Terpenoids from *Clerodendrum formicarum* Gürke (Lamiaceae) of Cameroon

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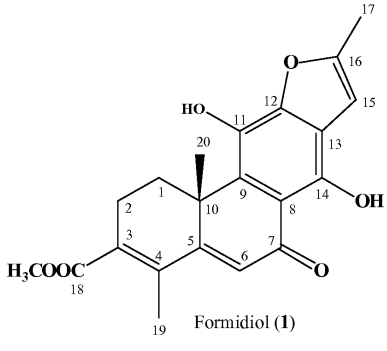
The ethanolic extract of the leaves of *Clerodendrum formicarum*, a Lameacious plant of Cameroon, afforded a new *abeo*-abietane diterpenoid named formidiol (**1**) together with some known di- and triterpenoids. They include 12,16-epoxy-11,14-dihydroxy-6-methoxy-17(15→16)-*abeo*-abieta-5,8,11,13,15-pentanene-3,7-dione (**2**), *trans*-phytol, friedelin and friedlan-3β-ol. Structures of all the isolated constituents have been characterized with the aid of 1D NMR spectroscopy while the structure of a new metabolite was elucidated *via* 2D NMR spectroscopic techniques.

Key words: Formidiol, *abeo*-Abietane, Leaves, *Clerodendrum formicarum*, Lamiaceae, Characterization, Spectroscopy

Introduction

Clerodendrum L. of the family Lamiaceae is a very large and diverse genus, and five hundred and eighty species including small trees, shrubs and herbs distributed in Asia, Australia, Africa, and America. The genus has been used as medicines specifically in India, China, Thailand, Korea, and Japan for the treatment of various life threatening diseases such as syphilis, typhoid, cancer, jaundice, and hypertension. Various species of the genus *Clerodendrum* are known to possess potent bioactivities. Hexane extracts of *C. colebrookianum* show strong antibacterial activities against *Staphylococcus aureus*, *S. haemolyticus*, *E. coli*, *Pseudomonas aeruginosa* [1]. The alcoholic extracts of *C. phlomidis* exhibit antimalarial activity against *Plasmodium falciparum* [2]. CNS-related activities were also observed in *C. phlomidis* showing tranquillizing, CNS-depressant and muscle-relaxant properties in experimental mice and rats [3]. A decoction of *C. phlomidis* (whole plant) has been reported to have antidiabetic activity [4]. *C. inerme* has been used as an antioxidant drug in various indigenous systems of medicines [5]. *C. bungei* show antitumor activity in hepatic cells of mice [6].

The major chemical components reported from the genus *Clerodendrum* are iridoids [7], iridoid glucosides [8], steroids [9], steroidal glycosides [10], terpenes [11], flavonoids [12], flavonoid glycosides [13], chalcone glycosides [14], and macrocyclic alkaloids [15]. The present communication describes the isolation and characterization of a new *abeo*-abietane diterpenoid named formidiol (**1**) together with some known di- and triterpenoids from the leaves of *Clerodendrum formicarum,* collected from Obili-Yaounde (Cameroon).



Formidiol (**1**)

Results and Discussion

The ethanolic extract of the leaves of *C. formicarum* afforded **1** as a red amorphous powder. The IR spectrum of **1** displayed three prominent absorptions. An absorption at 3697 cm^{-1} is due to the hydroxyl function/s in the molecule while the remain-

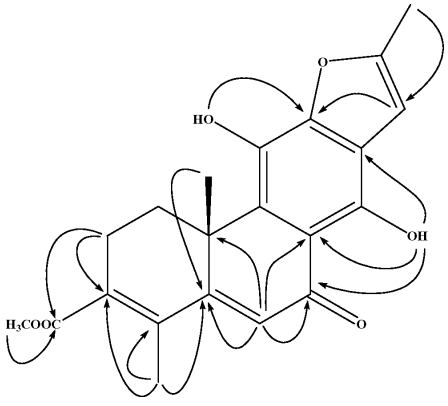


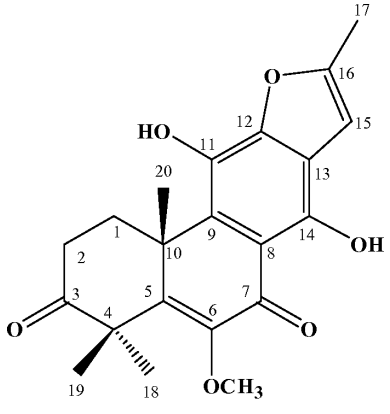
Fig. 1. Important HMBC connectivities in **1**.

ing two prominent bands at 1615 and 1721 cm^{-1} attest for an α,β-unsaturated ketone and an ester carbonyl function, respectively. The molecular ion peak observed in the EIMS at m/z = 368 and the formula associated with this peak was found as $C_{21}H_{20}O_6$ in the high-resolution mass spectrum showing the presence of twelve degrees of unsaturation in the molecule. A significant fragment at m/z = 308 is due to the loss of a methyl formate molecule from the molecule ion peak confirming the presence of a formate moiety in the molecule.

