FULL PAPER

Clerodendrumol, A New Triterpenoid from *Clerodendrum yaundense* GÜRKE (Lamiaceae)

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A new lupane-type triterpene named clerodendrumol (**1**), *i.e.*, (16α)-lupa-12,20(29)-dien-16-ol, together with five known compounds, octacosa-(5*Z*,9*Z*)-dienoic acid (**3**), tetracosanoic acid (**4**), genkwanin (**5**), (12*S*)-hydroxyoctadeca-(9*Z*,13*E*,15*E*)-trienoic acid (**6**), and (9*S*)-hydroxyoctadeca-(10*E*,12*E*)-dienoic acid (**7**), were isolated from the twigs of *Clerodendrum yaundense*. *O*-Acetylclerodendrumol (**2**), a derivative of **1**, was prepared by a standard acetylation procedure applied to compound **1**. The structure of the new compound was elucidated using spectroscopic analysis (NMR and MS) data, and chemical conversions (acetylation and deacetylation). The human breast cancer cell line (MDA-MB-231) was sensitive to all test samples, and clerodendrumol (**1**) as well as its derivative **2** had good activity (IC_{50} values 7.5 and 13.2 μM). Genkwanin (**5**) exhibited strong activity on HeLa cell line with IC_{50} 7.8 μM.

Introduction. – *Clerodendrum*, a member of the family Lamiaceae, is a very large and diverse genus with approximately 600 species distributed in Asia, Australia, America, and Africa [1]. Plant species of this genus are small trees, shrubs and herbs, and have several traditional medicine uses in Asia and Africa [2][3]. Previous phytochemical investigations on some species of the genus *Clerodendrum* reported the isolation of phenols, flavonoids, terpenoids, steroids, and cyanogenic glycosides [1][2][4–6].

Clerodendrum yaundense is a small tree of 2 to 4 m high with white flowers found in the western region of Cameroon where traditional healers used the leaves for the treatment of jaundice and infectious diseases. To the best of our knowledge, no phytochemical and biological studies have been reported on this plant species.

In part of our continuing search for bioactive constituents from plant species of *Clerodendrum* [4], we report the isolation and structure elucidation of a new triterpenoid (**1**) and a derivative **2** thereof, together with five known compounds (**3**–**7**) from the twigs of *C. yaundense*. The structure of the new compound was established based on spectroscopic analysis (NMR and MS), and chemical conversions. The antiproliferative activity of the crude extract, **1**–**3**, and **5** is also reported.

Results and Discussion. – The MeOH crude extract of the twigs of *Clerodendrum yaundense* was subjected to silica gel medium pressure liquid column chromatography (MPLC). Then, several attempts were made to seperate the mixture by column chromatography (*Sephadex LH-20*, normal-phase and reversed-phase SiO_2), but only chemical resolution by acetylation/deacetylation was successful to afford clerodendrumol (**1**) and *O*-acetylclerodendrumol (**2**) along with five known compounds, octacosa-(5*Z*,9*Z*)-dienoic acid (**3**) [7], tetracosanoic acid (**4**) [8], genkwanin (**5**) [9], (12*S*)-hydroxyoctadeca-(9*Z*,13*E*,15*E*)-trienoic acid (**6**) [10], and (9*S*)-hydroxyoctadeca-(10*E*,12*E*)-dienoic acid (**7**) [11] (*Fig. 1*). Compounds **1** and **3** were initially isolated as a mixture. The acetylation reaction of the mixture of **1** and **3** gave compounds **2** and **3**, while deacetylation of **2** furnished **1**. The structures of the known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

Clerodendrumol (**1**) was obtained as white powder and the IR spectrum had prominent absorption bands at 3438 and 1645 cm^{-1}, characteristic for a OH group and of non-conjugated C=C bonds. The HR-FAB-MS gave a *pseudo*-molecular ion peak ($[M+H]^+$) at *m*/*z* 425.3781 corresponding to the molecular formula $C_{30}H_{48}O$, consistent with seven degrees of unsaturation. The ^{1}H-NMR spectrum (*Table 1*) of **1** had signals (δ(H) 5.31 (br. *d*, *J* = 4.6), 4.74 (br. *s*), and 4.67 (br. *s*)) of a vinylic and of exocyclic CH_2 group H-atoms. A signal due to a H-atom of an O-bearing CH group was also observed at δ(H) 3.40–3.33 (*m*). The ^{1}H-NMR spectrum exhibited further signals of three H-atoms each, for seven *singlet*s between δ(H) 0.72 and 1.58, corresponding to one Me group attached to a vinylic C-atom, and six tertiary Me groups. The ^{13}C-NMR spectrum (*Table 1*) exhibited a total number of 30 C-atoms including seven C-atoms, six CH, ten CH_2, and seven Me. Important C-atom signals were observed at δ(C) 148.2 (C(20)), 142.2 (C(13)), 121.6 (C(12)), and 112.2 (C(29)) characteristic for a lupane-type triterpenoid [12][13]. The ^{13}C-NMR spec-

