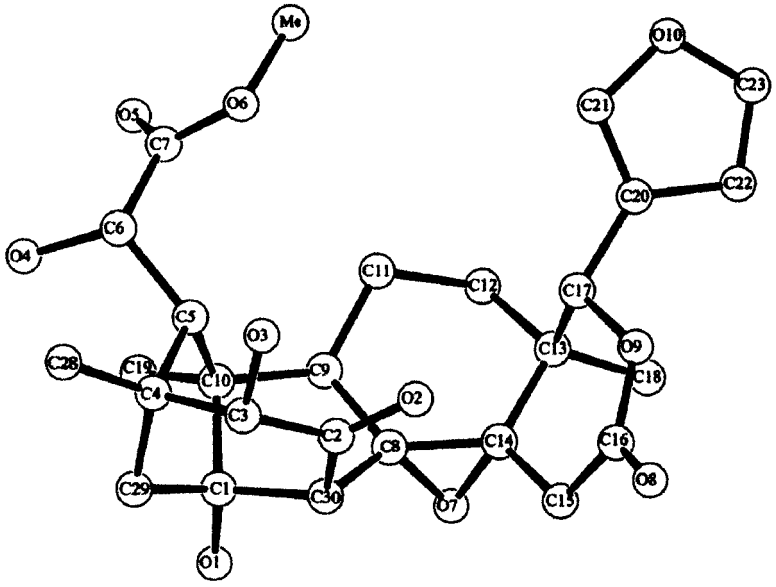


Figure 5. X-Ray crystal structure of **2**.

6) of **10**, the higher counterpart of a positive split benzoate CD was observed at 242 nm ($\Delta\epsilon$ +0.65: $\pi-\pi^*$ of benzoate), which suggested a positive twisting of the benzoate and furan chromophors to reveal the absolute configuration of the benzoyl group and furan moiety in **2**.[15] The interacting negative counterpart of this split CD being the furan chromophore (λ_{max} 206 nm) is masked by the Cotton effect of ester and lactone group.

In this study, the stereochemistry at C-6 of the rings B,C-seco limonoids isolated from *K. senegalensis* was first established as *S* unambiguously, which was particularly of interest in contrast to the *R* configuration in mexicanolides from *Swietenia*,[16,17] because this differentiation seems to be suggestive of the oxidation step of C-6 in the biosynthesis of rings B,D-seco limonoids.

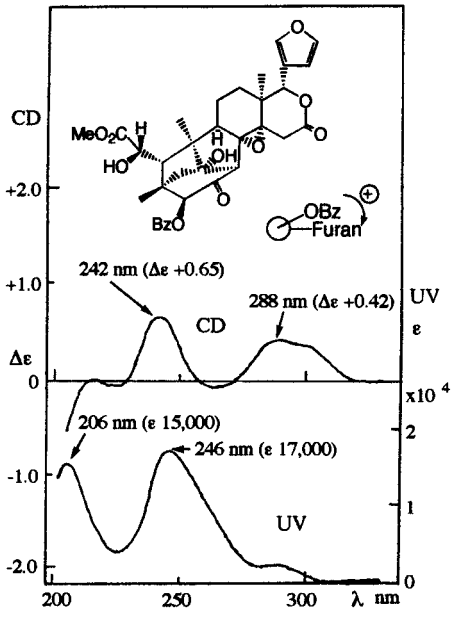


Figure 6. CD spectrum of **10**.

The rearranged phragmalin structure of khayanolide C (**3**), $C_{27}H_{32}O_{10}$; $[\alpha]_D$=+0.2° (*c* 0.38), was readily suggested from the spectral data. Compound **3** having the same molecular formula with khayanolide A (**2**) showed a similar IR spectrum to that of **2**, but in the UV spectrum a strong absorption band was observed at 207 nm (ϵ 12000) to suggest the presence of double bond other than furan ring. The ^{1}H and ^{13}C NMR spectra of **3** (Table 1) were similar to those of **2** except for the presence of an additional trisubstituted double bond [δ_C 165.4 (s) and 119.8 (d) and δ_H 6.14 (s)] instead of the epoxide in **2**. Although the chemical shifts of many signals in **3** were also somewhat different from those of **2**, all of the signals in the spectra were assigned to the structure shown by considering of a conformational change due to the lack of epoxy ring and the formation of C–C double bond. In particular, large down field shifts of the H-5β and H-11β signals from δ 1.77 and 1.04 in **2** to δ 2.43 and 1.65 in **3** were accounted for by an anisotropic effect of the 2-carbonyl group. On the other hand, irradiation of the 30-H signal at δ 3.24 (t, *J*=2.1 Hz) led to sharpen the H-3α and H-9 signals at δ 3.56 (br s) and 1.94 (m). These W-type long-range couplings were well explicable by the proposed structure including the stereochemistry. The HMBC spectrum of **3** also supported the structure well and the NOE correlations (Fig. 7) were observed between the H-30 signal and the

