Pergamon

Tetrahedron Letters 39 (1998) 719–722

TETRAHEDRON LETTERS

Khayalactone, a Novel Limonoid from *Khaya grandifoliola*

Marguérite-H.K. Tchuendem,[a] J. Foyere Ayafor,[a,*] Joseph D. Connolly[b] and Olov Sterner[c,*]

[a]Department of Chemistry, University of Dschang, Box 67, Dschang, Cameroon

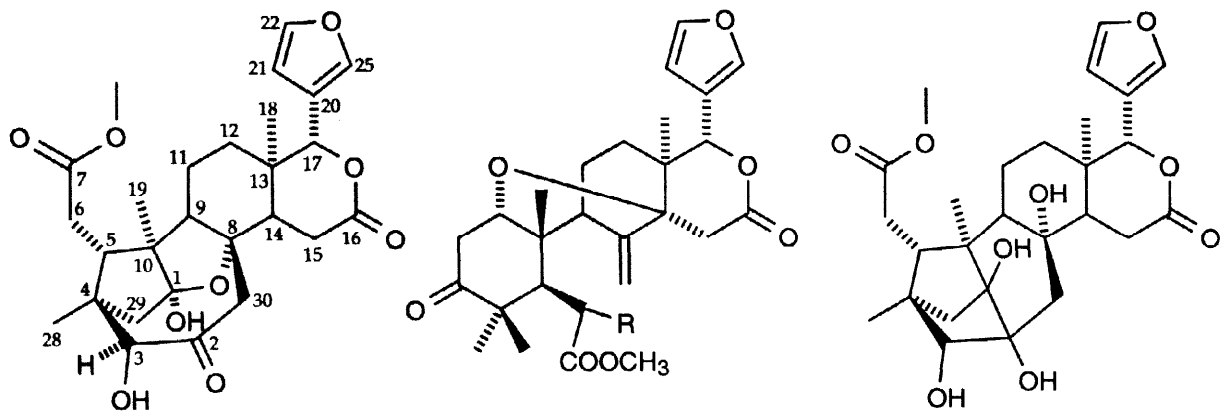
[b]Chemistry Department, The University, Glasgow G128QQ, Scotland

[c]Division of Organic Chemistry 2, University of Lund, P.O.Box 124, S-221 00 Lund, Sweden

Received 20 October 1997; accepted 14 November 1997

Abstract: The isolation and structure elucidation by spectroscopic methods of khayalactone (**1**) from extracts of *Khaya grandifoliola* is described. Khayalactone is a novel hexacyclic A,B,D-*seco* limonoid with a highly oxidised carbon framework.

The limonoids are modified triterpenes with, or derived from a precursor with, a 4,4,8-trimethyl-17-furanylsteroid skeleton found to date only in plants of the order Rutales.[1] Limonoids show a broad spectrum of biological activities. Some like azadirachtin from the neem tree *Azadirachta indica*[2] and harrisonin from *Harrisonia abyssinica*[3] show marked insect antifeedant and growth regulating activities, while the rubrins from *Trichilia rubra* are potent cell adhesion inhibitory agent.[4] In continuation of our investigations on Cameroonian medicinal plants,[5] we have examined the stem bark of *Khaya grandifoliola*. This paper reports the isolation and structure elucidation of a novel A,B,D-*seco* limonoid, khayalactone (**1**) (name proposed by us), obtained together with methyl angolensate (**2a**)[1] and methyl 6-hydroxyangolensate (**2b**).[1]



1 **2** **3**

Figure 1. a: R = H; b: R = OH.