DOI: 10.1002/hlca.201500217

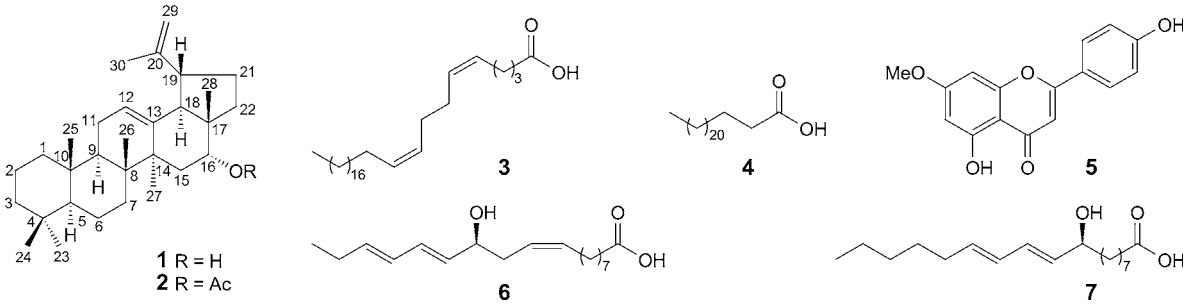

Fig. 1. *Chemical structures of compounds* **1**–**7**

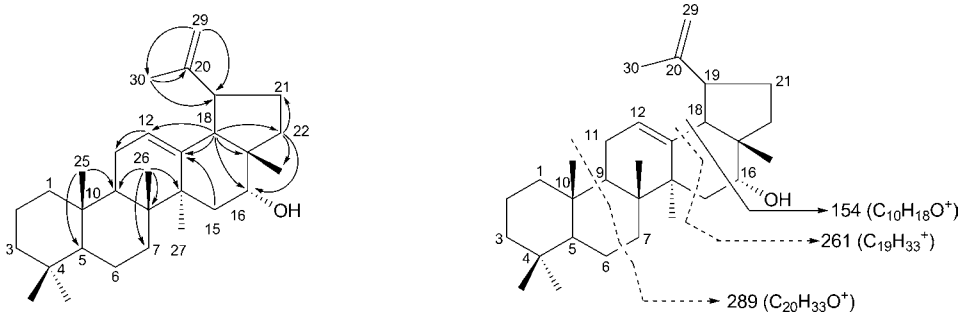

Fig. 2. *Key HMBCs* (H → C) *and MS fragments of clerodendrumol* (**1**)

trum had an additional and relevant C-atom signal at δ(C) 71.8 assignable to C(16). The HMBCs (*Fig. 2*) between H-atom signals at δ(H) 2.21 (H–C(18)), 1.83 – 1.81 (H_a–C(22)) and C-atom signal at δ(C) 71.8 (C(16)) was indicative for the OH group to be attached to C(16). The position of the OH group at C(16) was further supported by ion fragments observed in the mass spectrum (*Fig. 2*) at *m*/*z* 154 ($[M+H-275]^+$, $C_{10}H_{18}O^+$; C(13)–C(18) and C(15)–C(16) cleavage), 195 ($[M+H-428]^+$, $C_{14}H_{27}^+$; RDA cleavage), 261 ($[M+H-268+2H]^+$, $C_{19}H_{33}^+$; C(13)–C(18) and C(14)–C(15) cleavage), and 289 ($[M+H-140]^+$, $C_{20}H_{33}O^+$; C(6)–C(7) and C(9)–C(10) cleavage). Further HMBCs were also observed between the H-atom signal at δ(H) 2.21 (H–C(18)) and C-atom signals at δ(C) 142.2 (C(13)), 121.6 (C(12)), 34.6 (C(17)), and 37.4 (C(22)); and between H-atom signal at δ(H) 1.83 – 1.81 (H_a–C(22)) and C-atom signals at δ(C) 32.7 (C(21)) and 20.0 (C(28); *Fig. 2*). A NOESY correlation between H-atoms at δ(H) 3.40 – 3.33 (H–C(16)) and 0.86 (Me(26), *Fig. 3*) suggested the α-configuration of the OH group at position C(16). On the basis of the NMR data above and by comparison with reported lupane-type triterpenoids [12][14][15], the structure of clerodendrumol (**1**) was elucidated as (16α)-lupa-12,20(29)-dien-16-ol (**1**).