The ^{1}H NMR spectrum of **1** was very simple with only a few signals due to the fact that most of the carbon atoms in the molecule are quaternary in nature. The proton spectrum displayed four methyl singlets at δ = 2.45 (H-17), 2.18 (H-19), 1.55 (H-20), and δ = 3.80, the latter being due to the methoxyl moiety. The two olefinic protons appeared as singlets at δ = 6.47 and 6.60 assigned to H-6 and H-15, respectively. The hydroxyl protons resonated at δ = 5.20 (HO-11) and 13.83 (HO-14). The carbon spectrum of **1** showed altogether 21 carbon signals which were further sorted out with the aid of DEPT experiments into four methyls, two methylenes, and two methines with the remaining ones for quaternary carbons. The two methyls directly attached to olefinic quaternary-carbons resonated at δ = 14.0 (C-17) and 17.2 (C-19) while the methoxyl group appeared at δ = 52.0. Signals at δ = 122.8 and 101.6 attested for C-6 and C-15, respectively. An α,β-unsaturated ketonic carbon appeared at δ = 190.9 and the ester carbonyl function at δ = 169.1. The olefinic-quaternary carbon to which the formate moiety is attached was observed at δ = 132.9. Signals of hydroxyl-bearing carbons appeared at δ = 131.4 (C-11) and 117.3 (C-14). A complete picture of

Table 1. ^{13}C-NMR data of **1** and **2** (δ_C in ppm; multiplicity in parentheses; solvent $CDCl_3$).

Position	**1**	**2** [16]
1	25.0 (t)	27.2 (t)
2	29.2 (t)	33.1 (t)
3	132.9 (s)	214.0 (s)
4	128.8 (s)	49.5 (s)
5	163.4 (s)	155.7 (s)
6	122.8 (d)	146.1 (s)
7	190.9 (s)	186.8 (s)
8	109.4 (s)	109.3 (s)
9	136.0 (s)	127.5 (s)
10	39.0 (s)	41.5 (s)
11	131.4 (s)	130.8 (s)
12	148.8 (s)	148.6 (s)
13	117.3 (s)	117.4 (s)
14	151.8 (s)	151.7 (s)
15	101.6 (d)	101.5 (d)
16	155.1 (s)	155.4 (s)
17	14.0 (q)	14.0 (q)
18	169.1 (s)	25.9 (q)
19	17.2 (q)	22.5 (q)
20	21.6 (q)	20.5 (q)
OCH_3	52.0 (q)	60.1 (q)



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the carbon spectrum of **1** is given in Table 1. Assignments of various protons and their associated carbons in the NMR spectra of **1** were correlated *via* HMQC experiments and cross-checked *via* HMBC connectivities (Fig. 1).

A known abietane diterpenoid (**2**) which was previously isolated by Barros *et al.* from *Aegiphila lhotzkiana* [16] was also isolated by us from the same source. From a comparative NMR-spectral analysis of **1** and **2** (Table 1) it was concluded that **1** is also a diterpenoid of the same class except for a methyl (Me-18) migrated from C-4 to C-3 (*abeo*-abietane) [17]. Abietane [18] and *abeo*-abietane diterpenoids [18, 19] have already been isolated and re-

ported from various species of the genus *Clerodendrum*.

On the basis of the above spectral information, the structure of the compound discussed above is elucidated as **1** and named formidiol. This compound is a new addition in the series of natural *abeo*-abietane diterpenoids.

In addition to formidiol (**1**) and 12,16-epoxy-11,14-dihydroxy-6-methoxy-17(15→16)-*abeo*-abieta-5,8,11,13,15-pentanene-3,7-dione (**2**) [16], some more known di- and triterpenoids have also been obtained form the same source. They include *trans*-phytol, **3** [20], friedelin, **4** [21] and friedlan-3β-ol, **5** [22]. Their spectral data are given in the Experimental Section.

