Salacetal, an Oleanane-type Triterpene from *Salacia longipes var. camerunensis*

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Phytochemical investigation of the roots of *Salacia longipes var. camerunensis* led to the isolation of a new triterpenoid, salacetal (**1**), together with the known compounds mangiferin, 2-hydroxy-3-oxo-D:A-friedooleanan-29-oic acid, β-sitosterol, and stigmasterol. The structure of the new compound as well as those of the known compounds were established by means of spectroscopic methods and by comparison with reported data.

Key words: Salacia longipes var. camerunensis, Celastraceae, Oleanane-type Triterpene, Salacetal

Introduction

Salacia longipes var. camerunensis is a liana largely distributed in Africa and commonly in Cameroon and East of Gabon [1]. Different species of the *Salacia* genus have been used over the world in the treatment of several ailments. The roots of *Salacia* species are extensively consumed in Japan, the United States and other countries as food supplement for the prevention of diabetes and obesity [2]. *S. reticulata*, *S. oblonga* and *S. chinensis* have long been used in India, Sri Lanka and China in the treatment of rheumatism and skin diseases [3]. In Tanzania, the roots of *S. madagascariensis* are used in folk medicine for the treatment of fever, malaria and menorrhagia [4]. Previous phytochemical investigation of plants of the genus *Salacia* resulted in the isolation of quinonemethides [3], sesquiterpenes [4, 5], stilbenes [6], and triterpenes [5]. To the best of our knowledge, no phytochemical or pharmacological study has been reported on the species *S. longipes var. camerunensis*. In a continuing search for biologically active compounds from Cameroonian medicinal plants, we have investigated the dichloromethane/methanol extract of the roots of *S. longipes var. camerunensis*. We report herein on the isolation and the structure elucidation of a new oleanane-type triterpene, salacetal (**1**), along with four known compounds.

Results and Discussion

The dichloromethane/methanol extract of the roots of *Salacia longipes var. camerunensis* was fractionated and purified on a silica gel column to afford five

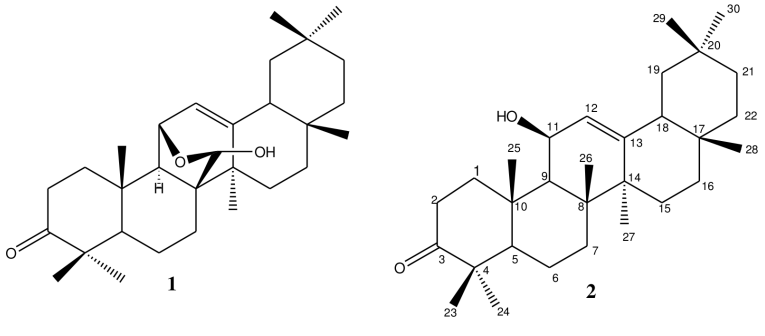


Fig. 1. Chemical structures of compounds **1** and **2**.

Table 1. ^{1}H (300 MHz) and ^{13}C (125 MHz) NMR data in $CDCl_3$ of **1**, and ^{13}C NMR data of **2** [8].

Attribution	**1**		**2**
	^{13}C	^{1}H	^{13}C
1	34.8	2.56, m	40.3
2	33.0	1.50 (m), 2.62 (m)	34.2
3	217.0	–	217.8
4	47.2	–	47.7
5	48.1	2.02 (m)	55.3
6	19.7	1.64 (m)	19.7
7	30.4	1.42 (m)	32.6
8	46.6	–	43.2
9	53.7	1.96 (d, J = 4.5 Hz)	48.5
10	37.8	–	37.5
11	72.1	4.45 (dd, J = 4.5; 4.2 Hz)	82.0
12	120.5	5.46 (d, J = 4.2 Hz)	121.2
13	152.8	–	152.8
14	41.7	–	42.3
15	26.7	0.86 (m), 2.04 (td, J = 13.4, 4.6 Hz)	27.9
16	26.6	1.06 (m), 1.84 (td, J =13.4, 4.6 Hz)	26.6
17	32.8	–	33.1
18	48.0	2.02 (m)	47.0
19	46.1	0.96 (m), 1.58 (dm, J = 13.7 Hz)	46.7
20	31.1	–	31.1
21	34.6	1.10 (m), 1.32 (m)	34.6
22	36.9	1.22 (m), 1.42 (m)	36.9
23	27.9	1.12 (s)	26.9
24	19.4	1.10 (s)	21.5
25	19.1	1.20 (s)	18.0
26	99.1	5.80 (d, J = 4.2 Hz)	16.6
27	23.6	1.08 (s)	24.7
28	28.7	0.83 (s)	28.5
29	33.3	0.86 (s)	33.2
30	23.7	0.87 (s)	23.6
OH	–	3.07 (d, J = 4.2 Hz)	–

compounds including salacetal (**1**), mangiferin [21], 2-hydroxy-3-oxo-D:A-friedooleanan-29-oic acid [22], β-sitosterol [23], and stigmasterol [24].

