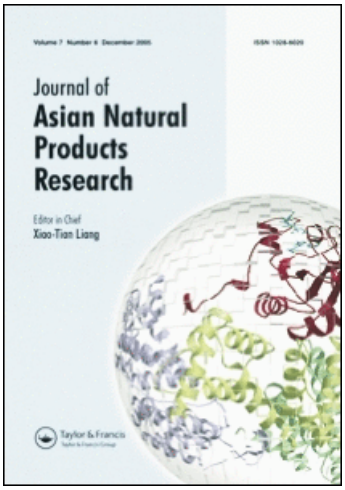

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ORIGINAL ARTICLE

Two new aromatic acids 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 two new salicylic acid derivatives named formoic acids A and B along with four known constituents which have been obtained for the first time from this source. They include flemingipanic acid, martynoside, verbascoside, and seguinoside K. Structures of all the isolated constituents have been elucidated with the aid of 1D and 2D NMR spectroscopic techniques.

Keywords: formoic acids A and B; aromatic acids; *Clerodendrum formicarum*; Lamiaceae

1. Introduction

Clerodendrum L. of the family Lamiaceae is a very large and diverse genus having 580 species distributed in Asia, Australia, Africa, and America. Members of the genus are being used as medicines in Indian, Chinese, Thai, Korean, Japanese systems of medicine for the treatment of life-threatening diseases such as syphilis, typhoid, cancer, jaundice, and hypertension. Many species of the genus *Clerodendrum* are known for potent bioactivities. Hexane extracts of *C. colebrookianum* show strong antibacterial activities against *Staphylococcus aureus*, *S. haemolyticus*, *Escherichia coli*, and *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 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* shows 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 glycoside [13], chalcone glycoside [14], and macrocyclic-alkaloids [15]. The present communication describes the isolation and characterization of two new natural salicylic acid-derivatives named as formoic acids A (**1**) and B (**2**) along with four

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known constituents flemingipanic acid (**3**) [16], martynoside [17], verbascoside [18], and seguinoside K [19] which have been obtained for the first time from the leaves of *Clerodendrum formicarum,* collected from Obili-Yaounde (Cameroon).

2. Results and discussion

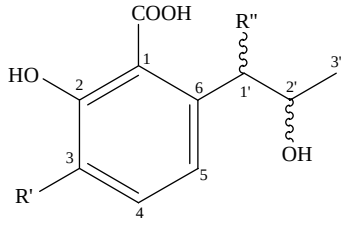
Fractions eluted with 15% ethyl acetate in hexane during silica gel column chromatography of ethanol extract from the leaves of *C. formicarum* collected from Obili-Yaounde (Cameroon) afforded **1**–**3** (Figure 1) as impure samples**,** which were further purified by HPLC as amorphous powders using normal phase column.

The IR spectrum of **1** displayed two prominent absorption bands at 3120 (broad) cm^{-1} attested for hydroxyl functions in the molecule and at 1680 cm^{-1} due to the acid carbonyl. A base peak at *m/z* 194 was observed in the EIMS due to the loss of a water molecule from the molecular ion peak, and the formula associated with this peak was found as $C_{10}H_{10}O_4$ with the aid of high resolution mass experiments, showing the presence of five degrees of unsaturation in the molecule.

The ^{1}H NMR spectrum of **1** was quite simple with only a few signals. The proton spectrum displayed a methyl doublet at δ 1.52 and a pair of double-doublets at δ 3.12 (1H, $J = 16.8$, 3.0 Hz, Ha-1′) and 2.66 (1H, $J = 16.8$, 11.4 Hz, Hb-1′) due to a methylene in the molecule. Another pair of mutually coupled doublets at δ 6.95 and 6.77 (1H each, $J = 9.0$ Hz) were assigned to aromatic protons H-4 and H-5, respectively. In addition to aromatic methines, a carbinylic-methine appeared as a multiplet at δ 4.68. A hydroxyl proton resonated at δ 10.60 (HO-1) due to the hydroxyl proton attached at *ortho* to acid function. These signals were further verified by ^{1}H–^{1}H COSY experiments (see Figure 2 and Table 1).

The carbon spectrum of **1** showed altogether 10 carbon signals which were further sorted out with the aid of DEPT experiments into a methyl, a methylene, three methines, and remaining quaternary carbons. The methyl and methylene signals appeared at δ 20.9 and 28.4, respectively. The carbinylic carbon resonated at δ 76.0 while two aromatic methines exhibited their resonances at δ 124.0 (C-4) and 116.0 (C-5). Among the five quaternary carbons, the most downfield signal at δ 169.9 was associated with the acid function in the molecule. The signals for two hydroxyl-containing quaternary carbons were located in the spectrum at δ 156.3 (C-2) and 143.5 (C-3). The last quaternary carbon signal resonated at δ 124.4 (C-6). A complete picture of carbon spectrum of **1** is given in Table 2.