0040-4039/98/$19.00
PII: S0040-4039(97)10644-X

A MeOH-CH_2Cl_2 (1:1) extract of the stem bark of *K. grandifoliola* was subjected to sequential partition extraction with CH_2Cl_2 and EtOAc. Flash chromatography of the combined CH_2Cl_2 and EtOAc extracts followed by MPLC chromatographic purification of the resulting fractions gave khayalactone (**1**).[6] The EI mass spectrum of khayalactone (**1**) showed a small peak at m/z 502, while a major peak (92 % of the base peak) was observed at m/z 459. FAB-MS measurements with and without the addition of sodium acetate confirmed that the molecular weight of **1** is 502, and HREI measurements indicated that the elemental composition is $C_{27}H_{34}O_9$. This is in agreement with the 1D NMR data (given in Table 1), khayalactone (**1**) consequently has an unsaturation index of 11 which with two carbon-carbon double bonds, one keto and two ester functions suggests the presence of six rings. The structure was determined by analysis of data from 2D NMR experiments (COSY, NOESY, HMQC and HMBC), and the correlations observed in the HMBC and NOESY spectra are summarised in Table 1. The furan moiety and its position could be established by the typical chemical shifts as well as the HMBC correlations from 17-H to C-20, C-21 and C-25, as well as from 21-H and 25-H to C-17. That C-17 is part of an lactone is shown by the correlations from 17-H to C-13, C-14 and C-16, and the HMBC correlations from the C-13 methyl protons (18-H_3), 8-H and 9-H together with the COSY correlations observed, establish the upper right part of the structure (as drawn in Figure 1). The remaining tricyclic system is strained and not previously reported in limonoids, but HMBC correlations from the two methyl groups (19-H_3 and 28-H_3) as well as from 6-H_2 and 30-H_2 indicate all carbon-carbon connectivities as well as how the two parts are joined together. According to their ^{13}C chemical shifts, C-1 could be an acetalic or hemiacetalic carbon and C-3 as well as C-8 should be oxygenated. 32 of the 34 protons are visible in the ^1H NMR spectrum recorded in $CDCl_3$-CD_3OD, and the two exchangeable protons appear as broad signals (a singlet and a doublet) in the spectrum recorded in DMSO-d_6. 3-OH couples with 3-H in DMSO-d_6, and also gives HMBC correlations to C-2, C-3 and C-4 which help to establish this part of the structure. Although no HMBC correlations could be observed from the second exchangeable proton in DMSO-d_6, it must be part of a hydroxyl group attached to C-1, which therefore is a hemiacetal, and the remaining oxygen bridge must be between C-1 and C-8. The relative stereostructure is suggested by the NOESY correlations summarised in Table 1, and it is in agreement, in applicable parts, with that reported for other limonoids.

Khayalactone (**1**) represents to our knowledge the only member of a new group of A,B,D-*seco* limonoids with a highly oxidised carbon framework. **1** could arise from a 1,2,3,8-tetrahydroxylated precursor such as **3** by cleavage of the 1,2-diol followed by formation of the hemiketal by addition of the 8α-hydroxyl group to the newly formed 1-carbonyl group. **3** has not been found in nature, but similar more highly oxygenated derivatives such as atomasins A and B have been reported.[7]

EXPERIMENTAL

Plant Material. The trunk bark of *Khaya grandifoliola* C. DC (Meliaceae) was collected in Foumban, Western Province of Cameroon, in January 1995. Plant identification was performed by Mr. Mezili Paul, a retired botanist of the Cameroon National Herbarium, and a voucher specimen (PM098/95) is deposited at the University of Dschang, Cameroon.

Extraction and isolation: The air-dried and powdered plant material (5 kg) was macerated in a mixture of MeOH-CH_2Cl_2 (1:1) for 24 h. Removal of the solvent from the filtrate under vacuum in a rotatory evaporator

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data, and HMBC and NOESY (in $CDCl_3$-CD_3OD 1:1) correlations for khayalactone (**1**) in $CDCl_3$-CD_3OD 1:1 and DMSO-d_6. The chemical shifts are given in ppm relative to the solvent signals (see Experimental), and the coupling constants (*J*) in Hz.