Compound **1** (Fig. 1) was obtained as a colorless powder. It gave a positive response with the Liebermann-Buchard test for triterpenes. Its molecular formula, $C_{30}H_{46}O_3$, with eight double bond equivalents, was determined by HR-ESIMS, which showed the *quasi*-molecular ion peak $[M+Na]^+$ at m/z = 477.3342 (calcd. m/z = 477.3339 for $C_{30}H_{46}O_3Na$). The ^{1}H NMR spectrum (Table 1) revealed the presence of singlet signals due to seven angular methyls (δ_H = 0.83, 0.86, 0.87, 1.08, 1.10, 1.12 and 1.20), a doublet of one methine proton (δ_H = 1.96, J = 4.5 Hz), and a double doublet due to an oxymethine proton (δ_H = 4.45, J = 4.5 and 4.2 Hz). The spectrum also exhibited three doublets due to an olefinic proton (δ_H = 5.46, J = 4.2 Hz), a dioxymethine proton (δ_H = 5.80, J = 4.2 Hz) and a hydroxy group (δ_H = 3.07, J = 4.2 Hz). The ^{13}C NMR (Table 1) and APT spectra of **1** displayed 30 carbon signals, which account for one ketone carbonyl (δ_C = 217.0), one double bond (δ_C = 152.8 and 120.5), one dioxymethine (δ_C = 99.1), and one oxymethine (δ_C = 72.1). In addition, three methines, nine methylenes, seven methyls and six quaternary carbons were observed. Based on these NMR data, compound **1** was assumed to be an olean-12-ene-type triterpenoid with one hydroxy group and one ketone carbonyl. In fact, signals at δ_C = 120.5 and 152.8 are characteristic of an olean-12-ene triterpenoid skeleton [7].

The NMR data of **1** were similar to those of 3-oxo-11β-hydroxyolean-12-ene (**2**) [8]. The differences were the disappearance of the 26-methyl and the 11-hydroxy groups in compound **1** and the appearance of a dioxymethine moiety and an additional double bond equivalent. The additional double bond equivalent in compound **1** may be derived from the formation of an additional ring between the 11-hydroxy and the 26-CH_3 groups (δ_C = 16.2) of compound **2**. In fact, couplings were observed in the ^{1}H-^{1}H COSY spectrum between the oxymethine (δ_H = 4.45, dd, J = 4.5 and 4.2 Hz) and the olefinic proton (δ_H = 5.46, d, J = 4.2 Hz) and the methine proton at δ_H = 1.96 (1H, d, J = 4.5 Hz). A further diagnostic coupling was seen between the dioxymethine (δ_H = 5.80, d, J = 4.2 Hz) and the hydroxy group (δ_H = 3.07, d, 4.2 Hz). In the HMBC spectrum (Fig. 2) the dioxymethine signal showed correlations with C-11 (δ_C = 72.1, oxymethine) and C-8 (δ_C = 47.2), confirming the existence of an additional ring in **1** (formed between the 11β-hydroxy and 26-methyl groups present in **2**). Additional correlations observed between the oxymethine proton H-11 and C-13 (δ_C = 152.8), C-12 (δ_C = 120.5), C-9 (δ_C = 53.7)

