New Triterpenoids from the Stem Barks of *Drypetes tessmanniana*

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The MeOH extract of the stem barks of *Drypetes tessmanniana* (Euphorbiaceae) afforded two new triterpene derivatives characterized as 3β-*O*-(*E*)-3,5-dihydroxycinnamoyl-11-oxo-olean-12-ene and 3β,6α-dihydroxylup-20(29)-ene together with seven known compounds. Their structures were established on the basis of spectral analysis.

Key words *Drypetes tessmanniana*; Euphorbiaceae; (*E*)-3,5-dihydroxycinnamoyl; acylated triterpene

Plants belonging to the *Drypetes genus* (Euphorbiaceae) are widely used in traditional medicine in West and Central Africa for the treatment of diverse infections such as sinusitis, swelling, boils, gonorrhoea and dysentery.[1—4] Diverse therapeutic applications of the *Drypetes* plants prompted us to carry out pharmacological and chemical studies on many species. In our previous results, we reported on the anti-inflammatory and analgesic actions of a crude extract and compounds isolated from *D. molunduana*,[5—7] phenolic constituents from *D. armoracia*[8] and the antileishmanial furanosesquiterpene and triterpenoids from *D. chevalieri*.[9] As a continuation of our search for compounds with biological activities from the *Drypetes* species, we studied the stem bark of *D. tessmanniana*, a small tree or shrub growing in the eastern forests of Cameroon. We isolated two new triterpene derivatives and seven known compounds. The known compounds were identified as lupeol (**3**),[10] friedelin (**4**),[10] 3,7-dioxofriedelane (**5**),[10] 3,15-dioxofriedelane (**6**),[10] friedelan-3-ol (**7**),[10] sitosterol (**8**)[7,8] and 3β-D-glucopyranosylsitosterol (**9**).[7,8] The structures of the new compounds, on the basis of spectroscopic analysis, have been determined as 3β-*O*-(*E*)-3,5-dihydroxycinnamoyl-11-oxo-olean-12-ene (**1**) and 3β,6α-dihydroxylup-20(29)-ene (**2**). In the present paper, their isolation and structural determination will be described.

Results and Discussion

The stem bark of *Drypetes tessmanniana* was powdered and extracted with MeOH, and the solvent was evaporated under vacuum. The crude extract was chromatographed on a column of silica gel eluted with *n*-hexane, EtOAc and MeOH in increasing polarities to afford compounds (**1**—**9**).

The novel compound (**1**) was obtained as a colourless amorphous solid. The molecular formula was deduced from the HR-TOF-MS ES$^+$ *m*/*z* 602.8491 (Calcd for $C_{39}H_{54}O_5$: 602.8506) and EI-MS (*m*/*z* 602 [M$^+$]), appropriate for 13 degrees of unsaturation. Its IR spectrum exhibited strong absorption bands due to free hydroxyl (3390 cm^{-1}), conjugated ester carbonyl (1702 cm^{-1}), enone carbonyl (1685 cm^{-1}), aromatic C=C (1605, 1514 cm^{-1}) and *trans*-disubstituted double bonds (1610, 975 cm^{-1}). The ^{1}H-NMR spectrum (Table 1) displayed eight singlet resonances for methyl groups (δ_H 0.82, 0.83, 0.88, 0.96, 1.00, 1.10, 1.29, 1.37) sug-

Table 1. ^{1}H- and ^{13}C-NMR (400, 100 MHz) Data[a] of Compounds **1** (C_5D_5N) and **2** ($CDCl_3$)

Attributions	**1**		**2**	
	δ_C	δ_H *J* (Hz)	δ_C	δ_H *J* (Hz)
1	39.1	1.18 m; 1.22 m	38.5	0.89 m; 1.62[b] m
2	24.2	1.83 m; 1.91 m	27.0	1.57 m; 1.67[b] m
3	80.2	4.90 dd (4.8; 11.1)	78.7	3.20 dd (5.6; 10.7)
4	38.5		39.1	
5	55.1	1.85 m	60.6	0.78 d (10.3)
6	18.8	1.40 m; 1.53 m	68.8	4.10 td (3.4, 10.3)
7	33.0	1.28 m; 1.60 m	46.7	1.40 m; 1.67[b] m
8	45.2		42.1	
9	61.9	2.45 s	49.9	1.27 m
10	37.4		39.3	
11	199.4		20.8	1.65 m; 1.66 m
12	128.3	5.75 s	25.0	1.03 m; 1.64 m
13	170.2		37.6	1.61[b] m
14	43.6		43.0	
15	26.7	1.07 m; 1.72 m	27.4	0.99 m
16	26.5	1.69 m; 2.03 m	35.5	1.34 m; 1.46[b] m
17	32.5		42.9	
18	45.5	1.67 s	48.3	1.46[b] m
19	47.7	2.09 m; 2.12 m	47.9	2.35 dt (10.3, 6.5)
20	31.1		150.8	
21	34.6	1.08 m; 1.35 m	29.8	1.20 m; 1.88 m
22	36.7	1.21 m; 1.43 m	39.9	1.16 m; 1.37 m
23	28.7	1.00 s	30.9	1.32 s
24	16.7	0.96 s	15.5	0.98 s
25	17.1	1.37 s	17.1	0.85 s
26	17.6	1.10 s	17.5	1.08 s
27	23.5	1.29 s	14.5	0.96 s
28	28.2	0.82 s	18.0	0.75 s
29	32.7	0.88 s	109.4	4.58 d (2.3)
				4.68 d (2.3)
30	23.6	0.83 s	19.3	1.68 s
1′	128.4			
2′	116.7	7.23 s		
3′	147.7			
4′	122.0	7.69 s		
5′	150.4			
6′	115.6	7.23 s		
7′	145.6	8.00 d (15.8)		
8′	115.9	6.66 d (15.8)		
9′	167.3			

