IDENTIFICATION OF TERPENOIDS FROM *KHAYA SENEGALENSIS*

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ABSTRACT

A certain bioactive compounds terpenoids was isolated from ethanol extract of the bark of *Khaya senegalensis* using NMR The isolation yielded three new triterpenoids of the mexicanolide type. These compounds were identified as 2-hydroxymexicanolide, 6-deoxydestigloylswietenine and 2, 6-dihydroxy-3-mexicanolide.

Keywords: *Khaya senegalensis*, terpenoids, NMR, ethanol extracts

INTRODUCTION

Khaya senegalensis (Desr.) A. Juss. is a large meliaceous tree native to the sub-Sahara savannah from Senegal to Uganda 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 antimalarial drug. 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 having a bicyclo[3.3.1]-ring system(1, 2). Several types of rings B,D-seco limonoids containing mexicanolides and their ring A bridged phragmalin limonoids have been also reported from *Khaya senegalensis* (3). These phytoconstituents comprises alkaloids, flavonoids, phenols, tannins, saponins and sterols. The phytoconstituents of *Khaya senegalensis* were analysed from various parts of the plant like the leaf root and shoot. The plant finds application in the treatment of diarrhoea and skin diseases. It is used in the treatment of catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation skin-ulcers and wounds. It is also used in the treatment of burns. It is used to calm cough and to treat laryngitis and Treacheries(1).

Terpenoids a large and diverse class of naturally occurring organic chemicals similar to terpenes, derived from five-carbon isoprene units assembled and modified in thousands of ways. Most are multicyclic structures that differ from one another not only in functional groups but also in their basic carbon skeletons. These lipids can be found in all classes of living things, and are the largest group of natural products (4, 5).

Plant terpenoids are used extensively for their aromatic qualities. They play a role in traditional herbal remedies and are under investigation for antibacterial, antineoplastic, and other pharmaceutical functions. Terpenoids contribute to the scent of eucalyptus, the flavours of cinnamon, cloves, and ginger, the yellow colour in sunflowers, and the red colour in tomatoes. Well-known terpenoids include citral, menthol, camphor, salvinorin A in the plant *Salvia divinorum*, the cannabinoids found in cannabis, ginkgolide and bilobalide found in *Ginkgo biloba*, and thecurcuminoids found in turmeric and mustard seed (6).

METHODOLOGY

Plant material

The bark samples of *Khaya senegalensis* were collected in January, 2014 from the Botanical garden of the Department of Biology, Umaru Musa Yar' adua University, Katsina, Nigeria and the species were identified at the herbarium unit of the same department. The herbarium voucher specimen was deposited. The root samples were immediately washed and shade-dried for four weeks to constant weights. The dried samples were pounded to fine powder using blender, and then stored in air-tight containers and then taken to Abertay University, Dundee, United Kingdom for extraction and the subsequent analysis.

Extraction

The plant material (769 g) was extracted with ethanol and the extract concentrated. The residue (6 g) was fractionated by VLC using petrol, petrol-CH_2Cl_2 (1:1), CH_2Cl_2, CH_2Cl_2-EtOAc (1:1), EtOAc, and MeOH as eluents. The fractions eluted with CH_2Cl_2-EtOAc (1:1) and EtOAc showed the highest antiparasitic activity and were further fractionated by repeated MPLC using petrol-EtOAc-MeOH (60:40:1) and CH_2Cl_2-EtOAc-HOAc (80:20:1) as eluents to give the three terpertanoids 1, 2 and 3.

Experimental work

The NMR spectra were recorded on a Varian Gemini 300, the mass spectra on a JEOL AX505W, and the CD spectra on a circular dichroic spectrometer built through a grant from the Danish Natural Science Research Council. Vacuum liquid chromatography (VLC) was performed on Silica-gel HF_{254} (10–40 μm), and medium pressure chromatography (MPLC) was performed on Silica-gel 60 (0.04–0.063 mm, Merck 1.09385) using a Bæckström Separo column and a FMI LAB pump model QD. TLC was performed on Silica-gel 60 (DC-Aluminium sheets, Merck 1.05549) and the spots visualized after spraying with a 5% EtOH solution of 4-dimethylaminobenzaldehyde by exposure of the sheet to HCl vapours.

RESULTS

2-hydroxymexicanolide

(1, 35 mg) was obtained as a colourless amorphous solid, $[\alpha]_D^{25}$ −80° (MeOH, *c* 1.105). CD $\Delta\epsilon_{286}$−3.7 ($CHCl_3$, *c* 0.14). HR-FABMS$^+$ found 545.2343, calculated for $C_{29}H_{36}O_8$ 545.2387. ^{1}H NMR and ^{13}C NMR (see Table 1).

6-deoxydestigloylswietenine

(2, 27 mg) was obtained as a colourless oil, $[\alpha]^{25}D$ −57° (MeOH, *c* 1.35). CD $\Delta\epsilon_{298}$−6.9 ($CHCl_3$, *c* 0.21). HR-FABMS$^+$ found 513.2452, calculated for $C_{29}H_{36}O_8$ 513.2488. ^{1}H NMR and ^{13}C NMR (see Table 1).