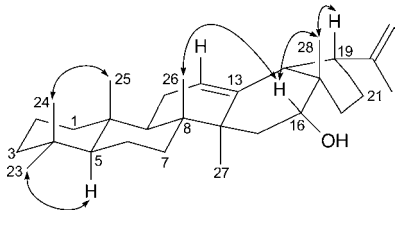

Fig. 3. *Key NOESY* (H ↔ H) *of clerodendrumol* (**1**)

Antiproliferative Activity. The antiproliferative activities of the crude extract, compounds **1**, **2**, **3**, and **5** were determined on five cancer cell lines (*Table 2*). The human breast cancer cell line (MDA-MB-231) was sensitive to all test samples. Clerodendrumol (**1**) and its derivative (**2**) had good activity (IC_{50} values 7.5 and 13.2 μM) on this cell line, while the crude extract (IC_{50} 39.2 μg/ml), and compounds **3** (IC_{50} 24.7 μM) and **5** (IC_{50} 38.4 μM) were only moderately active. Genkwanin (**5**) exhibited strong activity on HeLa cell line with IC_{50} (7.8 μM) comparable to that of the reference standard 5-fluorouracil (6.4 μM), whereas the crude extract had only weak activity (IC_{50} 92.6 μg/ml). The antiproliferative activity of genkwanin (**5**) is in agreement with the previous result obtained for a flavonoid on HeLa cancer cell line [16].

Lupane-type triterpenoids have been reported to possess antiporliferative activity on cancer cell lines [17 – 19]. Thus, our results on the isolation of an antiproliferative triterpenoid, clerodendrumol (**1**), from *Clerodendrum yaundense* strengthen the previous findings. The phytochemical study of others species of the same genus is in progress in our research group, as well as the characterization of more bioactive constituents.

Experimental Part

General. TLC: Pre-coated silica gel GF_{254} plates (SiO_2; *Merck*) with detection accomplished by visualizing with a UV light (254 and 365 nm), followed by spraying with 1% $Ce(SO_4)_2$/10% aq. H_2SO_4 and then heating to 150°. Column chromatography (CC): silica gel *60N*

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.) *for Clerodendrumol* (**1**) *and Acetylclerodendrumol* (**2**). δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)
1	1.95 – 1.93 (*m*), 1.99 – 1.97 (*m*)	32.6	1.50 – 1.48 (*m*), 2.00 – 1.98 (*m*)	33.9
2	1.53 – 1.51 (*m*), 1.55 – 1.53 (*m*)	20.2	1.07 – 1.05 (*m*), 1.59 – 1.57 (*m*)	21.5
3	1.76 – 1.74 (*m*), 1.79 – 1.77 (*m*)	32.9	0.95 – 0.93 (*m*), 1.00 – 0.98 (*m*)	33.7
4		36.4		36.2
5	1.02 (br. *s*)	57.7	1.01 – 0.99 (*m*)	56.7
6	1.58 – 1.56 (*m*), 1.71 – 1.69 (*m*)	23.8	1.17 – 1.15 (*m*), 1.34 – 1.32 (*m*)	24.3
7	1.30 – 1.28 (*m*), 2.03 – 2.01 (*m*)	29.0	0.98 – 0.96 (*m*), 1.32 – 1.30 (*m*)	33.9
8		51.3		56.7
9	1.08 (br. *s*)	57.0	0.94 – 0.92 (*m*)	50.0
10		46.8		42.3
11	1.47 (*dd*, *J* = 5.7, 3.5), 1.43 (*dd*, *J* = 6.3, 4.6)	38.3	1.14 – 1.12 (*m*), 1.86 – 1.84 (*m*)	37.0
12	5.31 (br. *d*, *J* = 4.6)	121.6	5.37 (*d*, *J* = 2.7)	122.7
13		142.2		139.7
14		43.2		45.8
15	2.03 (br. *d*, *J* = 3.4), 1.98 (*dd*, *J* = 5.7, 3.4)	40.8	2.32 – 2.30 (*m*), 2.33 – 2.31 (*m*)	38.1
16	3.40 – 3.33 (*m*)	71.8	4.60 (*t*, *J* = 4.9)	74.0
17		34.6		36.6
18	2.21 (*d*, *J* = 5.7)	43.4	1.60 (br. *s*)	31.9
19	1.88 – 1.86 (*m*)	50.4	1.86 (*d*, *J* = 10.9)	49.5
20		148.2		147.6
21	1.04 – 1.03 (*m*), 1.06 – 1.05 (*m*)	32.7	1.57 – 1.55 (*m*), 1.88 – 1.86 (*m*)	27.8
22	1.83 – 1.81 (*m*), 1.86 – 1.84 (*m*)	37.4	1.51 – 1.49 (*m*), 1.96 – 1.94 (*m*)	39.7
23	0.73 (*s*)	34.6	1.25 (*s*)	35.5
24	0.95 (*s*)	22.0	0.91 (*s*)	21.5
25	0.72 (*s*)	19.3	0.84 (*s*)	19.8
26	0.86 (*s*)	12.5	0.67 (*s*)	12.0
27	1.02 (*s*)	26.8	1.02 (*s*)	29.4
28	1.02 (*s*)	20.0	1.02 (*s*)	19.3
29	4.74 (br. *s*), 4.67 (br. *s*)	112.2	4.73 (br. *s*), 4.64 (br. *s*)	111.4
30	1.58 (*s*)	18.0	1.60 (*s*)	17.8
Me*C*=O	–	–		170.6
*Me*C=O	–	–	2.03 (*s*)	21.0