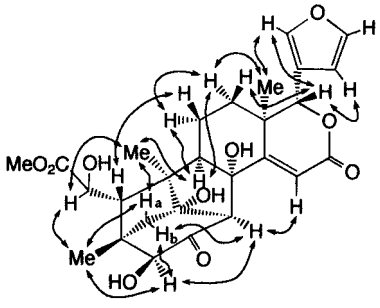


Figure 7. Significant NOE correlations in **3**.

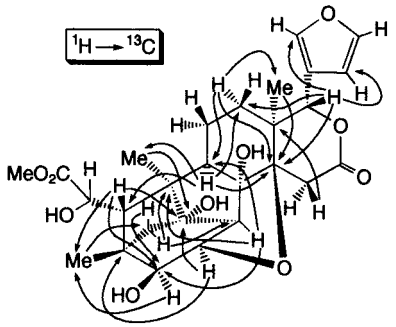
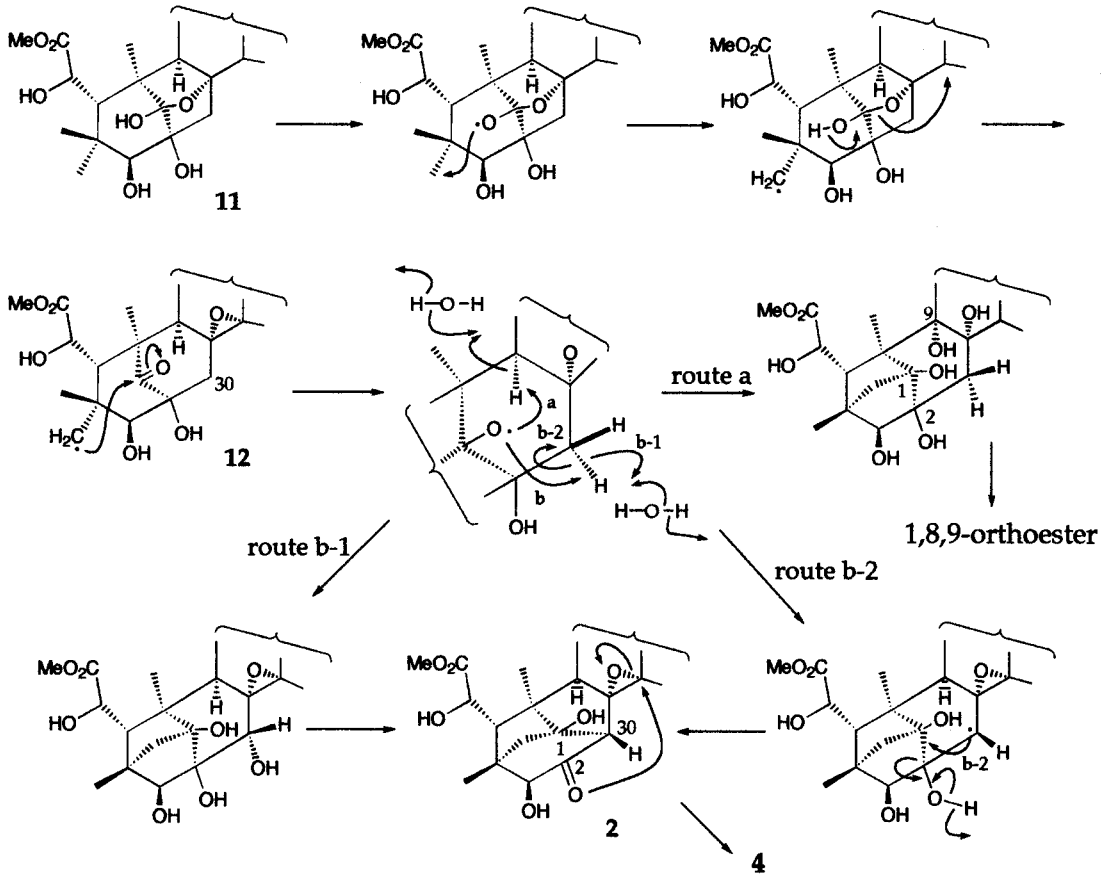