	$CDCl_3$-CD_3OD 1:1		DMSO-d_6			
Pos.	δH; mult.; *J*	δC; mult.	δH; mult.; *J*	δC; mult.	HMBC	NOESY
1	-	115.7; s	-	116.4; s		
2	-	203.9; s	-	203.4; s		
3	3.47; s	84.2; d	3.30; m	84.4; d	2,4,5,28	28,29β
4	-	43.9; s	-	44.3; s		
5	3.63; dd; 6.2, 9.4	36.1;	3.48; dd; 4.4, 11.0	36.5; s	1,3,4,6,7,10,19	6a,6b,11β,30β
6a	2.48; dd; 9.4, 16.2	34.1; t	2.46; dd; 11.0, 15.6	34.9; t	4,5,7,10	5,19,28
6b	2.43; dd; 6.2, 16.2	-	2.25; dd; 4.4, 15.6	-	4,5,7,10	5,19,28
7	-	174.1; s	-	173.8; s		
8	-	82.0; s	-	81.9; s		
9	2.12; m	52.8; d	2.07; dd; 7.9, 11.7	52.6; d	5,8,10,11,14,19	14,18,19
10	-	54.6; s	-	54.8; s		
11α	1.77; m	-	1.59; m	-	8,9,12,13	12α
11β	2.08; m	16.4; t	1.95; m	16.8; t	8,9,12,13	5
12α	1.22; ddd; 1.6, 8.4, 14.4	-	0.99; m	-	9,11,13,17,18	11α
12β	1.46; ddd; 9, 9, 14.4	29.0; t	1.36; ddd; 9, 9, 14.3	28.9; t	9,11,13,17,18	17,30β,30α
13	-	38.3; s	-	38.6; s		
14	1.91; dd; 6.1, 11.8	53.2; d	1.78; dd; 5.8, 12.7	52.9; d	8,9,13,15,16,17,18	9,15α,18
15α	2.62; dd; 6.1, 15.8	-	2.23; dd; 5.8, 15.1	-	8,13,14,16,17	14
15β	3.05; dd; 11.8, 15.8	28.5; t	3.09; dd; 12.7, 15.1	29.7; t	8,13,14,16,17	17,30α
16	-	173.6; s	-	172.6; s		
17	5.13; s	79.4; d	5.28; s	78.8; d	13,14,16,18,20,21,25	12β,15β,30α,21,25
18	1.05; s	22.9; q	0.94; s	24.0; q	12,13,14,17	9,14
19	0.96; s	17.7; q	0.83; s	18.8; q	1,5,9,10	6a,6b,9
20	-	119.6; s	-	120.6; s		
21	6.41; dd; 0.8, 1.7	109.2; d	6.47; m	110.5; d	17,20,22,25	17,22
22	7.44; dd; 1.7, 1.7	142.1; d	7.66; dd; 1.6, 1.6	143.1; d	20,21,25	21
25	7.47; m	140.3; d	7.63; m	141.1; d	20,21,22	17
28	1.08; s	23.4; q	0.98; s	24.8; q	3,4,5,29	3,6a,6b,29α
29α	1.83; d; 14.2	-	1.73; s	-	1,3,4,5,10,28	28
29β	1.94; d; 14.2	45.6; t	1.73; s	46.3; t	1,3,4,5,10,28	3
30α	2.36; d; 12.8	-	2.34; d; 12.7	-	2,3,8,14	12β,15β,17
30β	3.70; d; 12.8	37.0; t	3.42; d; 12.7	37.3; t	2,3,8,14	12β,5
7-OCH_3	3.69; s	50.7; q	3.59; s	51.4; q	7	19,28
1-OH	-	-	5.78; brs	-		
3-OH	-	-	6.02; d; 4.0	-	2,3,4	