Assignments of various protons and their associated carbons in the NMR spectra of **1** were correlated via HMQC experiments and cross-checked with the aid of HMBC correlations (Figure 2).



1 R' = OH, R" = H (formoic acid A)
2 R' = H, R" = OH (formoic acid B)
3 R' = H, R" = H (flemingipanic acid)

Figure 1. Structures of compounds **1**–**3**.

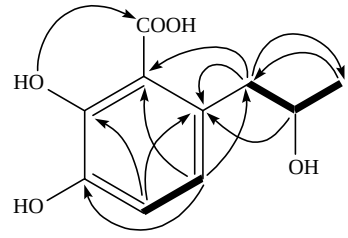


Figure 2. HMBC (⟶) and ^{1}H–^{1}H COSY (▬) correlations in **1**.

Table 1. The ^{1}H NMR (600 MHz) spectral data of compounds **1**–**3** in $CDCl_3$.

H No.	Formoic acid-A (**1**) δ ppm (J in Hz)	Formoic acid-B (**2**) δ ppm (J in Hz)	Flemingipanic acid (**3**) δ ppm (J in Hz)
3	–	6.98 (1H, br d, $J = 8.4$)	6.67 (1H, br d, $J = 7.2$)
4	6.95 (1H, d, $J = 9.0$)	7.53 (1H, dd, $J = 8.4, 7.2$)	7.38 (1H, dd, $J = 8.4, 7.2$)
5	6.77 (1H, d, $J = 9.0$)	7.02 (1H, br d, $J = 7.2$)	6.87 (1H, br d, $J = 8.4$)
1′	3.12 (1H, dd, $J = 16.8, 3.0$), 2.66 (1H, dd, $J = 16.8, 11.4$)	4.57–4.63 (2H, m, overlapped with H-2′)	2.92 (2H, br d, $J = 7.2$)
2′	4.68 (1H, m)	4.57–4.63 (2H, m, overlapped with H-1′)	4.72 (1H, m)
3′	1.52 (3H, d, $J = 6.6$)	1.50 (3H, d, $J = 6.0$)	1.50 (3H, d, $J = 6.1$)
OH-1	10.60 (1H, s)	10.98 (1H, s)	11.0 (1H, s)

On the bases of the above spectral information and comparison with flemingipanic acid (**3**) isolated from *Flemingia paniculata* [16] and also isolated by us from *C. formicarum* (see Tables 1 and 2), the structure of above discussed compound is elucidated as **1** and named formoic acid A. This compound is a new addition in natural salicylic acid derivatives.

The second compound **2** of the same formula was found quite similar when comparing with the NMR spectral data of **1** and **3**. The skeleton of **2** is based on di-*ortho*-substituted benzoic acid and the three aromatic protons appeared at δ 6.98 (br d, $J = 8.4$ Hz, H-3), 7.53 (dd, $J = 8.4, 7.2$ Hz, H-4), and 7.02 (br d, $J = 7.2$ Hz, H-5). The signals of their associated carbons were found in the ^{13}C NMR spectrum of **2** with the help of HMQC experiments at δ 117.9 (C-3), 136.9 (C-4), and 116.2 (C-5). The two adjacent carbinylic protons resonated in the range between δ 4.57 and 4.63 as an overlapped multiplet while their carbons appeared at δ 79.9 (C-1′) and 69.2 (C-2′) in the NMR spectra. The remaining data of **2** are described in Tables 1 and 2, and in the experimental section. The obtained NMR spectral data were finally attested with the aid of ^{1}H–^{1}H COSY and HMBC experiments (Figure 3). This compound is named formoic acid B and is another new addition in natural salicylic acid derivatives.

In addition to flemingipanic acid (**3**) [16], three more known constituents have been isolated for the first time from our investigated source. They include martynoside [17], verbascoside [18], and seguinoside K [19].

Figure 3. HMBC (⟶) and ^{1}H–^{1}H COSY (▬) correlations in **2**.

Table 2. The ^{13}C NMR (150 MHz) spectral data of compounds **1**–**3** in $CDCl_3$.