Figure 8. Selected HMBC correlations in **4**.

H-3α and H-15 (δ 6.14, s) signals and the H-5 signal and the H-11β signal to establish the stereochemistry of **3**.

The third compound, khayanolide B (**4**); $C_{27}H_{34}O_{10}$, $[\alpha]_D=-2.6°$, mp>300°C, was isolated as colorless needles from ethyl acetate. Compound **4** had 11 unsaturations different from **2** and **3**, but the IR and UV spectra were similar to those of **2**. The ^{13}C NMR spectrum of **4** showed three tertiary methyl resonances at δ 14.4, 17.7 and 19.2 and the ^1H NMR spectrum showed characteristic 29-methylene resonances at δ 1.37 and 1.88 (each d, *J*=12.2 Hz), confirming **4** to have the same rearranged phragmalin structure as **2** and **3**. The NMR data of **4** were similar to **2**, but differed in the absence of a ketone group at δ 210.4 in **2**. The ^1H and ^{13}C signals were elucidated in detail by using the HMQC and HMBC (Fig. 8) correlations. The presence of a methine proton at δ 4.50 (dd, *J*=9.5 and 6.8 Hz, H-2) coupling with the signals at δ 3.41 (d, *J*=6.8 Hz, H-3) and 2.60 (d, *J*=9.5 Hz, H-30), suggested that the ketone group at C-2 in **2** was changed to an oxymethine group in **4**. In addition to the above observation, the ^{13}C NMR spectrum of **4** showed the presence of three quarternary oxycarbon signals at δ 81.5, 84.3 and 87.0 not forming any epoxy ring different from **2**, which suggested the presence of an another ether linkage. The observed NOE correlations (Fig. 9) showed that **4** had the same relative stereochemistry as **1** and **2** at positions C-3, C-4, C-5, C-6, C-9 and C-30 of the tricyco [4.2.1.1]decane ring system. Another correlations of the H-2 signal with the H-3, $H_{pro\text{-}R}$-29 at δ 1.88 (d, *J*=12.2 Hz) and H-30 signals showing their α-orientation was also observed. In particular, NOE correlations of the H-2 and H-30 signals with the H-15β signal at δ 2.78 (d, *J*=18.8 Hz) strongly suggested the formation of an ether linkage between C-2 at δ 72.2 and C-14 at δ 81.5. The presence of the



Scheme 1. Proposed biogenetic pathway to khayanolides (**2** and **4**) from a mexicanolide (**12**).

2β,14β-ether linkage in **4** was also confirmed by the significant downfield shift for 5-H (**4**: δ 3.05; **2**: δ 1.77; **3**: δ 2.43) due to an anisotropic effect of the ether oxygen. On the other hand, an NOE observation of the H-5 signal with the H-12β signal at δ 1.85 (m) in **4** different from that between the H-5 and H-11β in **2** and **3** is noteworthy in the structure **4**.