Experimental Section

General

The melting points were recorded in glass capillary tubes using a Büchi 535 melting point apparatus and are uncorrected. Optical rotation was measured on a Jasco DIP-360 (Japan Spectroscopic Co. Ltd., Tokyo, Japan) digital polarimeter. The IR spectrum was recorded on a Shimadzu IR-460 instrument. The ^{1}H- and ^{13}C NMR spectra were recorded at 600/500 and 150/125 MHz, respectively, on Bruker AM 600 and AM 500 spectrometers using TMS as an internal standard. The EI mass spectrum was scanned on a Jeol-JMS HX-110 mass spectrometer.

Collection and identification

The leaves of *C. formicarum* were collected in June 2008, from Obili-Yaounde, Cameroon and identified by Mr. Nana Victor of National Herbarium of Yaounde, Cameroon, where a voucher specimen has been deposited (Herbarium # HNC-13658).

Extraction, isolation and characterization

The collected leaves were dried under shade for a week. The dried and powered material (6.0 kg) was then soaked in ethanol (12 L) for six days. The resulting extract was concentrated by means of evaporation under vacuum distillation (84.5 g) and subjected to silica gel column chromatography using hexane, hexane/ethyl acetate, ethyl acetate, and ethyl acetate/methanol as mobile phase.

*Formidiol (**1**)*

Fractions eluted with 10 % ethyl acetate in hexane afforded **1** as a red amorphous powder (4.5 mg). – UV ($CHCl_3$): $\lambda_{max}(\log\varepsilon)$ = 291.5 (3.76) nm. – $[\alpha]_D^{20}$ = +76.0 (c = 0.78, $CHCl_3$). – IR ($CHCl_3$): ν = 3697 (OH), 2925 (=CH), 1721 (C=O), 1615 (α,β-unsaturated ketone), 1208 (C-O) cm^{-1}. – MS (EI, 70 eV): *m/z* = 368 $[M]^+$, 353 $[M–CH_3]^+$, 308 $[M–H_3COOCH]^+$. – HRMS (EI): *m/z* = 368.1256 (calcd. 368.1260 for $C_{21}H_{20}O_6$), 353.1082 (calcd. 353.1025 for $C_{20}H_{17}O_6$), 308.1014 (calcd. 308.1049 for $C_{19}H_{16}O_4$). – ^{1}H NMR (600 MHz, $CDCl_3$, 29 °C, TMS): δ = 13.83 (1H, s, HO-14), 6.60 (1H, s, H-15), 5.20 (1H, s, HO-11), 6.47 (1H, s, H-6), 3.35 (1H, m, H-2β), 2.45 (3H, s, H-17), 2.18 (3H, s, H-19), 1.62 (1H, m, H-2α), 1.55 (3H, s, H-20), 3.80 (3H, s, OCH_3). – ^{13}C NMR (150 MHz, $CDCl_3$, 29 °C, TMS): see Table 1. – HMBC: see Fig. 1.

*12,16-Epoxy-11,14-dihydroxy-6-methoxy-17(15→16)-abeo-abieta-5,8,11,13,15-pentanene-3,7-dione (**2**)* [16]

Upon further elution with the same polarity, compound **2** was obtained as a yellow powder (5.0 mg). – M. p. = 245 °C. – IR ($CHCl_3$) ν = 3350 (OH), 1710 (C=O), 1640 (α,β-unsaturated ketone) cm^{-1}. – MS (EI, 70 eV): *m/z* = 370 $[M]^+$, 355. – HRMS (EI): *m/z* = 370.1397 (calcd. 370.1416 for $C_{21}H_{22}O_6$). – ^{1}H NMR (600 MHz, $CDCl_3$, 29 °C, TMS): δ = 13.60 (1H, s, HO-14), 6.49 (1H, s, H-15), 5.22 (1H, s HO-11), 3.90 (3H, s, OCH_3), 3.38 (1H, dt, J = 13.8 Hz, H-1β), 2.75 (2H, m, H-2), 2.45 (3H, d, J = 1.2 Hz, H-17), 1.55 (3H, s, H-19), 1.50 (3H, s, H-18), 1.47 (3H, s, H-20). – ^{13}C NMR (150 MHz, $CDCl_3$, 29 °C, TMS): see Table 1.