provided an organic extract (335 g). This extract was suspended in 80 % aqueous MeOH (3 l) and extracted three times with 500 ml of CH_2Cl_2 to afford a low polarity CH_2Cl_2 fraction (102 g). This fraction was subjected to vacuum liquid chromatography on SiO_2 (200-400 mesh) using hexane-EtOAc mixtures as eluent. 500 ml fractions were collected and grouped on the basis of their TLC profiles. The fractions eluted with hexane-EtOAc 9:1 (19 g) were further purified by repeated column chromatography on SiO_2 (70-230 mesh) and on Sephadex LH-20 (hexane-CH_2Cl_2 3:2) to yield β-sitosterol (3 g), methyl angolensate (**2a**) (4 g) and 6-hydroxy methyl angolensate (**2b**) (120 mg). The combined fractions eluted with pure EtOAc revealed, with the Erhlich reagent as detector on TLC, the presence of at least two limonoids. Further purification of this fraction (16 g) by MPLC using the Baeckström Separo Column (i.d. 15 mm) with a continous gradient of EtOAc-MeOH afforded crude khayalactone (**1**) (220 mg) and catechin (4 g). Final purification by LH-20 gel permeation chromatography with CH_2Cl_2 - MeOH 1:1 as eluent gave pure **1** (182 mg) as colourless needles.

Spectroscopy: ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temperature with a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with shielded gradient coil. The spectra were recorded in $CDCl_3$-CD_3OD 1:1 and DMSO-d_6, and the solvent signals (δH 3.31 (CHD_2OD) and δC 77.0 ($CDCl_3$) in $CDCl_3$-CD_3OD 1:1; δH 2.50 and δC 39.5 in DMSO-d_6) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (*J*) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH}$=145 Hz and $^nJ_{CH}$=10 Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (rev. 941001). Mass spectra were recorded with a Jeol SX102 spectrometer, while the UV and the IR spectra were recorded with a Varian Cary 2290 and a Perkin Elmer 298 spectrometer. The melting point (uncorrected) were determined with a Reichert microscope, and the optical rotation were measured with a Perkin-Elmer 141 polarimeter at 22 °C.

Acknowledgements: Financial support from the International Programs in the Chemical Sciences (IPICS), Uppsala, Sweden and the Swedish Science Research Council is gratefully acknowledged.

REFERENCES AND NOTES

1. Taylor, D.A.H in *Advances in Medicinal* Phytochemistry; Barton, Sir D.; Ollis W.D., Eds.; John Libbey: London, 1986; p. 179.
2. Champagne, D.E.; Koul, O.; Isman, M.B.; Scudder, G.G.E; Towers, G.H.N. *Phytochemistry* **1992**, *31*, 377-394.
3. Rajab, M.S.; Rugutt, J.K.; Fronczek, F.R.; Fischer, N.H. *J. Nat. Prod.* **1997**, *60*, 822-825.
4. Musza, L.L.; Killar, L.M.; Speight, P.; McElhiney, S.; Barrow, C.J.; Gillum, A.M.; Cooper, R. *Tetrahedron* **1994**, *50*, 11369-11378.
5. Tane, P.; Tsopmo, A.; Ngnokam, D.; Ayafor, J.F.; Sterner, O. *Tetrahedron* **1996**, *52*, 14989-14994.
6. Khayalactone (**1**) was obtained as colourless crystalline needles, mp 241-243 °C. $[\alpha]_D^{22}$ = +8 ° (*c* 0.4, $CHCl_3$-MeOH 1:1). MS, *m/z* (% rel. int.): 502.2209 (M^+, 7, $C_{27}H_{34}O_9$ requires 502.2203), 485 (5), 459 (92), 441 (25), 430 (14), 409 (11), 182 (100), 95 (38). UV (MeOH) λ_{max} (ε): No maxima above 210 nm. IR (KBr): 3400, 2940, 1720, 1700, 1460, 1430, 1380, 1300,1270, 1230, 1160, 1020, and 870 cm^{-1}. See Table 1 for ^1H and ^{13}C NMR data.
7. Tschouankeu, T.A.; Tsoamo, E.; Connolly, J.D.; Rycroft, D.S. *Phytochemistry* **1989**, *28*, 2855-2860.