The most interesting question about khayanolides (**2**–**4**) is how they are formed from mexicanolides in the plant. A possible pathway leading to the formation of khayanolides can now be proposed by modifying that proposed by Taylor for the biosynthesis of the phragmalin limonoid.[7] Formally, the C-29 methyl group of mexicanolide is oxidized to a radical like **12**, but the precursor of it has been suggested to be a ketal of the *Xyrocarpus* type such as **11**, which yields an oxygen radical (Scheme 1). This can oxidize C-29 methyl to the radical **12**, which may attack the C-1 ketone from the C-1 ketal to give the second oxygen radical. This radical can oxidize 9-methine (route a) or 30-methine (route b) to their carbon radicals, one (from route a) of which produces the 9-OH compound, giving an 1,8,9-orthoacetate. (i) A radical from route b produces the 30-OH compound (route b-1), giving a tricyclo[4.2.$1^{10,30}$.$1^{1,4}$]decane ring system (**2**) by pinacol-type rearrangement, or (ii) the C-30 radical attacks the 1,2-bond (route b-2), giving the same compound **2**, as shown in Scheme 1. In any event, a driving force to produce the rearranged tricyclo decane ring system should be a large ring strain in tricyclo[3.3.$1^{10,2}$.$1^{1,4}$]decane system. This strain can be taken away by the shift of $C_1 \rightarrow C_2$ bond to C-30. Subsequent *trans* attach of the resulting carbonyl oxygen to the 14-epoxide carbon will give the 2β,14β-oxide bridge in **4**.

Antifeedant activity of the isolated compounds was tested by a conventional leaf disk method against the third instar larvae of Japanese insect pest *Spodoptera littoralis* (Boisduval).[13] The most potent was seneganolide (**1**) and khayanolide A (**2**), which were active at 300 ppm, with 50 ppm corresponding to a concentration of ca. 1 μg/leaf-cm^2. The activity is weaker than that of well known limonoid antifeedant azadirachtins from *Melia azadirachta* indica,[18] but comparable to that of the second trichilins[1] and azedarachins[2] from *Trichilia roka* and *M. azedarach*. Khayanolide C (**4**) and methyl angolensates (**5**, **6** and **7**) also showed a moderate activity at 500 ppm, but khayanolide B (**3**) was active at 1000 ppm.

3. Experimental

^1H and ^{13}C NMR spectra were measured at 500 and 125 MHz at 40°C in $CDCl_3$ (compounds **5**–**7**) and $CDCl_3$ containing a small amount of CD_3OD (compounds **2**–**4** and **10**) on a JEOL FX-500 spectrometer. IR (KBr) and UV (MeOH) spectra were recorded on JASCO FT/IR 5300 and Shimadzu UV-210A spectrophotometers. Optical and CD spectra were measured in MeOH at 22° using JASCO DIP-370S and JASCO J-720 spectropolarimeters. HPLC was performed on Waters μBondapak C_{18} column by using 30–60% H_2O–MeOH as solvent.

3.1. Plant material

The stem bark was collected in January 1999 at Alexandria in Egypt.

3.2. Extraction and isolation

After defatting with hexane, the dried stem bark (910 g) was extracted with Et_2O (3 l) to yield 3.9 g of material, which was fractionated by DCCC using CH_2Cl_2–MeOH–H_2O (5:5:3) in descending mode to give 200 fractions. Using TLC, these fractions were rearranged to six limonoid fractions; fr 1 (Fr no. 40: 1.13 g), fr 2 (Fr no. 52–58: 45 mg), fr 3 (Fr no. 59–70: 74 mg), fr 4 (Fr no. 80–94; 21 mg), fr 5 (Fr no. 104–138; 38 mg) and fr 6 (Fr no. 140–174; 12 mg). The first fraction (1.1 g) was purified through HPLC with 55–65% MeOH as the solvent to give **5** (2.5 mg), **6** (33 mg) and **7** (9 mg). The second fraction (45 mg) was purified by HPLC using 45–50% MeOH solvent system to give **1** (5 mg). A similar purification of the fifth fraction using 40–50% MeOH give **2** (17 mg) and **3** (3 mg), and from the sixth fraction, compound **4** (4.5 mg) was also purified with 40–50% MeOH.

3.2.1. Seneganolide A (1). Colorless needles from acetone, mp 276–278°C; HRFABMS *m/z*: 471.2021 $[M+1]^+$, calcd for $C_{26}H_{31}O_8$, 471.2019; $[\alpha]_D=+62°$ (*c* 0.92); IR (KBr) ν_{max} cm^{-1}: 3580–3350, 1730, 1700, 1637 and 875; UV (MeOH) λ_{max} nm (ε): 210 (5000); CD (MeOH): $\Delta\epsilon_{255}$ −0.5, $\Delta\epsilon_{270}$ −0.4 and $\Delta\epsilon_{340}$ −0.2.