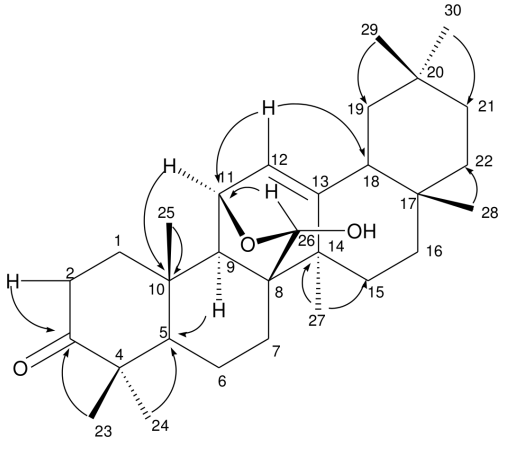
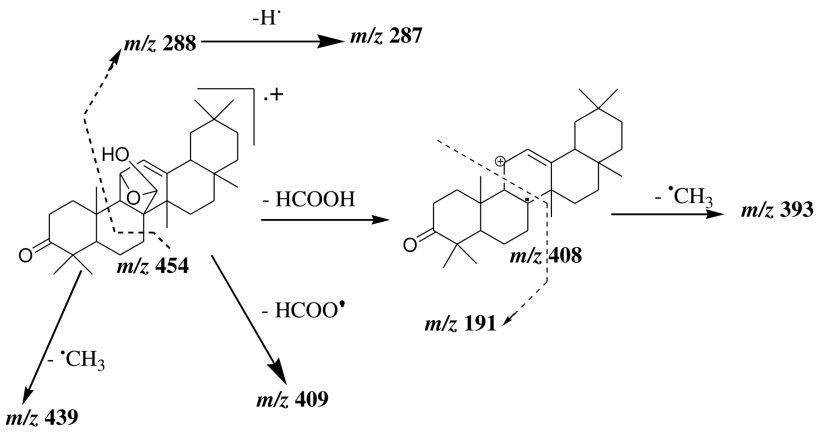


Fig. 2. Selected HMBC correlations of compound **1**.



Scheme 1. Characteristic fragment peaks in the EI-MS spectrum of compound **1**.

and C-10 (δ_C = 37.8) confirmed the position of the double bond. Cross peaks were also observed between Me-23 (δ_H = 1.10) and C-3 (δ_C = 217.0), indicating the location of the carbonyl group at C-3. This was further confirmed by a characteristic fragment ion peak at m/z = 191 (Scheme 1) obtained by a retro Diels-Alder fragmentation in the EIMS spectrum of **1**. In addition, the base peak observed at m/z = 408 $[M–HCOOH]^+$ due to loss of formic acid confirmed the presence of a hemi-acetal group in compound **1**.

Studies relative to the orientation of the oxygenated group at C-11 in several 11-hydroxy and -methoxy 12-oleane- and ursane-type triterpenes have been reported from different plant families (Labiatae [9 – 13], Burseraceae [14, 8] and Celastraceae [15 – 17]). In most cases, the hydroxy group was in the less hindered equatorial 11α-position, based on the large axial-axial $J_{9,11}$ values (8 – 11 Hz) of the coupling constant between H-9 and H-11. Two 11β-hydroxy-oleane-type triterpenes, 3-oxo-11β-hydroxy-olean-12-ene (**2**) [8] and 11β,21β-dihydroxy-olean-12-en-3-one [18], have also been reported. With respect to the B and C rings with the *trans* junction and chair conformation in oleanane triterpenes [19 – 20], the value of the coupling constant ($J_{11,9}$ = 4.5 Hz) observed between H-11 and H-9 clearly indicated that the oxygen at C-11 is in the axial position (β-oriented) in compound **1** (Fig. 3). Thus, compound **1** is 11β,26-epoxy-26-hydroxy-olean-12-en-3-one, named salacetal.

The isolated compounds were tested for their antibacterial potency against *Bacillus subtilis*, *Staphylococcus aureus* and *Escherichia coli*, and their antifungal activity against *Candida albicans* and *Mucor miehei*. No significant effects were detected in these bioassays. However, the preliminary tests carried out on the crude extract showed moderate effects against *Bacillus subtilis* and *Escherichia coli* with inhibition diameters of 11 and 12 mm at 40 μg per platelet, respectively. The crude extract was also tested *in vitro* for its preliminary cytotoxicity assay using *Artemia salina*. It exhibited a 40 % mortality of larvae at 10 μg/mL.

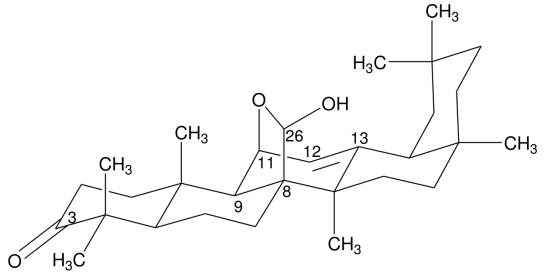


Fig. 3. Orientation of the hemi-acetal group in compound **1**.