a) Assignments were accomplished using HSQC, HMBC, ^{1}H–^{1}H COSY and NOESY experiments. *b*) Overlapping signals.

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gesting the triterpene skeleton, one singlet at δ_H 5.75 assigned to the proton of the enone group, and one doublet of doublet at δ_H 4.90 (1H, J=4.8; 11.1 Hz) assigned to the 3-oxymethine proton. It also exhibited two singlet resonances for three aromatic protons at δ_H 7.23 (2H) and δ_H 7.69 (1H) and two doublet signals at δ_H 6.66 (1H, J=15.8 Hz), and δ_H 8.00 (1H, J=15.8 Hz). These data suggested the presence of the 3,5-dihydroxycinnamoyl moiety in (**1**). The ^{13}C-NMR spectrum (Table 1) showed signals for 39 carbons including 30 carbons for the triterpene skeleton and 9 carbons for the cinnamoyl moiety. The structure of the 3,5-dihydroxycinnamoyl moiety was confirmed by the HMBC spectrum (Fig. 2) which displayed long range correlations from protons H-2′/H-6′ (δ_H 7.23; s) to carbons C-3′ (δ_C 147.7), C-5′ (δ_C 150.4) and C-7′ (δ_C 145.6). This group was placed at the C-3 position as deduced from the downfield shift observed for proton H-3 (δ_H 4.90) and the crossed peaks exhibited in the HMBC spectrum from proton H-3 (δ_H 4.90) to carbons C-1 (δ_C 39.1), C-24 (16.7) and C-9′ (167.3). The β-orientation of the ester group was determined using H-3α coupling constant (J_{trans}=11.1 Hz) and the NOESY spectrum which showed crossed peaks between both protons H-3 (δ_H 4.90) and H-5 (δ_H 1.85). The presence of the enone in (**1**) was confirmed by the carbon resonances at δ_C 199.4 (C-11), 128.3 (C-12) and 170.2 (C-13). The HMBC spectrum also displayed crossed peaks between proton H-12 (δ_H 5.75) and carbons C-9 (δ_C 61.9), C-11 (199.4) and C-13 (170.2). The ^{1}H- and ^{13}C-NMR signals of the triterpene moiety were similar to those of 3β-hydroxy-olean-12-en-11-one reported in the literature.[11] Thus compound **1** was established to be 3β-*O*-(*E*)-3,5-dihydroxycinnamoyl-11-oxo-olean-12-ene (Fig. 1). To the best of our knowledge, the compound reported here is one of the rare examples of acylated triterpenes with an unusual phenylpropanoid, (*E*)-3,5-dihydroxycinnamic acid moiety.[12] A number of studies have reported on the isolation of acylated triterpenes from plants and some have demonstrated diverse biological properties including cytotoxic,[13] antitumor[14,15] and anti-inflammatory[16] activities.