3.2.2. Khayanolide A (2). Colorless prisms from AcOEt, mp 237–239°C; HRFABMS *m/z*: 517.2092 $[M+1]^+$, calcd for $C_{27}H_{33}O_{10}$, 517.2074; $[\alpha]_D=+62°$ (*c* 0.92); IR (KBr) ν_{max} cm^{-1}: 3550–3350, 1735, 1720(sh), 1618, 1028 and 875; UV (MeOH) λ_{max} nm (ε): 211 (3000); CD (MeOH): $\Delta\epsilon_{210}$ +0.4 (π–π* of furan), $\Delta\epsilon_{245}$ −0.08 and $\Delta\epsilon_{294}$ +0.1 (π–π* of C=O).

3.2.3. Khayanolide C (3). A white amorphous powder, mp 200–202°C; HRFABMS *m/z*: 517.2079 $[M+1]^+$, calcd for $C_{27}H_{33}O_{10}$, 517.2074; $[\alpha]_D=+0.2°$ (*c* 0.38); IR (KBr) ν_{max} cm^{-1}: 3550–3300, 1730–1690, 1238, 1032 and 875; UV (MeOH) λ_{max} nm (ε): 207 (12000); CD (MeOH): $\Delta\epsilon_{212}$ +1.8, $\Delta\epsilon_{245}$ −4.3 and $\Delta\epsilon_{358}$ −0.7.

3.2.4. Khayanolide B (4). Colorless needles from AcOEt, mp >300°C; HRFABMS *m/z*: 519.2255 $[M+1]^+$, calcd for $C_{27}H_{35}O_{10}$, 519.2231; $[\alpha]_D=-2.6°$ (*c* 0.53); IR (KBr) ν_{max} cm^{-1}: 3580–3460, 1726, 1631, 1051, 1026 and 875; UV (MeOH) λ_{max} nm (ε): 211 (8000); CD (MeOH): $\Delta\epsilon_{252}$ −0.1.

3.2.5. Methyl angolensate (5). A white amorphous powder; $C_{27}H_{34}O_7$; FABMS *m/z*: 471 $[M+1]^+$; $[\alpha]_D=-40°$ (*c* 0.45); IR (KBr) ν_{max} cm^{-1}: 3080, 1745, 1720, 1618 and 875; UV (MeOH) λ_{max} nm (ε): 209 (6000); ^{13}C NMR ($CDCl_3$): δ 13.8q (C-18), 21.5q (C-29), 21.6q (C-19), 23.7t (C-11), 25.9q (C-28), 29.3t (C-12), 32.7t (C-6), 33.8t (C-15), 39.4t (C-2), 41.5s (C-13), 42.9d (C-5), 44.0s (C-10), 48.0s (C-4), 50.0d (C-9), 52.1q (OMe), 77.2d (C-1), 79.6d (C-17), 80.2s (C-14), 109.9d (C-22), 111.5t (C-30), 120.8s (C-20), 140.8d (C-21), 142.6d (C-23), 145.8s (C-8), 170.0s (C-16), 173.9s (C-7), 213.0s (C-3).

3.2.6. Methyl 6-acetoxyangolensate (6). An amorphous powder, mp 208–210°C; HRFABMS *m/z*: 529.2416 $[M+1]^+$, Calcd for $C_{29}H_{37}O_9$, 529.2438; $[\alpha]_D=-75°$ (*c* 0.75); IR (KBr) ν_{max} cm^{-1}: 3075, 1745, 1720, 1618 and 875; UV (MeOH) λ_{max} nm (ϵ): 209 (6000); ^{13}C NMR ($CDCl_3$): δ 13.8q (C-18), 21.1q (Ac), 22.7q (C-19), 23.8q (C-29), 24.0t (C-11), 24.8q (C-28), 28.7t (C-12), 33.7t (C-15), 39.1t (C-2), 41.4s (C-13), 44.5s (C-10), 46.5d (C-5), 48.8s (C-4), 50.8d (C-9), 53.0q (OMe), 72.4d (C-6), 78.1d (C-1), 79.5d (C-17), 80.6s (C-14), 109.9d (C-22), 111.7t (C-30), 120.7s (C-20), 140.8d (C-21), 142.8d (C-23), 145.8s (C-8), 169.9s (C-16), 170.1s (Ac), 170.9s (C-7), 211.2s (C-3).