Experimental Section

General

Optical rotation was determined on a Perkin-Elmer polarimeter (model 241). NMR spectra were recorded in $CDCl_3$ on Mercury 300 and Inova 600 spectrometers. The chemical shifts are given in ppm relative to TMS as internal reference. HR-ESIMS was performed on a microTOF 10237 spectrometer (Bruker). UV spectra were recorded in $CHCl_3$ on a Carry 300 spectrophotometer. Pre-coated TLC Polygram SilG/UV_{254} plastic sheets from Macherey-Nagel were used for thin-layer chromatography (0.2 mm layer thickness) and visualized under UV light (254 and 365 nm) or sulfuric anisaldehyde reagent (85 mL MeOH, 14 mL EtOAc, 1 mL H_2SO_4 and 1 mL 4-methoxybenzaldehyde). Column chromatography and flash chromatography were conducted using silica gel (Merck 230 – 400 and 70 – 230 mesh)

with different mixtures of *n*-hexane-ethyl acetate, and dichloromethane-methanol as eluents.

Plant material

Salacia longipes var. camerunensis was collected in December 2008 at Mount Kala (Yaoundé), in the Centre Region of Cameroon. The plant was identified by Mr. V. Nana, botanist at the National Herbarium of Cameroon, where a voucher specimen (N° 28963 / SRF/Cam) has been deposited.

Extraction and separation

The roots of *S. longipes var. camerunensis* (2.4 kg) were crushed and extracted at r. t. with a mixture of CH_2Cl_2-MeOH (1 : 1), (2 × 7 L, 48 h each). The extract was concentrated under vacuum to afford 70.6 g of a residue. 60.0 g of the extract was chromatographed on silica gel (Merck, 230 – 400 mesh) using mixtures CH_2Cl_2-MeOH of increasing polarity as eluent. 91 fractions of 300 mL each were collected and combined on the basis of TLC analysis to yield four main fractions labelled A (11.2 g), B (9.5 g), C (13.1 g), and D (18.0 g).

Fraction A (11.2 g) was subjected to column chromatography on silica gel (Merck, 70 – 230 mesh). Elution with *n*-hexane-EtOAc gradient mixtures resulted in the collection of 549 fractions of 25 mL each, which were combined on the basis of TLC analysis. Further purification of sub-fractions 16 – 32 afforded the mixture of β-sitosterol and stigmasterol (17.8 mg) while that of sub-fractions 183 – 189 gave salacetal (**1**, 6.2 mg). Chromatography of fraction B on silica gel (Merck, 70 – 230 mesh) and eluting with *n*-hexane-EtOAc gradient mixtures resulted in the collection of 107 fractions of 25 mL each, which were combined on the basis of TLC analysis. Further purification of sub-fractions 71 – 76 afforded 2-hydroxy-3-oxo-D:A-friedooleanan-29-oic acid (10.2 mg). Purification of fraction D with mixtures CH_2Cl_2-MeOH of increasing polarity as eluent afforded mangiferin (71.3 mg). Fraction C was a complex mixture and was not further studied.

Evaluation of antimicrobial activity

Three bacterial species, including *Bacillus subtilus*, *Staphylococcus aureus* and *Escherichia coli*, the yeast *Candida albicans* and the fungus *Mucor miehei* were used. *In vitro* assays were performed according to the methods described in previous reports by Latha et *al.* [24].

*Salacetal [11β,26-epoxy-26-hydroxy-olean-12-en-3-one] (**1**)*

Colorless powder. – $[\alpha]_D^{20}$ = +37 (c = 0.1, MeOH). – UV/Vis (MeOH): $\lambda_{max}(\log\varepsilon)$ = +246 (3.2), 332 (3.1) nm. – ^{1}H NMR ($CDCl_3$, 300 MHz) and ^{13}C NMR ($CDCl_3$, 125 MHz) spectroscopic data: see Table 1. – MS (EI, 70 eV): m/z (%) = 454 (40) $[M]^+$, 439 (19), 409 (33), 408 (100), 393 (30), 322 (38), 287 (13), 255 (25), 203 (12), 191 (20). – HRMS ((+)-ESI): m/z = 477.3342 (calcd. m/z = 477.3339 for $C_{30}H_{46}O_3Na$, $[M+Na]^+$).

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