[a]) Recorded in (D_6)acetone. [b]) Recorded in $CDCl_3$.

Table 2. *Antiproliferative Activity of Crude Extract and Compounds*[a]) *Isolated from* C. yaundense

Samples	IC_{50}				
	HeLa	MDA-MB 231	PSN-1	A549	PANC-1
Crude extract[b])	92.6	39.2	> 100	> 100	88.5
1[c])	> 100	7.5	> 100	> 100	> 100
2	> 100	13.2	> 100	> 100	> 100
3	> 100	24.7	> 100	> 100	> 100
5	7.8	38.4	88.9	> 100	49.3
5-Fluorouracil[d])	6.4	1.1	8.7	9.7	3.0

[a]) Compounds **4**, **6**, and **7** were not tested. [b]) μg/ml. [c]) IC_{50} in μM. [d]) Positive control, IC_{50} in μM.

spherical, neutral, 40 – 50 μm (*Kanto*, Japan), *Cosmosil 75C_{18}*-silica gel (*Kyoto*, Japan), and *Sephadex LH-20*. MPLC: *Büchi Sepacore* system (*Büchi Labortechnik AG*, Flawil, Switzerland). M.p.: *Yanaco*, *Micro MP* apparatus; uncorrected. Optical rotations: *Jasco P-2100* polarimeter. IR Spectra: *Jasco FT-IR 460* spectrometer (*Kyoto*, Japan); $\tilde{\nu}$ in cm^{-1}. NMR Spectra: *Bruker 500* instrument; δ in ppm rel. to Me_4Si as internal standard, J in Hz. FAB-MS: *Jeol JMS-AX505W* spectrometer; in m/z.

Plant Material. The twigs of *Clerodendrum yaundense* were collected in Dschang (Western-Region of Cameroon) in April 2015 and were identified by Mr. *Nana*, a retired botanist, from the Yaoundé National Herbarium where our sample was compared to the available specimen with voucher number 10842 SRF/Cam.

Extraction and Isolation. The twigs of *C. yaundense* (2.8 kg) were dried and milled to a powder before macerated in MeOH (10 l) for 24 h (repeated three times) to give a crude extract (102 g, 4% yield) after

filtration and removal of the solvent using rotative evaporator. Part of this extract (50 g) was subjected to a normal phase SiO_2 MPLC using hexane, AcOEt, and MeOH as eluent in gradient polarity to give 73 fractions of 300 ml each that were monitored by TLC to ten combined fractions (*Frs.* F_1–F_{10}). *Fr.* F_2 (500 mg, hexane/AcOEt 95 : 5) gave **4** (19 mg), while *Fr.* F_3 (900 mg, hexane/AcOEt 9 : 1) crystallized in acetone to give a mixture of **1** and **3** (197 mg). After unsuccessful separation of this mixture using *Sephadex LH-20* and silica gel reversed and normal phases column chromatography (CC), chemical reactions (acetylation and deacetylation) were used to finally obtain compounds **1**, **2**, and **3** in pure form. *Fr.* F_6 (240 mg, hexane/AcOEt 7 : 3) was subjected to the reversed phase SiO_2 CC using MeOH and H_2O in gradient polarity to give 74 fractions of 50 ml each that were further combined by comparative TLC to 17 fractions. *Frs.* F_{6-3}, F_{6-6}, and F_{6-8} were separately subjected to prep. TLC followed by *Sephadex LH-20* to afford compounds **5** (34.8 mg), **6** (8.3 mg), and **7** (2.4 mg), resp.