3.2.7. Methyl 6-hydroxyangolensate (7). An amorphous powder, mp 130–132°C; HRFABMS *m/z*: 487.2357 $[M+1]^+$, calcd for $C_{27}H_{33}O_8$, 487.2332; $[\alpha]_D=-56°$ (*c* 0.20); IR (KBr) ν_{max} cm^{-1}: 3600–3200, 1740–1710, 1618 and 875; UV (MeOH) λ_{max} nm (ϵ): 209 (6000); ^1H NMR ($CDCl_3$): δ 0.88 (3H, s, H_3-18), 1.03 (1H, m, H-12α), 1.06 (3H, s, H_3-28), 1.39 (3H, s, H_3-19), 1.46 (3H, s, H_3-29), 1.57 (1H, tt, *J*=14.7 and 5.3 Hz, H-11α), 1.73 (1H, dt, *J*=13.8 and 4.6 Hz, H-12β), 2.34 (1H, dd, *J*=14.2 and 2.7 Hz, H-2α), 2.34 (1H, dd, *J*= 14.2 and 2.7 Hz, H-9), 2.58 (1H, d, *J*=18.1 Hz, H-15β), 2.75 (1H, s, H-5), 2.90 (1H, d, *J*=18.1 Hz, H-15α), 3.09 (1H, d, *J*=2.0 Hz, OH), 3.10 (1H, dd, *J*=14.2 and 5.6 Hz, H-2β), 3.57 (1H, dd, *J*=5.6 and 2.7 Hz, H-1), 3.84 (3H, s, OMe), 4.42 (1H, d, *J*=2.0 Hz, H-6), 4.91 and 5.19 (each 1H, s, H_2-30), 5.63 (1H, s, H-17), 6.36 (1H, dd, *J*=1.7 and 0.8 Hz, H-22), 7.38 (1H, t, *J*=1.7 Hz, H-23), 7.42 (1H, br s, H-21); ^{13}C NMR ($CDCl_3$): δ 13.8q (C-18), 23.4q (C-29), 23.7 (C-19), 24.1t (C-11), 24.8q (C-28), 28.7t (C-12), 33.7t (C-15), 39.1t (C-2), 41.4s (C-13), 44.7s (C-10), 47.6d (C-5), 48.8 (C-4), 50.8d (C-9), 53.4q (OMe), 72.4d (C-6), 78.3d (C-1), 79.4d (C-17), 80.4s (C-14), 110.0d (C-22), 111.6t (C-30), 120.8s (C-20), 140.8d (C-21), 142.8d (C-23), 146.0s (C-8), 170.0s (C-16), 176.6s (C-7), 212.0s (C-3).