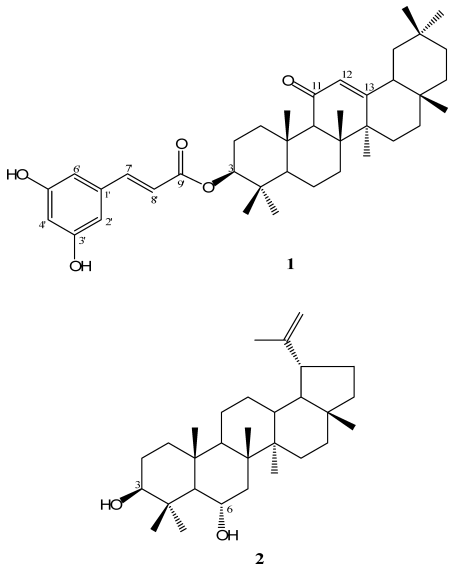

Fig. 1. Structures of Compounds **1** and **2**

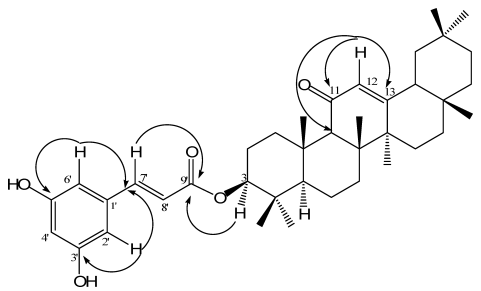

Fig. 2. Important HMBC Correlations in Compound **1**

Compound **2** was obtained as a colourless amorphous solid. The pseudo molecular ion peaks at m/z 443 $[M+H]^+$ and 460 $[M+NH_4]^+$ in its CI/NH_3 MS and the HR-TOF-MS ES^+ (m/z 442.3825) suggested its molecular formula to be $C_{30}H_{50}O_2$. The IR spectrum indicated the presence of hydroxyl (3400 cm^{-1}) and olefinic (1660 cm^{-1}) groups. The ^{1}H-NMR spectrum of **2** (Table 1) displayed six tertiary methyl singlet signals (δ_H 1.32, 1.08, 0.98, 0.96, 0.85, 0.75), one isopropenyl group [δ_H 1.68 (3H, s), 4.58 and 4.68 (1H each, d, J=2.3 Hz)] and two oxymethine protons (δ_H 3.20, 4.10), while the ^{13}C-NMR spectrum showed 30 carbon signals including seven methyls, nine methylenes, seven (two of which are oxygenated) methines, and six quaternary carbons (Table 1). On the basis of the analysis of the ^{1}H–^{1}H COSY, HMQC and DEPT spectra, **2** was suggested to be a lupane-type triterpene bearing two hydroxyl groups. The EI-MS of **2** exhibited important peaks at m/z 442 $[M]^+$, 424 $[M-H_2O]^+$, 409 $[M-H_2O-CH_3]^+$, 406 $[M-2H_2O]^+$, 236, 218, 205, 203, and 189. This fragmentation pattern supported the lup-20(29)-ene skeleton with the location of both hydroxyl groups on rings A and B. The positions of the two hydroxyl groups were established on the basis of the HMBC and NOESY (Fig. 3) experiments. The HMBC spectrum of **2** showed correlations between the oxymethine proton at δ_H 3.20 and the carbons C-2 (δ_C 27.0), C-4 (δ_C 39.1), C-5 (δ_C 60.6), C-23 (δ_C 30.9), and C-24 (δ_C 15.5), confirming the location of one hydroxyl group at C-3. The 3β-OH equatorial orientation was established using H-3α coupling constant (J_{trans}=10.7 Hz) and the interaction observed in the NOESY spectrum, between the two axial protons H-3α (δ_H 3.20) and H-5 (δ_H 0.78). Furthermore, the HMBC spectrum exhibited interactions between the second oxymethine proton at δ_H 4.10 and the carbons C-4 (δ_C 39.1), C-5 (δ_C 60.6), C-7 (δ_C 46.7), C-8 (δ_C 42.1), and C-10 (δ_C 39.3). These data suggested the second hydroxyl group to be located at C-6. The NOESY spectrum displayed important correlations between the proton H-6 (δ_H 4.10) and the methyl protons CH_3-24 (δ_H 0.98), CH_3-25 (δ_H 0.85), and CH_3-26 (δ_H 1.08), suggesting the β-axial orientation of the proton H-6. Consequently, OH-6 was deduced to have the α-equatorial orientation. Further arguments were given by the H-6β coupling constant (td, J=3.4, 10.3 Hz) which was similar to the reported values.[17]

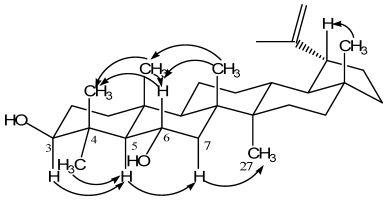

Fig. 3. Important NOESY Correlations in Compound **2**

In addition, the ^{13}C-NMR spectrum of **2** (Table 1) exhibited the important and characteristic signal for carbon C-5 at δ_C 60.6 that was higher (*ca.* 4—5 ppm) than some reported data for C-5 (*ca.* 55.4—56.6 ppm) in similar triterpenes with a 6β-OH group.[18—20] Therefore, the structure of compound **2** was established as 3β,6α-dihydroxylup-20(29)-ene (Fig. 1). The same structure was reported in the literature,[21] but, in the ^{13}C-NMR data published, the carbon C-5 was assigned at δ_C 55.4; this value should correspond, in agreement with the literature values, to the 6β-OH orientation, and not to the 6α-OH as attributed. Consequently, the reported structure might be wrong and should be revised.