*trans-Phytol (**3**)* [20]

Elution with 0.5 % ethyl acetate in hexane, afforded compound **3** as a light mobile oil (20.0 mg). – MS (EI, 70 eV): *m/z* = 296 $[M]^+$. – ^{1}H NMR (500 MHz, $CDCl_3$, 29 °C, TMS): δ = 5.38 (1H, br.t, J = 6.9 Hz, H-2), 4.13 (2H, d, J = 6.9 Hz, H-1), 1.64 (3H, s, H-3a), 0.81 (6H, d, J = 6.5 Hz, H-7^a & H-11), 0.84 (6H, d, J = 6.6 Hz, H-15^a & H-16). – ^{13}C NMR (125 MHz, $CDCl_3$, 29 °C, TMS): δ = 59.4 (C-1), 123.3 (C-2), 140.3 (C-3), 16.2 (C-3a), 39.9 (C-4), 25.7 (C-5), 36.8 (C-6), 32.8 (C-7), 19.7 (C-7a & 11a), 37.4 (C-8), 24.5 (C-9), 37.3 (C-10), 32.7 (C-11), 37.3 (C-12), 24.8 (C-13), 39.4 (C-14), 28.0 (C-15), 22.6 (C-15a), 22.7 (C-16).

*Friedelin (**4**)* [21]

Elution with 1 % ethyl acetate in hexane afforded compound **4** as colorless needles (6.5 mg). – M. p. = 259–261 °C. – IR ($CHCl_3$): ν = 1720 (C=O) cm^{-1}. – MS (EI, 70 eV): *m/z* = 426 $[M]^+$, 411, 314, 302, 287, 273, 257, 232, 218, 205, 163, 123. – HRMS (EI): *m/z* = 426.3821 (calcd. 426.3862 for $C_{30}H_{50}O$). – ^{1}H NMR (500 MHz, $CDCl_3$, 29 °C, TMS): δ = 2.38 (1H, m, H-2α), 2.27 (2H, m, H-2β, H-4), 1.95 (1H,m, H-1a), 1.18 – 1.92 (m, rest of the protons), 1.16 (3H, s, H-28), 1.03 (3H, s, H-27), 0.99 (6H, s, H-26 & H-29), 0.93 (3H, s, H-30), 0.86 (3H, d, J = 6.0 Hz,

H-23), 0.85 (3H, s, H-25), 0.70 (3H, s, H-24). – ^{13}C NMR (125 MHz, $CDCl_3$, 29 °C, TMS): δ = 22.2 (C-1), 41.5 (C-2), 213.0 (C-3), 58.2 (C-4), 42.1 (C-5), 41.3 (C-6), 18.2 (C-7), 53.1 (C-8), 37.4 (C-9), 59.5 (C-10), 35.6 (C-11), 30.5 (C-12), 39.7 (C-13), 38.3 (C-14), 32.8 (C-15), 36.0 (C-16), 30.0 (C-17), 42.8 (C-18), 35.3 (C-19), 28.2 (C-20), 32.4 (C-21), 39.2 (C-22), 6.8 (C-23), 14.7 (C-24), 17.9 (C-25), 20.3 (C-26), 18.7 (C-27), 32.1 (C-28), 31.8 (C-29), 35.0 (C-30).

*Friedlan-3β-ol (**5**)* [22]

By elution with 1% ethyl acetate in hexane, compound **5** was obtained as colorless needles (5.5 mg). – M. p. = 280 – 283 °C. – MS (EI, 70 eV): *m/z* = 428 $[M]^+$, 413, 395, 289, 275, 248, 220, 206, 149, 137, 125, 69. – HRMS (EI): *m/z* = 428.3996 (calcd. 428.4018 for $C_{30}H_{52}O$). – ^{1}H NMR (500 MHz, $CDCl_3$, 29 °C, TMS): δ = 3.71 (1H, br.s, H-3), 1.88 (1H, dt, *J* = 9.9, 2.0 Hz, H-2a), 1.71 (1H, m, H-6a), 1.56 – 1.25 (m, rest of the protons), 1.15 (3H, s, H-30), 0.98 (3H, s, H-26), 0.97 (6H, s, H-28), 0.96 (6H, s, H-27), 0.94 (3H, s, H-24), 0.92 (3H, s, H-29), 0.91 (3H, d, *J* = 7.2 Hz, H-23), 0.85 (3H, s, H-25). – ^{13}C NMR (125 MHz, $CDCl_3$, 29 °C, TMS): δ = 15.8 (C-1), 35.2 (C-2), 72.7 (C-3), 49.2 (C-4), 37.1 (C-5), 41.7 (C-6), 17.5 (C-7), 53.2 (C-8), 38.4 (C-9), 61.4 (C-10), 35.3 (C-11), 30.6 (C-12), 37.8 (C-13), 39.7 (C-14), 32.3 (C-15), 36.0 (C-16), 30.0 (C-17), 42.8 (C-18), 35.5 (C-19), 28.2 (C-20), 32.8 (C-21), 39.3 (C-22), 11.6 (C-23), 16.4 (C-24), 18.2 (C-25), 18.6 (C-26), 20.1 (C-27), 32.0 (C-28), 32.1 (C-29), 35.0 (C-30).

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