3.2.8. *p*-Bromobenzoylation of khayanolide A (2). Compound **2** (7 mg) was treated with *p*-bromobenzoyl chloride (22 mg) in pyridine (1 ml) at 50°C for 3 days. Purification of the product gave 2.5 mg of the 3-*O*-benzoate (**10**); $C_{34}H_{35}O_{11}Br$; FABMS *m/z*: 699 and 701 $[M+1]^+$; IR (KBr) ν_{max} cm^{-1}: 3600–3200, 1740–1710, 1618 and 875; UV (MeOH) λ_{max} nm (ϵ): 206 (15000) and 246 (17000); CD (MeOH); $\Delta\epsilon_{242}$ +0.65 (π–π^* of benzoate) and $\Delta\epsilon_{288}$ +0.42 (n–π^* of C=O); ^1H NMR ($CDCl_3$, 400 MHz): δ 1.07 (1H, br t, *J*=13 Hz, 11β-H), 1.08 (3H, s, Me-18), 1.17 (1H, br dd, *J*=14.5 and 2 Hz, 12β-H), 1.24 (1H, m, 11α-H), 1.25 (3H, s, Me-19), 1.31 (3H, s, Me-28), 1.50 (1H, br d, *J*=15 Hz, 12α-H), 1.97 (1H, dd, *J*=13.5 and 5.0 Hz, 9-H), 2.01 (1H, br, 1-OH), 2.15 (1H, d, *J*=5.1 Hz, 5-H), 2.30 and 2.47 (each 1H, d, *J*=12.8 Hz, 29-H_2), 2.53 (1H, d, *J*=5.8 Hz, 6-OH), 2.64 and 3.05 (each 1H, d, *J*=18.7 Hz, 15-H_2), 2.85 (1H, br s, 30-H), 3.80 (3H, s, CO_2Me), 4.42 (1H, dd, *J*=5.8 and 5.1 Hz, 6-H), 5.35 (1H, br s, 3-H), 5.46 (1H, s, 17-H), 6.39 (1H, m, 22-H), 7.42 (1H, br s, 21-H), 7.43 (1H, br t, *J*=1.6 Hz, 23-H), 7.63 (2H, dt, *J*=8.6 and 1.7 Hz, 3′- and 5′-H), 7.93 (2H, dt, *J*=8.6 and 1.7 Hz, 2′- and 6′-H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 16.0 (C-18), 18.0 (C-19), 19.0 (C-28), 19.2 (C-11), 31.6 (C-12), 36.2 (C-15), 37.2 (C-13), 42.0 (C-4), 44.9 (C-29), 46.2 (C-5), 52.8 (OMe), 54.8 (C-9), 57.4 (C-10), 62.0 (C-30), 63.8 (C-14), 71.9 (C-6), 75.9(C-8), 85.8 (C-3), 86.0 (C-1), 109.9 (C-22), 120.4 (C-20), 128.4 (C-1′), 128.7 (C-4′), 131.5 (C-2′ and 6′), 131.9 (C-3′ and 5′), 141.1 (C-21), 143.2 (C-23), 164.8 (*C*OBz), 169.3 (C-16), 174.5 (C-7), 201.5 (C-2).

3.3. Crystallographic studies

The X-ray data for **2** were collected on a Rigaku AFC7R four-circle diffractometer, using $\omega/2\theta$ scan mode. All calculations were performed with the crystallographic software package teXsan (Molecular Structure Corporation, 1985, 1992). The structure was solved by direct methods[19] and subsequent Fourier recycling, and refined by full-matrix least-squares refinement against $|F|$, with all hydrogen atoms fixed at the calculated positions except hydroxyl hydrogen. No absorption corrections were applied. The absolute configuration was not determined by the X-ray experiment.

3.4. Crystal data

$C_{27}H_{32}O_{10}$, orthorhombic, space group $P2_12_12_1$, MoK_α radiation, $2\theta_{max}=60°$, a=21.465(6), b=14.413(4), c=8.122(4) Å, U=2509(1) Å^3. D_c=1.367 g cm^{-3}, μ=1.04 cm^{-1}, reflection measured: $+h$, $+k$, $\pm l$, 7077 independent intensities, 5311 observed ($I>2.00\sigma(I)$), T=296 K, reflection/parameter ratio=15.0, weighting scheme=$1/[\sigma^2(F_o)^2+(0.06)^2F_o^2]^{-1}$, R=0.065, R_w=0.082, GOF=1.88, maximum residual electron density 0.39 eÅ^{-3}.

3.5. 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. Ten larvae were placed in a Petri dish with the five treated leaf disks with sample and the five untreated disks as control. The feeding assay terminated after the larvae had eaten approximately 50% of one of the disks. This choice test was done 200, 300, 500 and 1000 ppm concentrations to determine minimum inhibitory concentration for each of the compounds.

3.6. Antifeedant activity of the rings B,D-*seco* limonoids, 1–7

The antifeedant potential; seneganolide (**1**): 300 ppm, khayanolide A (**2**): 300 ppm, khayanolide B (**4**): 1000 ppm, khayanolide C (**3**): 500 ppm, methyl angolensate (**5**): 500 ppm, methyl 6-acetoxyangolensate (**6**): 500 ppm and methyl 6-hydroxy angolensate (**7**): 500 ppm. In this test, 50 ppm corresponds to a concentration of ca. 1 μg/leaf-cm^2.

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