Experimental

General The MPs were determined using a Kofler microhot stage apparatus. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. IR spectra (ν_{max} in cm^{-1}) were obtained from potassium pellets on a Nicolet 510 FT instrument. Mass spectra were recorded with ZQ 2000 Waters and Q-Tof1 Micromass spectrometers using electro spray ionization (ESI-MS: U_c=30 V), a Nermag R10-10C spectrometer and a HP-5973 Mass Selective Detector. ^{1}H-NMR (δ [ppm], J [Hz]) and ^{13}C-NMR spectra were recorded at 400 MHz and 100 MHz, respectively, using a Bruker AC 400 spectrometer and a Varian Gemini 400 MHz instrument. Multi-impulsional 2D NMR experiments (^{1}H–^{1}H COSY, ^{1}H–^{1}H NOESY, ^{13}C–^{1}H HSQC, ^{13}C–^{1}H HMBC) were performed using standard Bruker or Varian Gemini micro-programs. Silica gel 60 (70—230 mesh) was used for column chromatography at normal pressure while silica gel 60 H (5—40 μm) and 60 AC (20—40 μm) were used for column chromatography under compressed air (300 mbar). Precoated silica gel 60 F_{254} aluminium plates were used for thin layer chromatography and eluted with mixtures of solvents such as hexane/CH_2Cl_2 (9 : 1); CH_2Cl_2/MeOH (19 : 1); CH_2Cl_2/MeOH (9 : 1).

Plant Material The stem barks of *Drypetes tessmanniana* (Euphorbiaceae) were collected from the Dja forest (East Cameroon), in November 2005. The herbarium specimen documenting the collection has been deposited in the National Herbarium Cameroon, Yaoundé, under Ref. No. 5677/SRFCAM.

Extraction and Isolation The dried powdered stem bark (6 kg) of *Drypetes tessmanniana* was extracted with MeOH at room temperature and concentrated to dryness under reduced pressure to yield a brown semi-solid residue (200 g). Part of the crude extract (150 g) was subjected to CC (column chromatography) on silica gel (70—230 mesh). Elution was carried out with hexane, EtOAc and CH_3OH in increasing polarity. A total of 185 fractions, 200 ml each, were eluted and the TLC permitted to combine the resulted fractions into four series, A—D. Further CC of series **A** (30 g), fractions 1—29 using hexane and EtOAc in increasing polarity yielded compounds (**3**) (24 mg), (**4**) (15 mg), (**5**) (15 mg), (**6**) (10 mg), (**7**) (150 mg) and (**8**) (35 mg). Further CC of series **B** (30 g), fractions 30—121, on silica gel 60 AC (40—70 μm) eluting with hexane/EtOAc and EtOAc/MeOH in increasing proportions yielded compounds (**1**) (20 mg) and (**2**) (15 mg). Further CC of series **C** (20 g), fractions 122—154 on silica gel 60 AC (40—70 μm) using EtOAc/MeOH in increasing polarity yielded compound (**9**) (30 mg).

3β-*O*-(*E*)-3,5-Dihydroxycinnamoyl-11-oxo-olean-12-ene (**1**): Colourless amorphous solid; TLC *Rf*: 0.7 (CH_2Cl_2/MeOH; 9 : 1); yellow spot developed on spraying with aqueous H_2SO_4 (50%). ^{1}H- and ^{13}C-NMR spectral data (400, 100 MHz, C_5D_5N), see Table 1. IR (KBr) cm^{-1}: 3390, 1702, 1685, 1610, 1605, 1514, 975. HR-TOF-MS ES$^+$ *m/z*: 602.8491 (Calcd for $C_{39}H_{54}O_5$: 602.8506). EI-MS (70 eV) *m/z*: 602 (M^+), 601, 423, 273, 232, 231 and 163. $[\alpha]_D^{20}$ +34.5° (c=0.70, MeOH).

3β,6α-Dihydroxylup-20(29)-ene (**2**): Colourless amorphous solid; ^{1}H- and ^{13}C-NMR spectral data (400, 100 MHz, $CDCl_3$), see Table 1. IR (KBr) cm^{-1}: 3400, 3030, 1660, 1260, 1180, 890. HR-TOF-MS ES$^+$ *m/z*: 442.3825 (Calcd for $C_{30}H_{50}O_2$: 442.3811). $[\alpha]_D^{20}$ +24.5° (c=0.60, $CHCl_3$). CI/NH_3 MS, *m/z*: 443 $[M+H]^+$, 460 $[M+NH_4]^+$. EI-MS (70 eV) *m/z*: 442 (M^+), 424 $(M-H_2O)^+$, 409 $(M-H_2O-CH_3)^+$, 406 $(M-2H_2O)^+$, 236, 218, 205, 203, 189.

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