C No.	Formoic acid-A (**1**) δ ppm (mult.)	Formoic acid-B (**2**) δ ppm (mult.)	Flemingipanic acid (**3**) δ ppm (mult.)
C-1	108.4 (s)	106.6 (s)	108.3 (s)
C-2	156.3 (s)	162.2 (s)	162.2 (s)
C-3	143.5 (s)	117.9 (d)	116.2 (d)
C-4	124.0 (d)	136.9 (d)	136.1 (d)
C-5	116.0 (d)	116.2 (d)	117.8 (d)
C-6	124.4 (s)	141.0 (s)	139.4 (s)
C-1′	28.4 (t)	79.9 (d)	34.6 (t)
C-2′	76.0 (d)	69.2 (d)	76.0 (d)
C-3′	20.9 (q)	18.0 (q)	20.7 (q)
COOH	169.9 (s)	168.5 (s)	170.2 (s)

3. Experimental

3.1 General experimental procedures

Optical rotations were measured on a JASCO DIP-360 (Japan Spectroscopic Co. Ltd, Tokyo, Japan) digital polarimeter. The UV spectra were recorded on a Shimadzu UV-240 spectrophotometer (Shimadzu Corporation, Tokyo, Japan) while IR spectra on a Shimadzu IR-460 instrument. The ^1H NMR and ^{13}C NMR spectra were recorded at 600 and 150 MHz, respectively, on a Bruker AM 600 spectrometer using TMS as an internal standard. The mass spectra were scanned on a Jeol-JMS HX-110 mass spectrometer. Purification of compounds was performed on a recycling preparative HPLC (JAI, Model # LC-908W) with a normal phase preparative column (JAI, SIL, SH-043-15).

3.2 Plant material

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

3.3 Extraction and isolation

The collected leaves (13.5 kg) were dried under the shade for a week and then ground into a powder. The dried and powered material (6.0 kg) was then soaked in ethanol (12 liters) for 6 days at room temperature. The resulted extract was concentrated at low temperature on a rotary evaporator to avoid thermal decomposition of natural constituents. The obtained crude ethanolic extract (84.5 g) was subjected to silica gel column chromatography using hexane, hexane: ethyl acetate, ethyl acetate, and ethyl acetate: methanol as mobile phase. Fractions eluted with 15% ethyl acetate in hexane were pooled on the bases of same TLC profiles and further purified by a recycling preparative HPLC connected with a normal phase preparative column using hexane–ethyl acetate (3:17) as a mobile phase. Compounds **1**–**3** were obtained as amorphous powders. Fractions eluted with 5% methanol in ethyl acetate during silica gel column chromatography, were pooled on the bases of same TLC profiles and further purified by HPLC connected with a reversed phase column using water–methanol as a mobile phase. Martynoside [17] (4.5 mg), verbascoside [18] (10.0 mg), and seguinoside K [19] (5.1 mg) were obtained as gummy substances.

3.3.1 Formoic acid A (*1*)

(4.6 mg). $[\alpha]_D^{28}$: 10.2 (*c* 0.913, $CHCl_3$). UV ($CHCl_3$) λ_{max} (log ε): 246.7 (2.91) nm. IR ($CHCl_3$) ν_{max}: 3120 (OH), 2971 (aromatic

CH), 1680 (C═O) cm^{-1}. ^1H NMR spectral data ($CDCl_3$): see Table 1. ^{13}C NMR spectral data ($CDCl_3$): see Table 2. EI-MS *m/z*: 194 $[M - H_2O]^+$(100%), 176, 165, 83, 79, 71, 57. HR-EI-MS *m/z*: 194.0553 $[M - H_2O]^+$ (calcd for $C_{10}H_{10}O_4$, 194.0579).

*3.3.2 Formoic acid B (**2**)*

(3.9 mg). $[\alpha]_D^{28}$: 12.1 (*c* 0.624, $CHCl_3$). UV ($CHCl_3$) λ_{max} (log ε): 248.9 (3.35) nm. IR ($CHCl_3$) ν_{max}: 3400 (broad OH), 2927 (aromatic CH), 1675 (C═O) cm^{-1}. ^1H NMR spectral data ($CDCl_3$): see Table 1. ^{13}C NMR spectral data ($CDCl_3$): see Table 2. EI-MS *m/z:* 194 $[M - H_2O]^+$, 177, 121, 150, 79. HR-EI-MS *m/z:* 194.0572 $[M - H_2O]^+$ (calcd for $C_{10}H_{10}O_4$, 194.0579).

*3.3.3 Flemingipanic acid (**3**)*

(4.2 mg). UV (MeOH) λ_{max} (log ε): 334 (3.16) nm. IR (KBr) ν_{max}: 3200 (OH), 1680 (C═O) cm^{-1}. ^1H NMR spectral data ($CDCl_3$): see Table 1. ^{13}C NMR spectral data ($CDCl_3$): see Table 2. EI-MS *m/z*: 178 $[M - H_2O]^+$, 160, 134, 106, 78, 51 [18].

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