Acetylation of the Mixture of **1** *and* **3**. Acetic anhydride (2 ml) was added to a mixture of compounds **1** and **3** (100 mg) dissolved in pyridine (2 ml) and kept in the darkness at r.t. for 24 h. The reaction was quenched with 10% HCl and extracted with AcOEt to yield **1** (64.1 mg, 64%) and **2** (30.4 mg, 31%) after purification on SiO_2 CC using hexane/AcOEt (4 : 1).

Deacetylation of **2**. Compound **2** (50 mg) was dissolved in $CHCl_3$/MeOH 1 : 9 (10 ml), and a 2M soln. of K_2CO_3 (10 ml) was added. After stirring at r.t. for 4 h, the TLC (hexane/AcOEt 4 : 1) showed complete consumption of **2** with one different and more polar product at R_f 0.3. Neutralization with a sat. soln. of NH_4Cl (15 ml) followed by extraction using AcOEt and removal of residual H_2O by anh. Na_2SO_4 gave compound **1** (43.5 mg, 87% yield).

Clerodendrumol (=*(16α)-Lupa-12,20(29)-dien-16-ol*; **1**). White powder. M.p. 232–234°. $[\alpha]_D^{22} = -75.3$ ($c = 0.1$, acetone). IR: 3438, 2938, 1645, 1465, 1377, 1053, 886. ^{1}H- (500 MHz, (D_6)acetone) and ^{13}C-NMR (125 MHz, (D_6)acetone): see *Table 1*. FAB-MS: 425 (18), 411 (3), 395 (5), 307 (18), 289 (5), 261 (3), 217 (22), 195 (4), 154 (100), 136 (77). HR-FAB-MS: 425.3781 ($[M+H]^+$, $C_{30}H_{49}O^+$; calc. 425.3784).

O-*Acetylclerodendrumol* (=*(16α)-Lupa-12,20(29)-dien-16-yl Acetate*; **2**). White powder. M.p. 215–217°. $[\alpha]_D^{22} = -21.7$ ($c = 0.1$, acetone). IR: 2937, 1732, 1646, 1467, 1368, 1251, 1039, 886. ^{1}H- (500 MHz, $CDCl_3$) and ^{13}C-NMR (125 MHz, $CDCl_3$): see *Table 1*. FAB-MS: 467 (8), 453 (25), 395 (30), 255 (10), 159 (20), 105 (80), 81 (100). HR-FAB-MS: 467.38833 ($[M+H]^+$, $C_{30}H_{51}O_2^+$; calc. 467.38891).

In vitro *Antiproliferative Assay*. The cell lines used were A549 (human lung cancer), HeLa (human cervix cancer), PANC-1 and PSN-1 (human pancreatic cancer), and MDA-MB-231 (human breast cancer). α-Minimum essential medium with L-glutamine and phenol red (α-MEM, *Wako*) was used for the first three cell lines, whereas high-glucose *Dulbecco*'s modified *Eagle*'s medium with L-glutamine, phenol red, and sodium pyruvate (DMEM, *Wako*) were used for the latter ones. Both media were supplemented with 10% fetal bovine serum (*Nichirei Bioscience*) and 1% antibiotic antimycotic soln. (*Sigma–Aldrich*). The *in vitro* antiproliferative activity of the crude extract and isolated compounds was determined as described previously [20]. Briefly, each cell line was seeded in 96-well plates (2×10^3 per well) and incubated at 37° for 24 h either in α-MEM or in DMEM under 5% CO_2 and 95% air. Thereafter, cells were washed with PBS (*Nissui Pharmaceuticals*), and test samples in serial dilution were added. After 72 h incubation, cells were washed with PBS, and 100 µl of α-MEM or DMEM containing 10% WST-8 cell counting kit (*Kumamoto*, Japan) sol. was added to the wells. The absorbance was measured at 450 nm after additional 2 h incubation.

The different concentrations of serial dilutions of tested samples were 100–3.125 µg/ml for the crude extract, 100–3.125 µM for isolated compounds, and 10–0.3125 µM for the positive control (5-fluorouracil), resp. Cell viability was calculated from the mean values of data from three wells by using the following equation, and antiproliferative activity was expressed as IC_{50} (50% inhibitory concentration) value.

$$\%\ \text{Cell viability} = 100 \times (\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}})/(\text{Abs}_{\text{control}} - \text{Abs}_{\text{blank}})$$

This work was supported in part by a *Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology*, Japan (*H. M.*). We are grateful to the *Matsumae International Foundation* (*MIF*) for the Fellowship awarded to one of us (*M. D. A.*) to work at the Institute of Natural Medicine, University of Toyama (Japan).

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Received August 18, 2015
Accepted November 6, 2015