2, 6-dihydroxy-3-mexicanolide

(3, 5 mg) was obtained as a colourless amorphous solid, $[\alpha]^{25}D$ −12° (MeOH, *c* 1.105). HR-FABMS$^+$ found 529.2466, calculated for $C_{29}H_{36}O_8$ 529.2438. ^{1}H NMR and ^{13}C NMR (see Table).

Table 1. ^{1}H NMR and ^{13}C NMR data for compounds 1, 2, and 3.

Position.	1		2		3	
	δ (^{1}H)	δ (^{13}C)	δ (^{1}H)	δ (^{13}C)	δ (^{1}H)	δ (^{13}C)
1		217.2		218.1		n.o.
2		77.8	3.15 *m*	48.0	3.1*	49.4
3	4.90 *s*	86.9	4.95 *d* (10.5)	78.4	4.83 *d* (10)	80.2
4		39.3		38.3		38.4
5	3.11 *s*	45.1	3.22 *m*	40.8	3.30 *s*	46.4
6	4.53 *s*	73.1	2.38 *m*	33.4	4.58 *s*	73.5
7		175.2		174.3		n.o.
8		126.5		127.8	3.1 *m*	34.8
9	2.12*	53.2*	1.8*	52.2	1.8*	44.6
10		52.4		53.0		51.3
11 *α*	1.8*	18.8	1.7*	18.8	1.7*	18.6
B			1.8*		1.9*	
12 *α*	1.2*	29.6	1.0*	29.1	1.5*	26.7
B	1.8*		1.8*		1.8*	
13		38.2		38.1		38.2
14		132.7		131.8		
15 *α*	3.48 *dt* (21, 2.1)	33.6	3.48 *dt* (20, 2.5)	33.5	5.78 d (2.5)	113.0
B	3.78 *dm* (21)		3.80 *dm* (20)			
16		169.4		170.0		170.3

17	5.56 *s*	80.8	5.70 *s*	80.7	5.12 s	81.2
18	1.04 *s*	18.3	1.08 *s*	17.9	1.01 s	18.4
19	1.51 *s*	17.6	1.16 *s*	16.8	1.38 s	17.1
20		120.6		120.6		119.9
21	7.48 *d* 1.8	141.1	7.56 *dd*	142.9	7.48 d (1.8)	143.1
22	6.42 *d* (1.8)	109.7	6.48 *d* (1.8)	109.9	6.42 d (1.8)	109.9
23	7.43 t (1.8)	143.2	7.42 t (1.8)	141.8	7.43 t (1.8)	141.2
28	1.03 *s*	20.7	0.81 *s*	20.5	1.11 *s*	23.1
29	0.78 *s*	22.6	0.72 *s*	23.2	0.90 *s*	20.7
30 *α*	1.75*	44.6	2.05 *dm* 15	33.4	1.65*	35.4
B	3.21 *d* (14.5)		2.81 *dd* (15, 2.5)		2.25 *m*	
OMe	3.72 *s*	53.3	3.85 *s*	53.3	3.91 *s*	53.3
Ac		169.9		170.4		
CH_3	2.19 *s*	21.2	2.18 *s*	21.3	2.17 *s*	23.1

However, in the H NMR spectra coupling constants are given in Hz in parentheses. * Severe overlapping of signals prevents a detailed interpretation of the coupling pattern. n.o. Not observed.

DISCUSSION

The vaccum liquid chromatography of the chloroform extract over silica gel resulted in a complex mixture of terpenoids as visualized on TLC by Ehrlich's reagent. Repeated medium pressure column chromatography on silica gel yielded three terpenoids in pure state (7). The ^{1}H NMR and ^{13}C NMR spectra (Table 1) exhibited signals consistent with tetranortriterpenoids of the mexicanolide type. Unlike the other two isolated terpenoids, the signal originating in the H-3 proton in compound 1 appeared as a singlet at δ 4.90 in the ^{1}H NMR spectrum which is indicative of the presence of a hydroxyl group at C-2. This assignment was further confirmed by ^{13}C NMR spectrum (Table 1), whereas a NOESY experiment provided evidence about the stereochemistry of 1. Such an oxygenation pattern has already been reported among this series of terpenoids (6-8). However, a ^{13}C-^{1}H chemical shift correlation necessitates the reversal of previously published assignments of signals C-9, C-2, and C-5 as shown in Table 1. This observation is consistent with published data for structurally related compounds (1, 9) . The ^{13}C NMR spectral assignments of 3 were in complete agreement with published data for methyl 3*β*-acetoxy-6-hydroxy-1-oxomeliac-14-enoate 3. To date no report is available on the relative configuration of the stereogenic centre C-8 which is absent in most terpenoids (7). The large allylic coupling (^{4}J=2.2 Hz) between H-

15 and H-8 indicates that H-8 is*β*-disposed. The stereochemistry was further substantiated by the observed NOESY interaction between H-8 and H-5. CD spectroscopy revealed that 1 and 3 have a negative Cotton effect at 290 nm as is generally found in terpenoids (4, 5, 10).

CONCLUSION

Best on the basis of these bioactive compounds found from this plant, it is recommended that the juicy form of the bark of *Khaya senegalensis* could be given the patients with diarrhoea and skin diseases and possibly catarrh, epilepsy, insanity, hysteria, rheumatic pains, hemorrhoids, painful menstruation and ulcers.

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